

Electronic Supplementary Information

Efficient control of group I intron ribozyme catalysis by DNA constraints

Elena Zelin and Scott K. Silverman*

Department of Chemistry, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, IL 61801 (USA)

Details of Experimental Procedures

Synthesis of the *Tetrahymena* group I intron ribozyme with two attached DNA strands

To prepare the L–21 ScaI ribozyme form of the *Tetrahymena* group I intron, the ScaI-digested pT7L–21 plasmid was used as a transcription template for T7 RNA polymerase.¹ The transcription product was purified by 6% PAGE. The 16 nt DNA constraint sequences were as follows: strand 1, 5'-GGAAGAGAGTGCACC-3'; strand 2, 5'-GGGTCGCACTCTCTTC-3' (the 15 nt following each 5'-G are complementary to the analogous 15 nt of the other strand, thereby forming a 15 bp duplex). Strand 1 was attached at A146 or A192 of the intron, and strand 2 was attached at A97, A286, or A308. For the A97-A308 ribozyme, strand 1 was attached at A97, and strand 2 was attached at A308. When noncomplementary strands were attached to the ribozyme, strand 1 was placed at both sites.

The 9FQ4 deoxyribozyme was used to attach DNA to the ribozyme. For initial analytical-scale assays, the reported procedure was followed as described,² with inclusion of 100 equivalents of disruptor oligonucleotide relative to the RNA substrate when appropriate. The DNA strands were 5'-adenylated as described.³ Sequences of the 9FQ4 deoxyribozymes and disruptor oligonucleotides are provided in a section below.

For preparative-scale synthesis of the doubly DNA-modified ribozyme, the left-hand (L) ribozyme substrate was the limiting reagent relative to each 9FQ4 deoxyribozyme (E), disruptor (D), and right-hand (R) 5'-adenylated DNA substrate. A 34 μ L sample containing 100 pmol of L, 110 pmol of each E, 400 pmol of each D, and 150 pmol of each R was annealed in 5 mM HEPES, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice for 5 min. When the two attached DNA strands were complementary, each deoxyribozyme was separately annealed to the corresponding DNA while the ribozyme was annealed to the appropriate disruptors, and the three separate annealing samples were mixed immediately after cooling on ice. The sample was adjusted to final concentrations of 50 mM CHES, pH 9.0, 150 mM NaCl, and 2 mM KCl by addition of 10 μ L of an appropriate stock solution. The ligation reactions were initiated by addition of 6 μ L of 1 M MgCl₂ (final Mg²⁺ concentration of 120 mM; final total volume of 50 μ L). The sample was incubated at 37 °C for 2 h and quenched with 50 μ L of stop solution (80% formamide, 1 \times TBE [89 mM each Tris and boric acid and 2 mM EDTA, pH 8.3], 50 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol). The products were separated by 6% PAGE and visualized by UV shadowing; the desired product was extracted and ethanol-precipitated. The isolated yield of the doubly DNA-modified ribozyme was ~10 pmol (~10%).

Ribozyme catalytic cleavage assays

Each cleavage reaction was performed under single-turnover conditions, with 11 nt RNA oligonucleotide cleavage substrate 5'-³²P-CCCUCUAAAAA-3' as the limiting reagent. A 7.8 μ L sample containing 5 pmol of ribozyme and 10 pmol of inhibitor oligonucleotide was annealed in 100 mM HEPES, pH 7.2 with NaOH, and 100 mM NaCl (or, when appropriate, 50 mM MES, pH 6.0 with NaOH and without added NaCl) by heating at 95 °C for 3 min and cooling at room temperature (23 °C) for 10 min. The sample was brought to 50 °C, and 0.7 μ L (100 pmol) of removal oligonucleotide was added. The sample was incubated at 50 °C for 15 min, and 1.2 μ L of 100 mM MgCl₂ (final Mg²⁺ concentration of 10 mM) as well as 1.2 μ L of 10 mM GTP (final GTP concentration of 1 mM) were added. The sample was incubated at 50 °C for 15 min, and 0.7 μ L of either water or free complementary oligonucleotide (10 pmol; strand 1)

was added. The sample was incubated at 50 °C for 30 min and 30 °C for 5 min. The cleavage reaction was initiated at 30 °C by addition of 0.4 μL (0.2 pmol) of RNA substrate (final substrate concentration of 17 nM; final total volume of 12 μL). Aliquots of 1–2 μL were removed, quenched onto 8 μL of stop solution, and stored on dry ice until analysis by 20% PAGE. Sequences of the inhibitor and removal oligonucleotides are provided in a section below. Equivalent results were obtained when the order of addition of removal oligonucleotide and Mg²⁺/GTP was switched.

