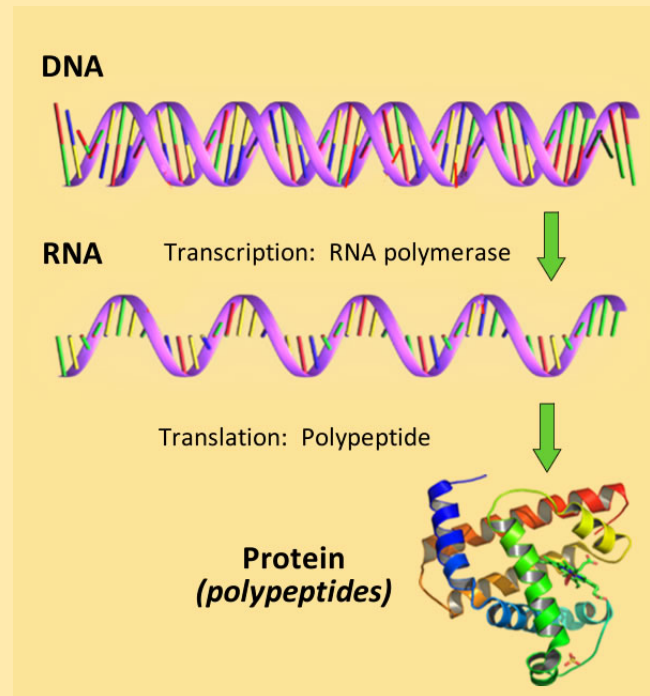


DNA TO PROTEIN OVERVIEW



Unit Overview

This unit provides information needed to understand how the digitally encoded information in DNA is translated into a functional protein that can be used for diagnostics, analysis and measurements in medical applications.

This information will help you to better understand the applications of MEMS (MicroElectroMechanical Systems) within the medical field.

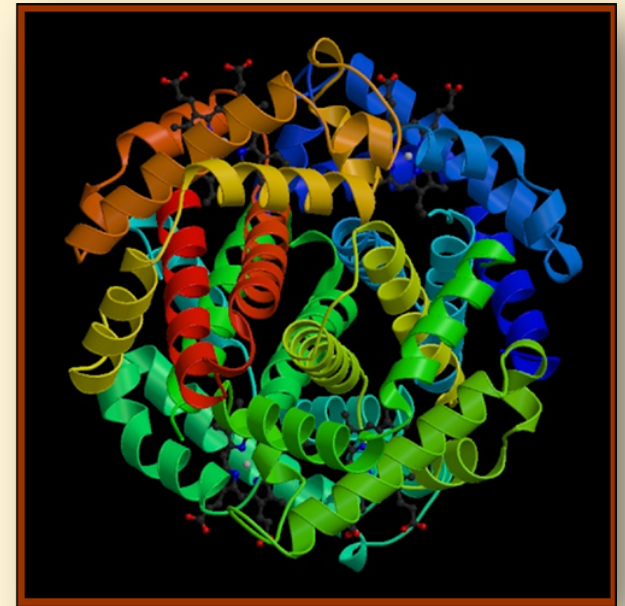
Objectives

- ❖ Describe the genetic code.
- ❖ Explain the "central dogma" of biology: DNA → RNA → Protein (polypeptide), and differentiate the separate parts that comprise the dogma.
- ❖ Describe the function of the components of Protein Translation.

Introduction

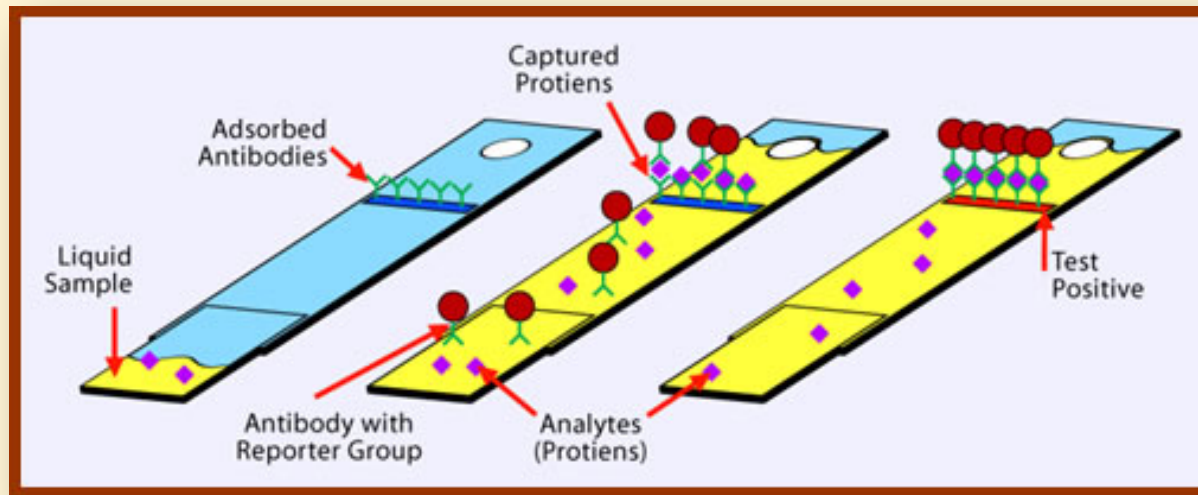
Most of the properties of living organisms ultimately arise from a class of molecules known as proteins (polymers composed of amino acids). These polymers fold into three-dimensional structures with unique functions.

