

## SUPPLEMENTAL INFORMATION FOR:

Wild-type is the optimal sequence of the HDV ribozyme  
under co-transcriptional conditions

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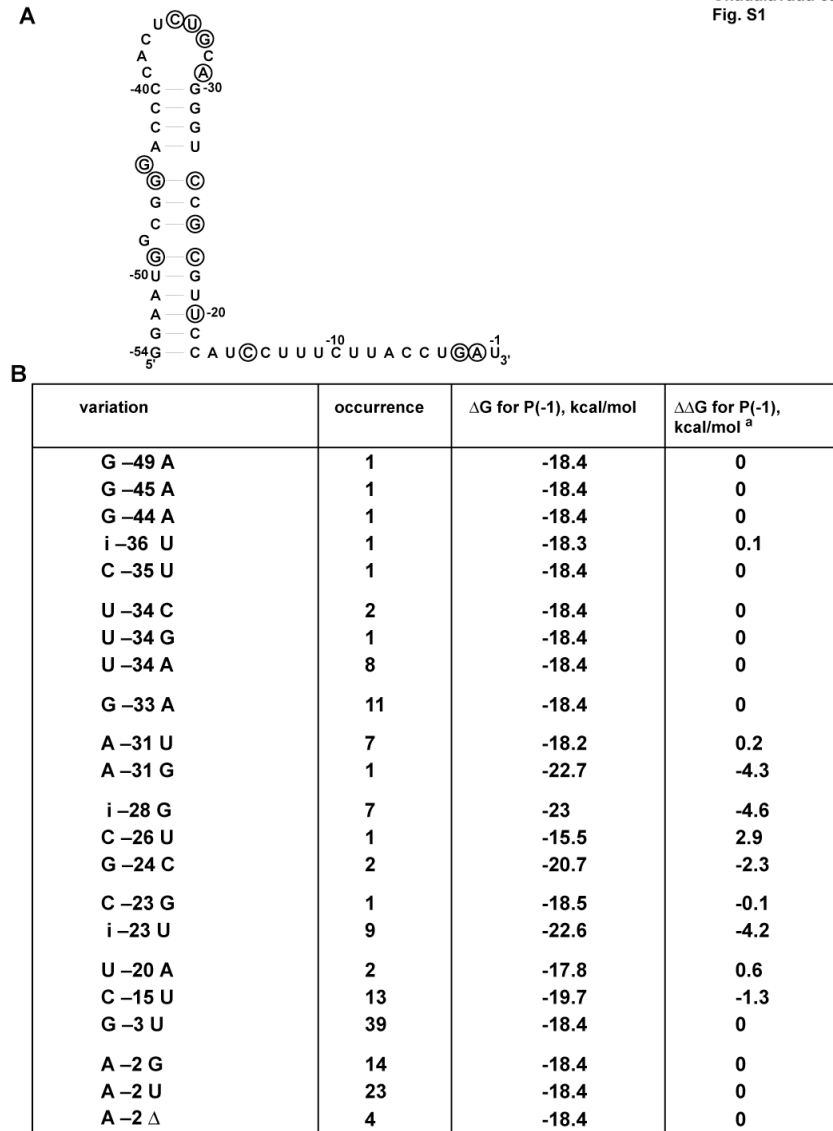
#### 1.) Three Supplemental Figures

**Figure S1.** Conservation of P(-1) in genomic HDV ribozyme variants

**Figure S2.** Relative rates of transcription as a function of NTP concentration and HDAg

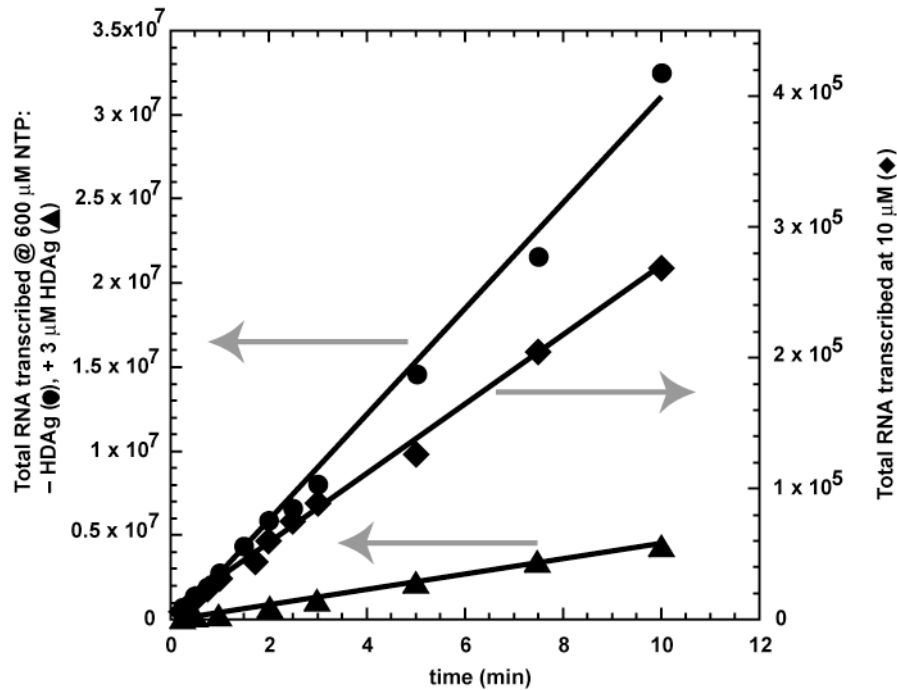
**Figure S3.** Basal rates of ribozyme self-cleavage in the presence of HDAg

