Supplementary Information for

Processing and translation initiation of non-long terminal repeat retrotransposons by hepatitis delta virus (HDV)-like self-cleaving ribozymes

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Supplementary Experimental Procedures

Constructs and primers for *in vitro* self-cleavage kinetics
Drosophila melanogaster (strain: W1118)

drz-Dmel-1-1 (R2)

Ribozyme construct:
5′aagcgctggtcaacggcgggagtaactatgactgtcttaaGGGGAGTCATGGGGTATTTGAGAGCAGAGG
GGGAGTATTCTTCTGTAATTCGTAAGTCATATCATATGATGTGCGGAAGGGGAATTT
TACTCTGTAACTCACAAGTCTCTCCTTTACTCAAGTCGACTCAAAACCTCCTCGTGGT
GGTCCCCGGTAATGCTAAACTTGTTTAGCAGCTAATTTGAGCGGCaaaactt

AL506: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGAAGCGCTGGTCAACGGCGGG
AL507: 5′AAGTTTTGCCGCTCAAATTAGCTGCTA
AL508 (inhibitor): 5′GACTCCCCTTAAGA

drz-Dmel-1-2 (R2)

Ribozyme construct:
5′ggcgagctgatcactgattggggtgactgcgcacgagttGGGGATCATGGGGTATTTGAGAGCAGAGGG
GGAGTATTCTTCTGTAATTCGTAAGTCATATCATATGGTGTGCGGAAGGGGAATTTT
ACTCTGTAACTCACAAGTCTCTCCTTTACTCAAGTCGACTCAAAACCTCCTCGTGGTG
GTCCCCGGTAATGCTAAACTCGTTTAGCAGCTAATTTGAGCGGCaaaac

AL527: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGGGCGAGCTGATCACTGATTGG
AL528: 5′GTTTTGCCGCTCAAATTAGCTGCTA
AL529 (inhibitor): 5′GATCCCCAACTCG

drz-Dmel-2-1 (Baggins)

Ribozyme construct:
5′gttttgtctccggattctccaattgttatttatttataatGGCCGCCATGACAGTAGGTATCACAAGGGGATCA
ACGCGCCACCACTACTGGAAGTGCAACTACACTCTCCACGCGAGGGCGGCTGGGAA
CAGGCTCTCAGTTAGGTCATGTCTCTGCTAAGAGTGCTGGCTAATCATAGTTGggct

AL512: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGGTTTTGTCTCCGGATTCTCCAA
AL565: 5′AGCCCAACTATGATTAGCCAGC
AL514 (inhibitor): 5′GGCGGCCATTAT

drz-Dmel-2-2 (Baggins)

Ribozyme construct:
5′cacgatatctacggatctcgcggtaacttaaaccaaaaaaGGCCGCCACGACAGTAGATATCACAAGGGG
ATCAACGCGCCACCAACGGTGGTACGCGCCGTAATTGTGAAACACTACTGGACGTG

CAACTACACTCTCCACGCGAGGGCGGCTGGAAACAGGCTTTTAGTAAGGTCATGTA
ACTGCTAAGAGTGCTGGCTAATCGTAGTTGggctgg

AL515: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGCACGATATCTACGGATCTCG
AL516: 5′CCAGCCCAACTACGATTAGCCA
AL517 (inhibitor): 5′GGCGGCCTTTTT

Drosophila simulans (strain: (Matzkin) Isosancruz-19)

drz-Dsim-1 (R2)

Ribozyme construct:
5′agtaagcgcgggtcaacggcgggagtaactatgactctctGAGGGATCTGGGGTAATTGCGAGCAGAGGG
GGAGTATTTTTCTGTAATTCGTAAGTCATATCATATGGTGTGCGGAAGGGGAATTTT
ACTCTGTAACTCACAAGTCTCTCCTTTACTCAAGTCGACTCAAAACCTCCTCGTGGTG
GTCCCCGGTAATGCTAAACTTGTTTAGCAGCTAATTTGAGCGGCaaaaactt

AL566: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGAGTAAGCGCGGGTCAACGGCG
AL567: 5′AAGTTTTTGCCGCTCAAATTAGCTGCT
AL568 (inhibitor): 5′GATCCCTCAGAGAG

Drosophila ananassae (strain: (BGS) SB18.8C)

drz-Dana-1 (R2)

