Supplementary data

Unstructured 5'-tails act through ribosome standby to override inhibitory structure at ribosome binding sites

Maaike Sterk, Cédric Romilly, and E. Gerhart H. Wagner*

Department of Cell and Molecular Biology, Biomedical Center,
Uppsala University, Box 596, S-75124 Uppsala, Sweden

Figure S1

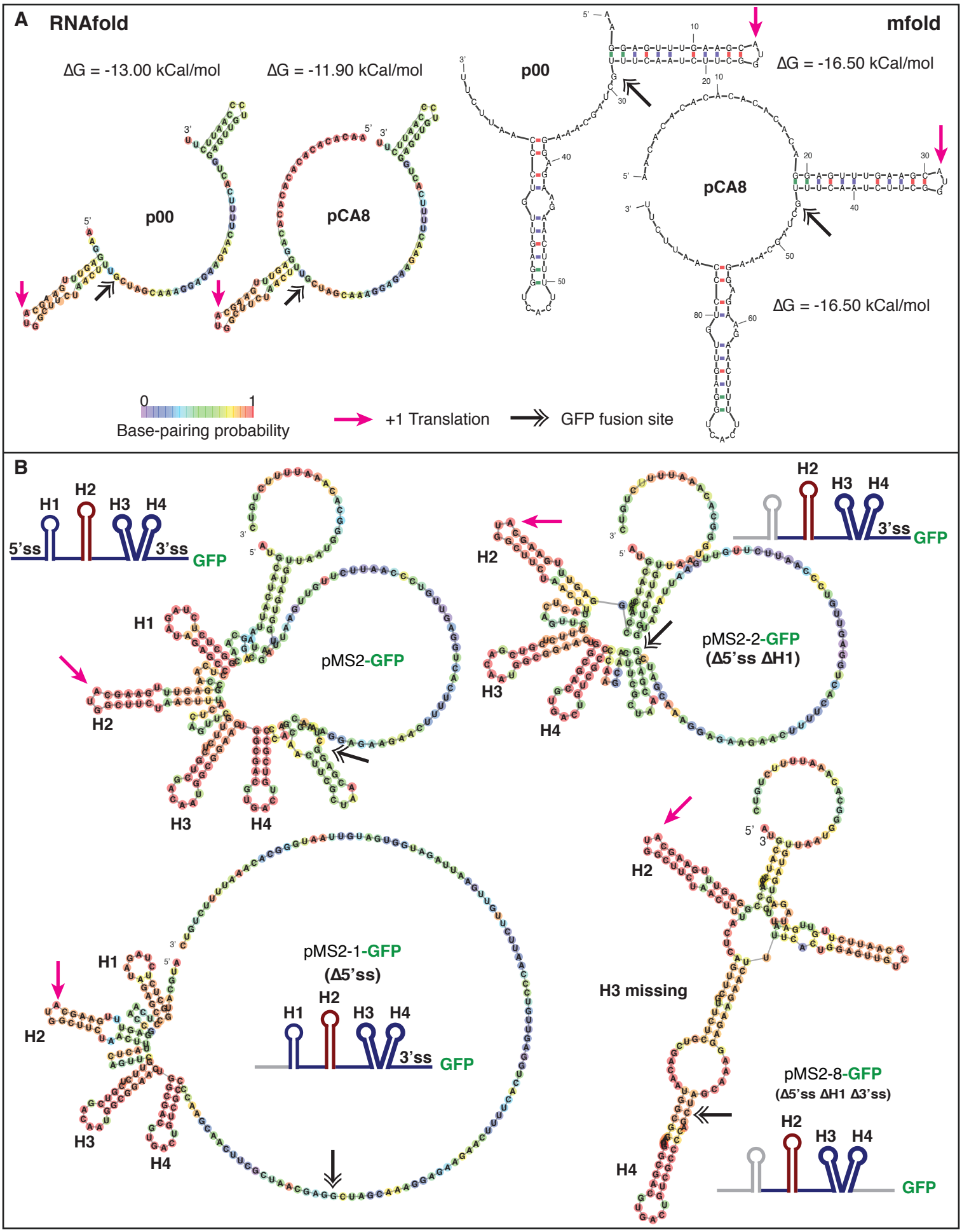

Figure S1: legend next page

Figure S1: RNA structure predictions of coat RBS structure ± standby regions in conjunction with flanking *gfp* sequences.

(**A**) Secondary structure prediction of mRNAs from constructs p00 and pCA8 including 60 nt of *gfp* sequences immediately downstream of the RBS stem-loop. Left: RNAfold predictions (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) (Gruber et al., 2008) using "centroid" image output. The structures are colored according to base-paring probability from 0 (purple) to 1 (red). If a nucleotide is in a single-stranded segment, the color denotes the probability of the said residue to be unpaired. Red arrow: translation start codon, black double head arrow: fusion site to *gfp.* Right: mfold predictions (http://unafold.rna.albany.edu/?q=mfold/rna-folding-form) (Zuker, 2003).

