Ribosomes: Machines that Synthesize Proteins

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Amino acids

Aminoacyl-tRNA synthetases

Essential part of the protein synthesis machinery

Protein Translation

Enzymes that ligate amino acids to tRNA

Translation

mRNA

E-TU

GTP

Ribosome

mRNA

2
Flow of Genetic Information

DNA → mRNA

Anticodon → Codon

AA → Amino acid

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### The Genetic Code

<table>
<thead>
<tr>
<th>First Position (5')</th>
<th>Second Position</th>
<th>Third Position (3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>PHE PHE</td>
<td>SER SER</td>
<td>TYR TYR</td>
</tr>
<tr>
<td>LEU LEU</td>
<td>STOP STOP</td>
<td></td>
</tr>
<tr>
<td>LEU LEU</td>
<td>PRO PRO</td>
<td>HIS HIS</td>
</tr>
<tr>
<td>LEU LEU</td>
<td>PRO PRO</td>
<td>GLN GLN</td>
</tr>
<tr>
<td>LEU LEU</td>
<td>PRO PRO</td>
<td></td>
</tr>
<tr>
<td>ILE ILE</td>
<td>THR THR</td>
<td>ASN ASN</td>
</tr>
<tr>
<td>ILE ILE</td>
<td>THR THR</td>
<td>LYS LYS</td>
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<tr>
<td>MET</td>
<td>THR THR</td>
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<tr>
<td>VAL VAL</td>
<td>ALA ALA</td>
<td>ASP ASP</td>
</tr>
<tr>
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<td>ALA ALA</td>
<td>GLU GLU</td>
</tr>
<tr>
<td>VAL</td>
<td>ALA ALA</td>
<td></td>
</tr>
</tbody>
</table>

*Singer & Berg*
Transfer RNA

Secondary structure

Tertiary structure

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The ribosome is an extremely large molecular machine

Bacterial ribosomes are comprised of:

- 2 subunits: 50S large subunit & 30S small subunit
- 3 RNA molecules >4500 nucleotides in length
- >50 different proteins
- Molecular weight of 2.5 million daltons
Secondary structure of large subunit ribosomal RNA from *Thermus thermophilus*
Peptidyl transferase reaction catalyzed by the ribosome

Biophysical techniques used to study the ribosome

- X-ray crystallography
- Cryo-electron microscopy
- smFRET
If the ribosome requires proteins to function, where did the proteins come from to make the first ribosome?
The ribosome is a ribozyme

The *H. marismortui* large ribosomal subunit

N Ban et al. Science 2000;289:905-920
Structure of the peptidyl transferase center in the 50S subunit of the ribosome

Peptidyl- & aminoacyl-tRNA substrate analogs

Transition state analog

Proposed proton shuttling mechanism for the peptidyl transferase reaction
Polypeptide tunnel

PT-peptidyl transferase center

Complete structure of the ribosome

Translation elongation cycle

Molecular mimicry by Elongation factors

Ribosome Translocation - a ratchet mechanism involving EF-G

A

Classical (pre)

<table>
<thead>
<tr>
<th>50S</th>
<th>E</th>
<th>P</th>
<th>Ac</th>
</tr>
</thead>
<tbody>
<tr>
<td>30S</td>
<td>E</td>
<td>P</td>
<td>A</td>
</tr>
</tbody>
</table>

Hybrid (pre)

<table>
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<th>50S</th>
<th>OH</th>
<th>Ac</th>
</tr>
</thead>
<tbody>
<tr>
<td>30S</td>
<td>Ac</td>
<td></td>
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</table>

Classical (post)

<table>
<thead>
<tr>
<th>50S</th>
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</thead>
<tbody>
<tr>
<td>30S</td>
<td></td>
<td></td>
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</table>

EF-G:GTP

B

C

Quartz Slide

Neutravidin/Biotin

The Nobel Prize in Chemistry 2009
“for studies of the structure and function of the ribosome”.

