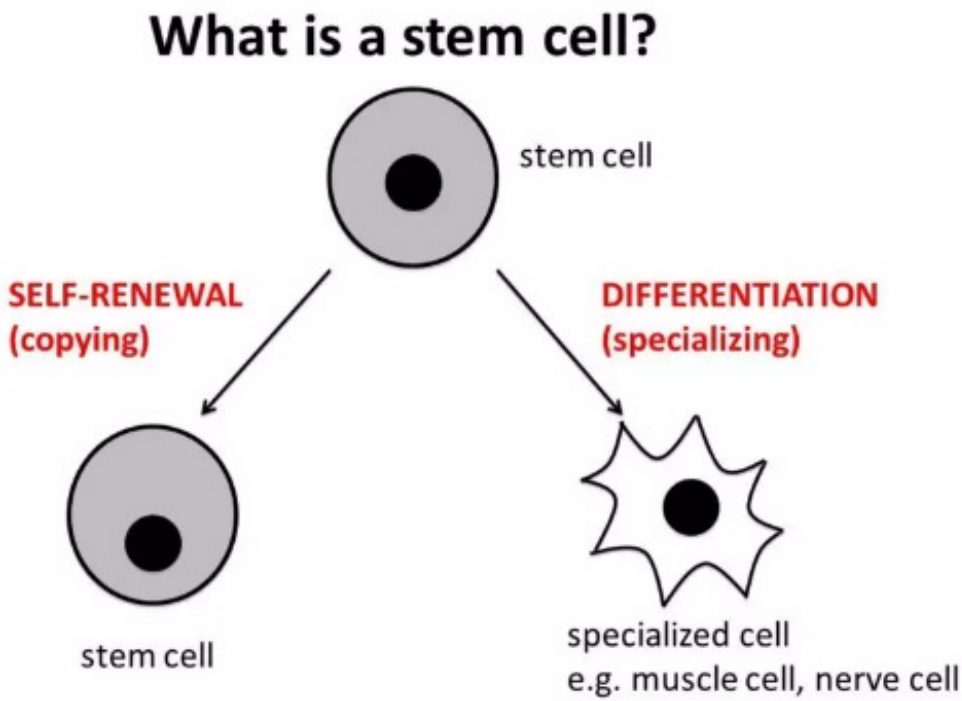
BB 101
Stem Cells, Cancer and Protein
Tutorial 6
15.02.2024

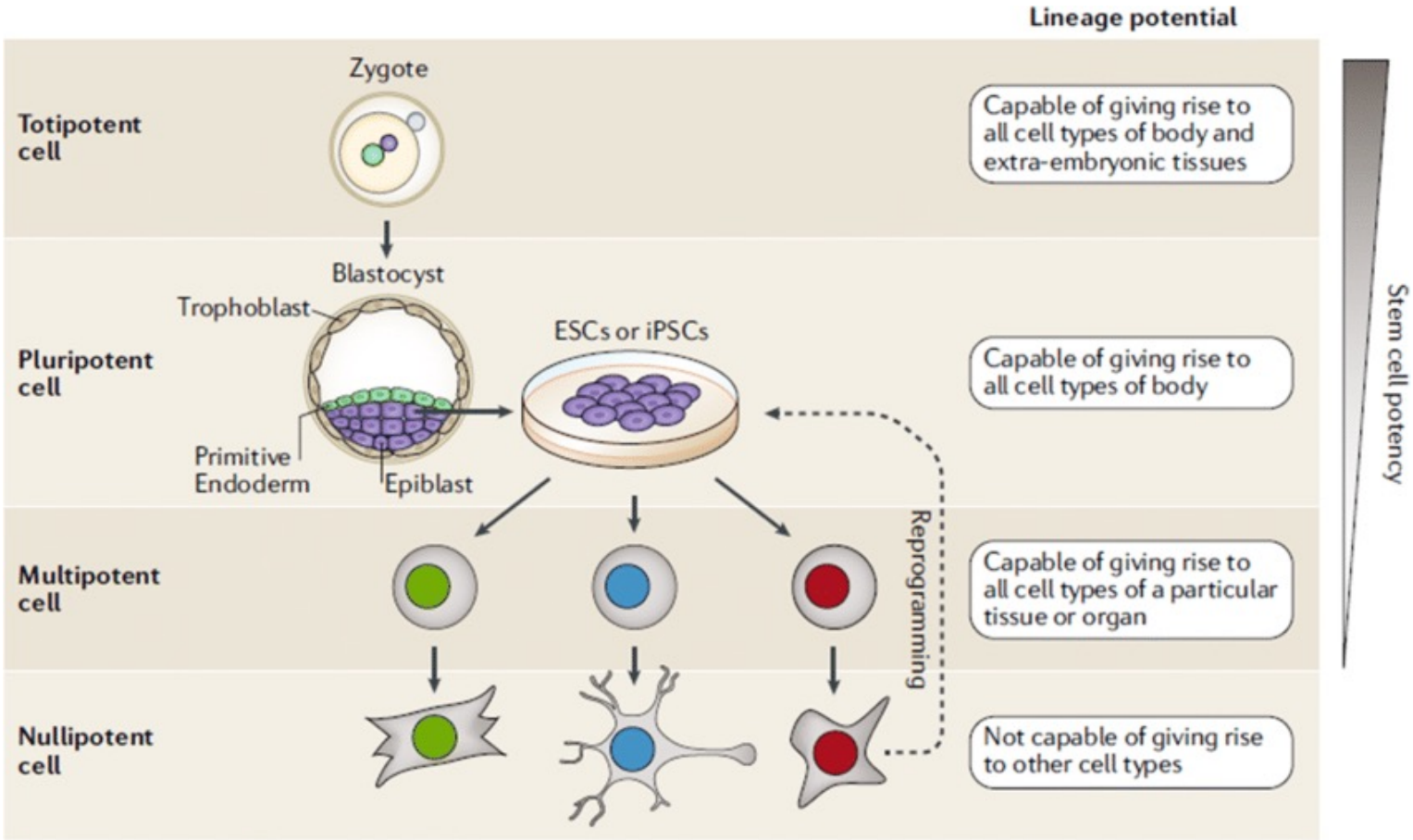
Stem Cell

- Undifferentiated or Unspecialized cells
- Ability to divide and differentiate
- Capable of self-renewal
- Form different types of specialized cells



