IMMUNOLOGY

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IMMUNOGLOBULINS

(Antibodies)

Structure & Functions

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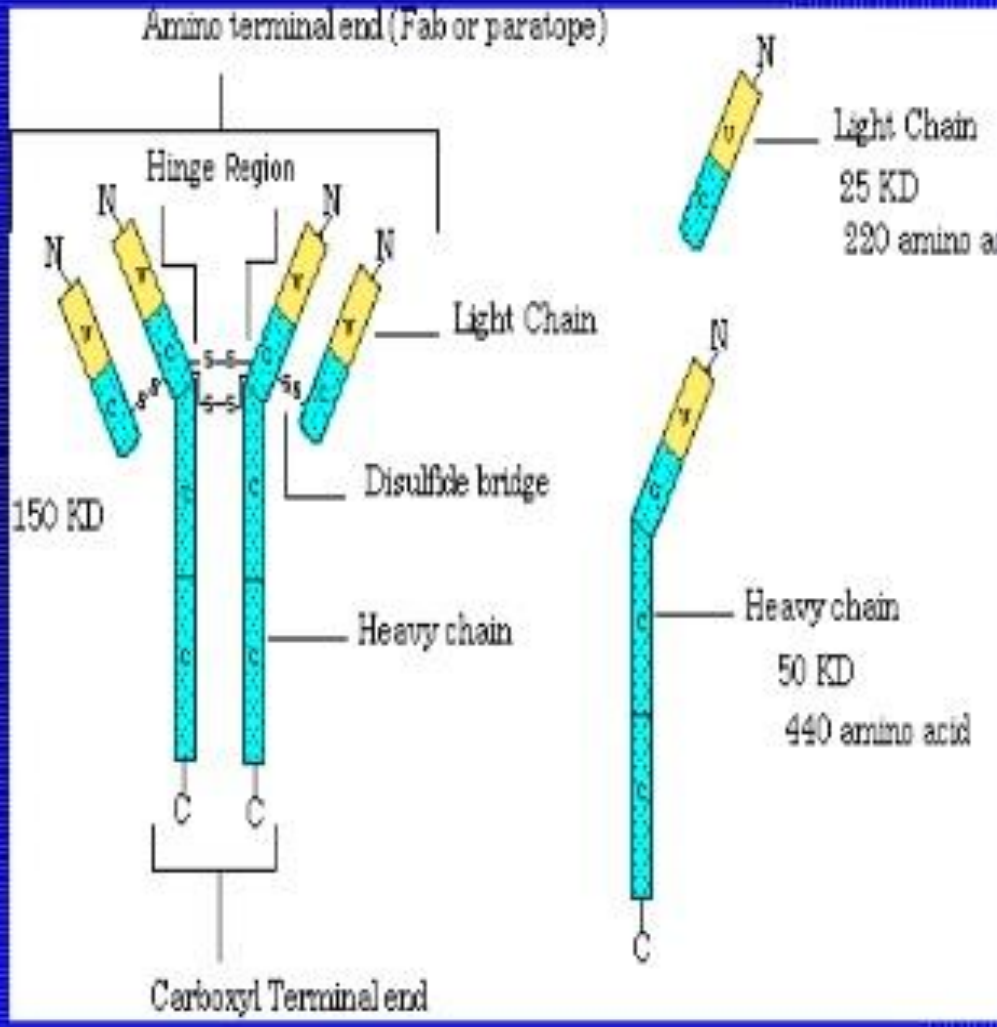
Immunoglobulin

Immunoglobulin is a glycoprotein that is made in response to an antigen and can recognize and bind to the antigen that caused its production.

- Are gamma globulins
- Synthesized by plasma cells
- Constitute 25-30 % of total serum proteins
- Antibodies are present in serum, tissue fluids and mucosal surfaces.
- All antibodies are immunoglobulins, but all immunoglobulins may not be antibodies

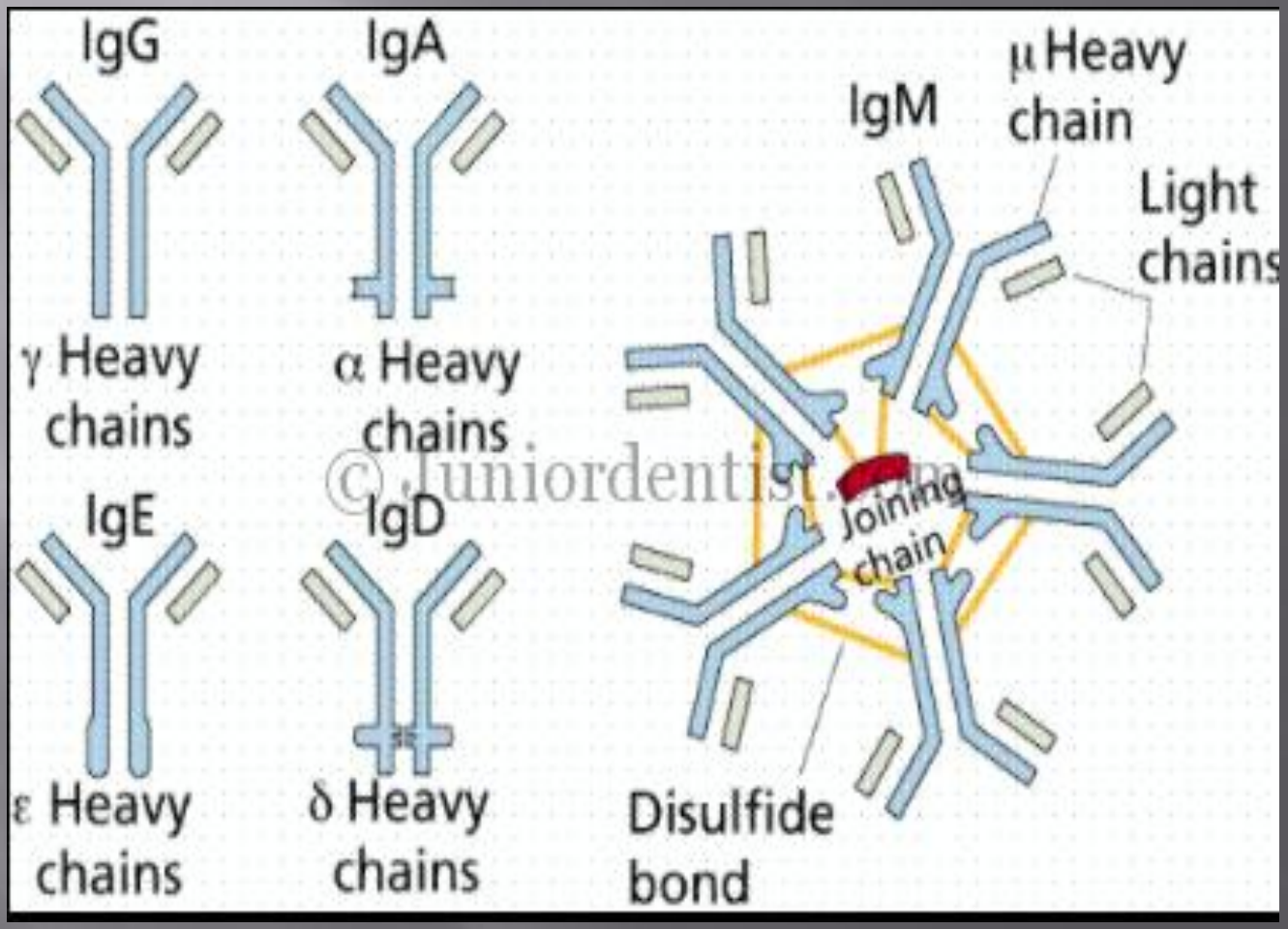
Basic structure

- Composed of 4 polypeptide chains.
- 2 identical light and 2 identical heavy chains
- Linked by disulphide bonds
- Light chains similar in all immunoglobulins
- Light chains occur in 2 varieties kappa and lambda
- Light and Heavy chains are subdivided into variable and constant region.
- Each heavy and light chain contains amino terminal in variable region carboxy terminal in constant region



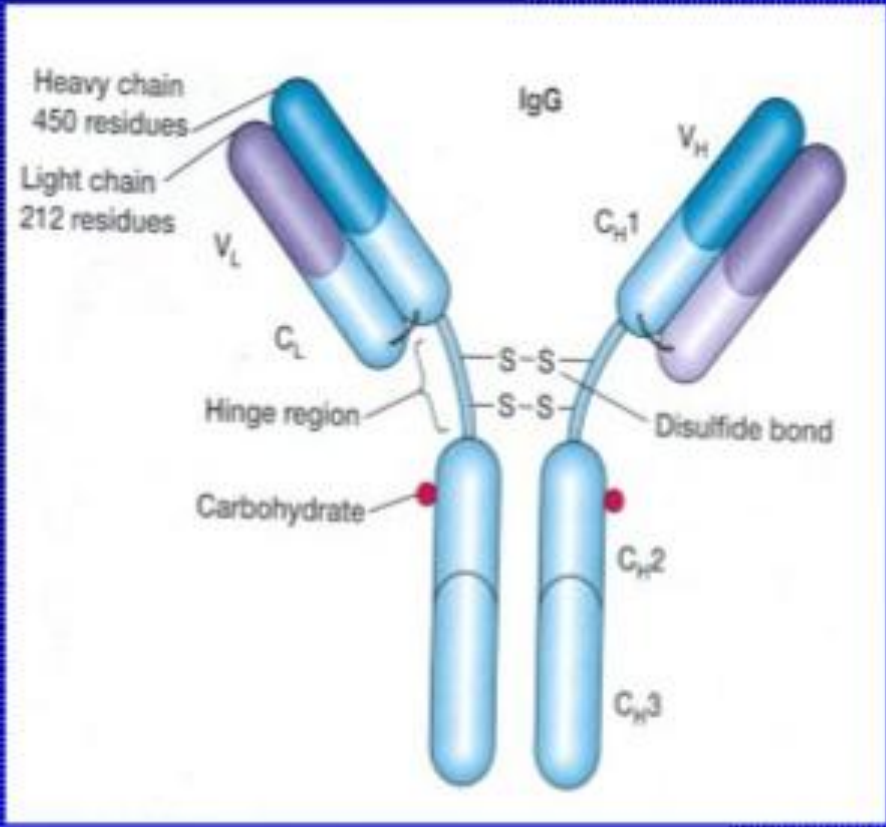
Classification

- Based on structure and antigenic nature of H chain the immunoglobulins are classified into 5 classes.
- Ig G- (gamma)
- Ig A- *(alpha)*
- *Ig M- (mu)*
- *Ig D- (delta)*
- *Ig E - (epsilon)*

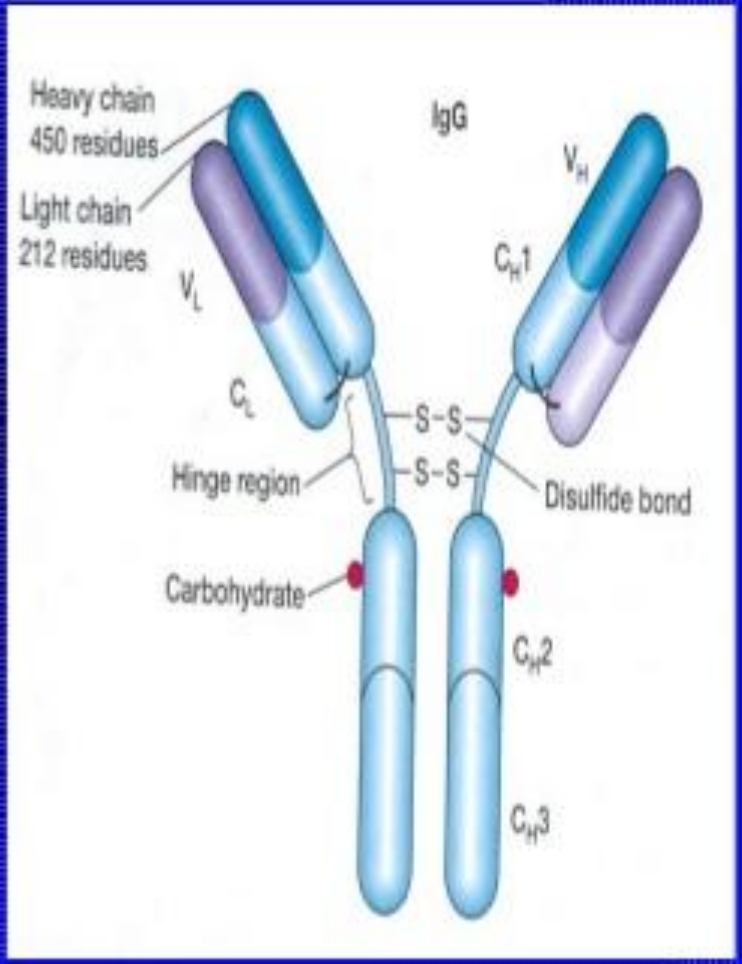


Immunoglobulin G (Ig G)

- Most abundant class in serum
- Constitutes 80% total immunoglobulin
- Present in blood, plasma and tissue fluids
- Contains less carbohydrate than other immunoglobulins
- It has a half life of 23 days: the longest of all of the immunoglobulin isotypes