Sequences of 9FQ4 deoxyribozymes

Each deoxyribozyme sequence is listed alongside the ribozyme nucleotide attachment site and the DNA constraint strand number (1 or 2). For each sequence, the two enzyme loops A and B are in boldface, and the P4 region is underlined.²

A146 (strand 1) 5'-GGTCGCACTCTCC**CGTAAGGCAAGGGC**TCCCC**GGCTCGGTTCC**GAGACTTGGT-3'
A159 (strand 1) 5'-GGTCGCACTCTCC**CGTAAGGCAAGGGC**CCATCG**GGCTCGGTTCC**CAAAGTTTCCCC-3'
A192 (strand 1) 5'-GGTCGCACTCTCC**CGTAAGGCAAGGGC**GTCCGG**CTCGGACCC**CAGCTTATTACC-3'
A97 (strand 1) 5'-GGTCGCACTCTCC**CGTAAGGCAAGGGC**GACGGGG**CTCGGACCC**CTTGCCCTTTTAAACCG-3'
A97 (strand 2) 5'-GAAGAGAGTGC**CGCGTAAGGCAAGGGC**GACGGGG**CTCGGACCC**CTTGCCCTTTTAAACCG-3'
A269 (strand 2) 5'-GAAGAGAGTGC**CGCGTAAGGCAAGGGC**GACAT**GGCTCGGACCC**TAGTCTGTGAACTGC-3'
A286 (strand 2) 5'-GAAGAGAGTGC**CGCGTAAGGCAAGGGC**ATAC**GGCTCGGACCC**CTTCCCCGACCG-3'
A308 (strand 2) 5'-GAAGAGAGTGC**CGCGTAAGGCAAGGGC**CCGACGG**CTCGGACCC**ATATCTTATGAGAAG-3'
A324 (strand 2) 5'-GAAGAGAGTGC**CGCGTAAGGCAAGGGC**CCCAT**GGCTCGGACCC**AAGGAGAGGTCCGAC -3'
A330 (strand 2) 5'-GAAGAGAGTGC**CGCGTAAGGCAAGGGC**CTAGCG**GGCTCGGACCC**CCCATTAAGGAGAGG-3'

Sequences of disruptor oligonucleotides used with the 9FQ4 deoxyribozymes

Each disruptor sequence is listed alongside the ribozyme nucleotide attachment site and the nucleotide range where the disruptor binds. In many cases, one or two strategic mutations (underlined) were made to disfavor unproductive binding of the disruptor to the deoxyribozyme.⁴

A146 (A152–A206) 5'-TAGGACCATGTCCGTCAGCTTATTACCATACCCCTTTGCAAGGCCATCTCAAAGTT-3'
A159 (A104–G147) 5'-CTGAGACTTGGTACTGAACGGCTGTTGACCCCTTTCCCGCAATT-3'
A192 (A105–C260) 5'-GTTTCCCCTGAGACTTGGTACTGAACGGCTGTTGACCCCTTTCCCGCAATT-3'
A97 (A28–U80) 5'-ATGCAATCTATTGGC**TTAC**AGACTAGCTACCAGGTGCATGCCTGATAACTTTT-3'
A269 (U287–U326) 5'-ATTAAGGAGAGGTCCGA**AT**TATATCTTATGAGAAGAATACA-3'
A286 (U292–U326) 5'-ATTAAGGAGAGGTCCGACTATATCTTATGAG**C**AGA-3'
A308 (A248–U292) 5'-AATACATCT**CCCC**GACCGACATTTA**AT**CTGTGAACTGCATCCAT-3'
A324 (A248–U292) 5'-AATACATCT**CCCC**GACCGACATTTA**AT**CTGTGAACTGCATCCAT-3'

Sequences of inhibitor and removal oligonucleotides

The two successful inhibitors oligonucleotides and their corresponding removal oligonucleotides are listed below, along with the six unsuccessful inhibitor oligonucleotides. In each case, the nucleotide range where the inhibitor binds is indicated; the 20 nt 3'-tail (boldface) is not complementary to any part of the ribozyme. Because certain regions in the ribozyme have internal secondary structure, portions of the removal oligonucleotides are directly complementary to the ribozyme. We therefore incorporated a small number of ribozyme-inhibitor Watson-Crick mismatches, where the ribozyme-removal interaction would also be weakened. The mutations to introduce these mismatches are underlined in the sequences below. The mutation at the 5'-terminus of the A161-C197 inhibitor sequence was inadvertent.