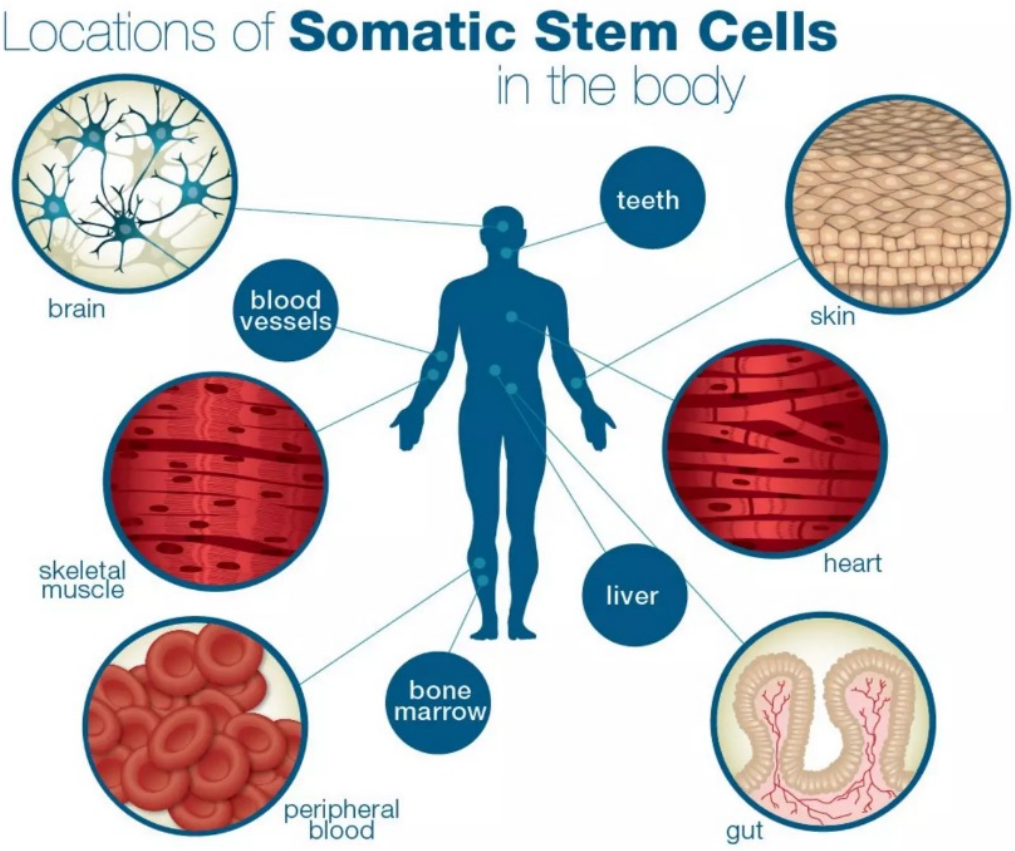
Types of Stem Cells

- Totipotent, pluripotent, and multipotent
- *Examples*- Embryonic SCs, Hematopoietic SCs, Mesenchymal SCs, Neural SCs, Epithelial SCs, Skin SCs



Source: Balistreri et al

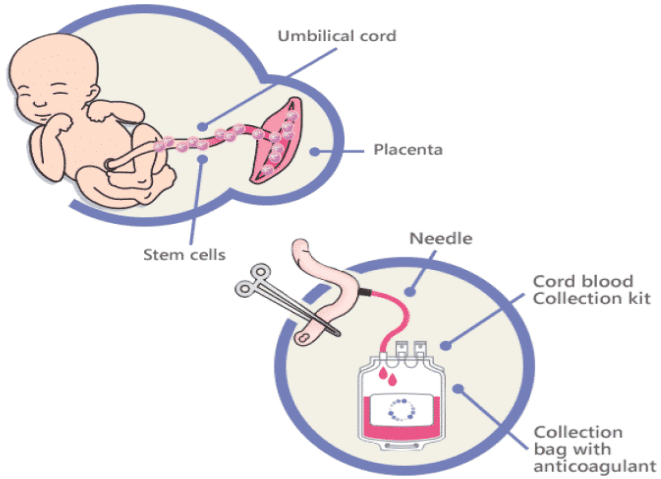
Importance of stem cells



Stem cell therapy, also known as **regenerative medicine**, promotes the repair response of diseased, dysfunctional, or injured tissue.

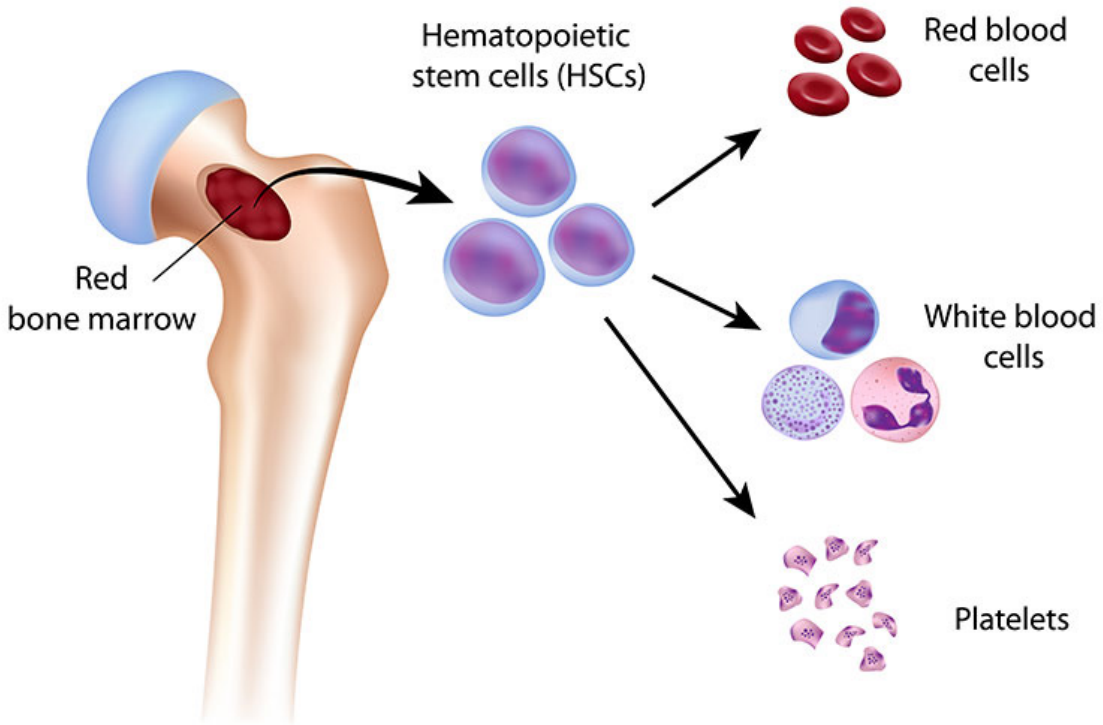
Example: For the treatment of leukemia (blood cancer)

- **Umbilical cord blood**



Stem Cell Therapy

- **Bone marrow transplant:** Doctors collect stem cells from marrow with a hollow needle.
- **Peripheral blood stem cell transplant**: PBSCs are collected from blood drawn with a small needle.



https://www.stemcellcareindia.com

Projects under trials....

1. Injecting modified stem cells directly into the brain after a stroke

2. Using stem cells to replace damaged cells in the inner ear that detect sound, helping to restore hearing

3. Altering the genes of stem cells to make them resistant to diseases, such as AIDS, and then inserting them into people with the disease

4. Cultivating stem cells to repair the fragile bones of people with osteoporosis

But…how do cells divide?

What happens to cell division when there is something wrong with the cell? And…how exactly do the cells know that something is wrong?

Cell cycle and its checkpoints

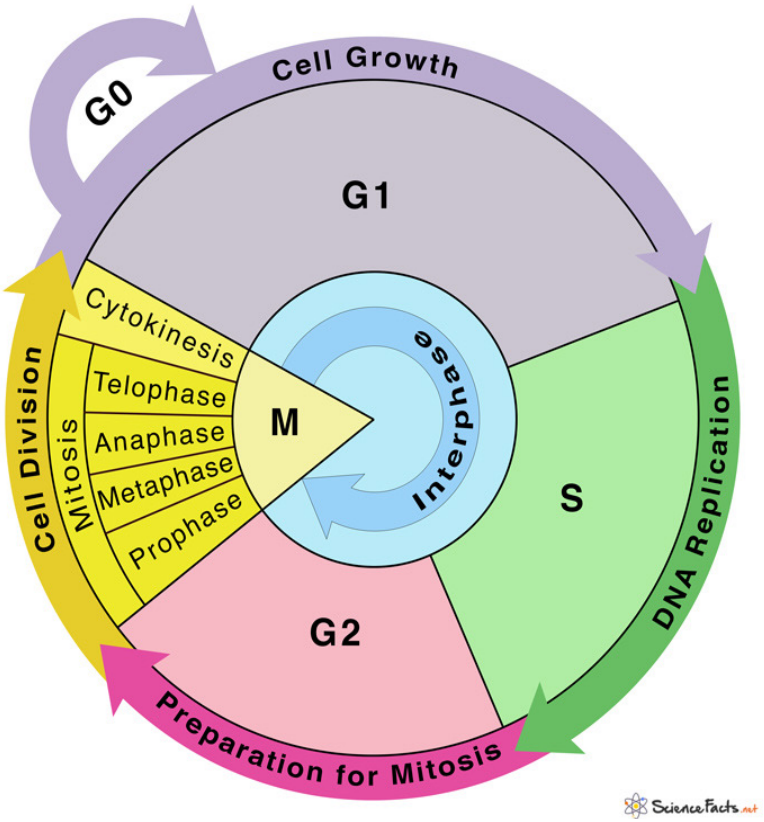


Fig.: Cell cycle

Important checkpoints:

1. G1 Checkpoint- Nutrients, growth factors, DNA damage
2. G2 Checkpoint- Cell size, DNA replication
3. M Checkpoint- Chromosome spindle attachment

Source: https://www.sciencefacts.net/cell-cycle.html

Is it possible for cells to show uncontrolled division?