The structure of the protein is dictated by the amino acids, and the amino acid order is dictated by the linear sequence of information found within a gene in an organism's DNA.



Protein Tertiary Structure
[Image courtesy of RSCB Protein
Data Bank]

Protein Applications



Home Pregnancy Test

The functions of proteins are taken advantage of in many ways.

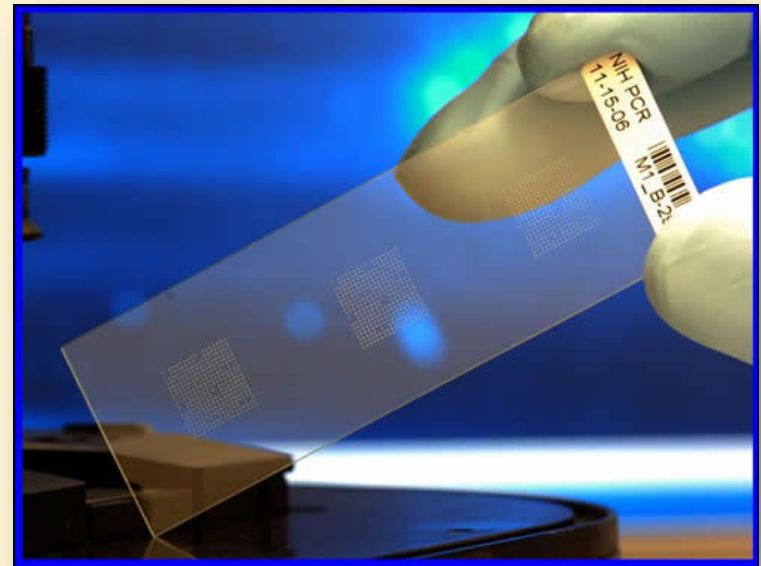
- ❖ Diabetics rely on glucometer strips that contain specific protein enzymes capable of catalyzing reactions that aid in determining blood glucose levels.
- ❖ Home pregnancy tests rely on proteins called antibodies found in the urine of pregnant women. These proteins react with the coating on the pregnancy test strip enabling diagnosis.

MEMS Applications

The emerging field of proteomics is analyzing the complex range of protein expression within cells.

Biologists use this information to create micro protein arrays on chips. These arrays investigate both the interactions of the arrayed proteins with other proteins as well as their potential for chemical modification.

This biochip was developed by Argonne National Laboratories. It has hundreds to thousands of gel drops on a glass, plastic or membrane support. The biochip system can identify infectious disease strains in less than 15 minutes when testing protein arrays and in less than two hours when testing nucleic acid arrays. [Image courtesy of Argonne National Laboratories]



Questions to be Answered

Protein microarrays and their electronic interface are microelectromechanical systems or MEMS or more specifically bioMEMS.

These methodologies rely on an understanding of the way in which information encoded digitally in DNA translates into a functional protein.

This unit will help to answer these questions:

- ❖ How did researchers crack this genetic code?
- ❖ Is the genetic code universal?
- ❖ What do we know about information flow within a cell?

Central Dogma of Biology

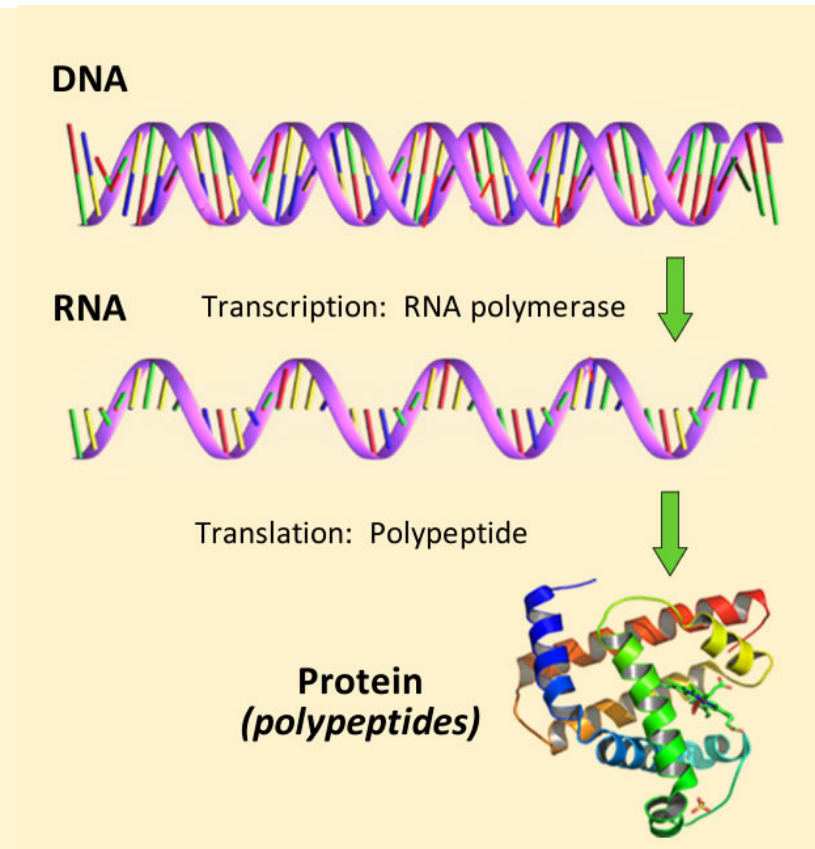
- ❖ In 1902, Archibald Garrod and his colleagues described a connection between a gene and a protein.
- ❖ In the 1940's, George Beadle and Edward Tatum's experiments showed that one gene specifies one enzyme.
- ❖ In 1957, Francis Crick proposed that genetic information flows in one-direction, from DNA to RNA to protein. He named this proposal the "Central Dogma" of biology.

