# Protein Analysis

## (Function, Structure and synthesis)

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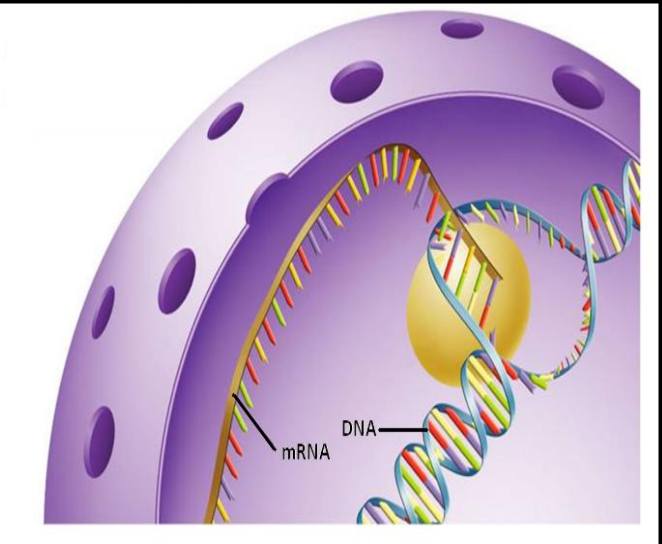
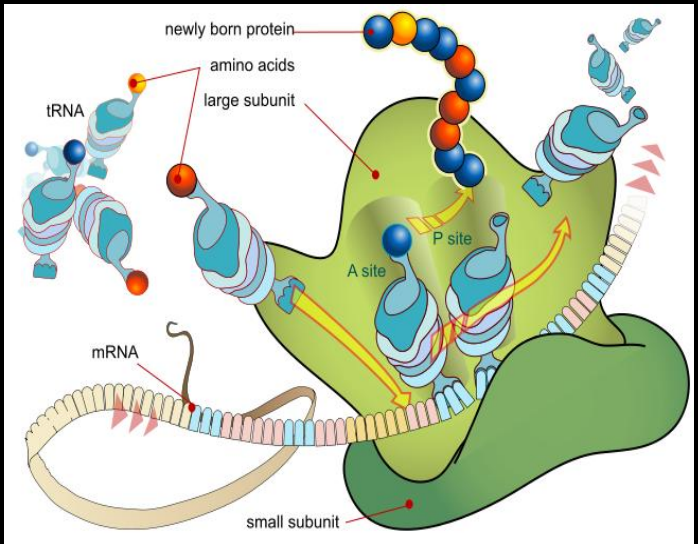
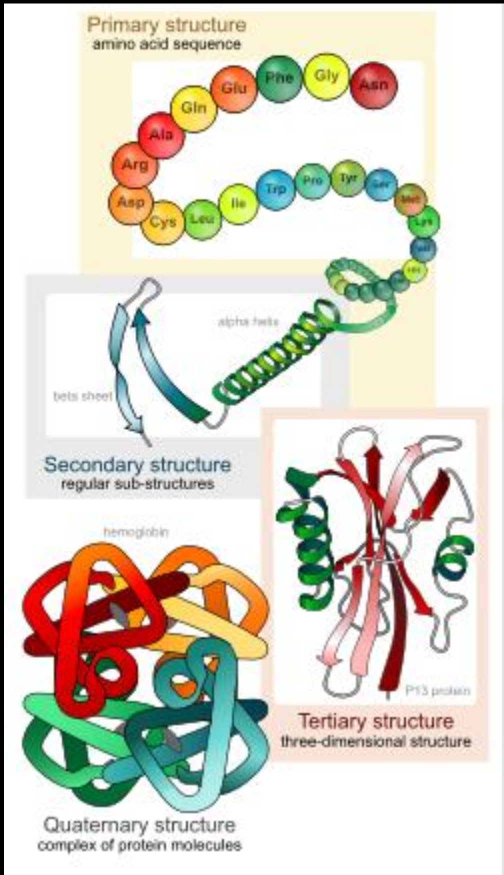
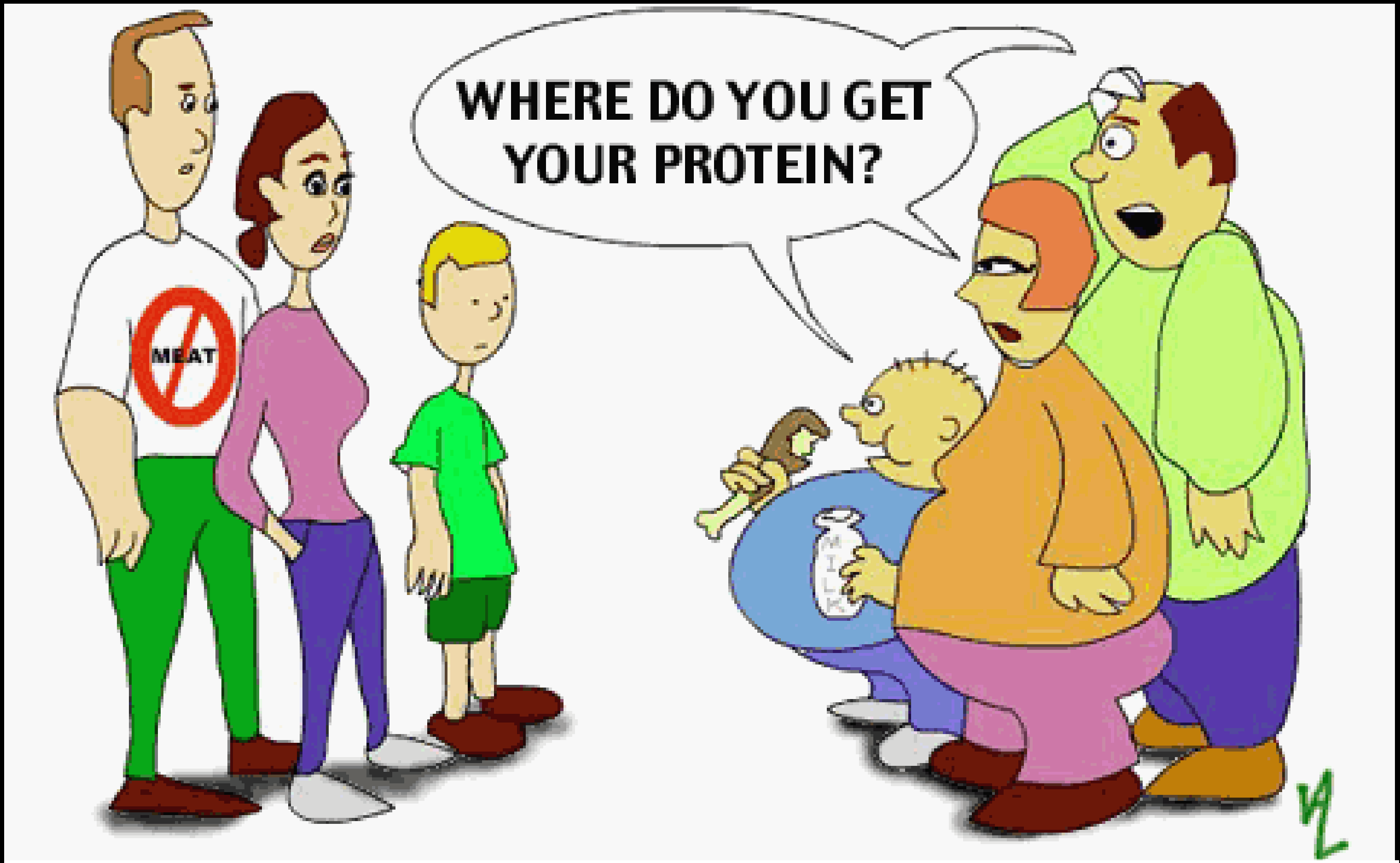


# Are proteins important?



## Hair and nails

Alpha keratin forms your hair and fingernails. Feathers, wool, claws, scales, horns and hooves

## Blood

Hemoglobin carries $O_2$ in blood to every part of the body

## Brain and Nerves

Ion channel protein control brain signaling by allowing molecules into and out of nerve cell

## Muscles

Actin and myosin enable all muscular movement from blinking to breathing to rollerblading

## Enzymes

Enzymes in saliva, stomach, and small intestine are proteins that help you digest food

## Cellular messengers

Receptor proteins stud the outside of the cells and transmit signals to partner proteins inside the cells.

