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Controlling orthogonal ribosome subunit interactions enables evolution of new function

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Titles and legends for Supplementary Figures and Supplementary Data Tables.

Supplementary Figure 1. Full gels for Figures. Full gels are shown. The Figure number that the gels correspond to is indicated, the molecular size standards are annotated and the area shown in the relevant Figure is indicated by a red outline.

Supplementary Data 1. Synthetic DNA sequences used in this study. The sequences of all primers grouped by their purpose.

Supplementary Data 2. Activity of O-stapled ribosomes and their MS2 variant. *In vivo* ribosome activities (mean of n=5) were measured from GFP production from an orthogonal sfGFP150TAG (O-sfGFP150TAG) reporter in cells expressing an intact or MS2-tagged linker length variant of O-ribo(h44H101) and the *M. mazei* pyrrolysyl-tRNA synthetase/tRNA_{CUA} (encoded by PylS/pylT) in the presence of 1 mM BocK. GFP fluorescence was normalized to that produced from a non-stapled O-ribosome.

Supplementary Data 3. In vitro translation of T7-O-GFP in S30 extracts containing O-stapled ribosome variants. The data for each O-stapled ribosome is normalized by the activity of the same S30 ribosome translating T7-WT-GFP. The number of biological replicates for each measurement performed for each O-stapled ribosome variant is indicated in the table.

Supplementary Data 4. In vitro translation of T7-O-GFP in S30 extracts containing O-stapled ribosome variants in the presence of 10 μ M spectinomycin and 50 μ M erythromycin (“+ES”). The +ES data for each O-stapled ribosome is normalized by the activity of that ribosome in the absence of spectinomycin and erythromycin. The number of biological replicates for each measurement performed for each O-stapled ribosome variant is indicated in the table.

Supplementary Data 5. Whole genome sequence of Δ 6-d2d8 parental strain.

Supplementary Data 6. Whole genome sequence of Δ 6-d2d8 A2 strain.

Supplementary Data 7. Whole genome sequence of Δ 6-d2d8 D2 strain.

Supplementary Data 8. Whole genome sequence of Δ 6-d2d8 D4 strain.

Supplementary Data 9. Whole genome sequence of Δ 6-d2d8 D8 strain.

Supplementary Data 10. Whole genome sequence of Δ 6-d2d8 E10 strain.

Supplementary Data 11. Comparison of the genomes for Δ 6-d2d8 derived strains. Genes with mutations identified in at least two independently mutated and fast-growing Δ 6-d2d8 strains, but not the non-mutagenized control strain A2. Whole-genome sequencing of four independently mutagenized and passaged strains revealed on average 303.5 nt mutations per strain in comparison to parental Δ 6-d2d8 strain (D2: 388, D4: 233, D8: 370, E10: 223); in contrast, a passaged strain that was not subject to mutagenesis contained 6 mutations. Comparing non-silent mutations between strains revealed 34 genes that were mutated in at least two independently evolved strains but not mutated in the passaged strain. For twenty operons, we found multiple genes within the operon mutated within at least three of the four

strains. And we found mutations in each of three operons (nanTRAM, ydiFNYSJ, hisHQBM) across the four evolved strains. Green cells mark strains with at least one mutation in the listed gene. The identification of genes and operons that are mutated in multiple independent strains, but not in the control strain, implicates these genes in the observed increases in growth rate.

Supplementary Data 12. O-stapled ribosomes selected for the intrinsic ability to translate polyproline stretches. Mutations in the 23S rRNA after selections on the peptidyl transferase center (PTC) and the exit tunnel libraries. Nucleotide positions within the PTC library are colored blue, while positions corresponding to the exit tunnel library are colored white. Mutant O-d2d8 ribosomes selected from the PTC library or the exit tunnel library are marked with PTC or ET respectively. Matched colors in the PTC mutation region show which parental d2d8PTC ribosome the subsequent d2d8ET was derived from.

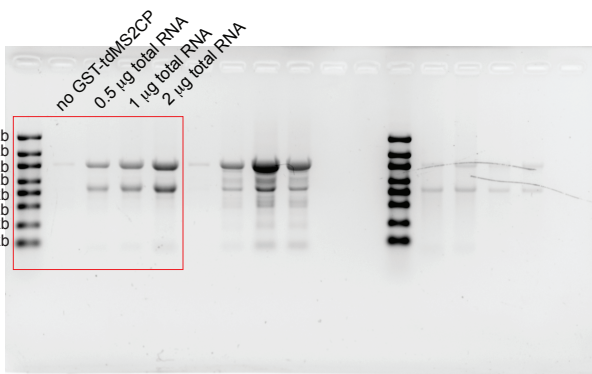
Supplementary note

Inter-ribosome distance in cells: Typical *E. coli* cells have dimensions of $\sim 1 \times 1 \times 4 \mu\text{m}^1$, and typical ribosome numbers in the log phase is $\sim 50000\text{-}70000^2$. If the ribosomes were uniformly distributed in the cytoplasm ($\sim 50\%$ of the cell volume³), ribosomes would make approximately a $30 \times 30 \times 60$ lattice within the cells. Therefore, the points that correspond to ribosome centroids would be 33 nm away from the nearest neighboring ribosomes. As the ribosome itself is a particle of radius ~ 10 nm, the distance from one ribosome edge to the other ribosome edge is ~ 13 nm for its nearest neighbors. This inter-ribosome distance is in the same order as the length of the original staple of O-ribo(h44H101), which was already designed to be significantly shorter than the inter-ribosome distance i.e. ~ 5 nm (2.8 Å rise per base pair in duplex RNA, 17 base pairs in the hinge).

