The Structural Basis of 5′ Triphosphate Double-stranded RNA Recognition by RIG-I C-terminal Domain

Cheng Lu[1], Hengyu Xu[2, 5], C. T. Ranjith-Kumar[3, 5], Monica Brooks[4], Tim Hou[1], Fuqu Hu[1], Andrew B. Herr[4], Roland K. Strong[2], Cheng Kao[3], and Pingwei Li [1]

Supplemental Materials

Table S1. Shows the sequences of RNA used for results presented in Figure 1, Figure S1, Figure 2, Figure 3, Figure 6, and Figure 7.

Figure S1. Shows additional binding studies by gel filtration chromatography related to data presented in Figure 1.

Figure S2. Shows the electron density map of the triphosphate dsRNA bound to RIG-I CTD as supplementary to Figure 3.

Table S2. Provide details of the interactions between RIG-I CTD and the 14-bp GC rich dsRNA as supplementary to Figure 4.

Figure S3. Comparisons of the structures of the RLR CTDs as supplementary to Figure 4.

Figure S4. RNA binding studies of RIG-I CTD mutants by gel filtration chromatography as supplementary to data presented in Figure 6.

Figue S5. IFN-β luciferase assays showing the relative signaling activities of three different forms of RNA used in cell-based assays as supplementary data for Figure 7.

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Table S1, related to Figure 1 and Figure 7. Sequences of RNA used in this study. The 5′ ppp RNAs are synthesized by in vitro transcription and purified by gel filtration chromatography or denaturing polyacrylamide gel electrophoresis. The blunt-ended dsRNA are chemically synthesized by Integrated DNA technologies (IDT). Sequences of one strand were shown for dsRNA with self-complementary sequences.

14-bp GC-rich 5′ ppp dsRNA	5′ pppGGCGCGCGCGCGCC 3′
12-bp AU-rich 5′ ppp dsRNA	5′ pppAUAUAUAUAUAU 3′
14-bp blunt-ended dsRNA	5′ GGCGCGCGCGCGCC 3′
13-nt 5′ ppp ssRNA	5′ pppGGCGCAUAGGCCG 3′
12-bp dsRNA with 5′ overhangs	5′ pppGAUAUGCGCGCGCGC 3′
12-bp dsRNA with 3′ overhangs	5′ pppGGCGCGCGCGCGCAUAU 3′
24-bp 5′ ppp dsRNA	5′ pppGGCGCGCGAGTCGACTCGCGCGC C 3′
27-nt 5′ ppp ssRNA	5′ pppGGUGCAGAUGAACUUCAGGGUCAGCUU 3′
27-bp blunt-ended dsRNA	5′ AAGCUGACCCUGAAGUUCAUCUGCACC 3′ 3′ UUCGACUGGGACUUCAAGUAGACGUGG 5′

