Cell Reports, Volume *37*

Supplemental information

Dual roles for piRNAs in promoting and preventing gene silencing in *C. elegans*

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Supplemental Figures

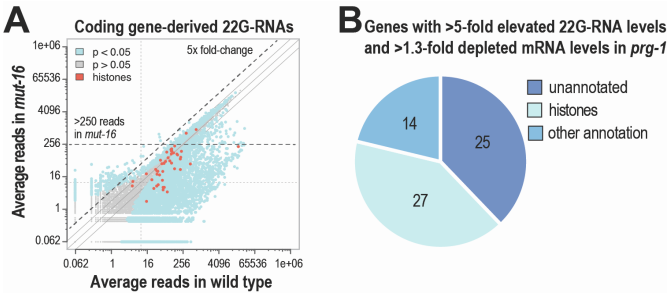

Figure S1. Gene classification of aberrant 22G-RNA targets in *prg-1* mutants. Related to Figure 1. (A) Scatter plot displaying each gene as a function of normalized log_2-transformed small RNA reads in wild type and *mut-16(pk710)* mutants. Note that the axes are reverse transformed to reflect non-transformed values. Hyperaccumulators were classified as genes that yielded an average of >250 normalized reads and which were upregulated >5 fold in *prg-1* mutants, as indicated by the dashed lines. Solid lines above and below the y = x line indicate 2 and -2 fold-changes. Libraries are from dissected distal gonads (n=3 biological replicates for each strain). (B) Pie chart classifying genes that produced >5-fold as many 22G-RNA reads in *prg-1(n4357)* mutants compared to wild type and for which the corresponding mRNA was downregulated >1.3-fold.

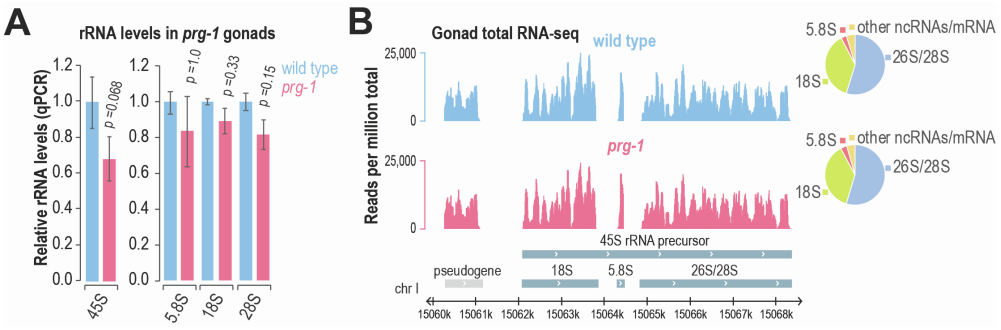

Figure S2. rRNA levels in *prg-1* mutants. Related to Figure 2. (A) Bar chart displaying mean relative rRNA levels in wild type and *prg-1(n4357)* mutant gonads, as determined by qRT-PCR. Error bars are standard deviation of the mean of 3 biological replicates. *rpl-32* or *act-1* levels were used for normalization. P values were calculated using two-sample t-tests and a Bonferroni correction was applied to account for multiple comparisons. (B) High-throughput sequencing read distribution across an rRNA locus (total RNA-seq from dissected gonads). One of three biological replicates for each strain is shown. The inset pie charts show rRNA and non-rRNA reads as a fraction of total reads. rRNA made up ~95% of all reads across each of the 6 libraries.

