Supporting Information for:

The Human HDV-like *CPEB3* Ribozyme is Intrinsically Fast Reacting

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1) Figures S1–S3

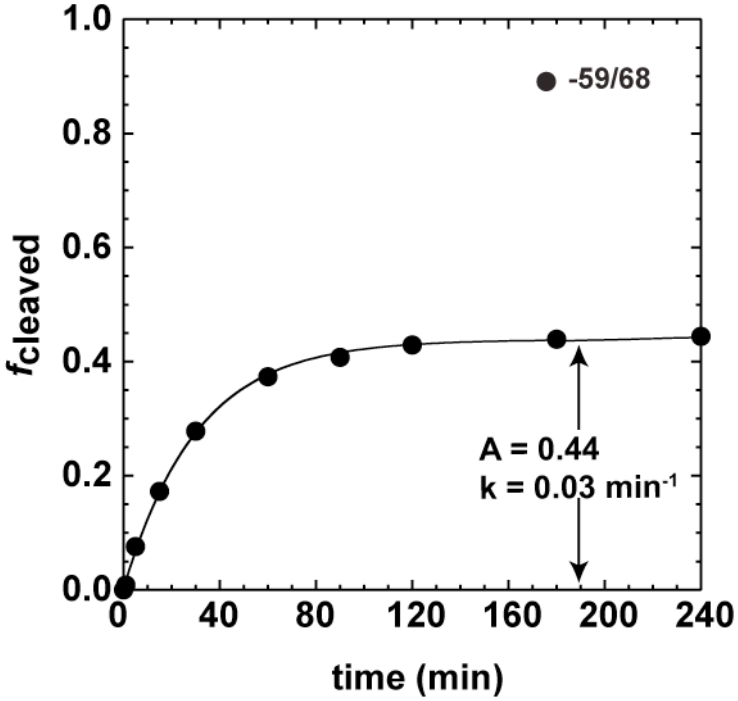

Figure S1. Self-cleavage of human *CPEB3* WT ribozyme. Self-cleavage reaction of full-length –59/68 construct that was transcribed, dephosphorylated, and subsequently labeled with [γ-^{32}P]ATP using conventional purification procedures ('two-step purification'). A plot of $f_{cleaved}$ *versus* time was generated, where each data point is the average of at least two trials. Data were well fit to single-exponential eq 1 ($R^2 = 0.999$) to give A = 0.44±0.003, B = –0.44±0.05, and k_{obs} = 0.03±0.001 min^{-1}. In contrast, self-cleavage assays carried out using RNA that were end-labeled during transcription (Figure 1D) had a fast reacting phase, suggesting that a fast-reacting phase had been lost during two-step handling, leaving behind just the slow and non-reacting species.

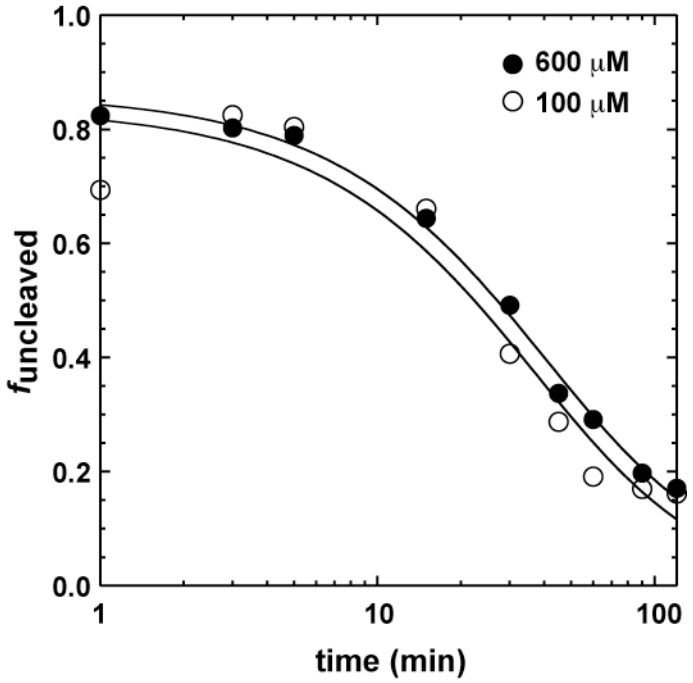

Figure S2. Noneffect of lowering the rate of transcription. Co-transcriptional self-cleavage for human –59/68 ribozyme, carried out using 600 μM (●) or 100 μM (○) NTPs. GTP concentrations were retained at 600 μM in both reactions. Plots of $f_{uncleaved}$ *versus* time were generated, where each data point is the average of at least two trials. Data for both reactions were fit to eq 3, and rates were nearly identical as revealed by superposition of the data.