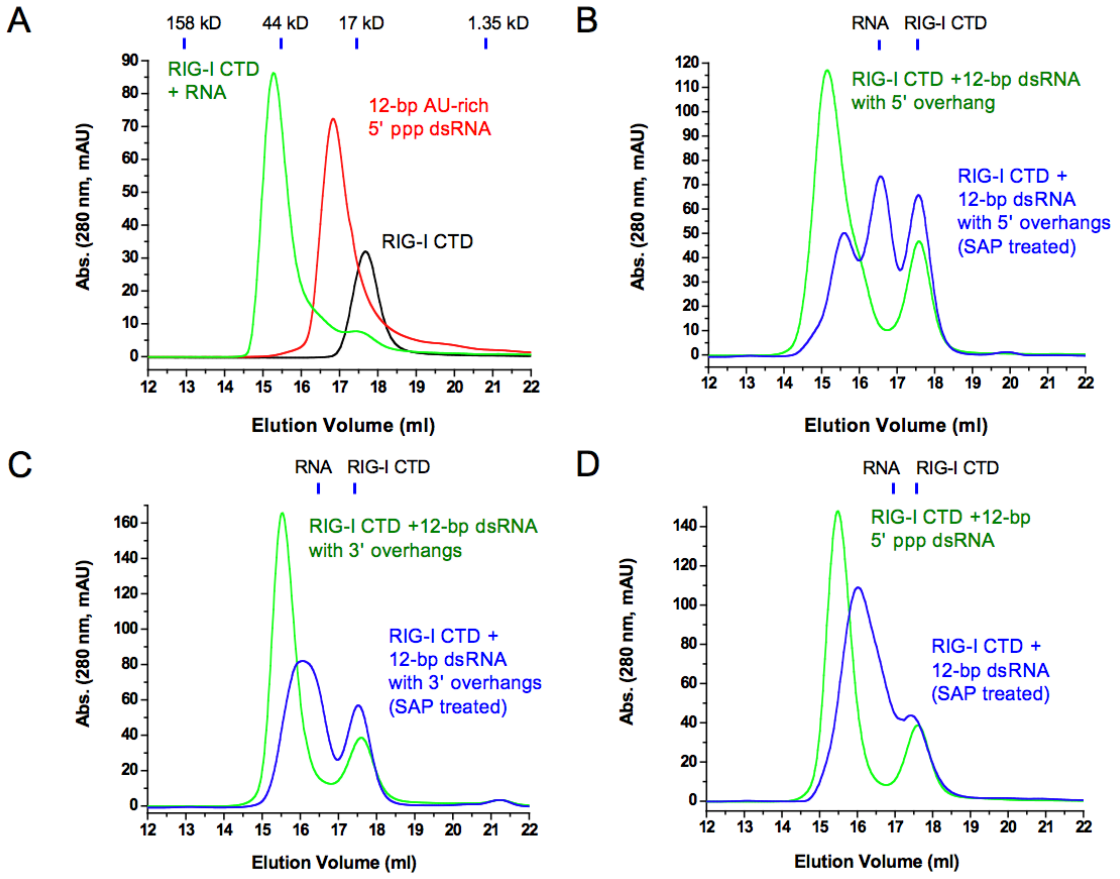


Figure S1, related to Figure 1. RNA binding studies by gel filtration chromatography. (A) Binding study of RIG-I CTD with the 12-bp AU-rich 5′ ppp dsRNA. (B) Binding studies of RIG-I CTD with a 12-bp dsRNA containing 5′ overhangs. Treatment of the dsRNA with shrimp alkaline phosphatase (SAP) reduced the binding between RIG-I CTD and the RNA. (C) Binding studies of RIG-I CTD with a 12-bp dsRNA containing 3′ overhangs. (D) Treatment of a blunt-ended 12-bp 5′ ppp dsRNA with SAP reduced its binding for RIG-I CTD.

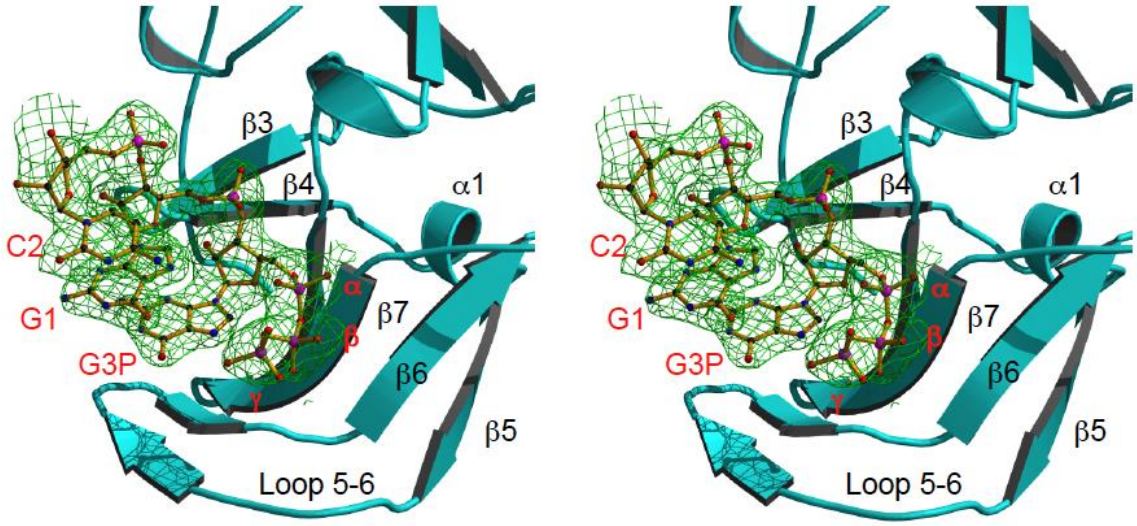


Figure S2, related to Figure 3. Stereo representation of the σ_A weighted $2F_o$-F_c map for the 5′ three nucleotides in the 14-bp GC-rich 5′ ppp dsRNA complex structure. The first nucleotide G3P contains a triphosphate group. RIG-I CTD is shown as cyan ribbons. The nucleotides are shown in ball-and-stick models.

Supplementary Table 2, related to Figure 4. Interactions between RIG-I CTD and the 14-bp GC-rich dsRNA.

