# Heterochromatin-dependent transcription of satellite DNAs in the *Drosophila* female germline

Xiaolu Wei[1], Danna G. Eickbush[2], Iain Speece[2], Amanda M. Larracuente[2]
[1]Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY 14642
[2]Department of Biology, University of Rochester, Rochester, NY 14627

## Abstract

Satellite DNAs (satDNAs) are tandemly repeated DNAs found primarily near centromeres, telomeres, and on sex chromosomes. They can make up to half or more of some eukaryotic genomes. Although typically thought of as junk DNAs, recent studies show that satDNAs play important roles in chromosome segregation, chromosome recognition, and maintaining genome stability. Abnormal satDNA activity is associated with chromosome missegregation, aging, and cancer. Despite its association with important phenotypes, we currently know little about satDNA maintenance at the chromatin level, or if satDNAs have specific functions. Previous studies have reported satDNA-derived transcripts. However, whether or not satDNA expression is regulated—and if so, how—remain open questions. Using the Drosophila germline as a model system, we characterized the expression pattern and regulatory network of satDNAs using a combination of genomic, cytological, and molecular approaches. Our data revealed that the satDNAs are transcribed into long noncoding RNAs (lncRNAs) and then processed into small RNAs in the germline, in a way resembling piRNAs (PIWI interacting RNAs), a subset of small RNAs that function to repress transposable elements (TEs) to maintain genome stability. Moreover, we found that the satDNA piRNA production is regulated by the same piRNA pathway components as the dual-strand cluster *42AB*. Taken together, our findings suggest that satDNAs are regulated by piRNAs originating from their own genomic loci. This novel mechanism for satDNA regulation provides insight into general features important for understanding the roles of satDNAs in the germline.

## Results

### Small RNA-seq data shows that SatDNA generates piRNAs in the germline.

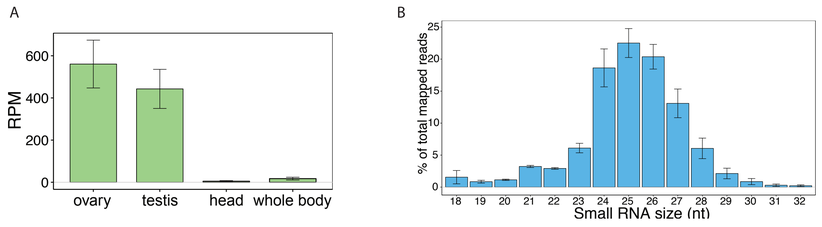
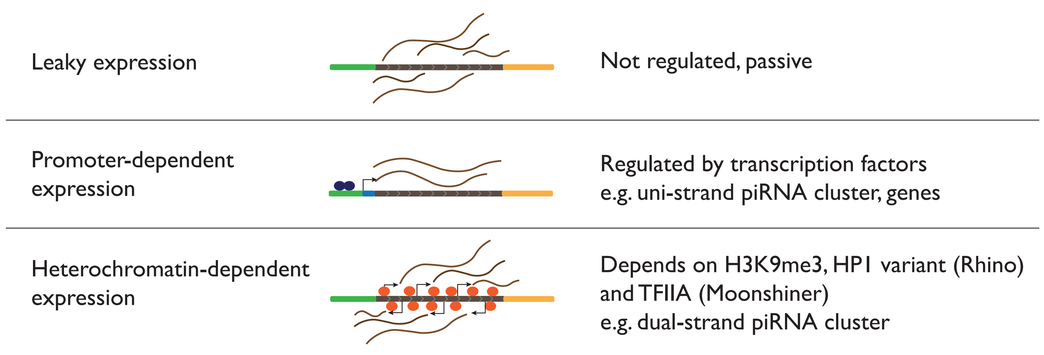



Fig1. (A) *Responder* (*Rsp*) satDNA small RNAs are mainly expressed in ovary and testis. RPM (Reads Per Million) is used to quantify expression levels. (B) Size distribution of *Rsp* small RNAs in ovary and testis shows majority of the small RNAs are piRNAs (data from Andersen et al., 2017; Fagegaltier et al., 2014; Ghildiyal et al., 2010; Mohn et al., 2014; Parhad et al., 2017; Rozhkov et al., 2010).

### Is satDNA expression regulated?




### Northern blot shows that *Rsp* satDNA expression level in different strains correlates with its genomic repeat number.