Central Dogma of Biology

Today, the Central Dogma is often revised to

- ❖ *DNA is transcribed to RNA, and*
- ❖ *RNA is translated to a polypeptide.*

This revision takes into account functional proteins that are composed of more than a single polypeptide.



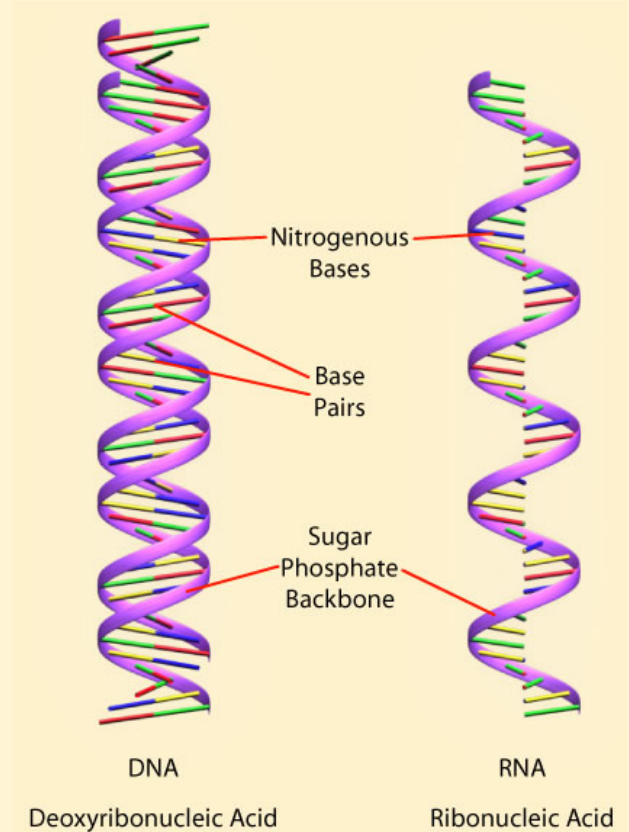
Exceptions to Central Dogma

Exceptions to The Central Dogma do occur.

One major exception to the flow of information is found in the family of Retroviruses. These viruses encode a polymerase, reverse transcriptase, which uses a RNA template to make a DNA copy.

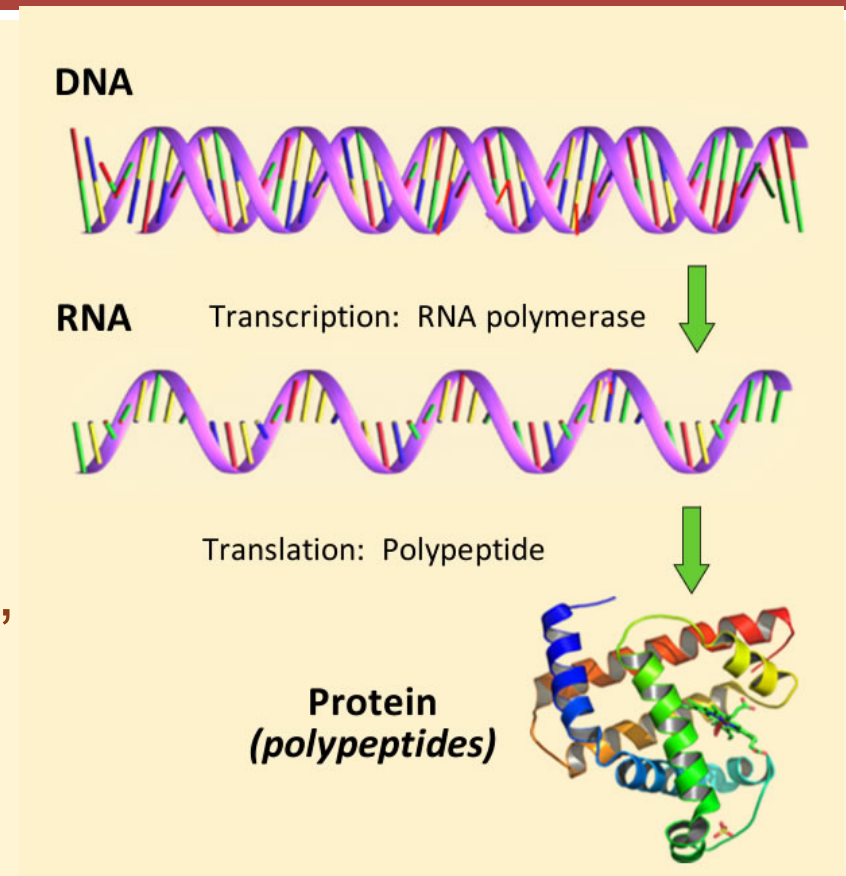
RNA Molecule

- ❖ RNA is a similar molecule to DNA with some very specific differences. Both RNA and DNA are long polymeric molecules with a ladder backbone and rungs of nitrogenous bases.
- ❖ In RNA, the sugar in the ladder is ribose rather than deoxyribose (as in DNA), and the nitrogenous base uracil replaces the thymine found in DNA.
- ❖ RNA is single-stranded and DNA is double-stranded.
- ❖ At least three types of RNA are known to be involved in the information flow from DNA to protein.



