Structural insights into aminoacyl-tRNA delivery by EF-Tu and translocation by EF-G

1. Structural Insights into Aminoacyl-tRNA Delivery by EF-Tu and Translocation by EF-G

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2. Elongation of the polypeptide chain by one amino acid

3. During the elongation cycle, two GTPases interact with the ribosome to catalyze tRNA incorporation and mRNA-tRNA translocation

- **Elongation Factor Tu (EF-Tu)**, in a ternary complex with GTP and aminoacyl-tRNA (aa-tRNA), binds to the ribosome and assists in the decoding process
- **Elongation Factor G (EF-G)**, in its GTP form, binds to the ribosome to effect the translocation of the tRNA-mRNA duplex from the A site to the P site

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X-ray crystallography showed EF-G and the ternary complex to be very similar, a discovery which led to the coinage of the term “molecular mimicry.”

Cryo-EM has shown that EF-G and the ternary complex bind to the same site of the ribosome.

We refer to this site, which involves multiple binding interactions with both subunits, as “the factor binding site.”

To observe the factors bound to the ribosome, we must stall the complex by the addition of an antibiotic, in this case fusidic acid or kirromycin.

With the help of cryo-EM, the binding sites of EF-Tu, EF-G, and tRNA have been mapped on the ribosome.

The following diagram depicts the binding configurations during the course of the elongation cycle.
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Decoding in two steps - introduction

- The decoding phase of the elongation cycle is divided into two steps, initial selection of the tRNA and proofreading, which are separated by an irreversible step of GTP hydrolysis.
- Only tRNAs satisfying cognate anticodon-codon match are in the end accommodated into the ribosomal A site.
- In the initial selection step, cognate aa-tRNAs lead to GTP hydrolysis and are thereby "passed" to the second step of proofreading; lower rates of GTP hydrolysis are observed for near-cognate aa-tRNAs.
- According to the widely accepted model of kinetic proof-reading by Hopfield, the high accuracy of decoding (better than 1 in 10,000) is achieved by higher forward rates for cognate aa-tRNAs than for near-cognate aa-tRNAs in the two steps, respectively.

Decoding: structural aspects of initial tRNA selection

- In structural terms, we can describe the initial tRNA selection as an interaction between the ternary complex - the complex formed by aminoacyl-tRNA, EF-Tu, and GTP – and the ribosome.
- An initial binding complex is formed, which allows the anticodon to interact with the codon at the A site of the small ribosomal subunit.
- Ternary complexes containing non-cognate aa-tRNAs are rejected since no stabilizing codon-anticodon interaction takes place.
- In the case of cognate aa-tRNAs, codon-anticodon interaction leads to a stabilization sufficient to send a conformational signal, presumably through the tRNA itself, to the site on the large subunit where GTP hydrolysis is initiated on EF-Tu.
- Near-cognate interaction (i.e., with one mismatch) may lead to a moderate degree of stabilization, which can also trigger GTP hydrolysis.

Decoding: structural aspects of initial tRNA selection

- According to the findings of Ramakrishnan at the Medical Research Council in Cambridge, England, stabilization of the codon-anticodon interaction is achieved by the action of two bases, A1492 and A1493, situated at the top of helix 44, base G530 of helix 18, and C1054.
- The helix 44 bases flip out of their stacked position, and bind tightly to the short Watson-Crick codon-anticodon helix formed between codon and anticodon in the cognate case.
- All these interactions are accompanied by a closing motion of the 16S rRNA around the decoding center, leading to further stabilization.
- In the near-cognate case, no stabilization occurs, and the ternary complex departs from the ribosome.

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Decoding: structural aspects of initial tRNA selection

GTP hydrolysis results in a large domain rearrangement in EF-Tu, which causes the factor to separate from the aa-tRNA, releasing the tRNA, and to depart from the ribosome (see next slide).

The conformational rearrangement causes the acceptor arm of aa-tRNA to be released from EF-Tu and accommodated in the peptidyl-transferase center, and EF-Tu to depart from the ribosome.

