

Supplemental information for

Inorganic phosphate blocks binding of pre-miRNA to Dicer-2 via its PAZ domain

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Four Supplemental Figures

Supplemental Figure S1. Purified Dicer-2 produces shorter RNA products from pre-miRNA than does purified Dicer-1

Supplemental Figure S2. Inorganic phosphate inhibits binding to Dicer-2 of pre-miRNA and short dsRNA, but not long dsRNA

Supplemental Figure S3. Dicer-2 requires a 5' monophosphate to efficiently cleave pre-miRNA

Supplemental Figure S4. Dicer-2 requires a 5' monophosphorylated, two-nucleotide 3' overhanging end and is inhibited by inorganic phosphate for processing of short, but not long, dsRNA

Supplemental Figure S5. Inorganic phosphate increased K_M and decreases k_{cat} of Dicer-2 for pre-*let-7* and 30 bp dsRNA, but not 104 bp dsRNA

Four Supplemental Tables

Supplemental Table S1. Sequencing statistics for small RNAs: reads

Supplemental Table S2. Sequencing statistics for small RNAs: species

Supplemental Table S3. Normalized small RNA abundance

Supplemental Table S4. Sequences of RNA oligonucleotides

Supplemental Figure Legends

Figure S1 Purified Dicer-2 produces shorter RNA products from pre-miRNA than does purified Dicer-1. 5' monophosphorylated pre-miRNAs (100 nM) were incubated with Dicer-1 or Dicer-2 (10 nM) for 2 h. Products were resolved by electrophoresis and detected by Northern hybridization. Sequences of DNA probes: *let-7*, 5'-TGA GGT AGT AGG TTG TAT AGT-3'; *let-7**, 5'-AGA AAG CTA GCA CAT TGT ATA-3'; miR-8, 5'-GAC ATC TTT ACC TGA CAG TAT-3'; miR-8*, 5'-CTA ATG CTG CCC GGT AAG ATG-3'; miR-79*, 5'-ATG CTT TGG TAA TCT AGC TTT-3'; miR-79*, 5'-ATA CAG CTA AAG CGC CAA AGC-3'.

Figure S2 Inorganic phosphate inhibits binding to Dicer-2 of pre-miRNA and short dsRNA, but not long dsRNA. The figure provides representative gel images for the quantitative data in Figure 3. For Dicer-1, ³²P-radiolabeled pre-*let-7* (30 pM) was incubated with Dicer with or without 25 mM phosphate for 20 min at 4°C, then irradiated with 254 nm light to cross link bound substrate to enzyme. For Dicer-2, ³²P-radiolabeled RNA (30 pM) containing 5-iodouridine as the penultimate nucleotide was incubated with Dicer with or without 25 mM phosphate for 20 min at 4°C, then irradiated with 302 nm light. Samples were resolved by SDS-PAGE.

Figure S3 Dicer-2 requires a 5' monophosphate to efficiently cleave pre-miRNA. pre-miR-307a (100 nM) bearing a 5' monophosphate or hydroxyl group were incubated with Dicer-2 (8 nM) with (red) or without (black) 25 mM inorganic phosphate. The substrate and product were detected by Northern hybridization using the DNA probe, 5'-TCGCTCACTCAAGGAGGTTG-3'. Data are mean ± SD for three independent trials.

Figure S4 Dicer-2 requires a 5' monophosphorylated, two-nucleotide 3' overhanging end and is inhibited by inorganic phosphate for processing of short, but not long dsRNA. Dicer-2 (8 nM) was incubated with 5' ³²P-radiolabeled 30, 38, 52, or 73 bp long dsRNA (100 nM) bearing a 5' monophosphate or hydroxyl group and a blunt or two-nucleotide, 3' overhanging end with or without 1 mM ATP in the presence (red) or absence (black) of 25 mM inorganic phosphate. Data are mean ± SD for three independent trials.

Figure S5 Inorganic phosphate increased K_M and decreased k_{cat} of Dicer-2 for pre-*let-7* and 30 bp dsRNA, but not 104 bp dsRNA. Michaelis-Menten kinetics were used to analyze Dicer-2 processing with (red) or without (black) 25 mM inorganic phosphate for (A) 5' ³²P-radiolabeled pre-*let-7* (6 nM Dicer-2); (B) 5' ³²P-radiolabeled 30 bp dsRNA with a 5' monophosphorylated, two-nucleotide, 3' overhanging end (6 nM Dicer-2); and (C) uniformly ³²P-radiolabeled 104 bp dsRNA with a 5' monophosphorylated, two-nucleotide, 3' overhanging end (1 nM Dicer-2). Reaction time courses for each substrate concentration are at right. Data are mean ± SD for three independent trials.

Supplementary Table S1. Small RNA sequencing statistics: reads. R1, R2, R3 indicate biologically independent replicates.

