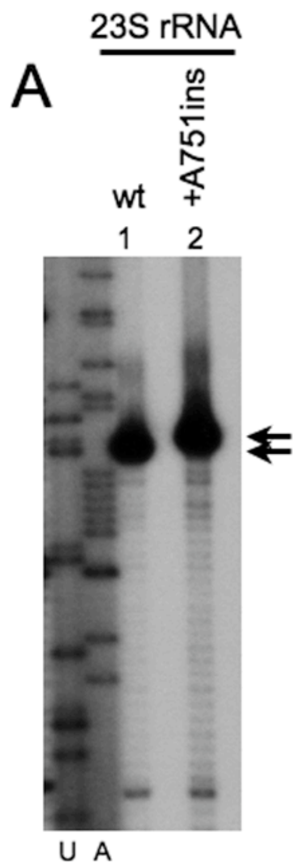
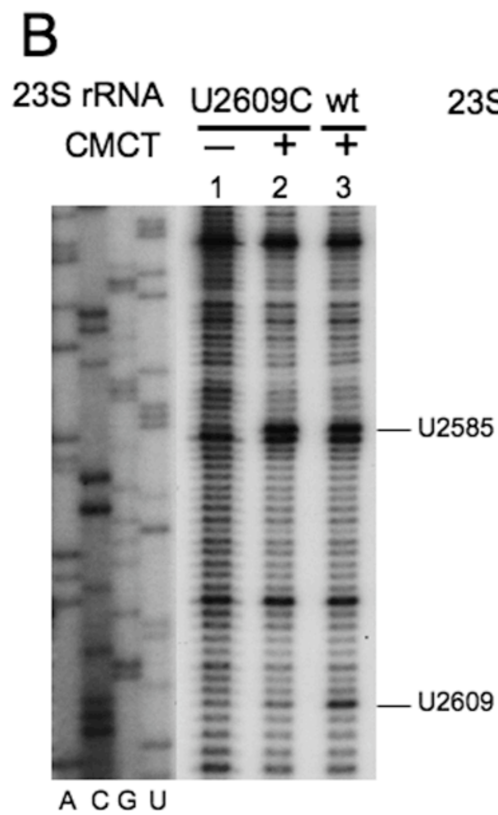
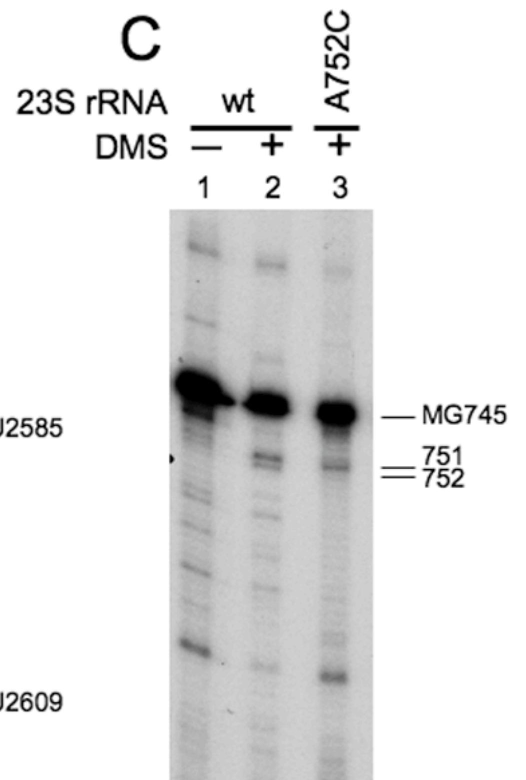
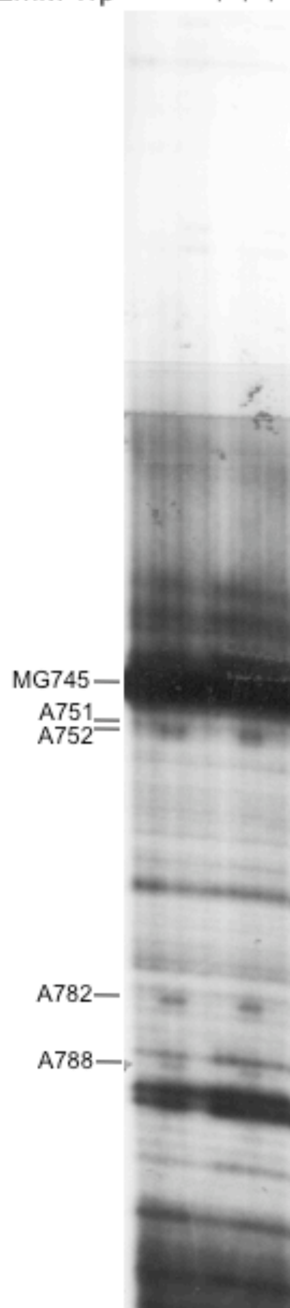
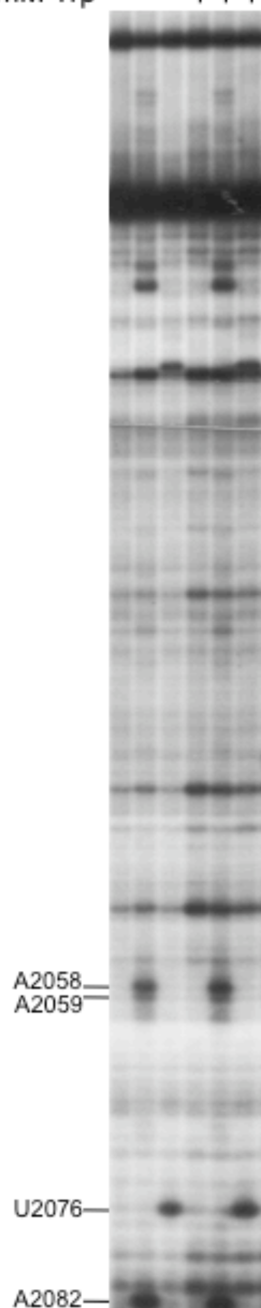
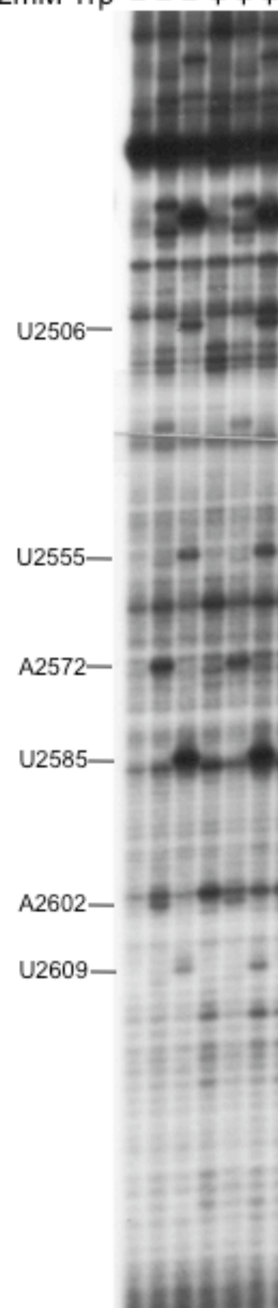
Supplementary Figure 1. Ribosomes were isolated from bacterial cells containing plasmids expressing the indicated 23S rRNAs. A) The presence of +A751ins 23S rRNA in the ribosomes could be detected using primer extension assays (Material and Methods). cDNA synthesis on 23S rRNA template obtained from wild-type ribosomes is usually stopped by the natural methylated G745 nucleotide (MG745) (43). 23S rRNA were extracted from the isolated ribosomes and used to performed primer extension assays with [^{32}P]-labeled oligonucleotides complementary to nucleotides 821-838 of 23S rRNA. The final products of the reaction were resolved using 6% urea-polyacrylamide gels. The position of a significant stall of cDNA synthesis is indicated with arrows. B) Isolated ribosomes were used to perform methylation protection assays (Materials and Methods). Ribosomes were exposed (+) or not (-) to the alkylating agent, CMCT, to methylate water-accessible uridines. 23S rRNAs were extracted and used to perform primer extension assays as indicated in part A). Primer extension was performed using [^{32}P]-labeled oligonucleotides complementary to 2654-2674 nucleotides of 23S rRNA. Nucleotides methylated by the presence of CMCT are indicated. C) Methylation protection assays were performed as indicated in part B). This time DMS was used as an alkylating agent. The primer extension assays were performed with [^{32}P]-labeled oligonucleotides indicated in part A). Nucleotides methylated in the presence of DMS are indicated.


