Figures and figure supplements

tRNA genes rapidly change in evolution to meet novel translational demands

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Figure 1. The growth defect associated with deletion of a singleton tRNA gene was rapidly rescued during the lab-evolution experiment. (A) Growth curve measurements of wild-type (WT) (green), ΔtRNA^[Arg]^[CCU] (blue) and the evolved deletion (red) are shown in optical density (OD) values over time during continuous growth on rich medium at 30°C. (B) The mutation that was found to recover the deletion phenotype in Figure 1. Continued on next page
Figure 1. Continued

The growth of MutΔtRNAArgCCU carrying the chimeric tRNA compared to wild-type (WT) under different conditions. (A) The sequence of the chimeric tRNA is drawn showing the scaffold of tRNAArgUCU with the mutated CCU anticodon. The anticodon triplet is marked with black circles. The evolved mutation is marked with a red circle. All 20 nucleotide differences between tRNAArgUCU and tRNAArgCCU are marked with blue background, next to which, in green letters, the original nucleotide of tRNAArgCCU are written. (B) Growth curve measurements of WT (green) and of MutΔtRNAArgCCU (magenta) are shown in OD_{600} values over time during continuous growth.

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Figure 2—figure supplement 1. Quadruple deletion of tRNA<sup>Ser<sub>GCU</sub></sup> is lethal. We perturbed the tRNA pool in a wild-type strain by deletion of an entire serine tRNA family. Here, the supply of tRNA<sup>Ser<sub>GCU</sub></sup> was eliminated by deletion of all four identical gene copies of this family located on chromosomes IV, VI, XII and X. A complete deletion of this gene family is lethal, indicating that the tRNA<sup>Ser<sub>GCU</sub></sup> is essential in <i>Saccharomyces cerevisiae</i>. To validate that the lethality is indeed due to the deletion of the tRNA<sup>Ser<sub>GCU</sub></sup> genes and not due to an unintentional perturbation of other putative genetic features in the vicinity of the deleted tRNA<sup>Ser<sub>GCU</sub></sup> copies, we introduced a plasmid with the tRNA<sup>Ser<sub>GCU</sub></sup> gene. As expected, the quadruple deletion strain was viable when supplemented with a plasmid carrying the tRNA<sup>Ser<sub>GCU</sub></sup> gene. (A and C) BY384 and tRNA<sup>Ser<sub>GCU</sub></sup> quadruple deletion strains, respectively, with a plasmid harboring a tRNA<sup>Ser<sub>GCU</sub></sup> gene grown on yeast extract/peptone/dextrose (YPD) plates. (B and D) BY384 and tRNA<sup>Ser<sub>GCU</sub></sup> quadruple deletion strains, respectively, grown on liquid medium that contains uracil (YPD) to allow the growth of cells that lost the plasmid and then plated on 5-fluoro-orotic acid (5-FOA) plates (see ‘Materials and methods’). As expected, normal size colonies were only observed in A, B and C. This means that the quadruple deletion strain with the plasmid could not lose the tRNA gene because of its essentiality.

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Figure 2—figure supplement 2. A chimeric serine tRNA can rescue the lethality of the quadruple deletion. Here, rather than inserting a plasmid with the original tRNA^Ser\_GCU gene, we complemented the tRNA^Ser\_GCU family deletion strain with a plasmid containing a chimeric serine tRNA with a GCU anticodon. The chimeric tRNA has an alternative scaffold derived from a different serine tRNA, tRNA^Ser\_CGA, that differs in 22 positions from the deleted serine tRNA family. This strain formed normal size colonies on a yeast extract/peptone/dextrose (YPD) plate.

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Figure 3. Anticodon switching is a widespread phenomenon in nature. (A) Number of species with at least one tRNA switching event in each domain of life. (B) The anticodon UUC convergently evolved in Mus musculus. A maximum likelihood phylogeny of tRNA sequences in M. musculus that decode glutamic acid (Glu) codons. Branch lengths express average nucleotide substitutions per site. Decimals on internal branches express branch support. (C) A comparison of nucleotide sequences for glutamic acid tRNA genes in M. musculus with anticodon UUC (top, tRNA1547 and tRNA359), ‘switched’ UUC tRNAs (middle, tRNA286 and tRNA754), and CUC tRNAs (bottom, tRNA1002, tRNA745, tRNA303, tRNA999, tRNA996 tRNA709, tRNA1001, tRNA1912 and tRNA81). The anticodon triplet is boxed in gray. Red vertical bars indicate differences between sequences. (D) The anticodon UAC convergently evolved in Homo sapiens. A maximum likelihood phylogeny of tRNA sequences in H. sapiens encoding for valine (Val) is shown. (E) A comparison of nucleotide sequences for H. sapiens tRNAs with anticodons UAC (top, tRNA6), a ‘switched’ UAC tRNA (middle, tRNA40), and an AAC tRNA (bottom, tRNA136). The number of genes is according to the tRNA database.