Cancer

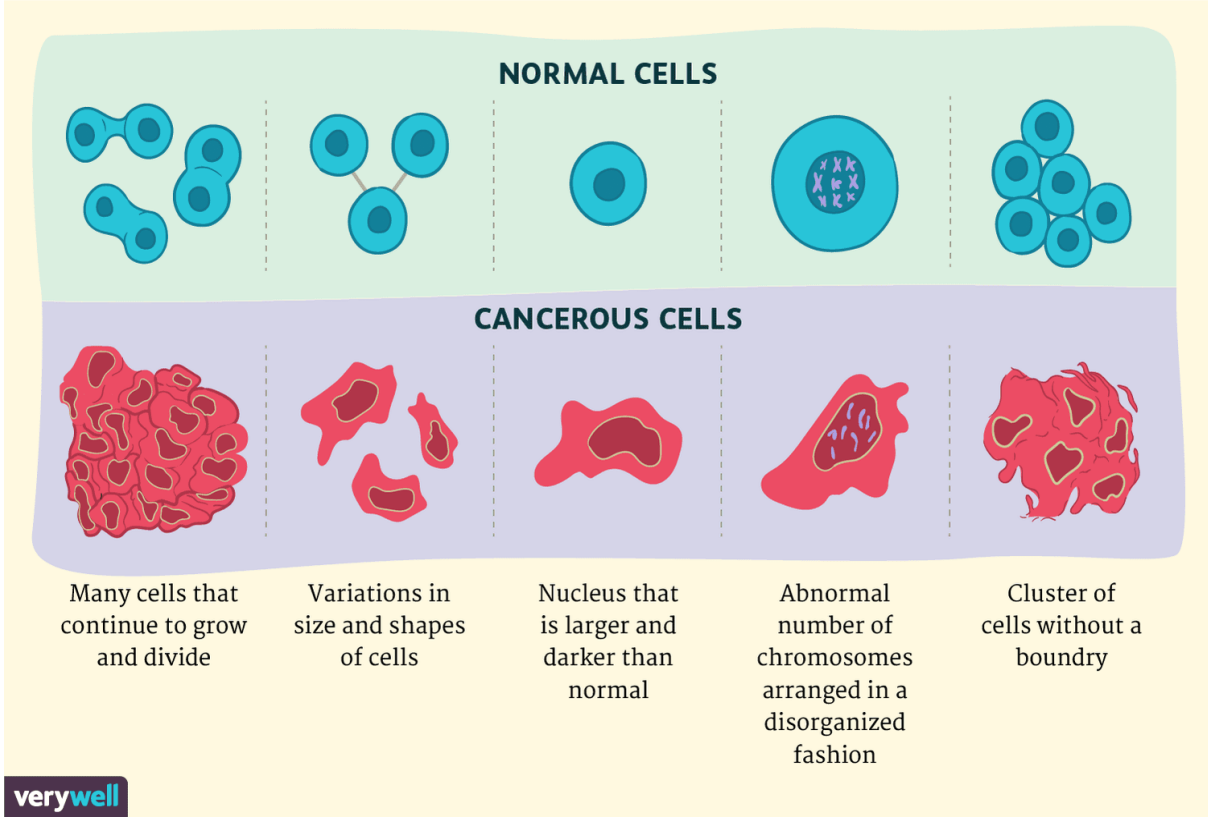


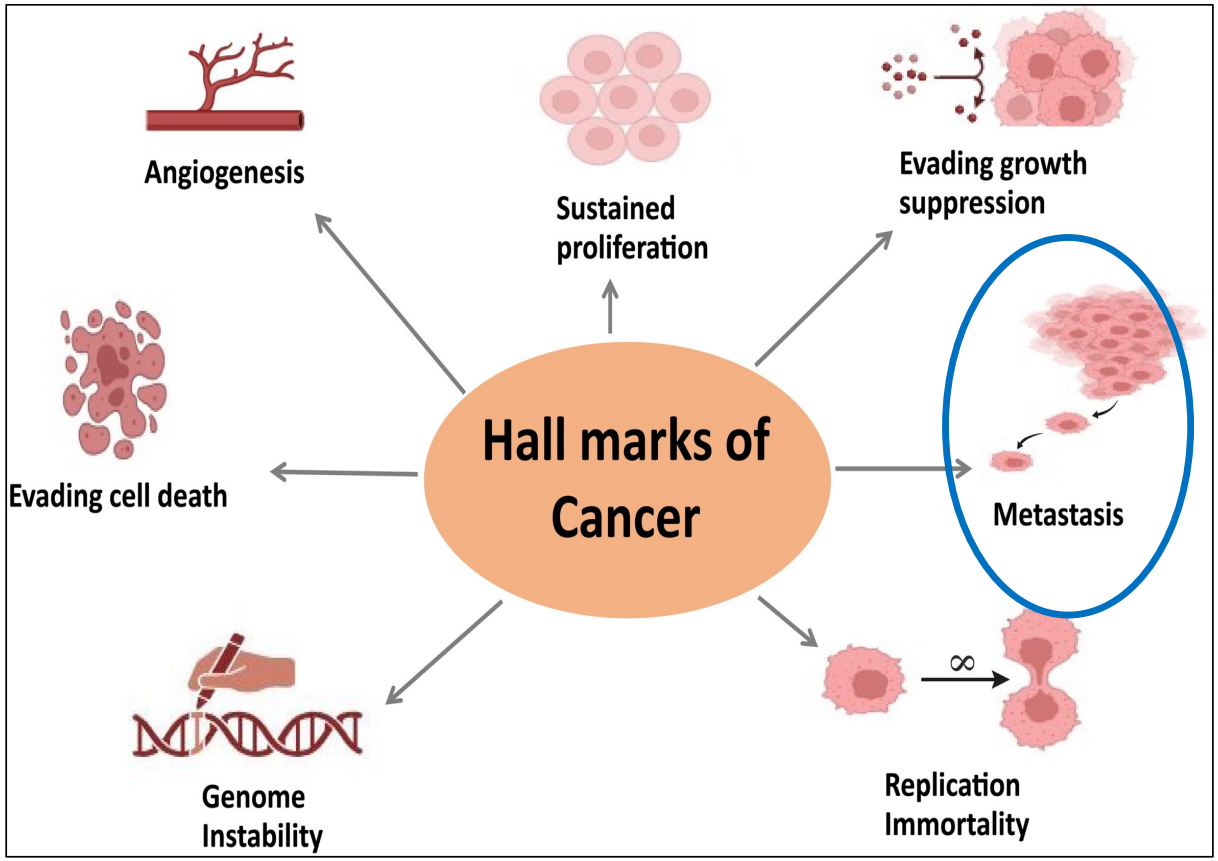
Fig.: Normal v/s Cancer cells

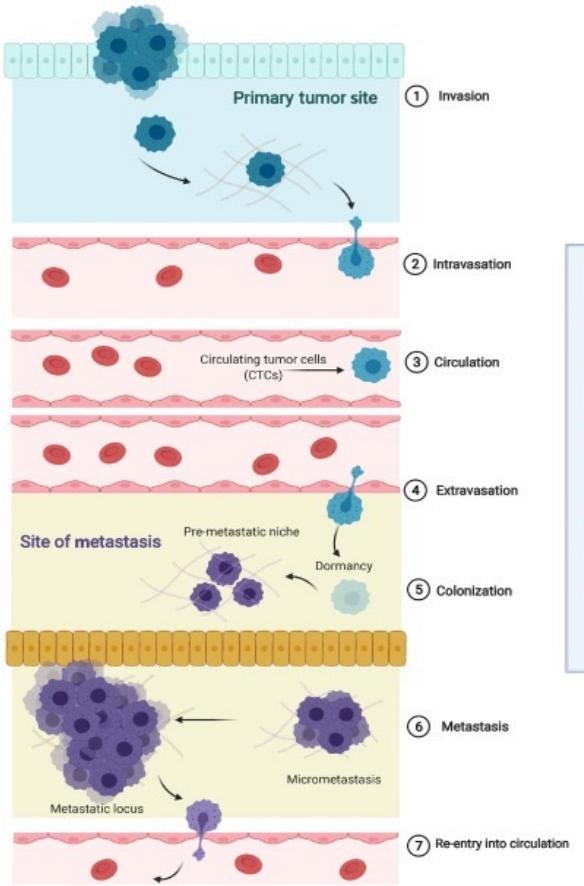
- Uncontrolled proliferation
- Bypass checkpoints
- Types:
  1. Benign
  2. Malign

