SUPPLEMENTARY MATERIAL

A minimized rRNA binding site for ribosomal protein S4 and its implications for 30S assembly

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Table S1. *E. coli* rRNAs designed and tested for S4 binding in this study.

RNA	16S rRNA nucleotides	Loop-closing sequences
16S[a]	1-1542	-
5'domain[b]	21-562	-
RNA(ΔH6-14)[b,c]	21-54, 357-562	cgcaag
RNA(ΔH5-14)[b,d]	21-46, 365-562	aga
5WJ[b,e]	21-46, 395-562	cucaa
5WJ_nt6 [b,f]	6-46, 395-562	cucaa
5WJ variants		
nt8-556 [f]	8-46, 395-556	cucaa
nt8-562 [f]	8-46, 395-562	cucaa
nt26-556	26-46, 395-556	cucaa
nt26-562	26-46, 395-562	cucaa
ΔH3	38-46, 395-562	cucaa
ΔH4	21-38, 404-562	-
H16trunc[b]	21-46, 395-409, 433-562	cucaa, uucg
H17trunc[b]	21-46, 395-446, 487-562	cucaa, uuuugcu
H18trunc	21-46, 395-515, 536-562	cucaa, uucg
BstH17[b]	21-46, 395-436, 500-562	cucaa
TthH17[b]	21-46, 395-436, 500-562	cucaa
G404U	21-46, 395-562	cucaa
U405C	21-46, 395-562	cucaa
G406A	21-46, 395-562	cucaa
U437A	21-46, 395-562	cucaa
A509,510C	21-46, 395-562	cucaa
G524C	21-46, 395-562	cucaa
C526A	21-46, 395-562	cucaa
5'domain variants		
G404U	21-562	-
U405C	21-562	-
G406A	21-562	-
U437A	21-562	-
A509,510C	21-562	-

The 16S rRNA nucleotides present in each construct is listed in the table, along with the sequences used to close loops for continuous transcription. BamHI and EcoRI sites were used to clone PCR products into pUC18 vector. Generally H1 and H2 were missing in all constructs except 16S rRNA, unless stated otherwise.

[a]16S rRNA was isolated from 30S subunits

[b]EcoRI-linearized plasmid used for transcription. For all others, a PCR template was used for transcription. Primer sequences are available upon request.

[c]RNA(ΔH6-14): Helices 6-14 of the 5'domain are deleted in this construct.

[d]RNA(ΔH5-14): Helices 5-14 of the 5'domain are deleted in this construct.

[e]5WJ: Helices 5-15 of the 5'domain are deleted in this construct.

[f]These constructs contain H1, in addition to H3, H4, H16-18.

Table S2. Relative free energies of S4 binding measured by competition against 5WJ RNA

RNA	K_{rel}	ΔΔG (kcal/mol)
16S rRNA	1.1 ± 0.4	-0.1 ± 0.3
5'domain	2.3 ± 0.4	-0.5 ± 0.1
5' and 3' ends		
5WJ_nt6	2.2 ± 1.2	-0.5 (-0.3, +0.5)
5WJ_nt8-556	0.5 ± 0.2 [a]	0.4 ± 0.3
5WJ_nt8-562	~ 5.3	~ -1.0
5WJ_nt26-556	> 63	< -2.6
5WJ_nt8-556	~ 4.9	~ -1.0
Internal deletions		
5WJΔH3	360 ± 130	-3.7 ± 0.3 [a]
5WJΔH4	≥ 280	≤ -3.5
5WJ:H16trunc	≥ 380	≤ -3.7
5WJ:H17trunc	4.1 ± 3.3	-1.0 (-0.4, +1)
5WJ:H18trunc	1.4 ± 0.7 [a]	-0.2 ± 0.4 [a]
Base substitutions		
5WJ:G404U	≥ 430	≤ -3.8
5WJ:U405C	400 ± 190 [a]	-3.8 ± 0.4
5WJ:G406A	≥ 730	≤ -4.1
5WJ:U437A	590 ± 430 [a]	-4.0 (-0.3, +0.8)
5WJ:A509,510C	660 ± 350 [a]	-4.1 (-0.3, +0.5) [a]
5WJ:G524C	42 ± 17 [a]	-2.3 ± 0.3 [a]
5WJ:C526A	1.9 ± 1.7	-0.4 (-0.4, +1.4) [a]
5'dom:G404U	> 1000	< -4.3
5'dom:U405C	> 1000	< -4.3
5'dom:G406A	> 1000	< -4.3
5'dom:U437A	≥ 333	< -3.6
5'dom:A509,510C	≥ 400	≤ -3.8

^{32}P-labeled 5WJ RNA and unlabeled competitor RNA were tested in a competition assay for their ability to bind Bst S4 in HKM4 at 42 °C, as described in Methods. Data were fit to Equation 1 to obtain the $K_{d\ Competitor}$. $K_{rel} = K_{d\ Competitor} / K_{d\ 5WJ}$, where $K_{d\ 5WJ} = 0.72 \pm 0.20$ nM. $\Delta\Delta G = -RT\ln K_{rel}$ at 315.15 K. The relative affinities and dissociation free energy values (two significant figures) were averaged from three or more experiments unless stated otherwise.

[a] Results from two experiments.

Table S3. Summary of base modification experiments on 5WJ RNAs with S4.