#### 2.) Supplemental References

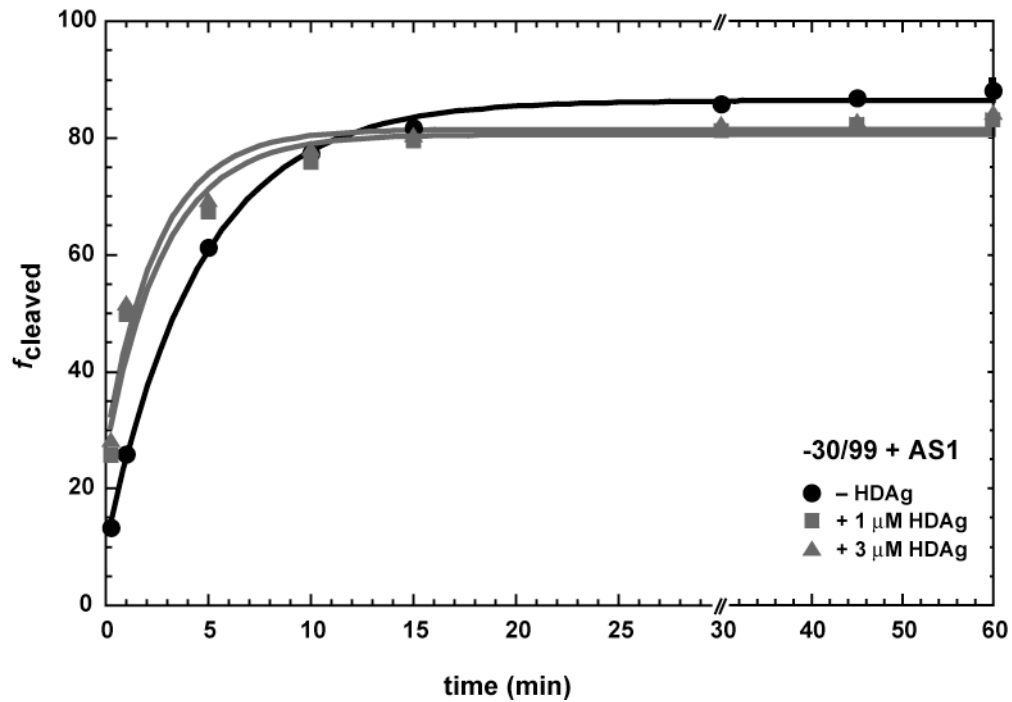


<sup>a</sup> WT P(-1) gave a  $\Delta G$  of -18.4 when folded using mfold v3.2

**Figure S1.** Conservation of P(-1) in genomic HDV ribozyme variants. (A) The secondary structure of the isolate used for wild-type, established by both experiments and calculations (Chadalavada et al. 2000), is shown. Positions at which substitutions occurred are circled. (B) Summary of variations found in genomic isolates. The variation and number of occurrences are provided, where ‘i’ represents an insertion and ‘ $\Delta$ ’ a deletion. In addition the predicted folding free energy (Mathews et al. 1999; Zuker 2003) and change in free energy relative to wild-type are provided in the last two columns.



**Figure S2.** Relative rates of transcription as a function of NTP concentration and HDAG. The total amount of transcript (cleaved plus uncleaved) was plotted versus time of transcription. The data fit well to a line, consistent with a constant rate of transcription as discussed in the Materials and Methods. The slopes were as follows:  $3.2 \times 10^6$  for 600  $\mu$ M NTPs and no HDAG (●),  $4.6 \times 10^5$  for 600  $\mu$ M NTPs and 3  $\mu$ M HDAG (▲), and  $2.6 \times 10^4$  for 10  $\mu$ M NTPs (600  $\mu$ M GTP) (◆); the first two plots refer to the left-hand y-axis, while the third plot refers to the right-hand y-axis. The slope for 600  $\mu$ M NTPs and 1  $\mu$ M HDAG was  $4.3 \times 10^5$  (data not shown).



**Figure S3.** Basal rates of ribozyme self-cleavage in the presence of HDAg. Full-length -30/99 wild-type ribozyme was mixed with HDAg and AS1. Reaction was initiated by addition of  $\text{Mg}^{2+}$  to a final concentration of 1 mM and reactions were fractionated by denaturing PAGE. Data were fit to equation 6 to give the following burst and  $k_{\text{obs}}$  values: 10% and  $0.22 \text{ min}^{-1}$  for no HDAg ( $\bullet$ ), 26% and  $0.35 \text{ min}^{-1}$  for 1  $\mu\text{M}$  HDAg ( $\blacksquare$ ), and 28% and  $0.39 \text{ min}^{-1}$  for 3  $\mu\text{M}$  HDAg ( $\blacktriangle$ ).

Supplemental References:

- Chadalavada, D. M., Knudsen, S. M., Nakano, S., and Bevilacqua, P. C. 2000. A role for upstream RNA structure in facilitating the catalytic fold of the genomic hepatitis delta virus ribozyme. *J. Mol. Biol.* **301**:349-367.
- Mathews, D. H., Sabina, J., Zuker, M., and Turner, D. H. 1999. Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *J. Mol. Biol.* **288**:911-940.
- Zuker, M. 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* **31**:3406-3415.