Cause: Change in genetic material (mutations)

Source: https://www.verywellhealth.com/cancer-cells-vs-normal-cells-2248794

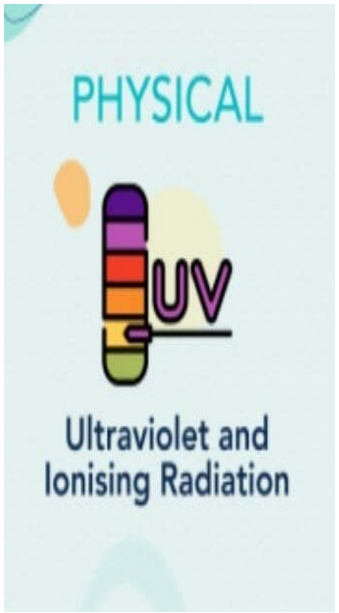
Hallmarks of Cancer

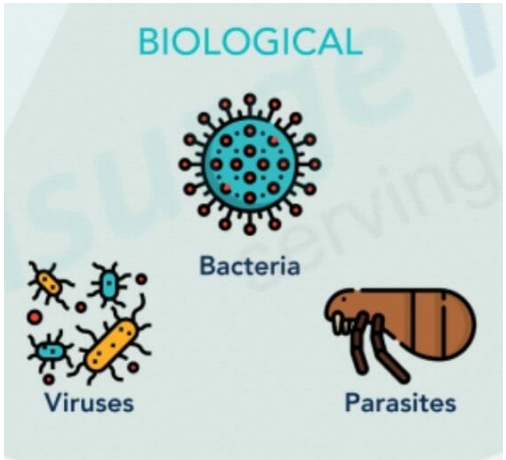




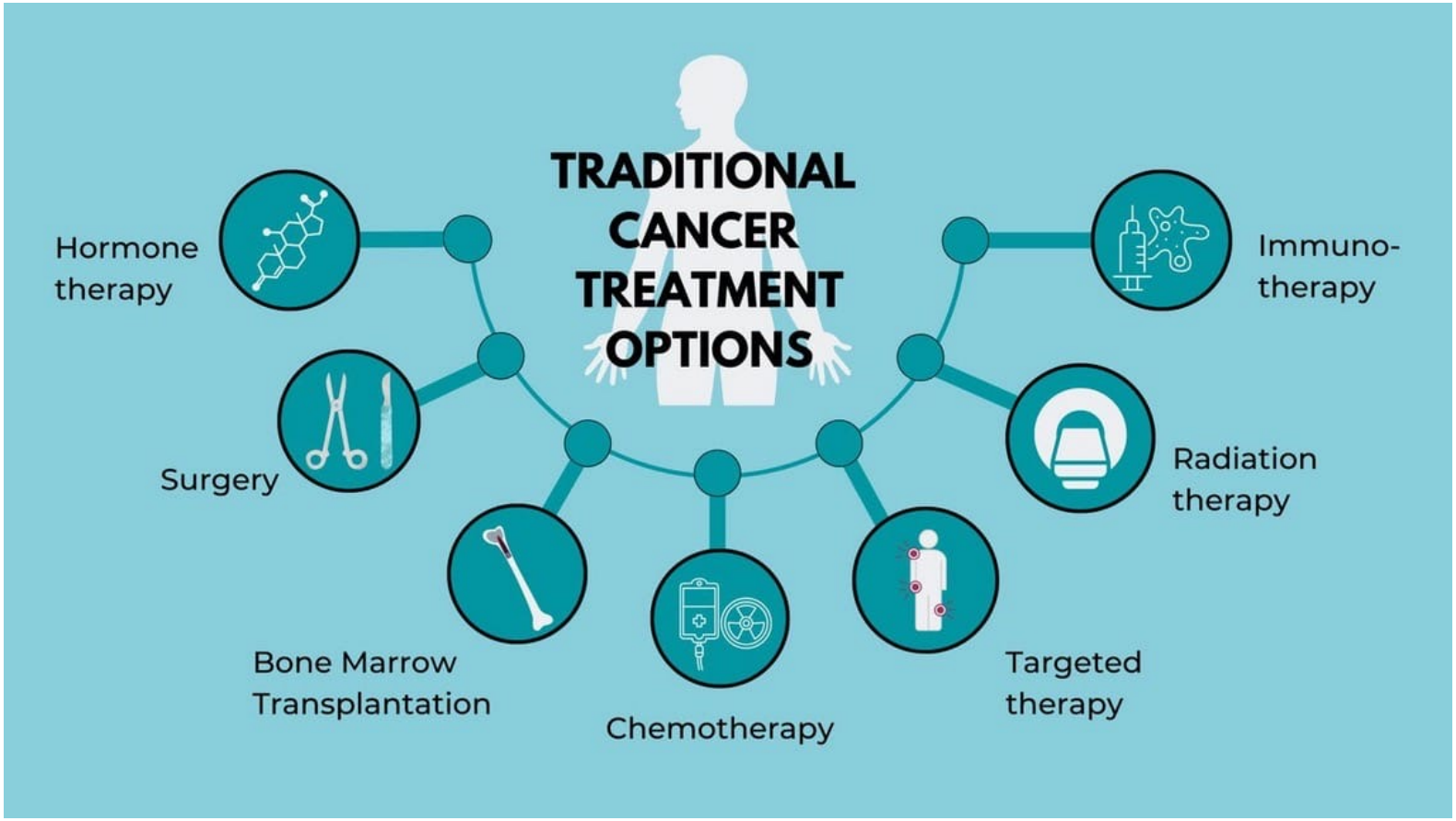
What causes mutations??

Cancer Causes





Cancer Treatment



Source: FLCCC Alliance

How can you help biologists ???

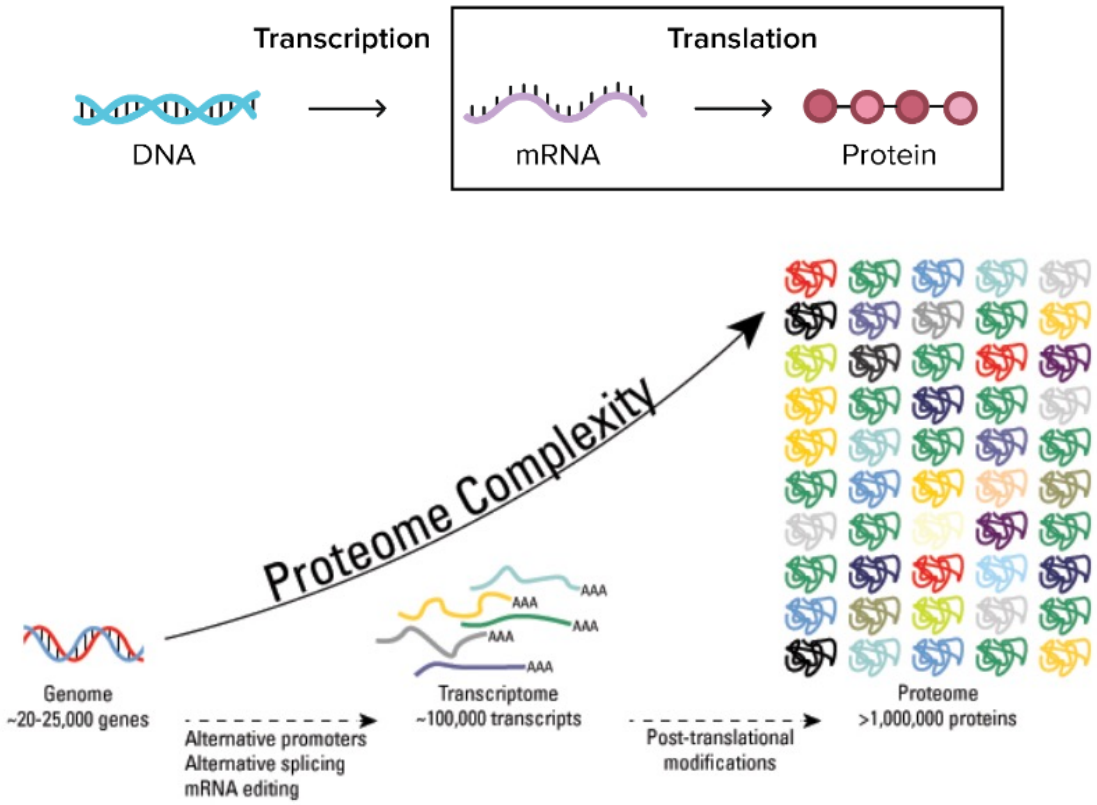
Working with biologists…..