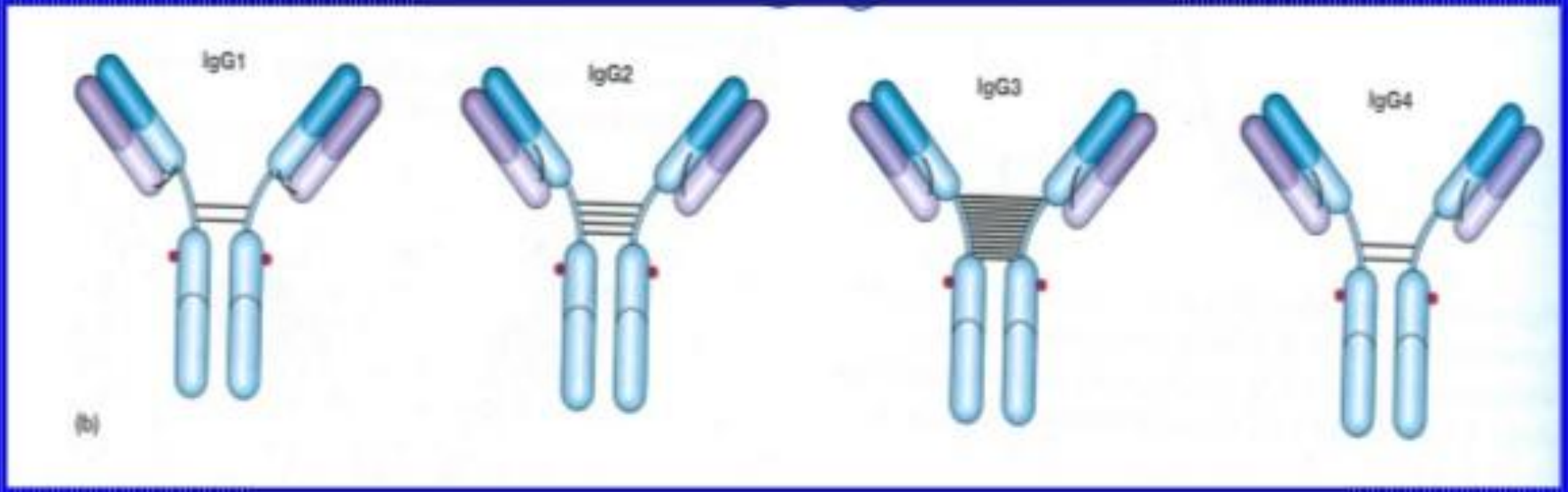


- Crosses placenta and provide natural immunity to foetus and neonate at birth
- Acts against bacteria and viruses by opsonizing
- Neutralize toxin
- Activate complement by classical pathway
- Catabolism of IgG is unique in that it varies with its serum concentration



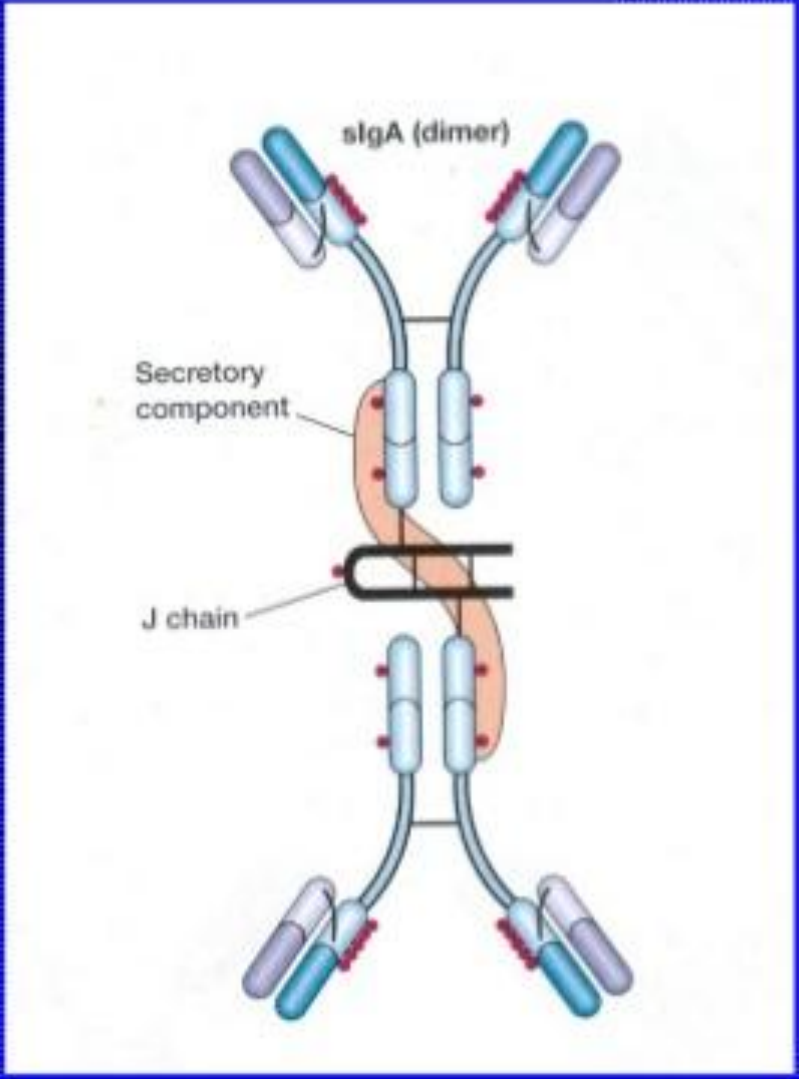
Sub classes of Ig G

- Ig G1, Ig G2, Ig G3, Ig G4.



Immunoglobulin A (Ig A)

- Constitutes 10-15 % of total immunoglobulins
- Present in milk, saliva, tears, mucous of respiratory tract, digestive tract and genitourinary tract.
- In serum exist as monomer
- In external secretions exist as dimer called secretory Immunoglobulin.
- Has 'J' chain and secretory piece.
- Half life: 6-8 days

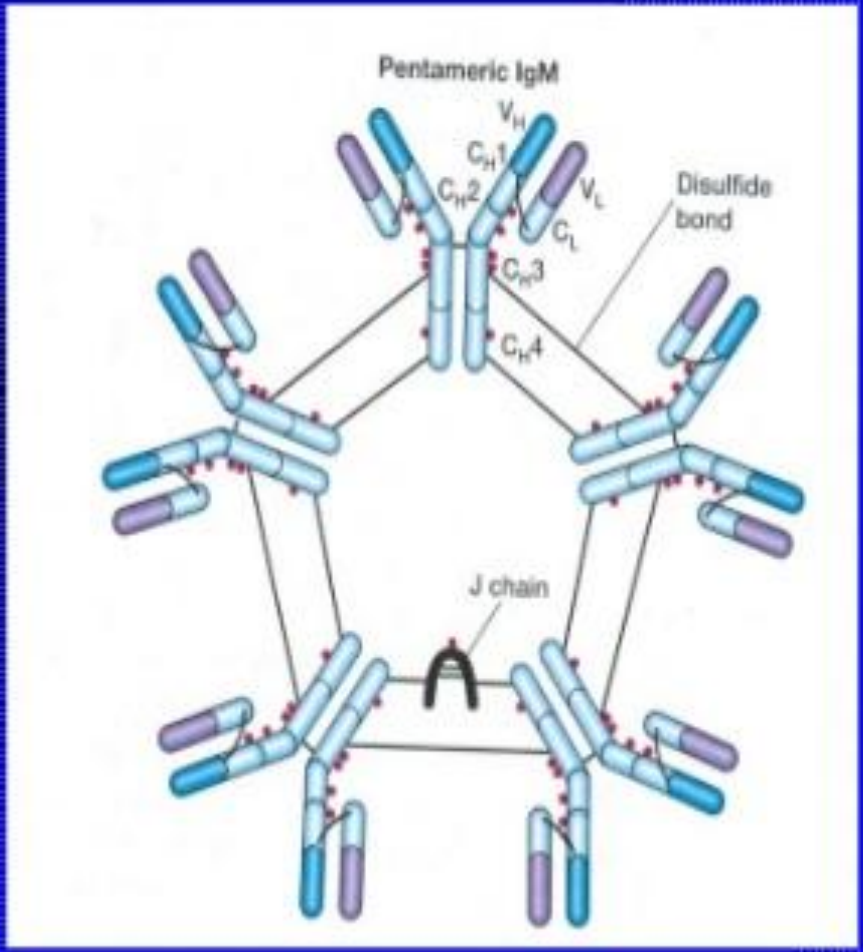


Functions

- Provides local immunity.
- Secretory Ig A binds to surface antigens of microorganism and prevent its attachment and invasion of the mucosal surfaces of respiratory and digestive tract- immune elimination.
- Secretory IgA provides important line of defense against *salmonella*, *Vibrio cholerae*, *N. gonorrhoeae*, influenza virus and poliovirus.
- Secretory IgA present in breast milk protects newborn during first months of life.
- Activates complement by the alternative pathway
- Promotes phagocytosis and intracellular killing of microorganisms

Immunoglobulin M (Ig M)

- Accounts for 5-10% of total serum proteins
- Polymer of five monomeric units (pentamer)
- Held together by disulfide bonds and 'J' chain
- Mol. Wt. of 900,000-10,00,000 (millionaire molecule)
- Half life: 5 days



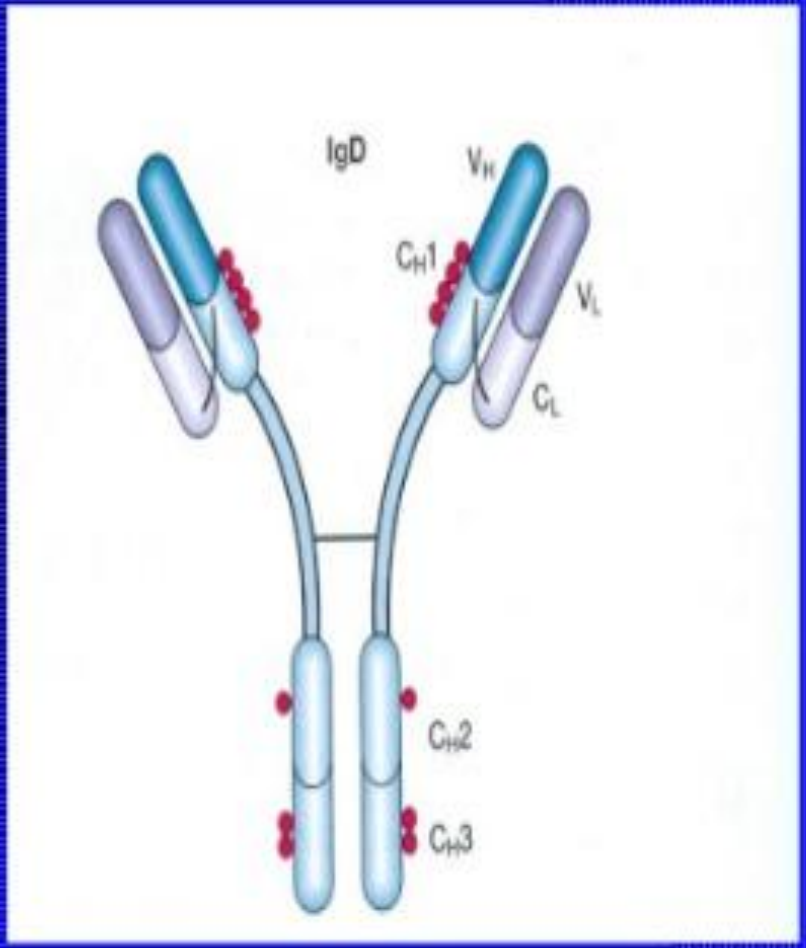
- Most of IgM (80%) present intravascularly
- Present in low concentration in intercellular tissue fluids
- Cannot cross placenta
- Presence of IgM antibody in serum of newborn indicate congenital infection.
- Earliest immunoglobulin to be synthesized by foetus (20 weeks)
- First immunoglobulin to be produced in primary response to antigen
- Relatively short-lived hence it's demonstration in the serum indicates recent infection
- Monomeric IgM appears on the surface of unstimulated B lymphocytes and act as receptors for antigens

Functions

- It agglutinates bacteria
- Activates complement by classical pathway
- Causes opsonization and immune heamolysis
- Believed to be responsible for protection against blood invasion by microorganisms

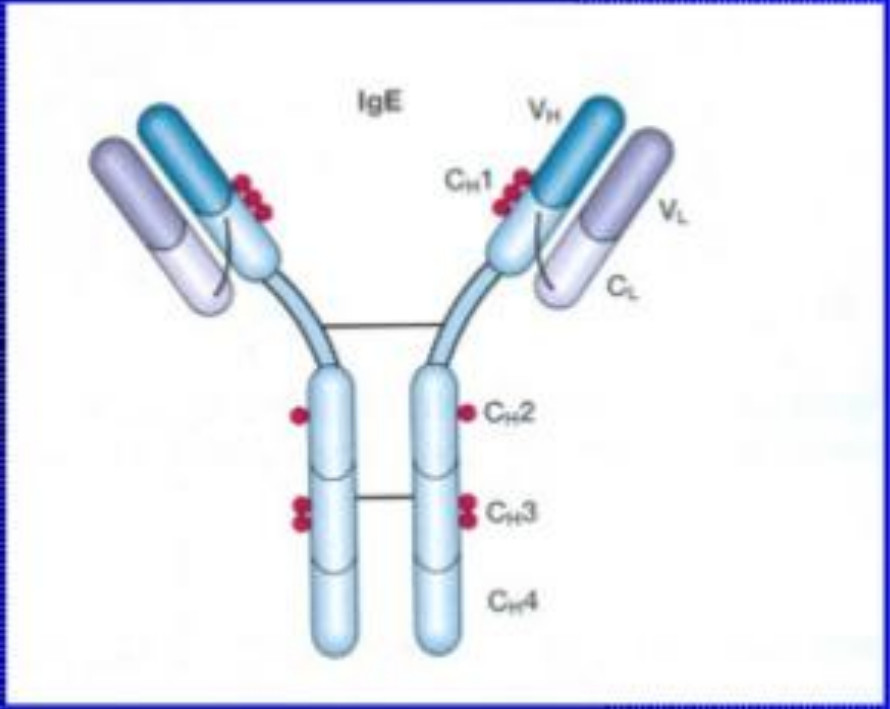
Immunoglobulin D (Ig D)

