

■ CELL BIOLOGY

The Mechanism of Double-stranded RNA Processing by Ribonuclease III: How Dicer Dices

Gan J, Tropea JE, Austin BP, Court DL, Waugh DS, and Ji X. Structural insight into the mechanism of double-stranded RNA processing by ribonuclease III. *Cell* 124: 355–366, 2006.

The ribonuclease III (RNase III) family is represented by bacterial RNase III and eukaryotic Rnt1p, Drosha, and Dicer, containing approximately 200, 500, 1400, and 1900 amino acid residues, respectively. They are double-stranded (ds) RNA-specific endoribonucleases, characterized by a 9-residue signature motif in their catalytic domains and a 2-nucleotide (nt) 3' overhang in their products. Dicer functions as an RNA-processing enzyme, producing small interfering RNA (siRNA) of approximately 22 nt in length, which mediates RNA interference (RNAi). Bacterial RNase III functions not only as a processing enzyme, but also as a protein that binds dsRNA without cleaving it. *In vitro*, both Dicer and RNase III can be used to produce siRNA cocktails that are effective mediators of gene silencing. Structurally, Dicer is the most complicated member of the family. Bacterial RNase III is much simpler (**Figure 1, part A**) and therefore serves as a model for the entire family.

Bacterial RNase III is composed of an endonuclease domain (endoND) followed by a dsRNA-binding domain (dsRBD). We determined the endoND structure of RNase III in 2001, revealing a symmetric endoND dimer. The dimerization creates a valley that can accommodate a dsRNA substrate. Eight negatively charged side chains are concentrated inside the valley, rendering the valley highly negatively charged (**Figure 1, part B**). Although biochemical data indicate that these residues are involved in the cleavage of dsRNA, we and others in the field were puzzled by the unfavorable interactions between negatively charged dsRNA and negatively charged side chains in the valley until 4 years later when we determined the crystal structure of the first catalytic complex of the entire RNase III family.

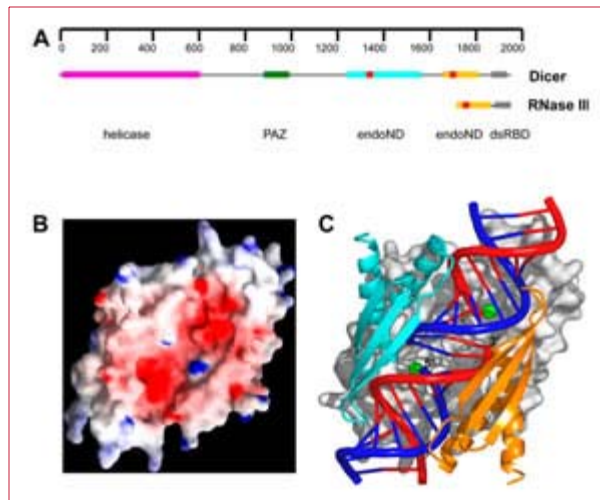


Figure 1. Structural features of RNase III and Dicer. *A)* Domain structure of *Aquifex aeolicus* RNase III (Aa-RNase III, SWISS-PROT O67082) and *Homo sapiens* Dicer (Hs-Dicer, SWISS-PROT Q9UPY3). Scale on top indicates the lengths of polypeptide chains; boxes in different colors represent individual domains. The red square on the endoND indicates the RNase III signature motif. *B)* Surface representation of the endoND dimer with the colors red and blue indicating negative and positive potential, respectively. *C)* Schematic view of the Aa-RNase III•dsRNA structure. The two endoNDs are shown as a molecular surface; the two dsRBDs are illustrated as ribbon diagrams (helices as spirals, β -strands as arrows, and loops as pipes) and colored in cyan and orange, respectively. The Mg^{2+} ions are indicated with green spheres; the two RNA strands are shown as tube-and-stick models in blue and red. endoND, endonuclease domain; dsRBD, dsRNA-binding domain; PAZ domain, an RNA-binding module found in Argonaute and some Dicer proteins (initially named for the protein families PIWI, Argonaute, and Zwilli).

The symmetric structure of Aa-RNase III•dsRNA (**Figure 1, part C**) is composed of two RNase III subunits, two dsRNA molecules, and two Mg^{2+} ions. The eight negatively charged side chains form the centers of two RNA cleavage sites. In each of the two sites, the 5' phosphate group of the RNA molecule is located in proximity to the Mg^{2+} ion. The entire structure resembles a clamp that cradles the dsRNA in the midst of the four domains. Mg^{2+} ions are a key factor in the binding of dsRNA inside the catalytic valley. We have demonstrated that dsRNA is bound outside of the valley if Mg^{2+} ions are absent. Dicer functions as a monomer. It has two endoNDs and one dsRBD (**Figure 1, part A**); the two endoNDs form an intramolecular dimer (MacRae IJ et al. *Science* 311: 195–198, 2006). Compared with the dimeric bacterial RNase III, one dsRBD is missing from monomeric Dicer. Studies on a number of dsRBD-containing proteins showed that a single dsRBD is sufficient to provide the proteins with clear specificity for target selection.

