Plasmid pLEPA was made by cloning the lepA gene and its native promoter into the low-copy vector pWSK29 (53), as described previously (7). The level of LepA produced from pLEPA is about twofold higher than that seen in WT cells, based on Western analysis (7). Plasmid pRSGA was constructed in an analogous way by amplifying the rsgA gene from BW25113 genomic DNA using primers rsgA-F (5′-GGATCTGGAGGTTGGGATC6GTCGACC-3′) and rsgA-R (5′-GCCCCGGGTCACCAGGGCATTTGGTTGC-3′) and cloning the resulting DNA fragment into the pWSK29 via Xho I and Xma I restriction sites.

For the LepA SILAC analysis, isogenic strains were constructed from a Lys− Arg− double auxotroph. Mutations ΔlysA::kan (JW2806) (11) and ΔargE::Tn10 (CAG12185) (54) were moved by P1 transduction into parental strain BW25113 [F−, Δ(lac phosphorase-araB)567, ΔlacZ4787::rrnB-3, Δ(rhaD-rhaB)568, rph-1, hsdR514] (11) to generate MRG01. Mutation ΔlepA::cat (4) was then moved by P1 transduction into MRG01 generating MRG02. Empty vector pWSK29 (53) was transformed into MRG01 and MRG02 to generate MRG03 and MRG04, respectively. Plasmid pLEPA was transformed into MRG02 to generate MRG05. MRG03 (WT, wild-type control), MRG04 (M, mutant ΔlepA), and MRG05 (C, complemented ΔlepA::lepA′) were used in the SILAC and SSU rRNA processing experiments. Plasmid pRB34 (4), identical to pRSGA except for the H81A codon change, was transformed into MRG02 to generate MRG102, which was included in the SSU rRNA processing experiments.

For the RsgA SILAC analysis, the kan marker in MRG01 was removed via flanking FRT sites, using pCP20-encoded FLP recombinase, as described (55). Mutation ΔrsgA::kan (JW4122) (11) was then moved by P1 transduction into this strain. These two strains were transformed with either pWSK29 or pRSGA to yield strains MRG06 (WT, control), MRG07 (M, ΔrsgA), and MRG08 (C, ΔrsgA::rsgA′), which were used in the SILAC and SSU rRNA processing experiments.

For growth measurements, prototrophic isogenic strains were constructed in E. coli BW21513. Mutations ΔlysA::kan and ΔlepA::kan (11) were each moved by P1 transduction into BW21513 to generate MRG33 and MRG35, respectively. Resistance markers were removed from these strains by FLP-mediated recombination, generating MRG42 and MRG45, respectively. Mutation ΔlepA::kan was moved into MRG42 to generate the double mutant MRG44. BW21513, MRG42, MRG45, and MRG44 were transformed with pWSK29, pLEPA, or pRSGA to obtain the strains used in growth rate measurements of Table 1.

Analyses of r Protein Composition of Ribosomal Particles. Cells were grown in M9 enriched minimal media plus glucose supplemented with light (unlabeled Arg and Lys; strains MRG03 and MRG06), medium (Arg13C6 and Lys13C6; strains MRG04 and MRG07), or heavy (Arg13C615N and Lys13C615N; strains MRG05 and MRG08) amino acids. In each case, strains were grown overnight in the appropriate M9 media and then diluted 1:100 in 50 mL of the same media. Cells were grown to OD600 ~ 0.5 and rapidly chilled, pelletted, and lysed as previously described (10). Clarified lysate (0.5 mL) was loaded onto an 11 mL 10%–60% sucrose gradient and subjected to ultracentrifugation at 150,000 × g for 4 h to separate ribosome complexes. Gradients were pumped using a syringe-pump system (Brandel) with in-line UV absorbance detector (UA-6, ISCO), and equivalent volume (0.5 mL) fractions were collected as indicated in Fig. S1. Corresponding fractions from WT, M, and C strains were mixed, and proteins were precipitated using 13% (wt/vol) trichloroacetic acid (TCA).

