

SUPPLEMENTARY DATA

Discharging tRNAs: a tug of war between translation and detoxification in *Escherichia coli*

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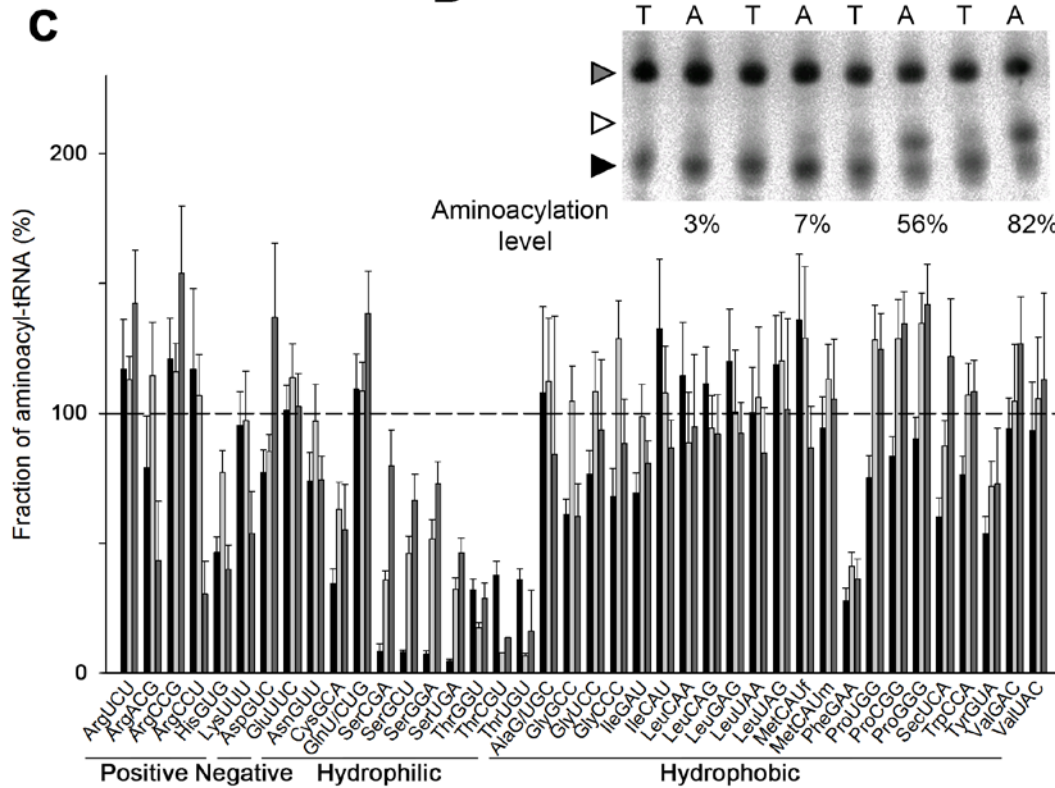
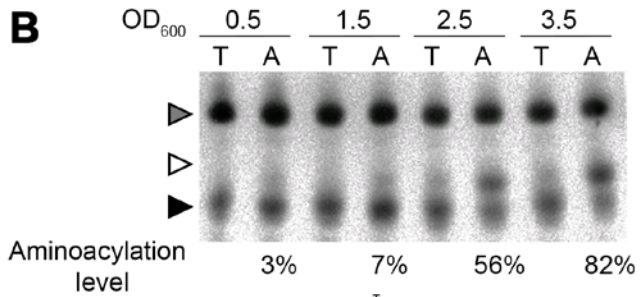
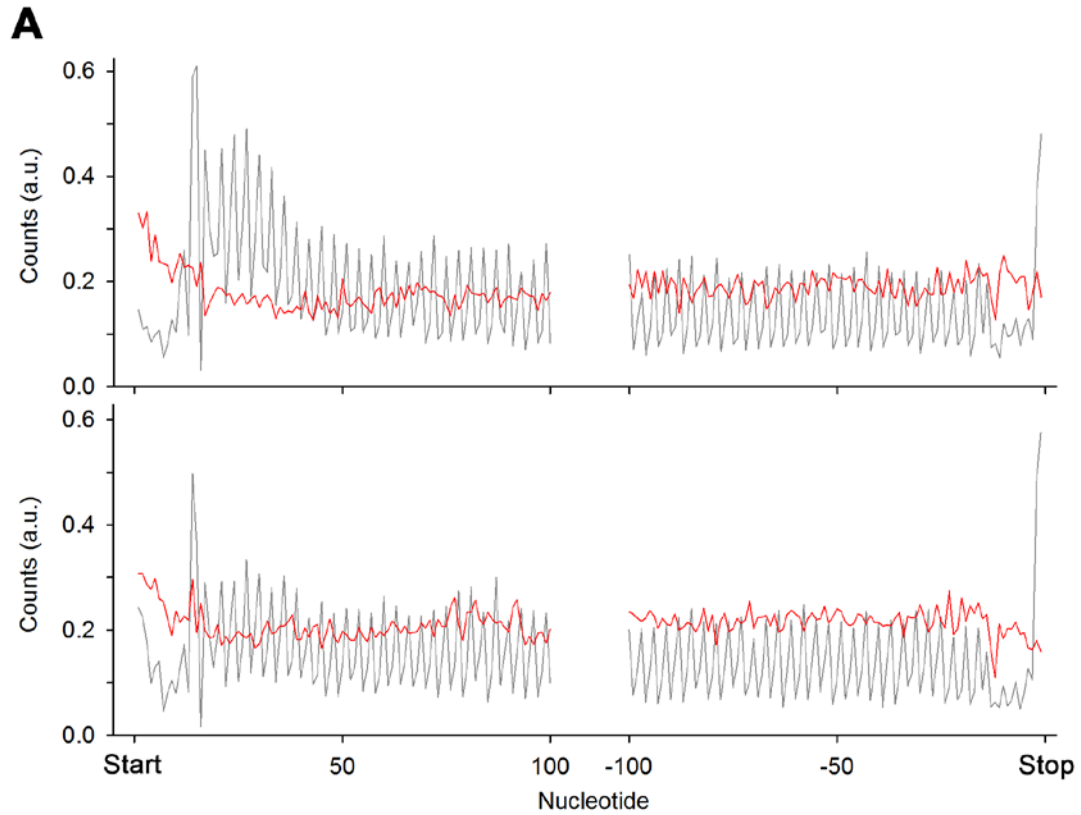
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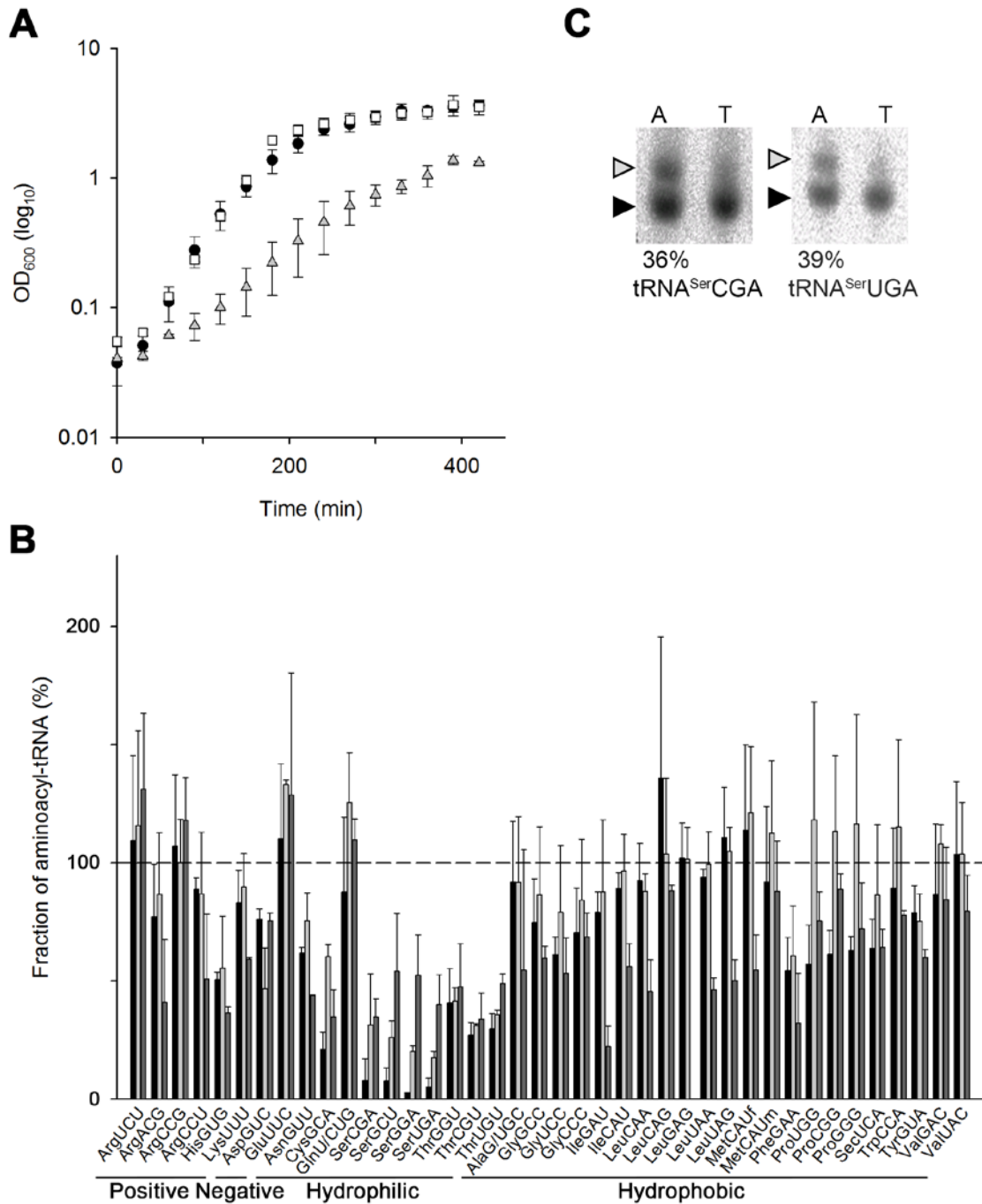
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tRNA isoacceptor. The numbers denote the percentage of the aminoacyl-tRNA from the total (aminoacylated and deacylated) in the corresponding lane. Black and gray arrows indicate the migration positions of deacylated and aminoacylated tRNAs, respectively. tRNA^{Ser}CGA reads UCG codons, tRNA^{Ser}GCU pairs to AGC/U codons and tRNA^{Ser}UGA reads UCA/U/G codons. tRNA^{Asp}GUC pairing to GAC/U, which is nearly 100% charged, is included for comparison. (B) Microarray of aminoacyl-tRNAs from *E. coli* BL21 (DE3) at OD₆₀₀=0.5 grown in LB at 37°C. For more details see the legend to Figure 1A. Data are means ± S.D. of two biological replicates. (C) Aminoacylation levels of all tRNAs^{Ser} in LB medium supplemented with 0.4% (w/v) glucose (dark gray) or 10 mM serine (light gray) and compared to growth in LB medium (black bars, panel A). Data are means ± S.D. of two biological replicates. For more details see the legend to Figure 1A.