- **Previous problem**: difficult to diagnose cancer at an early stage
- But, now, with the help of powerful techniques such as Mass Spectrometer one has huge proteomics data.
- With the help of computational biology and machine learning programs, biologists are working on targeting specific proteins for treatment.
- *For example*: Detection of **biomarkers**

Biomarker panel	Cancer prediction
OVA1™ (CA 125, prealbumin, apolipoprotein A-1, beta2 microglobulin, transferrin)	Prediction of ovarian cancer risk in women with adnexal mass
DCP and AFP-L3	Risk assessment for development of hepatocellular carcinoma
Risk of Ovarian Malignancy (ROMA)	Prediction of ovarian cancer risk in women with pelvic mass
PCA3 (Prostate Cancer Antigen 3)	Determination of need for biopsy or repeat-biopsy in patients at risk for prostate cancer
Overa (CA 125, apolipoprotein A-1, transferrin, follicle-stimulating hormone, human epididymis protein 4)	Prediction of ovarian cancer risk in women with adnexal mass

https://edrn.nci.nih.gov/about-edrn/fda-approved-tests/

Synthesis of Proteins from Gene

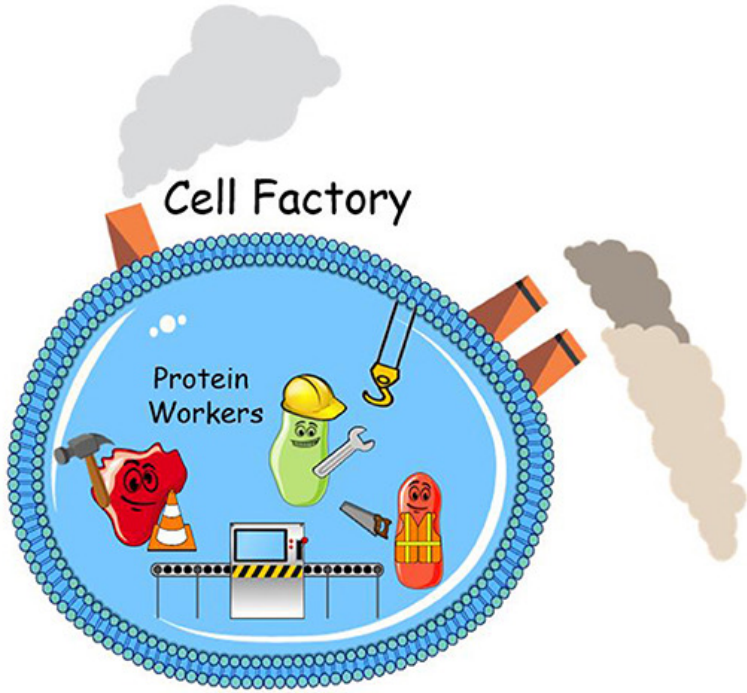


Post-transcriptional modification: The processes of polyadenylation, 5' capping, and splicing.

Post-translational modification: Covalent processing events that change the properties of a protein by proteolytic cleavage and adding a modifying group, such as acetyl, phosphoryl, glycosyl, and methyl, to one or more amino acids

What are Proteins?

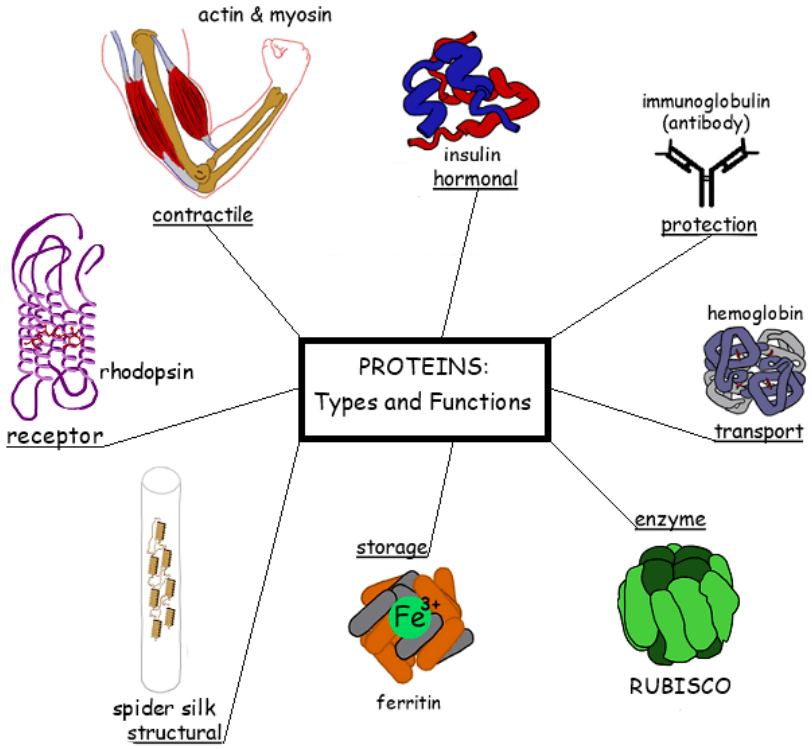
- Large biomolecules
- Found in every cell in the body
- Made up of chains of **amino acids**
- Peptides: fewer than 50 amino acids
  - Dipeptides: 2 amino acids
  - Tripeptides: 3 amino acids
  - Polypeptides: more than ten amino acids
  - Proteins: more than 50 amino acids
- The 20 amino acids commonly found as residues in proteins
- **Proteome-** complete set of proteins expressed by an organism



What are the different roles of Protein?

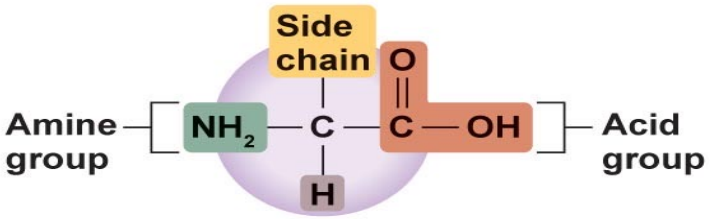
Proteins can be classified into several types based on their functions:

- Enzymes
- Structural Proteins
- Transport Proteins
- Hormones
- Antibodies (Immunoglobulins)
- Motor Proteins
- Storage Proteins
- Receptor Proteins
- Defensive Proteins



