TRANSLATION 1: SYNTHETASES, TRANSLATION CYCLE and FACTORS

IMPORTANT POINTS

1. Several different codons can specify a single amino acid. Some amino acids have more than one tRNA and these tRNAs are constructed so that they require accurate base-pairing only at the first two positions of the codon and can tolerate a mismatch (wobble).

2. tRNA molecules are adaptors (with a cloverleaf-like secondary structure). All tRNA are subject to a variety of chemical modification 1 in 10 nucleotides are modified: dihydrouridine, pseudouridine, inosine, etc).

3. Synthetases act as the genetic dictionary and thus have a dual recognition problem: matching amino acids with their cognate tRNAs. There is a different synthetase for each amino acid. Synthetases attach amino acids to the 3' end of the tRNA. The reaction is coupled to ATP hydrolysis and produces a high-energy bond between the tRNA and the aa. The energy of that bond is used at a later stage to link the amino acid to the growing polypeptide chain.

4. The aminoacylation of tRNA proceeds stepwise: (1) the synthesis of an aminoacyl-adenylate, as an active intermediate, from the amino acid and adenosine triphosphate (ATP), and (2) the transfer of the aminoacyl moiety from the adenylate to the 3'-terminal adenosine (3'-A) of tRNA. The correct recognition of substrates by aaRSs is essential for the accuracy of translation. However, the affinity difference is not large enough for the enzyme to discriminate strictly between similar amino acids (Pauling, 1957).

5. The tRNA synthetases have an editing activity that hydrolyzes incorrect products in a tRNA-dependent manner. Amino acids that are smaller than or isosteric to the cognate are edited by a hydrolytic editing mechanism. Distinguishing tRNAs requires only few "identity elements."

6. By footprinting and FRET experiments, it has been shown that a tRNA passes through six physically distinct states during its transit across the ribosome. In dramatic contrast to the classical, static two-site (A,P) model, the identification of hybrid sites provides an important link between decoding and translocation.
7. The structure of the ribosome was solved at 2.5 to 4.5 Å resolution. rRNA and not proteins determine the overall structure, its ability to position tRNAs on the mRNA and its catalytic activity in forming peptide bonds. The rRNA is folded into a highly compact core, which determines the overall shape of the ribosome. The proteins are generally located on the surface and fill gap of the folded RNA and appear to stabilize the RNA and form ant interface with the environment.

8. Elongation factors (EF-Tu and EF-G) enter and leave the ribosome in each cycle. The hydrolyze GTP to GDP and undergo conformational changes in the process. These changes in the elongation factors accelerate the transition between the different conformational states of the ribosome.

9. Similarly than for small enzymes, The ribosome is a highly dynamic molecular machine, specifically a processive Brownian motor. Evidence from smFRET and cryo-EM for spontaneous transitions between the macrostates has led to a revision of the role of EF-G in translocation, from instrumental to ancillary, accelerating a process structurally ingrained in the ribosome.
I. Reviews.


II. tRNA Synthetases are the Genetic Dictionary (biochemistry)


14. Fukai, S., Nureki, O., Sekine, S., Shimada, A., Tao, J. S., Vassylyev, D. G., and Yokoyama, S., Structural basis for double-sieve discrimination of L-valine
from L-isoleucine and L-threonine by the complex of tRNA(Val) and valyl-tRNA synthetase, *Cell, 103*, 793-803 (2000).


III. tRNA are adaptors


IV. tRNA in the ribosome


IV. The Elongation Factors


large conformational changes in the 70S ribosome. Nature Structural Biology 6, 643-7.


The translation machinery

The chemistry of peptide bond formation is fundamentally **SIMPLE**:

However the translation machinery is very **COMPLEX**?

**Challenges**

- Change of language but maintain the message/sequence
- Fidelity: selective amplification of Cognate vs. Non-Cognate aa:tRNA/mRNA:tRNA
- Reading frame maintenance
Major Constraint: tolerable error frequency

<table>
<thead>
<tr>
<th>Frequency of inserting an incorrect amino acid</th>
<th>Probability of synthesizing an error-free protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of amino acid residues</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>0.364</td>
</tr>
<tr>
<td></td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>0.915</td>
</tr>
<tr>
<td></td>
<td>0.840</td>
</tr>
<tr>
<td></td>
<td>0.368</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td>0.970</td>
</tr>
<tr>
<td></td>
<td>0.905</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>0.999</td>
</tr>
</tbody>
</table>

Tolerable error frequency is $10^{-4}$!