- Structure is similar to IgG
- Serum concentration 30 micrograms per ml
- Constitutes 0.2% of total immunoglobulins
- Half life: 3 days
- IgD together with IgM is major membrane bound immunoglobulin on unstimulated B lymphocytes-acts as recognition receptors for antigens



Immunoglobulin E (Ig E)

- Structure is similar to Ig G
- Has 4 constant region domains.
- Mol. Wt. 1,90,000
- Half life: 2 days
- Heat labile (inactivated at 56^{0}C in 1 hour)
- Normal serum concentration 0.3 ug/ml
- Mostly present extra cellularly
- Does not cross placenta



- Produced in the lining of respiratory and intestinal tract
- Known as reagin antibody
- Does not activate complement nor agglutinate antigens
- Binds to the Fc receptors on the membranes of blood basophils and tissue mast cells
- Mediates immediate hypersensitivity reaction and P.K. reaction
- Responsible for symptoms of anaphylactic shock, hay fever and asthma.
- Play a role in immunity against helminthic parasites

By: Olla BaEissa

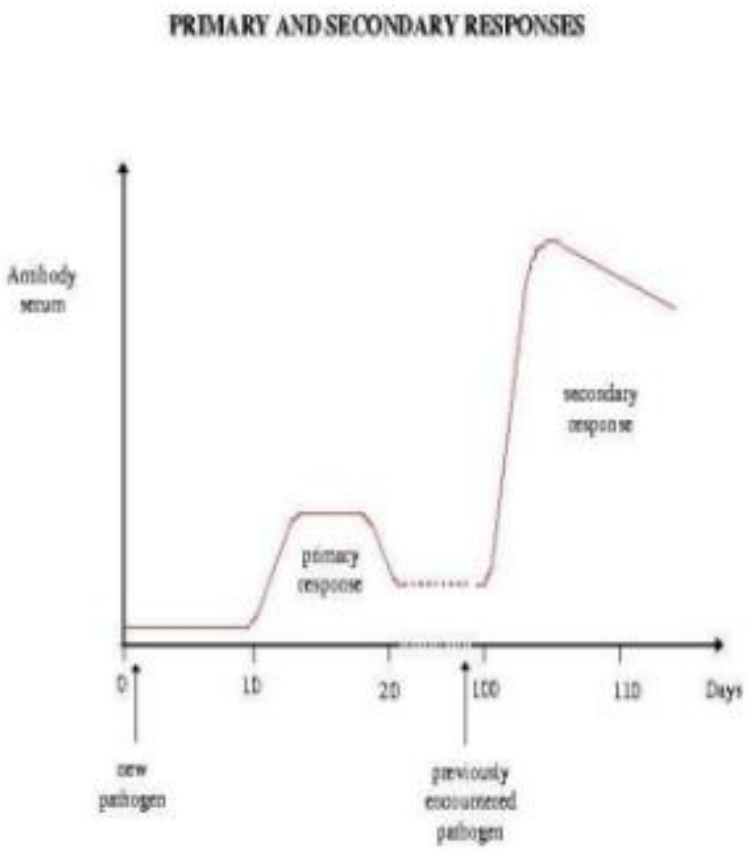
Types of immunoglobulins

Immunoglobin	heavy chain	structure	Function or roles	note
IgG	Gamma γ	Monomer only	1- have the ability to traverse the blood vessels to tissues 2- pass through the placenta to the fetus 3- important in the secondary response	They are the most abundant of the circulating antibodies
IgA	Alpha α	- monomer in serum (blood) - dimer in secretions as sweat, tears and saliva and in mucosal fluids	- protect the walls of bronchi and small intestine. - high concentration in breast milk - help in transferring immunity to the new born infants	
IgM	Mu μ	pentamer	They are produced in the primary immune response 2- They are restricted to the blood stream due to high mass 3- important in the primary response	Its subunits are held together by a glycoprotein called j-chain (joining chain).
IgD	Delta δ	monomer	1- present on the surface of B cells where they function as receptors for antigens 2-their function in serum is not yet defined	present on the surface of B cells
IgE	Epsilon ε	monomer	1- important in protection against parasites 2- They are involved mainly in the development of allergic responses 3- the release of histamine, 4- which produces contraction of smooth muscles and 5 - stimulates secretion of mucus.	

ANTIBODY IMMUNE RESPONSES

PRIMARY AND SECONDARY

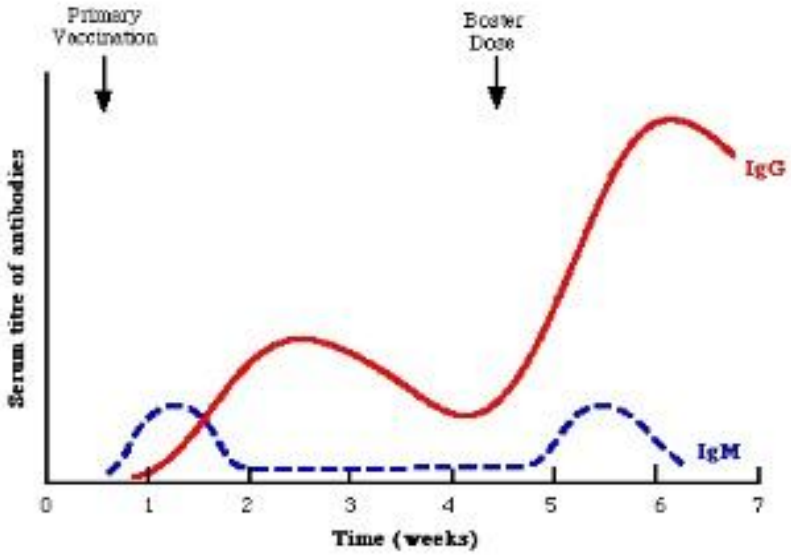
Primary and Secondary Immune Responses



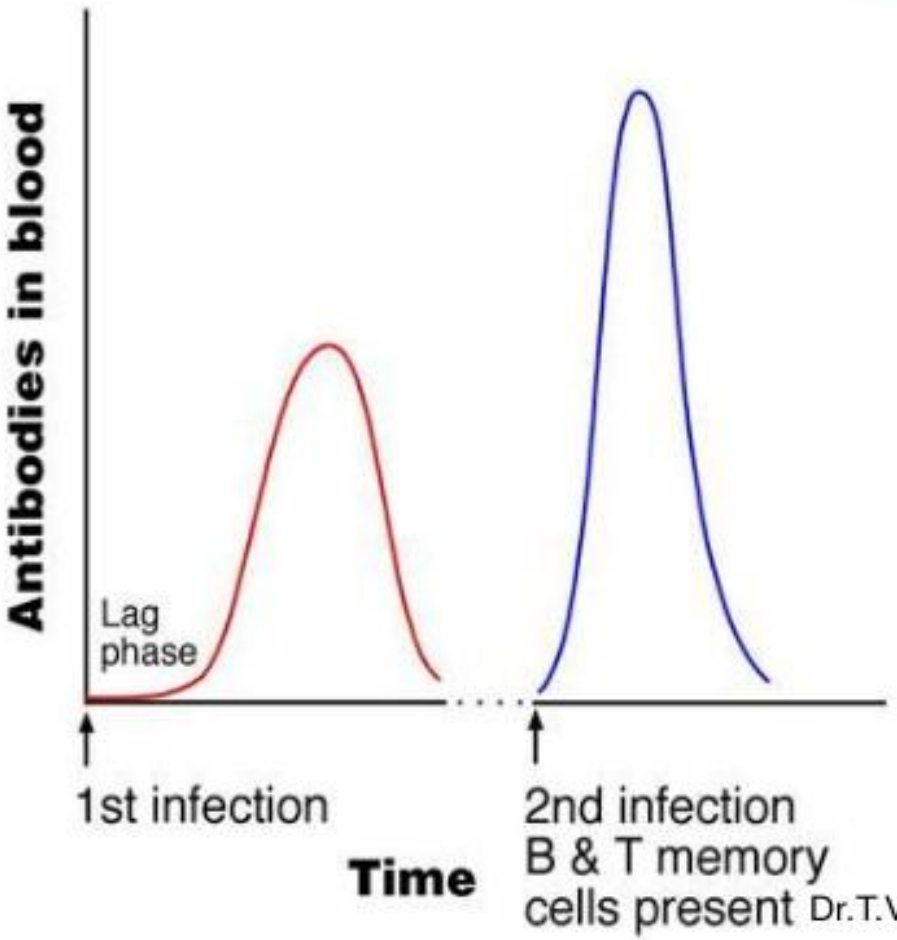
- A single injection of antigen helps in sensitizing or priming of immunocpompent cell producing particular antibody than in the actual elaboration of high levels of antibody.
- Effective levels of antibody are usually induced by only subsequent injection of antigens.

Booster Dose

- The antibody response to an initial antigenic stimuli differs qualitatively and quantitatively from response to subsequent stimuli with the same antigen
- The former primary response and later secondary response



Primary and Secondary Response



- The primary response is slow, sluggish and short lived with long lag phase and does not persist for long time
- The secondary response is prompt powerful and prolonged with short or negligible lag phase and with higher level of antibodies

Table 8.11. Differences between Primary and Secondary immune-response.

Character	Primary immune-response	Secondary immune-response
1. Period of occurrence	On first encounter with specific antigen.	On second or more time encounter with some antigen.
2. Speed	Slower, so takes more time.	Faster, so takes less time.
3. Intensity	Weaker response, as no memory cells present.	Strong response, as memory cells were present.
4. Longevity	Lasts for short period.	Lasts for longer period

THANK YOU….

BLOTTING TECHNIQUES

By
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What is blotting?

- Blots are techniques for transferring DNA , RNA and proteins onto a carrier so they can be separated, and often follows the use of a gel electrophoresis. The Southern blot is used for transferring DNA, the Northern blot for RNA and the western blot for PROTEIN.

TYPES OF BLOTTING TECHNIQUES

Blotting technique

Southern Blot

It is used to detect DNA.

Northern Blot

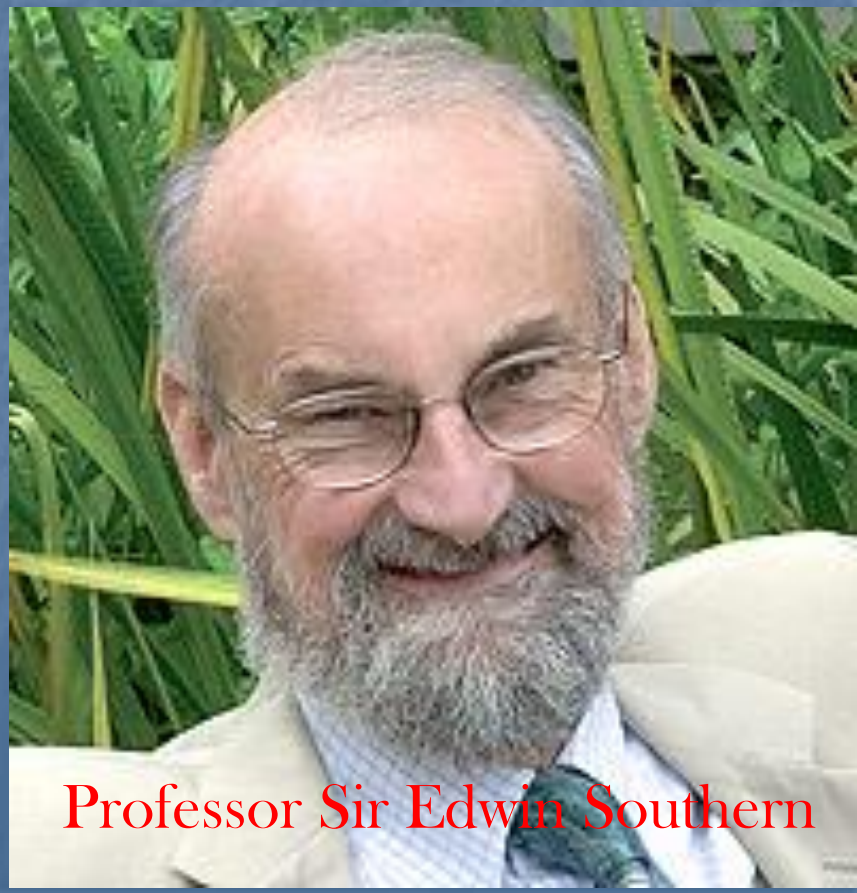
It is used to detect RNA.

Western blot

It is used to detect protein.

SOUTHERN BLOTTING

- Professor Sir Edwin Southern, Professor of Biochemistry and Fellow of Trinity developed this method in 1975.
- Southern won the Lasker Award for Clinical Medical Research prize for the method of finding specific DNA sequences he developed this procedure at Edinburgh University more than 30 years ago. The technique is known as DNA transfer or 'Southern blotting'


Professor Sir Edwin Southern

Cont….

- This method Involves separation, transfer and hybridization.
- It is a method routinely used in molecular biology for detection of a specific DNA sequence in DNA samples. The DNA detected can be a single gene, or it can be part of a larger piece of DNA such as a viral genome.