C102–G141 inhibitor 5'-CTTGGTACTTAACGGCTGTTGACCCCTTTCCTGCAATTCCCAGGTGAAGGCCGCTCTCCA-3'
 Removal oligonucleotide 5'-**TGGAGAGCGGCCTT**CACCTGGAATTGCAGGAAAGGGTCAACAGCCGTTAAGTACCAAG-3'

A161–C197 inhibitor 5'-CTCCGTTAGCTTATTACCATAACCCCTTTGCAAGGCCATCAGGTGAAGGCCGCTCTCCA-3'
 Removal oligonucleotide 5'-**TGGAGAGCGGCCTT**CACCTGATGGCCTTGCAAAGGGTATGGTAATAAGCTACCGGAG-3'

A161–G180 inhibitor 5'-CATACCCCTTTGCAAGGCCATCAGGTGAAGGCCGCTCTCCA-3'

G200–C229 inhibitor 5'-GACTTAGGACTTGGCTGTGTGGTTAGGACCCCAGGTGAAGGCCGCTCTCCA-3'

A113–G141 inhibitor 5'-CTTGGTACTTAACGGCTGTTGACCCCTTTCAGGTGAAGGCCGCTCTCCA-3'

G181–C197 inhibitor 5'-GTCCGTTAGCTTATTACCCAGGTGAAGGCCGCTCTCCA-3'

U177–A210 inhibitor 5'-TGGTTAGGACCATGTCCGTTAGCTTATTACCATCAGGTGAAGGCCGCTCTCCA-3'

A263–G282 inhibitor 5'-CCCCGATCGACATTTATTCTCAGGTGAAGGCCGCTCTCCA-3'

Secondary structure of the 388 nt L-21 ScaI ribozyme form of the *Tetrahymena* group I intron RNA

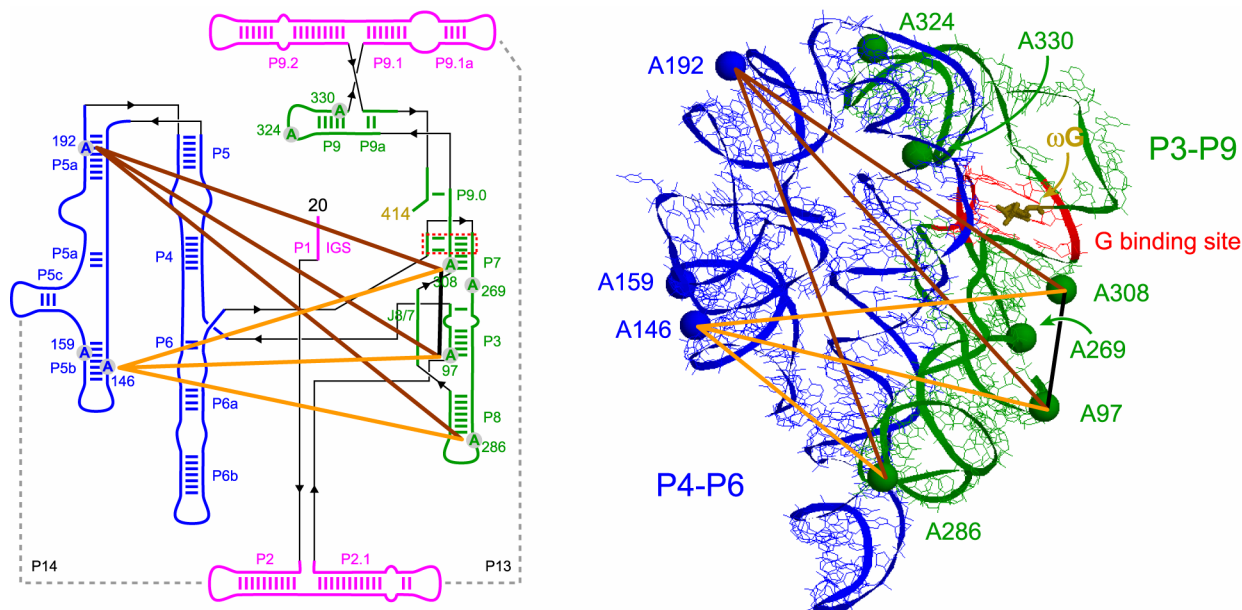


Figure S1. Secondary structure of the 388 nt L-21 ScaI ribozyme form of the *Tetrahymena* group I intron RNA,^{1,5} colored as shown in Fig. 2 (3D structure shown again on the right side of this figure to facilitate comparison).