[Ref. 1]

The discrimination problem

- $\Delta G$ of C vs. NC is only 10-100 x
  
  e.g. Ile/AUU Leu/CUU
       Val/GUU Phe/UUU

- Compounded by "Pool Bias": 20:1 / NC:C
  
  - a.a. @ synthetase site
  - amino acyl tRNA @ A site
tRNAs: the "adapters" used in decoding.

- **Codons**: 61 + 3 Stop codons
- **40 tRNAs** (20-24 in mitochondria)
- **Aminoacyl RNA synthetase (RS)**: ~20: one for each aa

The Wobble Position
Aminoacyl tRNA synthetase (RS): (the genetic dictionary)

Dual recognition problem:
1) amino acid (cognate vs. non-cognate)
2) tRNA (cognate vs. non-cognate)

Two classes of synthetases

RS synthetases are highly conserved
Classes are defined by two types of distinct architectures used to construct the catalytic sites.
Class 2 links amino acid to the 3’-OH group of the ribose, and class 1 links it to the 2’-OH.
Class 1 contact the acceptor stem at the minor groove, class 2 at the major groove.

[Ref. 5]
Amino acid discrimination: editing

Half of the synthetases have editing domains

Table 1  Editing substrates and domains of aa-tRNA synthetases

<table>
<thead>
<tr>
<th>Class</th>
<th>aaRS</th>
<th>Editing substrates</th>
<th>Cis-editing domain</th>
<th>Trans-editing factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>IleRS</td>
<td>Val, Hcy&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CP1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>ValRS</td>
<td>Thr, α-Abu&lt;sup&gt;a&lt;/sup&gt;, Hcy&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CP1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LeuRS</td>
<td>Hcy&lt;sup&gt;a&lt;/sup&gt;, γ-hLeu&lt;sup&gt;a&lt;/sup&gt;, Val, nLeu&lt;sup&gt;a&lt;/sup&gt;, Met, Ile</td>
<td>CP1/active site?</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>MetRS</td>
<td>Hcy&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Active site?</td>
<td>–</td>
</tr>
<tr>
<td>Class II</td>
<td>ThrRS</td>
<td>Ser</td>
<td>N2</td>
<td>ThrRS-ed</td>
</tr>
<tr>
<td></td>
<td>AlaRS</td>
<td>Gly, Ser</td>
<td>AlaX-like</td>
<td>AlaXp</td>
</tr>
<tr>
<td></td>
<td>ProRS</td>
<td>Ala, 4-Pro&lt;sup&gt;b&lt;/sup&gt;, Cys&lt;sup&gt;b&lt;/sup&gt;</td>
<td>INS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>YbaK, ProX</td>
</tr>
<tr>
<td></td>
<td>PheRS</td>
<td>Tyr, Ile</td>
<td>B3B4</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LysRS II</td>
<td>Hcy&lt;sup&gt;a&lt;/sup&gt;, Hse&lt;sup&gt;a&lt;/sup&gt;, Orn&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Active site?</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SerRS</td>
<td>SerHX&lt;sup&gt;a&lt;/sup&gt;, Thr, Cys</td>
<td>Active site?</td>
<td>–</td>
</tr>
</tbody>
</table>
Case study: amino acid discrimination (ThrRS)

Achieved by monitoring differences in size and chemistry

ThrRS structure

The monomer of E. coli ThrRS. A molecule of AMP is shown in the active site. The N-terminal domains are on the left. The arrow indicates where the truncation was made to produce N-ThrRS.

[Ref. 15]
Aminoacylation and Deacylation by ThrRS

Aminoacylation

![Graph showing aminoacylation](image)

Deacylation

![Graph showing deacylation](image)

The editing site and the tRNA

Superposition of the acceptor arm of tRNA\(^{\text{Gln}}\) (in blue) with the acceptor arm of tRNA\(^{\text{Thr}}\) (in pink) within ThrRS. The picture shows the CCA end of tRNA\(^{\text{Gln}}\) pointing toward the editing site. The catalytic domain of ThrRS is in green, the N2 domain in yellow.
tRNA recognition by synthetases

All tRNA must be recognized equally by EF-Tu and by the ribosomal A site. So, how can they be distinguished by RS?