Ribozyme construct:
5′taagcgcgggtcaacggcgggagtaactatgactctctttGGAGAATATGGATTTGATTGTGCAGAGGGGG
TGCTATACCGTAACTCGTAAGCCATGCAATCAGATCAAGTCGACTCAAAACCTCCTC
GTGGTATTCTCTGGGTGCCAGTATTTACTGGTAGCTGATTTGAGCGGCgaaag

AL581: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGTAAGCGCGGGTCAACGGC
AL581: 5′CTTTCGCCGCTCAAATCAGCT
AL583 (inhibitor): 5′ATATTCTCCAAAGAGA

Drosophila sechellia (strain: (BSC) 3529 line 15)

drz-Dsec-1 (R2)

Ribozyme construct:
5′agtaagcgcgggtcaacggcgggagtaactatgactcttaGGGGATCAGGGGTAATTGCGAGCAGAGGGG
GAGTATTTTTCTGTAATTCGTAAGTCATATCATATGGTGTGCGGAAGGGGAATTTTA
CTCTGTAACTCACAAGTCTCTCCTTTACTCAAGTCGACTCAAAACCTCCTCGTGGTGG
TCCCCGGTAATGCTAAACTTGTTTAGCAGCTAATTTGAGCGGCaa

AL798: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGAGTAAGCGCGGGTCAACGGC
AL799: 5′TTGCCGCTCAAATTAGCTGCTA

AL800 (inhibitor): 5′GATCCCCTAAGAG

Drosophila persimilis (strain: CC23)

drz-Dper-1 (R2B)

Ribozyme construct:
5′aagcgcgggtcaacggcgggagtaactatgactctcttaaGGAAGATATGGATCTGAACAATAGCGTAGA
AGGGGAGTCATTCCGTAATTCGTAAATCGTAAAAATTAGATCAAGTTGATTCAAGAC
CTCCTCGTGGTATCTTCTGGATGCTATTAGACTAAAGTTCTTTTGGTCTAATAGTAAC
TAACTTGAACagcgaaa

AL645: 5′ TTCCCGCGAAATTAATACGACTCACTATAGGGAGAAGCGCGGGTCAACGGCG
AL646: 5′ TTTCGCTGTTCAAGTTAGTTACTATT
AL644 (inhibitor): 5′ ATCTTCCTTAAGAGA

drz-Dper-2 (Baggins)

Ribozyme construct:
5′tcagcttgtccgatattgtagtagtttttaagttgacccaGGCCGTCAGCCATCGGGTGCTACAGGGGGACA
AACGGATGACGGGTCGGCTCGCACAACTGTGAACTCGGAACCCGCCGTAACTGTAC
ACACCGGTGGAAGTGCGACTATACTCTCCCCGTGAGGGCGGCTGGAAACAGGTCCT
GGCACGGTCATGTCTCTGCCAGGATTCTGGCGAATGGTAGTCGggcg

AL633: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGTCAGCTTGTCCGATATTGTAGTAG
AL634: 5′ CGCCCGACTACCATTCGCC
AL635 (inhibitor): 5′ GACGGCCTGGG

Papilio xuthus

drz-Pxut-1 (SARTPx)

Ribozyme construct:
5′aagcgcgggtcaacggcgggagtaactatgactctcttaaGGAAGATATGGATCTGAACAATAGCGTAGA
AGGGGAGTCATTCCGTAATTCGTAAATCGTAAAAATTAGATCAAGTTGATTCAAGAC
CTCCTCGTGGTATCTTCTGGATGCTATTAGACTAAAGTTCTTTTGGTCTAATAGTAAC
TAACTTGAACagcgaaa

AL1026:
5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGTTAGGTTAGGTTAGGTTAGGTTAGGTTAGGTAG
GGGTGCGGTGGCCGGTGACCTCCTCGTGGTGCA
AL1027:
5′CTCCCCGGCCGGTTGTTAGCCTCCCCACAGAAGGTGGGGAGTTTCCAGGGTGCACCACGAGGAGGTC
ACCGGCCACCGCACCCCTACCTAACCTAA
AL997 (inhibitor): 5′GCACCCCTACCT

Ascaris lumbricoides

drz-Alum-1 (R4)

Ribozyme construct:
5′ggtagccaaatgcctcgtcatctaattagtgacgcgcatgGGGGCCGGTGGGTTTACTCACTTCTGACCCA
CCACCAACGGAACGAGGGAAAGCAGAGCTGGGGCCCTCTTCCGATTGGCATGGAAC
CGACCTCCACGTGGTGGCCCTGGGCAACGGAATTCAAGAGAGGATTTAATCCTCTCT
ATCATTTGCAAGATGGATGAGATCGAGGTATCCGGCAAACAGGTTCCAAGTgagc