		5WJ RNA			
nt	**16S**	**WT**	**H18trunc**	**C526A**	**G524C**
G31 [a]	PP [b]	E	Bst P [b], Eco E [c]	E	Bst P, Eco E
G38	P	P	P	P	P
A397	P	P	N.D. [d]	N.D.	Bst P, Eco N.D.
G404	P	P	N.D.	N.D.	Bst N.D., Eco P
G413	P	Bst (E) [e], Eco P	N.D.	P	Bst P, Eco N.D.
A493	P	P	P	P	P
G497	PP	P	Bst PP, Eco P	Bst PP, Eco P	P
A498	PP	P	P	PP	Bst PP, Eco P
A499	P	PP	P	PP	PP
G505	P	Bst P, Eco (E)	Bst EE [c], Eco (E)	Bst P, Eco (E)	Bst P, Eco E
G506	P	Bst vary, Eco E	Bst P, Eco (E)	P	Bst P, Eco (E)
A509	P	PP	Bst PP, Eco P	PP	Bst PP, Eco P
A510	PP	PP	Bst PP, Eco P	PP	Bst PP, Eco P
G521	E	Variable	N.A. [f]	Bst E, Eco (E)	E
G524	E	E	N.A.	E	N.A
C525		no change	no change	no change	E
C526		no change	no change	N.A	E
G530	E	E	N.A.	(E)	E
A533	P	P	N.A.	(P) [g]	(P)
A546	E	N.D	N.D.	N.D.	N.D.

The RNAs alone and in complex with Bst and Eco S4 in HKM4 and HKM20, respectively, were modified using DMS and kethoxal. Complexes were formed at 42 °C and probed at 0 °C. Only nucleotides in helices 3, 4, 16-18 were examined, and compared to results for 16S rRNA-S4 complexes at 42 °C (Powers and Noller, 1995a).
Bst – with Bst S4; Eco – with Eco S4
[a] G31 should not be protected in the 5WJ constructs since they are missing H12
[b] P or PP – moderate or strong protection with S4
[c] E or EE – moderate or strong enhancement with S4
[d] N.D. – not determined due to pausing or lack of clarity
[e] (E) – moderate to strong modification in RNA alone, and no change with S4
[f] N.A. – nucleotide not present in this RNA
[g] (P) – very weak modification in RNA alone, and no change with S4

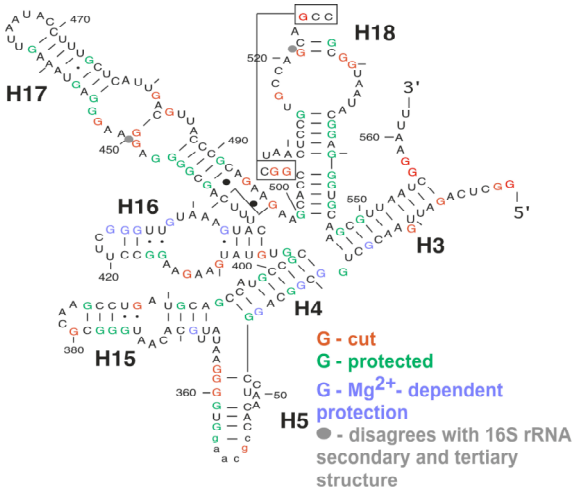

Figure S1. Secondary structure probing of RNA(ΔH6-14). Results of RNase T1 probing on RNA(ΔH6-14) at 42 °C in the presence of 25 mM K^+ Hepes, pH 7.5, and 0-20 mM $MgCl_2$. G's in green were protected from RNase T1 cleavage in buffer alone, while G's in blue required 0.5-4 mM $MgCl_2$ to form the correct secondary structure. Nucleotides in lower case reflect non-native sequence added to close helix 5. Symbols are described in the key.

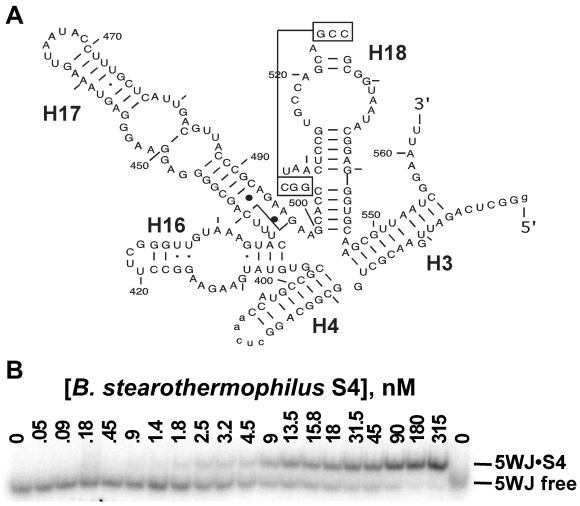

Figure S2. Titration of *B. stearothermophilus* S4 with ^{32}P-labeled *E. coli* 5WJ RNA. (A) Secondary structure of the 5WJ RNA showing its sequence. Nucleotides in lower case reflect non-native sequence used to close helix 4. **(B)** Nondenaturing gel mobility shift assay was used to determine K_d by incubating ^{32}P-5WJ RNA and S4 at 42 °C in HKM4 buffer, then loading an aliquot on a 6% TKM2 gel. Bound and free 5WJ RNAs are labeled. The data were

fit to the Langmuir binding isotherm and gave an average K_d of 13 ± 6 nM for the 5WJ•S4 complex.

Supplementary Reference

Powers, T., and Noller, H. F. (1995a). A temperature-dependent conformational rearrangement in the ribosomal protein S4.16 S rRNA complex. *J. Biol. Chem.,* **270**, 1238-1242.