Transcription

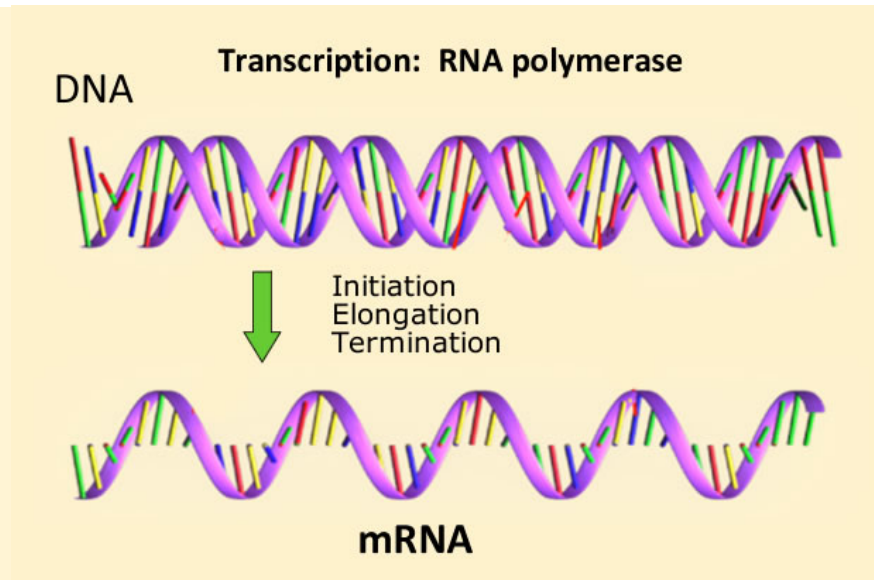
- ❖ The first step of central dogma (DNA→RNA) is transcription.
- ❖ Transcription can be defined as DNA-directed RNA synthesis.
- ❖ It requires a DNA template, RNA polymerase, and ribonucleotide subunits.



Transcription

Transcription occurs in three phases:

- ❖ Initiation
- ❖ Elongation
- ❖ Termination

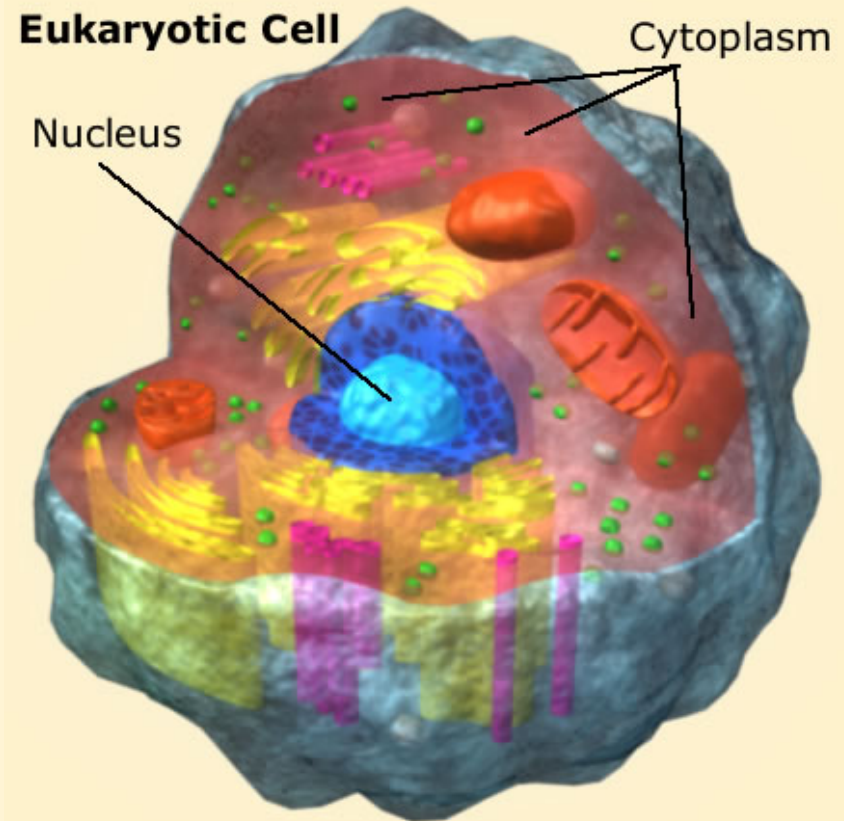


Initiation requires a promoter and RNA polymerase. The promoter contains the initiation site and tells RNA polymerase where to start, which strand to read, and which direction to take from the start. RNA polymerase then elongates the transcript until termination occurs at specific base sequences.

