The mechanism for activation of GTP hydrolysis on the ribosome

Supplementary materials and methods

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Materials and Methods

Ribosomes from *Thermus thermophilus* harboring a C-terminal truncation of protein L9(*1*) were purified as previously described(*2*) from cells grown at the Bioexpression and Fermentation Facility at the University of Georgia. mRNA with the sequence 5'GGCAAGGAGGUAAAAAUGUUCUGGAAA was purchased from Dharmcon (Thermo Scientific). Trp-tRNA^{Trp} was prepared as described (*3*).

Complexes of trp-tRNA^{Trp}-GDPCP-70S ribosome were prepared and purified by Ni-NTA affinity purification as described (*3*) with the exception that the antibiotic paromomycin was included in the reaction mixture to increase sample yield. The trp-tRNA^{Trp} used in this study contained a G24A mutation, which increased the reproducibility of crystal growth. On cognate mRNA, it is known that this tRNA behaves identically to wild type trp-tRNA^{Trp}(*4*) and crystal structures of G24A and tRNA^{Trp} and bound to the ribosome on the cognate codon are extremely similar (*3*).

Crystals were grown as described(*5*) by vapor diffusion in sitting drop trays by addition of 3 μL reservoir (100 mM MES pH 6.3, 60-100 mM KCl, 50 mM sucrose, 1% glycerol, and 5.3% (w/v) PEG20K) to 3 μL of the 70S-TC sample, and cryo-protected stepwise to a final solution of 100 mM MES pH 6.3, 100 mM KCl, 50 mM sucrose, 1.1% glycerol, 6.0% (w/v) PEG20K, 15 mM $MgOAc_2$, and 30% (w/v) PEG400 before being frozen by plunging into liquid nitrogen.

Data was collected at beamline ID 14-4 of the European Synchrotron Light Source(*6*), and integrated and scaled using XDS(*7*). These crystals are in space group $P2_1$, with unit cell dimensions a=197.6 b=274.9 c=282.5, β=91.8. This crystal form is related to our previous crystals (*5*) (*3*), which were also in $P2_1$, but the length of the a axis is doubled in the previous form. The two forms are related by a small translation of the ribosome, which converts the non-crystallographic symmetry in the larger unit cell into crystallographic symmetry, leading to only one ribosome molecule in this smaller asymmetric unit.

The structure was determined by molecular replacement in CNS(*8*), using a ligand-free ribosome as the search model. Iterative rounds of model building and refinement were carried out in coot(*9*) and CNS(*8*) as previously described(*5*). To avoid contamination of R_{free}, the R_{free} set was inherited from our previous data with the conversion $h_{new}=l_{old}/2$, $k_{new}=-k_{old}$, $l_{new}=h_{old}$ to account for the change in unit cell. All figures were made in Pymol(*10*).

