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Structurally Heterogeneous Ribosomes Cooperate in Protein Synthesis in Bacterial Cells

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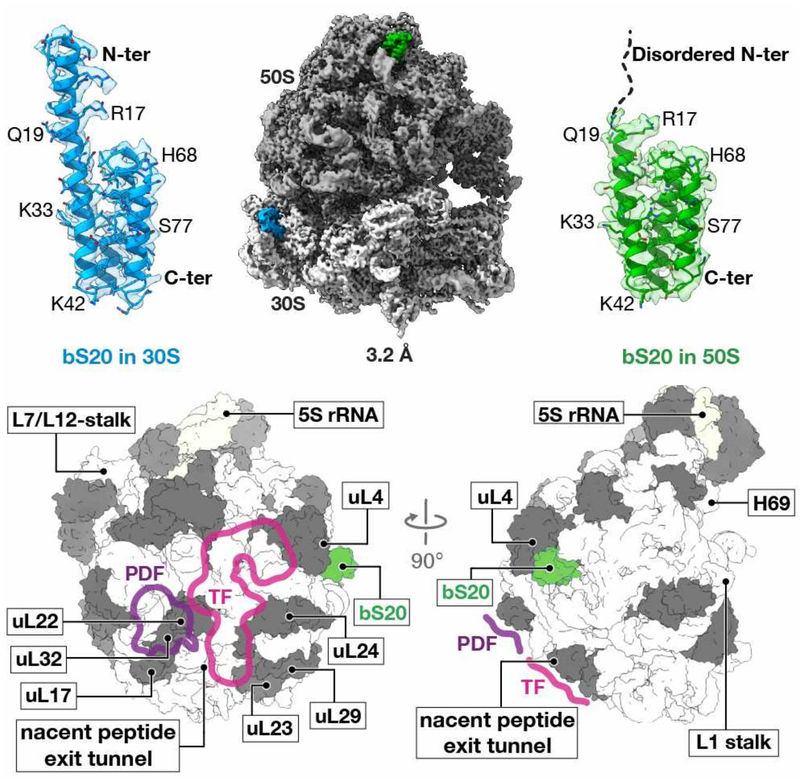
Even thought the structure and function of ribosomes is highly conserved across all domains of life, ribosomes have been shown to differ in their structure and composition between organisms, but also within a single organism or even single cells. Evoking the idea that structurally distinct pools of ribosomes might be fuctionally different and used to translate specific mRNAs. Whilst there is supporting evidence for conditional alterations in ribosomes structure, e.g. in response to environmental stimuli, it is unknown to what extent structural heterogeneity reflects genuine functional specialization rather than stochastic variations in ribosome assembly. Here, we combine high-resolution cryo-electron microscopy and *in situ* tomography to directly observe structurally distinct ribosomes during protein synthesis in single bacterial cells.

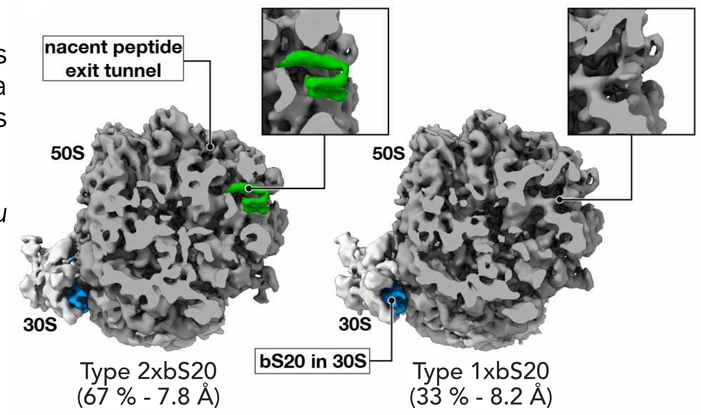
two distinct populations of *P. urativorans* 70S ribosomes observed *in vitro* and *in situ*



High resolution single particle analysis of purified 70S ribosomes and subtomogram averaging using cellular tomograms reveal a second binding site of bS20 on the 50S subunit that is substoechiometrically ocupied.

