Supporting Online Material for

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Insights into translational termination from the structure of RF2 bound to the ribosome

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Materials and methods

Purification of 70S ribosomes, tRNA, RF2 and mRNA

Thermus thermophilus ribosomes and *E. coli* $tRNA^{Phe}$ were purified as described previously (*S1*). His-tagged *T. thermophilus* RF2 was overexpressed in *E. coli* and purified as described previously (*S2*), and the His tag cleaved off using TEV protease. The cleaved tag, any remaining tagged RF2 and the TEV protease itself, were removed by passing the mixture through a Ni-NTA column. Mass spectrometry showed that only a small fraction of Q240 (<5-10%) was methylated. The mRNA used was chemically synthesized by Dharmacon and had the sequence 5' GGC AAG GAG GAG AAU AAA **UUC <u>UGA</u>** UAC A 3' which contained a UUC Phe codon in the P site (bold) and an RF2-specific UGA stop codon in the A site (underlined bold).

Crystallization of complexes

Complexes of RF2 with the ribosome containing $tRNA^{Phe}$ and mRNA with a UGA codon in the A site were crystallized as described previously for the ribosome-tRNA complex (*S1*). All complexes were formed in buffer G (5 mM HEPES pH 7.5, 10 mM MgAc, 50 mM KCl, 10 mM NH_4Cl, 6 mM 2-mercaptoethanol). To form an RF2 complex, 70S ribosomes at a final concentration of 4.4 μM were incubated with a 2-fold excess of mRNA and a 4-fold excess of $tRNA^{Phe}$ at 55°C for 30 min, before being further incubated with a 4-fold excess of RF2 for 30 min. The complex was left at room temperature for 30 min prior to crystallization. Crystals were grown in sitting drop vapor diffusion experiments in which 2.4 μl of ribosomal complex including 2.8 mM Deoxy Big Chap (Hampton Research, added directly before setup) was mixed with 2 μl reservoir solution containing 0.1M KCl, 0.1M Tris-HAc pH7, 3-4.5%(w/v) PEG20K, 3-4.5%(w/v) PEG550MME and left to equilibrate at 20°C. Crystals grew in 2-3 weeks to the size of up to 50x70x700 μm. The crystals were sequentially transferred to cryo-protecting solution (0.1 M KCl, 10 mM NH_4Cl, 10 mM MgAc, 0.1M Tris-HAc pH 7, 5% PEG 20K, 25% PEG550MME), and frozen by plunging into liquid nitrogen. All data collection was carried out at 100K.

Data collection, refinement and model-building

X-ray diffraction data were collected from five separate regions of a single crystal at beamline X10SA at the Swiss Light Source, Villigen. Data were integrated and scaled with the XDS package (*S3*). A starting model consisting of our previous high-resolution structure of the 70S ribosome (*S1*) with all its ligands omitted was used for refinement. Prior to its use in refinement, errors in the sarcin-ricin loop, a stem-loop of the L1 binding region of 23S RNA and in protein L28 were corrected. Refinement was carried out using CNS (*S4*) as described previously(*S1*) or using the program Phenix (*S5*) as follows: Initially, rigid body refinement of each of the two 70S molecules was done, followed by position and B-factor refinement. With Phenix, an additional step of TLS refinement was done after B-factor refinement, in which the TLS groups defined were the head, 5′ domain, platform and 3′ minor domain of the 30S subunit; the body and L1 stalk of the 50S subunit, and 5S RNA. The initial R/R_{free} of the model was 27.2/32.0. Sigma A weighted difference Fourier maps clearly showed the presence of the mRNA, tRNA and RF2 ligands. Initially P-site tRNA and codon were built in and the model re-refined. Using the high resolution *E. coli* structure (*S6*), a homology model for ribosomal protein L11 and the L11 binding region of 23S RNA was derived and built into the density. Then E-site tRNA, the domains of RF2 and the A-site codon were placed, but key regions such as the SPF loop, the GGQ loop, residues A1492, A1493 and G530 in the decoding center and the less well-ordered domain 1 of RF2 were left out, and a new round of refinement as above, was carried out. Finally, the missing regions were built in. The final model included RF2 residues 6-356, the entire E- and P-site $tRNA^{Phe}$, the A- and P-site codons and two bases of the E-site codon. The maps shown in all figures are derived from CNS.