Supplementary Figure 2. Read calibrations and aminoacylation level of tRNA isoacceptors in different growth phases. (A) Cumulative plots using 23-26 nt long sequencing reads for all ORFs detected over the threshold of 60 rpm (1). Start denotes the first nucleotide of the start codon; stop designates the last nucleotide of the stop codon. Upper and lower panels represent LB and MM, respectively. Ribosome-protected fragments are in black and mRNA reads from the RNA-seq are in red. (B) Northern blot analysis of the charging levels of tRNA^{Ser}GCU pairing to the UCG codon at different ODs of *E. coli* MC4100 grown in LB at 37 °C. A denotes aminoacyl-tRNA and T the total deacylated tRNAs. The numbers under the blot denote the percentage of the aminoacyl-tRNA from the total (aminoacylated and deacylated) in the corresponding lane. Specific ³²P-labeled DNA probes were used to visualize 5S rRNA and tRNA^{Ser}GCU. Dark gray, white and black arrows indicate the position of 5S rRNA, aminoacyl-tRNA and deacylated tRNA, respectively. (C) Microarray of aminoacyl-tRNAs from *E. coli* MC4100 grown in LB medium to OD₆₀₀=1.5 (black), OD₆₀₀=2.5 (light gray) and OD₆₀₀=3.5 (dark gray). Data are means ± S.D. For more details see the legend to Figure 1A.



Supplementary Figure 3. Growth curves and aminoacylation level of tRNA isoacceptors in different media. (A) Growth curves of cells grown in LB (black circle), MM (grey triangle) and MM+AA (open square). Data are means \pm S.D. of two biological replicates. (B) Microarray of aminoacyl-tRNAs from *E. coli* MC4100 grown in LB medium (black), MM+AA (light gray) and MM (dark gray) to OD₆₀₀=0.5. Data are means \pm S.D. of two-three biological replicates. For more details see the legend to Figure 1A. (C) Northern blot analysis of the charging levels of

tRNA^{Ser}CGA and tRNA^{Ser}UGA from *E. coli* MC4100 grown in MM+AA (OD₆₀₀ =0.5) at 37 °C. tRNA^{Ser}CGA reads UCG codons and tRNA^{Ser}UGA reads UCA/U/G codons. A denotes aminoacyl-tRNA and T the total deacylated tRNAs. The numbers denote the percentage of the aminoacyl-tRNA from the total (aminoacylated and deacylated) in the corresponding lane. Black and gray arrows indicate the positions of deacylated and aminoacylated tRNAs, respectively.

