

BIO240 Lecture Notes

Chapter 6: Translation

tRNA - clover leaf configuration [Figs. 5.20] formed by binding of complementary sequences in folding molecule

1. anticodon loop - contains anticodon sequence that matches genetic code codons of mRNA
2. Acceptor stem - 3' end of molecule binds specific amino acid (tRNA is **charged** when it contains the appropriate attached amino acid)

Charging of tRNA [Figs. 6.9 and 6.10]:

Transfer RNAs must exist for 20 amino acids. Sometimes the tRNA anticodon is recognized by specific enzyme associated with proper amino acids. Sometimes other portions of the tRNA act to guarantee interaction only with the proper enzyme. Enzymes responsible are called **aminoacyl-tRNA synthetases** and charged tRNA is also called **aminoacyl-tRNA**.

Charging tRNA requires ATP. Aminoacyl-tRNA synthetases first associate with proper amino acids and ATP. Amino acid is then associated with AMP (ATP loses two phosphates) forming aminoacyl-AMP. Next uncharged tRNA associates with enzyme such that 3' end is adjacent to amino acid. Synthetase then switches aminoacyl bond from AMP to tRNA so tRNA and amino acid to form proper **aminoacyl-tRNA** (or **aa-tRNA**). Both aa-tRNA and AMP then released from synthetase.

Translation: simplified as in prokaryotes

1. Large and small subunits of rRNA associate with a charged, specialized "initiator tRNA", GTP, Mg^{2+} , and several proteinaceous initiation factors.
2. Initiation codon of mRNA causes charged formylmethionyl tRNA to move to small subunit of ribosome - structure called the **initiation complex**
3. Reading frame is now set so that all following triplets are read in turn

Translation occurs in three stages similar to those of transcription: **initiation, elongation, and termination.**

Initiation: Prokaryotic Model

Formation of initiation complex: [Figure 6.11]

Small subunit of ribosome binds with initiation factors (IFs) IF1, IF2, IF3, and GTP (energy source).

Initiation Complex binds to **Shine Delgarno** sequence “5’ AGGAGG 3’ ” on leader sequence of mRNA (which sets the reading frame of initial AUG) and losses IF3.

Initiation codon of mRNA binds to fMet tRNA (formylmethionine tRNA). The Shine-Delgarno sequence, 5’ AGGAGG 3’, complements sequence 3’ NCCUCC 5’ of the 16S rRNA subunit of the ribosomal small subunit. Together, these components form the 30S initiation complex.

Thereafter, attachment of the 50S (large) subunit is facilitated through dephosphorylation of GTP to GDP and removal of IF1 and IF2. The 30S initiation complex and 50S ribosomal subunit complex together form a 70S initiation complex where fMet tRNA anticodon 3’ UAC 5’ complements mRNA start codon 5’ AUG 3’ in the P (peptidyl) site of the ribosome. (The 16S, 30S, 50S and 70S subunits / initiation complexes are designated by where they separate out in an increasing density sucrose gradient. The 16S subunit is least dense and separates out at 16 “S” units of sucrose gradient, the 30S separates out at 30 “S” units of gradient. Therefore, when you combine the 30S and 50S subunits, they together separate out at a higher gradient, but not at a gradient equal to their additive combined individual gradients).

The ribosome has two sites for binding of aminoacyl-tRNA (or “charged tRNA”) the

Peptidyl (or P) site and the Aminoacyl (or A) site.

Initiation: Eukaryotic Model

Eukaryotic initiator factor eIF-4E which includes cap-binding protein (CBP) binds to cap of mRNA – recall that prokaryotic mRNA has no cap.

40S ribosomal (small) subunit binds to Met-tRNA (unmodified methionine but a special initiator form of tRNA), additional eIFs and GTP forming a complex that moves along mRNA until it finds initial start codon, **AUG**, which is embedded in the Kozak sequence, 5' **ACCAUGG** 3'.

Both Kozak and Shine-Delgarno sequences greatly facilitate initial binding of mRNA to small subunit of ribosome. Next, this 40S initiation complex binds to the 60S (large) ribosomal subunit with help of GTP, and eIFs are displaced. This latter complex is the 80S initiation complex.

