Supplemental information

Materials and Methods

Purification of ribosomes, tRNA, and mRNA

Mutant 70S *Thermus thermophilus* ribosomes containing a C-terminal truncation of the L9 protein were obtained as described (*1*), submitted and purified as previously reported (*2*). tRNAs were purified from *E. coli* as described (*2*), and mRNA was obtained from Dharmacon of sequence

5′ GGCAAGGAGGUAAAAAUGUUCACCAAA 3′.

Complex formation and crystallization

Complexes were prepared with His-tagged EF-Tu as described by Schuette et al. (*3*). The isolated ternary complex (EF-Tu•GTP•Thr-$tRNA^{Thr}$) was assembled by incubation of EF-Tu (120 uM) with GTP (6 mM) and a tRNA charging reaction of $tRNA^{Thr}$ (26 uM), threonyl-synthetase (50 nM), threonine (0.4 mM), ATP (6mM), phosphoenolpyruvate (6 mM), pyruvate kinase (0.02 mg/ml), and pyrophosphatase (0.01 mg/ml) in a buffer of 10 mM K-HEPES pH 7.5, 15 mM $MgOAc_2$, 50 mM KCl, 10 mM NH_4Cl, and 6 mM β-mercaptoethanol, for 30 minutes at 37°C. Separately, an initiation complex was formed in the same buffer at 55° C containing ribosomes, mRNA, and $tRNA^{Phe}$ as described previously (*2*). The initiation complex was then cooled on ice and kirromycin and paromomycin were added at 50 and 100 μM, respectively. Paromomycin was included because it increases the stability of the TC-ribosome complex. Initiation and ternary complexes were combined and incubated for 30 minutes at 37°C. Purification of ribosomes bound to ternary complex by "pull-down" was as described (*4*), except that the elution from Ni^{+}-NTA was with 50 mM His adjusted to pH 6.5. The eluted fractions were pelletted through a 1.1 M sucrose cushion containing antibiotics and the buffer described above at pH 6.5, overnight at 86,000 g, to remove excess ternary complex.

The pelletted 70S complexes were resuspended at concentrations of 3.7-4 μM, in a buffer containing 10 mM K-HEPES pH 6.5, 13 mM $MgOAc_2$, 50 mM KCl, 10 mM NH_4Cl, and 1 mM tris(2-carboxyethyl)phosphine), 50 μM kirromycin, and 100 μM paromomycin. Following addition of equimolar $tRNA^{Phe}$ and the detergent HEGA-9 to a concentration of 46 mM, crystals were grown via vapor diffusion in sitting drop trays by addition of 3 μL reservoir (100 mM MES pH 6.3, 22-25 mM KCl, 50 mM sucrose, 1% glycerol, and 5.4% (w/v) PEG20K) to 3 μL of the 70S-TC sample. Crystals with needle or plate morphology were visible within hours and grew to full size (~1000 μm x 500 μm x 30 μm) within one week. Crystals were cryo-protected stepwise until reaching a final solution containing 100 mM MES pH 6.3, 33 mM KCl, 50 mM sucrose, 1.1% glycerol, 6.0% (w/v) PEG20K, 15 mM $MgOAc_2$, and 30% (w/v) PEG400, in which they were incubated overnight. Crystals were harvested and frozen by plunging into liquid nitrogen.

Data collection and refinement

The crystals are in space group $P2_1$, with unit cell dimensions a=289.566 b=268.363 c=403.884, β=91.011. Small variations in the crystallization conditions led to an alternative, related crystal form, also in $P2_1$, where the length of the c axis has been halved. The two forms are related by a small translation of the ribosome, which breaks the crystallographic symmetry, leading to two ribosome molecules in the larger asymmetric unit.

X-ray diffraction data were collected on ID 14-4 at the European Synchrotron Light Source, Grenoble, France. Data were collected in 10-30° wedges on 12 separate crystals due to the extreme radiation sensitivity of the crystals. Merging wedges of diffraction data likely led to an increase in R factors. Data were integrated and scaled using XDS (*5*). The structure was determined by molecular replacement using the program CNS (*6*) and the high-resolution structure of the 70S ribosome as a starting model (*2*) with corrections as reported (*7*). Refinement was carried out using the programs CNS (*6*) and Phenix (*8*) using the following general scheme: rigid body refinement of each of the 70S ribosomes in the asymmetric unit; followed by an additional rigid body refinement in which each of the rRNA domains, ribosomal proteins, ligands, and tRNAs were defined as separate rigid groups; and finally, rounds of energy minimization and B-factor refinement. Noncrystallographic restrains were used during refinement. Iterative rounds of building and refinement were carried out resulting in a final model containing P- and E-site tRNAs, mRNA, the ternary complex, and the antibiotic ligands. Parallel refinements that did not include TC were performed to yield unbiased density maps. All displayed electron density maps were calculated using the program CNS (*6*). Figures were generated with Pymol (Delano Scientific). Helical parameter measurements were obtained using the program Curves, and buried surface areas were obtained using the program CNS. Sequence conservation statistics for rRNA were obtained from The Comparative RNA Web Site (http://www.rna.ccbb.utexas.edu/), and for EF-Tu were calculated using sequences obtained from Microbial Genome Database for Comparative Analysis (http://mbgd.genome.ad.jp/) and the National Center for Biotechnology Information.