Nucleotide (ends) Atoms	**RIG-I CTD Atoms**	**Distances (Å)**	**Type of Interactions**
G3P (5′)			
O2G	Lys858 NZ	3.4	ES
O2B	Lys858 NZ	2.7	ES
O2B	His847 NE2	2.6	ES
O1B	Lys861 NZ	2.7	ES
O2A	Lys861 NZ	2.7	ES
O2A	Lys888 NZ	2.4	ES
C2	Phe853 CE2	3.2	HP
O2′	His830 ND1	2.5	HB
O5′	Gly874 CA	3.8	VDW
O3A	Ile875 CG2	2.9	VDW
O2′	Val886 CG1	3.9	VDW
O2′	Tyr831 N (S1)	3.0 / 3.0	sHB
O2′	Ile887 O (S1)	3.0 / 3.0	sHB
G1 (5′)			
C5′	Ser829 O	3.5	VDW
O4′	His830 CE1	3.3	VDW
O2P	Lys888 CB	3.5	VDW
O2P	Trp908 N (S21)	2.6 / 3.0	sHB
O2P	Ser906 O (S32)	3.0 / 2.9	sHB
O2P	Trp908 CD1	3.2	VDW
C2 (5′)			
O1P	Trp908 N (S21)	3.4/3.0	sHB
G3 (5′)			
O2P	Lys907 NZ	3.2	ES
C10 (3′)			
O1P	Lys849 NZ	2.7	ES (?)
C13 (3′)			
O2′	Ser854 OG	3.4	HB
N3	Phe853 CB	3.9	VDW

HB: hydrogen bonds
VDW: van der Waals contacts
HP: hydrophobic interactions
sHB: solvent mediated hydrogen bonds. Solvent distances to protein atoms and to dsRNA atoms are shown.
ES: electrostatic interactions

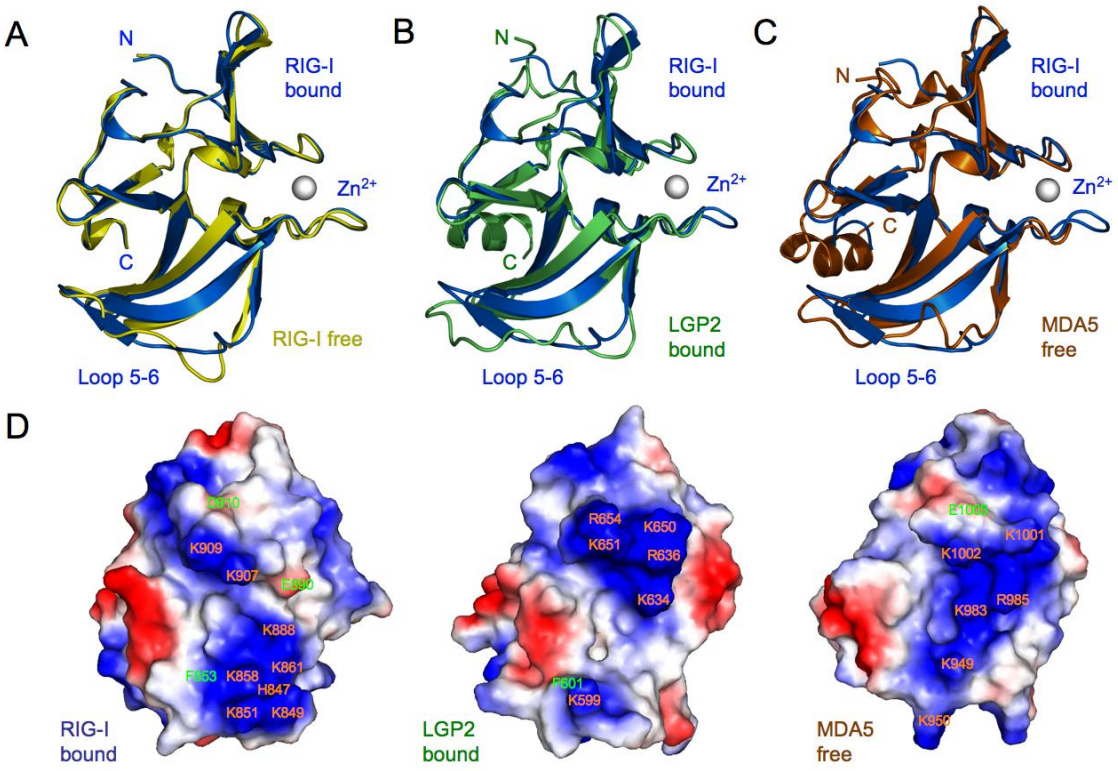


Figure S3, related to Figure 4. The structures of RLR CTDs are conserved but with distinct electrostatic surface potential. (A) Superposition of RIG-I CTD structures in isolation (yellow) and in complex with 5′ ppp dsRNA (blue). (B) Superposition of RNA bound RIG-I CTD (blue) and LGP2 CTD (green) structures. (C) Superposition of structures of RNA bound RIG-I CTD (blue) and MDA5 CTD in isolation (orange). (D) Electrostatic surface potential of the RNA binding surfaces of RIG-I, LGP2 and MDA5 CTDs. Positvely charged regions are colored blue, negatively charged regions are colored red.

