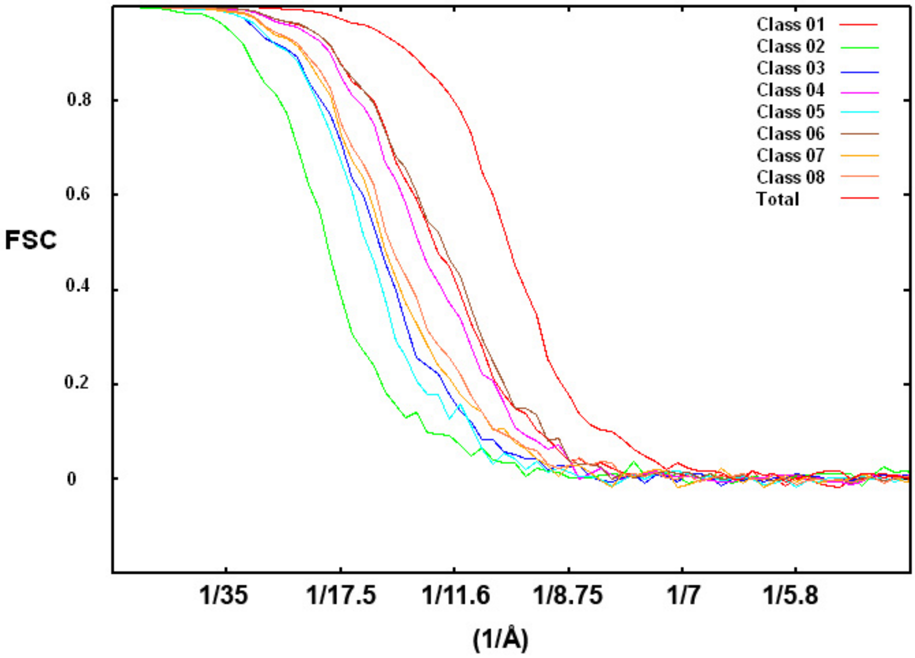
SUPPLEMENTARY INFORMATION



Class	Number of particles	FSC 0.143	FSC 0.5
Class 01	39,143	9.8 Å	12.4 Å
Class 02	6,388	13.8 Å	18.3 Å
Class 03	25,204	11.6 Å	15.0 Å
Class 04	33,458	10.1 Å	13.0 Å
Class 05	15,488	12.0 Å	15.7 Å
Class 06	34,799	9.6 Å	12.0 Å
Class 07	22,231	10.7 Å	14.8 Å
Class 08	28,791	10.8 Å	14.4 Å
Total	**205,502**	**8.9 Å**	**10.1 Å**

Fig S1 *Resolution curves.* Sorting using maximum likelihood analysis in the program ML3D subdivided the data set into eight subclasses. The resolutions of the maps for each of the classes were estimated using a cutoff of 0.143 in Fourier Shell correlation (FSC)[1]. Also, resolution estimations at cutoff 0.5 in the FSC are shown. The numbers in the appended table also indicate the number of particles assigned to each group by the sorting method.