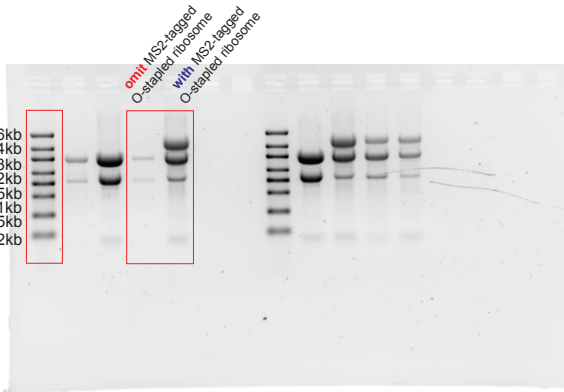
References

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- 2 Bremer, H. & Dennis, P. P. Modulation of Chemical Composition and Other Parameters of the Cell at Different Exponential Growth Rates. *EcoSal Plus* **3**, doi:10.1128/ecosal.5.2.3 (2008).
- 3 Wingreen, N. S. & Huang, K. C. Physics of Intracellular Organization in Bacteria. *Annu Rev Microbiol* **69**, 361-379, doi:10.1146/annurev-micro-091014-104313 (2015).

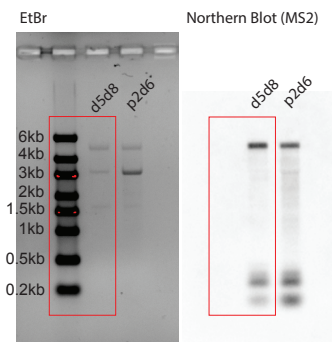
Extended data figure 2c



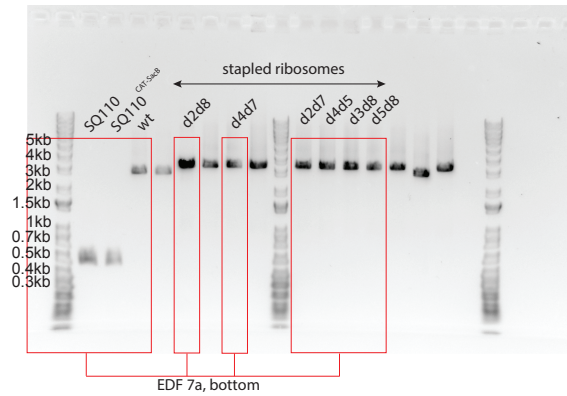
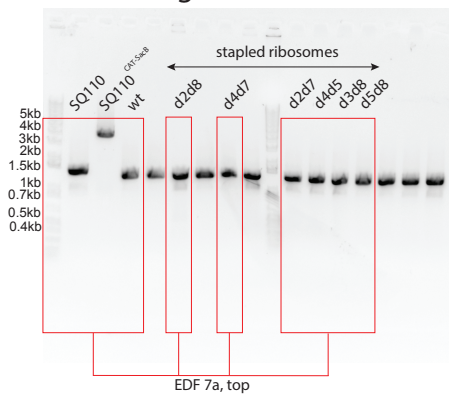
Extended data figure 2d



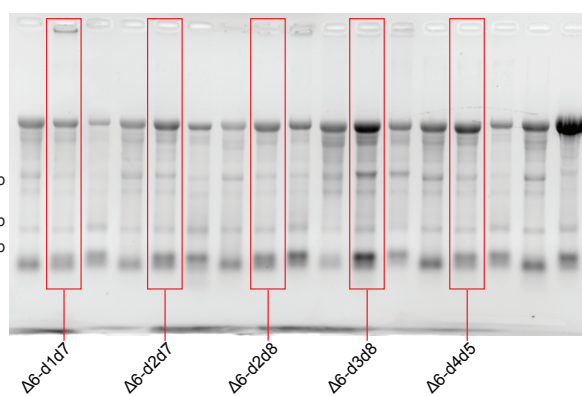
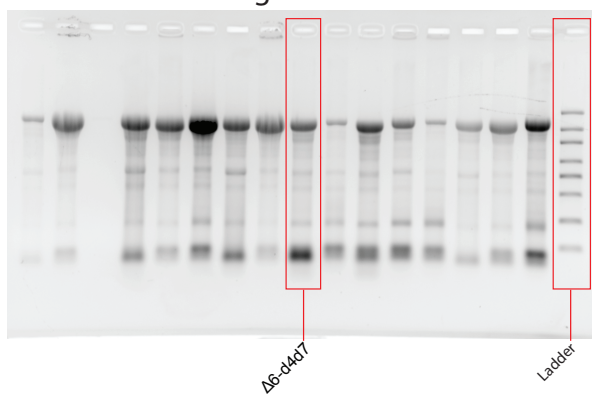
Extended data figure 2e



Extended data figure 7a

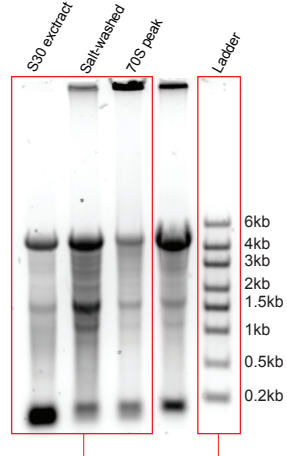
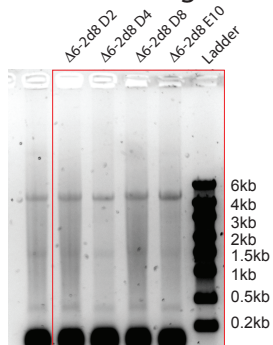


Extended data figure 7b



Extended data figure 8a

Extended data figure 7d



Extended data figure 9a

