Supplemental Information

The RNase PARN-1 Trims

piRNA 3′ Ends to Promote Transcriptome

Surveillance in *C. elegans*

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Supplemental Experimental Procedures

Brood Size and Fertility Test

Brood sizes of adult N2 and outcrossed mutants at generation twelve were scored as described (Batista et al., 2008; Wang and Reinke, 2008). Newly-hatched L1 larvae were placed singly on plates at 25 °C. Once worms reach the adult stage, the brood counts were conducted by transferring them daily to a fresh plate until egg production stopped.

Immunoprecipitation and RNA isolation

200,000 synchronous adult N2 and outcrossed mutants at generation twelve were collected. Samples were suspended in one volume of IP buffer (110 mM Potassium acetate, 2 mM magnesium acetate, 0.1% Tween 20, 0.5 % Triton, 1mM DTT, 20 mM HEPES-KOH, pH 7.5) with 5 X concentration cOmplete protease inhibitors (Roche) and homogenized in a steel homogenizer. Cell extracts were cleared by two rounds of centrifugation at 14,000 x g for 15 min. 12 μg anti-PRG-1(Batista et al., 2008), anti-GFP (mFX73, Wako), or anti-FLAG-antibodies (Sigma) were incubated with 60 μl Protein A/G dynabeads (Life Technologies) at 4°C for 1 h. 20 mg of lysate was added and samples were incubated on a rotator at 4°C for 2 h. Beads were washed three times with IP buffer with protease inhibitors and once with final wash buffer (150 mM NaCl, 2 mM magnesium acetate, 50 mM Tris-HCl pH 7.5). RNA was recovered by treatment with proteinase K (2.0 mg ml^{-1} in 0.5% (w/v) SDS, 40 mM EDTA, 20 mM Tris-HCl, pH 7.5) at 50°C for 10 min, followed by extraction with TRI Reagent (MRC) and ethanol precipitation. Isolated RNA was treated with Tobacco Acid Pyrophosphatase (TAP) (Epicentre) and subjected to small RNA cloning.

Data analysis

Small RNA sequencing data were analyzed essentially as described (Lee et al., 2012). In brief, 5' and 3' adaptor sequences were trimmed using a custom Perl script. Libraries were sorted into different bins based on barcode sequences. Reads of at least 17 nt in length were mapped to the *C. elegans* genome (WS215) and miRBase 16 using Bowtie 0.12.7. A custom Perl script that allows no mismatches with reads <19 nt, one mismatch for 19-21, two for 22-24, and three for ≥ 25 nt was used to perform a post-match analysis. To account for differences in sequencing volume between samples, reads were normalized to either the total number of miRNAs or nonstructural RNAs as described in the paper. Normalized reads were visualized in the UCSC genome browser. All scripts are available upon request.

21U-RNA target sites were predicted using three criteria. 1). piRNAs (rpm ≥ 2) from PRG-1 IP (N2) were selected; 2). The 21U-RNA sequences were first aligned to the annotated transcripts by allowing up to six mismatches using bwa with parameters (-n 6 -N -o 0) (Li and Durbin, 2009). Base-pairing at the first nucleotide of piRNAs was not taken into consideration; 3). The 22G-RNAs in *prg-1* mutants must be reduced by at least 2 fold compared to that in WT within 100 nt intervals centered on predicted piRNA targets. The densities of 22G-RNAs for WT and *parn-1* mutants were calculated from four biological replicates prepared by 5' Polyphosphatase or TAP cloning. The average abundance is calculated by taking the mean density along the 100 nt window.

Protein analysis

Protein lysate were generated from synchronized populations of gravid adults. Western blotting was performed as described (Batista et al., 2008). Antibodies used for Western blotting analysis were anti-PRG-1(Batista et al., 2008), anti-CSR-1 (Claycomb et al., 2009), anti-alpha-Tubulin (MCA77, Serotec), and anti-GFP (GenScript).

Northern blot analysis

RNA samples were separated on 15% polyacrylamide/7 M urea gels and transferred to Hybond-N+ nylon membrane (GE Healthcare) at 400 mA for 1 h in 1× TBE buffer. RNA was ultraviolet cross-linked (254 nm, 120 mJ) for three times in a Stratalinker (Stratagene). Hybridizations with radiolabelled probes were performed in ULTRAhyb buffer (Life Technologies) at 37°C overnight. RNAs were detected using the StarFire system (IDT) (Table S1).

RT-qPCR

For quantitative real-time RT-PCR, reverse transcription for DNase-treated total RNA was performed using antisense primers WT6 and WT213 (Table S1). Real-time PCR was performed in triplicate reactions using Power SYBR Green PCR Master Mix (Applied Biosystems). WT212 and WT213 were used to detect *parn-1* transcript. WT5 and WT6 were used to amplify *act-3*. All statistics were calculated in Microsoft Excel. Error bars in the graph represent the standard deviation.