Supplemental Figures

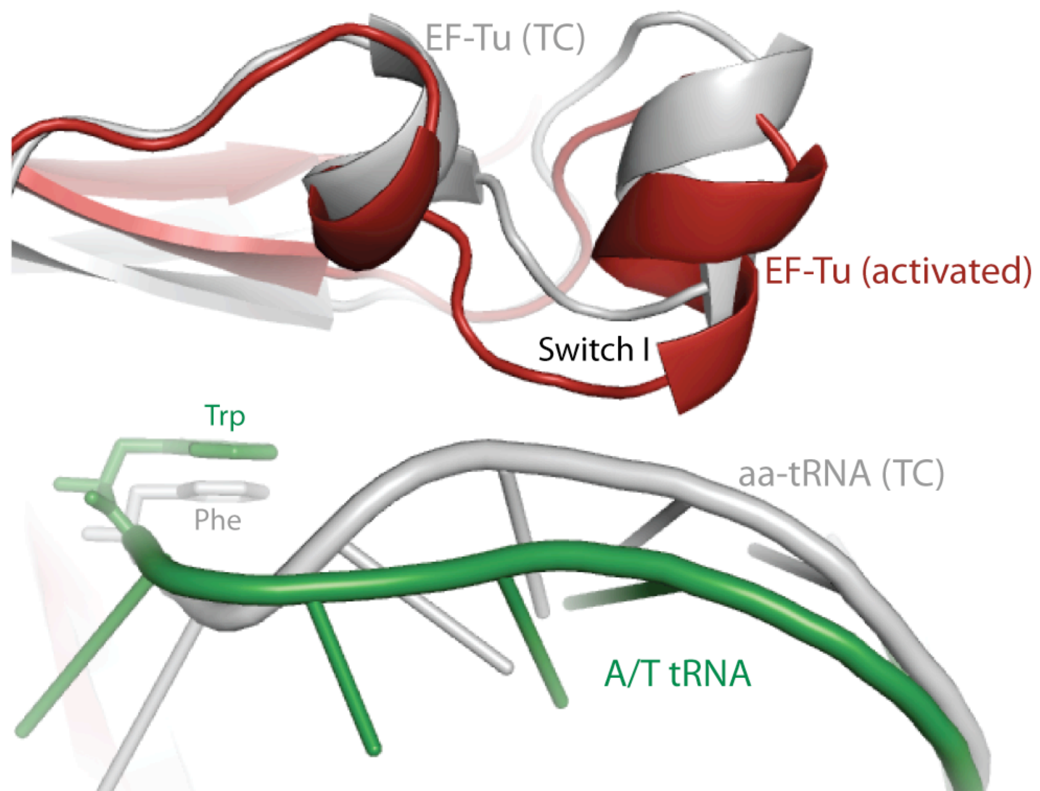

fig s1. Binding the ribosome induces a distortion in the 3' end of the aminoacyl-tRNA (green—activated structure, grey—isolated TC) that disrupts interactions between the Switch I loop of EF-Tu (red—activated, grey—TC) and the tRNA backbone.