## Antibodies

Proteins that help defend your body against foreign invaders such as bacteria and viruses

## Cellular construction workers

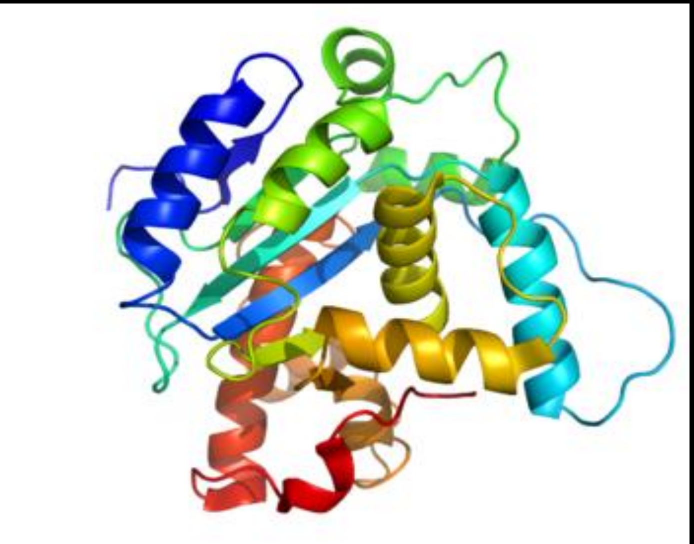
Huge clusters of proteins do cells' heavy work such as copying genes during cell division and making new proteins

# Function of proteins

1. Help fight disease (immune defense)
2. Build new body tissue (cell division and differentiation)
3. Enzymes used for digestion and other chemical reactions
4. Component of all cell membranes
5. Proteins serve to transport small molecules, ions, or metals.
6. They control blood homeostasis (blood clotting)
7. Control the coordination of movements by regulating muscle cells and the production and transmission of impulses within and between nerve cells.

# What is a protein?

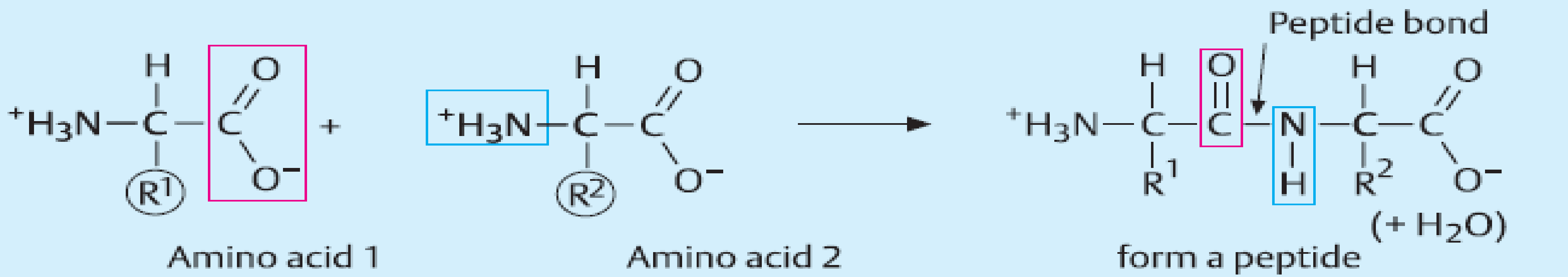
- Proteins are composed of amino acids – there are 20 different amino acids
- Different proteins are made by combining these 20 amino acids in different combinations
- Proteins are manufactured by the ribosomes
- There are over 10,000 proteins in our body

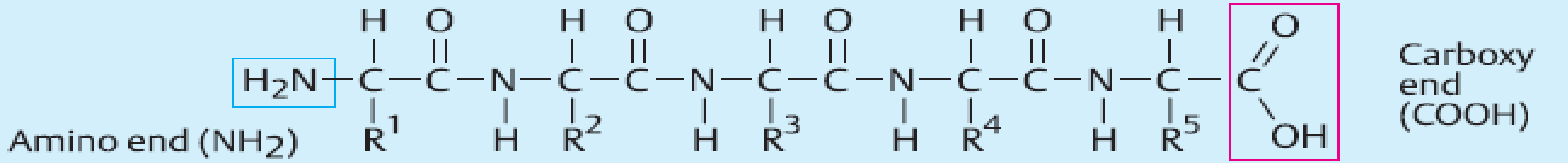


-The carboxyl group of one amino acid binds to the amino group of the next (a peptide bond or an amide bond).

-When many amino acids are bound together by peptide bonds, they form a polypeptide chain.

- Each polypeptide chain has a defined direction, determined by the amino group (—NH2) at the beginning, and the carboxyl group (—COOH) at the end of a peptide chain other.



Peptide composed of five amino acids (pentapeptide)

**A- Primary structure of a protein**

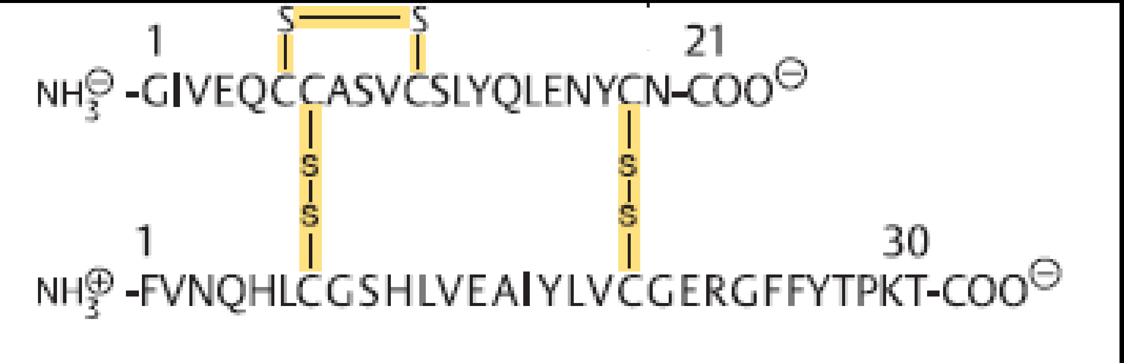
It refers to amino acid linear sequence of the polypeptide chain

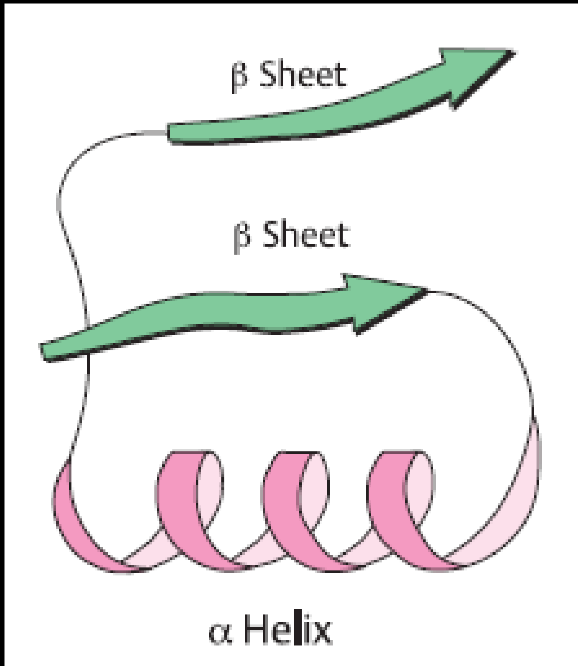
Amino acid sequence in a one dimensional plane