SUPPLEMENTARY REREFENCES

1. Del Campo, C., Bartholomaeus, A., Fedyunin, I. and Ignatova, Z. (2015) Secondary Structure across the Bacterial Transcriptome Reveals Versatile Roles in mRNA Regulation and Function. *PLoS Genet*, **11**, e1005613.
2. Bjork, G.R. and Hagervall, T.G. (2014) Transfer RNA Modification: Presence, Synthesis, and Function. *EcoSal Plus*, **6**.
3. Murphy, F.V.t. and Ramakrishnan, V. (2004) Structure of a purine-purine wobble base pair in the decoding center of the ribosome. *Nat Struct Mol Biol*, **11**, 1251-1252.
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Supplementary Table 1: Summary of the *E.coli* tRNAs with the corresponding codon they recognize. Modified nucleosides at the wobbling position in the anticodon are underlined.

| tRNA anticodon (5'-3') | Recognized codon (5'-3') | tRNA name |
|---|--------------------------|-----------------------------|
| tRNA ^{Ala} <u>UGC</u> , GGC ⁴ | GCU, GCA, GCG, GCC | Ala1B, Ala2 ¹ |
| tRNA ^{Arg} <u>ACG</u> ⁵ | CGU, CGC, CGA | Arg2 |
| tRNA ^{Arg} CCG | CGG | Arg3 |
| tRNA ^{Arg} UCU | AGA | Arg4 |
| tRNA ^{Arg} CCU | AGG | Arg5 |
| tRNA ^{Asn} GUU | AAC, AAU | Asn |
| tRNA ^{Asp} GUC | GAC, GAU | Asp1 |
| tRNA ^{Cys} GCA | UGC, UGU | Cys |
| tRNA ^{Gln} UUG, CUG | CAA, CAG | Gln1, Gln2 ¹ |
| tRNA ^{Glu} UUC | GAA, GAG | Glu2 |
| tRNA ^{Gly} CCC | GGG | Gly1 |
| tRNA ^{Gly} UCC | GGA, GGG | Gly2 |
| tRNA ^{Gly} GCC | GGC, GGU | Gly3 |
| tRNA ^{His} GUG | CAC, CAU | His |
| tRNA ^{Ile} GAU | AUC, AUU | Ile1 |
| tRNA ^{Ile} <u>CAU</u> ⁶ | AUA | Ile2 |
| tRNA ^{Leu} CAG | CUG | Leu1 |
| tRNA ^{Leu} GAG | CUC, CUU | Leu2 |
| tRNA ^{Leu} <u>UAG</u> ⁴ | CUA, CUU, CUG | Leu3 |
| tRNA ^{Leu} CAA | UUG | Leu4 |
| tRNA ^{Leu} UAA | UUA, UUG | Leu5 |
| tRNA ^{Lys} UUU | AAA, AAG | Lys |
| tRNA ^{Met} CAU | AUG | Metf1, Metf2 ^{1,2} |
| tRNA ^{MEt} CAU | AUG | Metm ³ |
| tRNA ^{Phe} GAA | UUC, UUU | Phe |
| tRNA ^{Pro} CGG | CCG | Pro1 |
| tRNA ^{Pro} GGG | CCC, CCU | Pro2 |
| tRNA ^{Pro} <u>UGG</u> ⁴ | CCA, CCU, CCG | Pro3 |
| tRNA ^{Sec} UCA | UGA | Sec |
| tRNA ^{Ser} <u>UGA</u> ⁴ | UCA, UCU, UCG | Ser1 |
| tRNA ^{Ser} CGA | UCG | Ser2 |
| tRNA ^{Ser} GCU | AGC, AGU | Ser3 |
| tRNA ^{Ser} GGA | UCC, UCU | Ser5 |
| tRNA ^{Thr} GGU | ACC, ACU | Thr1, Thr3 ¹ |
| tRNA ^{Thr} CGU | ACG | Thr2 |
| tRNA ^{Thr} <u>UGU</u> ⁴ | ACA, ACU, ACG | Thr4 |
| tRNA ^{Trp} CCA | UGG | Trp |
| tRNA ^{Tyr} GUA | UAC, UAU | Tyr1, Tyr2 ¹ |
| tRNA ^{Val} <u>UAC</u> ⁴ | GUA, GUG, GUU | Val1 |

| | | |
|-------------------------|----------|---------------------------|
| tRNA ^{Val} GAC | GUC, GUU | Val2A, Val2B ¹ |
|-------------------------|----------|---------------------------|

¹Note that the probe on the array recognizes two tRNAs.