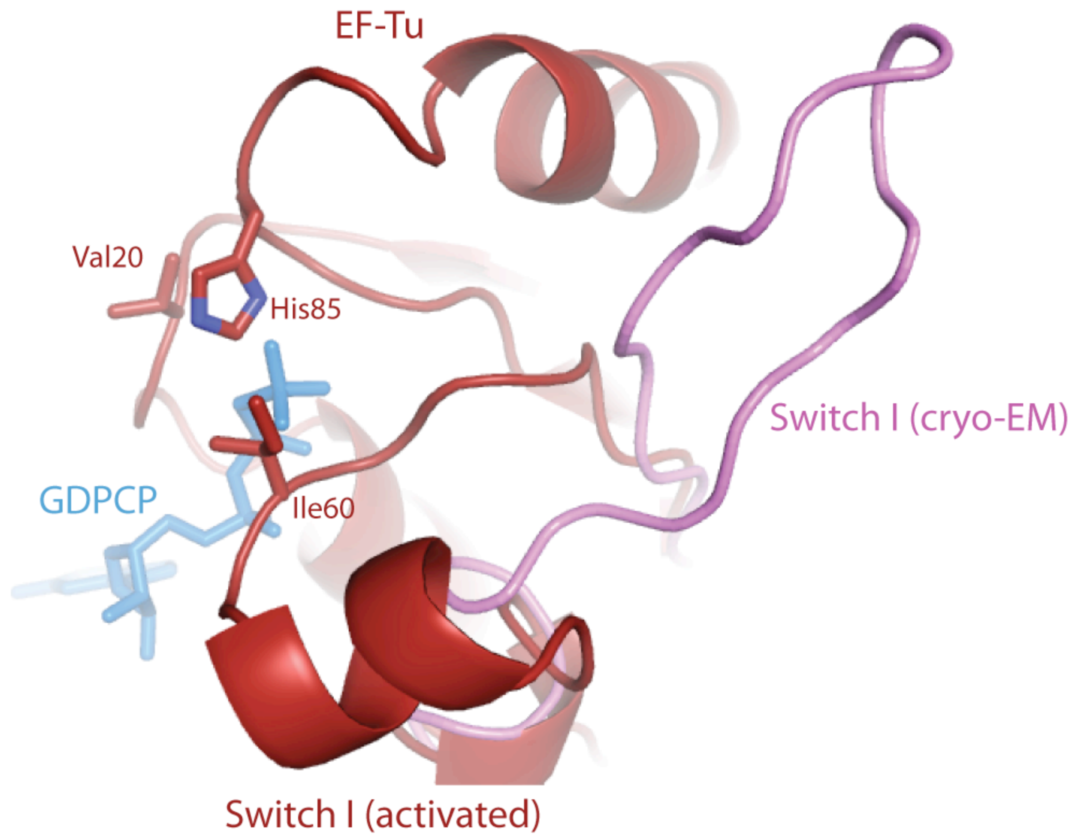

fig s2. In the activated structure of EF-Tu bound to the ribosome, the Switch I loop (red) is in a well ordered and located in the GTPase center. We see no evidence that a disordered or radically remodeled Switch I (pink)(*11*) is required for GTPase activation and hydrolysis on the ribosome.

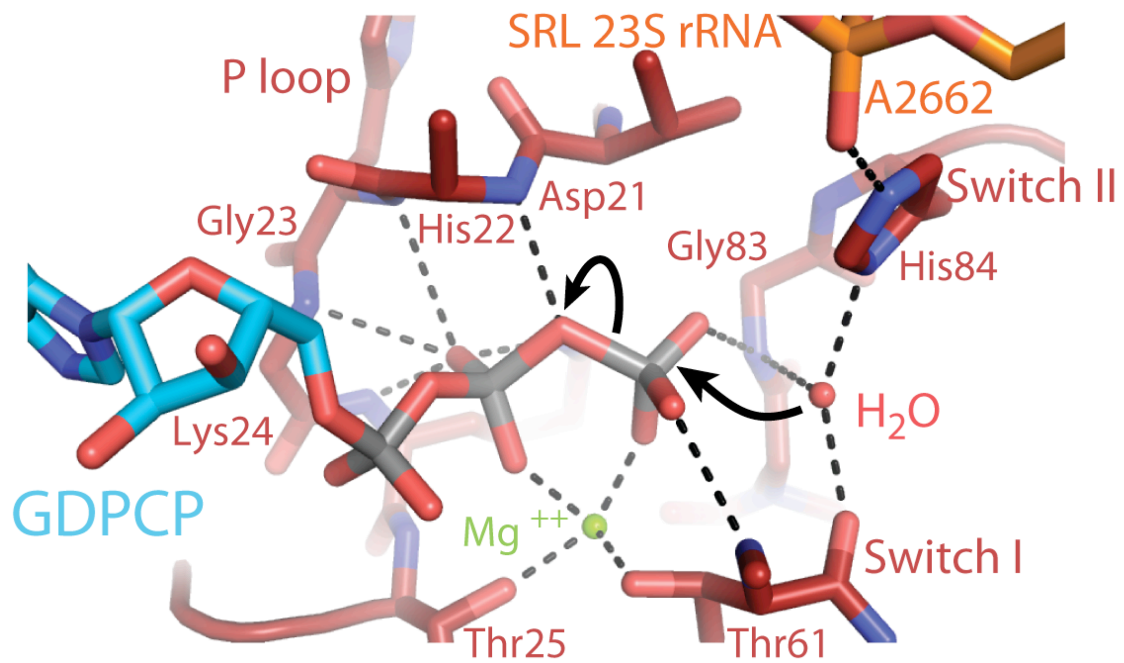

fig s3. Several interactions between EF-Tu and the GTP stabilize the β and γ phosphates that are important for GTP hydrolysis. Additionally, several interactions between the Switch I residue Thr61 and the γ phosphate explain how release of Pi results in the disordering of Switch I.