The A and the B chains are connected by two disulfide bridges joining the cysteines

**B- Secondary structural units**

Two basic units of global proteins are α Helix formation (α helix) and a flat sheet (β pleated sheet)



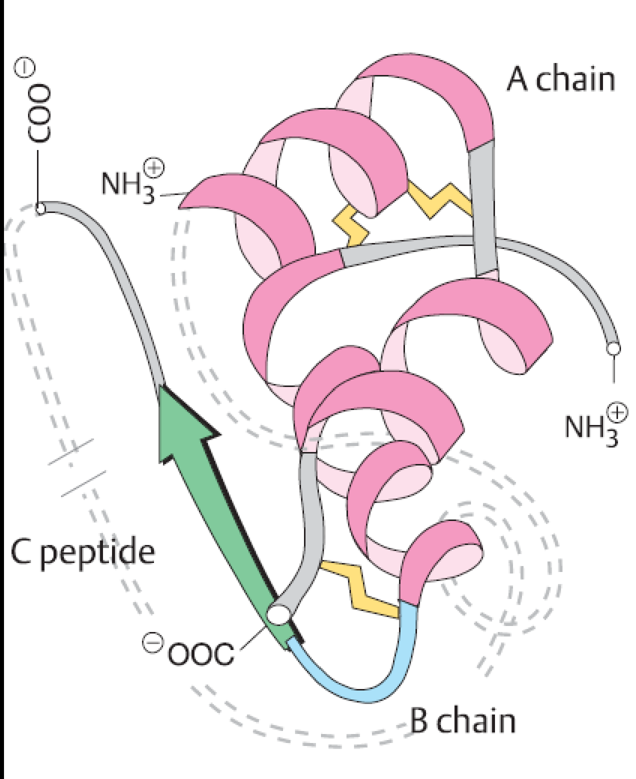



## B- Tertiary structural units

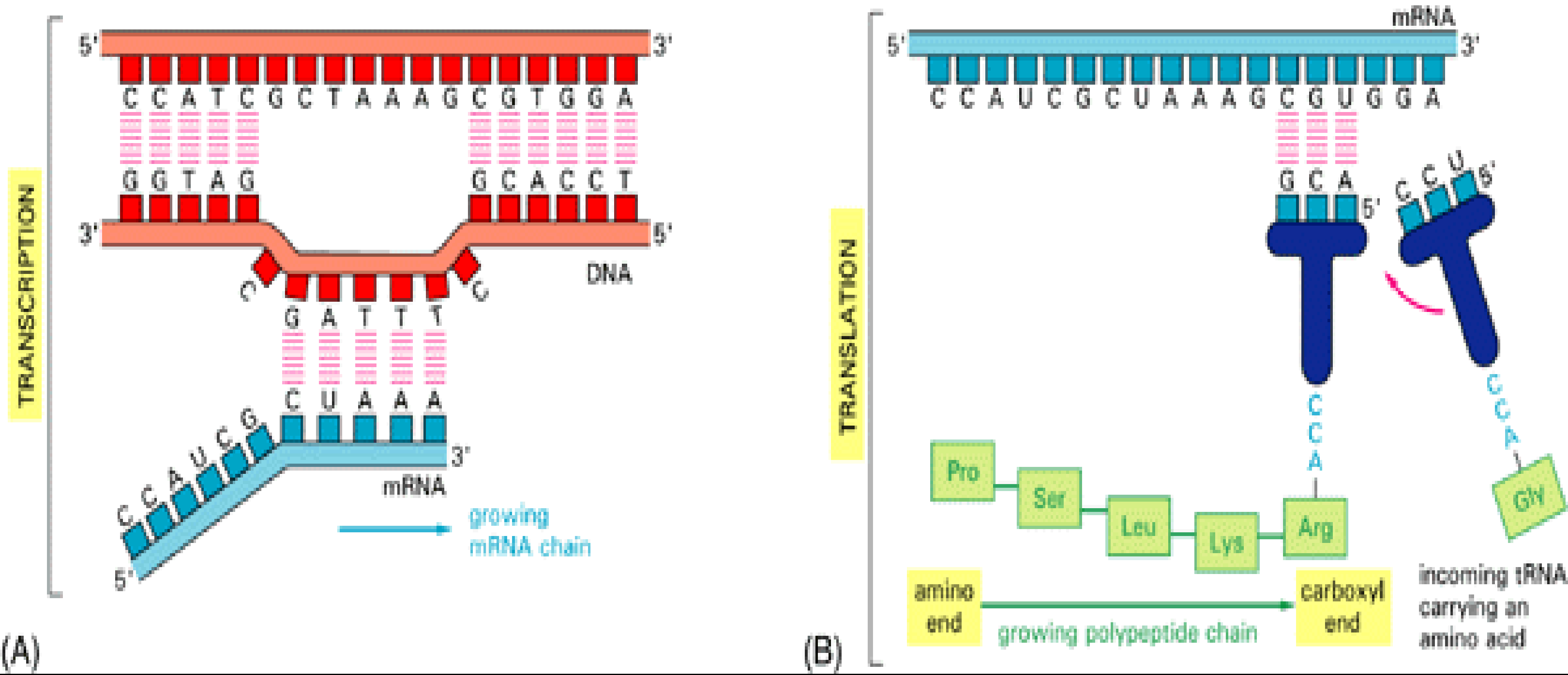
refers to three-dimensional structure of a single protein molecule
The alpha-helices and beta-sheets are folded into a compact globule
The folding is driven by the non-specific hydrophobic interactions

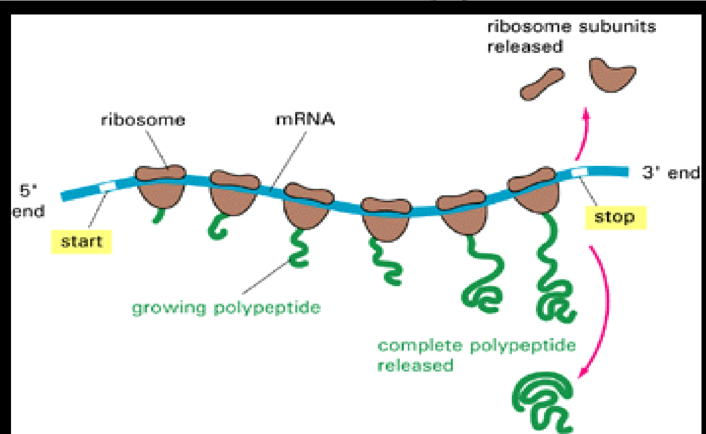
## D- Quaternary structural units

Three-dimensional structure of a multi-subunit protein
and how the subunits fit together



# How can cells manufacture proteins?



# Protein Synthesis

# Transcription

First step:

