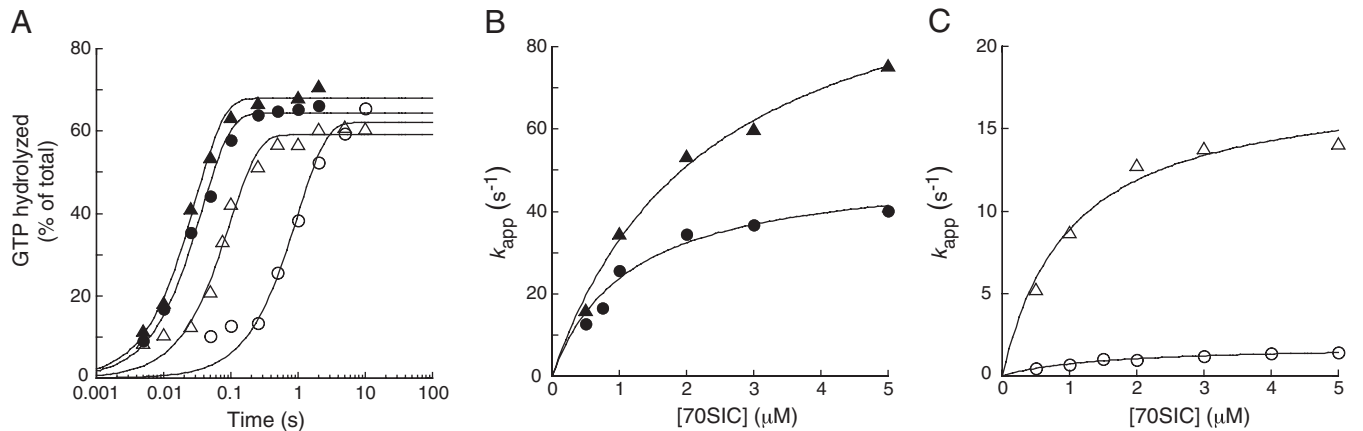# Supporting Information

Fig. S1. Effects of 16S rRNA mutation G299A on initial selection. The 70S initiation complexes (70SIC) programmed with either cognate UUU (closed symbols) or near-cognate CUU (open symbols) in the A site were rapidly mixed with elongation factor thermo unstable (EF-Tu)·[γ-^{32}P]GTP·Phe-tRNA^{Phe}, and rates of GTP hydrolysis were determined. Wild type, ●/○; G299A, ▲/△. (*A*) Examples of time courses at 1 μM 70SIC. Data were fit to a single exponential function to obtain the apparent rates of GTP hydrolysis (k_{app}). Apparent rates for cognate (*B*) and near-cognate (*C*) reactions plotted vs. [70SIC]. Data were fit to the equation $k_{app} = k_{GTPmax}$·[70SIC]/($K_{1/2}$ + [70SIC]), yielding the parameters shown in Table 1.
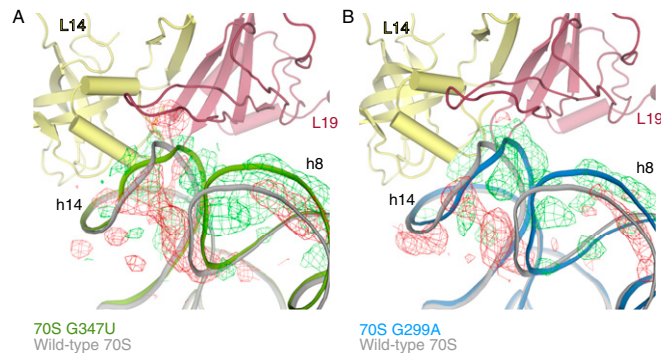

Fig. S2. Conformational changes of helices 8 (h8) and 14 (h14). (*A*) Original, unbiased difference F_o–F_c density of the 70S G347U structure shows positive density (green) and negative density (red; 2 σ) after crystallographic refinement using the wild-type 70S structure as the starting model [Protein Data Bank (PDB) ID code 2WDG]. The original rRNA is shown as a gray backbone, and the rebuilt h8 and h14 backbone of the 70S G347U structure is shown in green. (*B*) Original, unbiased difference F_o–F_c density of the 70S G299A structure using the wild-type 70S structure as the starting model with the same color scheme as in *A*. The rebuilt h8 and h14 backbone of the 70S G299A structure is shown in blue.
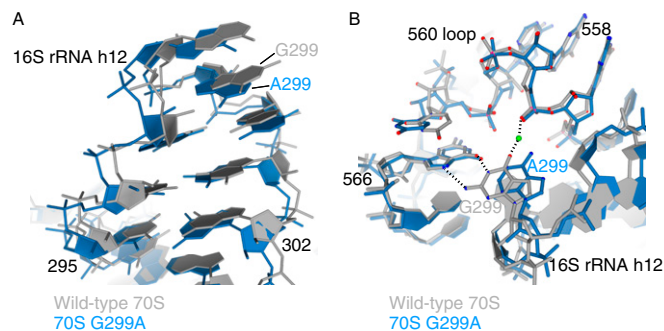