Table s1. Relationship between *Thermus thermophilus* and *E. coli* numbering of EF-Tu and ribosomal protein S12. The text refers to the *E. coli* numbering for ease of reference to the literature, but the pdb deposition is in native *T. thermophilus* numbering for EF-Tu and ribosomal proteins. rRNA has been converted to *E. coli* numbering for deposition.

EF-Tu	
E. coli	*T.thermophilus*
19	19
20	20
42	42
51	52
52	53
53	54
57	58
58	59
60	61
65	66
83	84
84	85
86	87
89	90
114	115
219	230
220	231
221	232
222	233
223	234
226	237
249	261
256	268
259	271
262	274
273	285
278	290
279	291
281	293
283	295
S12	
E. coli	*T.thermophilus*
74	78
76	80
119	123

Table s2. Summary of crystallographic data and refinement

Data collection	**70S-TC (merged from 12 crystals)**
Space Group	$P2_1$
Cell dimensions	
a, b, c (Å)	*a*=289.566 *b*=268.363 *c*=403.884
α, β, γ (°)	α=90.000 β=91.011 γ=90.000
Resolution (Å)	50-3.6 (3.9-3.6) *
R_{sym}	21.9 (76.9)
$I/\sigma I$	6.15 (1.73) *
Completeness (%)	93.6(85.7)
Redundancy	6.0 (5.2)
Refinement	
Resolution (Å)	50.0-3.6
No. unique reflections	665,418
R_{work}/R_{free}	28.3/30.8
No. atoms	
RNA	205,034
Protein	100,772
B-factors	
RNA	104
Protein	111
R.m.s deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.2

* $I/\sigma I$ =2 at 3.75 Å resolution.

Supplemental Figures

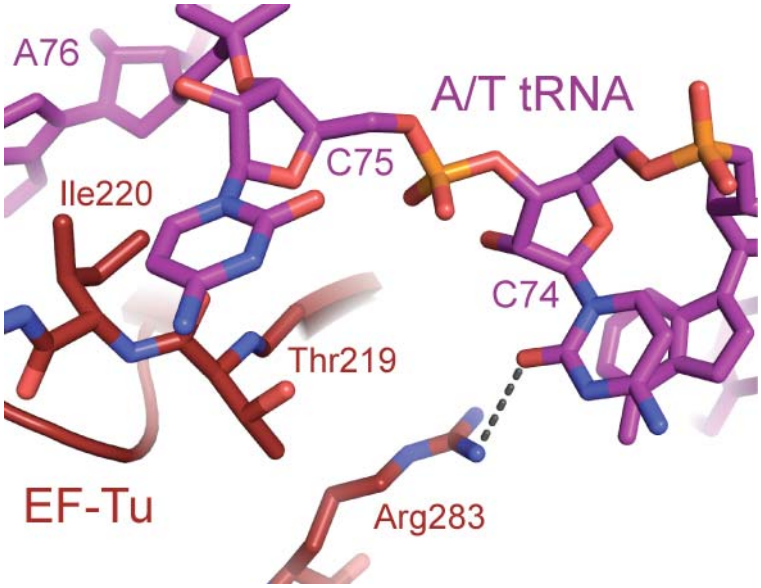

fig s1. Interactions of the 3' end of the A/T tRNA with EF-Tu. Upon binding of the TC to the ribosome, the 3' end of the aminoacyl-tRNA becomes distorted between residues 72-75. This distortion allows tRNA residues C74 and C75 to form stabilizing interactions with EF-Tu. More specifically, residue C75 packs against residues Ile220 and Thr219 of EF-Tu while C74 moves within hydrogen-bonding distance of Arg283.

