Protein synthesis II
Biochemistry 302

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Two idealized views of the 70S ribosomal complex during translation

These models show all three sites (A, P, E) occupied by tRNAs. This would never occur during protein synthesis.
Prokaryotic translation: Cyclic nature of chain elongation

• **A site (AA-tRNA binding, EF-Tu-GTP hydrolysis)**
  - Loading of new AA-tRNA joined to **EF-Tu-GTP**
  - Codon positioning of AA-tRNA assisted by GTP hydrolysis
  - Dissociation of EF-Tu-GDP and reloading of “free” EF-Tu with GTP via **EF-Ts exchange factor**

• **A,P sites (transpeptidation)**
  - α-amino group from A site AA-tRNA attacks the carbonyl carbon of P-site bound peptidyl-tRNA
  - Formation of new peptide bond at A/P hybrid-site
  - P-site tRNA (w/o peptide) - leaving group

• **A, P, E site (translocation, EF-G-GTP hydrolysis)**
  - Transfer of uncharged tRNA to E site and ejection
  - Translocation of peptidyl-(3’OH) tRNA from A site to P site
  - Ribosome movement 3’ to the next codon
Model for peptide chain elongation in prokaryotes

EF-Tu-GTP is regenerated for another cycle.

50S subunit (Peptidyltransferase ribozyme complex)

30S subunit (Proofreading occurs after the charged tRNA is in place and both before and after GTP hydrolysis by EF-Tu.)
Regeneration of GTP-EF-Tu by EF-Ts assisted nucleotide exchange

This species is now ready to bind AA-tRNA for another round.

Note that this exchange rxn does not require GTP hydrolysis.

after release from ribosome

Fig. 27.23
Peptidyl transfer and translocation likely involves hybrid ribosome states (an idea championed by Harry Noller)

Anti-codon ends remain fixed in 30S subunit while acceptor ends of tRNAs are free to move leftward in 50S subunit.
A look at the transition state of peptidyl transferase

Tetrahedral carbon intermediate resolves to yield a deacylated tRNA (P) and a peptidyl tRNA extended by one amino acid.

Peptidyl transferase inhibitors with P or A site ribosome binding sites.

Puromycin resembles 3′ end of amino-acylated tRNA.

Peptidyl transferase region of *Haloarcula marismortui*

No proteins near (~18 angstroms) of active site. Catalytic activity depends entirely on RNA.

Atoms belonging to 23S rRNA >95% conserved in all three kingdoms are red.

But what gives the RNA its catalytic power....making A2486 a stronger base via charge relay

Negative electrostatic charge originating from buried A2485 phosphate could be relayed to N3 of A2486 via the proposed mechanism to generate an imino tautomer.

Charge relay mechanism is important in serine protease catalysis.

Raising the pKa of A2486 makes the proximal α amino group of AA-tRNA a better nucleophile.

N3 represented as standard tautomer but is thought to function as a general base.

Tetrahedral carbon intermediate stabilized by H-bonding between protonated N3 and oxyanion.

Deacylation: Proton transfer from N3 to the peptidyl-tRNA 3’OH.

Termination of protein synthesis

- Signaled by arrival of stop codon in the A site
- No corresponding stop tRNA so release factor complex (RF1, RF2, RF3) binds to ribosome instead. RF3 is a GTPase.
- Peptidyltransferase transfers P-site peptide chain to a water molecule. Release of peptide chain and RFs is stimulated by RF3-mediated hydrolysis of GTP.
- Unstable 70S ribosome dissociates assisted by IF1 and IF3.
- 30S subunit stays attached to polycistronic messages.

Fig. 27.26
Counting the energy cost of translation (for a polypeptide of N residues)

- 2N: ATPs required to charge tRNAs (each charging reaction $\text{ATP} \rightarrow \text{AMP} + \text{PPi}$)
- 1: GTP needed for initiation
- N-1: GTPs required for N-1 peptide bonds during elongation mediated by EF-Tu-GTP
- N-1: GTPs required for N-1 translocations by EF-G-GTP
- 1: GTP required for termination
- Sum: 4N high-energy phosphate molecules must be hydrolyzed to complete a peptide chain of N residues
  - $\sim$160 kJ/mol per peptide bond
  - Entropy price paid by the cell for making a specific peptide sequence in a rapid and accurate way ($\sim20^N$ possibilities if process were random)
Translational efficiency enhanced by polyribosomes (elongation is rate-limiting)

Fig. 27.29

In *E. coli*, 15,000 ribosomes synthesizing @ 15 AA/sec \(\rightarrow\) 750 proteins of 300 AA/sec.

One ribosome, one mRNA model does not account for the total rate of protein synthesis per *E. coli* cell. As many as 50 ribosomes bound/RNA under certain conditions.
Summary of important differences in translation machinery in eukaryotes

• Ribosome
  – Additional 5.8S rRNA component in large 60S subunit
  – mRNA aligned on the small 40S subunit using 5′ cap (no Shine-Dalgarno sequence or fMet)
  – “Scanning” identifies correct start Met

• Initiation factors (multiple eIFs)
  – Many more required (11 vs 3)
  – Some bind mRNA, others attach to ribosomal subunits

• Elongation factors (eEFs)
  – No differences, all orthologs of prokaryotic EFs

• Termination (only one release factor)
  – eRF recognizes all stop codons (UAA, UAG, UGA)
Translation initiation in eukaryotes

40S subunit - eIF3, eIF4C preassembly → 43S complex

mRNA-eIF1, eIF1A, eIF4A, eIF4B, eIF4F preassembly

Scanning for AUG start by initiation complex requires ATP

60S subunit joins complex

eIF2-GTP-Met-tRNA preassembly

eIF2 recycling via eIF2B-mediated GDP-GTP exchange

eIF6 ribosome dissociation

Fig. 28.35
Translational control in eukaryotes

• mRNA level
  – Sequestration of specific mRNAs by mRNA-binding proteins (oogenesis and spermatogenesis)
  – Rapid degradation of specific mRNAs (cell cycle control)
  – Regulatory elements (stem loops) in 5′-UTR

• eIF2 kinases
  – Heme controlled inhibitor (HCI) – eIF2 kinase activated when heme levels are low (globin levels high) but inactivated when heme levels rise
  – Interferons (antiviral agents produced by immune cells in response to infection) – induce synthesis of eIF2 kinase to inhibit viral protein synthesis in infected cells
Translational control by eIF2 kinases (regulation of globin synthesis in rbc)

Fig. 28.37

Falling heme levels

eIF2 not available for translation initiation when ↓ heme
Many antibiotics are prokaryotic protein synthesis inhibitors

- **Streptomycin**
  - Binds to 30S subunit, inhibits codon:anticodon bp
- **Tetracycline (cannot pass cell membranes in euk)**
  - A site drug, inhibits tRNA-binding
- **Puromycin (also inhibits protein syn in euk too)**
  - A site drug, promotes transfer of nascent chain to drug
- **Chloramphenicol**
  - P site drug, inhibits the peptidyl transferase reaction
- **Erythromycin**
  - P site drug, inhibits the translocation step
Antibiotics that block protein synthesis

Blocks AA-tRNA: codon base-pairing

Inhibits AA-tRNA binding to ribosome

Looks like peptide bond

Binds 23S rRNA, blocks elongation

Looks like 3’ end of AA-tRNA

Fig. 27.28
Mutations conferring antibiotic resistance map to 23S rRNA peptidyltransferase loop

View down active site cleft

View from peptide exit tunnel

Yarus inhibitor: CCdAp-Puromycin