

LECTURE 24

TRANSLATION

A. Components of translation

1. Ribosomes: molecules that are roughly half ribosomal RNA (rRNA) and half protein, and are composed of two subunits (one small and one large).

a) In *E. coli*, the small (30S) and large (50S) subunits join to form the intact, functional ribosome (70S).

30S subunit: 16S rRNA + >20 proteins

50S subunit: 5S rRNA + 23S rRNA + >30 proteins

(i) the rRNA is transcribed as a 30S precursor (below) which is cleaved autocatalytically into four cleavage products (one of which is a tRNA molecule).

(ii) the 30S transcribed unit is organized as follows:

|16S rRNA | 4S tRNA | 23S rRNA | 5S rRNA |ⁿ

b) In eukaryotes, the small (40S) and large (60S) subunits join to form the intact, functional ribosome (80S).

40S subunit: 18S rRNA + >30 proteins

60S subunit: 5S rRNA + 5.8S rRNA + 28S rRNA + ≈ 50 proteins

(i) The rRNA is transcribed as a 45S precursor (below) which is cleaved autocatalytically into three cleavage products.

(ii) The organization of the eukaryotic rRNA cluster is as follows:

| NTS | ETS¹ | 18S rRNA | ITS¹ | 5.8S rRNA | ITS² | 28s rRNA | ETS² |ⁿ

(iii) The rRNA genes are tandemly arranged at one or more chromosomal sites called NORs (nucleolar organizer regions) and their transcription is via RNAP I. The 5S rRNA gene is located elsewhere in the genome and is transcribed by RNAP III.

c) Ribosomal binding sites occur on both the smaller and larger subunits

(i) The smaller ribosomal subunit (30S or 40S) contains the binding site for the mRNA.

(ii) The larger ribosomal subunit (50S or 60S) contains at least four binding sites.

- A site → aminoacyl site (site for incoming tRNA-amino acid)
- P site → peptidyl site (growing polypeptide site)
- E site → exit site (departing tRNA site)
- GTP site → energy site (hydrolysis of GTP)

2. Transfer RNAs: small molecules that are 4S or 70-90 nucleotides long.

a) There are from 1-4 tRNA genes for each of the common 20 amino acid molecules.

b) Amino acids are “loaded” onto tRNAs by amino acid-activating enzymes called amino-acyl tRNA synthetases. There is at least one amino-acid-activating enzyme for each of the 20 common amino acids.

(i) Each aa-tRNA synthetase also is capable of ‘proofreading’ to insure that the proper amino acid is ‘loaded’ on to the proper tRNA molecule

c) tRNAs exist in standard stem-and-loop structure, forming (in two dimensions) a three, cloverleaf shaped molecule.

d) Functions of tRNAs reside in each of the clover arms and the 3’ CCA terminus:

- (i) recognition of mRNA codon (anticodon)
- (ii) recognition of the appropriate amino-acid activating enzyme
- (iii) binding to appropriate sites on the ribosome
- (iv) CCA binding to the amino acid

Initiation of translation:

1) Initiation complex forms: complex includes 30S (40S) ribosomal subunit, a messenger RNA (mRNA), several initiation factors (proteins), and an initiating tRNA (tRNA_i).

2) The 50S (60S) subunit positions tRNA_i into the P site.

3) Initiation in prokaryotes:

a) tRNA_i^{f-Met} -- Initiator tRNA that recognizes the start codon (AUG) and “loads” a formylated methionine. This initiator tRNA also can recognize GUG (valine) when present (rarely).

b) Shine-Dalgarno sequence -- A polypurine tract (AGGAGG in *E. coli*) that is located on the mRNA about seven nucleotides *upstream* from the AUG start codon.

(i) The Shine-Dalgarno sequence is complementary to a sequence near the 3’ end of the 16S rRNA, and presumably is responsible for binding the mRNA to the

smaller subunit of the ribosome. It also is the site where the initiation complex forms to begin the search for an AUG start codon.

- (ii) Note that AUG start codons in prokaryotes (and eukaryotes) do not occur at the 5' end of the mRNA but rather are downstream (but not too far).

3. Initiation in eukaryotes:

- a) $tRNA_i^{Met}$ -- An initiator tRNA (like prokaryotes) but that does not carry a formylated methionine.
- b) There also is no Shine-Dalgarno sequence. The initiation complex forms at the 5' end of the mRNA and begins the search for an AUG start codon.
- c) A cap-binding protein (CPB) binds the 7-MG at the 5' end of the "processed" eukaryotic mRNA and is involved in formation of the initiation complex.

B. Elongation:

1. The initial step, subsequent to the binding of the $tRNA_i$ in the P site, is binding of an aminoacyl-tRNA to the A site via codon-anticodon pairing.
2. The growing polypeptide chain in the P site is then transferred to the amino acid on the tRNA at the A site.
3. The (now) uncharged $tRNA_i$ at the P site is moved (translocated) to the E site, and the tRNA with the growing polypeptide chain is translocated from the A site to the P site.
4. The entire ribosome moves 3'-ward (downstream) to the next codon, where a new aminoacyl-tRNA is bound via codon-anticodon pairing to the A site.
5. The process continues repeatedly until termination of translation.
 - a) Several ribosomes (polyribosomes) translate the same mRNA sequentially,

C. Termination:

1. Termination of translation occurs when a ribosome "hits" any of three stop codons (below). There are several *release factors* (proteins) that assist in termination of translation and the release of the amino acid chain (polypeptide).

Stop codons are: UAG – amber
 UAA – ochre
 UGA – umber

THE GENETIC CODE

- A. The genetic code is responsible for the incorporation of 20 common amino acids into the growing amino acid (polypeptide) chain during translation. The cracking of the code (along with other aspects of translation) led to several Nobel prizes.
- B. Reading the code is straightforward.
- C. Important properties of the code include the following:
- 1) The code is comprised of nucleotide triplets (codons), i.e., is a 3-base code.
 - 2) The code is non-overlapping and comma free.
 - 3) The code is punctuated with periods (stop codons).
 - 4) The code is nearly universal. Known exceptions are a few code words in mitochondria.
 - 5) There is co-linearity of gene (DNA and mRNA sequence) and protein (amino acid sequence).
 - 6) The code is buffered against single base-pair substitutions.
 - a) The code is degenerate, i.e., more than one code word calls for the same amino acid.
 - (i) the only “loners” are AUG (methionine) and tryptophan (UGG).
 - (ii) degeneracy exists in two-fold, four-fold, and six-fold states
 - (iii) transitions and transversions
 - b) Chemical buffering: where amino acid changes generally result in the new amino acid having similar properties chemically to the replaced amino acid
 - (i) “chemical” similarities: acidic, basic, hydrophobic, hydrophilic, etc.
 - (ii) examples include lysine and arginine (basic amino acids), aspartate and glutamate (acidic amino acids)
 - c) *Wobble Hypothesis*: buffering via tRNA

5' base in anticodon3' base in mRNA

G
C
A
U
I

U or C
G
U
A or G
A, U, or C