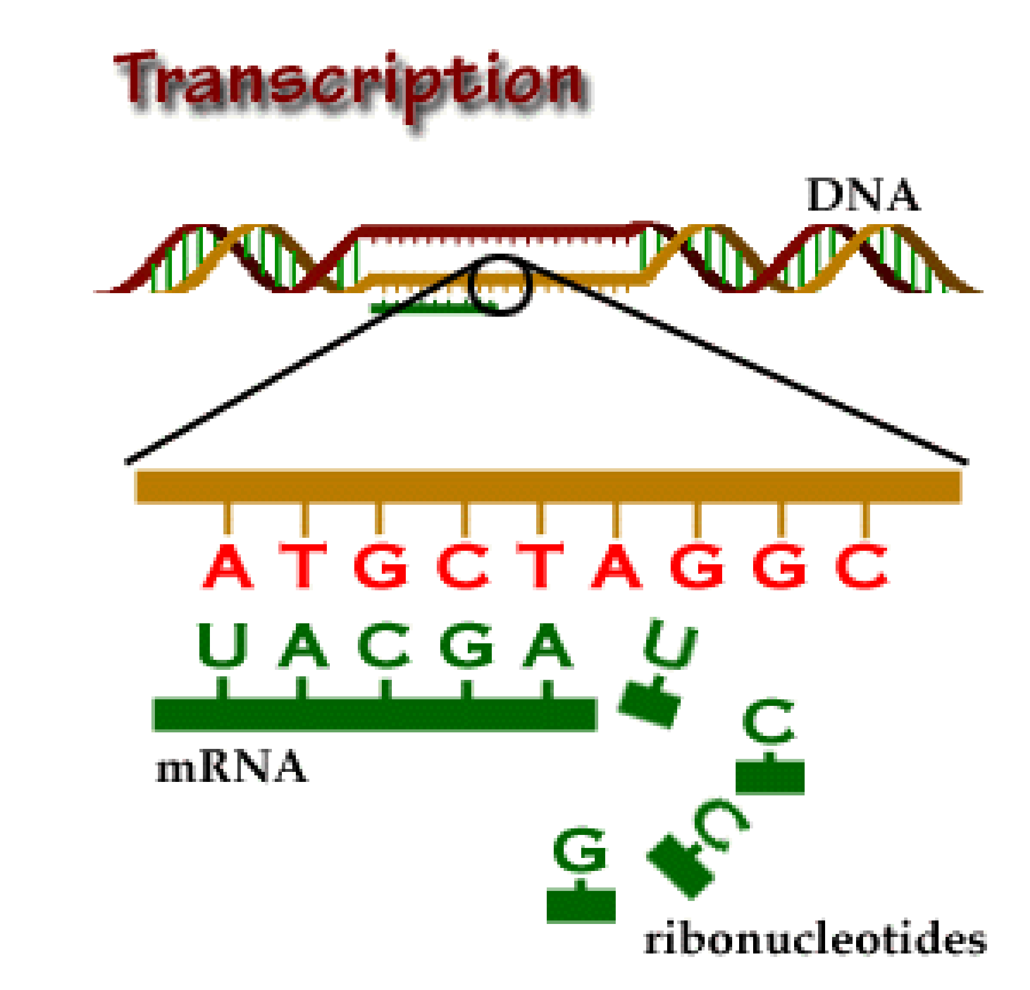
Copying of genetic information from DNA to RNA called Transcription

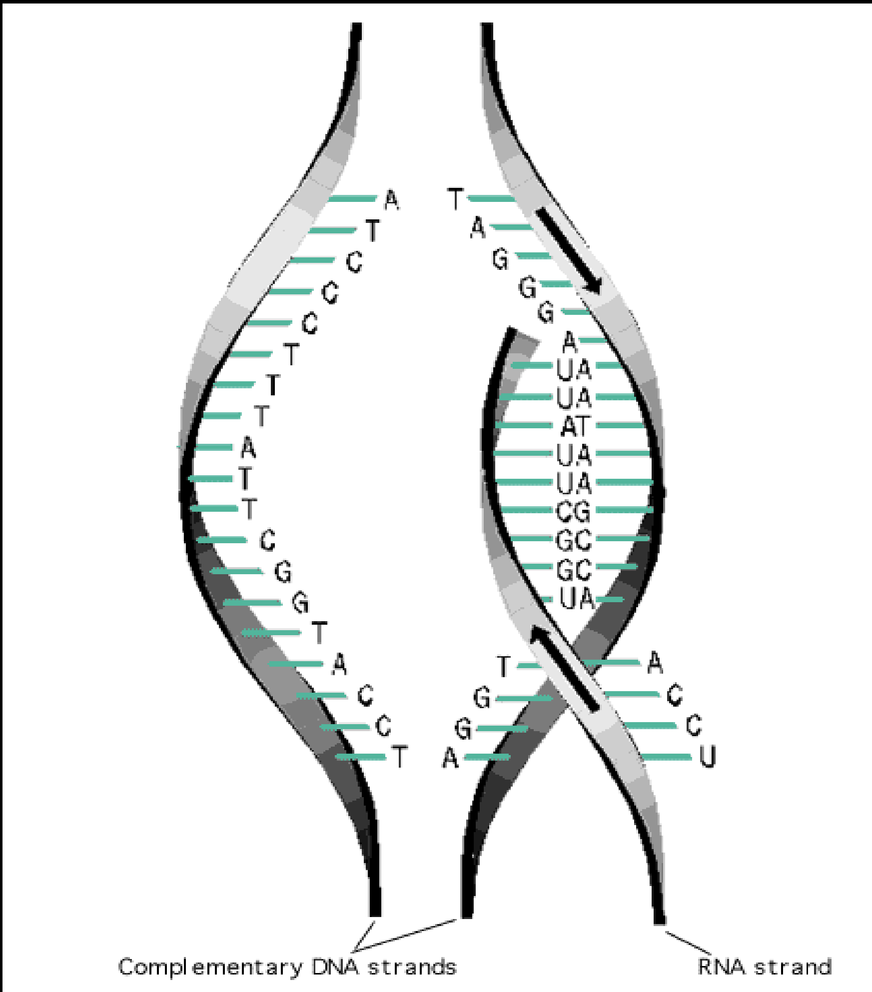
Why? DNA has the genetic code for the protein that needs to be made, but proteins are made by the ribosomes.

Ribosomes are outside the nucleus in the cytoplasm.