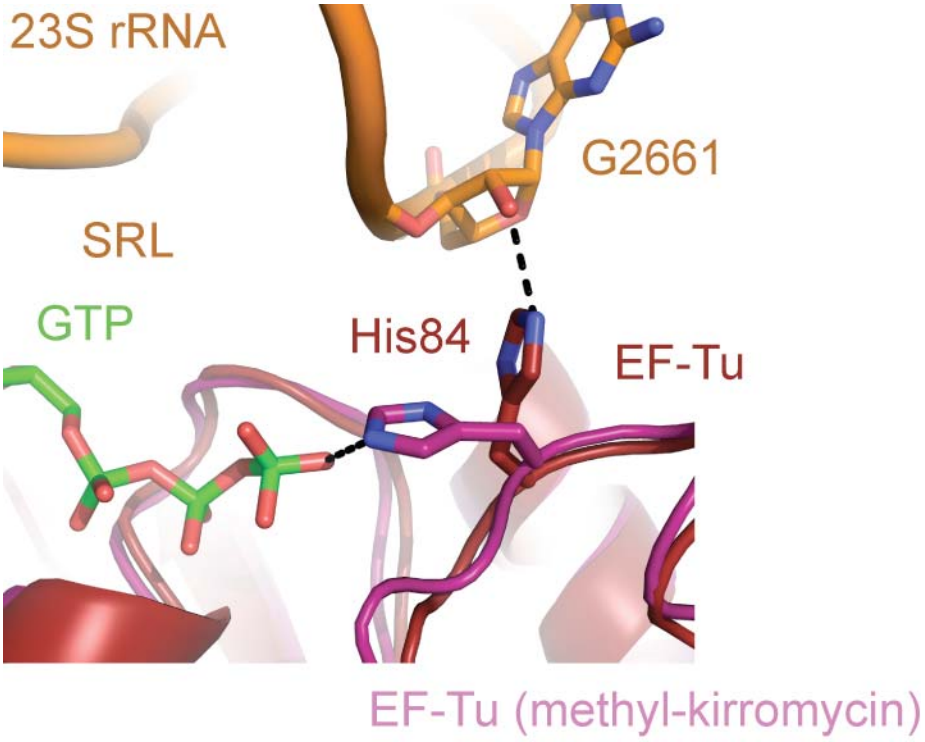

fig s2. Interaction of the EF-Tu catalytic residue His84 with SRL nucleotide G2661. His84 is within hydrogen bonding distance of the 2' OH of G2661 of the sarcin ricin loop. In the structure of EF-Tu in complex with aurodox, His84 is positioned towards the (modeled) γ-phosphate of GTP, and may aid in GTP hydrolysis (*9*) (*10*).

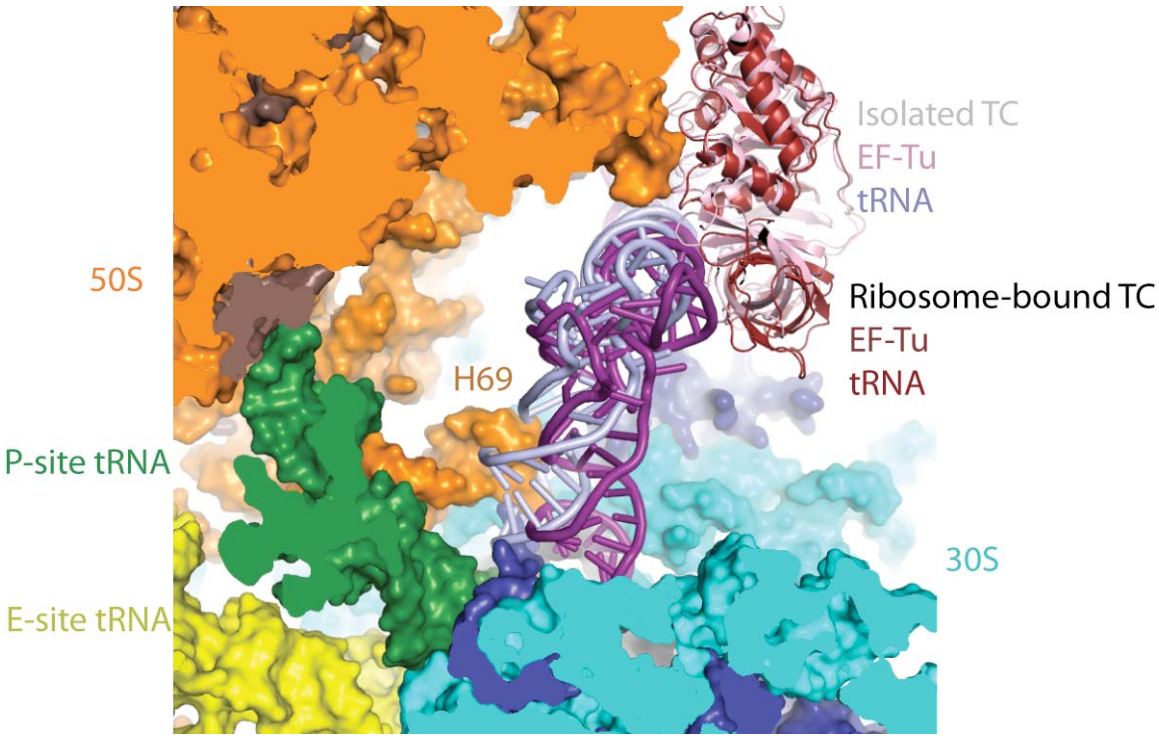

fig. s3. Superimposition of the isolated ternary complex and the ribosomal complex. When superimposed on domain 1 (GTPase domain), isolated TC (*11*) displays only minimal steric clashes with the ribosome. The most significant clash is with H69 of 23S rRNA, which is known to be mobile. Initial binding of TC is likely to involve a minimally distorted aminoacyl-tRNA conformation.

References for Supplemental Information

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