Genotype	Total reads	Genome-mapping reads	rRNA reads	Reads excluding rRNAs	miRNA reads	siRNA reads	cisNAT-mapping siRNA reads	Structured loci-mapping siRNA reads	Transposon-mapping siRNA reads
<i>w¹¹¹⁸</i> (R1)	27,971,451	23,645,223	1,350,642	22,294,581	8,790,430	375,562	4,894	39,753	592,055
<i>w¹¹¹⁸</i> (R2)	27,745,570	23,084,115	648,449	22,435,666	9,402,711	360,587	4,649	38,889	583,048
<i>w¹¹¹⁸</i> (R3)	30,637,561	25,112,967	1,402,637	23,710,330	9,614,787	460,696	5,294	46,624	710,349
<i>w¹¹¹⁸; dicer-2^{L811fsx}/CyO</i> (R1)	30,005,040	25,092,671	766,897	24,325,774	10,010,265	523,278	4,119	23,383	791,012
<i>w¹¹¹⁸; dicer-2^{L811fsx}/CyO</i> (R2)	31,265,161	25,745,474	872,130	24,873,344	11,089,351	542,957	4,262	22,105	811,123
<i>w¹¹¹⁸; dicer-2^{L811fsx}/CyO</i> (R3)	28,949,848	23,699,488	1,043,923	22,655,565	9,177,101	469,312	3,419	18,713	720,290
<i>w¹¹¹⁸; dicer-2^{L811fsx}</i> (R1)	26,797,201	22,187,718	998,314	21,189,404	7,907,956	279,306	653	2,854	525,773
<i>w¹¹¹⁸; dicer-2^{L811fsx}</i> (R2)	29,965,183	24,601,061	1,186,220	23,414,841	9,666,166	254,680	770	2,123	549,160
<i>w¹¹¹⁸; dicer-2^{L811fsx}</i> (R3)	27,808,652	21,233,397	1,147,334	20,086,063	8,024,274	245,927	667	3,640	486,807
<i>w¹¹¹⁸; dicer-2^{G31R}/CyO</i> (R1)	30,581,605	26,033,620	2,052,302	23,981,318	9,321,003	288,021	3,908	31,854	547,069
<i>w¹¹¹⁸; dicer-2^{G31R}/CyO</i> (R2)	30,363,738	25,468,822	2,314,633	23,154,189	9,516,400	373,111	4,153	30,249	598,487

<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{G31R} /CyO (R3)	32,044,414	26,380,139	1,034,677	25,345,462	9,787,488	453,207	4,586	35,367	714,553
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{G31R} (R1)	29,517,967	24,723,915	835,433	23,888,482	9,649,112	253,237	1,346	1,064	483,694
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{G31R} (R2)	31,448,489	24,630,926	1,164,686	23,466,240	9,664,843	214,567	1,204	1,005	463,176
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{G31R} (R3)	28,939,762	23,752,069	1,339,803	22,412,266	8,755,057	221,349	1,207	1,075	456,036

Supplementary Table S2. Small RNA sequencing statistics: species.

Genotype	Total species	Genome-mapping species	Species excluding rRNAs	miRNA species	siRNA species	cisNAT-mapping siRNA species	Structured loci-mapping siRNA species	Transposon-mapping siRNA species
<i>w</i> ¹¹¹⁸ (R1)	4,407,270	2,881,354	2,850,789	2,940	132,859	2,784	835	129,240
<i>w</i> ¹¹¹⁸ (R2)	4,089,341	2,527,884	2,501,626	2,667	111,000	2,308	721	107,971
<i>w</i> ¹¹¹⁸ (R3)	4,778,380	2,774,104	2,743,086	2,795	140,442	2,580	863	136,999
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{L811fsx} /CyO (R1)	4,087,835	2,620,307	2,587,370	2,769	145,215	1,919	721	142,575
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{L811fsx} /CyO (R2)	4,251,225	2,575,333	2,542,743	2,896	147,396	1,968	725	144,703
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{L811fsx} /CyO (R3)	4,449,139	2,563,125	2,526,974	2,725	133,757	1,681	659	131,417
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{L811fsx} (R1)	3,880,401	2,425,149	2,389,220	2,698	94,839	247	282	94,310
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{L811fsx} (R2)	3,645,626	2,109,692	2,077,833	2,480	68,770	189	215	68,366
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{L811fsx} (R3)	4,167,216	2,155,205	2,122,701	2,607	75,546	203	263	75,080
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{G31R} /CyO (R1)	4,184,046	2,962,878	2,504,442	2,721	97,325	1,895	664	94,766
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{G31R} /CyO (R2)	5,007,028	2,540,667	2,902,248	2,802	116,786	2,178	743	113,865

<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{G31R} /CyO (R3)	4,407,270	2,933,478	2,850,789	2,940	132,859	2,784	835	129,240
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{G31R} (R1)	4,162,316	2,353,870	2,367,493	2,659	68,357	542	154	67,661
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{G31R} (R2)	4,254,552	2,399,447	2,341,880	2,566	70,252	528	166	69,558
<i>w</i> ¹¹¹⁸ ; <i>dicer-2</i> ^{G31R} (R3)	4,579,558	2,371,450	2,928,774	2,548	84,149	1,843	571	81,735

Supplementary Table S3. Normalized small RNA abundance (ppm). Data are mean of three biologically independent replicates \pm SD.

