Bioinformatics analysis of small RNA libraries identifies Loquacious-PD as exclusively required for production of hairpin-derived, but not transposon or *cis*-NAT, siRNAs

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Small non-coding RNA molecules play important roles in normal genome regulation, pathologic misregulation, and genome defense. In *Drosophila melanogaster*, the biogenesis of small interfering RNAs (siRNAs) and microRNAs (miRNAs) requires the coupled activity of a Dicer RNAse and a partner dsRNA-binding protein (dsRBPs). MiRNAs processed through Dicer-1 often partner with the PB isoform of Loquacious (Loqs-PB) to tune Dicing specificity, while exogenous siRNA production couples Dicer-2 with R2D2. The PD isoform of Loquacious (Loqs-PD) has been shown to serve a distinct function in processing endogenous siRNAs, yet a complete understanding of endogenous siRNA biogenesis remains elusive because of potential involvement of both R2D2 and distinct Loqs isoforms across endo- and exo-siRNA pathways.

Through bioinformatics analysis of small RNA seq data from S2 Loqs-ORF KO cells rescued with individual Loqs isoforms (PA, PB, and PD), we show that production of endogenous hairpin siRNAs (hpRNAs), but not other endo-siRNAs, has a nearly absolute requirement for Loqs-PD. Loqs KO cells produced virtually no hpRNAs, and hpRNA production was exclusively rescued by the loqs-PD isoform. Libraries rescued with loqs-PD alone did not show a significant increase in transcripts associated with non-hairpin Transposon (TE), cis-natural antisense (*cis*-NAT) and other published endo-siRNA loci.

We then investigated other published small RNA datasets and confirmed that knockout of loqs drastically reduces the production of endogenous siRNA loci, while knockout of r2d2 result in a similar non-hairpin endo-siRNA profile as wild-type cells. Analysis of several data sets suggest a model in which the PD isoform of loqs is absolutely required for hpRNA production, but -- possibly in combination with other loqs isoforms or even r2d2 -- plays more of a helper role in the maturation of non-hairpin derived siRNAs.