AL1029:
5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGGGTAGCCAAATGCCTCGTCATCTAATTAGTGAC
GCGCATGGGGGCCGGTGGGTTTACTCACTTCT
AL1027:
5′CTCCCCGGCCGGTTGTTAGCCTCCCCACAGAAGGTGGGGAGTTTCCAGGGTGCACCACGAGGAGGTC
ACCGGCCACCGCACCCCTACCTAACCTAA
AL1000:
5′GCTCACTTGGAACCTGTTTGCCGGATACCTCGATCTCATCCATCTTGCAAATGATAGAGAGGATTAA
ATCCTCTCTTGAATTCCGTTGCCCAGGGCCACC
AL1001 (inhibitor): 5′GCATGAGGGGCC

Heliconius numata

drz-Hnum-1 (putatively R1)

Ribozyme construct:
5′ttaatagggttctctatgttaacctagatttaagaaatttGGGGTGCTATGTCGGTTACCTCCTCGTGGGGCA
CCCTGGGCAACGGGACGGGCAGCAGCATCACAGTCGTGGTGCTGCTGCTCGTCGCG
GCTAATAAACCGACAcgcg

AL1047:
5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGTTAATAGGGTTCTCTATGTTAACCTAGATTTAAG
AAATTTGGGGTGCTATGTCGGTTACCTCCTCG
AL1048:
5′CGCGTGTCGGTTTATTAGCCGCGACGAGCAGCAGCACCACGACTGTGATGCTGCTGCCCGTCCCGTT
GCCCAGGGTGCCCCACGAGGAGGTAACCGACAT
AL1050 (inhibitor): 5′GCACCCCAAATTT

Schistosoma mansoni

drz-Sman-1 (putative RTE)

Ribozyme construct:
5′ggggcaccacagggatGGGAGGCGAAATCCGACTCACACGTCCTCGTCGTGCCTCCTGGAT
CATGGGACTGATTCCATGACCTAACGTGGGCGGttaat

AL593:
5′TTCCCGCGAAATTAATACGACTCACTATAGGGGCACCACAGGGATGGGAGGCGAAATCCGACTCA
AL594:
5′ATTAACCGCCCACGTTAGGTCATGGAATCAGTCCCATGATCCAGGAGGCACGACGAGGACGTGTGA
GTCGGATTTCGCCTCCC
AL595 (inhibitor): 5′GCCTCCCATCCCTG

Anopheles gambiae

drz-Agam-3-1 (R6Ag3)

Ribozyme construct:
5′aggtcaccgaaacaacgttgtacctaaggtaacctgctgtGCCCGGCAAAGTCCGACCATCACCTCCTCGC
GGTGCCGGGCGGGGTAGGAGTTCGTCCTTGGACAGGCTCTGAGCTAACGATGGCggca
c

AL1126: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGAGGTCACCGAAACAACGTTG
AL1127: 5′GTGCCGCCATCGCTAGCTCA
AL943 (inhibitor): 5′CCGGGCACAGC

Aplysia californica

drz-Acal-1 (RTE)

Ribozyme construct:
5′aatatgagggtctgtgtgtattcaattgctgagaccttgtGGTGCGCATGTCCTGCCAAAAAGACGAGGAGG
ACAGGGATTTCAACCAACCCCTGTCCGGCCCAGTTTGGCGGGGCAAGTCATCCAGC
ACCTCTCCGTGGTGCGCACTAGTAACCGCTTTCTTGCGGGCTAAGCTGGATGagat

AL1340:
5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGAATATGAGGGTCTGTGTGTATTCAATTGCTGAG
ACCTTGTGGTGCGCATGTCCTGCCAAAAAGACG
AL1341:
5′CCACGGAGAGGTGCTGGATGACTTGCCCCGCCAAACTGGGCCGGACAGGGGTTGGTTGAAATCCCT
GTCCTCCTCGTCTTTTTGGCAGGACATGCGCACC
AL1342:
5′ATCTCATCCAGCTTAGCCCGCAAGAAAGCGGTTACTAGTGCGCACCACGGAGAGGTGCTGGATGACT
TG
AL1343 (inhibitor): 5′CGCACCACAAGG

Ciona intestinalis

drz-Cint-1 (R2)

Ribozyme construct:
5′tagaggatccctaaacggcgggagtaactatgactctcttGACTCTCTATGGTGGTCGCCTTCTCGTGGCGA
GAGTCGTAATTACCTCCGGCATAACTGCGAGCCCTCACAAGGCTGGCAGGGACTGC
GGGAGCGAAACCAAGAATCACCAcgcc