Transcription (continued)

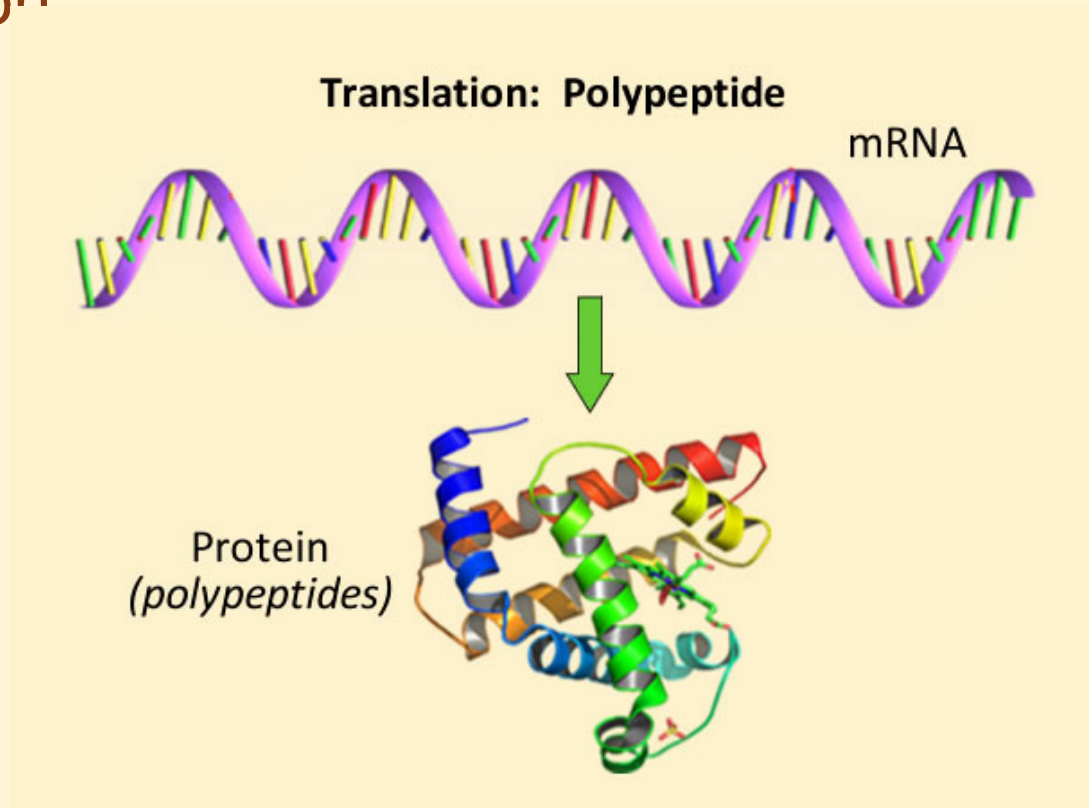
The RNA produced is complementary to the DNA and is called messenger RNA (mRNA). In eukaryotic cells, the mRNA leaves the nucleus and enters the cytoplasm (see *graphic*).

In the cytoplasm, the mRNA serves as the template for the creation of a polypeptide (protein).



Translation

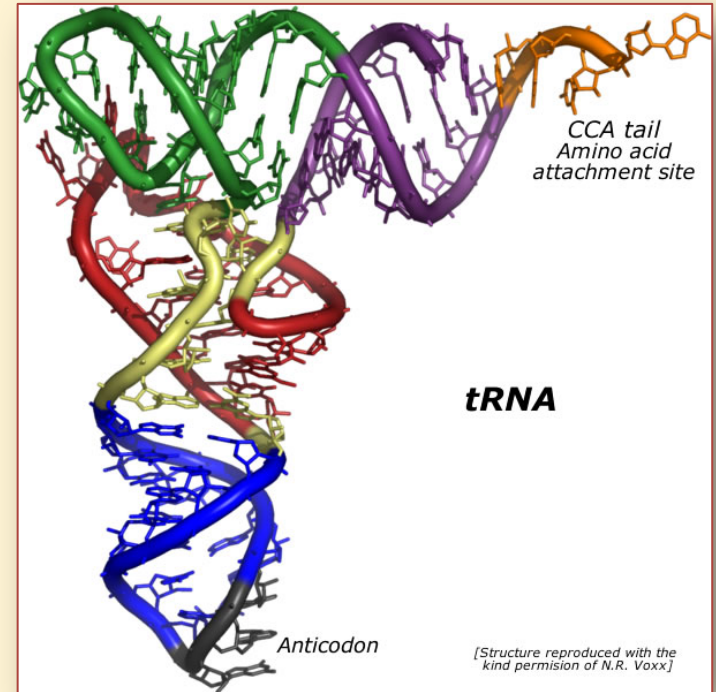
This second step, RNA→protein is known as translationⁿ



Translation

During translation another type of RNA, transfer RNA (tRNA), acts as an adapter molecule that recognizes the message of mRNA. The tRNA carries specific amino acids for incorporation into a growing polypeptide chain.

A third RNA, ribosomal RNA (rRNA) is part of the ribosome that is also essential for translation of the mRNA.



The Genetic Code

Although the flow of information was postulated in 1957, the genetic code of DNA was not deciphered until early 1960s. In 1961, artificial mRNAs were made and placed in a cell-free translation system. (Remember that the mRNA contains the information and instructions from the translation: *mRNA to Protein*).

The first mRNA consisted only of uridine. The translation product was a polymer of phenylalanine (*phe*).

Researchers concluded that the code was a triplet code.