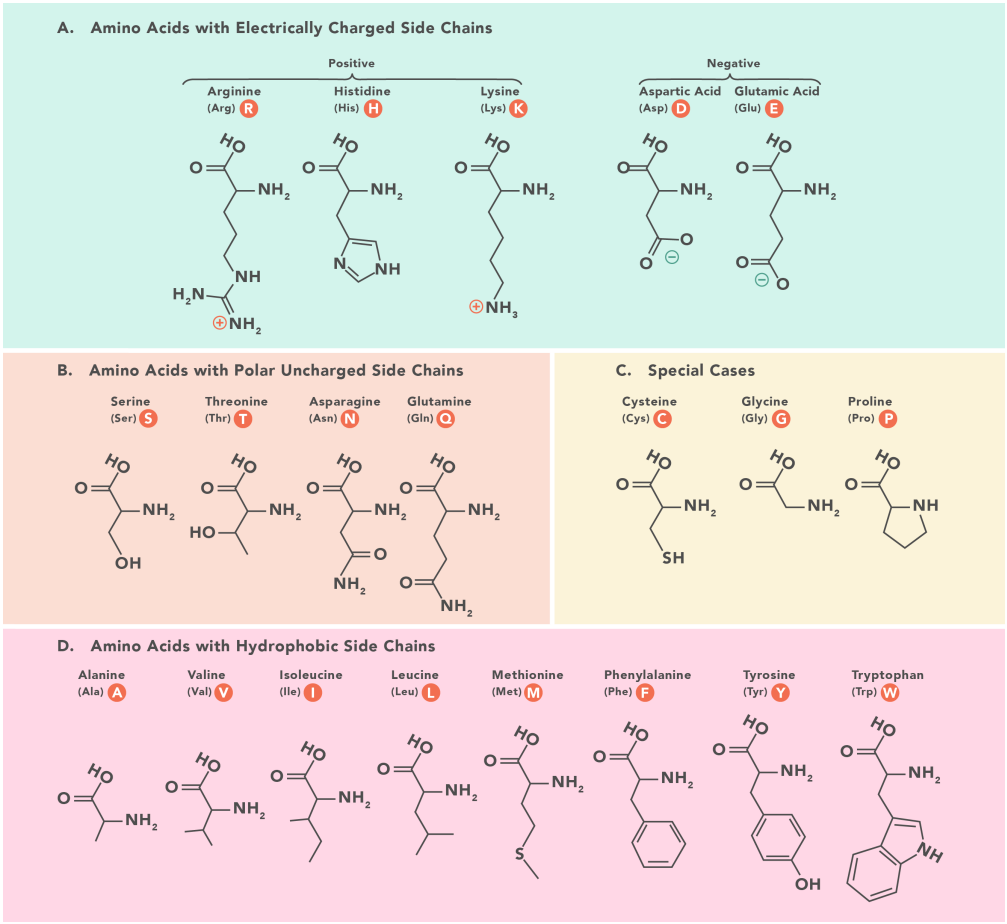
https://alevelbiology.co.uk/notes/functions-of-proteins/

Amino Acid



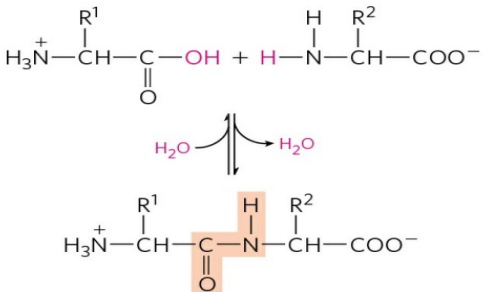
α-carbon atom is thus a chiral center.
> Exception?

Amino acids can be classified based on their **R group and Polarity**.

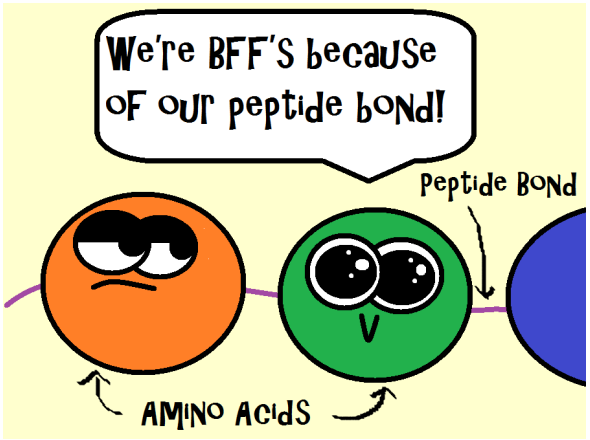


Peptide Bond

- Two amino acid molecules covalently joined through a substituted amide linkage, termed a peptide bond, to yield a dipeptide
- Linkage is formed by the removal of water (dehydration)



- Apart from peptide bonds, Ionic bonds, Disulfide bonds, Hydrogen bonds, and Hydrophobic Interactions are involved in protein folding

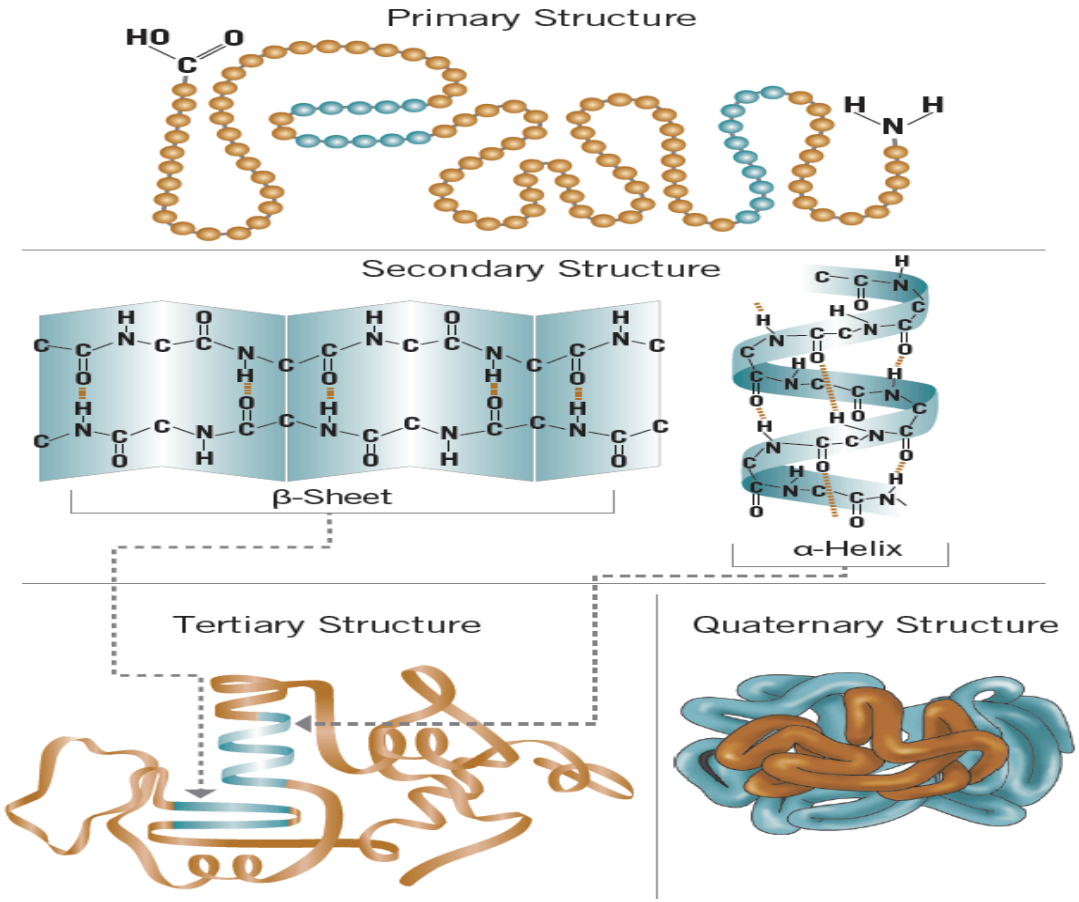


How many water molecules are released in forming a polypeptide containing seven amino acid residues?

Protein Structural Level

- Proteins can be described at several levels of complexity, arranged in a kind of conceptual hierarchy. Mainly four types.