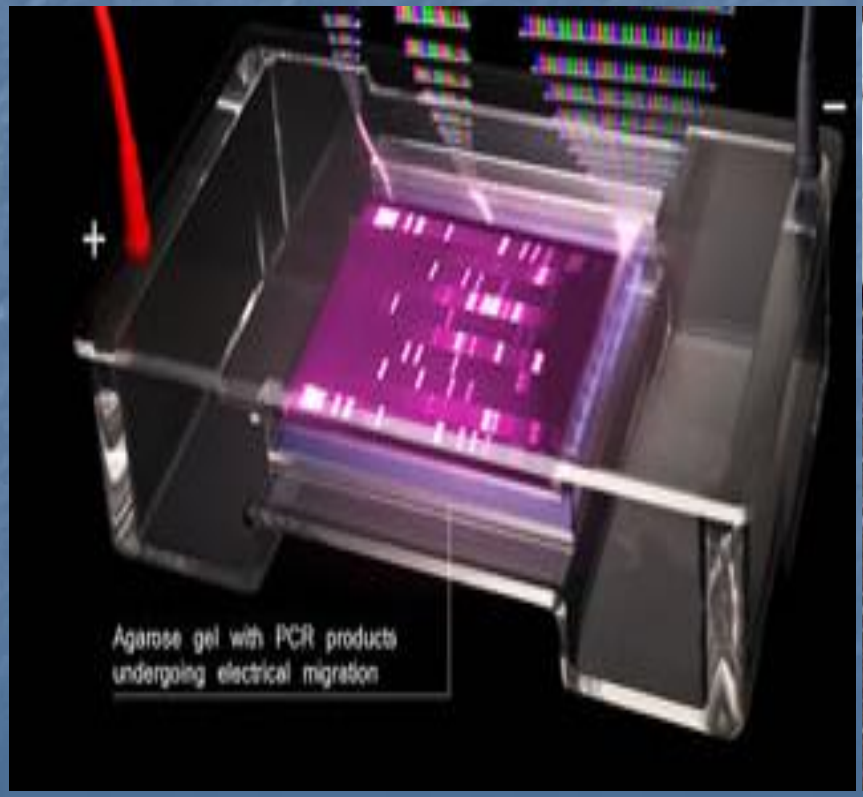
Cont….

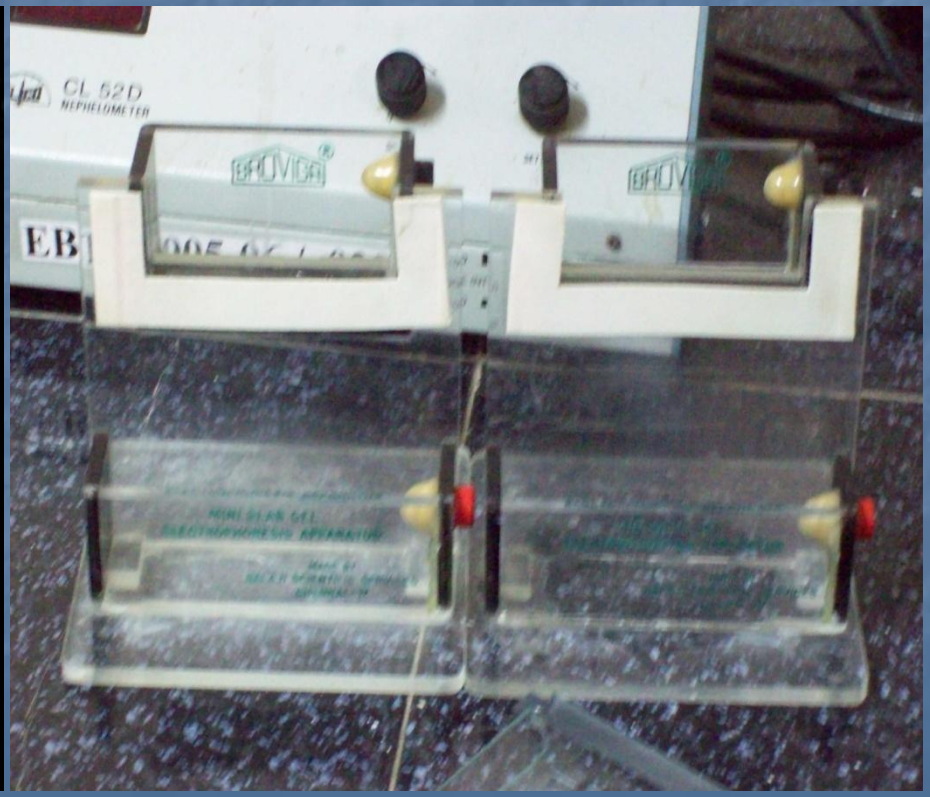
- Southern blotting combines agarose gel electrophoresis for size separation of DNA with methods to transfer the size separated DNA to a filter membrane for probe hybridization.
- The key to this method is Hybridization.
- Hybridization - Process of forming a double-stranded DNA molecule between a single-stranded DNA probe and a single-stranded target patient DNA.

PRINCIPLE

1. The mixture of molecules is separated.
2. The molecules are immobilized on a matrix.
3. The probe is added to the matrix to bind to the molecules.
4. Any unbound probes are then removed.
5. The place where the probe is connected corresponds to the location of the immobilized target molecule.

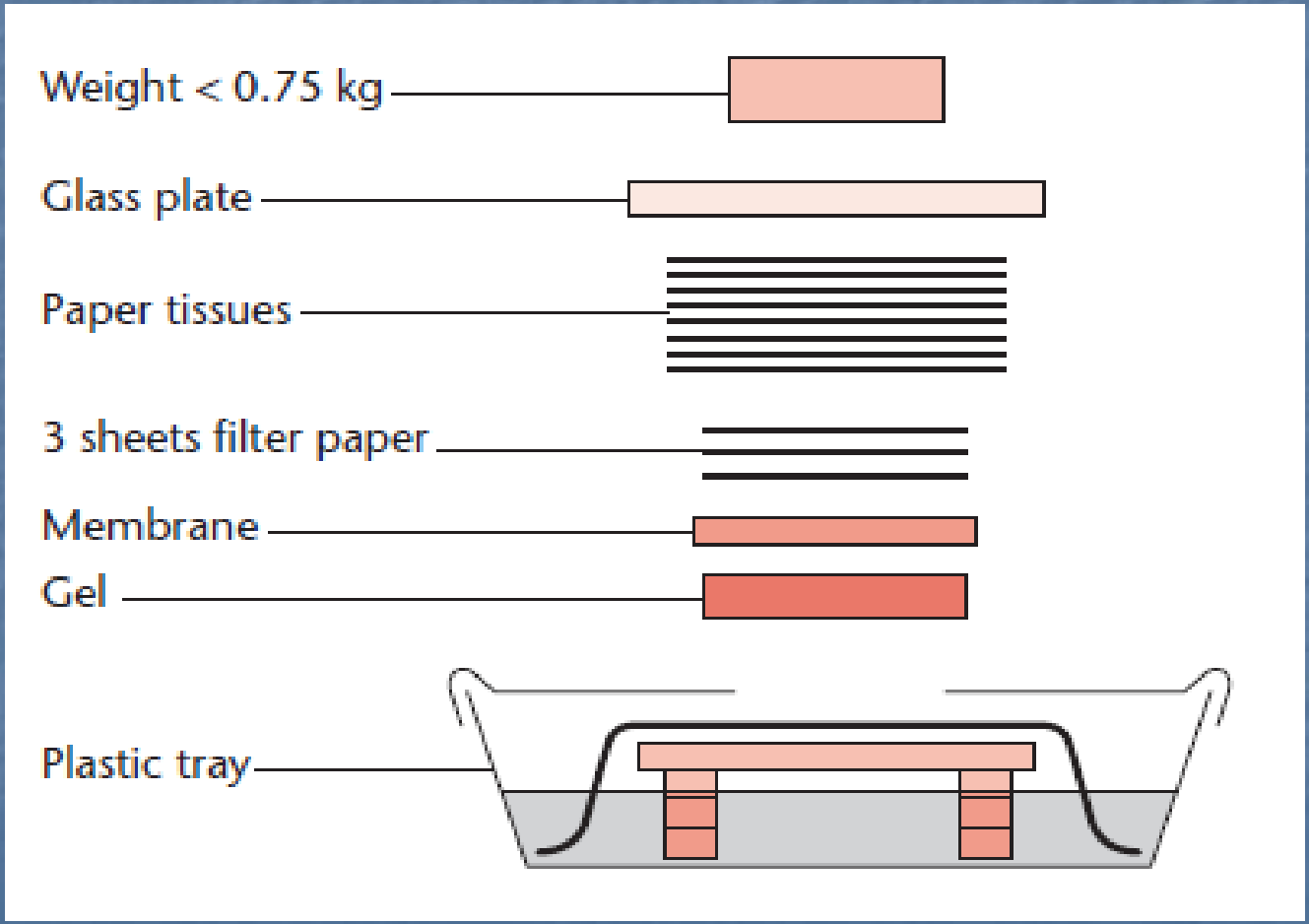
APPARATUS


+
−
Agarose gel with PCR products
undergoing electrical migration


CL 52D
NEPHELOMETER
BROVICA

Whatman 3MM paper

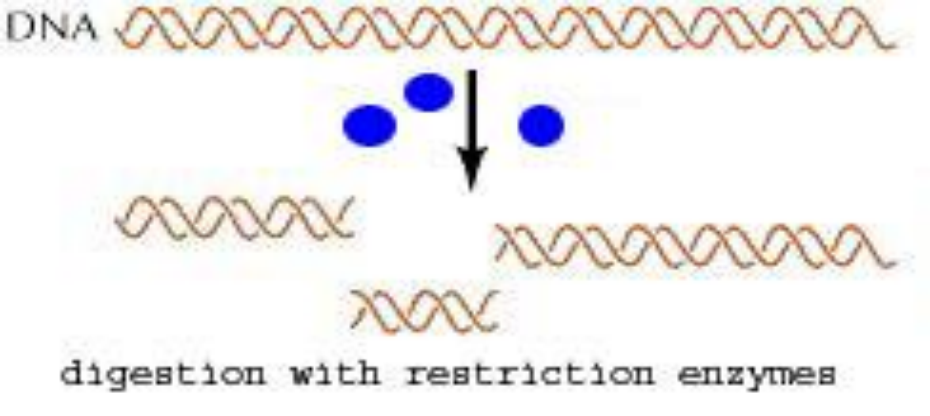
nitrocellulosemembrane



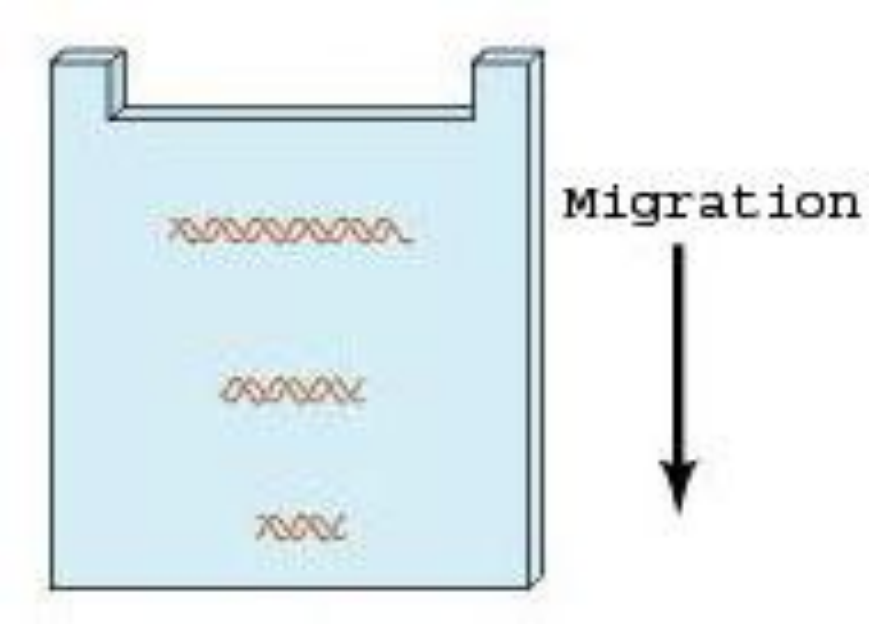
Capillary plotting apparatus

Steps in southern blotting

1. Digest the DNA with an appropriate restriction enzyme.



2. The complex mixture of fragments is subjected to gel electrophoresis to separate the fragments according to size.



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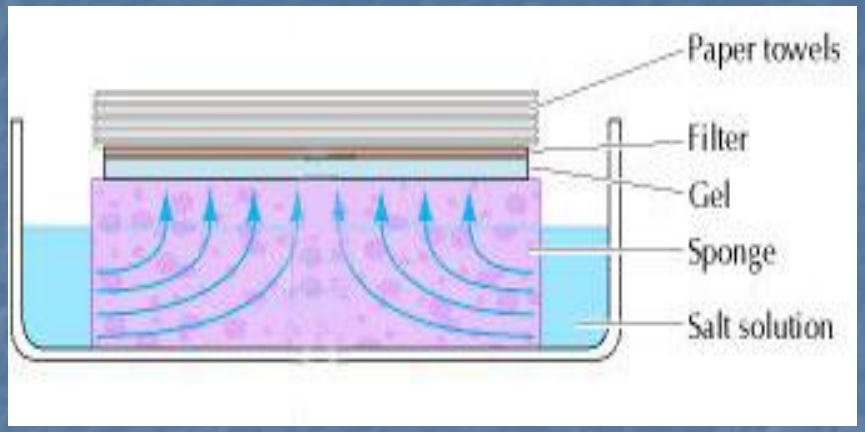
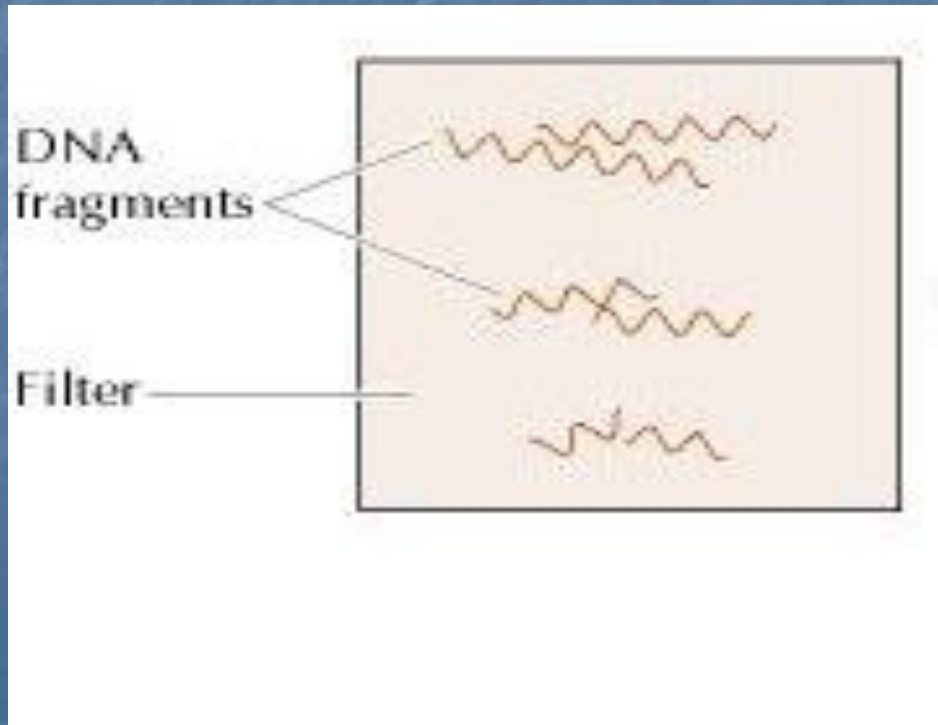
3.The restriction fragments present in the gel are denatured with alkali and transferred onto

4. a nitrocellulose filter or nylon membrane by blotting.



