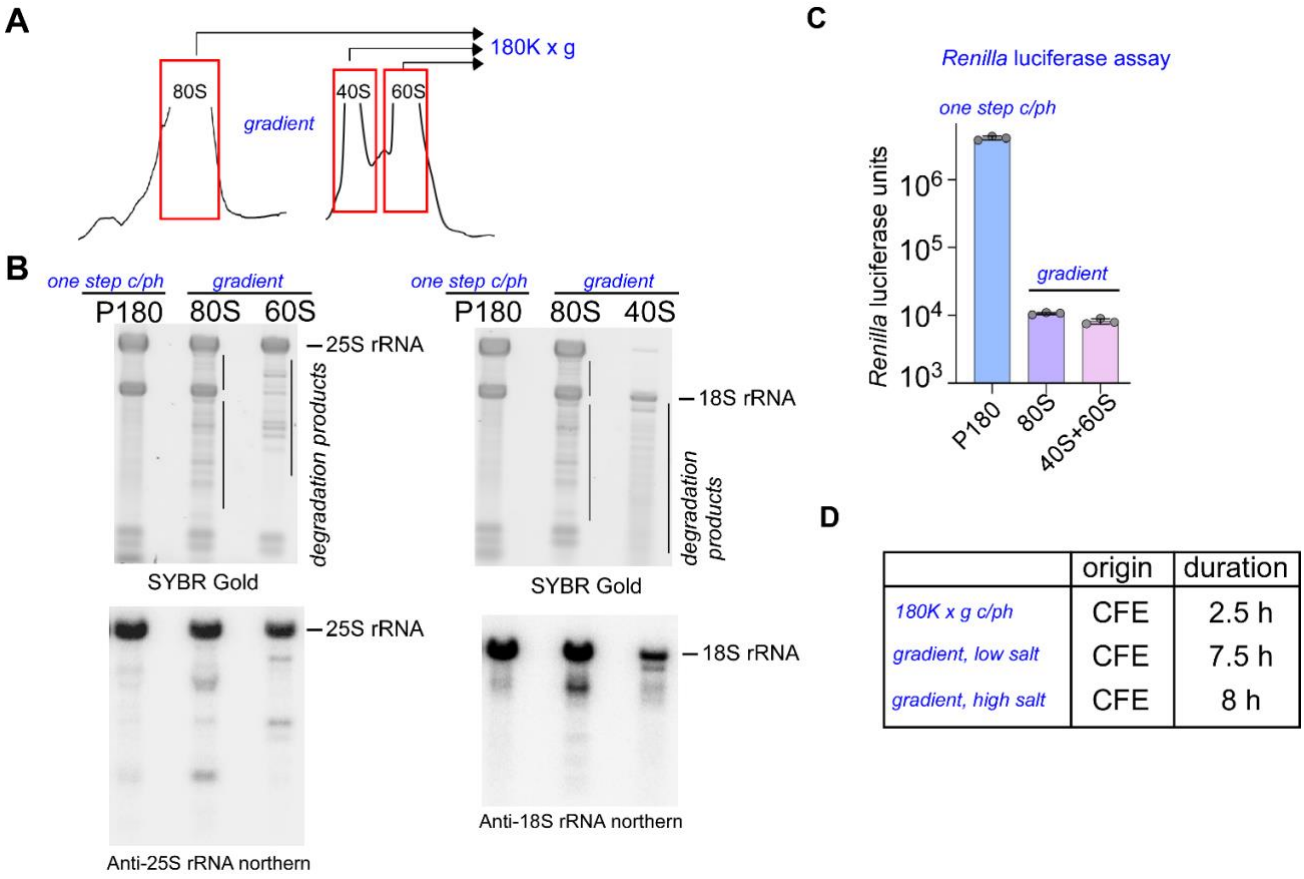
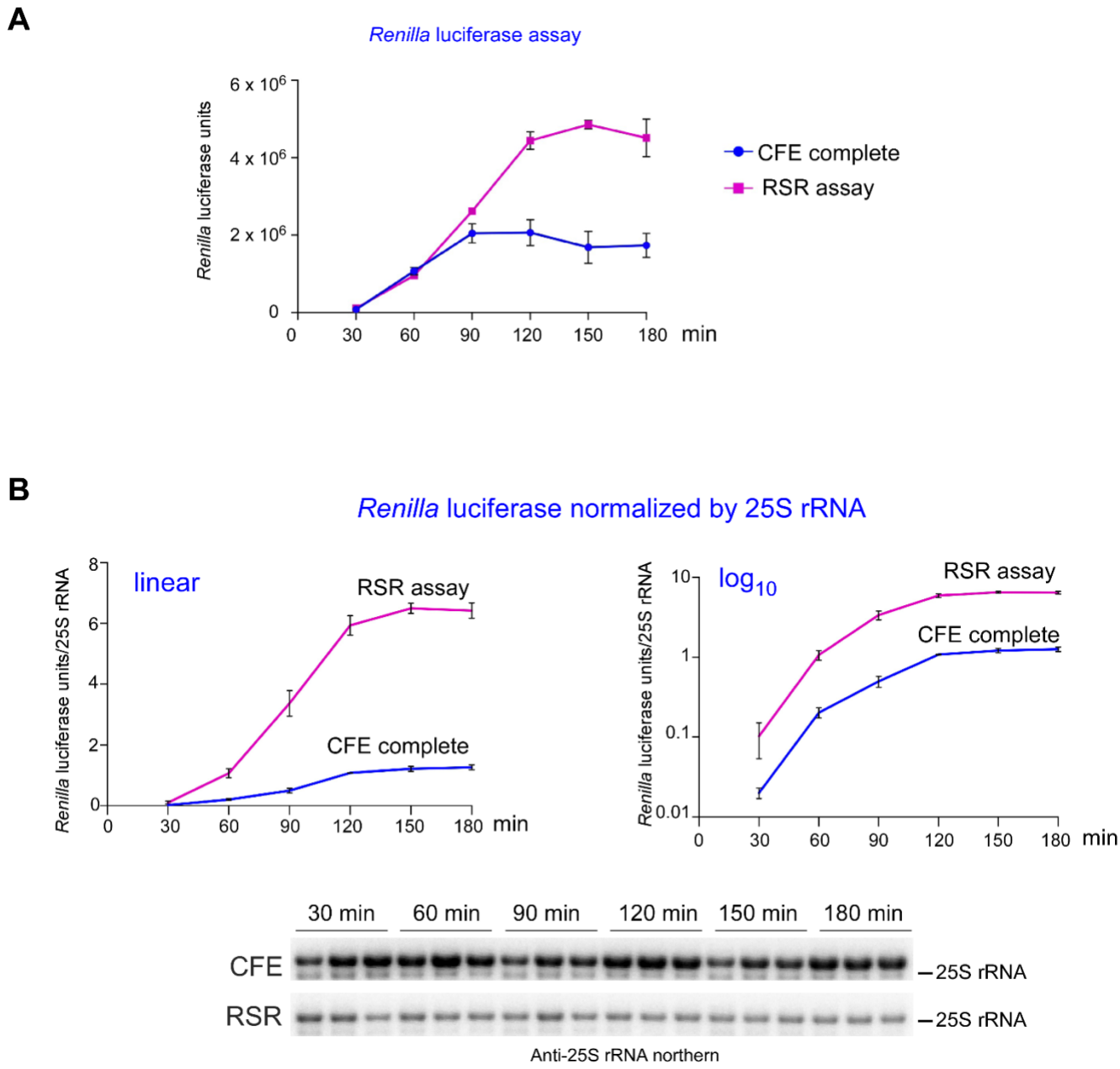
SUPPLEMENTAL FIGURES

