TRANSLATION 2: FIDELITY, CATALYSIS, INITIATION AND TERMINATION

MAJOR POINTS

1. Discrimination of cognate (C) from non-cognate (NC) tRNA at the ribosomal A site requires a large contribution from the ribosome to amplify the minimal differences in binding energy. Aminoacyl tRNAs enter the A site on the 30S ribosome in a ternary complex with EFTu.GTP. The anticodon stem-loop binds to the decoding site in 16S rRNA, but the aminoacyl moiety is prevented from entering the peptidyl transferase site in 23S rRNA until GTP has been hydrolyzed.

2. Kinetic proofreading, driven by induced fit, is used by the ribosome to discriminate between correct and incorrect aa-tRNA during both initial selection and proofreading. Kinetic proofreading is made possible by the action of the GTPase elongation factor (EF-Tu in bacteria) that delivers aa-tRNAs to the ribosome in a ternary complex with GTP, allowing for two sequential opportunities to reject an incorrect aa-tRNA, pre- and post-hydrolysis. 'Induced fit' describes conformational changes that are thought to be responsible for accelerating GTPase activation and tRNA accommodation upon recognition of cognate tRNA species. The ribosome recognizes the geometry of codon-anticodon base pairing at the first two positions but monitors the third, or wobble position, less stringently.

3. Codon recognition by cognate tRNA results in the hydrolysis of GTP by EF-Tu over 75 Å away. Binding energy of cognate tRNA is used to induce conformational changes in the ribosome that stabilize a transition state for GTP hydrolysis by EF-Tu and subsequently result in accelerated accommodation of tRNA into the peptidyl transferase center. In addition, the transition state for GTP hydrolysis is characterized, among other things, by a distorted tRNA.

4. The catalytic site is formed by the 23S rRNA with the nearest amino acid is located 1.8 nm away. The center is a highly structured pocket that precisely orient the growing chain and an aminoacyl tRNA. A ring nitrogen of adenine acts as a functional group in an acid-base reaction.

5. The initiator codon AUG is also used to specify methionine internally. The initiator methionyl tRNA is specialized to interact with an Initiation Factor (IF2) instead of EFTu and to bind to the P site instead of the A site.

6. In prokaryotes, the initiator codon is constrained to lie a short distance downstream of a polypurine-rich sequence which base-pairs with the 3' end of 16S rRNA (the Shine-Dalgarno (SD) interaction). Internal initiation sites can be used as independent ribosome entry sites (if they are not occluded).
7. In prokaryotes, mRNAs that encode a product whose primary function is to bind RNA are often autogenously regulated. When the primary target is saturated, the protein shuts off translation of its secondary target, its own mRNA.

8. Peptide release, the reaction that hydrolyzes a completed protein from the peptidyl-tRNA upon completion of translation, is catalyzed in the active site of the large subunit of the ribosome and requires a class I release factor protein. The ribosome and release factor protein cooperate to accomplish two tasks: recognition of the stop codon and catalysis of peptidyl-tRNA hydrolysis.

REFERENCES

I. Reviews


II. Fidelity of Decoding at the Ribosomal A site


III. The Ribosome


30. *Agirrezabala, X. et al. Visualization of the hybrid state of tRNA binding promoted by spontaneous ratcheting of the ribosome. Mol Cell 32, 190-7 (2008).*


IV Initiation: Ribosome binding site in Prokaryotes


Shine-Dalgarno interaction in vivo . "Dedicated ribosome"


V. The Termination Factors


The 30s subunit and its role in decoding

Fidelity of the aa-tRNA selection on the ribosome: kinetics

- the ribosome uses the same discriminatory interactions more than once in consecutive rejection steps (proofreading), as proposed by Hopfield and Ninio (Ref 1).

[Ref. 5,6]
Elemental rate constants (k) experimentally determined

<table>
<thead>
<tr>
<th>Step</th>
<th>Cognate</th>
<th>Near-cognate</th>
<th>Nocognate</th>
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<tr>
<td>Initial binding</td>
<td>$k_{1}$</td>
<td>110$^a$</td>
<td>110$^b$</td>
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<td></td>
<td>$k_{-1}$</td>
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<tr>
<td>Codon recognition</td>
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<td>$k_{-2}$</td>
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<td>GTPase activation and GTP hydrolysis$^d$</td>
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<td>GTP-GDP cof. change of EF-Tu</td>
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<td>70</td>
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<td>Accommodation of aa-tRNA</td>
<td>$k_{5}$</td>
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<td>and formation of peptide bond$^d$</td>
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<td>Dissociation of EF-Tu</td>
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<td>Rejection of aa-tRNA</td>
<td>$k_{7}$</td>
<td>&lt;0.3</td>
<td>6</td>
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</table>

$^a$Data are from References 21, 22, 23.
$^b$Kinetic steps and rate constants are defined in Figure 1. Poly(U)-programmed ribosomes were used with ternary complexes of Phe-tRNA$^{Phe}$ (cognate), Leu-tRNA$^{Leu}$ (GAG) (near-cognate), or poly(A)-programmed ribosomes with Phe-tRNA$^{Phe}$ (noncognate). Rate constants that determine the discrimination are in bold type.
$^c$GTPase activity.
$^d$Grouped for the analysis, because the former reaction is rate limiting.