(**B**) Secondary structure predictions of mRNAs for constructs pMS2, pMS2-1, pMS2-2 and pMS2-8 using RNAfold (centroid view). For each prediction, 87 nt of *gfp* sequence was included. The schematic structure of the elements in the MS2 standby side, based on previously published data (De Smit and Van Duin, 2003), is indicated in blue with key elements, namely 5'ss, H1, H2, H3, H4 and 3'ss (upper left corner). For the other three structures, the same kind of schematics is shown, but with grey elements indicating deleted segments (pMS2-1 (Δ5'ss), pMS2-2 (Δ5'ss ΔH1), and pMS2-8 (Δ5'ss ΔH1 Δ3'ss). The predicted relative ΔG^0-values are: pMS2-GFP (ΔG -51,7 kcal/mol), pMS2-1-GFP (ΔG -36.7 kcal/mol), pMS2-2-GFP (ΔG -39.1 kcal/mol), pMS2-8 (ΔG -45.1 kcal/mol). Base pairing probability scale and arrows as in A.

References:

Gruber AR, Lorenz R, Bernhart SH, Neuböck R, Hofacker IL. The Vienna RNA Websuite. *Nucleic Acids Res.* Jul 1;36((2008)

M. Zuker Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* 31, 3406-15, (2003)

De Smit MH and Van Duin J Translational Standby Sites: How Ribosomes May Deal with the Rapid Folding Kinetics of mRNA *J. Mol. Biol.* 33, 737-743 (2003)

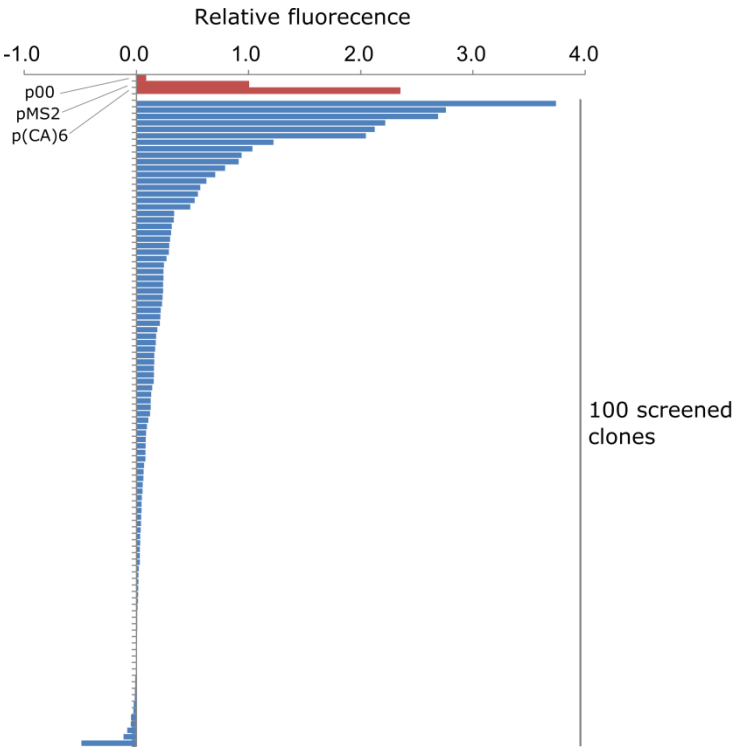

Figure S2: Relative *in vivo* expression of 100 constructs with an insert of 12 random nucleotides, similar to p(CA)6, as measured by plate reader. For comparison, values of p00, pMS2 and p(CA)6 are shown (in red). The majority of constructs confer expression levels close to background.