- **Primary Structure**: linking amino acid residues in a polypeptide chain
- **Secondary Structure**: stable arrangements of amino acid residues giving rise to recurring structural patterns
- **Tertiary Structure**: three-dimensional folding of a polypeptide
- **Quaternary Structure**: protein with two or more polypeptide subunits, their arrangement in space



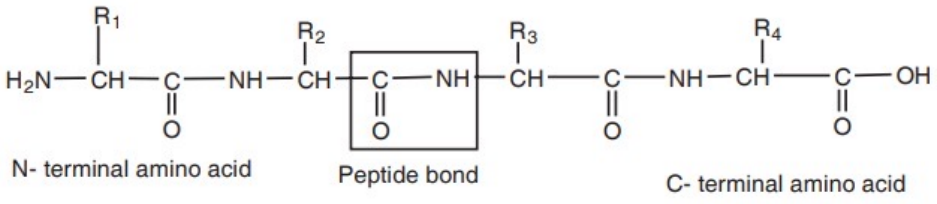
What makes one protein an enzyme, another a hormone, another a structural protein, and still another an antibody? How do they differ chemically?

The most obvious distinctions are **structural**. Each protein has a distinctive number and sequence of amino acid residues, That determines how it folds up into its unique three-dimensional structure, and this, in turn, determines the function of the protein.

Any alteration in the structure or sequencing changes the shape and function of the protein

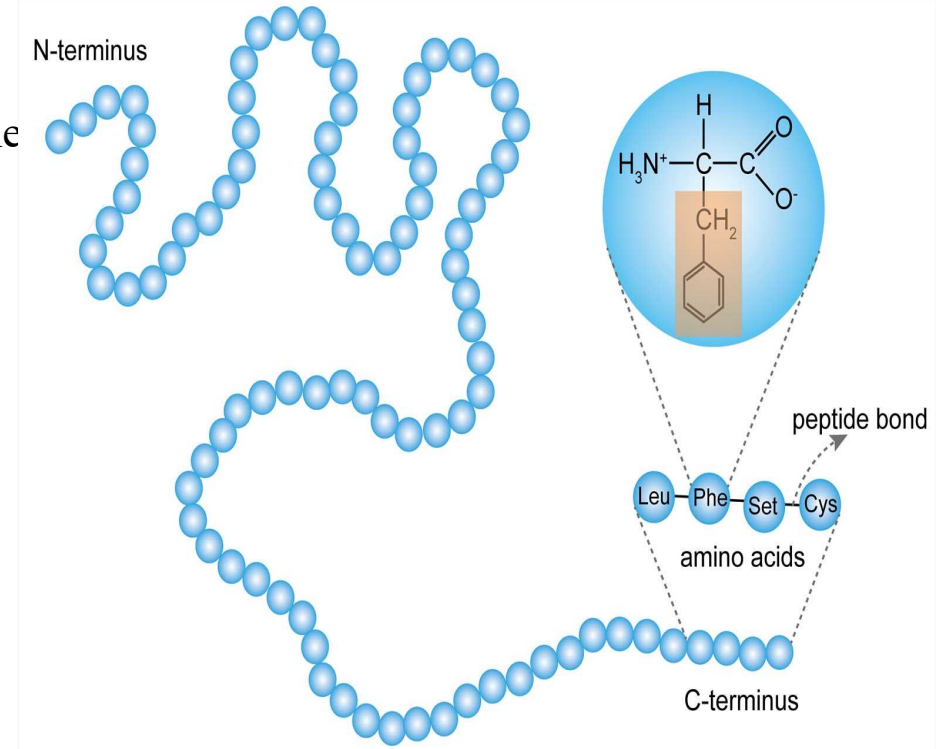
Primary Structure

- Ordering of amino acids to form their chains.
- Determines the final fold and, therefore, the function of the protein.
- Any change in the sequence changes the entire protein.



For example the primary structure of a protein can be written as

H_2N— gly — lys — leu — val — ala — glu — COOH
1 2 3 4 5 6



Anfinsen's Hypothesis

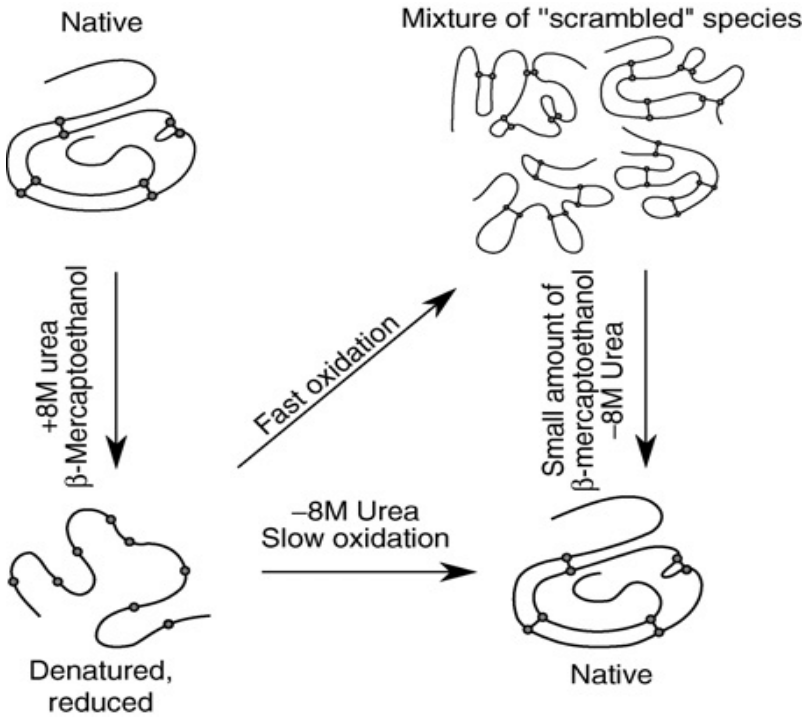
Ingredients: RNase A enzyme, β-mercaptoethanol, 8M Urea

Experiment 1: Addition of both β-mercaptoethanol and 8M Urea
Observation: Denaturation of Disulphide and Hydrogen bonds resulting in the complete unfolding of the protein.

Experiment 2: Simultaneous removal of both β-mercaptoethanol and 8M Urea.
Observation: Reformation of a disulphide linkage. **100% Activity**.

Experiment 3: Sequential removal of β-mercaptoethanol followed by 8M Urea.
Observation: Scrambled Protein formed. Biologically **not active.**

Experiment 4: Addition of small amount of β-mercaptoethanol.
Observation: **100% Activity.**



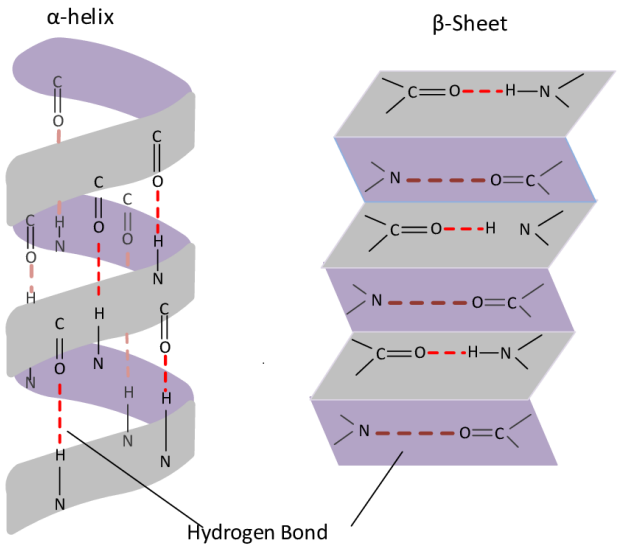
Inference: Primary structure determines the final conformation of the protein.

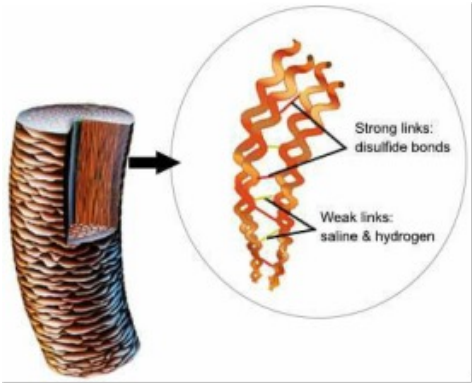
Secondary Structure

•Polypeptide chains usually fold due to the interaction between the amine and carboxyl group of the peptide link.

