RNA

Ribosome-guided piRNA production

The ribosome decodes messenger RNAs and constructs proteins based on the genetic blueprint. Ribosomes also associate with non-coding RNAs, such as PIWI-interacting RNA (piRNA) precursors, during the meiotic pachytene stage. Intriguingly, the ribosome mediates pachytene piRNA biogenesis by guiding endonucleolytic cleavage of piRNA precursors.

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PIWI-interacting RNA (piRNA) is the most diverse group of small non-coding RNA molecules found in animals. Highly expressed in germ cells, piRNAs protect the germline genome from transposons by interacting with the PIWI subfamily of Argonaute proteins[1]. By silencing transposons, piRNAs are essential for spermatogenesis from worms to humans. Previous studies have revealed that the sequences of piRNAs are highly diverse (over 1 million unique sequences) but poorly conserved across species[2]. Unlike microRNAs (miRNAs) and small interfering RNAs (siRNAs), piRNAs are generated via Dicer-independent processing[3]. Additionally, piRNAs are longer (24–35 nt) than miRNAs and siRNAs (21–22 nt). The mouse germ cells express two classes of piRNAs at the pre-pachytene and pachytene stages of meiosis. Pre-pachytene piRNAs contain repetitive sequences and are likely derived from transposons. As pachytene piRNA clusters lack transposon sequences or other repetitive elements, the biogenesis of pachytene piRNAs remains an open question.

The remarkable heterogeneity of piRNAs is consistent with the notion that most piRNAs are derived from long, continuous single-strand transcripts[4]. Their 5′-monophosphate ends are believed to be formed when their precursors are cleaved into tens of thousands of fragments. At least two highly related biogenesis pathways contribute to piRNA production[1]. First, production of piRNAs can be started by an initiator piRNA-guided endonucleolytic cut of complementary target transcripts[5]. This process is known as the ping-pong cycle, which amplifies pre-existing piRNAs. Second, the production of piRNAs follows a 5′ → 3′ stepwise manner by slicing precursors, thereby generating non-overlapping fragments[6,7]. The 'phased' production is thought to be triggered by the slicer activity of PIWI proteins directed by complementary initiator piRNAs. A mitochondria-associated endonuclease PLD6 is believed to mediate subsequent cleavage of piRNA precursors, generating the 5′ uridine bias characteristic of piRNAs[8]. Given the non-repetitive sequence feature of pachytene piRNAs, one important question is how the phased cleavage sites are designated on piRNA precursors.

Notably, pachytene piRNA precursors share many features with protein-coding RNAs[4]. Both are synthesised by RNA