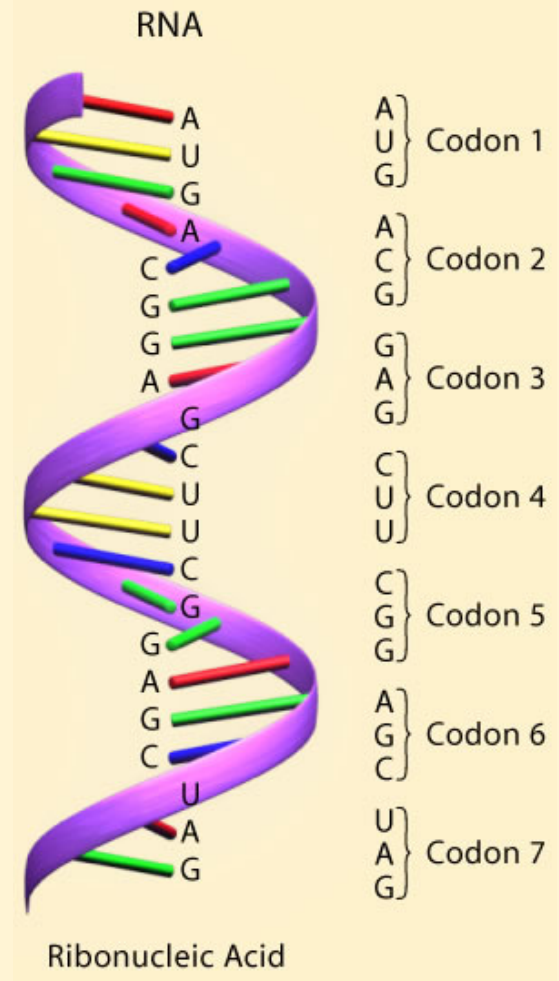
UUU encodes the amino acid *phe*, re-stated "*UUU is the codon for phe.*"

The Genetic Code

The remainder of the code was rapidly deciphered.

AUG is the "start" codon and specifies methionine (*met*). Three stop codons were identified: UAA, UAG, and UGA. These are also known as nonsense codons as they do not code for any amino acid.

The graphic illustrates the mRNA bases and the resulting codons.



The Genetic Code

Creating all possible triplet codons from the 4 bases (*U, C, A, G*) yields 64 codons, more than enough to encode the 20 common amino acids (*Phe, Leu, Ile, Met, Val, Ser...*).

Find the following codons in the genetic code table to the right.

"start" codon: *AUG (met)*.

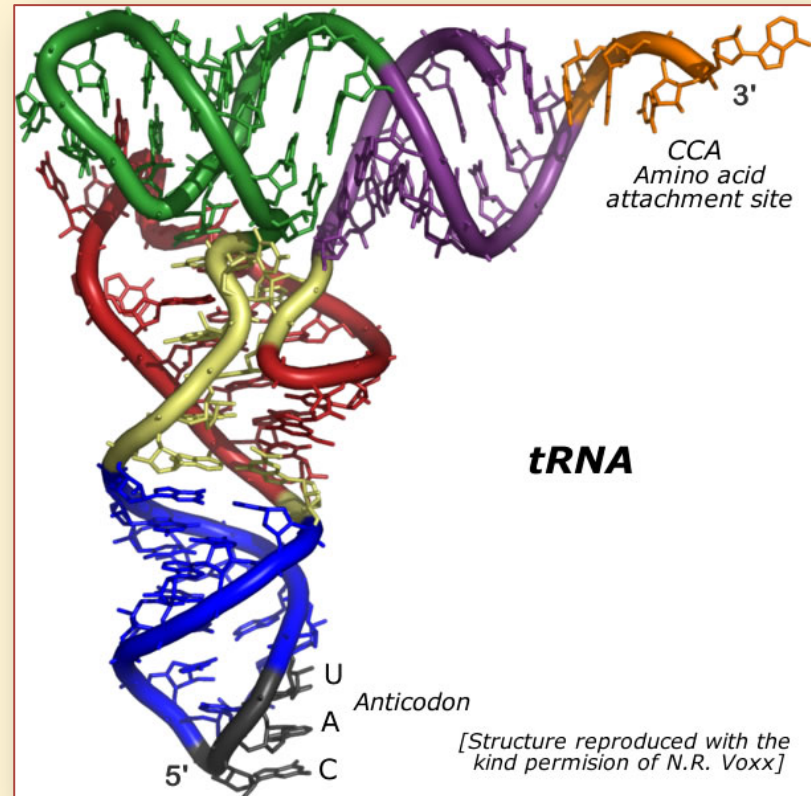
stop codons: *UAA, UAG, and UGA*.

Codons for the amino acids *valine* and *serine*.

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

The Role of tRNA in Translation

Translation links the mRNA, appropriate tRNAs and ribosomes to produce the final product, a polypeptide. The tRNA (*right*) contains an anticodon (*UAC*) and carries an amino acid (*CCA*). The conformation of tRNA allows it to interact with the ribosome.