- This procedure preserves the distribution of the fragments in the gel, creating a replica of the gel on the filter.



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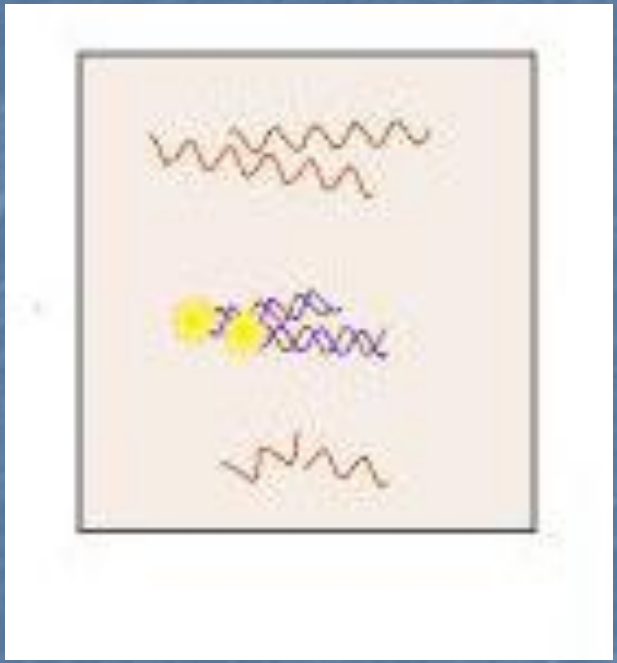
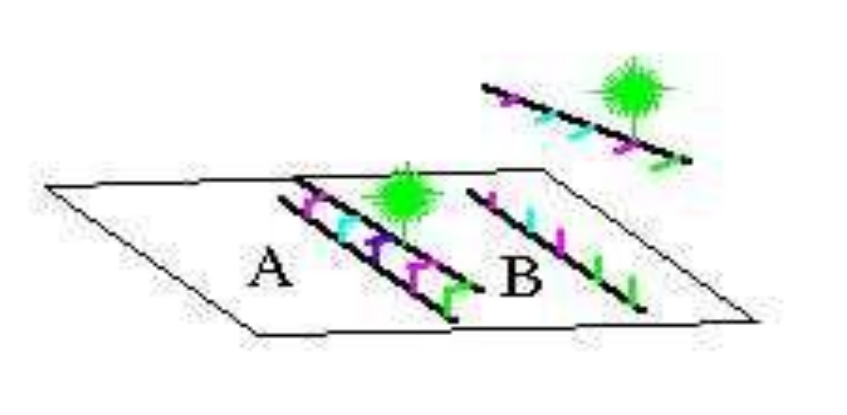
5.The filter is incubated under hybridization conditions with a specific radiolabeled DNA probe.



- The probe hybridizes to the complementary DNA restriction fragment.



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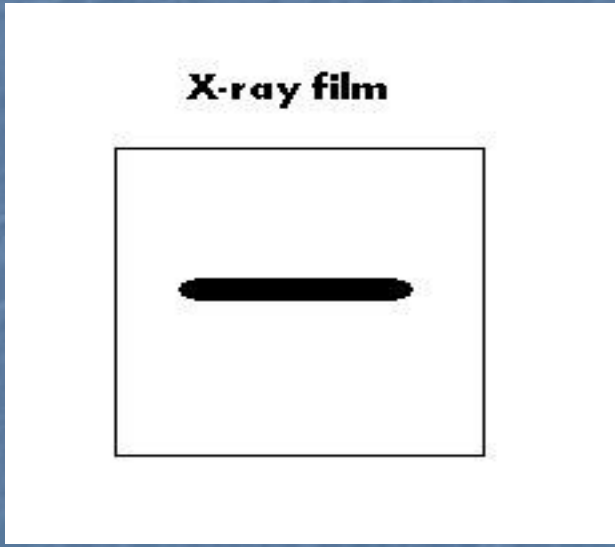
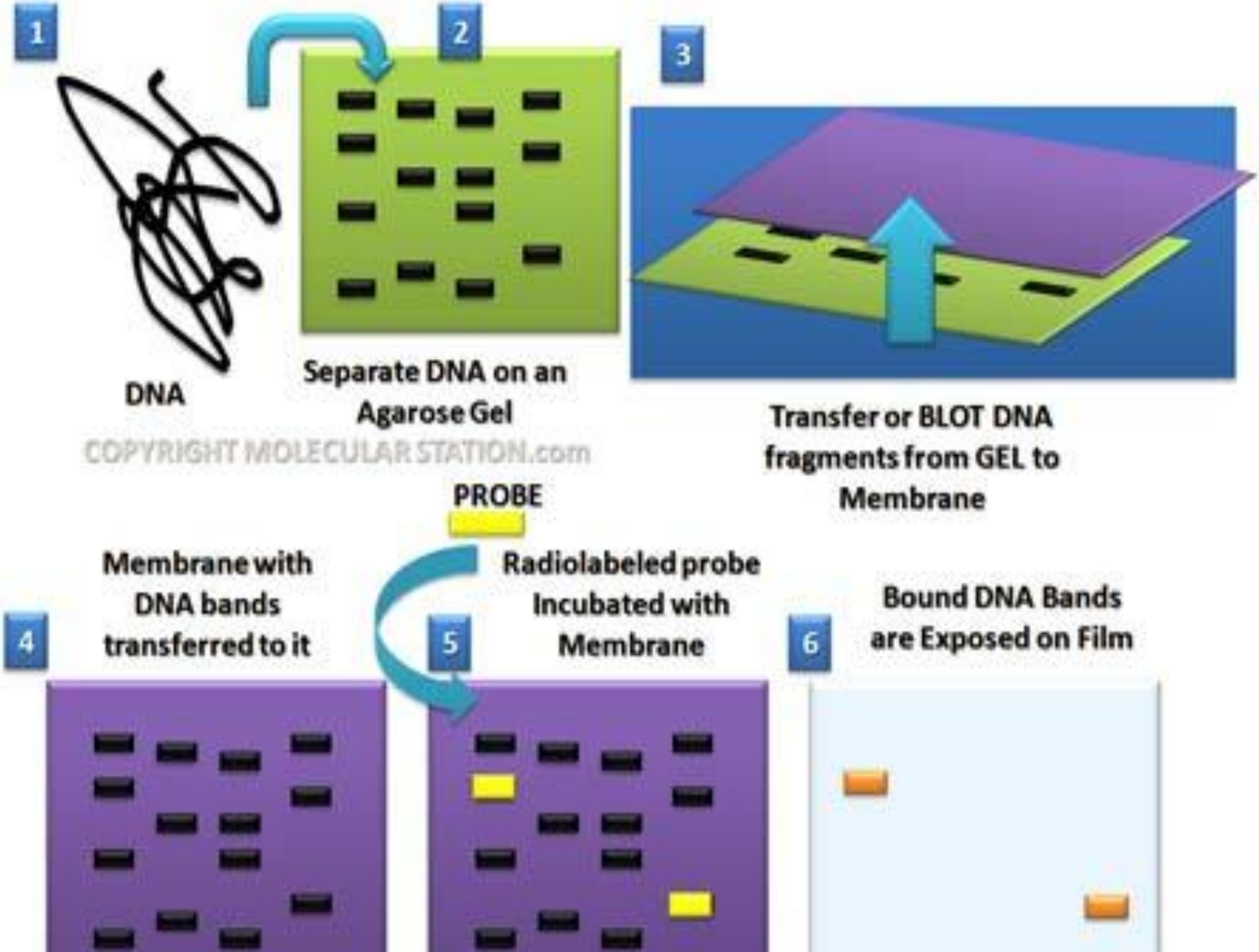
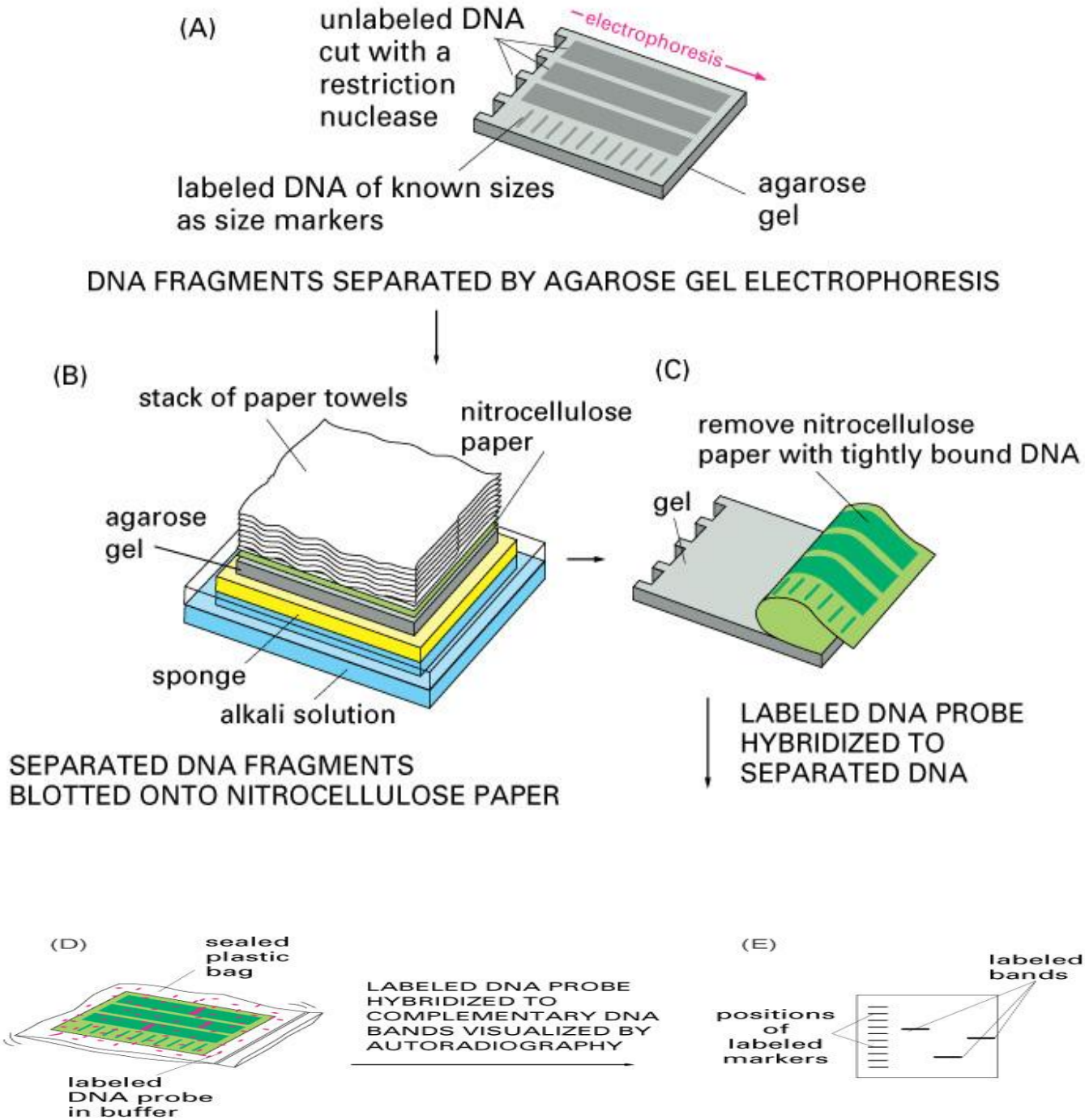
6. Excess probe is washed away and the probe bound to the filter is detected by autoradiography, which reveals the DNA fragment to which the probe hybridized.