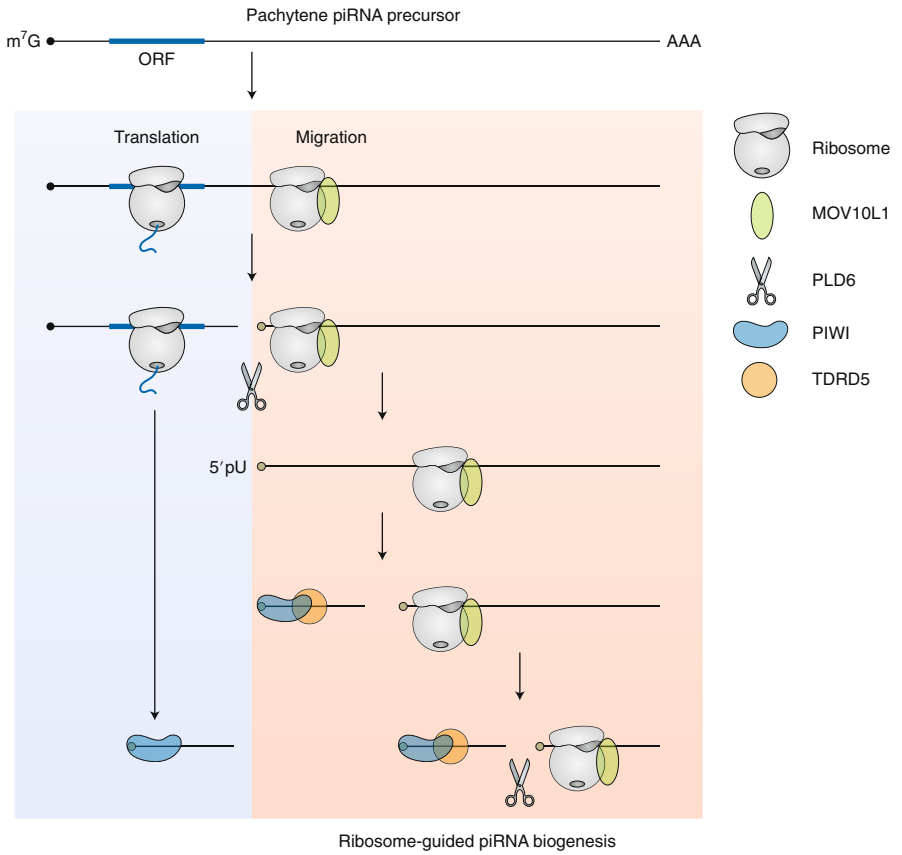

Fig. 1 | Ribosome-guided pachytene piRNA biogenesis. The pachytene piRNA biogenesis involves two distinct mechanisms depending on different regions of the precursor. Ribosomes load onto pachytene piRNA precursors and translate putative ORFs akin to conventional mRNAs. The terminating ribosomes migrate into the downstream region with the help of MOV10L1 helicase. The subsequent endonucleolytic cleavage by PLD6 generates 5′ phosphorylated uridine (5′ pU), which is followed by TDRD5-facilitated PIWI binding. Continuous ribosome migration gives rise to sequential production of non-overlapping piRNAs from the precursor. By contrast, piRNAs derived from upstream ORFs follow a mechanism independent of MOV10L1 and TDRD5. The common downstream piRNA maturation steps are not shown. ORF, open reading frame; MOV10L1, Mov10 like RISC complex RNA helicase I; PLD6, phospholipase D family member 6; TDRD5, tudor domain-containing 5.

polymerase II, followed by 5′ capping, splicing and polyadenylation. In mouse cells, pachytene piRNAs are bound by the two PIWI proteins MILI and MIWI. Early studies suggest an association of MIWI and the protein translation machinery[9], indicating a possible connection between pachytene piRNA biogenesis and ribosomes. In addition, the size of piRNAs is also reminiscent of ribosome footprints (~28 nt). In this issue of *Nature Cell Biology*, Sun et al.[10] demonstrate that ribosomes may guide piRNA biogenesis by defining the cleavage sites on pachytene piRNA precursors.

The first evidence came from the observation that pachytene piRNA precursors, and several proteins involved in piRNA biogenesis, were co-sedimented with ribosomes in polysome fractions separated by sucrose gradients. Notably, ribosome profiling (Ribo-seq) revealed abundant ribosome footprints mapped to piRNA precursors. However, great caution must be taken when interpreting these results, as some of the reads could be derived from PIWI-bound intermediates or other RNA–protein complexes. Sun et al. provide several lines of evidence supporting the active ribosome engagement on piRNA precursors[10]. First, piRNA-associated reads resembled the ribosome footprints generated from mRNAs. Second, those piRNA footprints were sensitive to harringtonine treatment, which leads to an accumulation of ribosomes at putative translation initiation sites. Third, by using RiboTag mice bearing hemagglutinin-tagged Rpl22, a ribosomal large subunit protein, the authors captured piRNA-associated reads from purified ribosomes. Therefore, the authors concluded that the piRNA footprints represent genuine ribosome-protected fragments, a strong indication of ribosome engagement on piRNA precursors.

