Figures and figure supplements

Organisms with alternative genetic codes resolve unassigned codons via mistranslation and ribosomal rescue

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Figure 1. A UAG-ending transcript in the genomically recoded organism (GRO) may produce proteins with multiple differing C-termini. (A) Unassigned codons arise when either the cognate tRNA or release factor recognizing a codon are removed. (B) Since the GRO lacks Release Factor 1 (RF1), ribosomal stalling at the UAG codons results in three possible fates for the nascent protein (blue): (1) suppression of the codon by a near-cognate or suppressor tRNA (yellow) and continued translation, (2) frameshifting of bases along the mRNA transcript into a new reading frame and continued translation (purple), or (3) ribosomal rescue by the ssrA-encoded tmRNA, ArfA, or ArfB proteins. If ribosomal rescue occurs via tmRNA, the resulting protein is tagged with a peptide sequence (red) for degradation, while rescue via ArfA or ArfB results in release of peptide without C-terminal modification.

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Figure 2. UAG codons in the genomically recoded organism elicit suppression, frameshifting, and tagging for degradation by the tmRNA. (A) Schematic of the GFP construct with a C-terminal 6x-His tag and a UAG stop codon, showing 102 nucleotides downstream of the UAG codon and the positions of other stop codons in the downstream tail. (B) Peptides identified from the C-terminus of a UAG-ending GFP construct expressed in the GRO (using libraries detailed in Supplementary file 3 and 4). Purified GFP protein was digested with trypsin, processed via MS/MS, and the resulting data were computationally searched using libraries encoding all possible suppressors and all possible subsequent reading frames. Peptides are mapped to the C-terminus of the original GFP construct and grouped by reading frame, with the number of bases skipped listed in the left column. Green text represents GFP, blue text represents the C-terminal 6x-His tag and unframedshifted readthrough, orange text represents the position of a UAG stop codon, purple text represents frameshifted readthrough, and red text represents the tmRNA tag. Black dashes represent ribosomal frameshifts (Figure 2—source datas 1 and 2). (C) MS-MS spectra for two peptides: the C-terminus of GFP with the appended degradation tag (LEHHHHHHAANDENYALDD) and the C-terminus of GFP demonstrating a +10 base skip in translation (LEHHHHHHGDPMVR). The other spectra validated from UAG-GFP expressing GRO.AA are shown in Supplementary file 2.

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Figure 3. Deletion of both ssrA and arfB restores protein production in the genomically recoded organism. (A) Comparison of doubling times for WT and GRO strains carrying listed deletions with and without GFP induction. Error bars show standard deviation centered at mean, n = 3; data were analyzed using Source code 1 (Figure 3—source datas 1 and 2). (B) Change in maximum optical density at 600 nm (OD$_{600}$) due to expression of UAG-GFP or UAA-GFP in wild-type (WT) and GRO strains carrying listed deletions. Error bars show standard deviation centered at mean, n = 3 (Figure 3—source datas 1 and 2). (C) Quantification of GFP abundance per 1 mL of cells at OD$_{600}$ of 2.5 via western blot from biological replicates of indicated strains (Figure 3—source datas 3–6). Error bars show standard deviation centered at mean, n = 3 (Figure 3—source datas 3–5). See Figure 3—figure supplement 1 for linear calibration curves used to quantify GFP abundance for each replicate experiment. Image of representative western blot is below the graph. p-values are calculated in relation to the GRO containing the UAG-ending GFP (GRO – UAG) and are as follows: * is $p \leq 0.05$, ** is $p \leq 0.01$, *** is $p \leq 0.001$, and **** is $p \leq 0.0001$. DOI: https://doi.org/10.7554/eLife.34878.009
**Calibration Curve:**
1 to 10 ng/lane

- Intensity (A.U.)
  - 0
  - 2
  - 4
  - 6
  - 8
  - 1x10^7
- Protein loaded (ng)
  - 0
  - 5
  - 10

**Calibration Curve:**
10 to 100 ng/lane

- Intensity (A.U.)
  - 0
  - 2
  - 4
  - 6
  - 8
  - 1x10^7
- Protein loaded (ng)
  - 0
  - 50
  - 100

**Figure 3—figure supplement 1.** Calibration curves used for quantification of GFP yields, as represented in Figure 3C, using GFP samples of known concentration. Replicate 1 corresponds to the western blot shown in Figure 3—source data 3; Replicate 2 corresponds to the western blot shown in Figure 3—source data 4; Replicate 3 corresponds to the western blot shown in Figure 3—source data 5.

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Figure 4. Deleting ssrA restores propagation of both viruses and conjugative plasmids in the genomically recoded organism. (A) Percent transfer of conjugative plasmid RK2 from a wild-type donor into wild-type (WT), GRO, or GRO with designated deletions (KO) as recipients. (Figure 4—source Ma et al. eLife 2018;7:e34878. DOI: https://doi.org/10.7554/eLife.34878)
Figure 4 continued

Data are obtained from technical triplicates generated from a single biological sample. (B) Percent increase in doubling time for strains carrying plasmid RK2 compared to strains lacking RK2 (Figure 4—source data 2 and 3). (C) Number of conjugation events for conjugative plasmid F from wild-type, GRO, or GRO with designated gene deletions as donors to a wild-type recipient (Figure 4—source data 4). Data are obtained from technical triplicates generated from a single biological sample. (D) Relative titer on wild-type, GRO, and GRO with designated deletions of phage λ (Figure 4—source data 5). Error bars show standard deviation centered at mean, n = 3. p-values are calculated in relation to the GRO condition and are as follows: * is p≤0.05, ** is p≤0.01, *** is p≤0.001, and **** is p≤0.0001. (E) Effects of sequential deletions of ribosomal rescue mechanisms on conjugative plasmid transfer efficiency. (F) Effects of sequential deletions of ribosomal rescue mechanisms on viral susceptibility.

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