Our cryo-EM map (next slide, center) shows the complex after GTP hydrolysis, but with an antibiotic present (kikromycin) that prevents the domain rearrangement in EF-Tu, its separation from the aa-tRNA, and its departure from the ribosome.

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**tRNA selection and accommodation: cryo-EM snapshots in three states**

- Post-initiation (post-translocation)
- "A/T" Phe-tRNA^* • EF-Tu • GDP • kir
- "A/A" 3OG

**Kinked, twisted high-energy conformation of aa-tRNA in the A/T state**

- The cryo-EM map shows the tRNA in an unusual conformation, characterized by a kink and twist in the anticodon stem, compared to the way it appears in the X-ray structure of the aminoacyl-tRNA • EF-Tu • GTP ternary complex.
- We believe that this conformational change occurs upon binding of a ternary complex containing cognate aa-tRNA to the ribosome, that it leads to a high-energy state which is maintained until the aa-tRNA is accommodated.

**Decoding: structural aspects of proofreading and accommodation**

- In the proofreading step, near-cognate aa-tRNAs are rejected, while the cognate aa-tRNA is allowed to proceed to be accommodated in the A site.
- In structural terms, the codon-anticodon interaction is weak for near-cognate aa-tRNAs, leading to rejection with high likelihood, whereas this interaction is strong for the cognate aa-tRNA; in the latter case, the aa-tRNA remains bound long enough to swing into the A site of the large subunit once GTP is hydrolyzed and EF-Tu has departed.

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Decoding: structural aspects of proofreading and accommodation

- A comparison of the cryo-EM density maps in the pre-accommodated (A/T) and accommodated (A) states has allowed us to conclude that accommodation into the A site is achieved by a conformational change of the aa-tRNA from the kinked and twisted high-energy form to the "normal" conformation known from X-ray crystallography.

- This conformational change results in the move of the acceptor (CCA) arm of the aa-tRNA with the attached aminoacyl group into the peptidyl-transferase center.

Aminoacyl-tRNA accommodation and peptidyl-transfer, followed by translocation

- Accommodation is rapidly followed by the transfer of the peptide from the P-site tRNA to the amino acid of the newly arrived A-site tRNA.

- The entire moiety formed by mRNA, the deacylated P-site tRNA, and the A-site tRNA bearing the nascent peptide now has to be moved, or translocated, so that the next codon may enter the decoding site.

- The second part of this talk will focus on the structural aspect of this process of mRNA-tRNA translocation, which is catalyzed by EF-G.
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**Elongation cycle of protein synthesis**

1. Initiation
2. Elongation
3. Translocation
4. Termination

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**mRNA-tRNA translocation in two steps**

The translocation phase of the elongation cycle is divided into two steps:

1. Translocation of mRNA-tRNA moiety along with the small subunit relative to the large subunit, and
2. Translocation of the mRNA-tRNA moiety relative to the small subunit

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**mRNA-tRNA translocation in two steps**

The translocation phase of the elongation cycle is divided into two steps:

- The first step is triggered by the binding of EF-G to the ribosome: EF-G binding, prior to GTP hydrolysis, induces a large conformational change in the ribosome termed ratchet motion; the small subunit rotates relative to the large subunit by up to 10 degrees, bringing the tRNAs into the hybrid states denoted as A/P and P/E
- The second step of translocation is triggered by GTP hydrolysis: a conformational change in EF-G causes the mRNA–tRNA moiety to be disengaged from the decoding center, allowing the small subunit to move relative to mRNA-tRNA

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Translocation step I: ratchet motion
Upon binding of EF-G, the small subunit rotates counter-clockwise relative to the large subunit

EF-G, upon binding to the ribosome, changes its conformation

X-ray structure of EF-G-GDP, altered such that domains III, IV, V are rotated for best fit with the cryo-EM map

"Induced fit" – upon binding of EF-G, both ribosome and EF-G undergo structural changes, such that a perfect match is achieved