1
DNA
2
Separate DNA on an Agarose Gel
3
Transfer or BLOT DNA fragments from GEL to Membrane
COPYRIGHT MOLECULARSTATION.com
PROBE
4
Membrane with DNA bands transferred to it
5
Radiolabeled probe Incubated with Membrane
6
Bound DNA Bands are Exposed on Film


(A)
unlabeled DNA cut with a restriction nuclease
electrophoresis
labeled DNA of known sizes as size markers
agarose gel
DNA FRAGMENTS SEPARATED BY AGAROSE GEL ELECTROPHORESIS
(B)
stack of paper towels
nitrocellulose paper
agarose gel
sponge
alkali solution
SEPARATED DNA FRAGMENTS BLOTTED ONTO NITROCELLULOSE PAPER
(C)
remove nitrocellulose paper with tightly bound DNA
gel
LABELED DNA PROBE HYBRIDIZED TO SEPARATED DNA
(D)
sealed plastic bag
labeled DNA probe in buffer
LABELED DNA PROBE HYBRIDIZED TO COMPLEMENTARY DNA BANDS VISUALIZED BY AUTORADIOGRAPHY
(E)
labeled bands
positions of labeled markers

APPLICATIONS

- Southern blots are used in gene discovery , mapping, evolution and development studies, diagnostics and forensics (It is used for DNA fingerprinting, preparation of RFLP maps)
- identification of the transferred genes in transgenic individuals, etc.

APPLICATIONS

- Southern blots allow investigators to determine the molecular weight of a restriction fragment and to measure relative amounts in different samples.
- Southern blot is used to detect the presence of a particular bit of DNA in a sample
- analyze the genetic patterns which appear in a person's DNA.

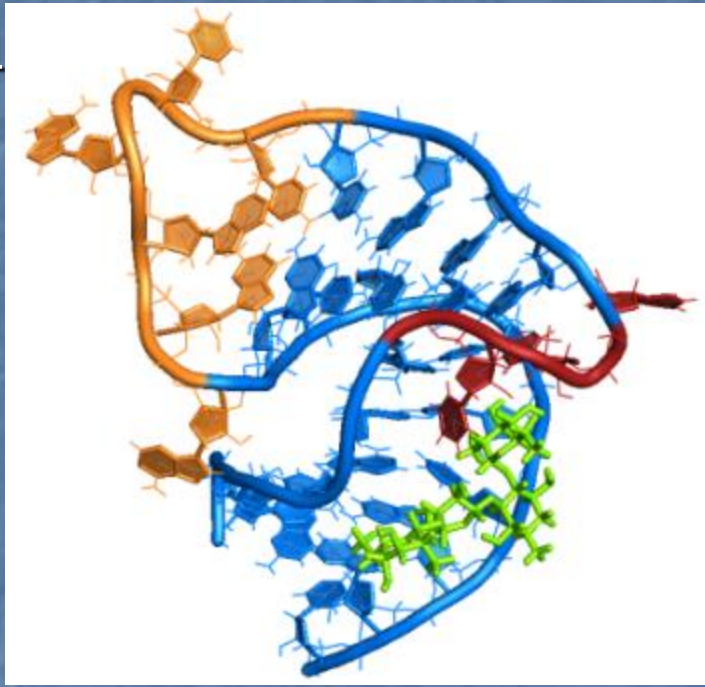
Northern Blotting

Northern blotting is a technique for detection of specific RNA sequences. Northern blotting was developed by James Alwine and George Stark at Stanford University (1979) and was named such by analogy to Southern blotting

Steps involved in Northern blotting

1. RNA is isolated from several biological samples (e.g. various tissues, various developmental stages of same tissue etc.)

   * RNA is more susceptible to degradation than DNA.



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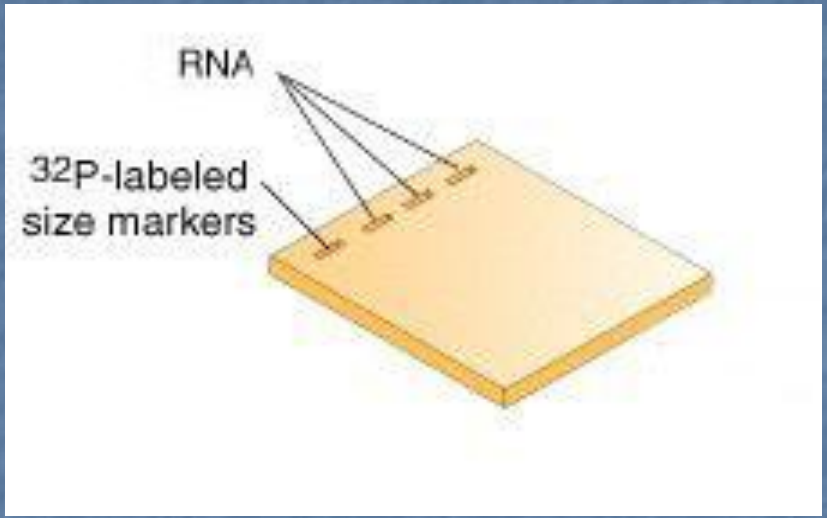
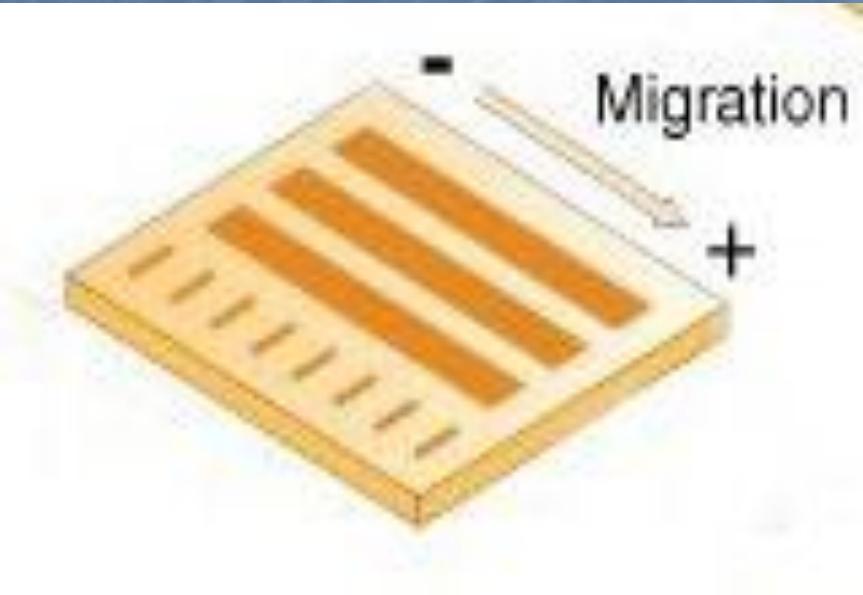
2. Sample's are loaded on gel and the RNA samples are separated according to their size on an agarose gel .



- The resulting gel following after the electrophoresis run.



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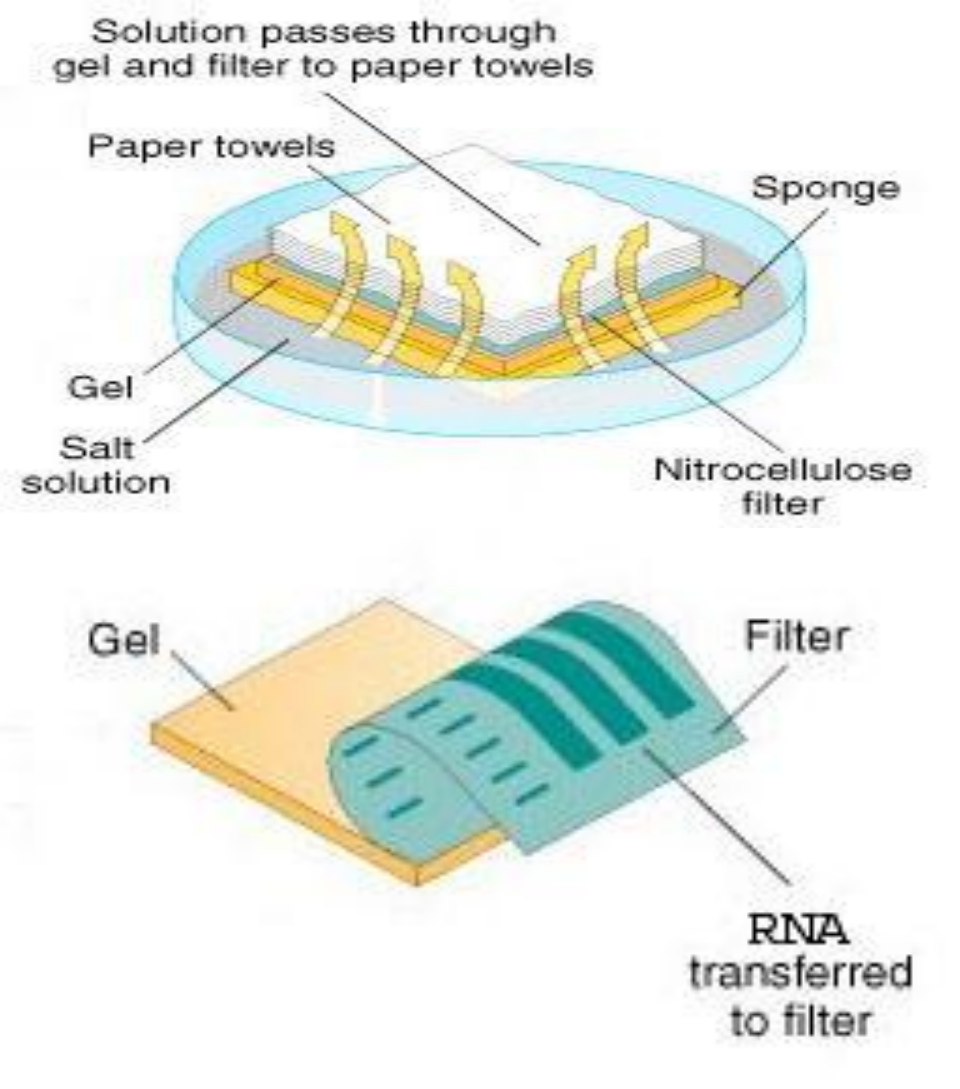
3. The gel is then blotted on a nylon membrane or a nitrocellulose filter paper by creating the sandwich arrangement.



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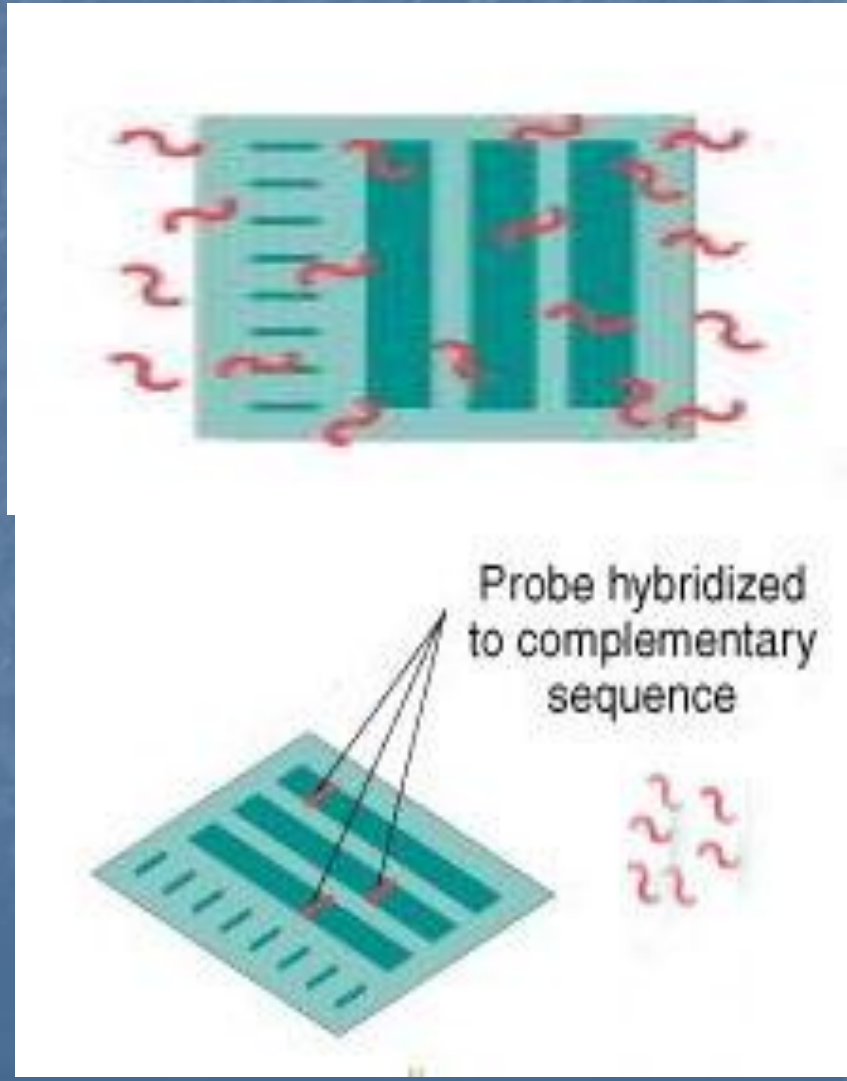
4. The membrane is placed in a dish containing hybridization buffer with a labeled probe.

- Thus, it will hybridize to the RNA on the blot that corresponds to the sequence of interest.

