Supplementary Material

Human Dicer preferentially cleaves dsRNAs at their termini without a requirement for ATP

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Materials and Methods

Preparation of dsRNA substrates

dsRNA substrates were prepared by annealing of RNAs obtained by the T7 RNA polymerase transcription of appropriate PCR-generated templates. To generate PCR fragments for synthesis of 40- to 130-nt RNAs, the pβact-eGFP plasmid (Billy et al., 2001) was used as a template. Following oligonucleotides (obtained from Microsynth; T7 promoter sequence is underlined) were used: T7GFPsense, (ATACGACTCAGCTCTAGGGAGAAGCCACTACCTGAGCACCAGT) and 3’GFPblunt (GGGAGAGTACAGCTCGTCCATGCCGAG), for the 130-nt sense strand, and T7GFPPanti (ATACGACTCAGCTCTAGGGAGATACAGCTCGTCCATGCCGAG) and 5’GFPblunt (GGGAGACCACTACCTGACCCGAGT), for the 130-nt antisense strand; oligonucleotides T7GFPsense and AGTGATCCCGGCGGCGGTCA, for the RNAshort-sense, and T7GFPPanti and CCGCCCTGAGCAAAGACCCC, for the RNAshort-antisens; oligonucleotides ATACGACTCAGCTCTAGGGAGAGGTCTTGGAGGTTCTGTGA and 3’GFPblunt for the 70-nt sense strand, and T7GFPPanti and GGGAGAGGTCTTGGAGGTTCTGTGA, for the 70-nt antisense strand; oligonucleotides T7GFPsense and GGGAGAGGTCTTGGAGGTTCTGTGA, for the 50-nt sense strand, and ATACGACTCAGCTCTAGGGAGAGGTCTTGGAGGTTCTGTGA and 5’GFPblunt, for the 50-nt antisense strand; oligonucleotides T7GFPsense and
GGGAGACAGGGCGGACTGGGT, for the 40-nt sense strand, and ATACGACTCACTATAGGGAGACAGGGCGGACTGGGT and GGGAGACCCTACTACCTGAGCACCC, for the 40-nt antisense strand. Templates for transcription of 30-nt sense and antisense RNAs were obtained by annealing of oligonucleotides

TTAATACGACTCTATAGGGAGACCCTACTACCTGAGCACCCATCTCCC and GGGAGATGGGTGCGTCAGGTAGTTGGTCTCCCTATAGTGAGTCGTATTAA, and

TTAATACGACTCTATAGGGAGATGGGTGCGTCAGGTAGTGGTCTCCC, and

GGGAGACCCTACTACCTGAGCACCCATCTCCCTATAGTGAGTGGTATTAA,

respectively.

RNAma and RNAdna substrates were generated by annealing of sense and antisense RNA strands shortened at their 3’ ends by 27 and 26 nt, respectively (referred to as RNAshort), as compared to the 130-nt RNAs. A twofold molar excess of oligoribonucleotides (obtained from Xeragon) or oligodeoxynucleotides complementary to the 5’-terminal 27- and 26-nt extensions was also added to the annealing mixture to yield RNAma and RNAdna, respectively. RNAtetra was obtained by the annealing of two RNAs, each containing a self-complementary region of 17 bp, topped by a GAAA tetra-loop. The two RNAs used were designed such that their combined double-stranded region was identical to that of the 130-bp dsRNA. DNA templates used for their synthesis were generated by annealing of 5’-phosphorylated oligonucleotides CGCGAAAATATACGACTCCTAGGGTG, CTCAGGTTAGGGAAAAACCCTACTAGGAGCA, CCCAGTCCGCTGAGAAAAGACCCCCACCG, AGAAGCGCGATCACCAGGTGCCTGGCTGAGT, TCGTGACCAGCCGGGGATCCTCCC,
GGGAGTGATCCCGGCGGCGGTCACGAACTCCAGCAGGACC,  
ATGTGATCGCGCTTCTCGTTGGGGTCTTTG,  
CTCAGGGCGGACTGGGTGCTCAGGTAGTGG,  
TTTCCCACTACCTGACACCCTATAGTGAGTCTAGTATTAATTTCCCG  
and  
oligonucleotides  
GGACGAGCTGTACGAGAATTTACGACTCAGCTCGTC,  
CATGCCGGGAGTGATCCCAGGCGGCGGTCAC,  
GAACTCCAGCAGGACCATGTGATCGCGCTT,  
CTCGTTGGGGTCTTTGCTCAGGCGGACT,  
AGTCCGCCCTGAGACAAAGACGAGCGAAGCGATCACAT,  
GGTCCTGGTGGATTCCGACGCGCCGGG,  
ATCACTCCCGGATGGAGCGAGCAGCTACTTTTC,  
and  
GTACAGCTGCTCCATGCCCTAGTGAGTGCTAGTATTAATTTCCCG.  
Following  
annealing, DNA cassettes were ligated by T4 DNA ligase (New England Biolabs) and  
PCR-amplified. Cassettes of the correct size were isolated on a low-melting agarose  
gel, verified by sequencing, and used for in vitro transcription reactions.  

Non-radioactive RNAs were synthesized using the Ambion T7  
MegashortScript kit following the manufacturer’s protocol. For preparation of  
radioactive RNAs, [α-32P]UTP (final specific activity 30 or 150 Ci/mmol; Amersham)  
was used. Samples were treated with DNase I and RNAs were purified by  
electrophoresis through a denaturating 10% PAGE. For annealing, RNA samples  
were heated at 95°C for 3 min, transferred to 75°C, and then slowly cooled down  
(over 4-6 h) to room temperature. During the annealing procedure for RNA tetra, 2  
mM MgCl2 was added when the temperature reached 50°C, to allow the proper  
folding of GAAA tetra-loops (Horton et al., 2000).
Additional references


Legends to Supplementary Figures

**Fig. A.** Effect of pH (left panel) and NaCl concentration (right panel) on activity of Dicer-HisC (25 ng). Reactions were incubated for 30 min. Sizes of RNA markers in lanes M are indicated. Lanes S, input 130-bp substrate. For pH optimum determination, a three-component buffer system containing 50 mM Bis-Tris, 50 mM triethanolamine, and 0.1 M acetic acid (Ellis and Morrison, 1982) was used. The buffer was adjusted to desired pH at 25°C.

**Fig. B.** Divalent-cation requirements of recombinant Dicer-HisC (25 ng). Indicated cations were added to 3 mM concentration as chloride salts. EDTA concentration was 1 mM. Lane S, input 130-bp dsRNA.

**Fig. C.** ProtK-treated Dicer cleaves dsRNA at 4°C. Indicated amounts of Dicer-HisC, preincubated without (upper panel) or with (lower panel) ProtK-agarose beads (1 μg of Dicer-HisC, 3 mg of beads containing 0.8 U of ProtK, 5 min at 37°C), were
incubated with the $^{32}$P-labelled 70-bp dsRNA for 30 min at either 4°C or 37°C. Lanes M, size markers.

**Fig. D.** Preincubation of Dicer-HisC with either CIP (left panel) or HK and glucose (middle panel) has no effect on cleavage of dsRNA. Right panel, activity of Dicer-HisC incubated under conditions identical to those in a left panel, but without HK or CIP addition. Times of incubation are indicated at the top, and quantification of the data is shown in the bottom panel. Sizes of RNA markers are indicated. Lanes S, input 130-bp substrate.
Zhang et al. Suppl. Figure A
Zhang et al. Suppl. Figure D