AL1344:
5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGTAGAGGATCCCTAAACGGCGGGAGTAACTATG
ACTCTCTTGACTCTCTATGGTGGTCGCCTTCTC
AL1345:
5′TGCCAGCCTTGTGAGGGCTCGCAGTTATGCCGGAGGTAATTACGACTCTCGCCACGAGAAGGCGACC
ACCATAGAGAGTC
AL1346:
5′GGCGTGGTGATTCTTGGTTTCGCTCCCGCAGTCCCTGCCAGCCTTGTGAGGGCTCGCAGT
AL1347 (inhibitor): 5′GAGAGTCAAGAGA

Primers for translation experiments
The primers listed below were used to amplify the wild-type ribozymes from genomic DNA. The constructs included a 300 bp leader sequence and a BamHI site downstream of the ribozyme.

drz-Dmel-1-2 (R2)
AL530: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGGCCCAGCAGGAGAGCACGT
AL1147: 5′TAGGGATCCGCGCCGCTCAAATTAGCTGCTAA

drz-Dsim-1 (R2)
AL937: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGTTAGTTACTTGTTCCCTGGATAGT
AL1145: 5′TAGGGATCCGCGCCGCTCAAATTAGCTGCTAA

drz-Dana-1 (R2)
AL938: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGTTGTAACTTGTTCCCCGGATAGT
AL1146: 5′TAGGGATCCGCGCCGCTCAAATCAGCTACCAG

drz-Agam-3-1 (R6Ag3)
AL941: 5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGCCACCACGTCGTGGCAAC
AL945: 5′AGTCGGTCTCGTCTTCGCCATCGTTAGCTCAGAGCC

To amplify the full length HCV IRES from a gifted plasmid, the following primers were used:
AL1199: 5′TTCCCGCGAAATTAATACGACTCACTATAGCTCCCCTGTGAGGAACTACTG
AL1200: 5′GGCGTCTTCCATGAGGATCCTTTTTCTTTGAGG

The entire luciferase gene was amplified from plasmid DNA using the primers below:
AL1149: 5′GCGCTGGATGGATCCATGGAA
AL1148: 5′AACTTATCGATTTTACCACATTTGTAG

To construct inactive ribozymes containing a single C to U mutation, the following oligonucleotides were used in with the QuikChange mutagenesis kit from Stratagene:

drz-Dmel-1-2 (R2)

AL1201: 5′CTAAACTCGTTTAGCAGTTAATTTGAGCGGCGCGG
AL1202: 5′CCGCGCCGCTCAAATTAACTGCTAAACGAGTTTAG

drz-Dsim-1 (R2)
AL1203: 5′CTAAACTTGTTTAGCAGTTAATTTGAGCGGCGCGG
AL1204: 5′CCGCGCCGCTCAAATTAACTGCTAAACAAGTTTAG

drz-Dana-1 (R2)
AL1205: 5′CCAGTATTTACTGGTAGTTGATTTGAGCGGCGCGG
AL1206: 5′CCGCGCCGCTCAAATCAACTACCAGTAAATACTGG

drz-Agam-3-1 (R6Ag3)
AL1207: 5′GGACAGGCTCTGAGTTAGCGATGGCAGCG
AL1208: 5′CGCTGCCATCGCTAACTCAGAGCCTGTCC

Primers for P1 extension DNA template preparation
The leader sequence variants shown in Figure 2 were made with PCR amplification from the drz-Dmel-1-2 plasmid using the specific forward primers and the universal reverse primer listed below. The universal forward primer was then added on to the constructs upstream of the original specific forward primer.