X-ray structure of EF-G-GDP
The EM density map shows that EF-G, when bound to the ribosome, is neither in its free GTP nor free GDP conformation

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Ratchet motion and translocation: experimental findings
- EF-G binding stabilizes the ratcheted conformation; in the cryo-EM map of the ribosome bound with EF-G, the P/E-site tRNA is visible.
- A crosslink placed across the interface between small and large subunit disables translocation (H. Nollet)

Summary: the ratchet motion leads to formation of the P/E position, and is necessary for translocation

L2-S6 crosslink inhibits translocation, by inhibiting the inter-subunit rotation

FRET studies, with a donor placed on the small subunit and two acceptors placed on the large subunit, on opposite sides of the donor, show anticorrelated distance changes (see next slide)

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Translocation of tRNA entails “hybrid states”

- tRNA proceeds "one step at the time":
  \[
  A/T \rightarrow A/A \rightarrow A/P \rightarrow P/P \rightarrow P/E \rightarrow E/E
  \]
  [position on small subunit] / [position on large subunit]

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Effect of the ratchet motion

- The ratchet motion has two important effects:
  1. mRNA and the tRNAs connected with it move along with the small subunit
  2. On the large subunit, each tRNA advances to the next site (P → E, A → P) while keeping its position on the small subunit, thus assuming a hybrid position (A/P and P/E, respectively)

Formation of the hybrid positions is required for translocation

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Basic concept: mRNA is first translocated along with the small subunit, relative to the large subunit, then it is translocated with respect to the small subunit.

Step I: mRNA moves along with 30S, relative to 50S (lock is closed)
Step II: 30S moves lock, relative to mRNA and 50S (lock is open)

As a result of the two steps, which involve opening and closing of a lock, mRNA is advanced by one codon.

This basic model requires some explanations and modifications:

1) What is the mechanism for closing and opening the lock?
2) The observed ratchet motion is not large enough to account for the ~20Å (~ three nucleotides) advance of the mRNA; how do we explain the rest?

1. What is the mechanism for closing and opening the lock between mRNA and the decoding center?

Answers:
- The mechanism for closing the lock is provided by the engagement of flipped-out bases A1492, A1493 and G530 with the codon-anticodon helix, which takes place for the cognate tRNA (Ramakrishnan’s results, Ogle et al., 2001)
- The mechanism for opening the lock is provided by a conformational change of EF-G triggered by GTP hydrolysis (Frank’s results, Taylor et al., 2007)
2) The observed ratchet motion is not large enough to account for the necessary ~20Å (= three nucleotides) advance of the mRNA with respect to the decoding center; how do we explain the rest?

Answer: an additional rotation of the small subunit head around the subunit’s long axis provides the additional advance.
Conclusions

- Cryo-EM reconstructions, interpreted by fitting of atomic models, have allowed us to step through the elongation cycle, and develop a mechanistic understanding of the dynamic processes of aa-tRNA selection and mRNA-tRNA translocation.
- Fundamental to the understanding of aa-tRNA selection and accommodation is the observation that the tRNA acts like a molecular spring stabilized to varying degrees by the interaction with mRNA and the decoding center.
- Fundamental to the understanding of mRNA-tRNA translocation is the observation that the architecture of the ribosome is unstable, allowing the ribosome to alternate between two different conformations related by the ratchet rotation.
- The role of elongation factor G is two-fold: (i) to stabilize one of these conformations temporarily, allowing key events of translocation to start, and (ii) to disengage the physical connection between the decoding center and the mRNA-tRNA moiety, allowing this moiety to advance relative to the decoding center.
- Thus, we are at the point now, thanks to seminal discoveries by cryo-EM, X-ray crystallography, and kinetic studies, where the entire mechanism of protein synthesis on the ribosome can be described in molecular detail—at least in broad strokes; refinement of this model will require higher-resolution cryo-EM density maps and X-ray structures of ribosome-elongation factor complexes.