Supporting Information

Jin et al. 10.1073/pnas.1112185108

Gel Lane	1	2	3	4	5	6	7	8
T. Th 70S ribosome	+		+		+			+
T. Th RF1	-		+		-			-
E. Coli RF3	-		+		+			+
GDPCP	-		+		+			-
GDP	-		-		-			+
A-site UAA stop codon	-		-		+			+
A-site UUC Phe codon	-		+		-			-
P-site AUG codon	-		+		+			+

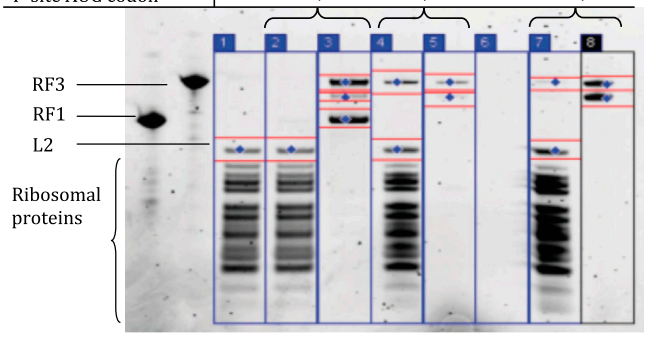

Fig. S1. Stop codon specific-binding of the *Escherichia Coli* release factor 3 and guanosine 5′-β,γ-methylenetriphosphate (RF3•GDPCP) to the *Thermus thermophilus* 70S ribosome. Ribosomal complexes were formed as described in *Materials and Methods*. Binding of RF3 to the 70S complexes in the presence of either guanosine diphosphate (GDP) or GDPCP, and in the presence of a stop codon UAA or a Phe codon UUC at the ribosomal A site were assayed by size-exclusion chromatography on a Superdex™ 200 (10/300) column (Amersham/GE Healthcare) and analyzed by SDS-PAGE. Gel lanes numbered 1, 2, 4, and 7 show the protein components of the complex that were eluded together with the 70S ribosome from the gel filtration experiment described. Gel lanes numbered 3, 5, and 8 show the excess amount of protein ligands that are unbound to the ribosome. Our results shows (*i*) release factor 1 (RF1) and RF3•GDPCP do not bind to the 70S ribosome when a Phe codon UUC is programmed in the ribosome A site (shown in gel lane 2 and 3); (*ii*) *E. Coli* RF3•GDPCP binds to the 70S ribosome with a stop codon UAA in the ribosome A site (shown in gel lane 4 and 5); and (*iii*) *E. Coli* RF3•GDP binds to the *T. thermophilus* 70S ribosome with very low affinity (shown in gel lane 7 and 8).