• They are found to exist in two different types of structures α – helix and β – pleated sheet structures.

• This structure arises due to the regular folding of the backbone of the polypeptide chain due to hydrogen bonding between the -CO group and -NH groups of the peptide bond.





Permanent Hair Waving is Biochemical Engineering!!

- Moist heat stretches α-keratin α-helices to a β conformation, reverting upon cooling.
- Disulfide bond manipulation with reducing and oxidizing agents creates lasting curls or waves, though not truly permanent due to hair growth.

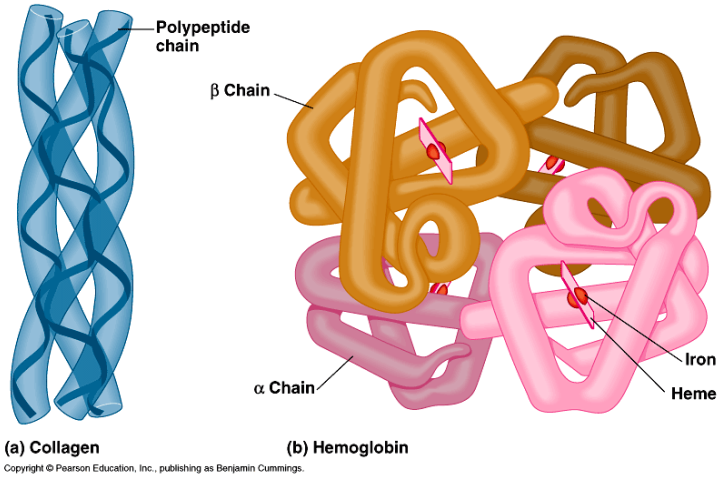
Tertiary and Quaternary Structure

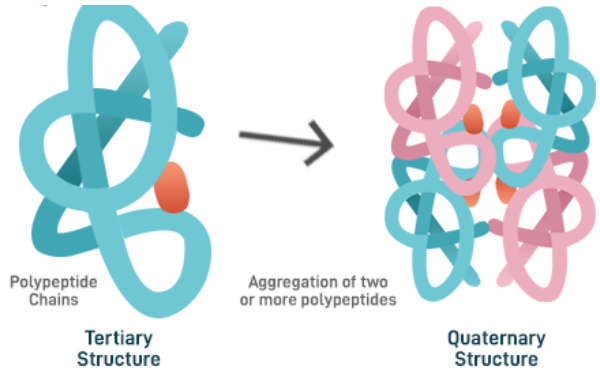
- This structure arises from further folding of the secondary structure of the protein.
- H-bonds, electrostatic forces, disulfide linkages, and Vander Waals forces stabilize this structure.
- It gives rise to two major molecular shapes called **fibrous and globular**.

Fibrous Protein- Keratin

Globular Protein- Albumin

- The spatial arrangement of various tertiary structures gives rise to the **quaternary structure**





Protein Folding

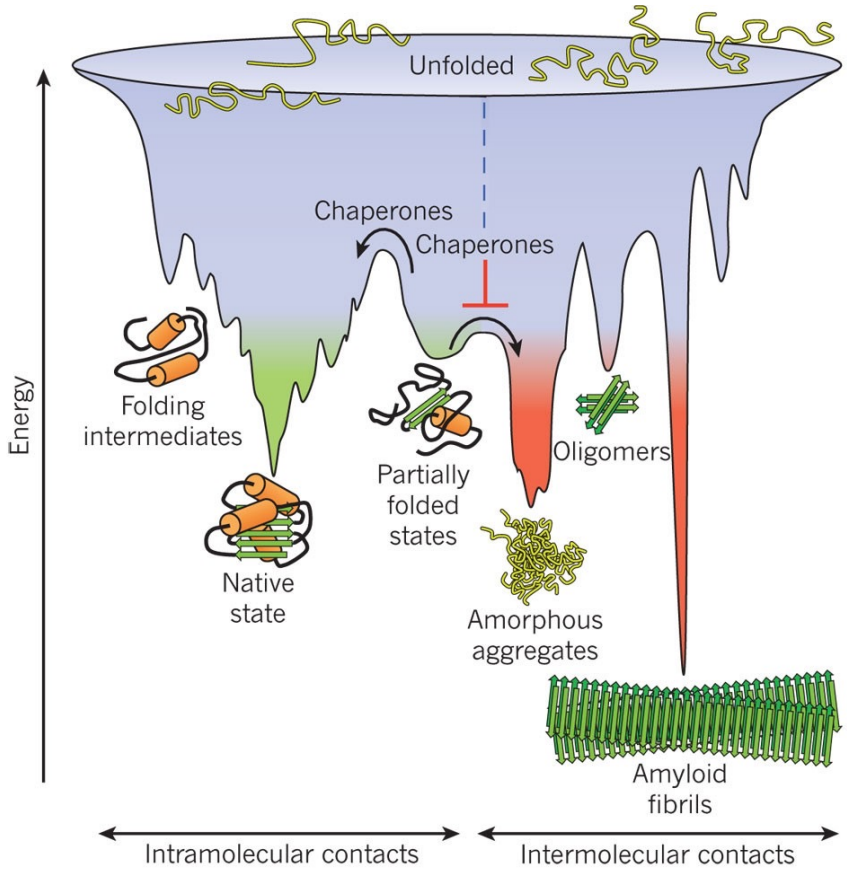
Why do Proteins need to fold?

To carry out their function, for instance, as enzymes or antibodies.

Proteins fold on a defined pathway (or a small number of alternative pathways); they don't randomly search all possible confirmations until they arrive at the most stable (lowest free energy) structure.

Chaperons are a group of proteins that assist a misfolded protein in refolding properly in its native state.
Eg: Bacterial Heat Shock protein

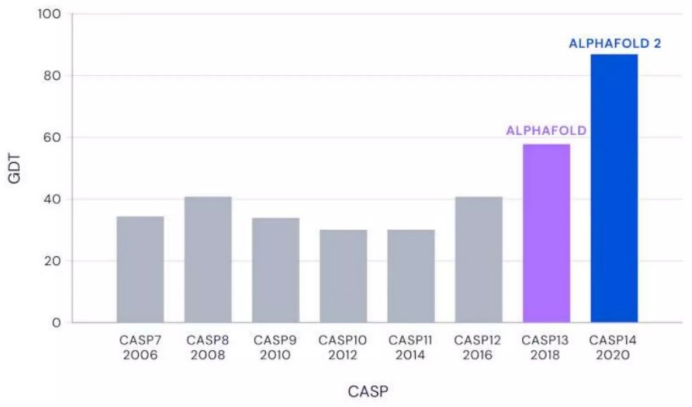
Diseases caused by misfolding of proteins:
Alzheimer's, Cystic Fibrosis, Parkinson's Disease

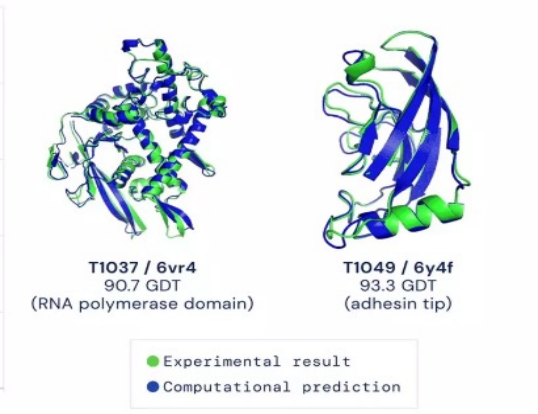


Protein Structure Prediction

Goal: Protein 3D Structure Prediction using Artificial Intelligence
Data: PDB (Publicly available)
Experimental Structure Prediction:

- NMR
- X-Ray crystallography
- Cryo-Electron Microscopy





DeepMind

BBC

AI breakthrough could spark medical revolution

By Paul Rincon
Science editor, BBC News website

22 July | Comments

nature

NEWS | 30 November 2020

'It will change everything': DeepMind's AI makes gigantic leap in solving protein structures

https://www.nextbigfuture.com/2020/12/expert-impressions-of-deep-mind-alphafold-protein-folding-advance.html