5. The membrane is washed



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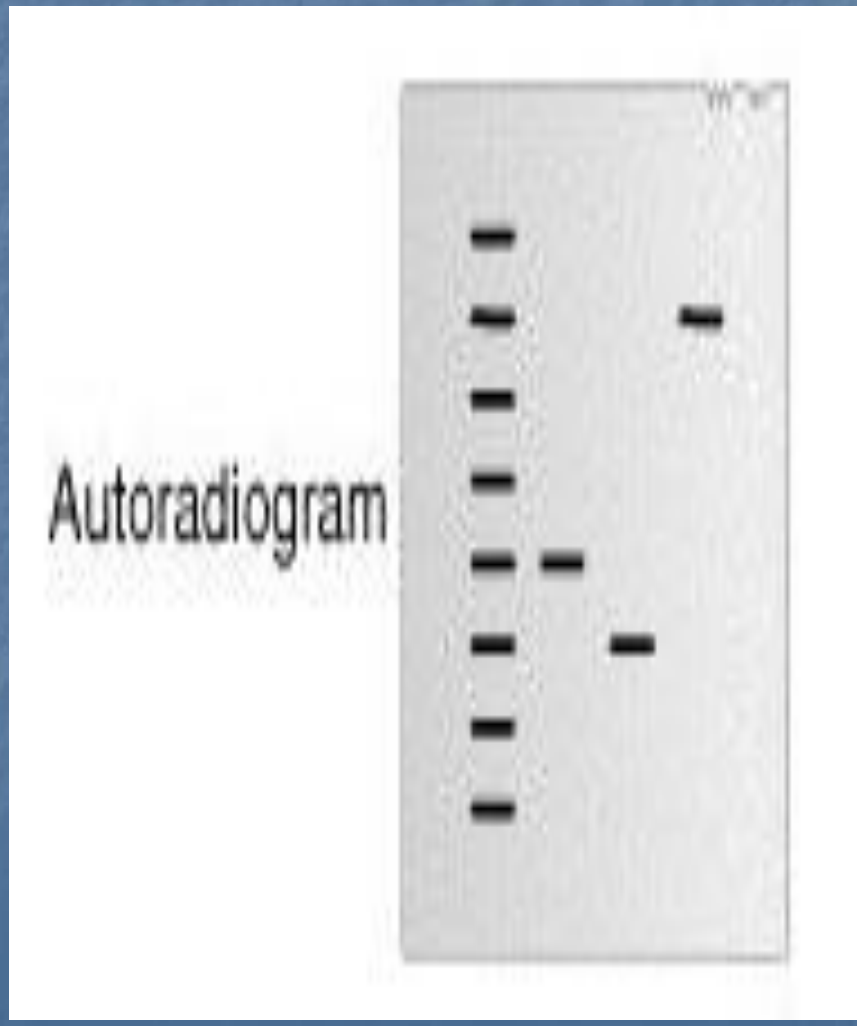
6. The labeled probe is detected via autoradiography or via a chemiluminescence reaction (if a chemically labeled probe is used). In both cases this results in the formation of a dark band on an x-ray film.

- Now the expression patterns of the sequence



APPLICATIONS

- A STANDARD FOR THE STUDY OF GENE EXPRESSION AT THE LEVEL OF MRNA (MESSENGER RNA TRANSCRIPTS)
- DETECTION OF MRNA TRANSCRIPT SIZE
- STUDY RNA DEGRADATION
- STUDY RNA SPLICING
- STUDY RNA HALF-LIFE
- OFTEN USED TO CONFIRM AND CHECK TRANSGENIC / KNOCKOUT MICE (ANIMALS)

Disadvantage of Nourthern plotting

1.The standard northern blot method is relatively less sensitive than nuclease protection assays and RT-PCR

2. Detection with multiple probes is a problem

3. If RNA samples are even slightly degraded by RNases, the quality of the data and quantitation of expression is quite negatively affected.

Western blotting

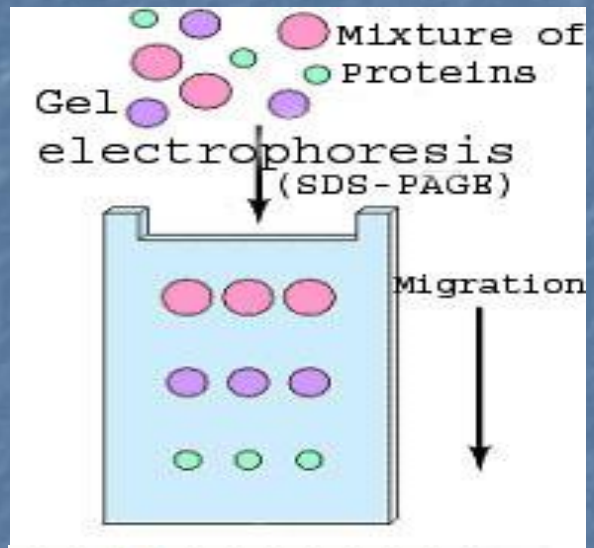
- Western blotting (1981) is an immunoblotting technique which rely on the specificity of binding between a protein of interest and a probe (antibody raised against that particular protein) to allow detection of the protein of interest in a mixture of many other similar molecules.

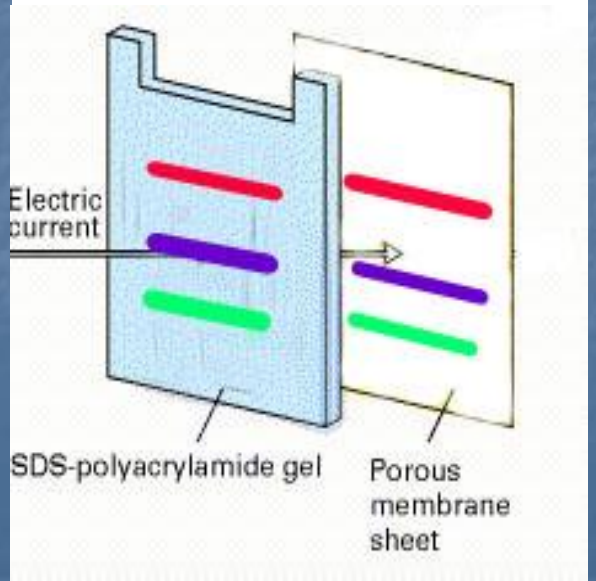
- The SDS page technique is a prerequisite for western blotting .

Steps in western blotting

1. A protein sample is subjected to electrophoresis on an SDS-polyacrylamide gel.

2. Electroblotting transfers the separated proteins from the gel to the





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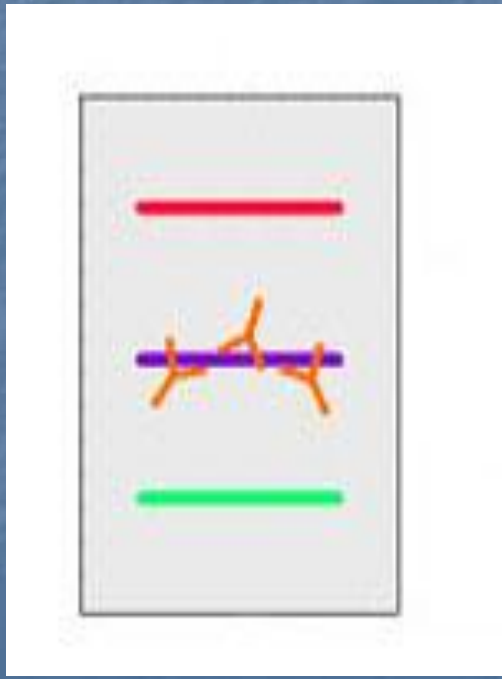
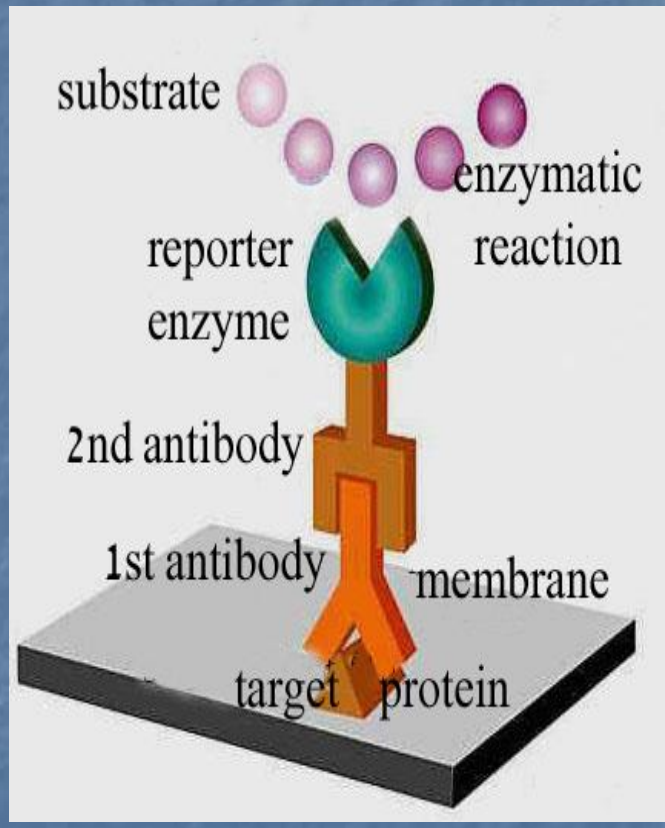
3. The blot is incubated with a generic protein (such as milk proteins or BSA) which binds to any remaining sticky places on the nitrocellulose.

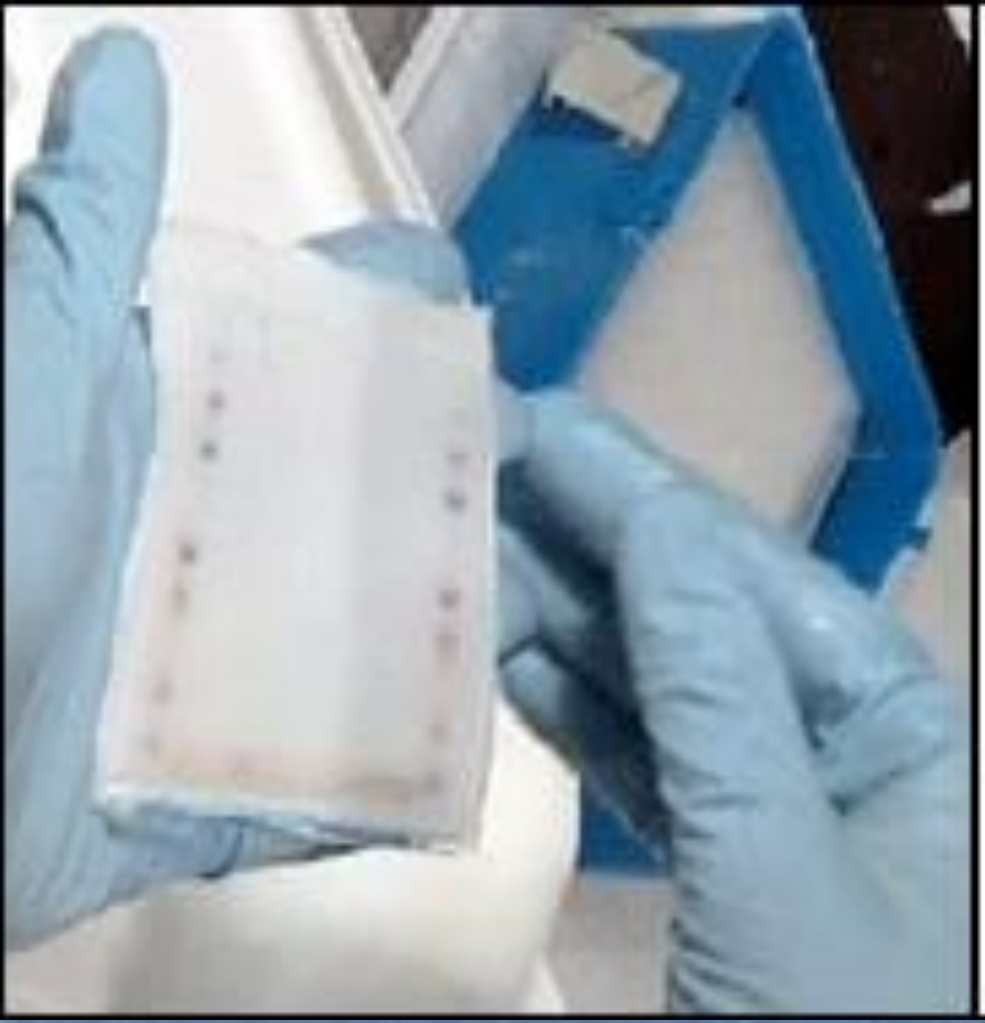


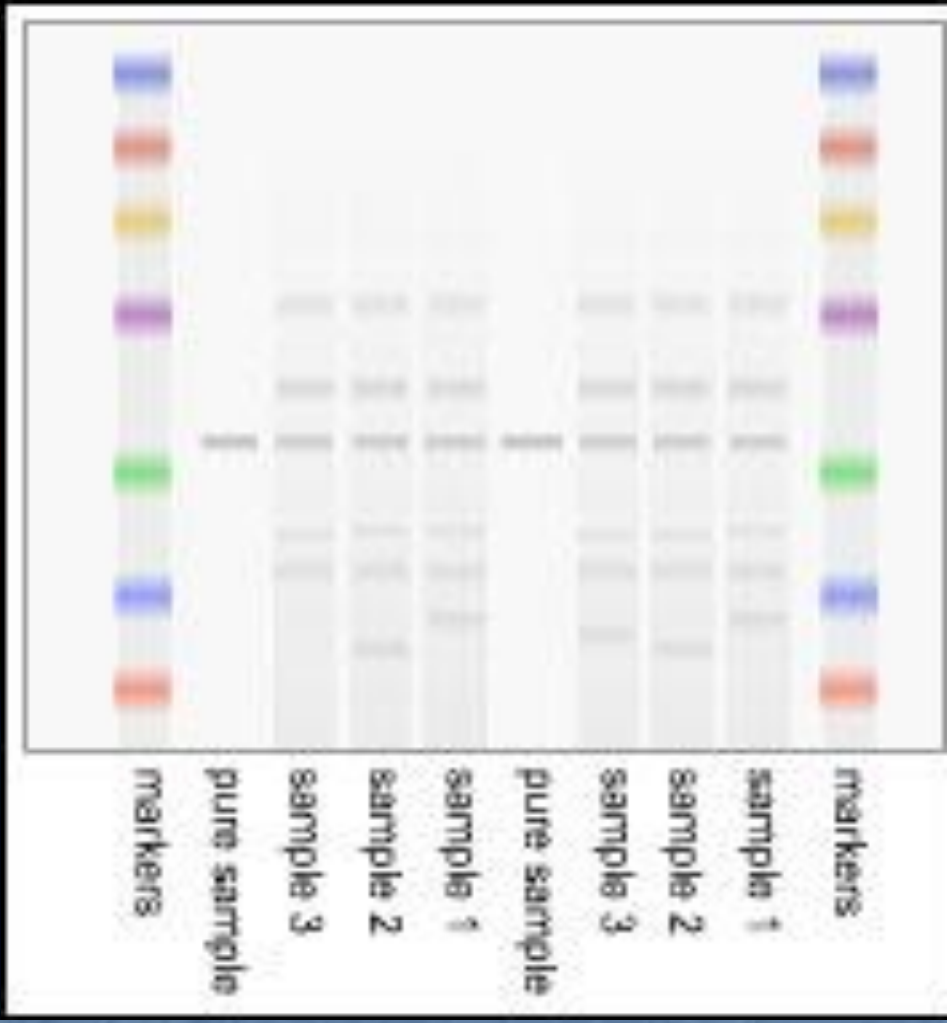
4. An antibody that is specific for the protein of interest (the primary antibody - Ab1) is added to the nitrocellulose sheet and reacts with the antigen. Only the band

