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Probing a piRNA paradox

As a common mechanism to protect genome stability, transcription of repetitive DNA (such as transposons) typically results in processing into small RNAs to initiate sequence-specific heterochromatinization of those elements genome-wide. However, a paradox then emerges as to how the resultant heterochromatin (which is largely compacted and transcriptionally repressed) can still serve as a source of small RNAs to maintain the heterochromatic state. A new study in *Drosophila melanogaster* shows how heterochromatin histone marks can recruit specialized transcription machinery to generate PIWI-interacting RNAs (piRNAs).

Andersen *et al.* sought to understand how piRNA cluster loci in *D. melanogaster* are bidirectionally transcribed despite lacking overt promoter DNA sequences and being in heterochromatin that harbours the classic repressive mark histone H3 lysine trimethylation (H3K9me3). Deleting promoters in DNA flanking the 42AB and 80F piRNA clusters indicated that these piRNA clusters were not transcribed via readthrough from flanking regions. Instead, cap sequencing (Cap-seq) to pinpoint the 5′ end of transcripts revealed pervasive initiation of transcription from many sites within the piRNA clusters.

To identify potential molecular mediators of this transcription initiation within piRNA clusters, the authors mined data from a published transposon derepression screen, focusing on hits that have sequence similarity to known transcription initiation machinery. They identified CG12721 as a potential ovary-specific paralogue of the large subunit of transcription factor IIA (TFIIA-L). Its role as a driver of piRNA cluster transcription was supported by its enrichment at piRNA clusters, its interactions with other transcription initiation proteins and, because its knockout led to depleted piRNA expression from the 42AB and 80F piRNA clusters, transposon derepression and fly sterility. Based on this function, the authors named CG12721 'Moonshiner' in reference to its activity in the face of the transcriptional 'prohibition' of the heterochromatin environment.

Further investigations revealed a more complete mechanistic understanding of Moonshiner-mediated transcription at piRNA clusters. Compared with TFIIA-L, Moonshiner lacks the carboxy-terminal domain that would allow it to be recruited to promoters bound by TATA-box binding protein (TBP). Instead, the authors characterized a physical interaction between Moonshiner and the Rhino–Deadlock complex as a mechanism to recruit Moonshiner to piRNA cluster loci. Using a variety of genetic manipulations and tethering experiments the investigators showed that Moonshiner can then recruit the TBP homologue TRF2 to form an alternative TFIIA–TRF2 complex to drive piRNA transcription.

The identification of Rhino-mediated Moonshiner recruitment helps to explain the apparent paradox of how heterochromatin can be transcribed to produce piRNAs. Rhino is a heterochromatin protein 1 (HP1) paralogue that binds to H3K9me3, hence transcription is triggered by a heterochromatic histone mark, rather than requiring DNA-binding transcription factors to bind to their inaccessible DNA target motifs in compacted heterochromatin.

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Furthermore, Rhino acts to divert transcripts into piRNA processing pathways rather than mRNA maturation, thus minimizing the potential genome instability threat of generating full-length transposon transcripts from piRNA clusters.

It will be interesting to determine how evolutionarily pervasive it is to specify small-RNA source loci through chromatin marks rather than DNA sequence motifs. Andersen *et al.* note that although Rhino is not conserved beyond fruit flies, SHH1 has a similar role in plants by binding to methylated H3K9 to recruit RNA polymerase IV for the transcription of small-RNA precursors in the RNA-directed DNA methylation (RdDM) pathway. As further support that this principle may be widespread, a recent separate study by Jih *et al.* demonstrated that in the fission yeast *Schizosaccharomyces pombe* the subdomains of heterochromatin marked by H3K9me2 (rather than H3K9me3) are sources of small RNAs for heterochromatin formation.

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> " transcription is triggered by a heterochromatic histone mark "

ORIGINAL ARTICLES Andersen, P. R. *et al.* A heterochromatin-dependent transcription machinery drives piRNA expression. *Nature* http://dx.doi.org/10.1038/nature23482 (2017) | Jih, G. *et al.* Unique roles for histone H3K9me states in RNAi and heritable silencing of transcription. *Nature* **547**, 463–467 (2017)
FURTHER READING Holoch, D. & Moazed, D. RNA-mediated epigenetic regulation of gene expression. *Nat. Rev. Genet.* **16**, 71–84 (2015)

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