THE SOLUTION: tRNA "Identity Sets"

1. Anti-codon is sole (major) determinant
   e.g. met
2. Acceptor stem is sole (major) determinant
   e.g. ala (G-U)
3. Complex (anti-codon + acceptor + variable pocket)
   e.g. phe

[Ref. 5, 16]
A comparison of the structures of procaryotic and eucaryotic ribosomes.

The ribosome is an allosteric enzyme.
The peptidyl-transferase site is the ACTIVE SITE

The Prokaryotic Cast
(in eukaryotes similar but more complex)

- Ribosome \( \sim 3 \times 10^6 \), 250 Å (50S + 30S = 70S)
- Factors: IF 1, 2, 3  
  EF-Tu, EF-Ts, EF-G  
  RF 1, 2, 3, RRF  
  GTP hydrolysis

Initiation  
Elongation  
Release
Crystal structure

Figure 6-70 Molecular Biology of the Cell 5/e (© Garland Science 2008)

RNA

Figure 6-84a, Molecular Biology of the Cell 5/e (© Garland Science 2008)

Figure 6-84b, Molecular Biology of the Cell 5/e (© Garland Science 2008)
Large subunit proteins

Translation Elongation Cycle

1. Initial selection
   - Codon recognition
   - Activation of GTPase
   - GTP hydrolysis
   - Accommodation

2. Proofreading
   - tRNA rejection (start over)

B

- Peptidyl Transferase
- EF-G,GTP Binding
- GTP hydrolysis
- Translocation
- EF-G Release

Key:
- 30S
- 50S
- mRNA
- P-site tRNA
- A-site tRNA
- EF-Tu-GTP-tRNA (ternary complex)
- EF-Tu-GDP
- EF-G

[Ref. 7** ]
Hybrid States model for the elongation cycle

Chemical probing/footprinting/cross-linking

[Ref. 18-21*]
EF-TU and GTP/GDP

- EF-Tu: binds aa-tRNA and delivers to A-site
  \[ K_{ass} \text{ Tu-GTP-aa-tRNA} > 10^5 K_{ass} \text{ Tu-GTP-tRNA} \]
  \[ K_{ass} \text{ Tu-GTP-aa-tRNA} > 10^6 K_{ass} \text{ Tu-GDP-aa-tRNA} \]
- EF-Tu GAP is the ribosome
  - (10^5 increase in GTPase rate)
- Tu binds GDP tightly (10^2 Kass GTP)
- GEF is required: EF-Ts: disrupts Mg binding

[Ref. 39]
THE ELONGATION FACTORS

- Elongation factors \textit{EF-Tu} and \textit{EF-G} are required for efficient translation rates
- They are weak GTPases
- Each contains a ras-like domain [Ref. 25-37]

\[
\begin{array}{c}
\text{Ribosome} \\
\text{aa-tRNA (GAC)}
\end{array}
\begin{array}{c}
\text{EF-Tu.GDP} \\
\text{EF-Tu.GTP}
\end{array}
\begin{array}{c}
\text{Pi} \\
\text{H}_2\text{O}
\end{array}
\begin{array}{c}
\text{EF-Ts (GEF)} \\
\text{EF-Tu/EF-Ts}
\end{array}
\begin{array}{c}
\text{GTP} \\
\text{GDP}
\end{array}
\]

THE ROLE OF EF-TS

[Ref. 37]
EF-G: Translocation Factor

Similar to EF-Tu in domains 1+2 (GTP-binding)
But also has domains 3, 4, 5: form mimic of t-RNA in EF-Tu
Mimic: shape and charge (q^− in domain 4)
Model: EF-G-GTP binds to 50S at Tu binding site (GAP in loop in 23S)
EF-G-GDP displaces the pept-tRNA from A-site in 16S
mRNA moves with codon-pept-tRNA (block reverse movement)

[Ref. 20, 21, 23]

The ribosome is highly dynamic, processive
Brownian motor: EF-G determines direction

• EF-G-dependent GTP hydrolysis greatly accelerates, the
  rearrangement of the ribosome that leads to translocation.

• Domain IV of the EF-G structure is crucial for both rapid
  translocation and subsequent release of the factor from the
  ribosome.

• By coupling the free energy of GTP hydrolysis to translocation,
  EF-G drives the directional movement of transfer and
  messenger RNAs on the ribosome.

[Ref. 6]
During translocation, EF-G experiences an extensive reorientation.

After translocation, domain 4 reaches into the decoding center. The factor assumes different conformations before and after translocation. [Ref. 44]