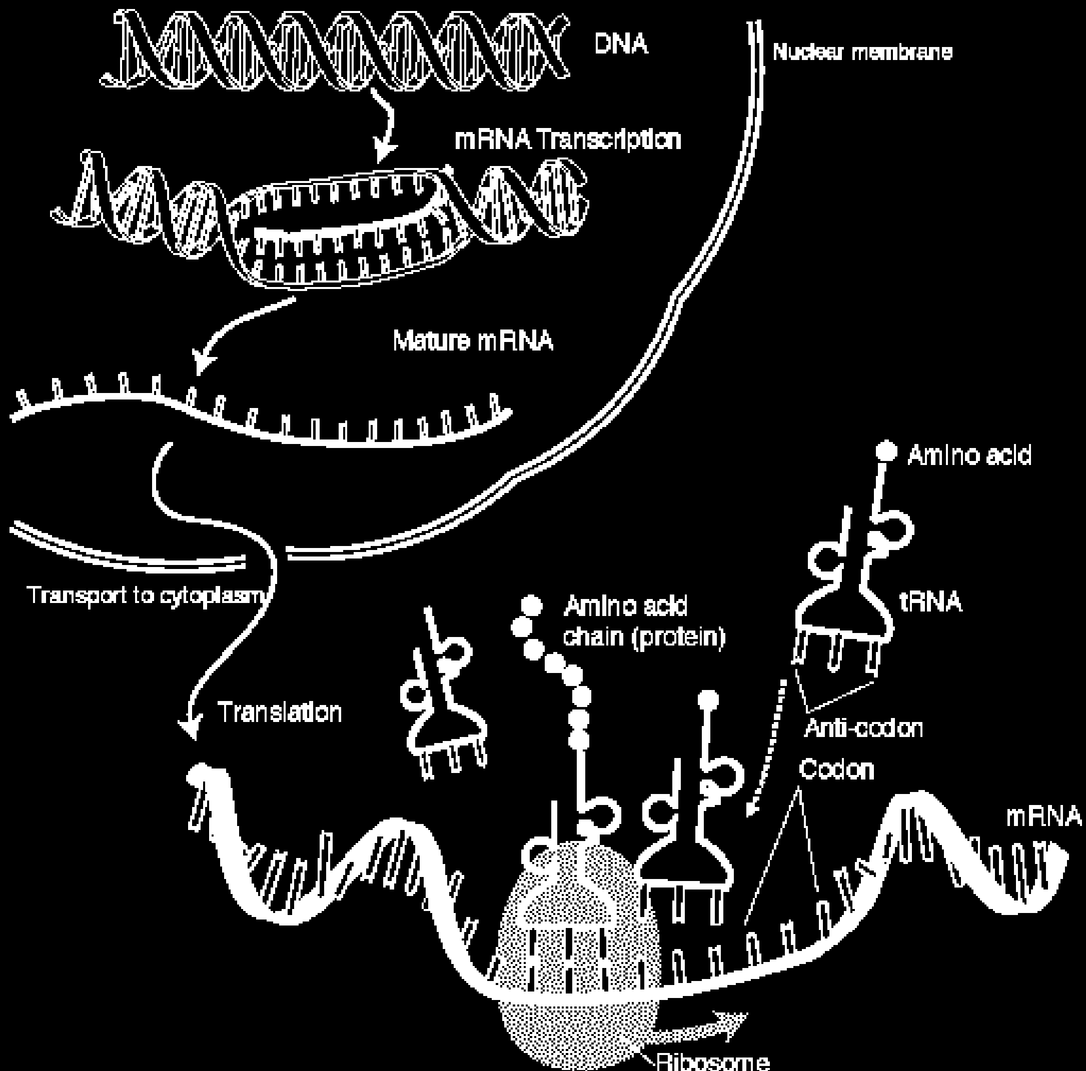
DNA is too large to leave the nucleus (double stranded), but RNA can leave the nucleus (single stranded).

- Part of DNA temporarily unwind and is used as a template to assemble complementary nucleotides into messenger RNA (mRNA).



- mRNA then goes through the pores of the nucleus with the DNA code and attaches to the ribosome.

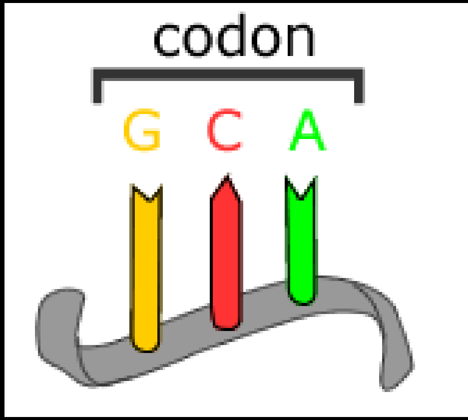


# Translation

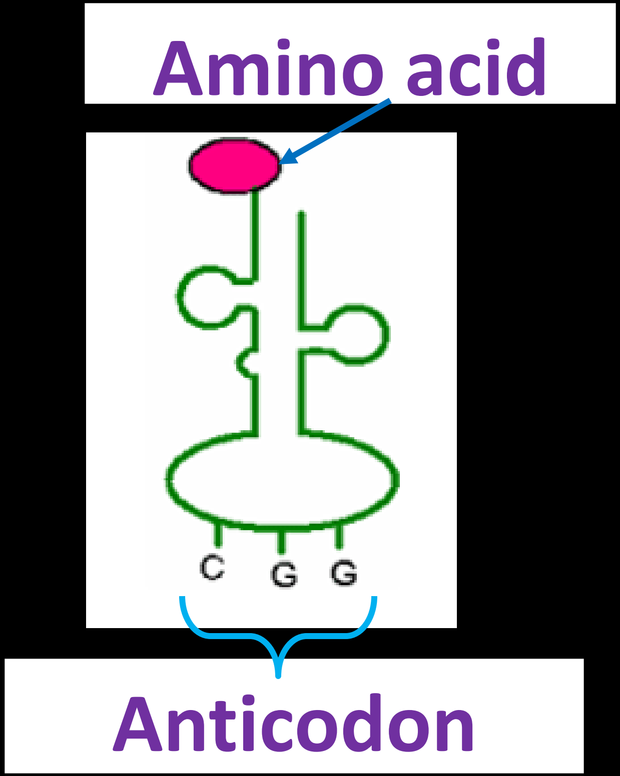
Second step:

- Decoding of mRNA into a protein is called Translation.
- Transfer RNA (tRNA) carries amino acids from the cytoplasm to the ribosome.
- mRNA carrying the DNA instructions and tRNA carrying amino acids meet in the ribosomes.
- Amino acids are joined together to make a protein.

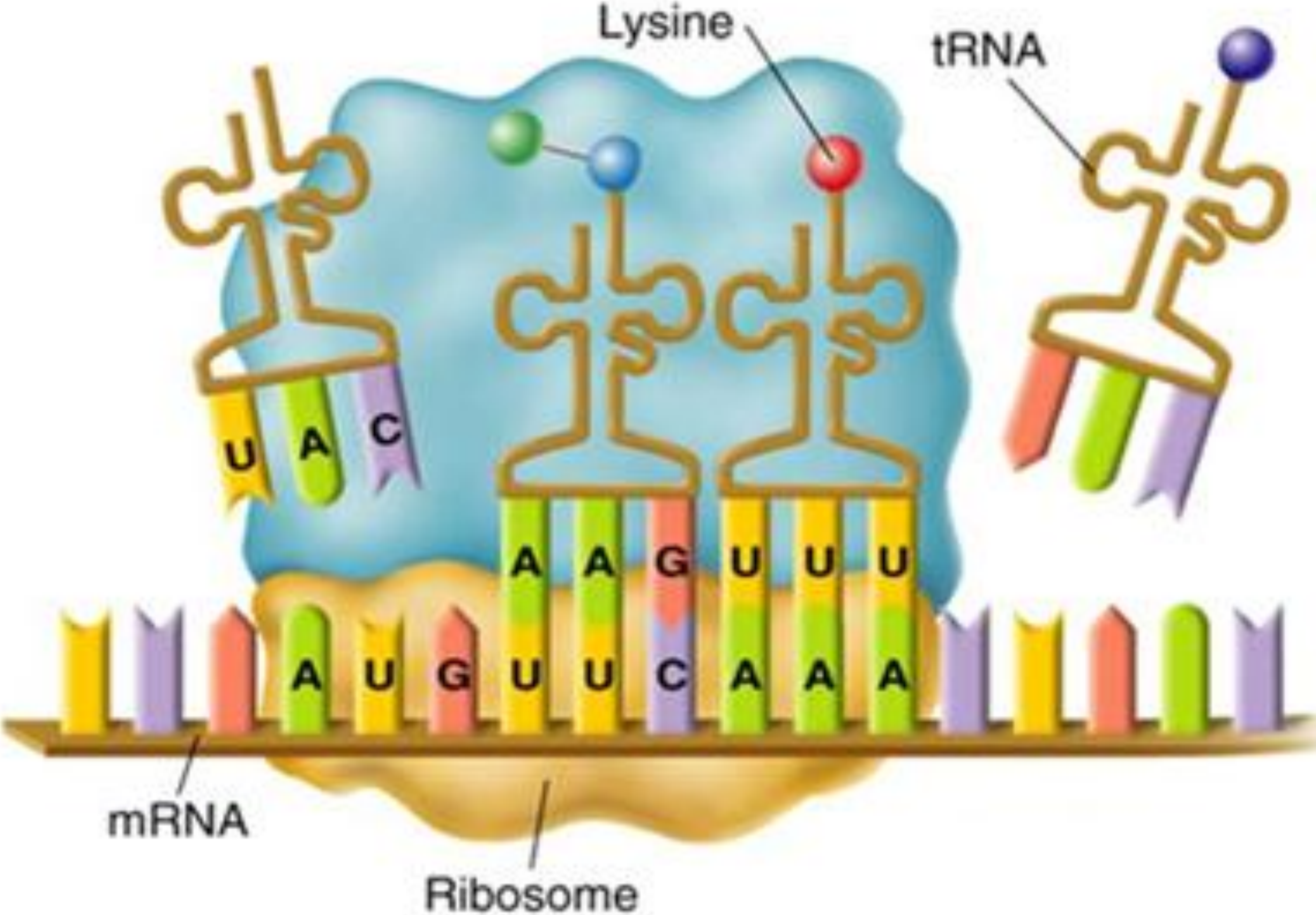
- **Codon** a series of three adjacent bases in an mRNA molecule codes for a specific amino acid.



