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Misprocessed tRNA response targets piRNA clusters

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Germline PIWI-interacting RNA (piRNA) clusters have a characteristic transcriptional status; although they do not have their own promoters, both genomic strands are transcribed, while splicing and 3′ processing signals are neglected. How this transcription is maintained remains unknown. Molla-Herman *et al* (2015) discovered that mutations in a tRNA processing factor cause the loss of transcription at some piRNA clusters, leading to sterility in *Drosophila melanogaster*. This defect in piRNA cluster transcription is restored by mutations in genes required for the DNA damage checkpoint or replication.

See also: **A Molla-Herman *et al*** (December 2015)

Growing cells are at risk of genome instability during replication. One source of this instability is replication fork blockage, because this can induce collapse of the replisome, leading to a double-strand break (DSB) (Branzei & Foiani, 2010). A DSB is the most deleterious form of DNA damage; however, to affect repair, cells are equipped with the DNA damage response pathway, which consists of elaborate machinery and includes proteins such as Chk2 (Ciccia & Elledge, 2010). This pathway triggers the DNA repair pathway and simultaneously arrests the cell cycle through p53 activation. DSBs are occasionally triggered by transcription–replication interference mediated by R-loop formation (Helmrich *et al*, 2013). An R-loop is a RNA–DNA hybrid structure enriched at RNA polymerase-pausing sites. This structure causes genome instability, possibly by blocking replication fork progression. Interestingly, mutations in several RNA-processing factors such as mRNA 3′-processing factors cause the accumulation of R-loops and concurrent DNA damage (Helmrich *et al*, 2013).

tRNA gene clusters are highly transcribed regions, making these sites collision hot spots between Pol III and the replication machinery. This conflict causes a frequent replication fork arrest, which can result in DNA lesions, namely DNA fragile sites (Helmrich *et al*, 2013). However, it is not known whether defects in tRNA processing affect the genome stability.

During the tRNA maturation process, 5′ leaders are removed from initial transcripts by RNase P and 3′ trailers are cleaved off by RNase Z (Jarrous & Gopalan, 2010). In this issue of *The EMBO Journal*, Molla-Herman and colleagues report that mutations in *D. melanogaster Rpp30*, a conserved subunit of RNase P, cause defects in oogenesis, leading to sterility (Molla-Herman *et al*, 2015). *Rpp30* mutations affect tRNA processing, resulting in the accumulation of tRNA transcripts with both 5′ leader and 3′ trailer sequences. However, the same mutations do not appear to affect the size and quantity of most mature tRNAs. This suggests that the observed defects in oogenesis are not caused by a shortage of mature tRNAs but rather result from the accumulation of misprocessed tRNAs. Mice that are mutant for the pre-tRNA splicing factor, CLP1, accumulate misprocessed tRNA fragments, leading to a progressive loss of motor neurons, while cells are sensitized to p53 activation in response to oxidative stress (Hanada *et al*, 2013). These findings prompted Molla-Herman *et al* (2015) to observe that the arrest of oogenesis in *Rpp30* mutants is partially rescued by inactivating p53, although double mutant flies remain sterile. In *Drosophila*, DNA damage activates ATM/Chk2 signaling, which in turn activates p53. Fertility is restored in *Rpp30; chk2* (a.k.a., *mnk*) double-mutant flies, suggesting that accumulation of misprocessed tRNAs in *Rpp30* mutants triggers the activation of DNA damage checkpoints.

Interestingly, Molla-Herman *et al* (2015) observed that PCNA, a key component of the replication machinery, was mostly lost from the nucleus in *Rpp30* mutants, suggesting a collapse of replication forks. Pol III also formed aggregates in *Rpp30* mutant nuclei, which may also cause replication fork collapse and replication stress. This was corroborated with the finding that mutations in *claspin*, a gene involved in the replication stress checkpoint (Ciccia & Elledge, 2010), partly rescue the oogenesis defect in *Rpp30* mutants. From these results, Molla-Herman *et al* (2015) conclude that *Rpp30* mutations increase DNA replication stress in the ovary.

RNA-seq analysis then revealed a surprise. The number of piRNA cluster transcripts was decreased in *Rpp30* mutants, which coincided with cluster loci having decreased levels of H3K9me modification, a well-characterized repressive histone mark, and de-repression of some transposable elements (TEs). piRNAs are processed in a Dicer-independent manner from long (~150 Kb) precursor transcripts and protect the genome in animal gonads by repressing the threat of TE-mediated insertion mutagenesis (Iwasaki *et al*, 2015). They are transcribed from special genomic regions, called piRNA clusters, which contain a large number of truncated TEs and are mostly located in heterochromatic regions. In *Drosophila*, two major types of piRNA cluster exist: some are transcribed uni-directionally ("uni-strand" clusters) and many others are transcribed from both genomic strands ("dual-strand" clusters) (Iwasaki *et al*, 2015). Transcription from uni-strand clusters