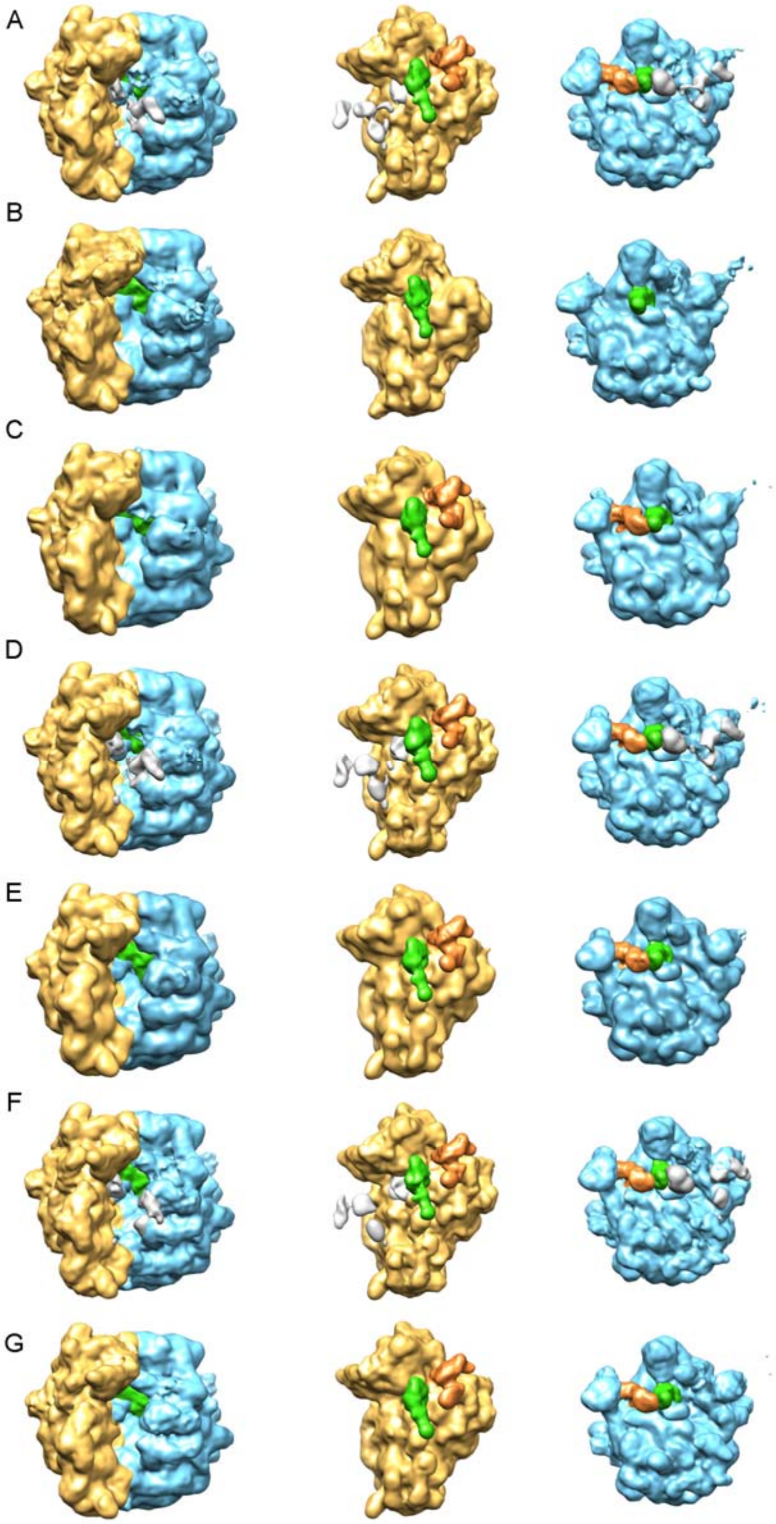


Fig S2 *Overview of the reconstructions.* A gallery of images is shown for 70S-RelA cryo-EM maps of classes 1 to 7 (**A** to **G**). In the renderings 30S subunit (yellow), 50S subunit (blue), and the P- and E-site tRNAs (green and orange, respectively) are shown. Densities (if any) attributable to the deacylated A-site tRNA and RelA are shown in grey.

