

Molecular Biology

Lecture 6

Translation

The genes in DNA encode protein molecules, which are the "workhorses" of the cell, carrying out all the functions necessary for life. For example, enzymes, including those that metabolize nutrients and synthesize new cellular constituents, as well as DNA polymerases and other enzymes that make copies of DNA during cell division, are all proteins.

In the simplest sense, expressing a gene means manufacturing its corresponding protein, and this multilayered process has two major steps. In the first step, the information in DNA is transferred to a messenger RNA (mRNA) molecule by way of a process called transcription.

During translation, which is the second major step in gene expression, the mRNA is "read" according to the genetic code, which relates the DNA sequence to the amino acid sequence in proteins. Each group of three base pairs in mRNA constitutes a codon, and each codon specifies a particular amino acid (hence, it is a triplet code). The mRNA sequence is thus used as a template to assemble—in order—the chain of amino acids that form a protein.

		Second nucleotide					
		U	C	A	G		
First nucleotide	U	UUU Phe	UCU	UAU Tyr	UGU Cys	Third nucleotide	U
		UUC	UCC Ser	UAC	UGC		C
		UUA Leu	UCA	UAA STOP	UGA STOP		A
		UUG	UCG	UAG STOP	UGG Trp		G
	C	CUU	CCU	CAU His	CGU		U
		CUC Leu	CCC Pro	CAC	CGC Arg		C
		CUA	CCA	CAA Gln	CGA		A
		CUG	CCG	CAG	CGG		G
	A	AUU	ACU	AAU Asn	AGU Ser		U
		AUC Ile	ACC Thr	AAC	AGC		C
		AUA	ACA	AAA Lys	AGA Arg		A
		AUG Met	ACG	AAG	AGG		G
	G	GUU	GCU	GAU Asp	GGU		U
		GUC Val	GCC Ala	GAC	GGC		C
		GUA	GCA	GAA Glu	GGA		A
		GUG	GCG	GAG	GGG		G

- **The start codon is AUG.** Methionine is the only amino acid specified by just one codon, AUG.

- **The stop codons are UAA, UAG, and UGA.** They encode no amino acid. The ribosome pauses and falls off the mRNA.
- The stretch of codons between AUG and a stop codon is called an **open reading frame (ORF)**. Computer analysis of DNA sequence can predict the existence of genes based on ORFs.
- **Other amino acids** are specified by more than one codon--usually differing at **only the third position**.

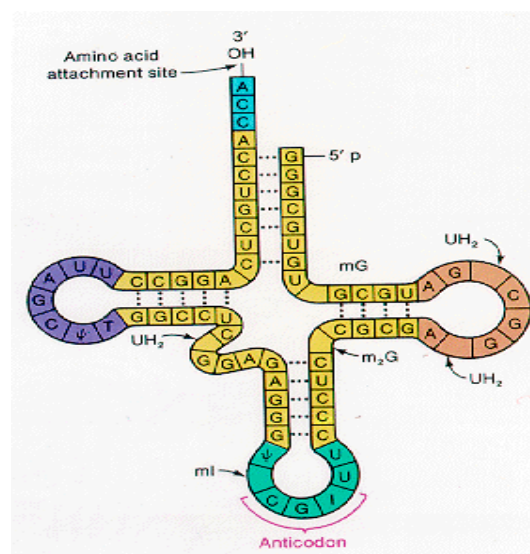
Where Translation Occurs

Within all cells, the translation machinery resides within a specialized organelle called the ribosome. In eukaryotes, mature mRNA molecules must leave the nucleus and travel to the cytoplasm, where the ribosomes are located. On the other hand, in prokaryotic organisms, ribosomes can attach to mRNA while it is still being transcribed. In this situation, translation begins at the 5' end of the mRNA while the 3' end is still attached to DNA.

In all types of cells, the ribosome is composed of two subunits: the large (50S) subunit and the small (30S) subunit (S, for svedberg unit, is a measure of sedimentation velocity and, therefore, mass). Each subunit exists separately in the cytoplasm, but the two join together on the mRNA molecule. The ribosomal subunits contain proteins and specialized RNA molecules—specifically, ribosomal RNA (rRNA) and transfer RNA (tRNA). The tRNA molecules are adaptor molecules—they have one end that can read the triplet code in the mRNA through complementary base-pairing, and another end that attaches to a specific amino acid.