RNA trimming assay

All in vitro trimming assays were conduced at 25°C. For time course experiments, the recombinant protein was incubated with 5' fluorescent labeled RNAs in a total of 10 μl reaction. The products were separated in 15% polyacrylamide/7M urea gel and imaged under ImageQuant LAS 4000 Imager (GE Healthcare). Bands from at least three independent experiments were quantified using ImageQuant TL Software (GE Healthcare). The ratio of the processed product and unprocessed substrates during time course was calculated and fitted as one curve. The

following synthetic RNA substrates (IDT) were used in the trimming assays: 21mer, 14mer, 14mer-PS-Terminal, 14mer-PS-Center, and 14-OME (Table S1).

To carry out trimming assays from PRG-1 complexes, PRG-1 was immunopurified from *parn-1* and *parn-1*; *henn-1* mutants using the anti-PRG-1 antibody and anti-FLAG (Sigma) antibody as described above. Dynabeads (Life Technologies) with the PRG-1 complex were washed twice with RNA trimming buffer and aliquoted equally into two or three tubes. 10 pmol recombinant PARN-1 protein and 1 pmol fluorescent labeled RNA (14mer) were added. The reaction was performed in a thermomixer (Eppendorf) with constant shaking (1000 rpm) at room temperature. At different time points, the reaction was stopped by proteinase K treatment and RNA was isolated by TRI Reagent (MRC) extraction and ethanol precipitation. 10% of purified RNA resolved by electrophoresis and imaged under ImageQuant LAS 4000 Imager (GE Healthcare). The rest of the sample was subjected to Northern blot analysis using the probe against 21UR-1949.

Immunostaining and Microscopy

Gonads of adult animals were extruded by decapitating worms on poly-L-lysine-coated glass slides. Gonads were fixed with 1% formaldehyde and permeabilized by freeze-cracking. After blocking for one hour in TBSB (1% BSA, 50 mM Tris-HCl, 150 mM NaCl, pH 7.5) at room temperature, samples were incubated with primary antibodies (anti-PRG-1 and anti-GFP (mFX73, Wako)) overnight at 4°C. Slides were washed 3 times with TBS (50 mM Tris-HCl, 150 mM NaCl, pH 7.5) and incubated with Cy-3 anti-mouse or FITC anti-rabbit secondary antibodies (Jackson Immunoresearch) at room temperature for 1 h. Slides were washed 3 times with TBS and mounted in Vectashield (Vector Labs). Confocal images were acquired using a Zeiss LSM510 Meta Confocal microscope and Openlab software.

To obtain epi-fluorescence images, all transgenic worms were mounted in 0.2 mM levamisole on RITE-ON glass slides (Becton, Dickinson and Company). Images were collected using the Axioplan2 Microscope and processed using AxioVision software (Zeiss).

Supplemental tables

Table S1. Oligonucleotides used in this study for RT-qPCR, Northern blotting and in vitro trimming assays, Related to the Experimental Procedures.

Oligos for RT-qPCR:

Oligo name	Sequences 5' to 3'	Targets
WT5	GGCCCAATCCAAGAGAGGTATCC	*act-3* Forward
WT6	GGGCAACACGAAGCTCATTGTA	*act-3* Reverse
WT212	TGGATAGCGGAAAGAAATGG	*parn-1* Forward
WT213	ATCTTGGTGGCTTGGTTTTG	*parn-1* Reverse

Oligos for Northern blotting analysis:

Oligo name	Sequences 5' to 3'	Targets
WT86	TTCACATCCCTAATCAGTGTCATGT/3StarFire/	miRNA-66
WT87	TGCACGGTTAACGTACGTACCAT/3StarFire/	21UR-1
WT329	TCCATTCTTTGTCACCCTCGTAT/3StarFire/	21UR-1949
WT529	TCCAACTGAATCATTATAAAGTCT/3StarFire/	22G-RNA (Reporter)
CMO12896	AAGATCCCAATTTCGAATACAATCGGTATCCAGTC/3 StarFire/	22G-RNA (f37d6.3)
CMO12136	CTCAAACTTGGGTAATTAAACC/3StarFire/	SL1 leader

Oligos for in vitro trimming assays:

Oligo name	Sequences 5' to 3'	Note
WT418	/56-FAM/rUrCrArUrGrCrArUrGrCrArUrArUrGrArCrUrCrArU	21mer
WT411	/56-FAM/ rUrCrArUrGrGrCrArUrGrCrArUrArU	14mer
WT417	/56-FAM/rUrCrArUrGrGrCrArUrGrC*rA*rU*rA*rU	14mer-PS-Terminal
WT504	/56-FAM/rUrCrArUrGrGrCrA*rUrGrCrArUrArU	14mer-PS-Center
WT412	/56-FAM/rUrCrArUrGrGrCrArUrGrCrArUrAmU	14-OME
Asterisk represents phosphorothioate bond. m represents 2'–O–methylation modification.		

Supplemental References

Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics *25*, 1754-1760.