A growing body of evidence suggests that more than half of the long non-coding RNAs (lncRNAs) in the cytoplasm associate with ribosomes[11]. These ribosomes stabilise lncRNAs, or translate short open reading frames (ORFs) embedded on lncRNAs. Supporting active translation of piRNA precursors, Sun et al.[10] report that ribosome footprints mapped to putative ORFs of piRNAs show strong 3-nt periodicity. The active translation of piRNAs was further confirmed by micropeptide detection in the testes of Flag-knockin mice created via CRISPR–Cas9 gene editing. Unexpectedly, the authors observed prevailing ribosome footprints in the downstream region of the ORFs. Intriguingly, ribosome density in the ORF downstream region positively correlated with piRNA abundance. Depletion of piRNA-engaged ribosomes using puromycin treatment reduced the abundance of piRNA generated from the downstream region of the ORFs. As the 5′ end of the ribosome footprints in the ORF downstream region corresponded to the 5′ position of piRNAs, the authors proposed that the ribosomes define the position of pachytene piRNAs.

What could be the mechanism underlying non-canonical ribosome engagement in the ORF downstream region? As the reads uncovered from this region lack the 3-nt codon periodicity, it is unlikely the consequence of stop codon readthrough. A previous study has reported that MOV10L1, a 5′→3′ ATP-dependent RNA helicase, binds to piRNA precursors and is required for endonucleolytic cleavage of piRNA precursors[12]. Following this lead, the authors found that MOV10L1 is responsible for continuous ribosome migration after ORF translation. Indeed, the density of ribosome footprints in the downstream region of the ORFs decreased in the absence of MOV10L1. This unexpected finding suggests that pachytene piRNA biogenesis may involve two distinct mechanisms, depending on the region of the piRNA precursors. In support of this notion, production of piRNAs derived from the ORF is not affected by MOV10L1 depletion. The dichotomy of pachytene piRNA biogenesis is in line with the finding that tudor domain-containing 5 (TDRD5) is not required for the 5′ end processing of pachytene piRNA precursors but is indispensable for downstream processing[13]. Similarly, in *Tdrd5* mutant mice, piRNA footprints derived from the ORF downstream region were largely depleted. Collectively, a ribosome-centered working model in pachytene piRNA biogenesis emerges (Fig. 1).

It is worth noting that the non-repetitive pachytene piRNAs from long intergenic regions are only found in vertebrates. In addition to mice, the authors nicely show that similar piRNA processing also exists in roosters and green lizards. Without transposon sequences, pachytene piRNAs may not function to silence transposons as originally suggested for pre-pachytene piRNAs. A previous study reported that pachytene piRNAs promote the clearance of targeted protein-coding RNAs during late spermiogenesis[14]. It is possible that the evolutionary divergence of piRNAs necessitates a more creative way for their biogenesis. The repurposing of ribosomes might be a solution, as pachytene piRNA precursors resemble mRNAs in many aspects.

The unconventional function of ribosomes in pachytene piRNA biogenesis is exciting, but the work of Sun et al.[10] also brings up a wealth of questions. For instance, it is currently unclear how MOV10L1 prevents ribosome dissociation after translation. One possibility is that MOV10L1 acts on ribosome recycling at the post-termination stage. Inefficient recycling of ribosomes at the stop codon could lead to re-initiation of translation in the downstream region. It will be interesting to explore whether MOV10L1 promotes re-initiation in non-germ cells. Another interesting question is whether ribosome-phased endonucleolysis occurs on mRNAs. A similar process called ribothrypsis has been reported in mammalian cells as part of the RNA decay pathway[15]. However, neither the identity of ribosome-associated endonucleases nor the cellular function of RNA fragments is known. Clearly, the ribosome, as one of the largest RNA–protein complexes, has a much broader cell biological role than previously thought. A growing body of evidence indicates that ribosome serves as a hub in mediating RNA surveillance, monitoring protein quality control, coordinating signalling pathways, and controlling cellular metabolism. A full understanding of the intricate ways in which all those processes are interconnected and orchestrated by ribosomes will be a worthwhile endeavor of the next decade or two. ❐

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Competing interests

The authors declare no competing interests.