Fig. 2b:
AL1250: 5′AGTAACTATGACTCTCTTAAGGGGGGATCATGGGGTATTTGAGAGCAGAG

Fig. 2c:
AL1251: 5′AGTAACTATGACTCTCTTAGGGGATCATGGGGTATTTGAGAGCAGAGGGG

Fig. 2d:
AL1252: 5′AGTAACTATGACTCTCTTAAGGGGATCATGGGGTATTTGAGAGCAGAGGGG

Fig. 2e:
AL1253: 5′AGTAACTATGACTCTCTTCGGGGATCATGGGGTATTTGAGAGCAGAGGGG

Fig. 2f:
AL1254: 5′AGTAACTATGACTCTCTCCGGGGATCATGGGGTATTTGAGAGCAGAGGGG

Fig. 2g:
AL1336: 5′AGTAACTATGACTCTCACCGGGGATCATGGGGTATTTGAGAGCAGAGGGG

Universal reverse:
AL1248: 5′GCGTCTTCCATGGATCCGCGCCGCTCAAATTAGCTGCTAAAC

Universal forward:
AL1247:
5′TTCCCGCGAAATTAATACGACTCACTATAGGGAGAAATTCAAGTAAGCGCTGGTCAACGGCGGGAG
TAACTATG

Transfection qPCR

The nucleic acid content of the transfected S2 cell lysates was analyzed *via* phenol-chloroform extraction followed by quantitative RT-PCR using the primers listed below. The MiniOpticon Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) was used to perform and monitor the reactions for 45 cycles. Maxima® SYBR Green/Fluorescein qPCR Master Mix (Fermentas) was combined with diluted cDNA or isolated DNA (no RT reaction) and 0.5 μM primer mixtures in each reaction.

AL1646: 5′TTGGAGCACGGAAAGACGATGA
AL1647: 5′ATCTTTCCGCCCTTCTTGGCCT

Negative control for translation assays

An inhibitory DNA oligonucleotide (shown below) was used to block translation of the drz-Dsim-1 luciferase construct as a negative control. The oligonucleotide was introduced into both *in vitro* and *in vivo* translation assays at two times the number of moles of RNA. After two 5 minute incubations at 55 °C and then 65 °C, the DNA-RNA mixture cooled on ice and used in the *in vitro* translation reaction and transfection assay as described in the main text.

AL1379: 5′GCGTCTTCCATGGATCCGC

Supplementary Table S1. Kinetic analysis of retrotransposon-associated ribozymes.

Ribozyme	10 mM Mg^{2+}, 37ºC Fast Rate Constant ($1/hr^{-1}$)	Amplitude	Slow Rate Constant ($1/hr^{-1}$)	Amplitude	Fraction Uncleaved	1 mM Mg^{2+}, 25ºC Fast Rate Constant ($1/hr^{-1}$)	Amplitude	Slow Rate Constant ($1/hr^{-1}$)	Amplitude	Fraction Uncleaved
drz-Dmel-1-1	37 ± 2	0.42 ± 0.08	1.4 ± 0.1	0.33 ± 0.05	0.21 ± 0.09	6.0 ± 1.0	0.16 ± 0.01	0.31 ± 0.17	0.48 ± 0.11	0.35 ± 0.12
drz-Dmel-1-2	0.37 ± 0.03	0.82 ± 0.08	—	—	0.10 ± 0.06	0.074 ± 0.016	0.34 ± 0.03	—	—	0.66 ± 0.03
drz-Dsec-1	90 ± 6	0.49 ± 0.13	3.1 ± 1.1	0.25 ± 0.02	0.24 ± 0.14	10 ± 1	0.50 ± 0.01	0.65 ± 0.11	0.34 ± 0.02	0.16 ± 0.02
drz-Dsim-1	16 ± 9	0.49 ± 0.16	0.020 ± 0.016	0.33 ± 0.26	0.13 ± 0.13	0.58 ± 0.07	0.58 ± 0.04	—	—	0.42 ± 0.06
drz-Dana-1	169 ± 13	0.18 ± 0.02	0.59 ± 0.02	0.11 ± 0.04	0.19 ± 0.01	57 ± 7	0.51 ± 0.02	3.5 ± 1.1	0.26 ± 0.05	0.20 ± 0.02
drz-Cint-1	ND	ND	ND	ND	ND	101 ± 43	0.21 ± 0.09	0.86 ± 0.37	0.24 ± 0.10	0.52 ± 0.09
drz-Dper-1	29 ± 9	0.12 ± 0.03	1.8 ± 0.7	0.15 ± 0.02	0.72 ± 0.02	1.6 ± 0.2	0.22 ± 0.03	—	—	0.75 ± 0.03
drz-Alum-1	ND	ND	ND	ND	ND	1.1 ± 0.1	0.38 ± 0.03	0.055 ± 0.040	0.22 ± 0.02	0.40 ± 0.01
drz-Agam-3-1	ND	ND	ND	ND	ND	31 ± 6	0.30 ± 0.02	2.0 ± 0.1	0.48 ± 0.01	0.22 ± 0.02
drz-Dmel-2-1	0.07 ± 0.01	0.67 ± 0.03	—	—	0.33 ± 0.03	ND	ND	ND	ND	ND
drz-Dmel-2-2	11 ± 2	0.25 ± 0.05	1.1 ± 0.3	0.72 ± 0.01	0.04 ± 0.04	0.055 ± 0.015	0.60 ± 0.05	—	—	0.40 ± 0.05
drz-Dper-2	ND	ND	ND	ND	ND	13 ± 4	0.21 ± 0.04	0.21 ± 0.08	0.31 ± 0.03	0.459 ± 0.001
drz-Acal-1	ND	ND	ND	ND	ND	0.25 ± 0.05	0.54 ± 0.11	—	—	0.46 ± 0.12
drz-Pxut-1	ND	ND	ND	ND	ND	0.067 ± 0.001	0.45 + 0.01	—	—	0.53 ± 0.01
drz-Hnum-1	ND	ND	ND	ND	ND	63.0 ± 0.5	0.45 ± 0.01	2.1 ± 0.92	0.23 ± 0.01	0.33 ± 0.01
drz-Leri-2	0.029 ± 0.007	0.81 ± 0.13	—	—	0.21 ±0.13	ND	ND	ND	ND	ND
drz-Lmen-1	0.54 ± 0.01	0.690 ± 0.003	—	—	0.31 ± 0.01	ND	ND	ND	ND	ND
drz-Sman-1	0.063 ± 0.003	0.79 ± 0.03	—	—	0.24 ± 0.04	ND	ND	ND	ND	ND