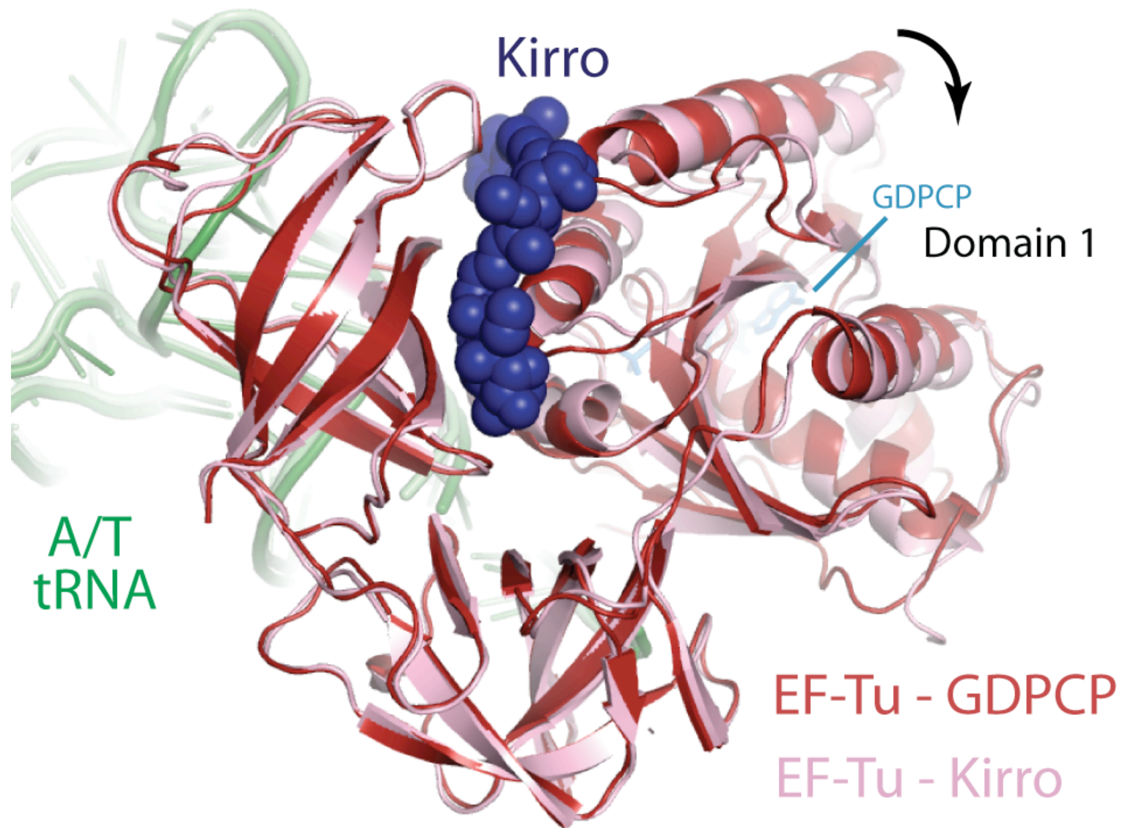

fig s4. A small rotation in the G-domain of EF-Tu relative to domains 2 and 3 is observed between the GDPCP (red) and kirromycin (pink) stabilized structures bound to the ribosome.

Table s1. Summary of crystallographic data and refinement

Data collection	**70S-TC-GDPCP (merged from 3 crystals)**
Space Group	$P2_1$
Cell dimensions	
a, b, c (Å)	*a*=197.6 *b*=274.9 *c*=282.5
α, β, γ (°)	α=90.0 β=91.8 γ=90.0
Resolution (Å)	50-3.1 (3.2-3.1) *†
R_{sym} (%)	22.8 (126.3)
$I/\sigma I$	6.98 (1.16) *
Completeness (%)	98.8(89.6)
Redundancy	5.2 (4.3)
Refinement	
Resolution (Å)	50.0-3.1†
No. unique reflections	537024
R_{work}/R_{free}	23.1/26.8
No. atoms	
RNA	102,537
Protein	50,602
B-factors	
RNA	83
Protein	87
R.m.s deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.2

* $I/\sigma I$ = 1.88 at 3.2 Å resolution (using a bin from 3.4-3.2 Å resolution)

† R_{work}/R_{free}= 33.2/35.4 for data from 3.2-3.1 Å resolution

References

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