Characteristics of the Genetic Code: [Fig. 6.7]

1. linear form using RNA bases that compose mRNA its "letters". The mRNA sequence is derived from the complementary (coding) strand of DNA.
2. each "word" of the code contains three mRNA letters. Each group of three ribonucleotides is called a **codon** and the code is a **triplet**
3. The code is **unambiguous**, so that each codon specifies only one amino acid.
4. The code is **degenerate**, so that several different codons may specify the same amino acid

5. The code contains "start" and "stop" codons indicating where translation should be initiated on the DNA and terminated, respectively.
6. There is no internal punctuation between start and stop codons, translation of mRNA continues reading one codon after another until a stop is reached
7. The code is **nonoverlapping**. Once translation commences, any single ribonucleotide at a specific location within the mRNA is part of only one triplet
8. The code is nearly **universal** i.e. it is the same code for nearly all living things on the planet.

Elongation: Prokaryotes and Eukaryotes

Each codon passes in sequence through ribosome (small and large subunits necessary) and binds temporarily with a complementary code on transfer RNA (tRNA) called the anticodon. Each tRNA contains only one anticodon and each such type of tRNA carries only one type of amino acid. As each anticodon binds temporarily with each codon passing linearly through the ribosome, each associated amino acid is linked together in sequence. Messenger RNA is read from 5' to 3'.

The "wobble" hypothesis states that the third codon position is less bond specific and less critical therefore in attracting the associated anticodon. This explains the particular degenerate nature observed in the genetic code where several codons that differ only in the third position will code for the same tRNA type and its associated amino acid.

Specific events of elongation [Fig. 6.13]:

Next sequential charged tRNA is complexed with elongation factor – Tu (**Ef-Tu**) and GTP and bound to the A-site of the ribosome. Peptide bond forms via **peptidyl**

transferase (a ribozymes, part of the 23S subunits of ribosome) between fMet (or Met) and next amino acid. Linked amino acids attached to tRNA in A-site (a peptidyl-tRNA).

Ribosome employs **Ef-G / GTP complex (eEf-2 / GTP complex** in eukaryotes) to move along mRNA to next codon – **translocation**. Uncharged tRNA moves from P-site to E-site. Peptidyl-tRNA moves from A-site to P-site. Uncharged tRNA is released from E-site.

Next sequentially charged tRNA complexed with Ef-Tu and GTP moves into A-site and cycle repeats. Initiation site of mRNA also now free to complex with another ribosome such that prokaryotic and eukaryotic translation can occur at multiple ribosomes simultaneously forming **polyribosome** or **polysome**.

Eukaryotes employ more elongation factors and elongation events occur in slightly different sequence compared to prokaryotes.

Termination:

Briefly, termination or stop codons UAG, UAA, UGA signal GTP dependent release factor actions that cleave polypeptide chain from terminal RNA. There are no tRNAs with anticodons that complement these triplets. Ribosomal interaction with a stop codon triggers stepwise termination:

1. release of polypeptide from the tRNA in P-site through reaction catalyzed by peptidyltransferase.
2. release of tRNA from ribosome,
3. disassociation of small and large subunits and of the RF from the mRNA

[Fig. 6.16].

In both prokaryotes and eukaryotes, the initiating amino acids (fMet and Met, respectively) are usually cleaved from the polypeptide chain as protein is modified for use by cell.

Distribution of Products of Translation [Fig. 6.17]:

Signal Sequences: bind to ER before translation is completed. These signal sequences allow modification and transport mechanisms specific to the end product to be initiated. Signal sequences (which are not universal to all gene products) are recognized by **signal recognition particles (SRPs)** an RNA-protein complex which then binds to docking protein of ER membrane. After signal sequence fully passes into cisternal space of ER it is removed by **signal peptidase**.

Golgi apparatus sorts modified products and packages them in membrane-bound vesicles for transport to specific destinations.

Proteins:

Four levels of structure. Final, three-dimensional configuration is determined by linear sequence of amino acids (primary structure). The same sequence of amino acids constructed under the same cellular conditions will take on the same three dimensional configuration. Structures that function efficiently are selected for. Thus, genes that produce efficient products are selected for.