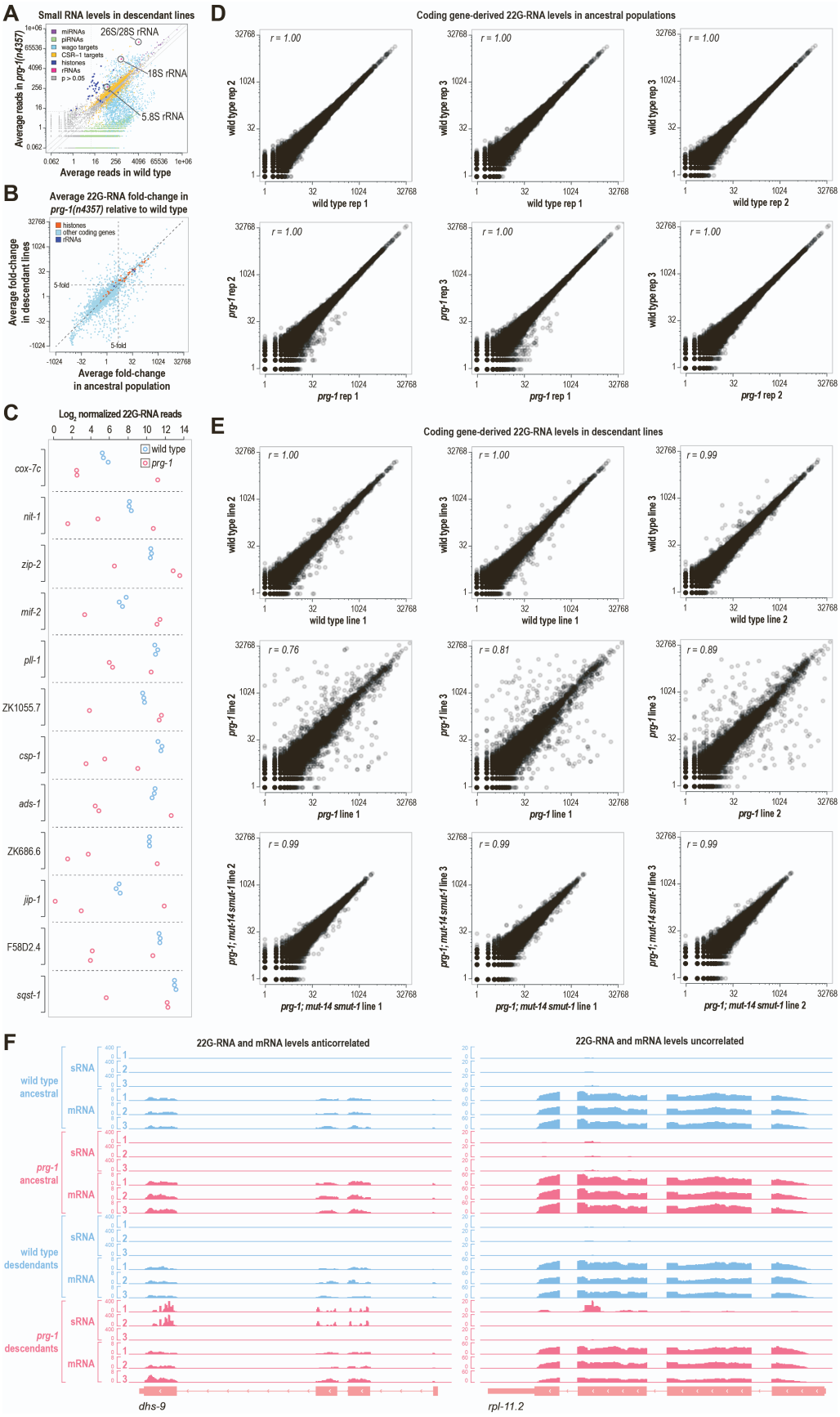
A
Small RNA levels in descendant lines
miRNAs
piRNAs
wago targets
CSR-1 targets
histones
rRNAs
p > 0.05
26S/28S rRNA
18S rRNA
5.8S rRNA
Average reads in prg-1(n4357)
Average reads in wild type
B
Average 22G-RNA fold-change in prg-1(n4357) relative to wild type
histones
other coding genes
rRNAs
5-fold
Average fold-change in descendant lines
Average fold-change in ancestral population
C
Log2 normalized 22G-RNA reads
wild type
prg-1
cox-7c
nit-1
zip-2
mif-2
pll-1
ZK1055.7
csp-1
ads-1
ZK686.6
jip-1
F58D2.4
sqst-1
D
Coding gene-derived 22G-RNA levels in ancestral populations
wild type rep 2
wild type rep 1
wild type rep 3
prg-1 rep 2
prg-1 rep 1
prg-1 rep 3
r = 1.00
E
Coding gene-derived 22G-RNA levels in descendant lines
wild type line 2
wild type line 1
wild type line 3
prg-1 line 2
prg-1 line 1
prg-1 line 3
prg-1; mut-14 smut-1 line 2
prg-1; mut-14 smut-1 line 1
prg-1; mut-14 smut-1 line 3
r = 1.00
r = 0.99
r = 0.76
r = 0.81
r = 0.89
F
22G-RNA and mRNA levels anticorrelated
22G-RNA and mRNA levels uncorrelated
wild type ancestral
prg-1 ancestral
wild type descendants
prg-1 descendants
sRNA
mRNA
dhs-9
rpl-11.2

Figure S3. Widespread stochasticity in 22G-RNA production in *prg-1* mutants. Related to Figure 3. (A) Scatter plot displaying each small RNA feature colored by class as a function of normalized log_2-transformed high-throughput sequencing reads in wild type and *prg-1(n4357)* mutants at 50 generations of continuous growth (whole gravid adult animals, n=3 biological replicates for each strain). Note that the axes are reverse transformed to reflect non-transformed values. Solid lines above and below the y = x line indicate 2 and -2 fold-changes. (B) Scatter plot displaying each coding gene and rRNA as a function of the log_2-transformed fold-change in *prg-1(n4357)* relative to wild type at 1 (ancestral population) and 50 generations (descendant lines) of continuous growth (axes show non-transformed values). Dashed lines mark y = x and the 5 fold-change cutoff used to classify hyperaccumulators. (C) Plots displaying normalized log_2-transformed 22G-RNA reads for several genes in each wild type and *prg-1(n4357)* mutant line after 50 generations of continuous growth (y-axis shows log_2-transformed values). (D-E) Pairwise scatter plots comparing normalized log_2-transformed 22G-RNA reads for each coding gene across biological replicates from the ancestral population (D) or the descendant lines (E) of wild type and *prg-1(n4357)* (y-axis shows log_2-transformed values). *prg-1(n4357); mut-14(mg464) smut-1(tm1301)* comparisons are shown as a control in (E). Pearson correlation values (r) are reported for non-transformed data (degrees of freedom = 20,474, $p < 2.2e-16$ for all comparisons). (F) mRNA and small RNA read distribution across representative gene loci, *dhs-9* and *rpl-11.2*. Three biological replicates or lines are shown for wild type and *prg-1(n4357)*.