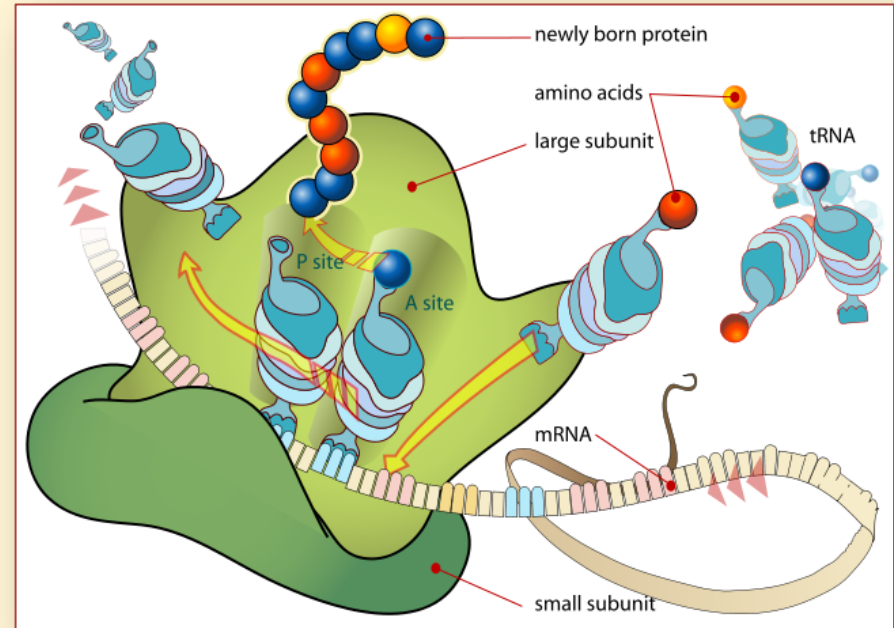


Protein Translation

During protein translation amino acids are linked together to form a polypeptide chain which will later be folded into a protein.

The ribosome is the workbench or factory for protein translation. It consists of a large and a small subunit (see *graphic – Protein Translation*).

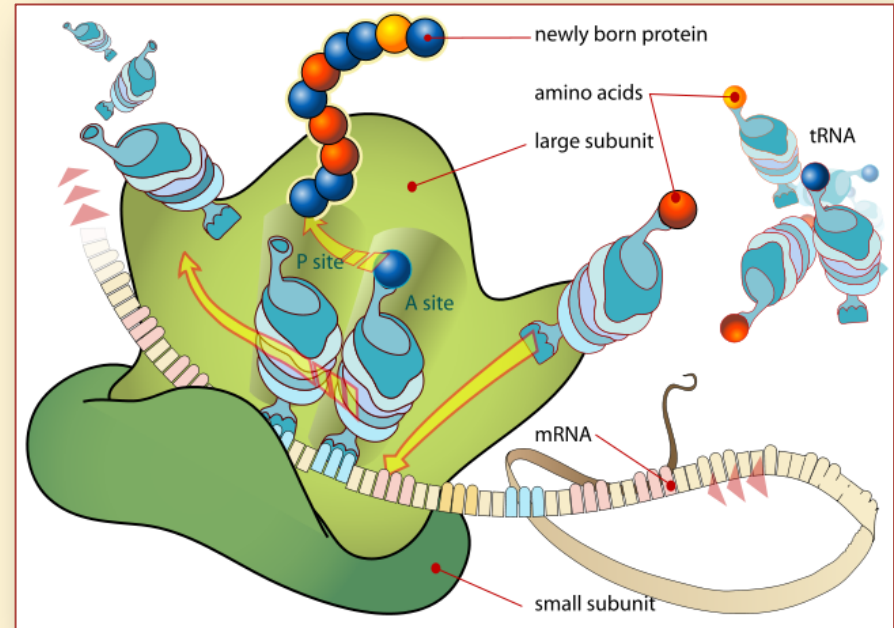
A ribosome can use any mRNA and all species of tRNA to make a polypeptide product.



Protein Translation
Diagram showing the translation of mRNA and the synthesis of proteins by a ribosome
[Image courtesy of LadyofHats]

Translation Steps

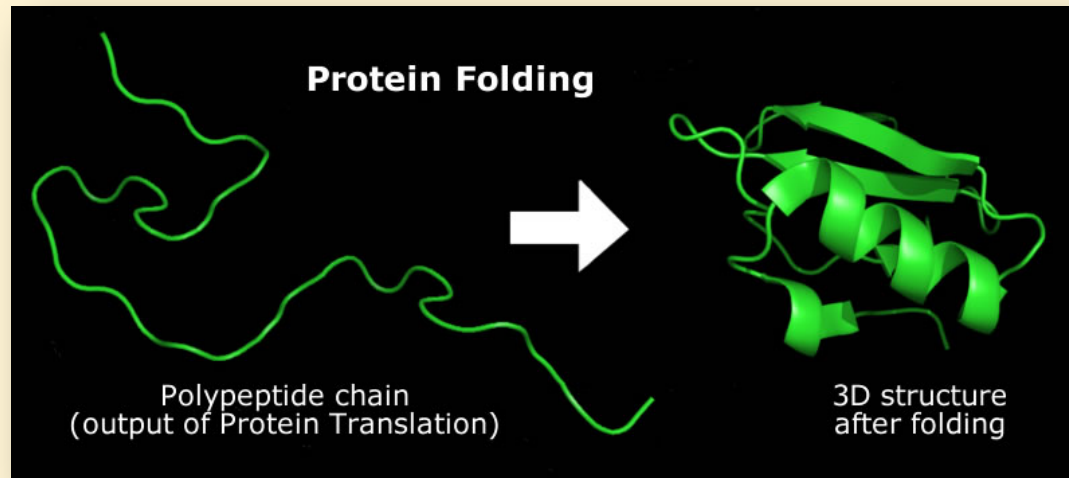
- ❖ Formation of an initiation complex consisting of the small ribosomal subunit, mRNA and tRNA^{met}.
- ❖ The large subunit binds and elongation begins.
- ❖ The anticodon of a tRNA molecule forms chemical bonds (i.e. base pair), with the mRNA's three letter codon. tRNA acts as translator between mRNA and protein by bringing the specific amino acid coded for by the mRNA codon.
- ❖ Stop codon is reached, a protein release factor breaks the bond between the polypeptide and the tRNA on the ribosome.