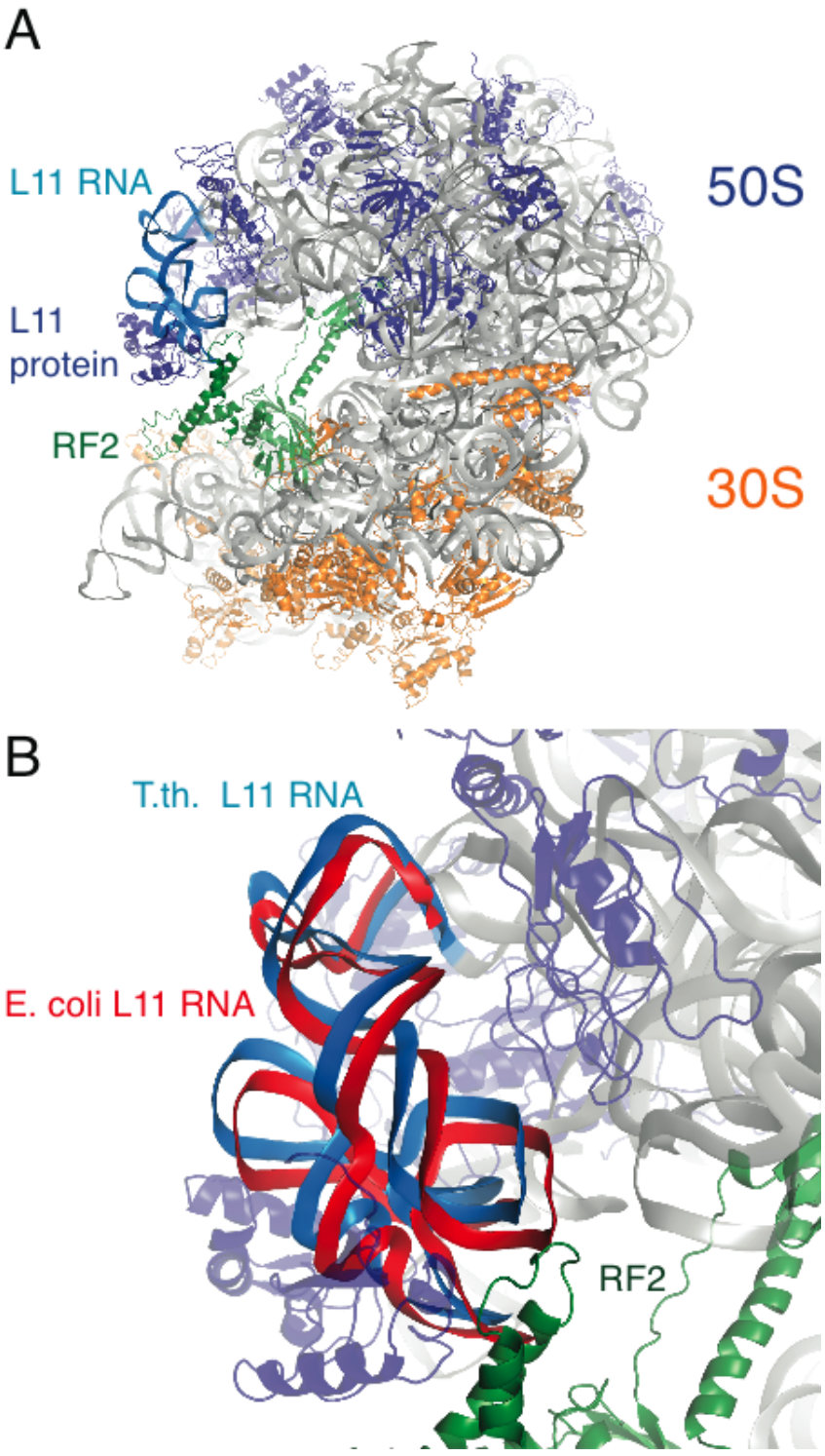

Figure S1. Interaction of Domain 1 of RF2 with the L11 region of the ribosome. A. Overview showing the interaction of Domain 1 of RF2 (green) with the L11-RNA region of the 50S subunit. B. Details showing the changes in the conformation of the L11-RNA region in the RF2-bound form (blue) with its conformation in the empty *E. coli* ribosome (red, from ref. *S6*). Domain 1 of RF2 consists of a bundle of 4 helices,

including an additional N-terminal helix not present in RF1. Although this region is poorly ordered the helices of RF2 are visible in maps calculated to 4-6Å resolution, allowing the domain to be placed as a rigid body. As seen in previous low-resolution studies (*S2, S7, S8*), Domain 1 interacts with the L11 region of the ribosome. This interaction is made possible by movements of both Domain 1 relative to the isolated RF2 crystal structure (*S9*) (Fig. 1C) and of the L11/RNA region relative to the structure of an empty ribosome (*S6*). As a result, the L11-RNA complex and, to a lesser extent, protein L10 were better ordered than in the high-resolution 70S structure in the absence of RF2 (*S1*). There is no evidence for a change in conformation of the N-terminal domain of L11 relative to the rest of the protein or its RNA region as suggested by earlier low-resolution studies (*S2*). Differences in interaction with this region between RF1 and RF2 may be functionally important. For example, deletion of L11 preferentially reduces RF1 function (*S10*) whereas mutations in the L11-binding RNA region affected only RF2 function (*S11*). However, a recent study showed that mutations in both domains of L11 affected both factors equally (*S12*).