A
23S rRNA
wt
+A751ins
1
2
U
A

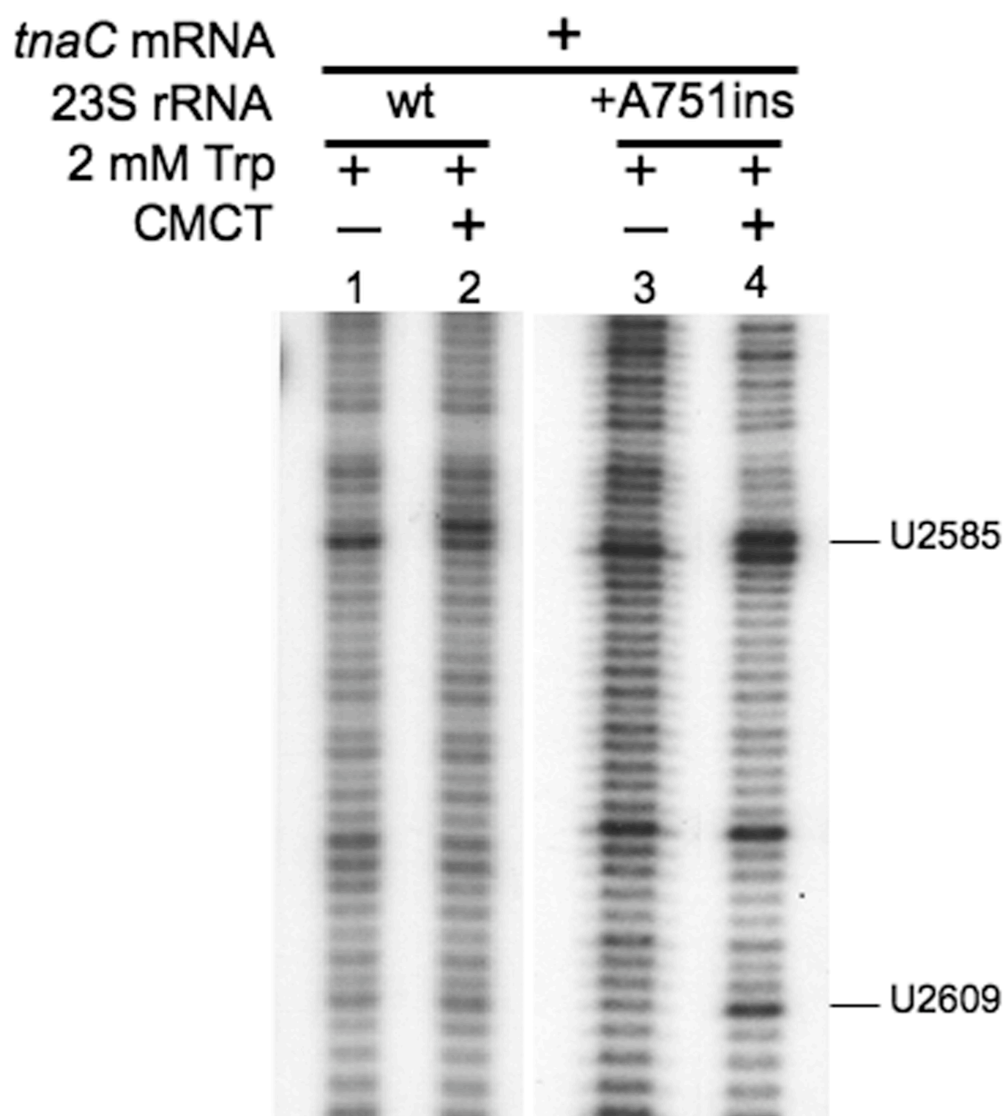

B
23S rRNA
U2609C
wt
CMCT
—
+
+
1
2
3
U2585
U2609
A
C
G
U


C
23S rRNA
wt
A752C
DMS
—
+
+
1
2
3
MG745
751
752

Supplementary Figure 2. Methylation pattern of wild type ribosomes exposed to Trp. Wild type ribosomes not engage in translation (vacant) were incubated in the presence (+) or absence (-) of Trp at 37°C during 10 min. The final mixes were exposed (+) or not (-) to alkylating agents either DMS or CMCT, the 23S rRNA was extracted and primer extensions were performed as indicated in Materials and Methods. Primer extensions were performed using [^{32}P]-labeled oligodeoxynucleotides which reveal methylation of nucleotides corresponding to indicated regions. For 620-820 region we used the oligo 5'-GGCGCTACCTAAATAGCT-3' (19); for 1900-2100 region we used the oligo 5'-CTATCCTACACTCAAGGCTC-3' (44); and for 2450-2650 region we used the 5'-TCCGGTCCTCTCGTACT-3' (44).


23S rRNA
region
620-820
CMCT
DMS
2mM Trp
MG745
A751
A752
A782
A788

23S rRNA
region
1900-2100
CMCT
DMS
2mM Trp
A2058
A2059
U2076
A2082

23S rRNA
region
2450-2650
CMCT
DMS
2mM Trp
U2506
U2555
A2572
U2585
A2602
U2609

Supplementary Fig 3. 23S rRNA nucleotides that are protected by the TnaC nascent peptide. Methylation protection assays were performed with ribosomes containing the indicated 23S rRNA alleles. Ribosomes translating (+) messengers containing the *tnaC* gene sequences were analyzed in a buffer with 2 mM Trp. The ribosomes were exposed (+) or not (-) to the indicated alkylating agents. Nucleotides methylated are indicated. These assays were performed with [^{32}P]-labeled oligonucleotides indicated in supplementary Fig 1B.



tnaC mRNA
+
23S rRNA
wt
+A751ins
2 mM Trp
+
+
+
+
CMCT
—
+
—
+
1
2
3
4
U2585
U2609