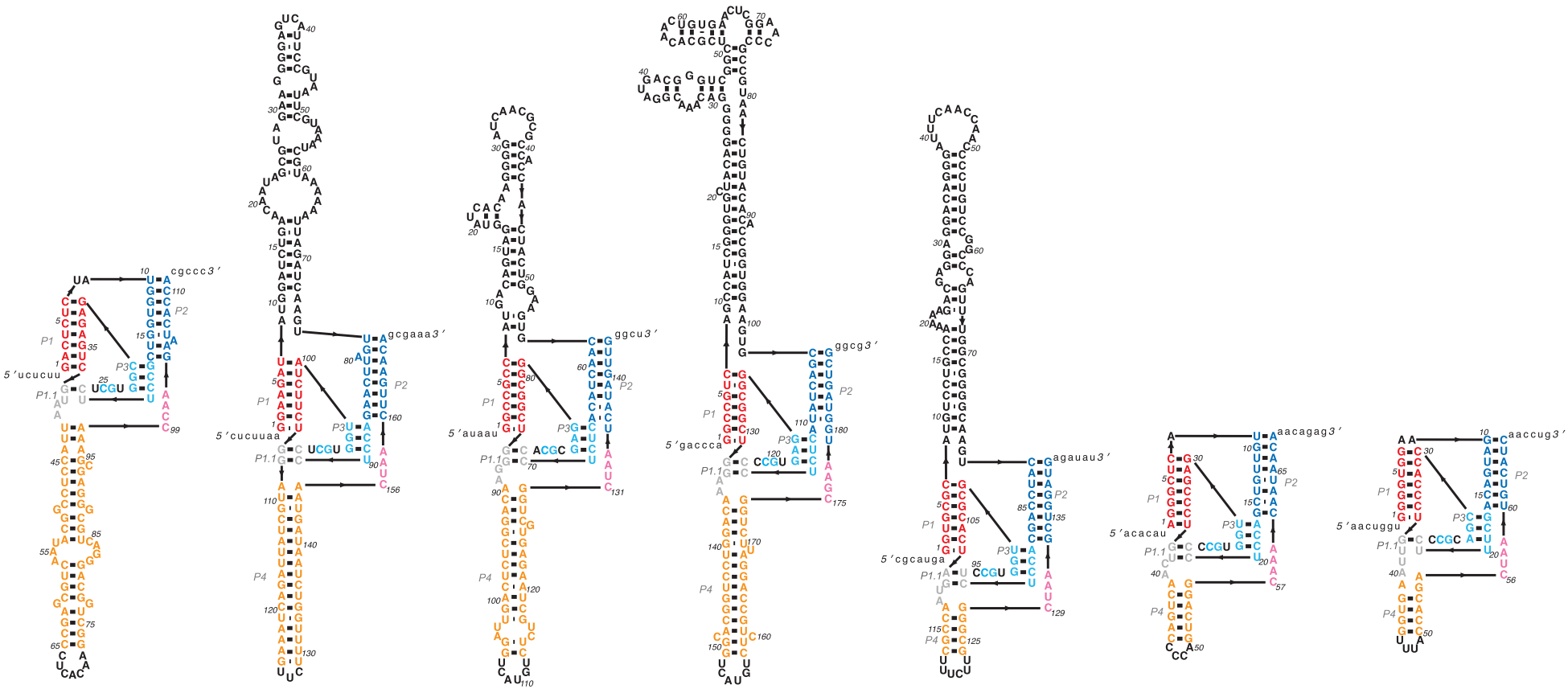
Supplementary Table S2. Kinetic analysis of drz-Dmel-1-1 in different divalent ions.