Mass Spectrometry. Protein precipitates were boiled in SDS sample buffer and run on a short 10% (wt/vol) SDS/PAGE gel. Proteins were visualized by colloidal coomassie (56) and digested out of the gel as described (50). Peptide samples were purified by solid phase extraction on C-18 STop And Go Extraction (STAGE) Tips (57) and analyzed by a quadrupole–time of flight mass spectrometer (Impact II; Bruker Daltonics) coupled to an Easy nano LC 1000 HPLC (ThermoFisher Scientific) using an analytical column that was 40–50 cm long, with a 75-μm inner diameter fused silica with an integrated spray tip pulled with P-2000 laser puller (Sutter Instruments) and packed with 1.9 μm diameter Reprosil-Pur C-18-AQ beads (Maisch, www.Dr-Maisch.com). Buffer A consisted of 0.1% aqueous formic acid, and buffer B consisted of 0.1% formic acid and 80% (vol/vol) acetonitrile in water. A standard 90-min peptide separation was done, and the column was washed with 100% buffer B before re-equilibration with buffer A. The Impact II was set to acquire in a data-dependent auto-MS/MS mode with inactive focus fragmenting the 20 most abundant ions (one at the time at a 18-Hz rate) after each full-range scan from m/z 200 to m/z 2,000 at 5 Hz rate. The isolation window for MS/MS was 2–3 depending on the parent ion mass to charge ratio, and the collision energy ranged from 23 to 65 eV depending on ion mass and charge. Parent ions were then excluded from MS/MS for the next 0.4 min and reconsidered if their intensity increased more than five times. Singly charged ions were excluded from fragmentation.

Data Analysis. Analysis of mass spectrometry data was performed using MaxQuant 1.5.1.0. The search was performed against a database comprised of the protein sequences from Uniprot’s E. coli K12 entries plus common contaminants with variable modifications of methionine oxidation, and N-acetylation of the proteins, in addition to the heavy amino acids used for quantitation. Only those peptides exceeding the individually calculated 99% confidence limit (as opposed to the average limit for the whole experiment) were considered as accurately identified. Raw isotope ratios (M/WT or C/WT) for S2–S21 and L1–L35 were normalized to the median value of all SSU or LSU proteins, respectively, in each sample. Isotope ratios were in some cases not obtained, depending on the particular sample and protein. Consequently, the number of measurements used to calculate a given value was sometimes smaller than the number of independent experiments. One-sample Student’s t test was used to evaluate the mean value (m) using the null hypothesis m = 1.0, and P values were calculated using online software (QuickCals, GraphPad).

Growth Rate Measurements. Overnight cultures were diluted 1:100 and grown in 50 mL LB media at 37 °C. Growth was monitored by measuring the turbidity of the culture, and log2 (OD600) values were plotted versus time. The linear portion of the plot was identified and fitted to a linear function, the slope of which defined growth rate (doublings/min), the inverse of doubling time.

Analysis of SSU rRNA Processing. Strains MRG03, MRG04, MRG05, and MRG07 were grown in glucose M9 media supplemented with Lys and Arg. Cells were lysed, and the clarified lysates were subjected to sucrose gradient sedimentation as described above. Ribosomes from selected sucrose gradient fractions (5–8, 13, 14) were precipitated with ethanol, pelletted, and dissolved in 200 μL extraction buffer (0.3 M NaOAc, pH 6.5, 0.5% SDS, 5 mM EDTA). At this point, fractions 13 and 14...
(containing the 70S peak) were pooled. RNA was extracted twice with water-saturated phenol and twice with CHCl₃/isoamyl alcohol (24:1), precipitated with ethanol, and pelleted. RNA pellets were dissolved in water and subjected to denaturing PAGE as described (14). Gels were stained with SYBR-Gold Nucleic Acid Stain (ThermoFisher, Invitrogen) and scanned using a Typhoon FLA 9000 fluorescence imager (GE Healthcare Life Sciences). The relative intensities of bands corresponding to 17S and 16S rRNA were quantified using ImageQuant version 5.2 (Molecular Dynamics), and the percent 17S was calculated, after correcting for the size difference between 16S and 17S, as \[
\frac{17S}{16S + 17S} \times 100%.
\]