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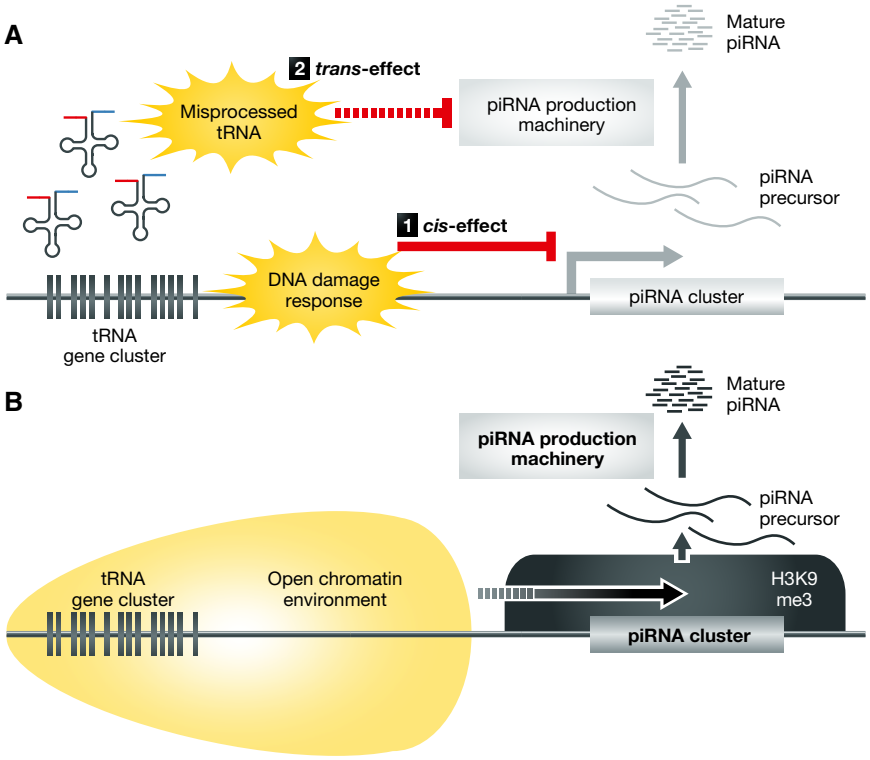

Figure 1. How tRNA misprocessing affects piRNA clusters.
(A) *cis*-effect (1): In *Rpp30* mutants, the DNA damage response triggered by replication fork collapse inhibits piRNA cluster transcription. *trans*-effect (2): In *Rpp30* mutants, misprocessed tRNAs themselves inactivate the piRNA production machinery, leading to piRNA loss. (B) In wild type, transcription at tRNA genes confers a chromatin environment suitable for piRNA transcription.

including *flamenco* locus is initiated from discrete promoter regions, which gives rise to long precursor transcripts with short motifs that recruit Yb, a core component of the piRNA-processing machinery (Ishizu *et al*, 2015). In contrast, dual-strand clusters do not have their own promoters but instead appear to be transcribed as read-through products from flanking genes (Mohn *et al*, 2014). While it is counterintuitive, dual-strand cluster transcription requires loci to be H3K9me modified, which is recognized by a germline-specific HP1 family protein, Rhino (Klattenhoff *et al*, 2009).

Molla-Herman *et al* (2015) also found that the levels of piRNAs derived from dual-strand clusters were dramatically decreased in *Rpp30* mutants, while uni-strand piRNAs were produced normally. piRNA production from dual-strand clusters was restored in *Rpp30; chk2* double mutants. Intriguingly, dual-strand clusters, but not uni-strand clusters, often reside near tRNA gene clusters. This correlation led Molla-Herman *et al* (2015) to propose that replication stress at tRNA gene loci may cause a *cis*-effect on nearby dual-strand piRNA clusters to arrest their transcription, probably by affecting chromatin structure (Fig 1A). Wild-type tRNA gene clusters may, therefore, promote a permissive transcription conformation for dual-strand piRNA clusters located in heterochromatin (Fig 1B) (Yamanaka *et al*, 2014). Supporting this model, tRNA gene loci have been implicated in various large-scale gene regulatory processes, including insulator activity and establishment of chromatin boundaries (Noma *et al*, 2006). In this regard, it will be interesting to see whether the expression of genes located near tRNA gene loci is affected in *Clp1*-mutant mice, particularly in their brains where misprocessed tRNA fragments accumulate (Hanada *et al*, 2013). However, the possibility remains that the misprocessed tRNAs may act in *trans* to specifically affect factors required for dual-strand cluster transcription, such as Rhino (Fig 1A). In this case, it is difficult to explain how piRNA production from dual-strand clusters is restored in *Rpp30; chk2* double mutants. For either scenario, the findings of Molla-Herman *et al* (2015) provide clues as to how dual-strand piRNA clusters are transcribed and will help to answer one of the most outstanding questions in the piRNA research field.

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