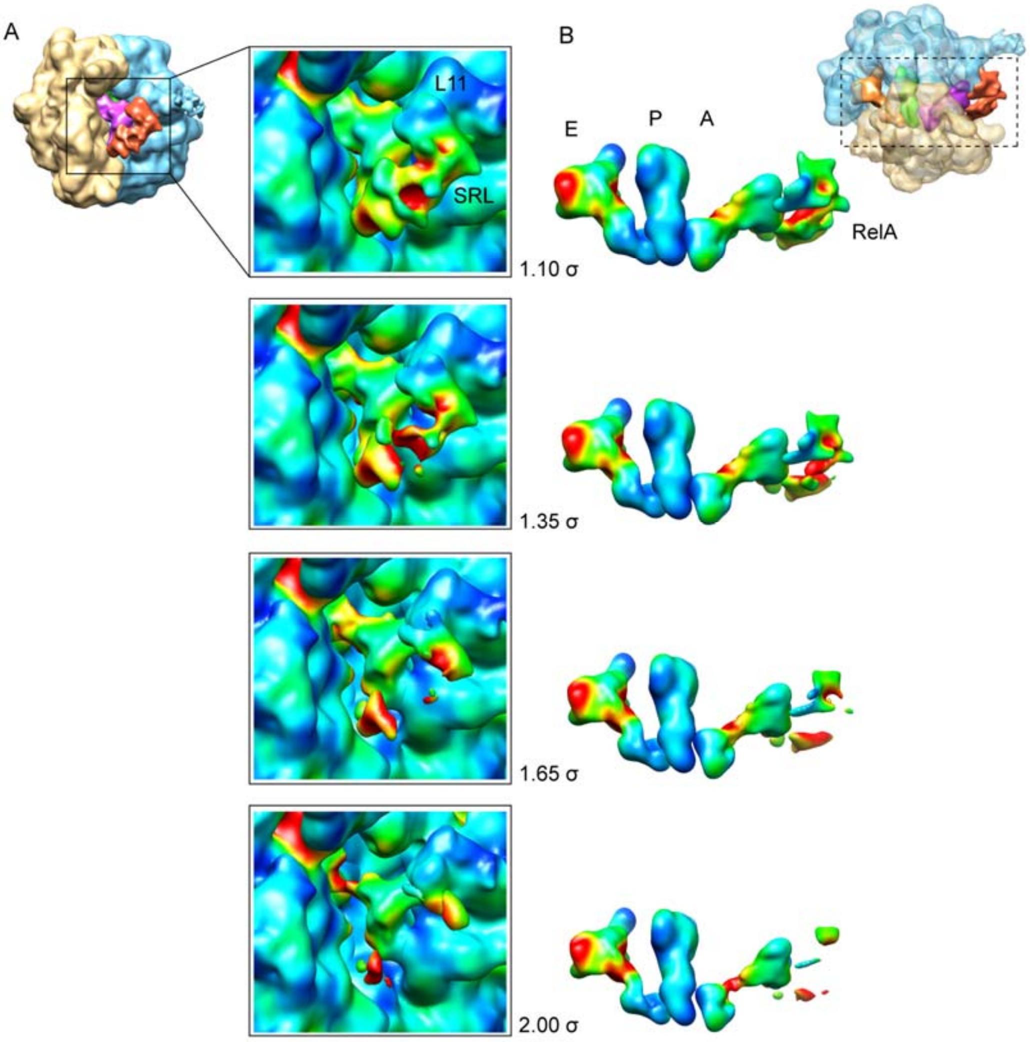


Fig S3 *Analysis of 3D reconstruction densities for 70S-RelA complex after classification.* (**A**, **B**) Different sets of threshold values (1.1, 1.35, 165 and 2.0σ) are used for comparison. In each panel, the orientation of the complex is shown as a thumbnail. The regions of higher variability are shown in red. Based on the density analysis, it can be concluded that the E- and A/T-tRNAs are the ones that have comparable occupancies, whereas the P-tRNA apparently shows a higher occupancy as judged by the density behavior. Based on the measurement of the average density values of the isolated maps, and the subsequent cross-comparison with other nucleic acid parts of the map, we can estimate that the occupancy of the A/T tRNA is ~70%.

70S		−	+	+	+	+	+	+
RelA		+	+	+	+	+	+	+
mRNA		−	+	+	+	+	+	+
P-tRNA(1x)		−	−	+	+	+	+	+
A-tRNA(1x)		−	−	−	+	−	−	−
A-tRNA(3x)		−	−	−	−	+	−	−
NH-A-tRNA(1x)		−	−	−	−	−	+	−
NH-A-tRNA(3x)		−	−	−	−	−	−	+

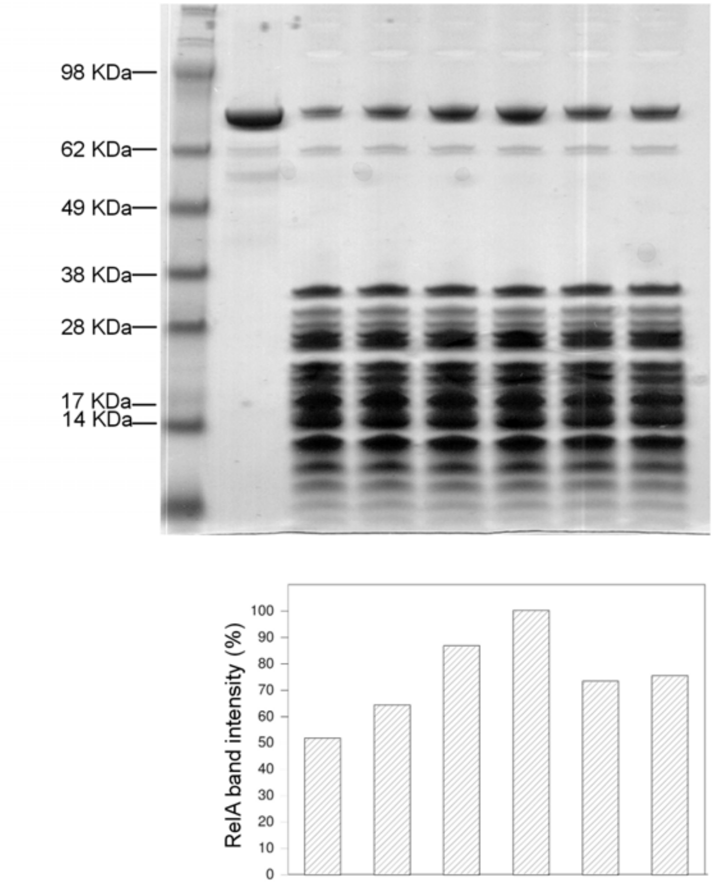


Fig S4 *Binding experiment using non-hydrolizable Phe-NH-tRNA^{Phe}*. 4-12% SDS-PAGE loaded with the pull-downs of the full-length RelA with different programmed states of the 70S. NH-A-tRNA refers to Phe-NH-tRNA^{Phe}. The presence of deacylated tRNA promotes the maximum binding of RelA to the ribosome. The observed binding of RelA in the absence of deacylated A-site tRNA is regarded as the initial survey of the ribosome complex by the stringent factor to sample the A site. It is also possible that the preparations of tRNAs used in the assay contain contamination of deacylated tRNAs that would interfere with the signal.

Reference

1. Rosenthal PB, Henderson R (2003) Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J Mol Biol* **333:** 721-745