Genotype	esi-1.1	esi-1.2	esi-2.1
<i>w¹¹¹⁸</i>	20.4 \pm 1.9	49.8 \pm 14.4	26,698 \pm 1,312
<i>w¹¹¹⁸; dicer-2^{L811fsx}/CyO</i>	10.4 \pm 1.0	10.0 \pm 2.3	12,170 \pm 1,360
<i>w¹¹¹⁸; dicer-2^{L811fsx}</i>	0.1 \pm 0.1	0.2 \pm 0.2	174.8 \pm 27.2
<i>w¹¹¹⁸; dicer-2^{G31R}/CyO</i>	12.5 \pm 0.4	33.7 \pm 1.5	29,024 \pm 10,024
<i>w¹¹¹⁸; dicer-2^{G31R}</i>	0.1 \pm 0.1	0.2 \pm 0.0	201.3 \pm 17.3

Supplementary Table S4. Sequences of RNA oligonucleotides.

Deoxynucleotide.

pre- <i>let-7</i> wild type	5'-UGA GGU AGU AGG UUG UAU AGU AGU AAU UAC ACA UCA UAC UAU ACA AUG UGC UAG CUU UCU-3'
pre- <i>let-7</i> blunt end	5'-AGU GAG GUA GUA GGU UGU AUA GUA GUA AUU ACA CAU CAU ACU AUA CAA UGU GCU AGC UUU CU-3'
pre- <i>let-7</i> perfect match stem	5'-UGA GGU AGU AGG UUG UAU AGU AGU AAU UAC ACA UCA UAC UAU ACA ACC UAC UAC CUC UCU-3'
pre- <i>let-7</i> tetraloop	5'-UGA GGU AGU AGG UUG UAU AGU AGA AAU ACU AUA CAA UGU GCU AGC UUU CU-3'
pre- <i>let-7</i> perfect match stem and tetraloop	5'-UGA GGU AGU AGG UUG UAU AGU AGA AAU ACU AUA CAA CCU ACU ACC UCU CU-3'
Strand 1 of 5'-PO ₄ , 3' overhang 25 bp dsRNA	5'-ACC CUG AAG UUC AUC UGC ACC ACC <u>AG</u> -3'
Strand 1 of 5'-PO ₄ , blunt 27 bp dsRNA	5'-UGA CCC UGA AGU UCA UCU GCA CCA <u>CCG</u> -3'
Strand 2 of 25 bp or 27 bp dsRNA	5'-CGG UGG UGC AGA UGA ACU UCA GGG UCA-3'
Strand 1 of 30 bp dsRNA	5'-GGA GGU AGU AGG UUG UAU AGU AGU AAG <u>ACC</u> -3'
Strand 2 of blunt 30 bp dsRNA	5'-GGU CUU ACU ACU AUA CAA CCU ACU ACC <u>UCC</u> -3'
Strand 2 of 3' overhang 30 bp dsRNA	5'-GGU CUU ACU ACU AUA CAA CCU ACU ACC UCC <u>AC</u> -3'
Strand 1 of 38 bp dsRNA	5'-GGA GGU AGU AGG UUG UAU AGU AGU AAG ACC AGA CCC <u>UA</u> -3'
Strand 2 of blunt 38 bp dsRNA	5'-UAG GGU CUG GUC UUA CUA CUA UAC AAC CUA CUA CCU CC-3'

Strand 2 of 3' overhang 38 bp dsRNA	5'-UAG GGU CUG GUC UUA CUA CUA UAC AAC CUA CUA CCU CCA C-3'
Strand 1 of 52 bp dsRNA	5'-GGA GGU AGU AGG UUG UAU AGU AGU AAG ACC AGA CCC UAG ACC AAU UCA UGC <u>C</u> -3'
Strand 2 of blunt 52 bp dsRNA	5'-GGC AUG AAU UGG UCU AGG GUC UGG UCU UAC UAC UAU ACA ACC UAC UAC CUC C-3'
Strand 2 of 3' overhang 52 bp dsRNA	5'-GGC AUG AAU UGG UCU AGG GUC UGG UCU UAC UAC UAU ACA ACC UAC UAC CUC CAC-3
Strand 1 of 73 bp dsRNA	5'-GGA GGU AGU AGG UUG UAU AGU AGU AAG ACC AGA CCC UAG ACC AAU UCA UGC CAC CGA AGC AAA UUU CAA UGC <u>C</u> -3'
Strand 2 of blunt 73 bp dsRNA	5'-GGC AUU GAA AUU UGC UUC GGU CGC AUG AAU UGG UCU AGG GUC UGG UCU UAC UAC UAU ACA ACC UAC UAC CUC C-3'
Strand 2 of 3' overhang 73 bp dsRNA	5'-GGC AUU GAA AUU UGC UUC GGU CGC AUG AAU UGG UCU AGG GUC UGG UCU UAC UAC UAU ACA ACC UAC UAC CUC CAC-3'