Translation

Dr. Kevin Ahern
Central Dogma

DNA Polymerase
DNA Replication (DNA to DNA)

Reverse Transcriptase
Reverse Transcription (RNA to DNA)

Transcription (DNA to RNA)

RNA Polymerase

RNA-Dep. RNA Polymerase
RNA Replication (RNA to RNA)

Ribosomes
Translation (RNA to Protein)

Ssense RNA

Nonsense RNA

Protein
mRNA Structure

Bacteria
<table>
<thead>
<tr>
<th>1st base</th>
<th>2nd base</th>
<th>3rd base</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>AGC</td>
<td>U</td>
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<tr>
<td>A</td>
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<td>G</td>
</tr>
<tr>
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<td>A</td>
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<td>A</td>
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<td>A</td>
</tr>
<tr>
<td>A</td>
<td>ACA</td>
<td>C</td>
</tr>
<tr>
<td>A</td>
<td>ACC</td>
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<tr>
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<tr>
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<td>C</td>
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<td>UUU</td>
<td>U</td>
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<td>UGA</td>
<td>A</td>
</tr>
<tr>
<td>U</td>
<td>UGC</td>
<td>C</td>
</tr>
</tbody>
</table>

- **64 Codons**
- **3 Stops**
- **1 Start**
- **2 Unique for AA**
- **59 Redundant**
Translation Steps

1. Amino acid activation
2. Chain initiation
3. Chain elongation
4. Repeated many times
5. Chain termination
Central Dogma

Eukaryotes

DNA
mRNA Transcription
Mature mRNA

Nucleus

Transport to cytoplasm for protein synthesis (translation)
mRNA

Cell membrane

Cytoplasm
Translation

Amino Acids: Ala, Ile, Arg, Leu, Gly, Tyr, Ser, Ala, Cys, Ile, His, Val, Ala, Ile, Arg

tRNA:

 anticodon: CGAUAUUCCGAUCCCAUGUCACGUACGUAGUGUCAGUAUCG

mRNA:

 codons: CGUAUAAGCGUAGCUAGGUAACUGCAUCGUAACUGCUAGCG

Protein
Simple View of Translation

Start Codon

Stop Codon

https://commons.wikimedia.org/wiki/File:Ribosomer_i_arbete.png
Structure

E-site  P-site  A-site

large ribosomal subunit

small ribosomal subunit

mRNA-binding site
tRNAs - Translator Molecules

Amino Acid Attachment Point
tRNAs - Translator Molecules
Amino Acid Activation - Charging of tRNAs

Aminoacyl tRNA-synthetase

Enzyme reads anti-codon and attaches proper amino acid

Type 1 - AA on 2’ OH
Type 2 - AA on 3’ OH
“Charging” of tRNAs
fMet Synthesis in Prokaryotes

Met-tRNA\textsuperscript{fmet}$\quad$\xrightarrow{\text{Met-tRNA}^\text{fmet formyl transferase}}$\quad$N$^\text{10}$-formyl-THF $\quad$THF $\quad$N-formyl-met-tRNA\textsuperscript{fmet}

Note similarity to peptide grouping
Wobble Base
## Wobble Base

<table>
<thead>
<tr>
<th>Base at 5’ End of Anticodon</th>
<th>Base at 3’ End of Codon</th>
</tr>
</thead>
<tbody>
<tr>
<td>I*</td>
<td>A, C, or U</td>
</tr>
<tr>
<td>G</td>
<td>C or U</td>
</tr>
<tr>
<td>U</td>
<td>A or G</td>
</tr>
<tr>
<td>A</td>
<td>U</td>
</tr>
<tr>
<td>C</td>
<td>G</td>
</tr>
</tbody>
</table>

* I = hypoxanthine.

Note that there are no variations in base pairing when the wobble position is occupied by A or C.
Small Ribosomal Subunit (30S Prokaryotes)
Large Ribosomal Subunit (50S Prokaryotes)
50S Ribosomal Subunit
<table>
<thead>
<tr>
<th>rRNA Name</th>
<th>Prokaryotes</th>
<th>Eukaryotes</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>5S</td>
<td>Large Subunit</td>
<td>Large Subunit</td>
<td>tRNA binding?</td>
</tr>
<tr>
<td>5.8S</td>
<td>Large Subunit</td>
<td>Large Subunit</td>
<td>Translocation?</td>
</tr>
<tr>
<td>16S</td>
<td>Small Subunit</td>
<td></td>
<td>mRNA alignment</td>
</tr>
<tr>
<td>18S</td>
<td>Small Subunit</td>
<td></td>
<td>mRNA alignment</td>
</tr>
<tr>
<td>23S</td>
<td>Large Subunit</td>
<td></td>
<td>Peptide bond formation</td>
</tr>
<tr>
<td>28S</td>
<td>Large Subunit</td>
<td></td>
<td>Peptide bond formation</td>
</tr>
</tbody>
</table>
Simple Overview
Overview