Fig. S3. Rearrangements in h12 due to the G299A mutation. (*A*) Displacement of h12 resulting from G299A (blue) compared with the wild-type 70S structure (PDB ID code 2WDG; gray). (*B*) The G299A mutation results in loss of the coordinated Mg^{2+} ion (green sphere) and the 566–299 purine–purine interaction, but does not cause an overall loss of tertiary structure.
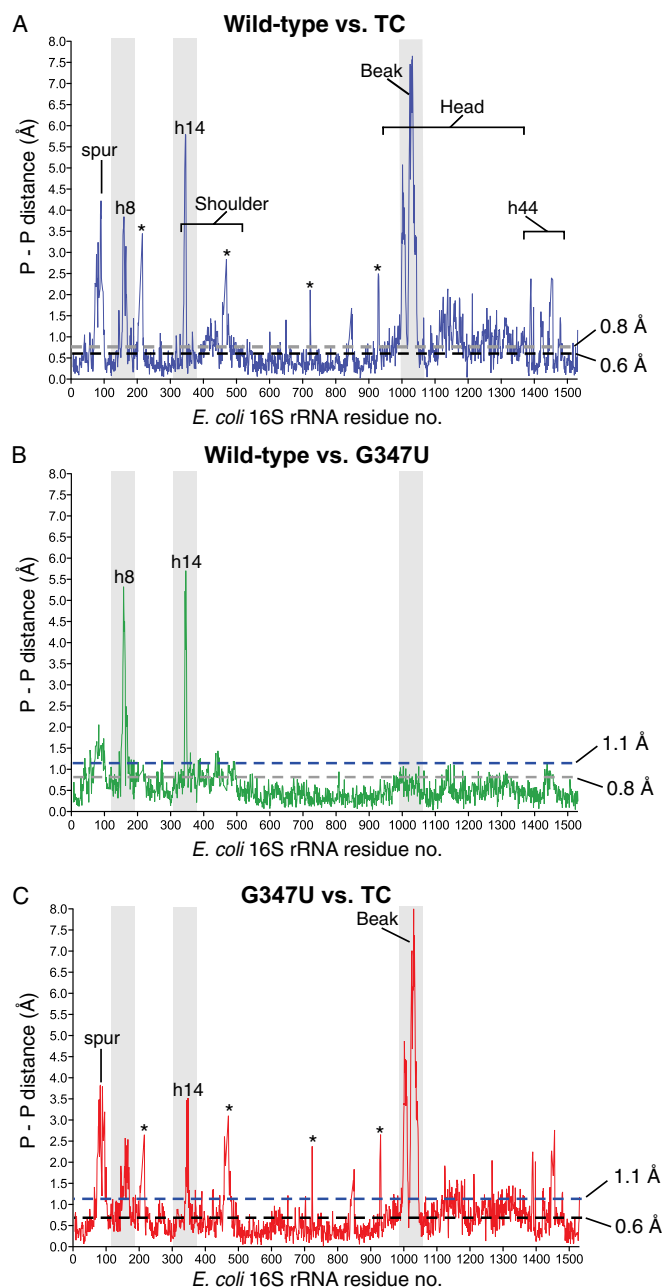
Fig. S4. Comparison of the 70S G347U structure with other 70S structures. (*A*) Comparison of the 16S rRNA phosphate–phosphate backbone differences between wild-type 70S (PDB ID code 2WDG) and 70S ternary complex (TC; PDB ID code 2XQD). The coordinate error for the wild-type 70S (0.8 Å) and 70S TC structures (0.6 Å) are shown as gray and black dashed lines, respectively. The asterisks denotes rRNA adjacent to the mobile spur regions that moves presumably because of different crystal forms used in the 2WDG and 2XQD structures (as not seen in *B*). (*B*) Same comparison as in *A* of wild-type 70S and 70S G347U. Differences are almost exclusively in the h8 and h14 regions. The coordinate error for wild-type 70S (0.8 Å) and 70S G347U structures (1.1 Å) are shown as gray and blue dashed lines, respectively. (*C*) Same comparison as in *A* of 70S TC and 70S G347U. Smaller differences in h8 and h14 seen in *B* are absent. The coordinate error for the 70S G347U structure (1.1 Å) and the 70S TC structure (0.6 Å) are shown as blue and black dashed lines, respectively. Additional differences (∼8 Å) between the G299A and G347U mutant structures and the TC-bound 70S structures are seen at the beak (part of the small subunit head domain), a region known to move upon TC binding (1–3).