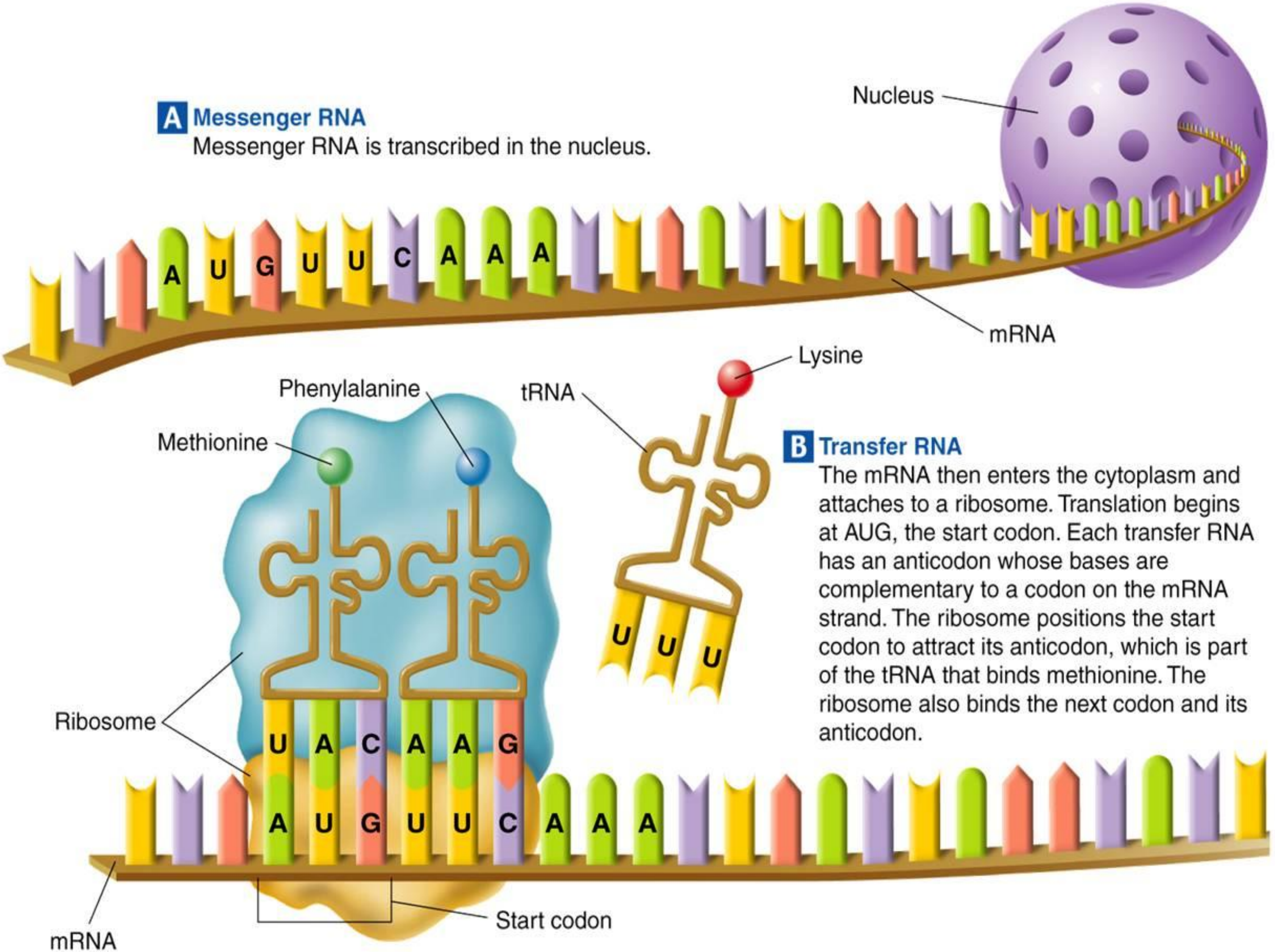

- **Anticodon** a triplet of nucleotides in tRNA that is complementary to the codon in mRNA.




- Each tRNA codes for a different amino acid.