Table S1: Primers used for strain constructions

Name	Sequence	Fw/Rev	Used for
EHO-444	GGAGUUUGAAGCAUGGCUUCUAACUUUGCTAGCAAAGGAGAAGAACTTTTCACTGGAGT	fw	pEH108
EHO-445	GCTTTTTTTATTGATCGCACACCTGACAGCTGC	rev	pEH108
MS119	ATATATGCATCATTAATCAGGCAACGGC	fw	pMS2; pMS2-4; pMS2-5; pMS2-6; pMS2-7
MS120	TATAGCTAGCCTCGTTAGCGAAGTTGCTTG	rev	pMS2; pMS2-1; pMS2-2; pMS2-3
MS148	ATATATGCATGGCTCTCTAGATAGAGCC	fw	pMS2-2
MS149	ATATATGCATCTCAACCGGAGTTTGAAG	fw	pMS2-2; pMS2-9; pMS2-11
MS150	ATATATGCATGGAGTTTGAAGCATGGCTTC	fw	pMS2-3
MS151	TATAGCTAGCTGGGGCGACAGTCACG	rev	pMS2-4; pMS2-8
MS152	TATAGCTAGCAGTTCCGCCATTGTCGAC	rev	pMS2-5; pMS2-9
MS153	TATAGCTAGCGAACTGAGTAAAGTTAGAAGC	rev	pMS2-6; pMS2-10
MS154	TATAGCTAGCAAAGTTAGAAGCCATGCTTC	rev	pMS2-7; pMS2-11
MS182	[P]GGAGTTTGAAGCATGGCTTCTAACTTTG[a)]	fw	pXX-series
MS183	[P]TGTGTGTTGTGCTCAGTATCTCTATCACTG	rev	p(CA)3
MS184	[P]TGTGTGTTGTGCTCAGTATCTCTATCAC	rev	p(CA)4
MS185	[P]TGTGTGTGTTGTGCTCAGTATCTCTATC	rev	p(CA)5
MS186	[P]TGTGTGTGTGTTGTGCTCAGTATCTC	rev	p(CA)6
MS187	[P]TGTGTGTGTGTGTTGTGCTCAGTATC	rev	p(CA)7
MS188	[P]TGTGTGTGTGTGTGTTGTGCTCAGTATC	rev	p(CA)8
MS189	[P]TGTGTGTGTGTGTGTGTTGTGCTCAGTATC	rev	p(CA)9
MS248	[P]ATATATTTGTGCTCAGTATCTCTATCACTG	rev	p(AU)3
MS249	[P]ATATATATTTGTGCTCAGTATCTCTATCAC	rev	p(AU)4
MS250	[P]ATATATATATTTGTGCTCAGTATCTCTATC	rev	p(AU)5
MS251	[P]ATATATATATATTTGTGCTCAGTATCTC	rev	p(AU)6
MS252	[P]ATATATATATATATTTGTGCTCAGTATC	rev	p(AU)7
MS253	[P]ATATATATATATATATTTGTGCTCAGTATC	rev	p(AU)8
MS254	[P]ATATATATATATATATATTTGTGCTCAGTATC	rev	p(AU)9
MS262	[P]TTTTTTTTGTGCTCAGTATCTCTATCACTG	rev	p(AA)3
MS263	[P]TTTTTTTTTTGTGCTCAGTATCTCTATCAC	rev	p(AA)4
MS266	[P]TTGTGCTCAGTATCTCTATCACTGATAG	rev	p00
MS268	[P]AAAAAATTGTGCTCAGTATCTCTATCACTG	rev	p(UU)3
MS269	[P]AAAAAAAATTGTGCTCAGTATCTCTATCAC	rev	p(UU)4

MS295	GGAGTTTGAAGCATGGCATCTAACTTTGCTAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCC	fw	p(XX)-M1
MS296	GGAGTTTCAAGCATGGCATCTAACTTTGCTAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCC	fw	p(XX)-M2
MS299	[P]TGTGTGTGTTGTGCTCAGTATCTCTATCACTGATAGGGATGTCAATCTC	rev	p(CA)4
MS300	[P]TGTGTGTGTGTGTGTGTTGTGCTCAGTATCTCTATCACTGATAGGGATGTCAATCTC	rev	p(CA)8

a) [P] indicates a 5’ phosphate

Table S2: Primers used for purposes other than strain constructions

Name	Sequence	fw/rev	Used for
EHO-715	GATGCCTCTAGATTTAAATGCTCGAAT	rev	T7 in vitro transcription
EHO-828	GAATTGGGACAACTCCAGTG	rev	reverse transcription (structure probing)
MS073	TATTGATCGC	-	control oligo for antisense experiments
MS213	TGTGTGTGTG	rev	DNA oligo antisense to CA-series
MS165	GaaattaatacgactcactatagTGCATCATTAATCAGGCAACGGC[a)]	fw	pMS2 T7 in vitro
MS198	GaaattaatacgactcactatagACACACAGGAGTTTGAAGCATGG	fw	(CA)3 T7 in vitro
MS199	GaaattaatacgactcactatagACACACACAGGAGTTTGAAGCATG	fw	(CA)4 T7 in vitro
MS200	GaaattaatacgactcactatagACACACACACAGGAGTTTGAAGC	fw	(CA)5 T7 in vitro
MS201	GaaattaatacgactcactatagACACACACACACAGGAGTTTGAAG	fw	(CA)6 T7 in vitro
MS202	GaaattaatacgactcactatagACACACACACACACAGGAGTTTG	fw	(CA)7 T7 in vitro
MS203	GaaattaatacgactcactatagACACACACACACACACAGGAG	fw	(CA)8 T7 in vitro
MS204	GaaattaatacgactcactatagACACACACACACACACACAGGAG	fw	(CA)9 T7 in vitro
MS255	GaaattaatacgactcactatagAATATATGGAGTTTGAAGCATGG	fw	(AU)3 T7 in vitro
MS256	GaaattaatacgactcactatagAATATATATGGAGTTTGAAGCATG	fw	(AU)4 T7 in vitro
MS257	GaaattaatacgactcactatagAATATATATATGGAGTTTGAAGC	fw	(AU)5 T7 in vitro
MS258	GaaattaatacgactcactatagAATATATATATATGGAGTTTGAAG	fw	(AU)6 T7 in vitro
MS259	GaaattaatacgactcactatagAATATATATATATATGGAGTTTG	fw	(AU)7 T7 in vitro
MS260	GaaattaatacgactcactatagAATATATATATATATATGGAG	fw	(AU)8 T7 in vitro
MS261	GaaattaatacgactcactatagAATATATATATATATATATGGAG	fw	(AU)9 T7 in vitro
MS274	GaaattaatacgactcactatagAGGAGTTTGAAGCATGG	fw	00 T7 in vitro
MS267	TCACCTTCACCCTCTCCACTG	rev	strcture probing
MS294	AATTGGGACAACTCCAG	rev	reverse transcription (toeprint)