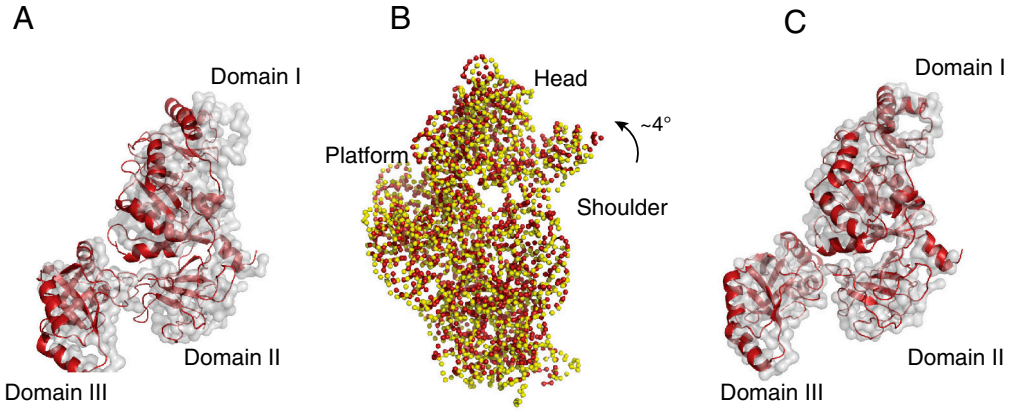

Fig. S2. Conformation comparison of the *Thermus thermophilus* 70S-bound release factor 3 and guanosine 5′-β,γ-methylenetriphosphate (RF3•GDPCP) with the *Escherichia Coli* 70S-bound RF3 with guanosine 5′-β,γ-imidotriphosphate (RF3•GDPNP) obtained by cryoelectron microscopy (cryoEM) study. (*A*) An alignment based on the 23S rRNA backbone of the structure from this study and the previous cryoEM structure (1), [Protein Data Bank (PDB) ID code 2O0F and 3DG5] shows a small difference in the orientation of 70S bound RF3. (*B*) The difference of the 30S subunit rotations of two studies. The difference of the RF3•GDPCP orientation can be explained by an approximately 4° further counterclockwise rotation of the 30S in this study compared to the cryoEM structure (1), (PDB ID code 3DG5). The 30S phosphate backbone was colored in red (this study) and in yellow for the cryoEM structure (PDB ID code 3DG5). (*C*) Superposition of the RF3•GDPCP (red) with the *E. Coli* 70S-bound RF3•GDPNP (gray) (1) (PDB ID code 2O0F) showing similar domain orientations.

1 Gao H, et al. (2007) RF3 induces ribosomal conformational changes responsible for dissociation of class I release factors. *Cell* 129:929–941.

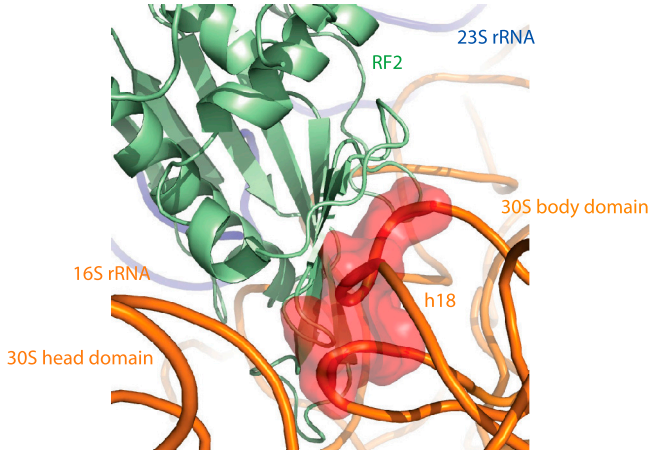

Fig. S3. A possible steric clash between the domain II and VI in release factor 2 (RF2) and helix h18 in 30S subunit as the result of intersubunit rotation. Intersubunit rotation results in larger than 7 Å movement in the decoding center of the ribosome. Superposing the 50S subunit of the RF2-bound (1) and the release factor 3 and guanosine 5′-β,γ-methylenetriphosphate (RF3•GDPCP)-bound 70S structures shows steric clashes (shown as surface in red) between domain II and IV of the RF2 (lime green) with the residue 516 to 531 region in helix h18 of the 30S body domain (orange). This structural incompatibility suggests that RF2 may not be able to accommodate the large conformational changes in the ribosome that results from the binding of the RF3•GDPCP.

1 Jin H, Kelley AC, Loakes D, Ramakrishnan V (2010) Structure of the 70S ribosome bound to release factor 2 and a substrate analog provides insights into catalysis of peptide release. *Proc Natl Acad Sci USA* 107:8593–8598.

Table S1. Summary of crystallographic data and refinement

	70S-RF3•GDPCP complex (Data are merged from nine crystals)
Data collection	
Space group	$P2_1$
Cell dimensions	
a, *b*, *c* (Å)	$a = 204.7$, $b = 229.3$, $c = 307.0$
α, β, γ (°)	$\alpha = \gamma = 90.0$; $\beta = 90.17$
Resolution (Å)	45–3.8 (3.9–3.8)*
R_{sym}	17.9 (174.9)
$I/\sigma I$	6.42 (1.06)
Completeness (%)	98.7 (99.0)
Redundancy	7.44 (7.29)
Refinement	
Resolution (Å)	45–3.8
No. unique reflections	274830
R_{work}/R_{free}	29.31/35.14
No. atoms	
RNA	99189
Protein	51385
Ligand	32
B-factor	
RNA	150
Protein	141
Ligand	108
Bond lengths (Å)	0.010
Bond angle (°)	1.5

*$I/\sigma I = 3.03$ at 4.0 Å; 1.31 at 3.9 Å.