Supporting Information

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DNAS Nd

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Fig. S1. Phosphate removal from A8 and A8XL by *Bacillus subtilis* RppH. In vitro transcribed A8 bearing a γ^{-32} P radiolabel and an internal fluorescein label was mixed with labeled A8XL and treated with purified RppH or RppH-E68Q (8 nM), and the radioactivity (P-32) and fluorescence (Fluor) of each RNA were monitored as a function of time by gel electrophoresis.



Fig. S2. Sequence and electrophoretic mobility of RppH substrates. Doubly labeled (A) A8, A4, A3, A2, A1, and A1+3 or (B) G8, G4, G3, G2, G1, and G0 were synthesized by in vitro transcription, gel purified, and subjected to electrophoresis on a 13.5% (wt/vol) polyacrylamide/8 M urea gel, and their radioactivity (P-32) and fluorescence (Fluor) were visualized.



Fig. S3. Relative reactivity of A8 and G8. Phosphate removal from A8 and G8 by equal amounts of RppH was monitored as in Fig. 2A and quantified by normalizing the radioactivity remaining in each RNA to the corresponding fluorescence intensity. Each time point is the average of two or more independent measurements.

DN A S

Table S1. DNA oligonucleotides used in these studies

PNAS PNAS

Name	Sequence	Purpose
A8-5	AATTCCTGCAGTAATACGACTCACTATTAGAACAACGGCG	Template for A8XL and A8
A8XL-3	mUmCCCGGGTTTCCCCGGGTCGCGTTTCCGCGTGGCGCCGCGTTACCGC	Template for A8XL
	GGCGCCGTTGTTCT	
A8-3	mUmCGCGTTTCCGCGTGGCGCCGCGTTACCGCGGCGCCGTTGTTCT	Template for A8
A4-5	AATTCCTGCAGTAATACGACTCACTATTAGAAGGCGC	Template for A4 _{AGAA}
A4-3	mUmCGCGTTTCCGCGTGGCGCCGCGTTACCGCGGCGCCTTCTAATA	Template for A4 _{AGAA}
A3-5	AATTCCTGCAGTAATACGACTCACTATTAGAGGCGC	Template for A3
A3-3	mUmCGCGTTTCCGCGTGGCGCCGCGTTACCGCGGCGCCTCTAATAG	Template for A3
A2-5	AATTCCTGCAGTAATACGACTCACTATTAGGGCGC	Template for A2
A2-3	mUmCGCGTTTCCGCGTGGCGCCGCGTTACCGCGGCGCCCTAATAGT	Template for A2
A1-5	AATTCCTGCAGTAATACGACTCACTATTAGGCGC	Template for A1 and A1+3
A1-3	mUmCGCGTTTCCGCGTGGCGCCGCGTTACCGCGGCGCCTAATAGTG	Template for A1
A1+3-3	mUmUCTCGCGTTTCCGCGTGGCGCCGCGTTACCGCGGCGCCTAATAGTG	Template for A1+3
A4 _{ACAA} -5	AATTCCTGCAGTAATACGACTCACTATAACAAGGCGC	Template for A4 _{ACAA}
A4 _{ACAA} -3	mUmCGCGTTTCCGCGTGGCGCCGCGTTACCGCGGCGCCTTGTTATA	Template for $A4_{ACAA}$
A44UAA-5	AATTCCTGCAGTAATACGACTCACTATAATAAGGCGC	Template for $A4_{A11AA}$
Α4 _{ΔUΔΔ} -3	mUmCGCGTTTCCGCGTGGCGCCGCGTTACCGCGGCGCCTTATTATA	Template for A4
A4 _{AGGA} -5	AATTCCTGCAGTAATACGACTCACTATTAGGAGGCGC	Template for A4
A4AGGA-3	mUmCGCGTTTCCGCGTGGCGCCGCGTTACCGCGGCGCCTCCTAATA	Template for A4
A4AGCA-5	AATTCCTGCAGTAATACGACTCACTATTAGCAGGCGC	Template for A4
A4 _{AGCA} -3	mUmCGCGTTTCCGCGTGGCGCCGCGTTACCGCGCGCCTGCTAATA	Template for A4 _{AGCA}
A4 _{AGUA} -5	AATTCCTGCAGTAATACGACTCACTATTAGTAGGCGC	Template for A4 _{AGUA}
A4 _{AGUA} -3	mUmCGCGTTTCCGCGTGGCGCCGCGTTACCGCGCGCCTACTAATA	Template for A4 _{AGUA}
G8-5	AATTCCTGCAGTAATACGACTCACTATAGGAACAACGGCG	Template for G8
G8-3	mUmCGCGTTTCCGCGTGGCGCCGCGTTACCGCGCGCCGTTGTTCC	Template for G8
G4-5	AATTCCTGCAGTAATACGACTCACTATAGGAAGGCGC	Template for G4GGAA
G4-3	mUmcGcGTTTCCGCGTGGCGCGCGCGTTACCGCGCGCCTTCCTATA	Template for G4 _{CCAA}
G3-5	AATTCCTGCAGTAATACGACTCACTATAGGAGGCGC	Template for G3
G3-3	mlmcgcgtttccgcgtggcgcgcgttaccgcgcgcctcctatag	Template for G3
G2-5	AATTCCTGCAGTAATACGACTCACTATAGGGGCGC	Template for G2
G2-3	mlimcgcgTTTCCGCGTGGCGCGCGCGTTACCGCGCGCCCCTATAGT	Template for G2
G1-5	AATTCCTGCAGTAATACGACTCACTATAGGGCGC	Template for G1
G1-3	mlimcgcgTTTCCGCGTGGCGCGCGCGTTACCGCGCGCCCCTATAGTG	Template for G1
G0-5	AATTCCTGCAGTAATACGACTCACTATAGGCGC	Template for G0
G0-3	mUmCGCGTTTCCGCGTGGCGCCGCGTTACCGCGCGCCCTATAGTGAG	Template for G0
G4caaa-5	AATTCCTGCAGTAATACGACTCACTATAGAAAGGCGC	Template for G4 _{CAAA}
G4 _{CAAA} -3	mlimcgcgTTTCCGCGTGGCGCCGCGTTACCGCGCGCCCTTTCTATA	Template for G4
G4 _{GGAA} -5	AATTCCTGCAGTAATACGACTCACTATAGCAAGGCGC	Template for G4 _{CCAA}
G4 _{GCAA} -3	mImCGCGTTTCCGCGTGGCGCCGCGTTACCGCGCGCCCTTGCTATA	Template for G4 _{CCAA}
G4 _{GUAA} -5		Template for G4 _{GUAA}
G4 _{GUAA} 3	mlimCCCCTTTCCCCCTCCCCCCCCCCTTACCCCCCCTTACTATA	Template for G4 _{GUAA}
		Northern blot probe for mini $vhxA$ -a/pP mRNA
TO DEEN IN	CCATTCCAACCCCCCATAAACC	Northern blot probe for tRNA ^{Cys} (internal standard)
RACE 1		DNA-RNA chimera used for RACE lightion
PRR733		Beverse transcription and first round of posted PCP
RACEntus		First round of nested PCR
DRR737		Second round of nested PCP and DNA conversion
PACEnort		Second round of nested PCP
RACENEST	GGAUAUTGAUATGGAUTGAAGGAGTA	Second round of nested PCK

All sequences are in the 5'-to-3' orientation. mU or mC, 2'-O-methyl nucleotide; rA, ribonucleotide. Complementary regions in the oligonucleotide pairs used to prepare each in vitro transcription template are underlined.