Conditions	Fast Rate Constant ($1/hr^{-1}$)	Amplitude	Slow Rate Constant ($1/hr^{-1}$)	Amplitude	Fraction Uncleaved
1 mM Mg^{2+}, 25°C	6 ± 1	0.16 ± 0.01	0.31 ± 0.17	0.48 ± 0.11	0.35 ± 0.12
1 mM Ca^{2+}, 25°C	13 ± 2	0.12 ± 0.01	0.49 ± 0.38	0.21 ± 0.13	0.55 ± 0.12
1 mM Mn^{2+}, 25°C	8 ± 4	0.13 ± 0.03	0.85 ± 0.38	0.47 ± 0.05	0.39 ± 0.01

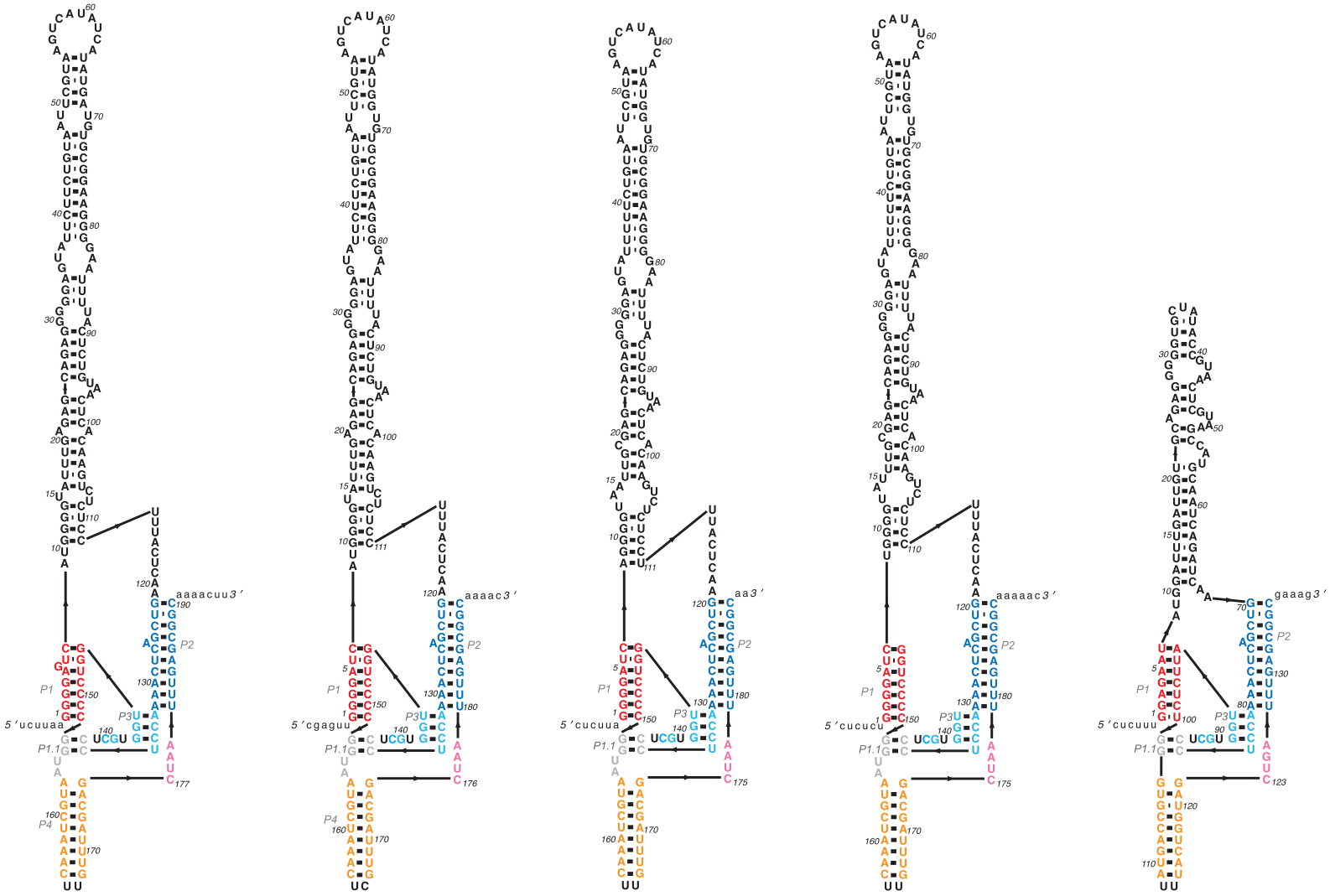
Supplementary Table S3. Kinetic analysis of drz-Dmel-1-1 leader sequence variants.