Venki Ramakrishnan  
Tom Steitz  
Ada Yonath

Big questions in protein translation

What is the origin of the ribosome?

How did the genetic code evolve?

Which amino acids came first in proteins?
Role of Aminoacyl-tRNA Synthetases in Protein Biosynthesis

Proper translation of the genetic code

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The 20 aminoacyl-tRNA synthetases all share a common mechanism

(1) $E + AA + ATP \rightleftharpoons E(AA-AMP) + P Pi$

(2) $E(AA-AMP) + tRNA \rightleftharpoons E + AA-tRNA + AMP$
Reaction Catalyzed by AARSs

Aminoacyl-tRNA
(terminal 3’ nucleotide of appropriate tRNA)

Adenine

Aminoacyl-tRNA

3'       2'
Aminoacyl-tRNA synthetases enzymes can be divided into 2 different classes.

Class I:
- MetRS
- ValRS
- LeuRS
- IleRS
- CysRS
- ArgRS
- GluRS
- GlnRS
- TyrRS
- TrpRS

Class II:
- SerRS
- ThrRS
- AlaRS
- GlyRS
- ProRS
- HisRS
- AspRS
- AsnRS
- LysRS
- PheRS

Ribas de Pouplana & Schimmel (2001)
Role of Aminoacyl-tRNA Synthetases in Protein Biosynthesis

Amino acids → Aminoacyl-tRNA synthetases → Aminoacyl-tRNAs

Proper translation of the genetic code

Errors in protein synthesis

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Protein Translation Studies: practical applications

- Development of Antibiotics

- Expanding the genetic code

P.G. Schultz, TSRI
Plant tumour biocontrol agent employs a tRNA-dependent mechanism to inhibit leucyl-tRNA synthetase

Shaileja Chopra¹,*; Andrés Palencia²,*; Cornelia Virus¹; Ashutosh Tripathy³; Brenda R. Temple⁴; Adrian Velazquez-Campoy⁵; Stephen Cusack² & John S. Reader¹
Pathogenic *Agrobacterium tumefaciens* causes crown gall tumors in plants

Mullins et al. 2001

Pathogenic *Agrobacterium tumefaciens*

Agrocinopine

Infected plant cell

Agrobacterium *radiobacter* (Biocontrol)
A. radiobacter (plant biocontrol agent) produces Agrocin 84 to compete with A. tumefaciens (pathogen)
TM84 is a potent inhibitor of leucyl tRNA synthetases (LeuRSs)

LeuRS Reaction:

1) Aminoacyl adenylate formation:

\[ \text{LeuRS + Leu + ATP} \rightleftharpoons \text{LeuRS (Leu-AMP) + PPI} \]

2) Aminoacyl transfer:

\[ \text{LeuRS (Leu-AMP) + tRNA}^{\text{Leu}} \rightleftharpoons \text{LeuRS} + \text{Leu-tRNA}^{\text{Leu}} + \text{AMP} \]

\[ K_{i_{\text{app}}} = 0.26 \pm 0.13 \text{ nM} \]
TM84 closely resembles Leu-AMP

TM84 (Toxic Moiety 84)

Leu-AMP
Hypothesis:

TM84 binds and acts as a stable Leu-AMP mimic to inhibit aminoacylation. IC₅₀ = 25 µM.

tRNA^{Leu} is essential for the tight-binding of TM84!

% Dose Response

\[
\text{log}_{10} [\text{TM84}] \text{ nM}
\]

LeuRS_{At} + TM84

IC₅₀ = 25 µM

LeuRS_{At} + tRNA + TM84

IC₅₀ = 1 nM
Utilizing Isothermal Titration Calorimetry (ITC) to dissect the mechanism of inhibition by TM84:

Unbound ligand

Bound ligand
tRNA^{Leu} is essential for tight-binding of TM84
TM84 binds to *E. coli* LeuRS-tRNA$^\text{Leu}$ in the aminoacylation-like conformation!