SUPPLEMENTARY INFORMATION FOR:

Activation of PKR by RNA misfolding: HDV ribozyme dimers activate PKR

Laurie A. Heinicke[1,2] and Philip C. Bevilacqua[1,†]

[1]Department of Chemistry, Center for RNA Molecular Biology, The Pennsylvania State University, University Park, PA 16802.

[2]**Present Address:** Department of Medicine, University of Pennsylvania School of Medicine, 415 Curie Blvd , Philadelphia, PA 19104

[†] To whom correspondence should be addressed:

PCB: Phone: (814) 863-3812; Fax: (814) 865-2927; e-mail: pcb5@psu.edu

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Contents:

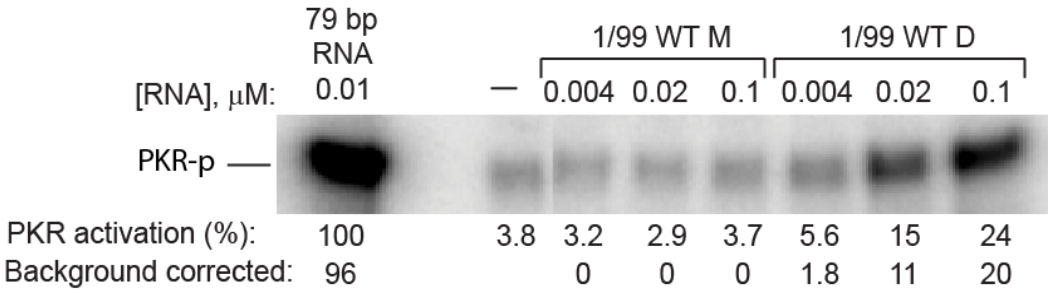


Figure S1. PKR activation by low RNA concentrations of isolated and purified 1/99 WT HDV monomer and dimer. A no-RNA lane is provided, and phosphorylation activities are normalized to 0.01 μM 79 bp RNA. Both no-RNA and 79 bp RNA lanes contain 100 mM NaCl and 5 mM $MgCl_2$. The no-RNA lane was subtracted from each lane in order to provide RNA-dependent activation values.