a) Lowercase letters correspond to the T7 promoter sequence.

Table S3: Strain construction by conventional cloning

Plasmid	Template	fw primer	rev primer
pMS2	pK2	MS119	MS120
pMS2-0	pK2	MS150	MS154
pMS2-1	pK2	MS148	MS120
pMS2-2	pK2	MS149	MS120
pMS2-3	pK2	MS150	MS120
pMS2-4	pK2	MS119	MS151
pMS2-5	pK2	MS119	MS152
pMS2-6	pK2	MS119	MS153
pMS2-7	pK2	MS119	MS154
pMS2-8	pK2	MS149	MS151
pMS2-9	pK2	MS149	MS152
pMS2-10	pK2	MS149	MS153
pMS2-11	pK2	MS149	MS154

Table S4: Strain construction by amplification of vector

Plasmid	Template	fw primer	rev primer
p00	pEH108	MS182	MS266
p(AA)3	pEH108	MS182	MS262
p(AA)4	pEH108	MS182	MS263
p(UU)3	pEH108	MS182	MS268
p(UU)4	pEH108	MS182	MS269
p(AU)3	pEH108	MS182	MS248
p(AU)4	pEH108	MS182	MS249
p(AU)5	pEH108	MS182	MS250
p(AU)6	pEH108	MS182	MS251
p(AU)7	pEH108	MS182	MS252
p(AU)8	pEH108	MS182	MS253
p(AU)9	pEH108	MS182	MS254
p(CA)3	pEH108	MS182	MS183
p(CA)4	pEH108	MS182	MS184
p(CA)5	pEH108	MS182	MS185
p(CA)6	pEH108	MS182	MS186
p(CA)7	pEH108	MS182	MS187
p(CA)8	pEH108	MS182	MS188
p(CA)9	pEH108	MS182	MS189
p00-M1	pEH108	MS295	MS266
p00-M2	pEH108	MS296	MS266
p(CA)4-M1	pEH108	MS295	MS299
p(CA)4-M2	pEH108	MS296	MS299
p(CA)6-M1	pEH108	MS295	MS186
p(CA)6-M2	pEH108	MS296	MS186
p(CA)8-M1	pEH108	MS295	MS300
p(CA)8-M2	pEH108	MS296	MS300

Table S5: DNA templates for transcription

RNA	Template	fw primer	rev primer	Purpose
00	p00	MS274	EHO-715	Translation
MS2	pMS2	MS165	EHO-715	Translation
(AU)3	p(AU)3	MS255	EHO-715	Translation
(AU)4	p(AU)4	MS256	EHO-715	Translation
(AU)5	p(AU)5	MS257	EHO-715	Translation
(AU)6	p(AU)6	MS258	EHO-715	Translation
(AU)7	p(AU)7	MS259	EHO-715	Translation
(AU)8	p(AU)8	MS260	EHO-715	Translation
(AU)9	p(AU)9	MS261	EHO-715	Translation
(CA)3	p(CA)3	MS198	EHO-715	Translation
(CA)4	p(CA)4	MS199	EHO-715	Translation
(CA)5	p(CA)5	MS200	EHO-715	Translation
(CA)6	p(CA)6	MS201	EHO-715	Translation
(CA)7	p(CA)7	MS202	EHO-715	Translation
(CA)8	p(CA)8	MS203	EHO-715	Translation
(CA)9	p(CA)9	MS204	EHO-715	Translation
00	p00	MS274	MS267	Structure probing
(CA)4	p(CA4)	MS199	MS267	Structure probing
(CA6)	p(CA)6	MS201	MS267	Structure probing
(CA)8	p(CA)8	MS203	MS267	Structure probing