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Supplemental Data

A High-Throughput Screen for Synthetic Riboswitches Reveals Mechanistic Insights into Their Function

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Supplemental Experimental Procedures

Results from Other Riboswitches Not Discussed in Text

The sequences, Miller Units in the absence and presence of theophylline (1 mM), activation ratios, and predicted folds for the synthetic riboswitches (see Figure 4 in text for secondary structure models).

Clone	Linker Sequence	Miller Units (0 mM)	Miller Units (1.0 mM)	Activation Ratio	Folds Like
5.1*	*AGCGCC* **C** ACGGC	70	1046	15	Fig. 4A.
5.2	TGCCA	64	284	4	Fig. 4A.
5.3	GGTAT	38	90	2	Fig. 4B.
5.4	CGCCA	38	65	2	Fig. 4A.
5 base parent	TATAA	898	4691	5	
6.1	CAAGGG	96	2687	28	Fig. 4B.
6.2	CTGTCA	82	1152	14	Fig. 4A.
6.3	GCGTGC	72	934	13	Fig. 4A.
6.4	CGCTGC	93	1162	12	Fig. 4A.
6.5	ATGTCA	103	1280	12	Fig. 4A.
7.1	TGCTGTC	44	874	20	Fig. 4A.
7.2	CTGTCAA	37	711	19	Fig. 4A.
7.3	TCTTGTC	35	458	13	Fig. 4A.
7.4	CTCTGTC	158	1842	12	Fig. 4A.
7.5	CAAGGCG	148	1306	9	Fig. 4B.
8.1	CCGCTGCA	51	1820	36	Fig. 4A.
8.2	CAAGGCGC	43	1420	33	Fig. 4B.
8.3	AGCTGCAA	141	3611	26	Fig. 4A.
8.4	CAGCTGTC	78	1785	23	Fig. 4A.
8.5	CGCTGTCC	51	910	18	Fig. 4A.

*Clone 5.1 contained an A->G mutation in the *aptamer sequence* and had an extra base (**C**) inserted.

Computational RNA Folding Protocol

Secondary structures of riboswitches were determined using the RNA mFold web server (http://www.bioinfo.rpi.edu/applications/mfold/rna/form1.cgi). Sequences stretching from 5'-end of the theophylline aptamer to 3'-end of the AUG start codon of riboswitches 8.1 and 8.2 were entered and secondary structures were calculated without constraints at 37 ºC with 50% suboptimality yielding 9 and 8 structures respectively. Extending the sequence to the transcription start site increased the number of suboptimal folds, but did not change the structure of the lowest-energy fold.

***In vivo* NMIA Modification Reaction**

An overnight culture harboring clone 8.2 (400 µL) was used to inoculate two 50 mL cultures of LB/ampicillin (50 µg/mL) supplemented with either no small molecule or theophylline (1 mM). Cultures were shaken at 37ºC until the OD_{600} was 0.4 and were split into two 10 mL volumes in 50 mL Falcon tubes. A solution of NMIA in DMSO (1 mL, 300 mM NMIA, ~30 mM final concentration) was added to one tube and DMSO alone (1 mL) was added to the other. The tubes were shaken at 37 ºC for 45 min. (~5 hydrolysis half lives for NMIA). Cells were pelleted by centrifugation after NMIA modification. Total RNA was extracted from the cells by the addition of H_2O (1.5 mL) and phenol (1.5 mL, pH 4.3) followed by vortexing and heating (55 ºC, 5 min). The mixture was centrifuged at 13000 g for 10 min. at room temp. The aqueous layer was removed, extracted with phenol (1.5 mL, pH 4.3) and centrifuged. The aqueous layer was separated and the RNA was precipitated with ethanol. Total RNA was resuspended in 50 µL of DEPC-treated H_2O.

Reverse transcription reactions were setup using total RNA as a template. NMIA modified or unmodified total RNA (2.5 µg) was added to a tube with 3 µL folding buffer (333 mM NaCl; 333 mM HEPES, pH 8.0; 33.3 $MgCl_2$) with 3 µL (~0.2 pmol) 5'-[^{32}P] labeled primer SKD432 (which anneals 25 bp 3' of the start codon) and DEPC-H_2O to obtain a total volume of 13 µL.. Tubes were heated to 65 ºC for 5 min. then set at 35 ºC for 20 min. Reverse transcription buffer (6 µL; 167 mM Tris, pH 8.3; 250 mM KCl; 10 mM $MgCl_2$; 1.67 mM each dNTP; 16.7 mM DTT) was added to each tube. Tubes were heated to 48 ºC for 1 min. followed by addition of 1 µL Superscript III (Invitrogen). Tubes were set at 48 ºC for 2 min. and the reactions were stopped by the addition of NaOH (1 µL, 4 M) followed by heating (95 ºC, 5 min).

Reactions were mixed with 29 μL of neutralizing gel loading solution (29 μL; 4:25 (v/v) 1 M unbuffered Tris-HCl and stop dye [formamide, 0.5x TBE, 50 mM EDTA 0.01% (w/v) bromophenol blue].

An in vitro transcribed RNA corresponding to the riboswitch-containing region of clone 8.2 (~4 pmol) was used as a sequencing standard as described.[1] The reverse transcription reactions and sequencing reactions were separated using denaturing gel electrophoresis (7 M urea; 8% (29:1) acrylamide: bisacrylamide), (0.4 mm x 30 cm x 32 cm) and were imaged using a phosphorimager.

Reactivity profiles for the (+) and (-) NMIA reactions for total RNA from cultures grown in the presence and absence of 1 mM theophylline were compared using a pixel intensity plot generated using NIH Image that was plotted in relation to the sequencing ladder.

Supplemental References

1. Merino, E.J., Wilkinson, K.A., Coughlan, J.L., and Weeks, K.M. (2005). RNA structure analysis at single nucleotide resolution by selective 2'-hydroxyl acylation and primer extension (SHAPE). J. Am. Chem. Soc. *127*, 4223-4231.