Within the ribosome, the mRNA and aminoacyl-tRNA complexes are held together closely, which facilitates base-pairing. The rRNA catalyzes the attachment of each new amino acid to the growing chain.

The structure of transfer RNA (tRNA):



Transfer RNA (tRNA) has the following structure:

- 3'OH end esterifies with COOH of amino acid:

This process, called charging, is catalyzed by a tRNA transferase, or aminoacyl tRNA synthetase, specific to the tRNA type. There are one or more tRNA types, specified by different genes, for EACH amino acid.

- Anticodon loop, capable of complementary base pairing to a codon on the message. May contain the unusual base inosine, which is capable of binding to more than one base. The "wobble hypothesis," by Francis Crick in the '60s, first showed how inosine could enable one tRNA to recognize more than one codon. Otherwise, the cell would need more than 60 different tRNAs.

Ribosome

The **ribosome** is a large and complex molecular machine, found within all living cells, that serves as the primary site of biological protein synthesis (translation). Ribosomes link amino acids together in the order specified by messenger RNA (mRNA) molecules. Ribosomes consist of two major components — the small ribosomal subunit which reads the RNA, and the large subunit which joins amino acids to form a polypeptide chain. Each subunit is composed of one or more ribosomal RNA (rRNA) molecules and a variety of proteins. The ribosomes and associated molecules are also known as the *translational apparatus*.

The ribosomal subunits of prokaryotes and eukaryotes are quite similar.^[8]

The unit of measurement is the Svedberg unit, a measure of the rate of sedimentation in centrifugation rather than size, and this accounts for why fragment names do not add up (70S is made of 50S and 30S).

Prokaryotes have 70S ribosomes, each consisting of a small (30S) and a large (50S) subunit. Their small subunit has a 16S RNA subunit. The large subunit is composed of a 5S RNA subunit, a 23S RNA subunit and 31 proteins.

Eukaryotes have 80S ribosomes, each consisting of a small (40S) and large (60S) subunit. Their 40S subunit has an 18S RNA and 33 proteins.^{[12][13]} The large subunit is composed of a 5S RNA, 28S RNA, a 5.8S RNA subunits and 46 proteins