**Fig. S1.** Fractionation of cell lysates by sucrose gradient sedimentation. Representative \(A_{254}\) traces of sucrose gradients from mutant \(\Delta lepA\) (A), complemented \(\Delta lepA\) (pLEPA) (B), mutant \(\Delta rsgA\) (C), and complemented \(\Delta rsgA\) (pRSGA) (D) strains. Peaks corresponding to subunits (30S, 50S), monosomes (70S), and polysomes (2x, 3x) are indicated. Arrows denote the top and bottom of the gradient; note, polysomes > 3x are pelleted under the conditions used. Numbers listed below the traces correspond to the fraction numbers discussed throughout the article.
**Fig. S2.** Ribosomal protein composition of LSU particles in the mutant ΔlepA strain. Shown are normalized isotope ratios (mutant versus wild type; M/WT), indicating the relative abundance of each protein (as indicated) in LSU particles contained in fractions 9–16 of the sucrose gradient. Fractions 10–11 and 13–14 encompass the 50S and 70S peaks, respectively (as indicated with braces). Five independent experiments were performed, and the data represent the mean ± SEM. Red and black bars indicate values deemed <1.0 and >1.0, respectively, based on Student’s t test (uncorrected P < 0.05). Striped bars denote cases where only one measurement was obtained. The blue line marks 1.0.

**Fig. S3.** Ribosomal protein composition of SSU particles in the complemented ΔlepA/lepA+ strain. Shown are normalized isotope ratios (complemented versus wild type; C/WT), indicating the relative abundance of each protein (as indicated) in SSU particles contained in fractions 6–16 of the sucrose gradient. Fractions 7–8, 10–11, and 13–14 encompass the 30S, 50S, and 70S peaks, respectively (as indicated with braces). Three independent experiments were performed, and the data represent the mean ± SEM. Red and black bars indicate values deemed <1.0 and >1.0, respectively, based on Student’s t test (uncorrected P < 0.05). Striped bars denote cases where only one measurement was obtained. The blue line marks 1.0.
Fig. S4. Mutation ΔlepA confers a synthetic growth defect in the absence of RsgA. Representative growth curves of strains (as indicated) are shown. Strains were grown in LB media at 37 °C, and turbidity of the culture (OD<sub>600</sub>) was monitored as a function of time.

Fig. S5. Ribosomal protein composition of LSU particles in the mutant ΔrsgA strain. Shown are normalized isotope ratios (mutant versus wild type; M/WT), indicating the relative abundance of each protein (as indicated) in LSU particles contained in fractions 9–18 of the sucrose gradient. Fractions 10–11, 13–14, and 17–18 encompass the 50S, 70S, and disome (2x) peaks, respectively (as indicated with braces). Six independent experiments were performed, and the data represent the mean ± SEM. Red and black bars indicate values deemed <1.0 and >1.0, respectively, based on Student’s t test (uncorrected P < 0.05). Striped bars denote cases where only one measurement was obtained. The blue line marks 1.0. N.D., not determined.
Fig. S6. Ribosomal protein composition of SSU particles in the complemented ΔrsgA/rsgA<sup>+</sup> strain. Shown are normalized isotope ratios (complemented versus wild type; C/WT), indicating the relative abundance of each protein (as indicated) in SSU particles contained in fractions 6–18 of the sucrose gradient. Fractions 7–8, 10–11, 13–14, and 17–18 encompass the 30S, 50S, 70S, and disome (2×) peaks, respectively (as indicated with braces). Four independent experiments were performed, and the data represent the mean ± SEM. Red and black bars indicate values deemed <1.0 and >1.0, respectively, based on Student’s t test (uncorrected P < 0.05). Striped bars denote cases where only one measurement was obtained. The blue line marks 1.0.

Fig. S7. Predicted rates of production of r proteins are similar in the presence and absence of LepA. (A) Relative rates of protein production were estimated from ribosome profiling data (4), by calculating average ribo-seq coverage per codon for each of the indicated r protein genes. (B) ARD values, calculated as ribo-seq coverage divided by RNA-seq coverage (4), for each of the same genes. N.D., not determined; the S17 gene contains the promoter of the downstream spc operon, which complicates the measurement of ARD. Data represent the mean ± SD for control WT (blue), mutant ΔlepA (orange), and complemented ΔlepA (pLEPA) (green) strains. AU, arbitrary unit.
Predicted rates of synthesis of 30S biogenesis factors are similar in the presence and absence of LepA. (A) Relative rates of protein production were estimated from ribosome profiling data (4), by calculating average ribo-seq coverage per codon for each of the assembly factor genes. (B) ARD values, calculated as ribo-seq coverage divided by RNA-seq coverage (4), for each of the same genes. Data represent the mean ± SD for control WT (blue), mutant ΔlepA (orange), and complemented ΔlepA (pLEPA) (green) strains. AU, arbitrary unit.