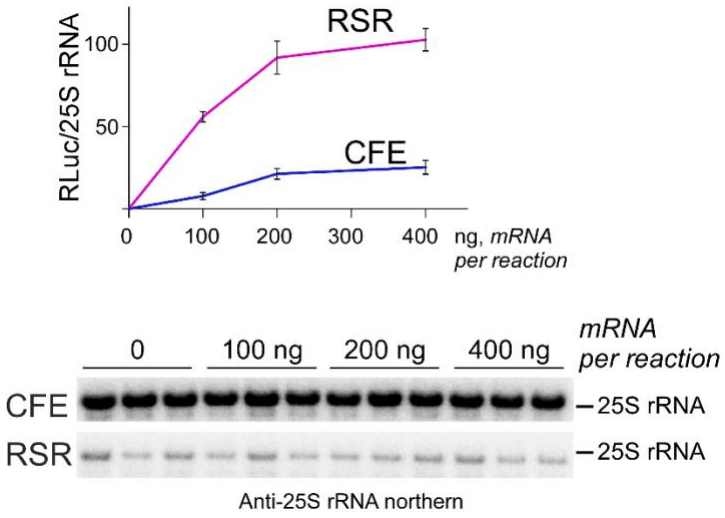


	origin	duration
180K x g c/ph	CFE	2.5 h
gradient, low salt	CFE	7.5 h
gradient, high salt	CFE	8 h

