Lost in translation: Mutating the ribosome active site *in vitro*

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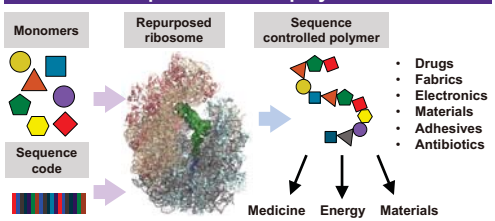
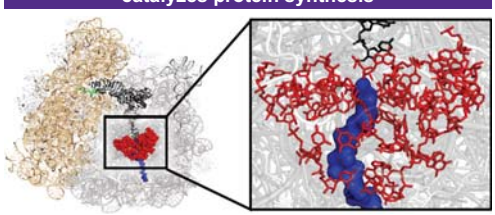
Background & Methods

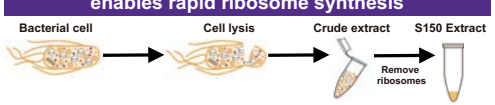
Ribosome engineering will enable the synthesis of sequence defined polymers

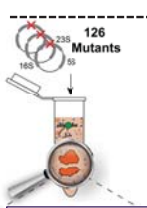


The ribosome's peptidyl transferase center (PTC) catalyzes protein synthesis



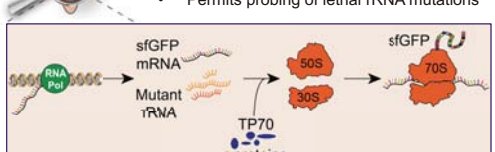
Integrated synthesis, assembly, & translation (iSAT) enables rapid ribosome synthesis





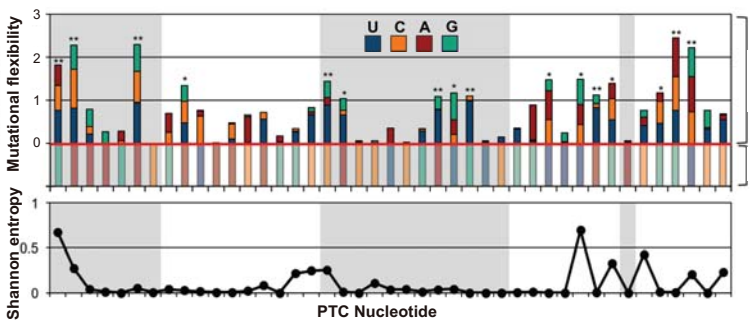
Advantages of iSAT:

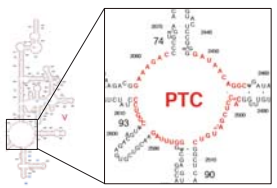
- Open test tube environment permits control of components
- Extract mimics cellular environment
- Transcription and translation occur in the same compartment
- Easily build and test ribosomal variants
- Permits probing of lethal rRNA mutations

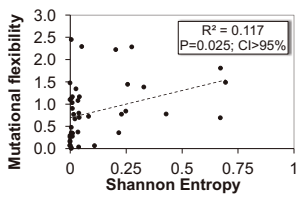


Results

In vitro iSAT assay for mutant ribosomes' translation activity

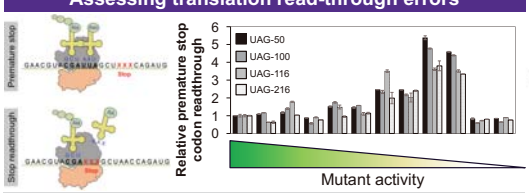






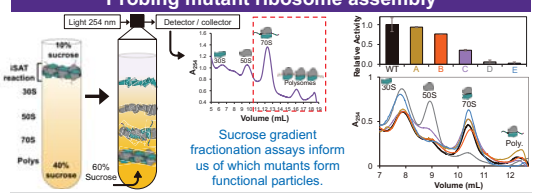
Despite the highly conserved nature of the ribosome's PTC active site, there still exists a range of mutational flexibility for a variety of bases at each nucleotide position. Furthermore, nucleotide conservation explains only a minor fraction of the observed variation in mutational flexibility.

Assessing translation read-through errors



Some PTC nucleotide mutants have high readthrough compared to WT.

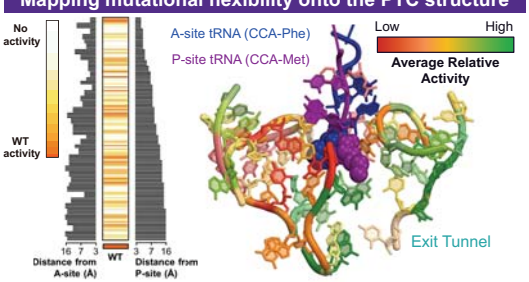
Probing mutant ribosome assembly



Sucrose gradient fractionation assays inform us of which mutants form functional particles.

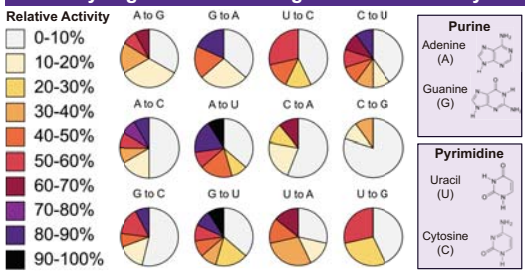
Regardless of activity, all but one mutant assemble into functional units.

Mapping mutational flexibility onto the PTC structure



PTC nucleotides critical to translation reside in a pocket adjacent to the exit tunnel, while mutationally flexible ones surround the exit tunnel.

Analyzing nucleotide change *vs* mutant activity



Nucleotide changes from C to A/G and G to C have the lowest activity. While changes from G to A, C/A/G to U exhibit the highest activity.

Summary & Conclusions

- Despite the high conservation of the ribosome's PTC, there is still incredible plasticity within its catalytic core.
- Permissive mutation pockets reside around both the A-site and P-site.
- Mutations of highly conserved PTC nucleotides impact translation kinetics and fidelity.
- Basic science studies of the ribosome's PTC may provide insights into engineering the active site.

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