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Figure 3—figure supplement 1. A comparison of discrepancy proportions at the anticodon triplet vs control triplets. Six control triplets were chosen; their locations are marked on a tRNA structure. (A–F) A comparison of the proportion of switched tRNAs for each species when the anticodon was masked versus the proportion of alternative discrepancies when the control triplet was masked. Each point corresponds to one species. A tRNA was identified as ‘switched’ if its nucleotide sequence phylogenetically clustered with other tRNAs with dissimilar mask triplets (see ‘Materials and methods’). DOI: 10.7554/eLife.01339.009
**Figure 4.** WTmultiCCU experiences a growth defect compared to WTmultiUCU and demonstrates higher levels of misfolded proteins. (A) Growth curve measurements of WTmultiControl (blue), WTmultiUCU (brown) and WTmultiCCU (khaki) are shown in optical density (OD) values over time during continuous growth. The WTmultiCCU strain carrying a high copy number plasmid harboring tRNA^Arg^{CCU} demonstrates slower growth compared to cells with an empty plasmid or with a tRNA^Arg^{UCU} plasmid that is mainly characterized by a longer growth delay (lag phase). (B) A demonstration of a WTmultiCCU cell in which the mCherry-Von Hippel–Lindau (VHL) proteins appear with a punctum phenotype when the protein quality control machinery is saturated with misfolded proteins. (C) A demonstration WTmultiUCU cell in which the quality control machinery is not occupied with other proteins; mCherry-VHL is localized to the cytosol. (D) WTmultiCCU, WTmultiUCU and WTmultiControl were transformed with a VHL-mCherry containing plasmid and visualized under the microscope; 1000 cells per strain were counted for either cytosolic or punctum localization of the VHL protein. The fold change in the number of cells containing puncta was then deduced by normalization to the WTmultiControl population. The 95% confidence interval is indicated. (E) The mRNA fold change of six representative heat-shock genes measured by real-time quantitative PCR (RT-qPCR). Presented values are the mean of two biological repetitions ± SEM. The significance of the fold change differences was examined using a t test, with *p<0.001 or **p<0.0001. DOI: 10.7554/eLife.01339.010
Figure 4—figure supplement 1. Multiple copies of rare tRNA\textsuperscript{Ser}\textsubscript{CGA} gene are deleterious compared to abundant tRNA\textsuperscript{Ser}\textsubscript{AGA}. Growth curve measurements of WTmultiControl (blue), WTmultiAGA (brown) and WTmultiCGA (khaki) are shown in optical density (OD) values over time during continuous growth on rich medium at 30°C. The WTmultiCGA strain carrying a high copy number plasmid harboring tRNA\textsuperscript{Ser}\textsubscript{CGA} demonstrates slower growth compared to cells with an empty plasmid or with a tRNA\textsuperscript{Ser}\textsubscript{AGA} plasmid.

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Figure 4—figure supplement 2. Multiple copies of the rare tRNA\textsuperscript{Gln,CUG} gene are deleterious compared to abundant tRNA\textsuperscript{Gln,UUG}. Growth curve measurements of WT\textit{multiControl} (blue), WT\textit{multiUUG} (brown) and WT\textit{multiCUG} (khaki) are shown in optical density (OD) values over time during continuous growth on rich medium at 30°C. The WT\textit{multiCUG} strain carrying a high copy number plasmid harboring tRNA\textsuperscript{Gln,CUG} demonstrates slower growth compared to cells with an empty plasmid or with a tRNA\textsuperscript{Gln,UUG} plasmid.

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Figure 4—figure supplement 3. Addition of low copy number tRNA^{Arg}_{CCU} is deleterious compared to low copy number tRNA^{Arg}_{UCU} when grown in heat. We created two strains harboring low copy number (CEN) plasmids carrying either tRNA^{Arg}_{UCU} or tRNA^{Arg}_{CCU} in addition to the endogenous copy. We termed these strains WTextraUCU and WTextraCCU, respectively. Growth curve measurements of WTextraUCU (green) and WTextraCCU (red) are shown in optical density (OD) values over time during continuous growth on rich medium at 39°C. Cells with the centromeric tRNA^{Arg}_{CCU} plasmid showed a modest growth defect compared to cells with the centromeric tRNA^{Arg}_{UCU} plasmid.

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