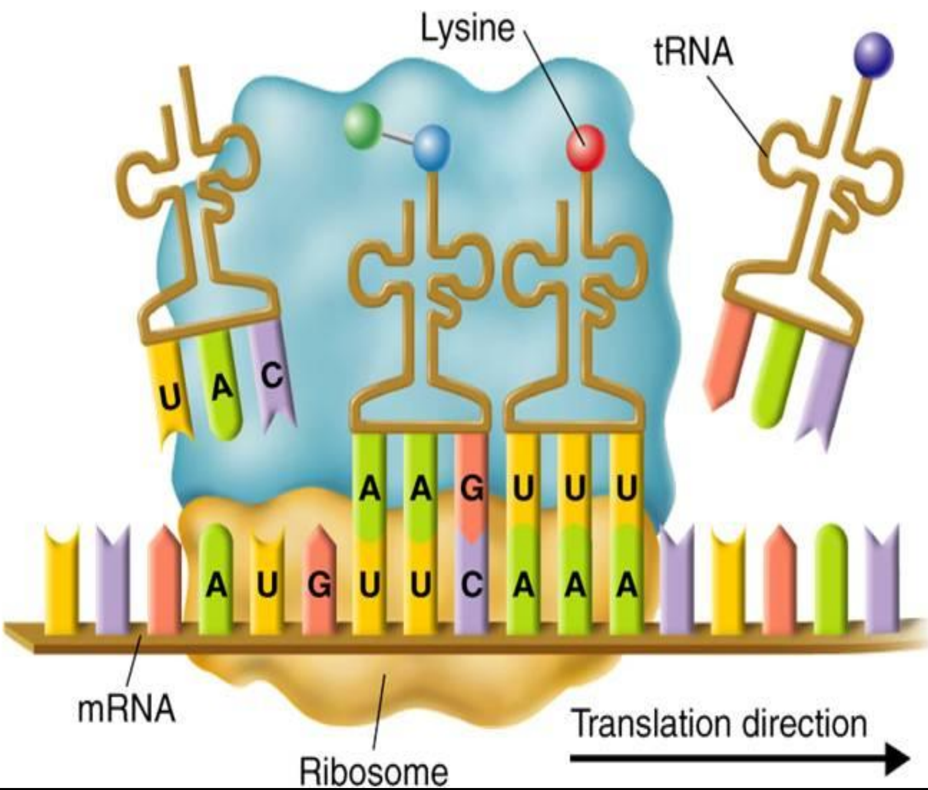


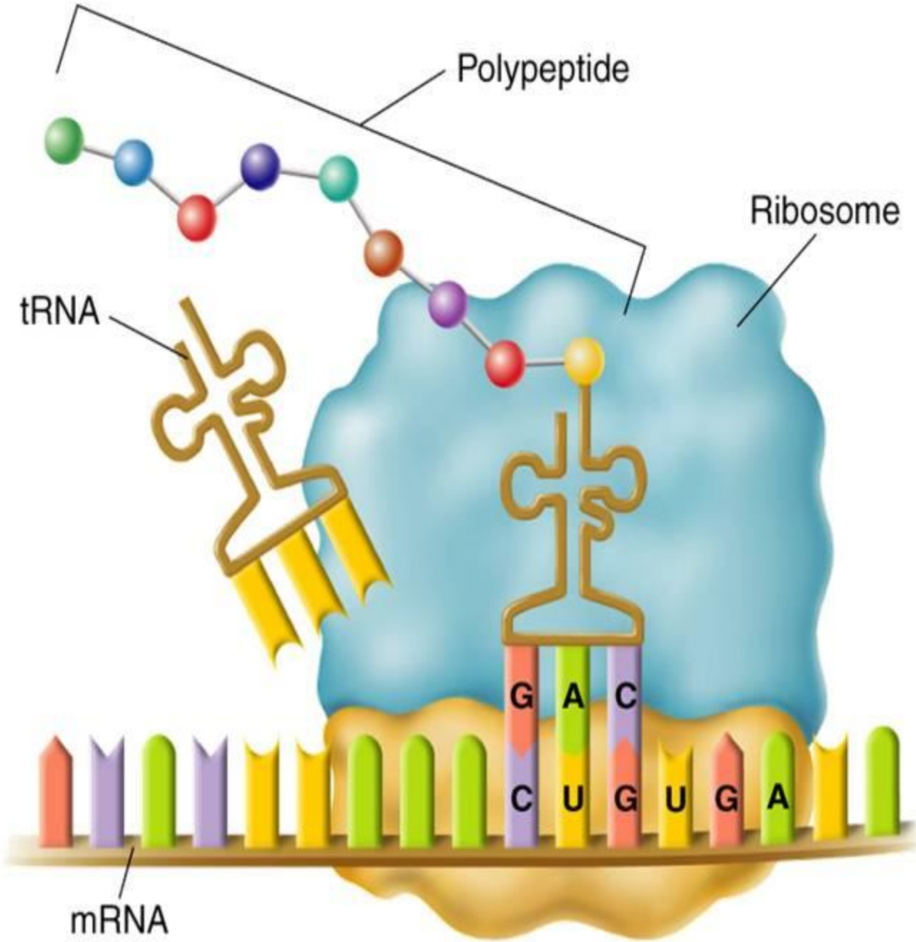
Lysine
tRNA
U
A
C
A
A
G
U
U
U
A
U
G
U
U
C
A
A
A
mRNA
Ribosome


A Messenger RNA
Messenger RNA is transcribed in the nucleus.
Nucleus
A U G U U C A A A
mRNA
Lysine
Phenylalanine
tRNA
Methionine
B Transfer RNA
The mRNA then enters the cytoplasm and attaches to a ribosome. Translation begins at AUG, the start codon. Each transfer RNA has an anticodon whose bases are complementary to a codon on the mRNA strand. The ribosome positions the start codon to attract its anticodon, which is part of the tRNA that binds methionine. The ribosome also binds the next codon and its anticodon.
U U U
Ribosome
U A C A A G
A U G U U C A A A
mRNA
Start codon

## C The Polypeptide "Assembly Line"

The ribosome joins the two amino acids—methionine and phenylalanine—and breaks the bond between methionine and its tRNA. The tRNA floats away from the ribosome, allowing the ribosome to bind another tRNA. The ribosome moves along the mRNA, binding new tRNA molecules and amino acids.




## D Completing the Polypeptide

The process continues until the ribosome reaches one of the three stop codons. The result is a complete polypeptide.

Polypeptide = **Protein**

# Different codons for amino acids

2nd Base

1st Base (rows) / 3rd Base (right column)

| | U | | C | | A | | G | | |
|---|---|---|---|---|---|---|---|---|---|
| U | UUU | Phenylalanine | UCU | Serine | UAU | Tyrosine | UGU | Cysteine | U |
| | UUC | Phenylalanine | UCC | Serine | UAC | Tyrosine | UGC | Cysteine | C |
| | UUA | Leucine | UCA | Serine | UAA | Stop | UGA | Stop | A |
| | UUG | Leucine | UCG | Serine | UAG | Stop | UGG | Tryptophan | G |
| C | CUU | Leucine | CCU | Proline | CAU | Histidine | CGU | Arginine | U |
| | CUC | Leucine | CCC | Proline | CAC | Histidine | CGC | Arginine | C |
| | CUA | Leucine | CCA | Proline | CAA | Glutamine | CGA | Arginine | A |
| | CUG | Leucine | CCG | Proline | CAG | Glutamine | CGG | Arginine | G |
| A | AUU | Isoleucine | ACU | Threonine | AAU | Asparagine | AGU | Serine | U |
| | AUC | Isoleucine | ACC | Threonine | AAC | Asparagine | AGC | Serine | C |
| | AUA | Isoleucine | ACA | Threonine | AAA | Lysine | AGA | Arginine | A |
| | AUG | Methionine (Start) | ACG | Threonine | AAG | Lysine | AGG | Arginine | G |
| G | GUU | Valine | GCU | Alanine | GAU | Aspartic Acid | GGU | Glycine | U |
| | GUC | Valine | GCC | Alanine | GAC | Aspartic Acid | GGC | Glycine | C |
| | GUA | Valine | GCA | Alanine | GAA | Glutamic Acid | GGA | Glycine | A |
| | GUG | Valine | GCG | Alanine | GAG | Glutamic Acid | GGG | Glycine | G |

Exercise:-

How can you translate these codons?

**CAC / CCA / UGG / UGA**

Histidine / Proline / Tryptophan / Stop

# Protein Analysis

## (Quantification)

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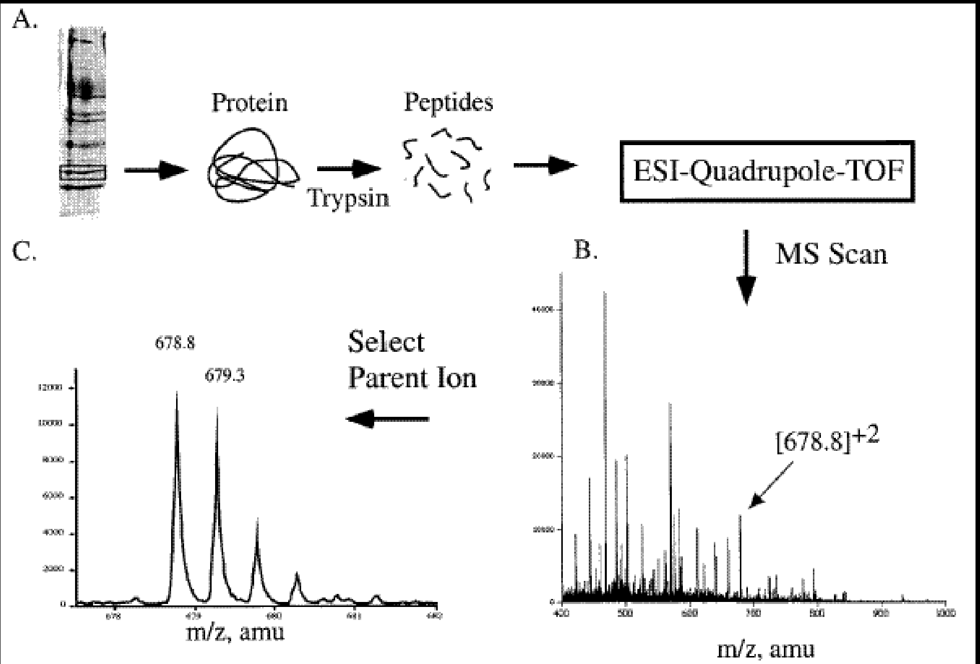
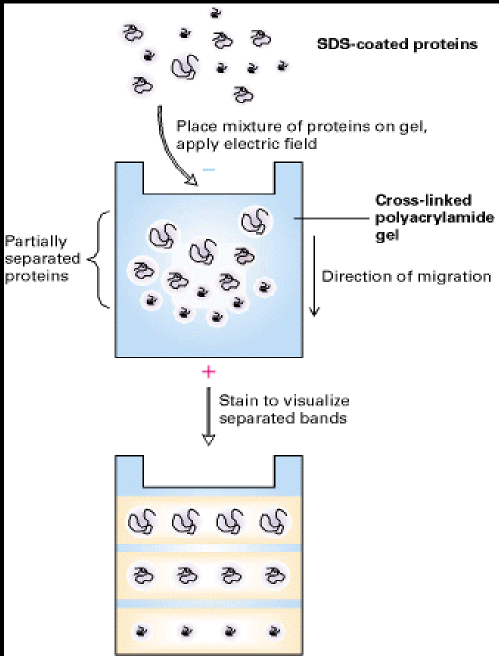