drz-Dmel-1-1 variant	Fast Rate Constant ($1/hr^{-1}$)	Amplitude	Slow Rate Constant ($1/hr^{-1}$)	Amplitude	Fraction Uncleaved
2b	19 ± 4	0.19 ± 0.08	0.35 ± 0.21	0.24 ± 0.03	0.57 ± 0.05
2c	90 ± 8	0.30 ± 0.19	0.83 ± 0.42	0.20 ± 0.06	0.57 ± 0.05
2d	86 ± 3	0.22 ± 0.07	1.15 ± 0.47	0.14 ± 0.05	0.63 ± 0.07
2e	0.36 ± 0.16	0.20 ± 0.04	—	—	0.69 ± 0.03
2e*	138 ± 40	0.19 ± 0.05	2.85 ± 0.66	0.19 ± 0.07	0.58 ± 0.10
2f	0.17 ± 0.05	0.32 ± 0.05	—	—	0.62 ± 0.04
2g	0.14 ± 0.01	0.37 ± 0.01	—	—	0.62 ± 0.02

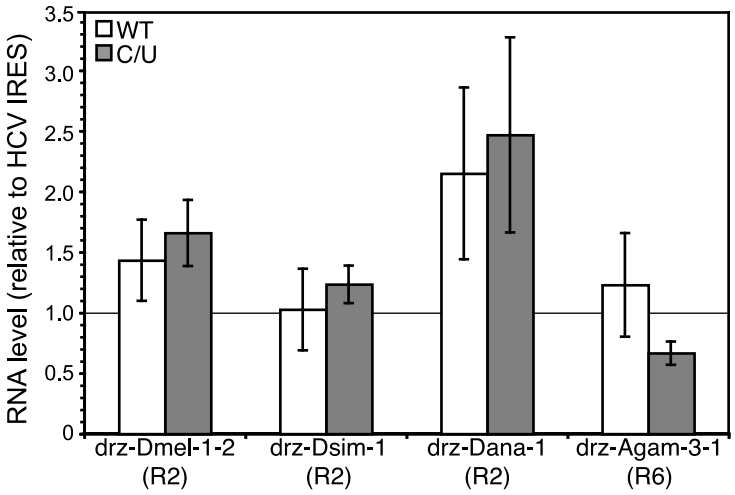
*Faster self-cleavage is observed in a different preparation of RNA.



Supplementary Figure S1. Secondary structures of other *in vitro* confirmed retrotransposon HDV-like ribozymes. From left to right, these include the *C. intestinalis* R2 drz-Cint-1, the *D. persimilis* R2B drz-Dper-1, the *D. melanogaster* Baggins drz-Dmel-2-1, the *D. persimilis* Baggins drz-Dper-2, the *A. californica* RTE drz-Acal-1, the *L. erinacea* RTE (putative) drz-Leri-1, and the *L. menadoensis* RTE (putative) drz-Lmen-1. Core elements are colored by region corresponding to the HDV ribozyme (Ferre-D'Amare et al., 1998).



Supplementary Figure S2. Secondary structures of the *in vitro* confirmed *Drosophila* R2 retrotransposon HDV-like ribozymes. From left to right, these include the *D. melanogaster* R2 drz-Dmel-1-1, the *D. melanogaster* R2 drz-Dmel-1-2, the *D. sechellia* R2 drz-Dsec-1, the *D. simulans* R2 drz-Dsim-1, and the *D. ananassae* R2 drz-Dana-1. These RNAs were used for the consensus secondary structure in Figure 2. Core elements are colored by region corresponding to the HDV ribozyme (Ferre-D'Amare et al., 1998).



Supplementary Figure S3. Level of ribozyme-terminated RNA relative to HCV IRES-terminated RNA. Quantitative RT-PCR of the luciferase coding region was performed on S2 cell lysates 24 h post-transfection to measure RNA levels. Equal amounts of RNA were used for transfections. All data are average values ± average deviations.