Suppression of catalysis with DNA constraints at A146-A97, A146-A286, or A146-A308 at pH 6.0

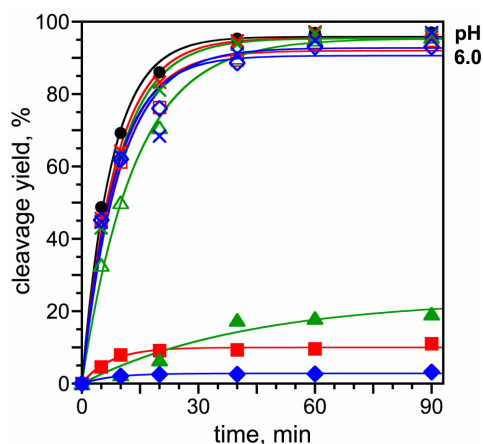


Figure S2. Suppression of group I intron ribozyme catalysis by DNA constraints at A146-A97, A146-A286, or A146-A308 (single-turnover substrate cleavage assays, 50 mM MES, pH 6.0, 10 mM Mg²⁺, and 1 mM GTP at 30 °C).

Suppression of catalysis with DNA constraints at A192-A97, A192-A286, A192-A308, or A97-A308

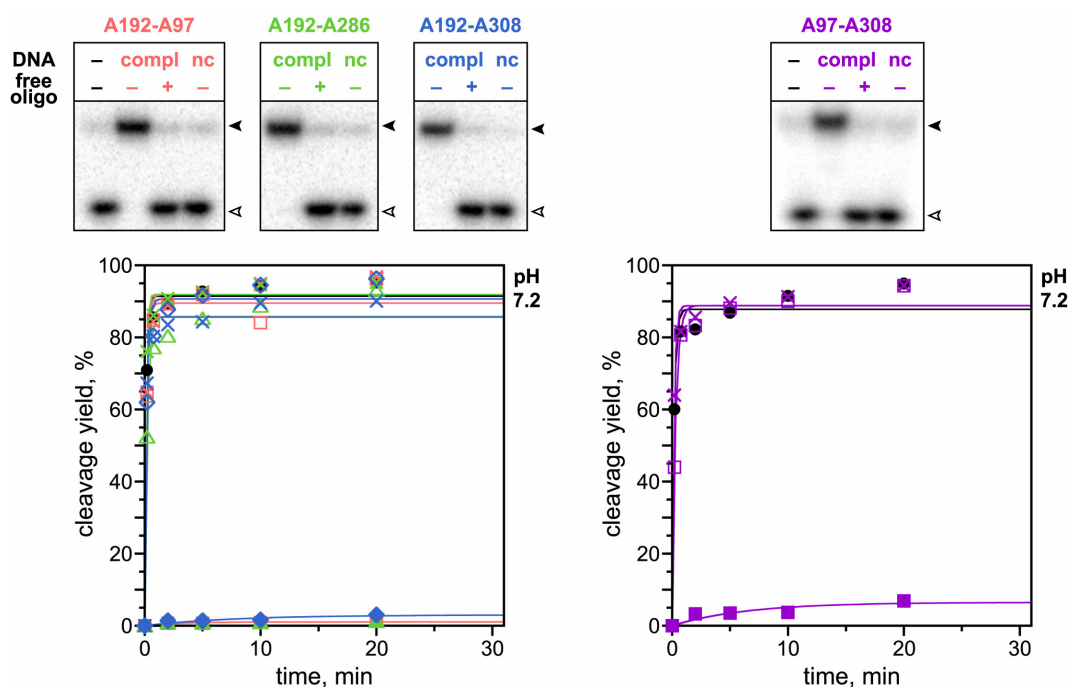


Figure S3. Suppression of group I intron ribozyme catalysis by DNA constraints at A192-A97, A192-A286, A192-A308, or A97-A308 (single-turnover substrate cleavage assays, 100 mM HEPES, pH 7.2, 10 mM Mg²⁺, 100 mM NaCl, and 1 mM GTP at 30 °C).

References for Supplementary Information

1. A. J. Zaig, C. A. Grosshans and T. R. Cech, *Biochemistry*, 1988, **27**, 8924-8931.
2. E. Zelin and S. K. Silverman, *ChemBioChem*, 2007, **8**, 1907-1911.
3. M. P. Patel, D. A. Baum and S. K. Silverman, *Bioorg. Chem.*, 2008, **36**, 46-56.
4. Y. Wang and S. K. Silverman, *Angew. Chem. Int. Ed.*, 2005, **44**, 5863-5866.
5. A. J. Zaig and T. R. Cech, *Science*, 1986, **231**, 470-475.