1. An enzyme called **aminoacyl tRNA synthetase** (not shown) attaches amino acids to their corresponding tRNA molecules using energy from ATP. Each amino acid has its own tRNA molecule with the anticodon for that amino acid.

2. A small ribosomal subunit attaches itself to the 5' end of an mRNA strand.

3. It moves along the mRNA until it finds a start codon. There, the first tRNA and the large ribosomal subunit join it.

4. Other tRNAs with anticodons matching the mRNA codons fall into place in the ribosome.

5. The first tRNA drops off its amino acid, breaks off and leaves to pick up another amino acid. The second moves over to make room for another tRNA.

6. tRNA after tRNA drop off their amino acids. They form a chain of amino acids linked by peptide bonds.

7. When the ribosome reaches a stop codon, it releases the finished polypeptide.
**Prokaryotes - Shine-Dalgarno Sequence**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>araB</td>
<td>U U U G G A U G G A G U G A A A C G A U G G C G</td>
</tr>
<tr>
<td>galE</td>
<td>A G C C U A A U G G A G G C G A A U U A U G A G A</td>
</tr>
<tr>
<td>lacI</td>
<td>C A A U U C A G G G U G G U G A U U G U G A A A A</td>
</tr>
<tr>
<td>lacZ</td>
<td>U U C A C A C A G G A A A C A G C U A U G A C C</td>
</tr>
<tr>
<td>trpE</td>
<td>C A A A A U U A G A G A A U A A C A A U G C A A</td>
</tr>
<tr>
<td>trpL leader</td>
<td>G U A A A A A G G G U A U C G A C A A U G A A A</td>
</tr>
</tbody>
</table>

Shine-Dalgarno sequence (purine-rich ribosome binding site)
Shine-Dalgarno Sequence

16S rRNA Sequence

AGGAGGUUUGACCUAUG

rRNA complementary to SD-sequence
Initiation

Small ribosomal subunit + fMet tRNA

Alignment of mRNA with 16S rRNA of subunit

Pairing of fMet tRNA to AUG codon

Large subunit joins fMet tRNA in P-site

Second tRNA pairs with codon in A-site

Peptide bond formed between AA#1 & AA#2, ribosome translocates
Initiation
Structure

Protection Against Hydrolysis

EF-Tu
Elongation
Elongation
Termination
Translation in Prokaryotes

1. Binding of mRNA by small subunit
2. Sliding to identify start codon (Shine-Dalgarno)
3. Base pairing of anticodon of initiator tRNA to AUG in mRNA (3 IFs)
4. Binding of large subunit
5. Entry of second tRNA into A-site (EF-Tu)
6. Peptide bond formation - peptide on tRNA in A-site
7. Translocation (requires GTP & EFG)
8. Exit of used tRNA, entry of new charged tRNA
9. Repeat 5-8 to STOP Codon
10. Entry of release factor with water
Translation in Eukaryotes

Differences
1. mRNA structure
2. Ribosome sizes (60S & 40S)
3. rRNA sizes (extra rRNA)
4. No Shine-Dalgarno
5. No fMet
Translation in Eukaryotes - Initiation

Kozak Sequences
Eukaryotic Translation
Eukaryotic Translation

Bonding to mRNA

Premature Stopping of Translation

Degradation of mRNA
Membrane Proteins
Structure

- mRNA
- SRP
- Ribosome
- Signal Peptide
Membrane Protein Synthesis

SRP binds to the Signal Sequence

Advantages of Membrane Proteins:
- Membrane proteins are involved in transporting molecules across cell membranes.
- They play a crucial role in various cellular processes, including signal transduction, nutrient uptake, and secretion.

Delivery of Soluble Protein
Delivery of Integral Membrane Protein

Cytoplasm
ER Lumen

mRNA
Ribosome
SRP
Signal Sequence
Membrane Channel
SRP Receptor
Signal Sequence Peptidase
Cleaved Signal Sequence