Model of the 30S subunit of the ribosome in the neighborhood of S21. Ribosomal protein S21 binds the platform domain of the subunit, near the E site, and lines a portion of the 5′ mRNA binding channel, where the SD–ASD helix forms. The image in A is based on a cryo-EM structure of the *E. coli* ribosome [PDB ID code 5AFI (52)]. In this structure, the mRNA (yellow) was unresolved 5′ of the E codon, although this region normally pairs with the ASD (green; 16S nucleotides 1535–1540) during initiation. S21 is shown in orange, as a surface-rendered model. The image in B is based on an X-ray crystal structure of the *Thermus thermophilus* ribosome [PDB ID code 4V4Z (58)], in which the SD–ASD helix is completely modeled. Ribosomes from *T. thermophilus* lack r protein S21. The perspective is identical in the two panels, so the relative positions of the ribosomal components can be readily compared.
Table S1. Amount of precursor 17S rRNA in various ribosomal fractions of the strains analyzed

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Description*</th>
<th>No. 5, pre-30S</th>
<th>No. 6, pre-30S</th>
<th>No. 7, 30S</th>
<th>No. 8, 30S</th>
<th>Nos. 13/14, 70S</th>
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<tr>
<td>MRG03 WT</td>
<td></td>
<td>58.0 ± 5.9 (n = 4)</td>
<td>42.3 ± 2.9 (n = 5)</td>
<td>35.6 ± 3.6 (n = 5)</td>
<td>29.9 ± 3.9 (n = 5)</td>
<td>7.6 ± 2.4 (n = 5)</td>
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<tr>
<td>MRG07 ΔrsgA</td>
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<td>75.3 ± 4.1 (n = 3)</td>
<td>75.1 ± 3.6 (n = 3)</td>
<td>71.7 ± 3.6 (n = 3)</td>
<td>75.5 ± 5.2 (n = 3)</td>
<td>37.2 ± 2.7 (n = 3)</td>
</tr>
<tr>
<td>MRG08 ΔrsgA (pRGSA)</td>
<td></td>
<td>67.2 ± 4.9 (n = 3)</td>
<td>43.1 ± 8.5 (n = 3)</td>
<td>28.9 ± 8.5 (n = 3)</td>
<td>28.9 ± 5.7 (n = 3)</td>
<td>9.3 ± 0.5 (n = 3)</td>
</tr>
<tr>
<td>MRG04 ΔlepA</td>
<td></td>
<td>69.8 ± 2.3 (n = 5)</td>
<td>54.4 ± 2.1 (n = 5)</td>
<td>49.1 ± 4.3 (n = 5)</td>
<td>37.2 ± 5.9 (n = 5)</td>
<td>9.7 ± 3.6 (n = 5)</td>
</tr>
<tr>
<td>MRG05 ΔlepA (pLEPA)</td>
<td></td>
<td>65.9 ± 4.8 (n = 6)</td>
<td>40.5 ± 3.4 (n = 6)</td>
<td>35.2 ± 4.4 (n = 6)</td>
<td>21.0 ± 4.3 (n = 6)</td>
<td>7.2 ± 1.1 (n = 6)</td>
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<tr>
<td>MRG102 ΔlepA (pRB34)</td>
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<td>73.9 ± 1.3 (n = 3)</td>
<td>56.1 ± 0.7 (n = 3)</td>
<td>43.8 ± 2.6 (n = 3)</td>
<td>35.7 ± 2.8 (n = 3)</td>
<td>10.1 ± 0.7 (n = 3)</td>
</tr>
</tbody>
</table>

Data correspond to percent of total SSU rRNA (mean ± SEM), with the number of independent replicas (n) indicated.

*All strains are isogenic and carry either the plasmid indicated or the empty vector.