Supplementary for: A conundrum of r-protein stability: unbalanced stoichiometry of r-proteins during stationary phase in *Escherichia coli*

Supplementary methods

Colony forming unit assay

Escherichia coli MG1655-SILAC (F-, λ-, rph-1, ΔlysA, ΔargA) strain was grown in MOPS medium (1). At mid-log ($A_{600} \approx 1$), 2 mg/ml of unlabeled arginine (Arg0) and lysine (Lys0) were added to the culture. Culture was divided into 8 separate batches and growth was continued for a maximum of 14 days. Samples were taken 3, 24, and 48 hours after start of growth (from here on referred to as mid-log, day one, and day two respectively), and subsequently on days 4, 6, 8, 10, 12, and 14. Samples were plated to LB agar plates to determine the number of colony forming units (CFU).

Dot-plot assay

For RNA dot-blot analysis, 0.5 µg of total RNA from each timepoint were dot-blotted onto a Hybond N+ membrane (Amersham Biosciences, Little Chalfont, UK), using vacuum manifold according to the manufacturer's protocol. Two parallel membranes were prepared, one to be probed with a 5'-labeled 16S RNA-specific probe (5'-GCCAGCGTTCAATCTGAG-3') and another with a 23S RNA-specific probe (5'-GCTTTCTTTAAATGATGGCTGCTT-3'). RNA was UV-crosslinked, and hybridization was performed as follows. Prehybridization was performed at 65°C for 2 h in 30 mL of hybridization solution (5x SSPE, 5x Dennhardt's solution, 0.5% SDS, 0,1 mg/ml Salmon sperm carrier DNA), after which radioactively labeled oligonucleotides were added to the hybridization solution. Hybridization was performed at 45°C for 12 h, after which membranes were washed for 10 min with 2x SSPE and 0.1% SDS at 45°C, and 3 times for 10 min with 1x SSPE and 0.1% SDS at 45°C. Washed membranes were visualized by auto-radiography.

Supplementary figure legends

Figure S1 – Number of viable cells during stationary phase culture. *E.coli* was grown in MOPS medium as described in main text. Cells were plated to LB plates 3, 24, and 48 hours after the start of growth (from here on referred to as mid-log, day one, and day two respectively) and subsequently on days 4, 6, 8, 10, 12, and 14. (A) The number of colony forming units (CFU) was determined. (B) Absorbance at 600 nm (A_{600}) of the culture on given timepoints. Values shown in the figure are the mean of three independent biological experiments with standard deviation (n=3; mean ± SD)

Figure S2 – Ribosome analysis of stationary phase cells using sucrose gradient centrifugation. This figure is related to Figure 2 in the main text. Cells were harvested from the stationary phase over the course of 14 days, lysates were loaded onto 15-25% sucrose gradients and analyzed by ultracentrifugation at 56000g for 16 hours. The ribosome patterns are normalized to 70S peak. The direction of sedimentation is from right to left. Later stages show the accumulation of intermediate particles between the 50S and 30S peaks (red arrows). Early stationary phase 70S peak has a notable shoulder (green asterisk), that is shown as 100S in figure S2. On the top are shown (3 identical copies for comparison) an example of ribosome profile from cells grown to mid-log phase (A_{600}~1.0) (gray background).

Figure S3 – Presence of 100S particles in stationary phase using sucrose gradient centrifugation. This figure is related to Figure 2 in the main text. Cells were harvested from the stationary phase over the course of 14 days. Cells were suspended in B100S buffer (25mM Hepes pH 7.5; 100mM KOAc; 10mM $Mg(OAc)_2$; 1mM DTT; 1mM PMSF) lysates were loaded onto 10-40% sucrose gradients (buffer used in gradients was B100S) and analyzed by ultracentrifugation at 56000g for 16 hours. The direction of sedimentation is from right to left.

Figure S4 – Total RNA content in cells decreases during the stationary phase. This figure is related to Figure 3 in the main text. Samples were collected over the course of 14 days. Cell samples were collected after 3h, 6h ,12, 24h (day 1), 48h (day 2), and subsequently in 48h intervals over the next 12 days. Total RNA was extracted as described in materials and methods. RNA concentration was determined by measuring absorbance at 260 nm and normalized to corresponding value on day 1 (Y-axis). Data was fitted into one-phase decay

model, where black line represents a mean and dotted line 95% confidence interval. Values shown in the figure are the mean of three independent biological experiments with standard deviation (n=3; mean ± SD).

Figure S5 – 16S and 23S rRNA quantification in total RNA. Samples were collected over the course of 14 days. Total RNA was extracted from cells and 16S and 23S rRNA were quantified by dot-plot hybridization (panel B). Shown on panel A are 16S (blue star) and 23S (red circle) rRNA quantity normalized to day 1. Shown on panel C are ratios between 16S and 23S rRNA quantities during stationary phase. Values shown in the figure are the mean of three independent biological experiments with standard deviation (n=3; mean ± SD).