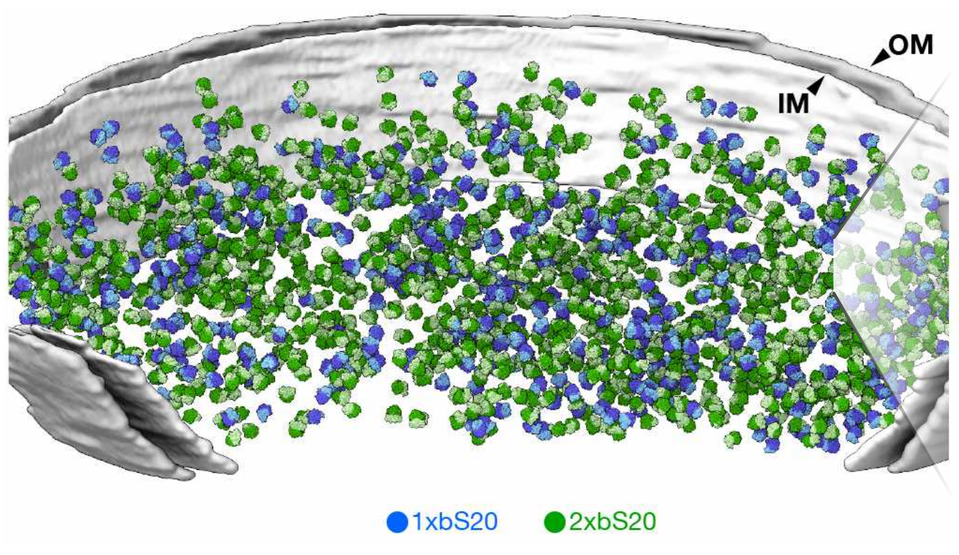
Two disitinct populations can be observed *in vitro* and *in situ* depending on the number of bS20 bound to the ribosome:

1xbS20 (30%)
2xbS20 (70%)

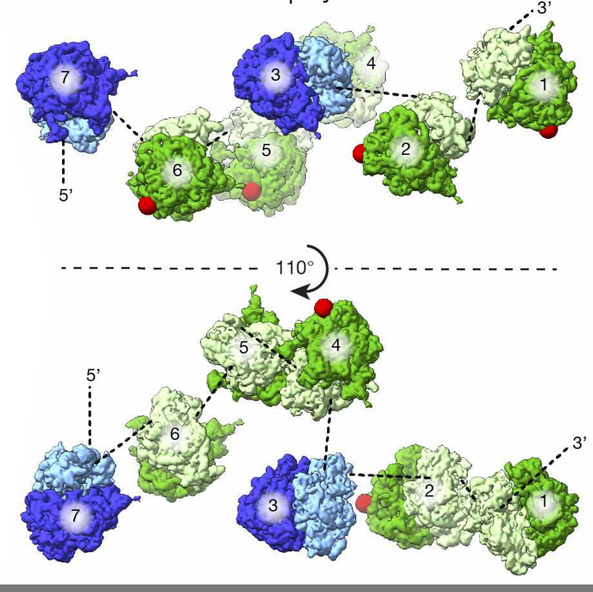


both types of ribosomes are randomly distributed within a single cell

distribution of two types of ribosomes in the cytosol:

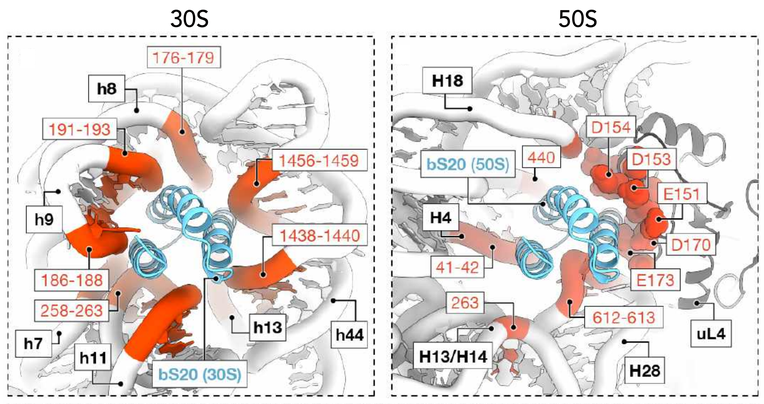


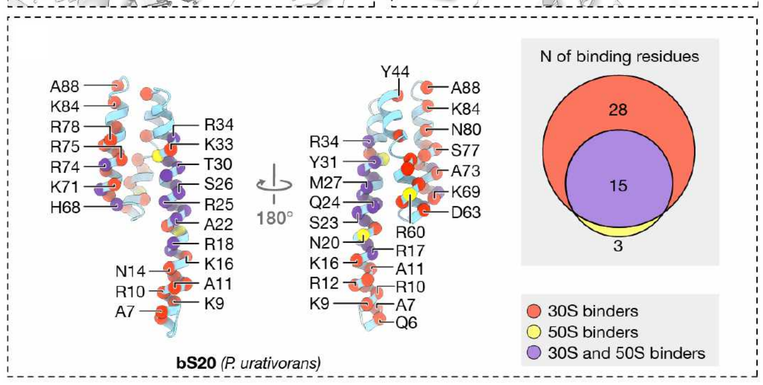
distribution of two types of ribosomes on traced polysomes:



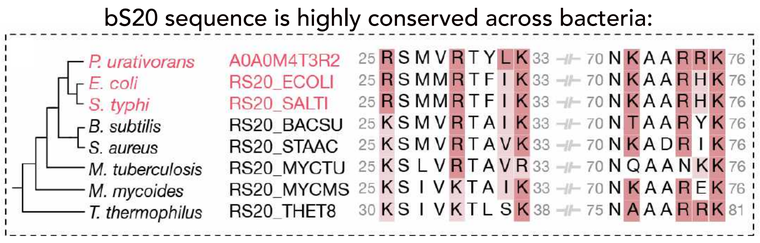
bS20 binding site via uL4 is conserved in proteobacteria

Comparison of the bS20-binding site

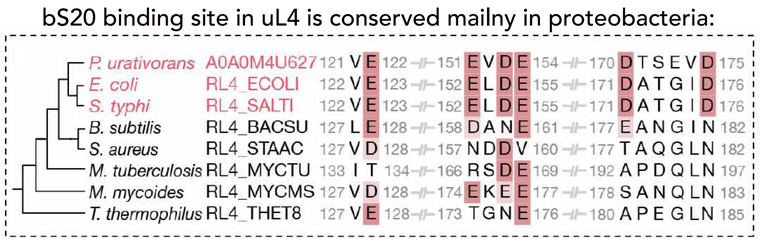




bS20 sequence is highly conserved across bacteria:

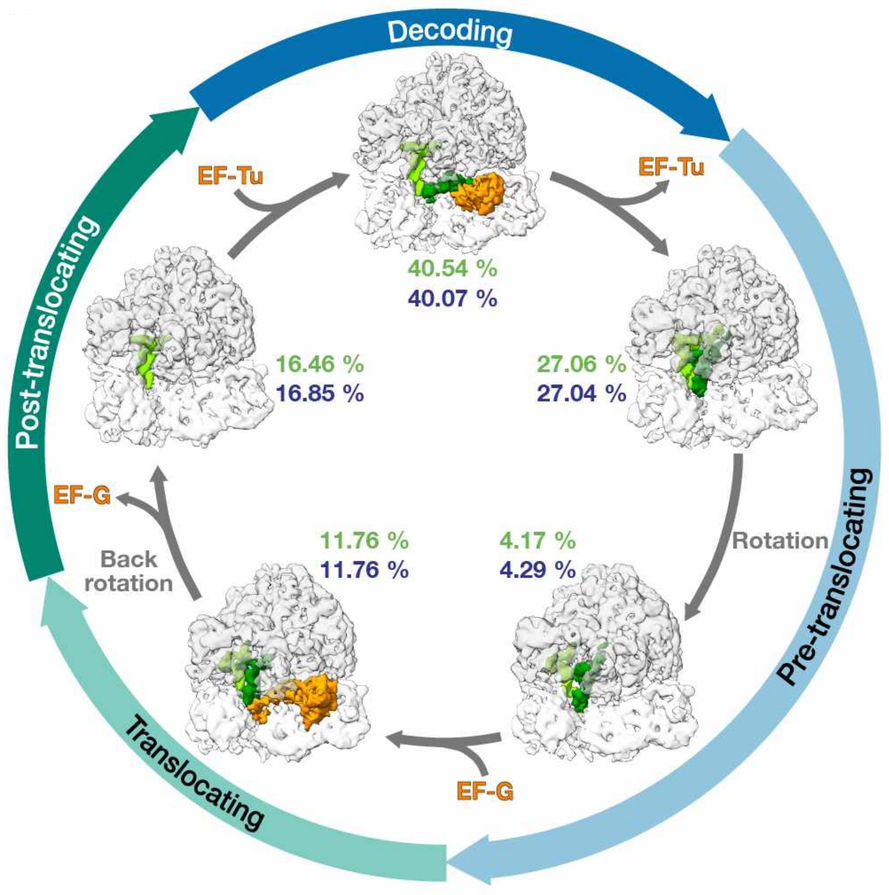


bS20 binding site in uL4 is conserved mailny in proteobacteria:

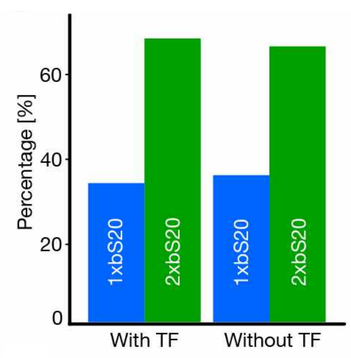


1xbS20 and 2xbS20 ribosomes are functionally invariant

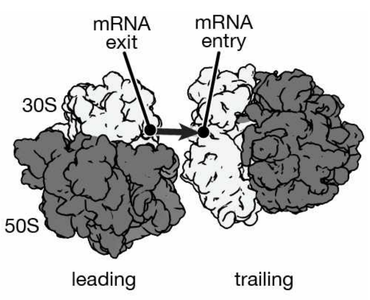
distribution of functional states:

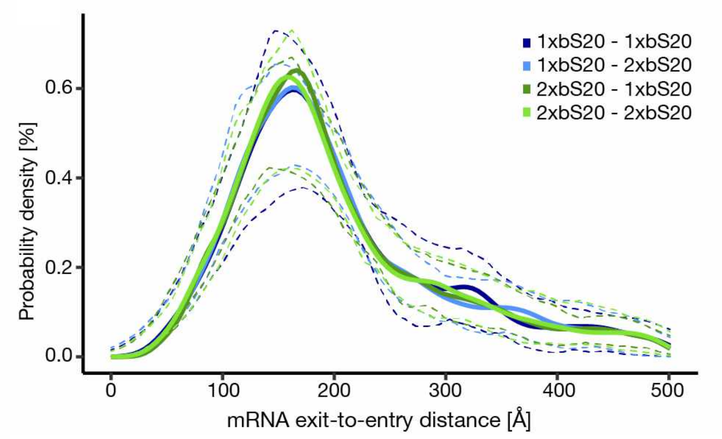


association with trigger factor:



distribution of mRNA exit-to-entry distances for two types of ribosomes:





- *P. urativorans* 70S ribosomes show heterogeneity in the stoichiometry of bS20 bound to the 50S subunit
- bS20 stoichiometry does not influence core the translational function of *P. urativorans* 70S ribosomes
- binding of bS20 to the large subunit via uL4 is conserved in proteobateria and migh act as a buffer for excess bS20
- structural heterogeneity can be functionally neutral and unrelated to targeting of specific mRNA

Helena-Bueno, K., Rybak, M.Y., Ekemezie, C.L. et al. A new family of bacterial ribosome hibernation factors. Nature 626, 1125–1132 (2024). https://doi.org/10.1038/s41586-024-07041-8