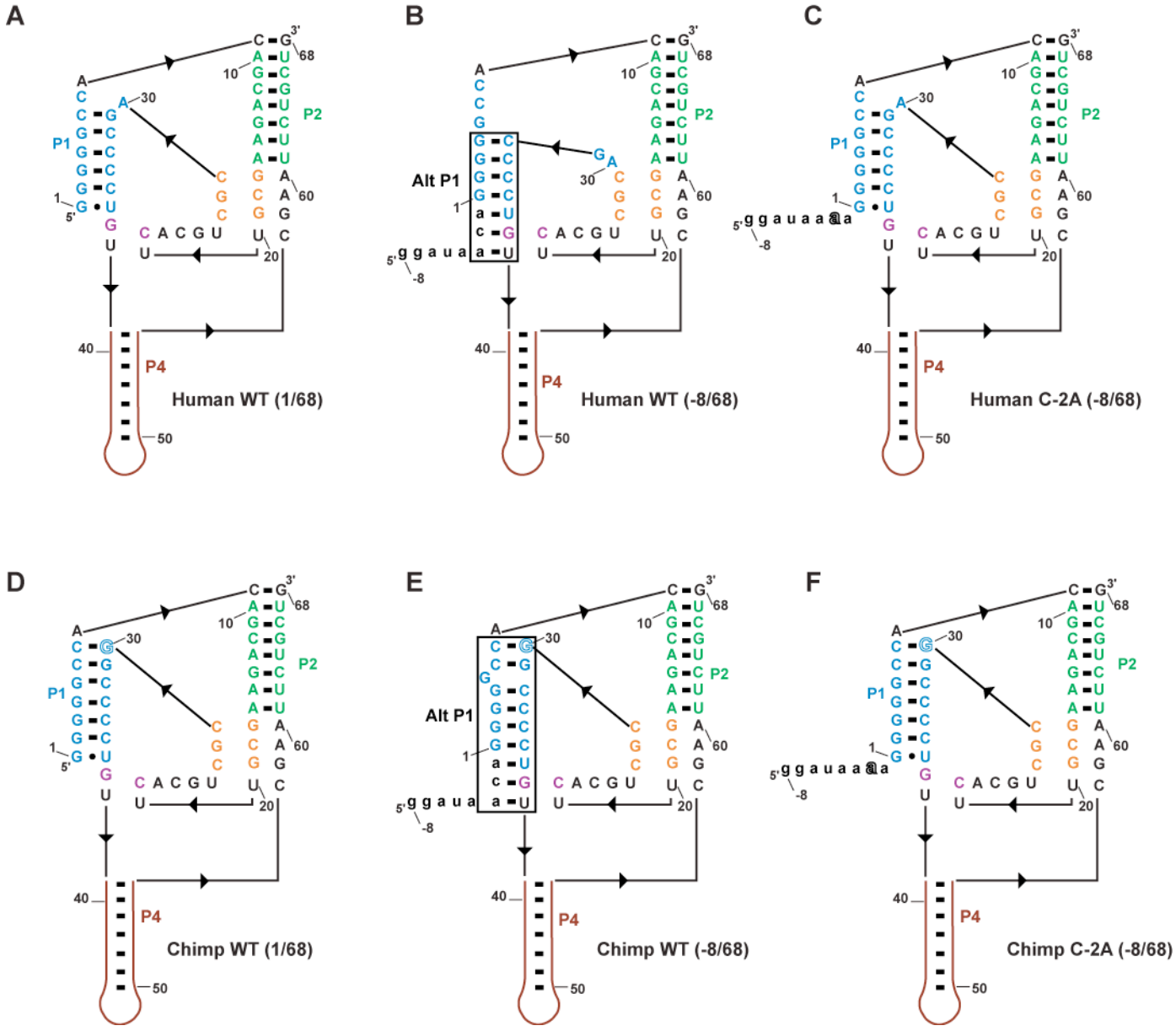
A
B
C
D
E
F
P1
P2
P4
Alt P1
Human WT (1/68)
Human WT (-8/68)
Human C-2A (-8/68)
Chimp WT (1/68)
Chimp WT (-8/68)
Chimp C-2A (-8/68)

Figure S3. Predicted secondary structures for WT and mutant *CPEB3* ribozymes using the ILM algorithm (*1, 2*). Non-native 3–4 bp helices containing less than two G–C base pairs are not provided because these are unlikely to be stable. Base changes are represented in outline font. P4 is depicted as a line for simplification and flanking sequence is in lower case font. Coloring is the same as in the native pairings in Figure 1B. (A) Cleaved 1/68 WT ribozyme. Native P1, P2, and P4 were predicted, while P1.1 and P3 were absent. (B) Precursor –8/68 human RNA. Native pairings P2 and P4 were observed, but P1, P1.1, and P3 were absent. An alternative pairing (Alt P1) (*3*) involving upstream nucleotides and much of P1 nucleotides is boxed here and in panel *E*. (C) Precursor –8/68 human C–2A RNA, which folds with a native P1 similar to panel A. (D), (E), and (F) are of the corresponding –8/68 chimp ribozyme, which contains a native G at position 30 (*4*).

2) Supporting References

(1) Ruan, J., Stormo, G. D., and Zhang, W. (2004) An iterated loop matching approach to the prediction of RNA secondary structures with pseudoknots. *Bioinformatics 20*, 58-66.

(2) Ruan, J., Stormo, G. D., and Zhang, W. (2004) ILM: a web server for predicting RNA secondary structures with pseudoknots. *Nucleic Acids Res. 32*, W146-149.

(3) Chadalavada, D. M., Senchak, S. E., and Bevilacqua, P. C. (2002) The folding pathway of the genomic hepatitis delta virus ribozyme is dominated by slow folding of the pseudoknots. *J. Mol. Biol. 317*, 559-575.

(4) Salehi-Ashtiani, K., Luptak, A., Litovchick, A., and Szostak, J. W. (2006) A genomewide search for ribozymes reveals an HDV-like sequence in the human CPEB3 gene. *Science 313*, 1788-1792.