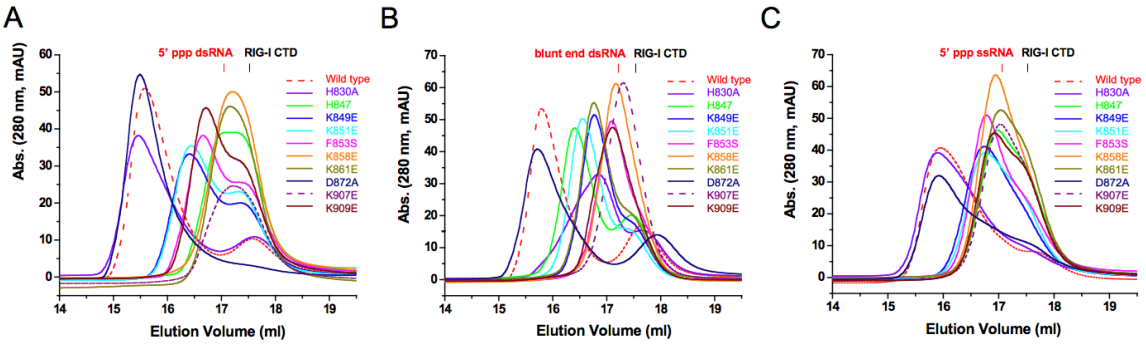
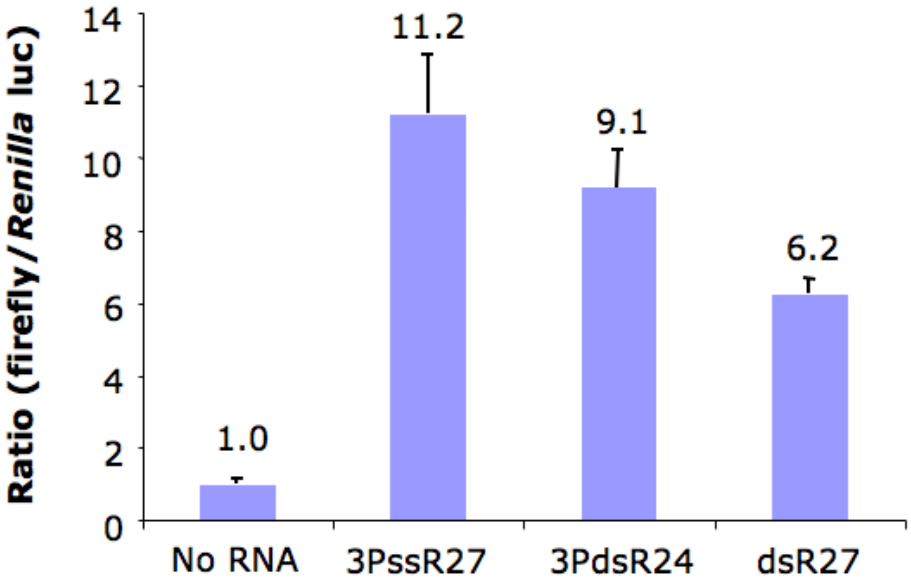


Figure S4, related to Figure 6. Mutations of key residues in RIG-I CTD affects the binding of three different forms of RNA. (A) Binding studies of wild type and mutants of RIG-I CTD for the 14-bp 5′ ppp dsRNA by gel filtration chromatography. Each of the chromatograms represents the elution profile of a 3:1 molar ratio mixture of RIG-I CTD or its mutants with the RNA. Elution volume of the protein and the RNA alone are shown above the chromatogram. Shift of the peak positions of mutant protein and RNA complexes relative to that of the wild type protein or the RNA alone reflects how the mutation affect RNA binding. Mutants with no peak shift (such as K907E) do not bind RNA. RNA binding is not affected for mutant with peak shift (such as D872A) similar to that of the wild type protein. (B) Binding studies of wild type and mutants of RIG-I CTD for the 14-bp blunt-ended dsRNA. (C) Binding studies of RIG-I CTD mutants for the 13-nt 5′ ppp ssRNA.



Figue S5, related to Figure 7. IFN-β luciferase assays showing the relative signaling activities of three different forms of RNA. 3PssR27 represents the 27-nt ssRNA with triphosphate; 3PdsR24 represents the 24-bp dsRNA with 5′ triphospahte; dsR27 represents the 27-bp blunt-ended dsRNA.