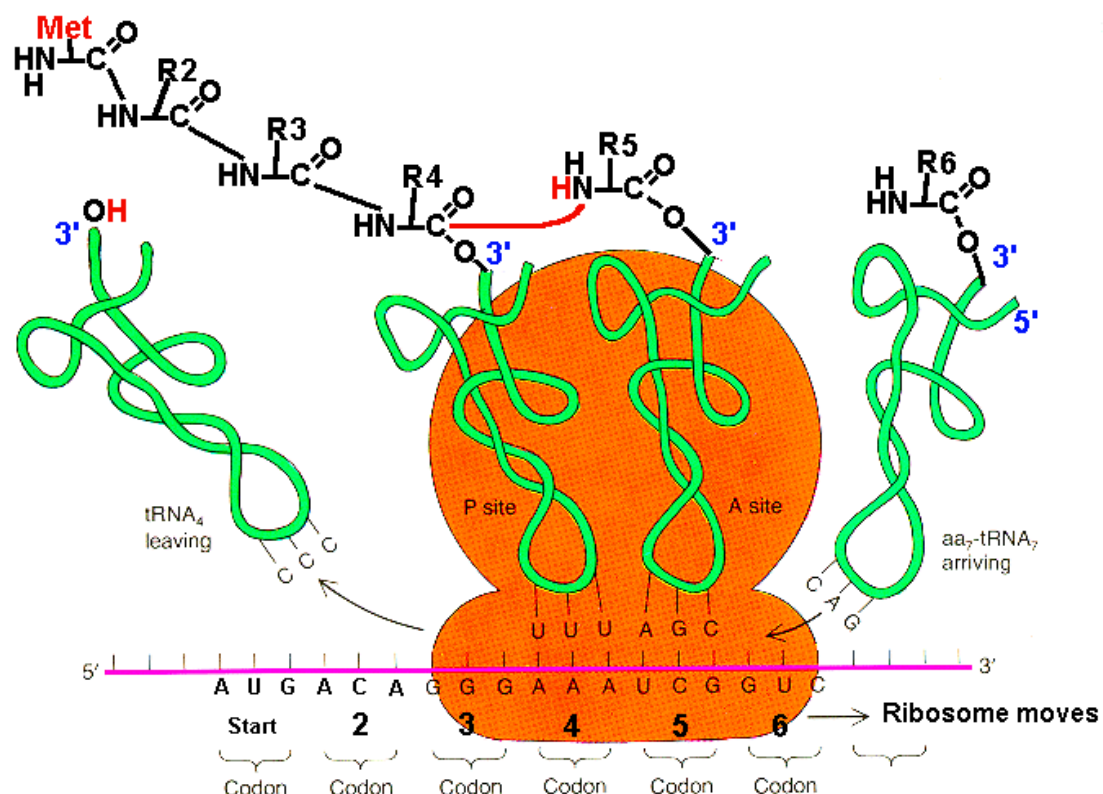
Translation of mRNA into polypeptide

Translation requires initiation, elongation, and termination. Translation is performed by the ribosome, an organelle composed of more than fifty different proteins plus two structural rRNAs,

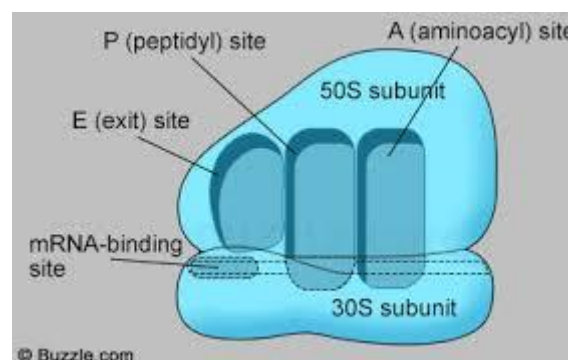
(1) Initiation occurs by binding of the 30s subunit to the mRNA. In bacteria, the mRNA binds by hybridization of a special sequence to the Shine-Dalgarno sequence of the 16s rRNA, part of the 30s subunit. The ribosome then finds the first AUG sequence on the mRNA, where it binds the anti-codon of a Met-tRNA, at the P site.

(2) Elongation occurs by successive amidation of the nascent (growing) chain. The 50s subunit now binds, creating the A site. Each new aminoacyl-tRNA enters at the A site, where it transfers the amino end of its amino acid to the carboxylic end of the nascent chain. The entire ribosome now "translates" over one codon position, so that the nascent chain is now bound to the P site. Elongation requires energy provided by GTP.

(3) Termination occurs when the A site reaches a stop codon. Since no tRNA exists with an anticodon complementary to the stop codon, the ribosome "pauses" until at last it "falls off" the mRNA, and the polypeptide chain terminates. This process is facilitated by a release factor protein that binds into the ribosomal A site containing a stop codon to help with protein release.



Modified from Griffiths et al., AN INTRODUCTION TO GENETIC ANALYSIS, 6th Ed., W.H. Freeman & Co., 1996.



Comparing Eukaryotic and Prokaryotic Translation

The translation process is very similar in prokaryotes and eukaryotes. Although different elongation, initiation, and termination factors are used, the genetic code is generally identical. As previously noted, in bacteria, transcription and translation take place simultaneously, and mRNAs are relatively short-lived. In eukaryotes, however, mRNAs have highly variable half-lives, are subject to modifications, and must exit the nucleus to be translated; these multiple steps offer additional opportunities to regulate levels of protein production, and thereby fine-tune gene expression.

Posttranslational modification (PTM) : is a step in protein biosynthesis. The posttranslational modification of amino acids extends the range of functions of the protein by attaching it to other biochemical functional groups (such as acetate, phosphate, various lipids and carbohydrates), changing the chemical nature of an amino acid (e.g. citrullination), or making structural changes (e.g. formation of disulfide bridges).

Also, enzymes may remove amino acids from the amino end of the protein, or cut the peptide chain in the middle. For instance, the peptide hormone insulin is cut twice after disulfide bonds are formed, and a propeptide is removed from the middle of the chain; the resulting protein consists of two polypeptide chains connected by disulfide bonds. Also, most nascent polypeptides start with the amino acid methionine because the "start" mRNA also codes for this amino acid. This amino acid is usually taken off during post-translational modification.