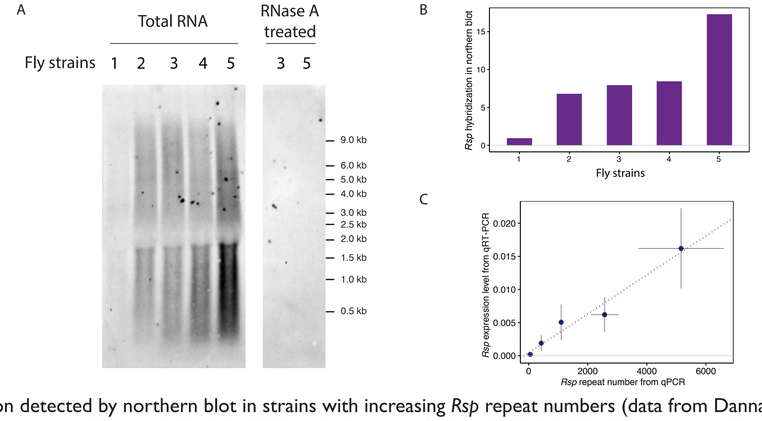



Fig2. *Rsp* expression detected by northern blot in strains with increasing *Rsp* repeat numbers (data from Danna G. Eickbush). Fly strains (*Rsp* copy number): 1. ZW144 (238), 2. Ral357 (640); 3. Iso-1 (1100); 4. Ral380 (2339); 5. ltpkcnbw (4086). The quantification of signal in northern blot is shown in (B). qPCR quantification of *Rsp* copy number and expression level for strains used in northern blot is shown in (C). *Rsp* expression level correlates with its copy number in the genome (Pearson's correlation coefficient $r^2$=0.93, p-value=0.02 for northern blot result in (B), and $r^2$=0.98, p-value=0.003 for qPCR result in (C)).

### ChIP-seq and Small RNA-seq analysis of piRNA pathway components: satDNAs are regulated by heterochromatin-dependent piRNA pathway.

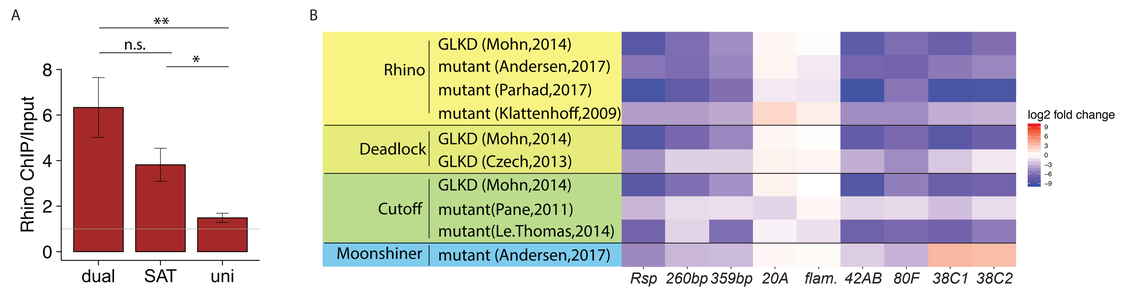
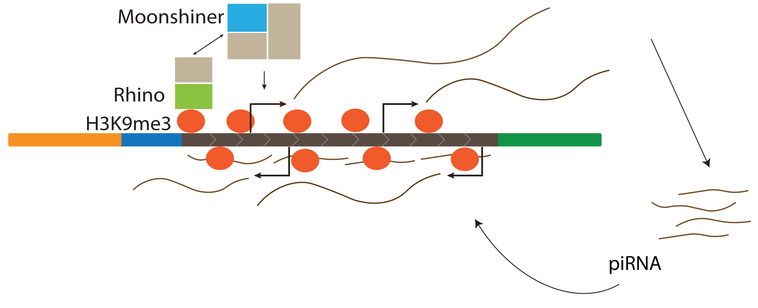



Fig3. SatDNA transcription shows a similar profile to the dual-strand piRNA cluster *42AB*. (A) Rhino ChIP-seq result showing the ChIP/input enrichment scores for dual-strand (dual) and uni-strand (uni) piRNA clusters, and satDNAs (SAT), indicates that satDNAs are enriched for Rhino. P-values are calculated by pairwise t-test with Hochberg adjusted for multiple comparison. * p-value<0.05, ** p-value<0.01 (data from (Parhad et al., 2017; Zhang et al., 2014)). (B) Heatmap showing the quantification of log2 fold change in small RNA-seq data from mutants of *rhino*, *cutoff*, *deadlock*, and *moonshiner* for satDNAs (*Rsp* and *260bp*, *359bp* satellite) and piRNA clusters (*20A*, *flamenco*, *42AB* and *38C1/2*), normalized by miRNA level, indicates satDNAs expression is regulated by heterochromatin-dependent piRNA pathway. GLKD: germline knockdown (data from (Mohn et al., 2014; Andersen et al., 2017; Parhad et al., 2017; Klattenhoff et al., 2009; Czech et al., 2013; Pane et al., 2011; Le.Thomas et al., 2014)).

## Model




SatDNAs are expressed into piRNAs in the germline, under the regulation of heterochromatin-dependent machinery, and these piRNAs may function to establish or maintain heterochromatin at the satellite genomic loci.

## Future Directions

1. Systematically characterize the regulatory pathway of satDNA expression.
2. Study the nature of sperm dysfunction associated with *Rsp* satDNA misregulation.

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