²Metf denotes initiator tRNA.

³Metm denotes elongator tRNA.

⁴(m)cmo⁵U at U34 of a tRNA enables base pairing with U, A and G at the third positions of codons (2,3)

⁵Inosine enables base pairing with U, C and A at the third positions of codons (3,4)

⁶k²C enables pairing with A at the third positions of (3,4)

Supplementary Table 2. Intracellular amino acids concentration in exponentially growing *E. coli* MC4100. Concentrations are mean of two biological replicates \pm S.D. n.d., not determined.

| Amino acid | Concentration, mM | |
|------------|--------------------|-----------------|
| | MM | MM+AA |
| Ala | 0.027 \pm 0.005 | 1.21 \pm 0.53 |
| Asn | 0.002 \pm 0.0004 | 0.32 \pm 0.01 |
| Gln | n.d. | 0.21 \pm 0.01 |
| Gly | 0.062 \pm 0.005 | 0.36 \pm 0.05 |
| His | 0.008 \pm 0.002 | 0.13 \pm 0.03 |
| Ile | 0.009 \pm 0.006 | 0.29 \pm 0.01 |
| Lys | 0.024 \pm 0.021 | 0.25 \pm 0.03 |
| Phe | 0.012 \pm 0.005 | 0.27 \pm 0.01 |
| Ser | 0.057 \pm 0.005 | 4.00 \pm 0.30 |
| Thr | 0.008 \pm 0.006 | 0.34 \pm 0.01 |
| Trp | 0.065 \pm 0.005 | 0.77 \pm 0.03 |
| Tyr | 0.011 \pm 0.004 | 0.21 \pm 0.01 |
| Val | 0.185 \pm 0.027 | 3.00 \pm 0.63 |

Supplementary Table 3: Alterations of the onset of exponential growth by some amino acids added to MM. The duration of the lag phase was measured from the growth curves in Figure 3. n.t., not tested; g.i., growth inhibition. Gray shadowed cells represent the concentration of each amino acid in MM+AA (see also Materials and Methods section).

| Amino acid | tRNA | Aminoacylation level (%) in: | | | Lag phase duration, h | | | | | | | | | | |
|------------|--------|------------------------------|-----------|-----------|---|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| | | LB | MM+AA | MM | Concentration of the corresponding amino acid in the medium, mM | | | | | | | | | | |
| | | | | | 0 | 0.05 | 0.1 | 0.2 | 0.4 | 0.5 | 1 | 5 | 10 | 15 | 30 |
| Ser | SerCGA | 8 ± 9.3 | 31 ± 21.8 | 35 ± 7.7 | 3.6 | <i>n.t.</i> | <i>n.t.</i> | <i>n.t.</i> | <i>n.t.</i> | 13.6 | <i>g.i.</i> | <i>g.i.</i> | <i>g.i.</i> | <i>g.i.</i> | <i>g.i.</i> |
| | SerGCU | 8 ± 5.5 | 26 ± 6.9 | 54 ± 24.4 | | | | | | | | | | | |
| | SerGGA | 3 ± 0.2 | 20 ± 2.4 | 52 ± 17.2 | | | | | | | | | | | |
| | SerUGA | 5 ± 3.8 | 18 ± 2.7 | 40 ± 12.4 | | | | | | | | | | | |
| Thr | ThrGGU | 41 ± 14.4 | 41 ± 5.6 | 48 ± 1.8 | 3.1 | <i>n.t.</i> | <i>n.t.</i> | 3.6 | 3.6 | 3.6 | 4.1 | 8.8 | 14.2 | 17.5 | 18.4 |
| | ThrCGU | 27 ± 5.4 | 31 ± 0.8 | 34 ± 1.1 | | | | | | | | | | | |
| | ThrUGU | 30 ± 6.5 | 36 ± 1.9 | 49 ± 4.2 | | | | | | | | | | | |
| His | HisGUG | 50 ± 3.2 | 55 ± 22 | 36 ± 2.6 | 3.1 | <i>n.t.</i> | 3.9 | 3.5 | <i>n.t.</i> | 3.9 | 4.8 | 8.5 | 12.1 | 15 | 19.4 |
| Cys | CysGCA | 21 ± 7.3 | 60 ± 5.2 | 35 ± 11.5 | 3.1 | 10.9 | 21.8 | <i>n.t.</i> | <i>n.t.</i> | <i>g.i.</i> | <i>g.i.</i> | <i>g.i.</i> | <i>g.i.</i> | <i>g.i.</i> | <i>g.i.</i> |