Figure S6 – The r-protein quantity in the 70S ribosomes does not change during the stationary phase. This figure is related to Figure 4 in the main text. Cells were collected over the course of 14 days and 70S ribosomes were isolated using sucrose gradients by centrifugation. 70S ribosomes were mixed in a 1:1 ratio with the reference ribosomes containing medium-heavy labeled arginine and lysine and analyzed using LC-MS/MS. R-protein relative quantity is presented as an (L+H)/M ratio (L+H = Sample; M = Reference). 50S r-protein (L+H)/M ratios are normalized against the average of (L+H)/M ratio of all 50S r-proteins. 30S r-protein (L+H)/M ratios are normalized against the average of (L+H)/M ratio of all 30S r-proteins. The blue box marks ±10% range of (L+H)/M ratio. Values shown in the figure are the means of three independent biological experiments with standard deviation (n=3; mean ± SD).

Figure S7 – bL17, uL22, and bS6 bind non-specifically to ribosomes during isolation. This figure is related to Figure 4 in the main text. Cells were collected after 10 days of growth and 70S ribosomes were isolated using sucrose gradients by centrifugation. 70S ribosomes were sedimented by centrifugation and resuspended in OV-10 buffer. Half of the 70S ribosomes were disassociated into 50S and 30S using sucrose gradients by centrifugation. 70S, 50S, and 30S ribosomes were mixed in a 1:1 molar ratio with the reference 70S ribosomes containing medium-heavily labeled arginine and lysine and analyzed using LC-MS/MS. R-protein relative quantity is presented as an (L+H)/M ratio (L+H = Sample; M = Reference). 50S r-protein (L+H)/M ratios are normalized against the average of (L+H)/M ratio of all 50S r-proteins. 30S r-protein (L+H)/M ratios are normalized against the average of (L+H)/M ratio of all 30S r-

proteins. The blue box marks ±10% range of (L+H)/M ratio. Values shown in the figure are representative to one control experiment. (n=1).

Figure S8 – Individual 50S r-proteins H/M ratio changes in proteome during stationary phase. This figure is related to Figure 5B in the main text. Cells were collected over the course of 14 days, lysed, and total protein from samples was mixed in a 1:1 ratio with total protein from the reference cells containing medium-heavy labeled arginine and lysine. Samples were analyzed using LC-MS/MS. Normalized H/M ratio describes r-proteins relative quantities against day 1. Values shown in the figure are the means of three independent biological experiments with standard deviation (n=3; mean ± SD).

Figure S9 – Individual 30S r-proteins H/M ratio changes in proteome during stationary phase. This figure is related to Figure 5B in the main text. Cells were collected over the course of 14 days, lysed, and total protein from samples was mixed in a 1:1 ratio with total protein from the reference cells containing medium-heavy labeled arginine and lysine. Samples were analyzed using LC-MS/MS. Normalized H/M ratio describes r-proteins relative quantities against day 1. Values shown in the figure are the means of three independent biological experiments with standard deviation (n=3; mean ± SD).

Figure S10 – R-proteins L/M ratio in the proteome during the stationary phase. This figure is related to Figure 5B in the main text. Cells were collected over the course of 14 days, were lysed, and total protein from samples was mixed in a 1:1 ratio with total protein from the reference cells containing medium-heavy labeled arginine and lysine. Samples were analyzed using LC-MS/MS. Heatmap representing r-protein stoichiometry in the total proteome. Normalized L/M ratio describes r-proteins relative quantities against day 1. Values shown in the figure are the median of three independent biological experiments (n=3; median).

Figure S11 – 50S subunits r-protein degradation kinetics. This figure is related to Figure 7 in the main text. Normalized H/M ratio values measured over 14 days were analyzed using non-linear regression. Data was fitted into two models: plateau followed by one phase decay and straight line. The fit of models was compared using Akaike's information criteria (2). Statistics of the analysis are presented in table S6. Shown on the figure are r-proteins with best-fitting models. On Y-axis is the normalized H/M ratio and on X-axis is time in days. The black line

represents best-fit values and the dotted line represents confidence bands (90%). All calculations were based on data obtained from 3 individual biological experiments (n=3). Black circles with error bars shown in the figure are the means of three independent biological experiments with standard deviation (n=3; mean ± SD).

Figure S12 – 30S subunits r-protein degradation kinetics. This figure is related to Figure 7 in the main text. Normalized H/M ratio values measured over 14 days were analyzed using non-linear regression. Data was fitted into two models: plateau followed by one phase decay and straight line. The fit of models was compared using Akaike's information criteria (2). Statistics of the analysis are presented in table S6. Shown on the figure are r-proteins with best-fitting models. On Y-axis is the H/M ratio normalized to early stationary phase (day 1) and on X-axis is time in days. The black line represents best-fit values and the dotted line represents confidence bands (90%). All calculations were based on data obtained from 3 individual biological experiments (n=3). Black circles with error bars shown in the figure are the means of three independent biological experiments with standard deviation (n=3; mean ± SD).

REFERENCES

1. Neidhardt FC, Bloch PL, Smith DF. 1974. Culture medium for enterobacteria. J Bacteriol 119:736-47.
2. Sugiura N. 1978. Further analysts of the data by akaike' s information criterion and the finite corrections. Communications in Statistics - Theory and Methods 7:13-26.