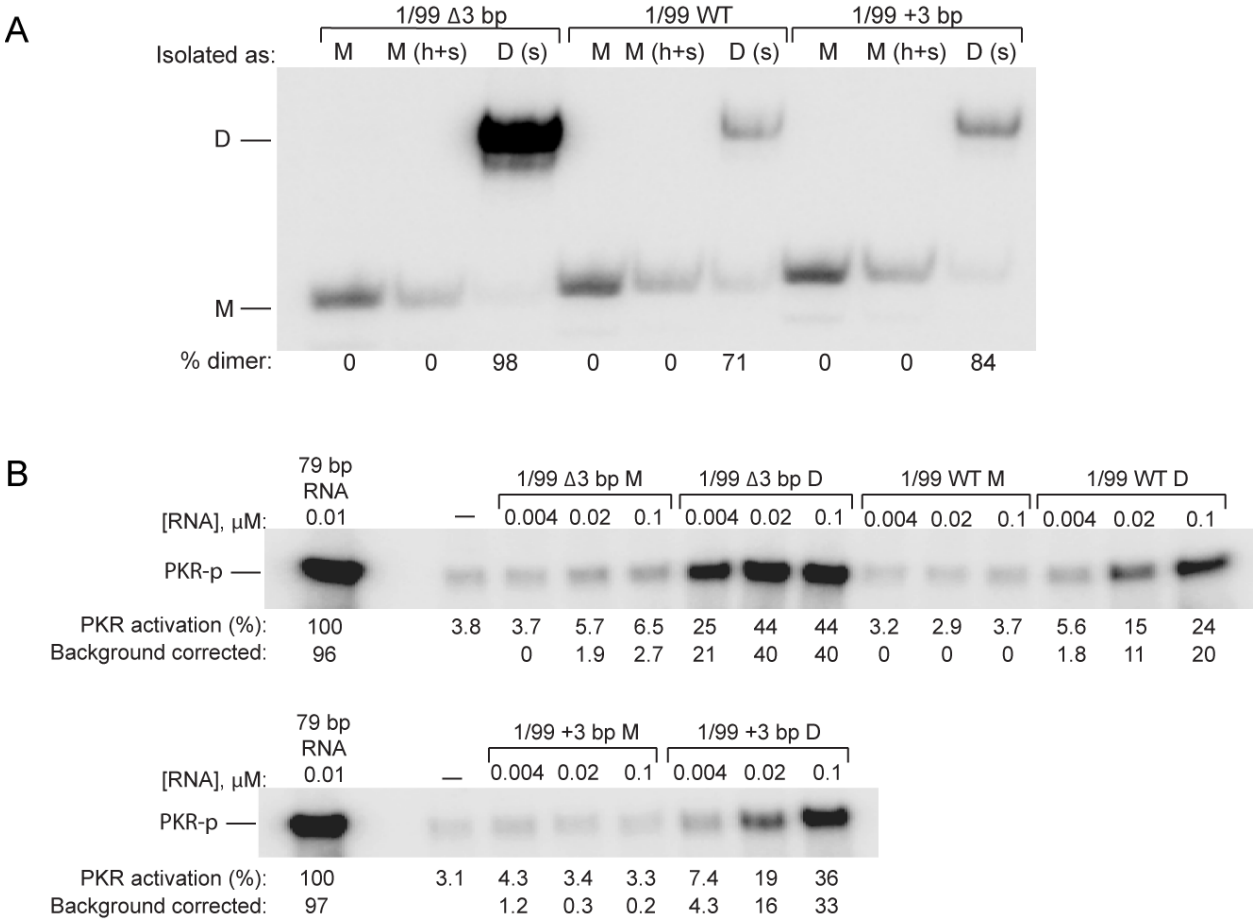
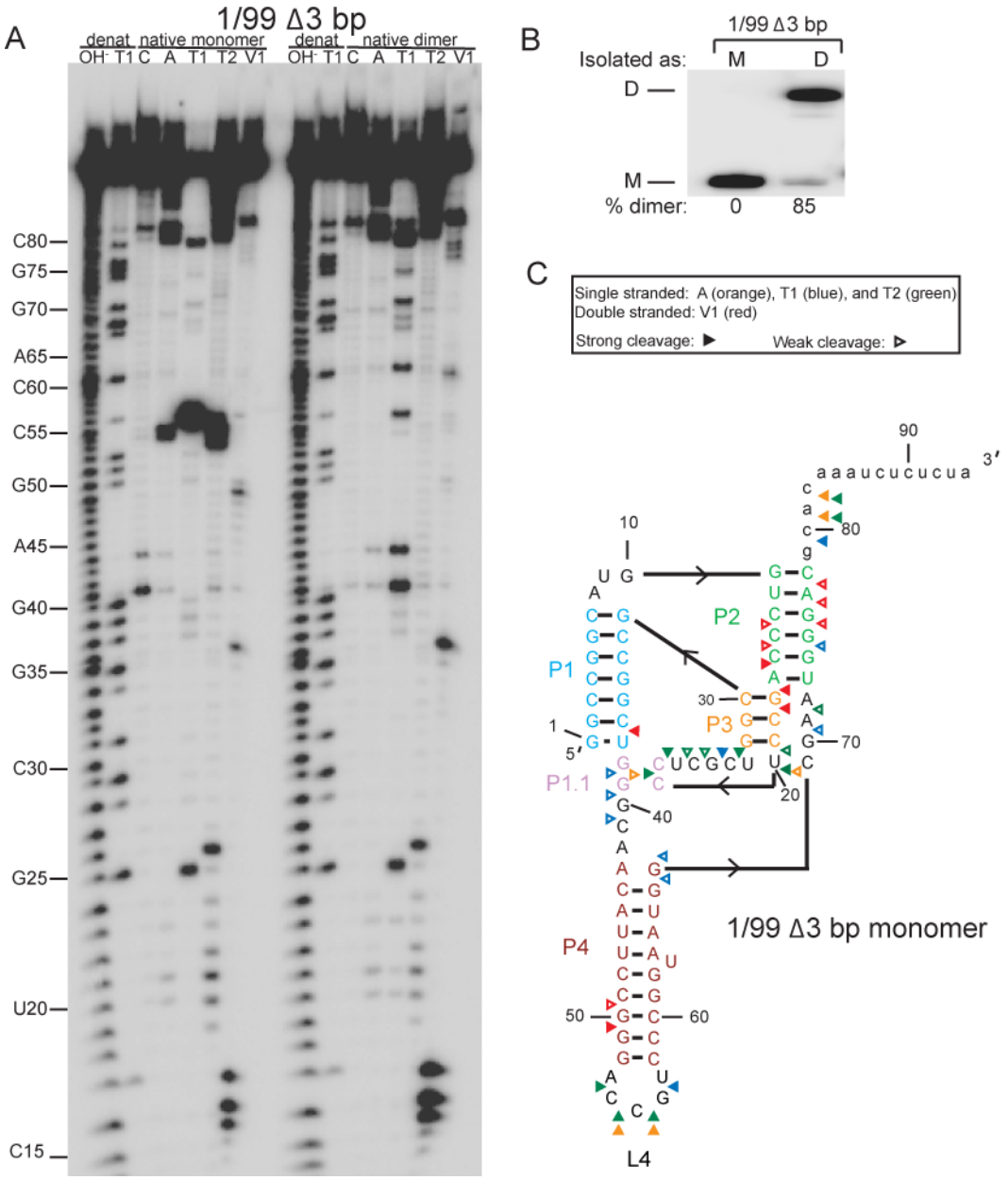


Figure S2. HDV dimers are activators of PKR. (A) Native gel analysis of isolated and purified monomer and dimer HDV ribozyme variants. 20 μM RNA (with trace 5'-end labeled RNA) was renatured in 1x TE (1/99 Δ3 bp) or 1x TEN_{100} (1/99 wt and 1/99 +3 bp) by incubation at 95 ^{o}C for 4 min, followed by slow cooling to 40 ^{o}C over 1.5 h. Presence of salt and slow cooling slightly enhanced dimer formation. Monomer and dimer RNAs were purified from native gel (gel not shown) and placed in 1x TE. Then, certain 4x[RNA] monomer and dimer samples were added to an equal volume of $1xTEN_{400}M_{20}$ (indicated with "s" for salt), followed by incubation at room temperature for 10 min. Prior to adding salt, certain monomer samples were renatured (indicated with "h" for heat), to remove possible dimer contaminants, by incubating at 90 ^{o}C for 1 minute, followed by incubation at 55 ^{o}C for 10 min. (B) PKR activation by HDV monomers and dimers. See Materials and Methods for details.