# Quantification of protein

## Detection and Assay of Proteins

- It is not possible to isolate a protein without a method of determining whether it is present.

- An assay, either quantitative or at least semi-quantitative, indicating which fraction contains the most the desired protein is essential.

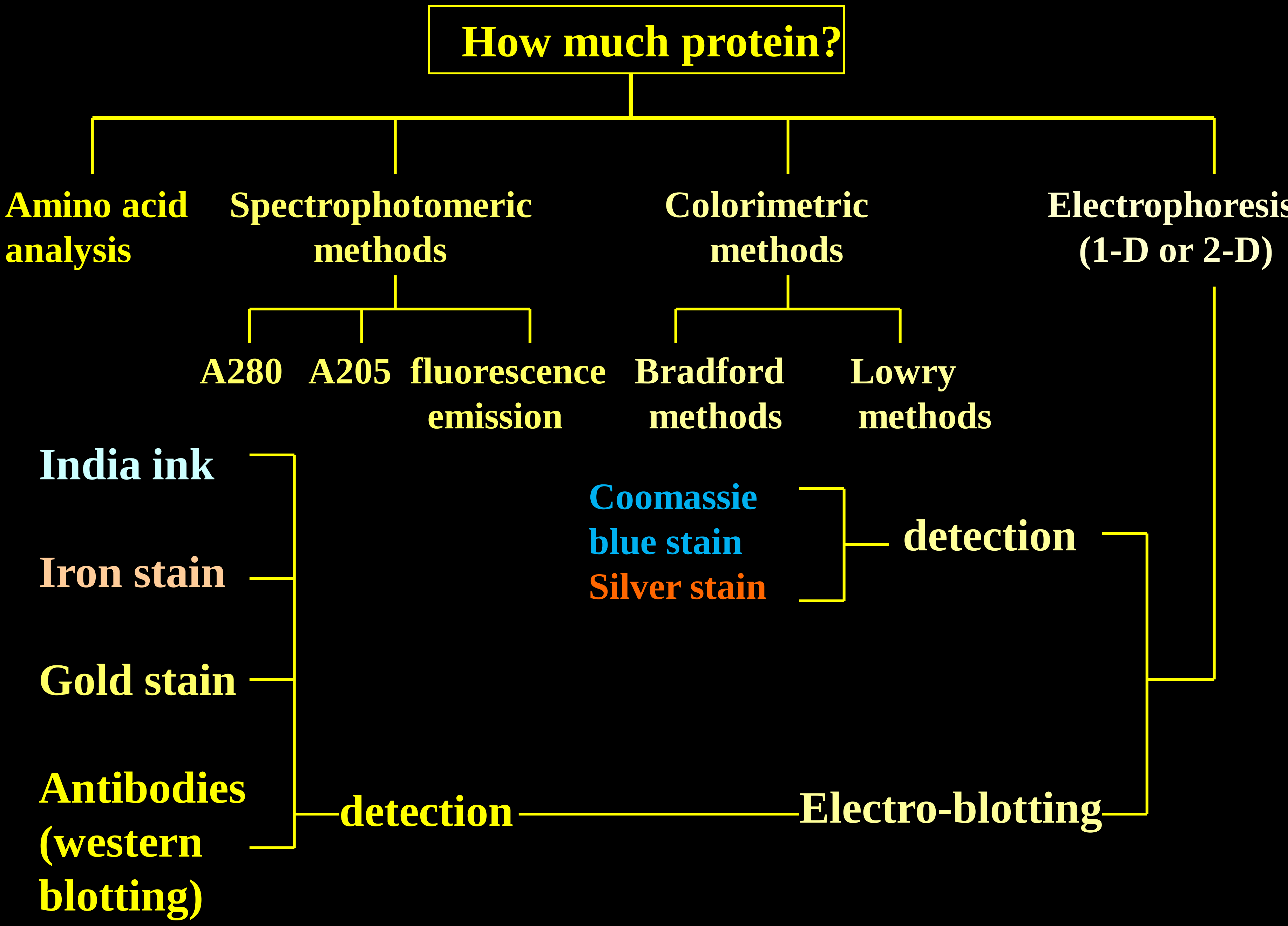
-There is no completely satisfactory single method to determine the conc. of protein in any given sample.

-The choice of the method depends on:-

  -The nature of the protein and the other components

  - Desired speed

  - Accuracy and sensitivity of assay


How much protein?
Amino acid analysis
Spectrophotomeric methods
Colorimetric methods
Electrophoresis (1-D or 2-D)
A280
A205
fluorescence emission
Bradford methods
Lowry methods
India ink
Iron stain
Gold stain
Antibodies (western blotting)
Coomassie blue stain
Silver stain
detection
detection
Electro-blotting

# I- Spectrophotometric methods

- It is used for measuring the conc. of a protein in solution

A- 280 nm (*A*280)

- Calculate protein conc. by comparison with a standard curve.
- This method is the most commonly used

-20 to 3000 µg/ml.

B- 205 nm (*A*205)

-Calculate the protein conc.

- Can detect lower conc. of protein
- It is useful for dilute protein samples

-1 to 100 µg/ml protein

These methods are simple and rapid

Used to quantitate total protein in crude lysates and purified or partially purified protein

## C- Fluorescence Emission

- Measuring fluorescence intensity of the protein sample solution based on fluorescence emission by the aromatic amino acids tryptophan, tyrosine, and/or phenylalanine

-5 to 50 µg/ml

-The advantages of this method are that the sample is not destroyed and that it is very rapid

# II- Colorimetric

## 1- Bradford method

- Based upon binding of the dye Coomassie brilliant blue to an unknown protein and comparing this binding to that of different amounts of a standard protein, usually BSA.
- 1 to 10 µg protein

## 2- Lowry method

- Which measures colorimetric reaction of Folin-Ciocalteu phenol reagent with the tyrosyl residues in an unknown protein and comparing this binding to that of different amounts of a standard protein, usually BSA.
- 1 to 20 µg protein

## III- Amino Acid Analysis

-The difficulties involved in obtaining reliable amino acid data

-The advent of colorimetric dye-binding assays

-Many proteins do not bind dyes in the same way as standards

-Identifying the nature of the amino acids present in a sample

-It is the most accurate method to determine protein in a sample

- The amount of protein required for detection could be as low as 0.05 nmol or 2.5 ug.