Supplemental information for

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

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Four Supplemental Figures

Four Supplemental Tables

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, ^{32}P-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, ^{32}P-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′ ^{32}P-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′ ^{32}P-radiolabeled pre-*let-7* (6 nM Dicer-2); (**B**) 5′ ^{32}P-radiolabeled 30 bp dsRNA with a 5′ monophosphorylated, two-nucleotide, 3′ overhanging end (6 nM Dicer-2); and (**C**) uniformly ^{32}P-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.