*Protein Translation
Diagram showing the translation of mRNA and the
synthesis of proteins by a ribosome
[Image courtesy of LadyofHats]*

After Translation

Signal sequences often target the growing polypeptide to a specific cellular location. For example, a membrane protein is directed to the cell membrane as it would not want to be in the cytoplasm of the cell.

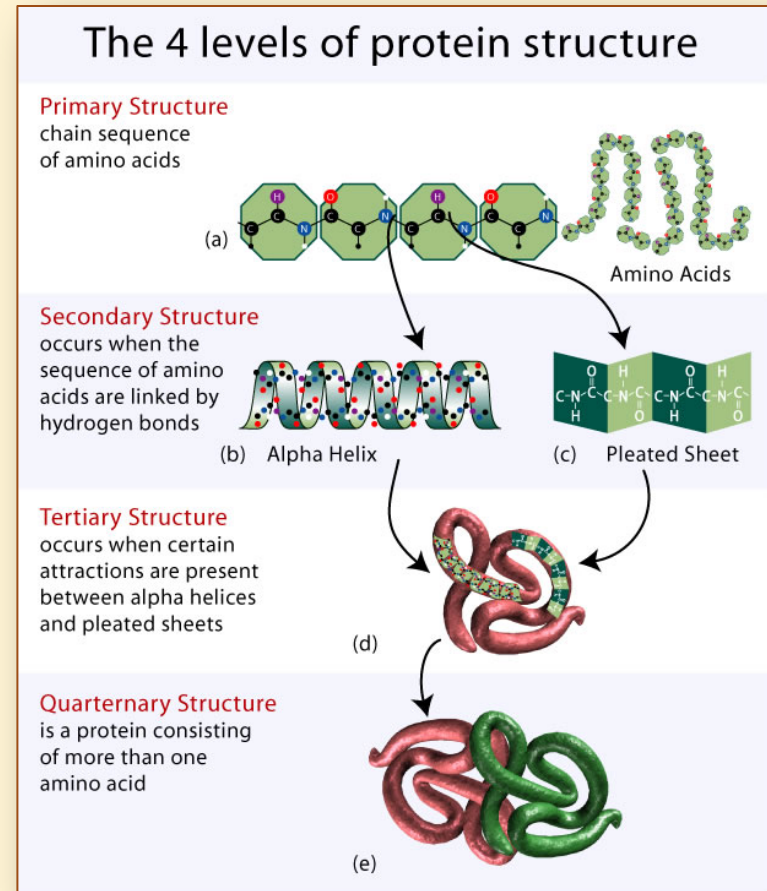


Most illustrations show a spaghetti-like strand of amino acids flowing away from a ribosome, but proteins begin folding as they are translated. Protein Folding is the process which converts a polypeptide into its characteristic and functional three-dimensional structure.

Protein Folding

Protein folding is described at four levels.

1. Polypeptide chain of amino acids
2. Formation of alpha-helix and beta-pleated sheet s
3. Additional folding and interactions between specific R-groups on amino acids, including disulfide bond formation, aggregation of hydrophobic side chains, van der Waals forces and ionic bond formation.
4. Proteins are defined that consist of multiple polypeptide subunits.



The Four Levels of Protein Structure
[Modified from freely available illustration provided by the Talking Glossary of Genetics]

Importance of Protein Folding

- ❖ Proper folding is essential for protein function.
- ❖ If an enzyme misfolds, the active site may not form, and the enzyme would not be able to function as a biocatalyst.
- ❖ In similar ways, if a structural protein misfolds, it also would not be able to carry out a normal structural function.

BioMEMS, DNA, and Protein

MEMS technology and our knowledge of biomolecules have provided the ability to study protein-protein interactions on the basis of binding events.

Such devices include

- ❖ peptide-loaded beads,
- ❖ microplates,
- ❖ pins and
- ❖ other flat micro-surfaces like membranes and chips.

ELISA

- ❖ One such device is ELISA (Enzyme-Linked Immuno-Sorbent Assay), a sensitive immunoassay that uses an enzyme linked to an antibody or antigen as a marker for the detection of a specific protein.
- ❖ It is widely used for detection and quantification of biological agents (mainly proteins and polypeptides).
- ❖ Its high selectivity and sensitivity draw great attractions in clinical, food safety, and environmental applications.



BioLOC's CD-ELISA™
[Printed with permission of BioLOC LLC]

Food for Thought

- ❖ *How does RNA differ from DNA?*
- ❖ *DNA is composed of 2 strands, of which only one strand is used as a template for RNA synthesis for a specific gene. By what mechanism is the correct strand chosen?*
- ❖ *What is a codon?*
- ❖ *How are proteins directed to the correct compartment within a cell?*
- ❖ *Given the types of chemical interactions that promote and maintain protein structure, can you think of ways to disrupt or break those interactions?*

Summary

- ❖ The central dogma of biology states that genetic information flows from DNA to RNA to protein.
- ❖ Deciphering the genetic code has allowed scientists to translate the sequence of DNA into the sequence of amino acids that comprise the primary structure of a protein or polypeptide.
- ❖ Protein structure is related to function, and proteins fulfill a wide diversity of functions within cells.
- ❖ Proteins are also an integral part of many diagnostic aids and devices.

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