1. Schmeing TM, et al. (2009) The crystal structure of the ribosome bound to EF-Tu and aminoacyl-tRNA. *Science* 326(5953):688–694.
2. Voorhees RM, Schmeing TM, Kelley AC, Ramakrishnan V (2010) The mechanism for activation of GTP hydrolysis on the ribosome. *Science* 330(6005):835–838.
3. Voorhees RM, Weixlbaumer A, Loakes D, Kelley AC, Ramakrishnan V (2009) Insights into substrate stabilization from snapshots of the peptidyl transferase center of the intact 70S ribosome. *Nat Struct Mol Biol* 16(5):528–533.
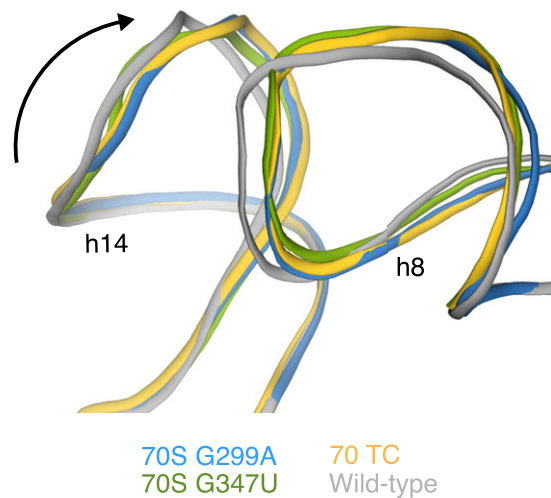
Fig. S5. Structural rearrangements of h8 and h14 resemble the conformational changes that result upon TC binding to the ribosome. Zoomed-in view of how both 16S rRNA mutations, G299A (blue) and G347U (green), result in the movement of h8 and h14, disrupting the intersubunit B8 interaction in a similar manner as observed when TC binds to the ribosome (PDB ID code 2XQD; gold).

Table S1. Growth rates of bacterial strains containing control or mutant ribosomes

Strain	Description	Growth rate*
Escherichia coli		
KLF2674	Δ7 prrn (wild-type)	1.38 ± 0.06
KLF4004	Δ7 prrn (G299A)	0.84 ± 0.07
KLF4006	Δ7 prrn (G347U)	1.30 ± 0.08
Thermus thermophilus		
HB8	Wild-type	1.14 ± 0.08
KLF1212	*rrsB (G299A) ΔrrsA::htk1*	0.72 ± 0.08
KLF1211	*rrsB (G347U) ΔrrsA::htk1*	1.06 ± 0.02

*In units of doublings per hour. Data represent the mean ± SEM from three or more independent experiments.

Table S2. Summary of crystallographic data and refinement

	G299A	G347U
Data collection		
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, b, c, Å	209.95, 445.55, 620.21	210.29, 445.45, 622.11
α, β, γ, °	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution, Å	50.0–3.5 (3.7–3.5)	50–3.9 (4.1–3.9)
R_{merge}, %	43.8 (130.3)	35.4 (140.9)
R_{pim}, %	13.8 (43.8)	13.4 (53.8)
I/σI	6.3 (1.8)	5.7 (1.8)
Completeness, %	99.9 (99.9)	95.0 (90.9)
Redundancy	11.5 (9.6)	6.6 (6.3)
Refinement		
Resolution, Å	50.0–3.5	50.0–3.9
No. of reflections	719,893	496,744
R_{work}/R_{free}	21.0/24.9	25.5/27.0
No. of atoms	294,835	292,549
Rmsd		
Bond lengths, Å	0.009	0.008
Bond angles, °	1.05	1.20

Values in parentheses are for highest-resolution shell.