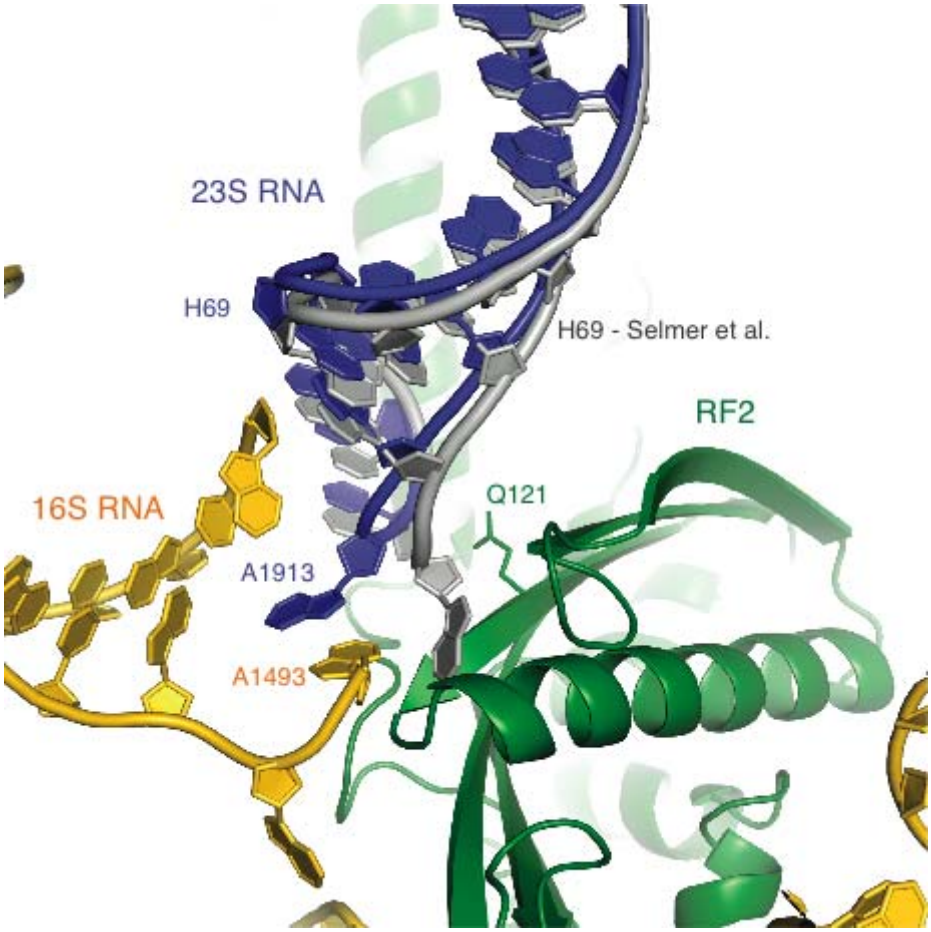

Figure S2. Changes in the conformation of helix 69 of 23S RNA (blue) relative to its conformation in a ribosome with a tRNA in the A site (gray, from ref. *S1*).

Table S1. Summary of crystallographic data and refinement

	70S – RF2 complex
Data collection	
Space Group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	*a*=211.3 *b*=450.9, *c*=614.0
α, β, γ (°)	α=β=γ=90
Resolution (Å)	50-3.45 (3.5-3.45)*
R_{sym}	17.5 (83.1)
$I / \sigma I$	8.19 (1.74)
Completeness (%)	99.6 (99.5)
Redundancy	5.46 (3.63)
Refinement	
Resolution (Å)	50.0-3.45
No. unique reflections	759,980
R_{work} / R_{free}	21.3/26.2 (19.2/24.3)**
No. atoms	
RNA	99689 (per molecule)
Protein	50328 (per molecule)
Ions	549 (per molecule)
B-factors	
RNA	82.5
Protein	99.8
Ions	54.7
R.m.s. deviations	
Bond lengths (Å)	0.007 (0.007)**
Bond angles (°)	1.2 (1.6)**

* $I / \sigma I$ = 2.33 at 3.5 Å.

**Values in parenthesis represent refinement results when using phenix and multiple TLS groups

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