Figure S3



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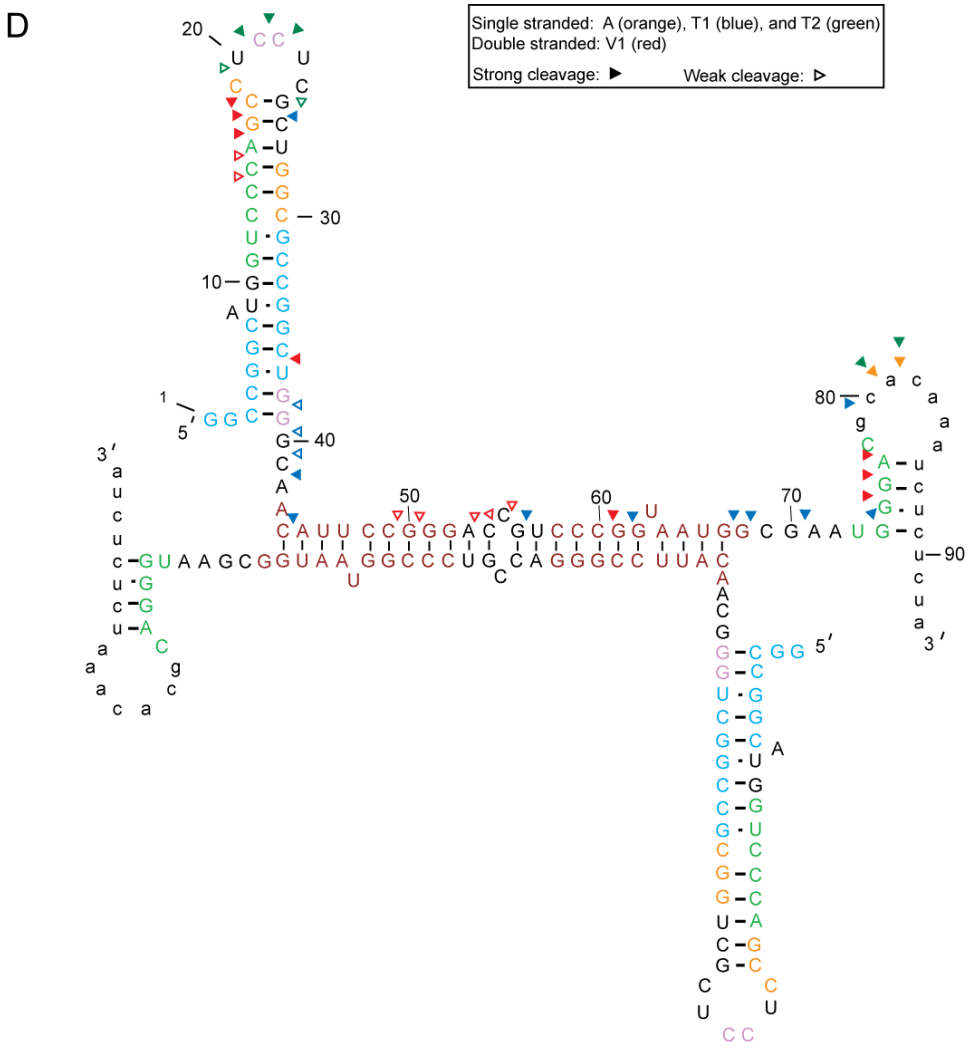

Figure S3. Enzymatic structure mapping of 1/99 Δ3 bp HDV. (A) Structure mapping of 1/99 Δ3 monomer and dimer. Monomeric and dimeric RNAs were prepared as described in the Materials and Methods sections "Enzymatic structure mapping of RNA"; purification gel not shown. Sequencing lanes for G (T1) and all nucleotides (OH^-) are provided to the left of each data set. The secondary structures were determined using RNases A (C, U-specific), T1 (G-specific), T2 (single stranded-specific), and V1 (double strand-specific). (B) Analytical native gel analysis of RNAs used for structure mapping. Gel is 10% native PAGE run in 0.5x TBE. This gel confirms that monomer identity has been retained and dimer identity has been largely retained. (C) Monomer cleavage data superimposed on the monomer secondary structure are consistent with published 1/99 HDV ribozyme structure. (D) Dimer cleavage data superimposed on an extended dimer secondary structure model. In panels (C) and (D), secondary structures are mapped with RNases A (orange), T1 (blue), T2 (green), and V1 (red). Closed and open triangles represent stronger and weaker cleavages, respectively.