Our structure indicates that a single RNA cleavage event occurs on each strand, which creates a terminal phosphate group at the 5' end of the strands, and the two RNA cleavage events together create the 2-nt 3' overhang (**Figure 1, part C**). The 3'-hydroxyl and 5'-phosphate groups and the 2-nt 3' overhang are hallmarks of RNase III reaction products, which are essential for the incorporation of siRNAs into the RNAi pathway. Underlining the importance of endoND dimerization, the hydrolysis of each RNA strand involves both

endoNDs: Residues from one endoND select the scissile bond, whereas those from the partner endoND carry out the hydrolysis of the phosphodiester bond. The structure reveals a wealth of information about the mechanism of dsRNA processing, which can be extrapolated to other RNase III family members.

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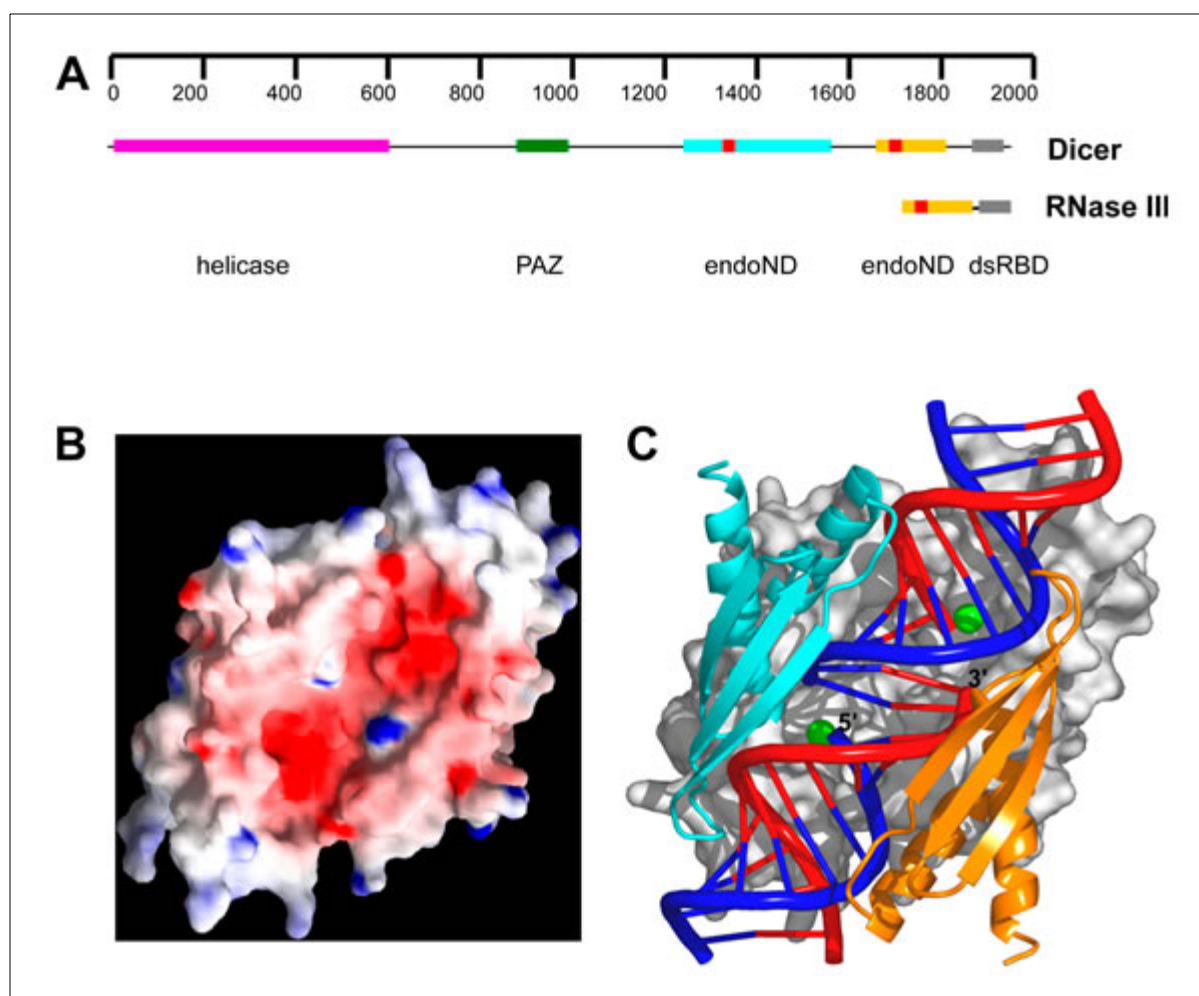


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