Kinetics and induced fit

- Kinetic proofreading, driven by induced fit, is the strategy used on the ribosome to discriminate between correct and incorrect aa-tRNA during both initial selection and proofreading. Binding ($k_{-2}$ and $k_{-7}$) and rate-limiting steps of rearrangement ($k_{3}$ and $k_{5}$)

- Induced fit implies that the binding of the correct substrate causes the structure of the catalytic center to change toward the active state, whereas binding of an incorrect substrate does not.

[Ref. 5,6, 9-12]
Decoding center and signal transduction

- In the ribosome likely candidate to form direct interactions with the codon-anticodon complex is helix 44 of 16S rRNA (around the 1500 loop) (footprinting and mutational analyses).

- Decoding center is likely to change when interactions between ribosomal residues and the codon-anticodon duplex are formed, thus creating a conformational signal that should be communicated to other parts of the ribosome, notably the GTPase and peptidytransferase centers on the 50S subunit.

- Two models for communication:
  1) Structural rearrangements of 23S rRNA and/or 16S rRNA are implicated in transmission of the GTPase activating signal to EF-Tu.

- 2) Mediated through the tRNA, which in the codon recognition complex interacts with both the mRNA and EF-Tu.

The 16S Ribosomal RNA Secondary Structure

![Diagram of the 16S Ribosomal RNA Secondary Structure]
Decoding center in the ribosome

Figure 6-65 Molecular Biology of the Cell Site (© Garland Science 2008)

Interactions at the Decoding Center

Interactions at a UG mismatch from a Leu near cognate ASL at the first codon position.

[Ref. 6]
Additional steps controlling translation accuracy

1- EF-Tu/tRNA interaction

*EF-Tu binds misacylated tRNAs over a much wider range of affinities (more strongly or weakly) than it binds the corresponding correctly acylated tRNAs.
*The thermodynamic contributions of the amino acid and the tRNA body to the overall binding affinity are independent of each other and compensate for one another when the tRNAs are correctly acylated. [LaRiviere et al Science 2001]

2- Quality control by the ribosome after peptide bond formation

Zaher&Green Nature 2009
Catalysis: is the Ribosome a Ribozyme?

Suggestive hints:

a) rRNA very conserved and important for function
b) RNA's can be catalytic (primitive RNA world)
c) Peptidyl-transferase inhibited by chloramphenicol: resistant mutations and footprinting map to highly conserved loop in 23S RNA. [Ref. 5]

Puromycin experiments

Experiment 1:
purified 50S subunit + puromycin + tRNA[^5S.fMet] + 33% methanol
Experiment 2:

50S Thermophilus aquaticus + PK in SDS --> phenol --> RNA + 5%protein. [Ref. 20]

BUT: T7 transcript: NO activity (modified bases?)

The puromycin group occupies the same location in both structures, and there are no proteins near that site.

[Ref. 28]
The catalytic center

The CCA portion of the mini-helix bound to the A-site and CCdA-p-Puro bound to the A- and P-sites. The base-pairing interactions between the P-site C74 and C75 and the P-loop of 23S rRNA on the left and the A-site C75 with the A-loop of 23S rRNA on the right.

Proposed chemical reaction for peptide bond formation
The 2’OH of the P-site tRNA directly assists in catalysis

[Ref. 32***]

DANCING AT CATALYTIC SITE OF THE RIBOSOME
Initiation of Translation in Prokaryotes

- Initiation is presumed to be the rate-limiting step in translation
- Initiation process serves to purposes:
  1. A particular mRNA is selected
     *(translation regulation)*
  2. The correct reading frame on the mRNA is selected
     *(translation accuracy)*

How to specify the Initiator tRNA

- The code is comma-free
- In vitro, ribosomes will translate all 3 frames
- There is only 1 Met codon (AUG), yet Met is used for initiation and elongation
Regions of E. coli initiator tRNA important for specifying its various properties highlighted in color

- Met is formylated
- Recognized by IF2 not EF-Tu
- Binds to P site (on 30S) not A site (on 70S)

[Ref. 38]

Anti-Shine-Dalqarno on the 16S rRNA

Complementary to Shine-Dalqarno on the mRNA
Special region of mRNA: RBS

Identified by:
1. RNase T1 protection
2. "toeprinting"
E. Coli 5’UTRs signature: "AGGAGG"
("dedicated ribosome")

[Ref. 39]

How to regulate the Efficiency of Initiation? (translation control)

Strength of cis-acting signals

1. Match to consensus S.D.
2. Spacing SD to AUG
3. 2° and 3° structure (occluding signals in hairpin)
Reconstruction of the initiation complex

Simonetti et al. Nature 2008

Termination

Termination codon moves to the A-site

No tRNA

3 factors: RF1: UAA/UAG and RF2: UAA/UGA: no GTPase domain homology to domains 3/5 EF-G

RF3: GTPase domain ~Tu and G. Non-codon specific

[Ref. 2]
Crystal structure of human eukaryotic release factor eRF-1

[Ref. 33-36]

E. coli RF1

in solution

bound to the ribosome

[Ref. 37]
RF1 interactions with the ribosome

Schematic of the Reactions Catalyzed at Peptidyl Transferase Center of the Ribosome

(A) Transsterification reaction.

(B) Proposed scheme for hydrolysis of the peptidyl-tRNA bond in site P by a catalytic water molecule coordinated by Glu-185 of the eRF1 GGQ motif in site A.

Mutations in RNAs of both subunits of the Escherichia coli ribosome caused defects in catalysis of peptidyl-tRNA hydrolysis in a realistic in vitro termination system. [Ref. 45]
Decoding center during initiation, elongation and termination

Termination reaction