Supplemental Figure S1. Comparison of the one-step 180K-centrifugation and sucrose gradient-based procedures to isolate ribosomes. **(*A*)** To purify 80S ribosomes by sucrose gradient centrifugation, complete CFE (150 μg of RNA) was loaded onto 15–45% (w/v) sucrose gradients prepared in 10 mM Tris–HCl (pH 7.4), 100 mM KOAc, and 4 mM $MgCl_2$ (low salt gradient). To purify 40S and 60S ribosomal subunits, KOAc was added to complete CFE (150 μg of RNA) to a final concentration of 400 mM. High salt-containing CFE was incubated at 21°C for 30 min with shaking in an Eppendorf Thermomixer at 1200 rpm and loaded onto 15–45% (w/v) sucrose gradients prepared in 10 mM Tris–HCl (pH 7.4), 400 mM KOAc, and 4 mM $MgCl_2$ (high salt gradient). The volume of each gradient was 11 mL. Gradients were centrifuged at 160,000 × g at 4°C for 4 h in a Beckman SW41Ti rotor (36,000 rpm) and fractionated using a Beckman fraction recovery system connected to an EM-1 UV monitor (Bio-Rad) to visualize 80S, 60S and 40S peaks (boxed in red). **(*B*)** Low salt gradient-derived 80S-containing fraction and high salt gradient-derived 40S and 60S fractions were collected (~ 1 mL) into 1.5 mL polypropylene ultracentrifuge tube (Beckman) and centrifuged at 180,000 × g for 2 h at 4°C in a Beckman TLA55 rotor. Likewise, ribosomes were pelleted from complete CFE by one-step 180K-centrifugation to obtain P180. All ribosomal pellets (P180, 80S, 60S and 40S) were resuspended in buffer A and 2 μg of RNA was analyzed by northern hybridization using indicated probes. Prior to hybridizations, gel was stained with SYBR Gold. **(*C*)** Translation reactions were assembled with S180 and 400 ng of capped-*TAP-RLuc* mRNA reporter. Ribosomes were added as follows (μg values refer to RNA amounts): 4 μg of P180 ribosomes, 4 μg of 80S, and 2 μg of each of the 60S and 40S. Reaction products were analyzed by a *Renilla* luciferase assay; luciferase units were graphed using a $\log_{10}$ scale. Error bars represent standard error of the mean (SEM) of 3 experiments. **(*D*)** Total time required to complete one-step centrifugation (*180K × g c/ph*) and gradient-based fractionation of ribosomes.



Supplemental Figure S2. Time course of the CFE and RSR translation reactions. **(*A*)** Translation reactions were assembled with complete CFE (blue) or with ribosomes purified from CFE via 180K-centrifugation (RSR, magenta). Both reactions were programmed with 400 ng of capped *TAP-Renilla* mRNA reporter; the final reaction volume was 30 μL. The complete CFE reaction contained 60 μg of the total RNA, while the RSR reaction contained 180K-pelleted ribosomes (18 μg of RNA). Total reporter synthesis was measured using a *Renilla* luciferase assay in 4.5 μL reaction aliquots collected at the indicated time points. Graph shows raw values that were not normalized by rRNA. **(*B*)** Equal volumes of each luciferase reaction were analyzed by northern blotting with a 25S rRNA specific probe. The radioactive signal corresponding to full-length 25S rRNA was converted to phosphorimaging units and used as a normalizer of the luminescent values from (A). The resulting RLuc/25S rRNA ratios are presented as both linear and $\log_{10}$ graphs. Representative northern blots are shown below the graphs. Error bars represent standard error of the mean (SEM) of 3 experiments.



Supplemental Figure S3. mRNA dose-dependence of the CFE and RSR translation reactions. Total RNA extracted from the luciferase reactions shown in Figure 4D was analyzed by northern hybridizations with a 25S rRNA-specific probe. Radioactive signal corresponding to the full-length 25S rRNA was converted to phosphorimaging units and used to obtain the RLuc/25S rRNA ratio in each sample. Representative northern blots are shown below the graphs. Error bars represent standard error of the mean (SEM) of 3 experiments.