Cont…

5. After washing for removal of non-specifically bound Ab1, second antibody (Ab2)is added, which specifically recognizes the Fc domain of the primary antibody and binds it. Ab2 is radioactively labeled, or is covalently linked to a reporter enzyme, which allows to visualize the protein-Ab1-Ab2 complex






markers
sample 1
sample 2
sample 3
pure sample
sample 1
sample 2
sample 3
pure sample
markers

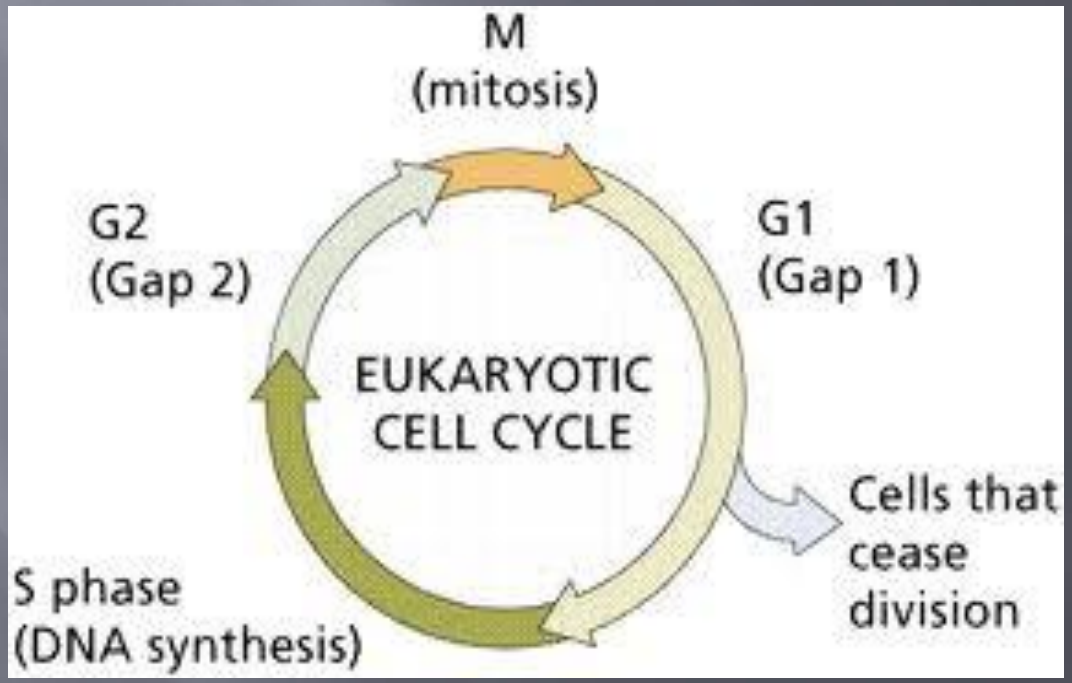
An example

Application

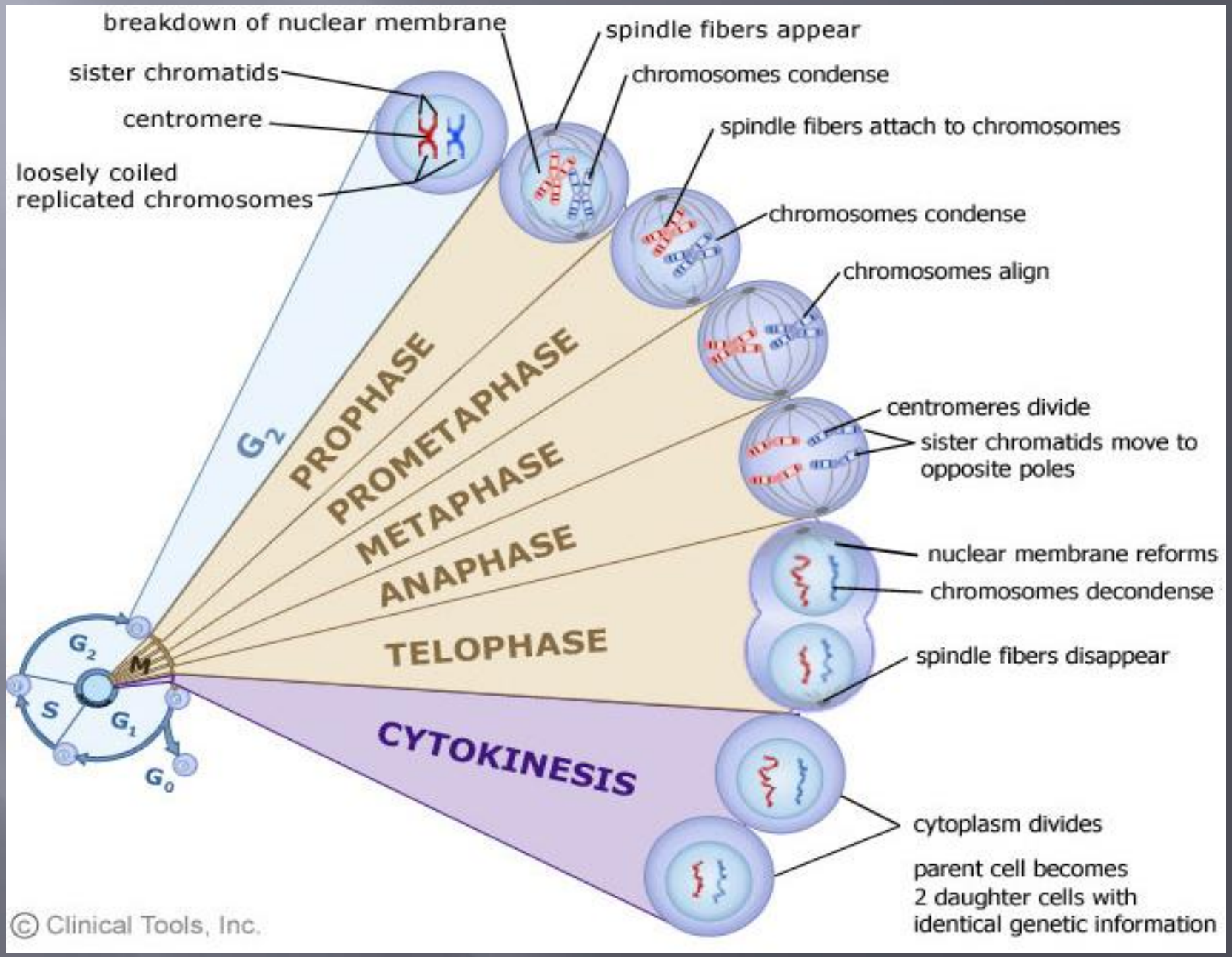
1. The confirmatory HIV test
2. Western blot is also used as the definitive test for Bovine spongiform encephalopathy (BSE)
3. Some forms of Lyme disease testing employ Western blotting.


Thank you!

CELL CYCLE


M
(mitosis)
G2
(Gap 2)
G1
(Gap 1)
EUKARYOTIC
CELL CYCLE
Cells that
cease
division
S phase
(DNA synthesis)

- All cells are produced by division of pre existing cells.
- Continuity of life depends on cell division.
- A cell born after division, proceeds to grow by macro molecular synthesis, reaches a species-determine division size and then divides.
- This cycle acts as a unit of biological time and defines life history of a cell.
- A cell cycle may be defined as the sequence of events happening between successive cell divisions.


breakdown of nuclear membrane
spindle fibers appear
sister chromatids
chromosomes condense
centromere
spindle fibers attach to chromosomes
loosely coiled
replicated chromosomes
chromosomes condense
chromosomes align
G2
PROPHASE
PROMETAPHASE
METAPHASE
ANAPHASE
TELOPHASE
CYTOKINESIS
centromeres divide
sister chromatids move to
opposite poles
nuclear membrane reforms
chromosomes decondense
spindle fibers disappear
G2
M
S
G1
G0
cytoplasm divides
parent cell becomes
2 daughter cells with
identical genetic information
© Clinical Tools, Inc.

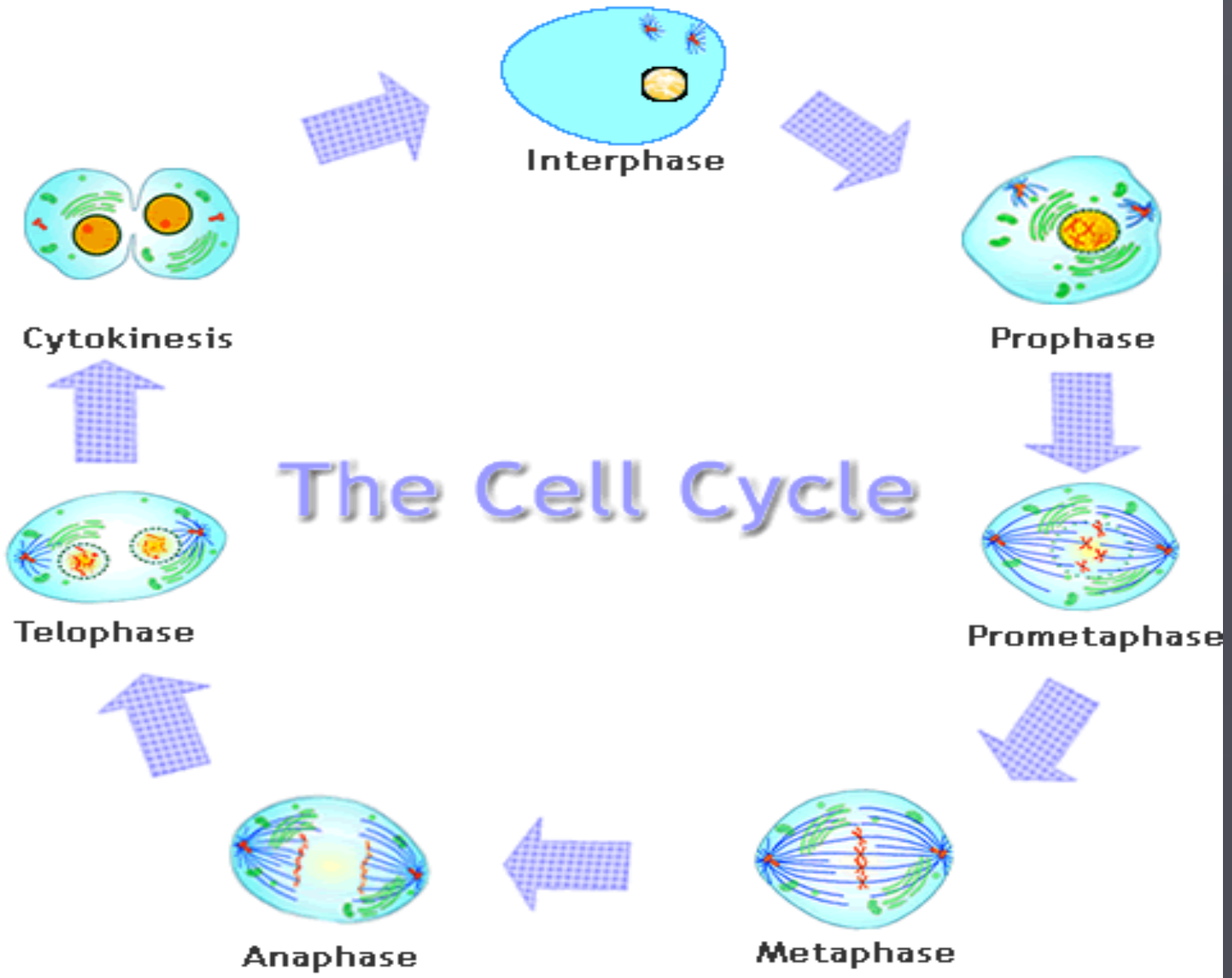
- The cell cycle comprises of the following events:

1. Chromosomal or nuclear cycle:

- In this DNA synthesis alternates with mitosis.
- During DNA synthesis, each double helical DNA molecule is replicated into two identical daughter DNA molecules.
- The duplicated copies of the genome are ultimately separated during mitosis.

2. Cytoplasmic or cell division cycle:

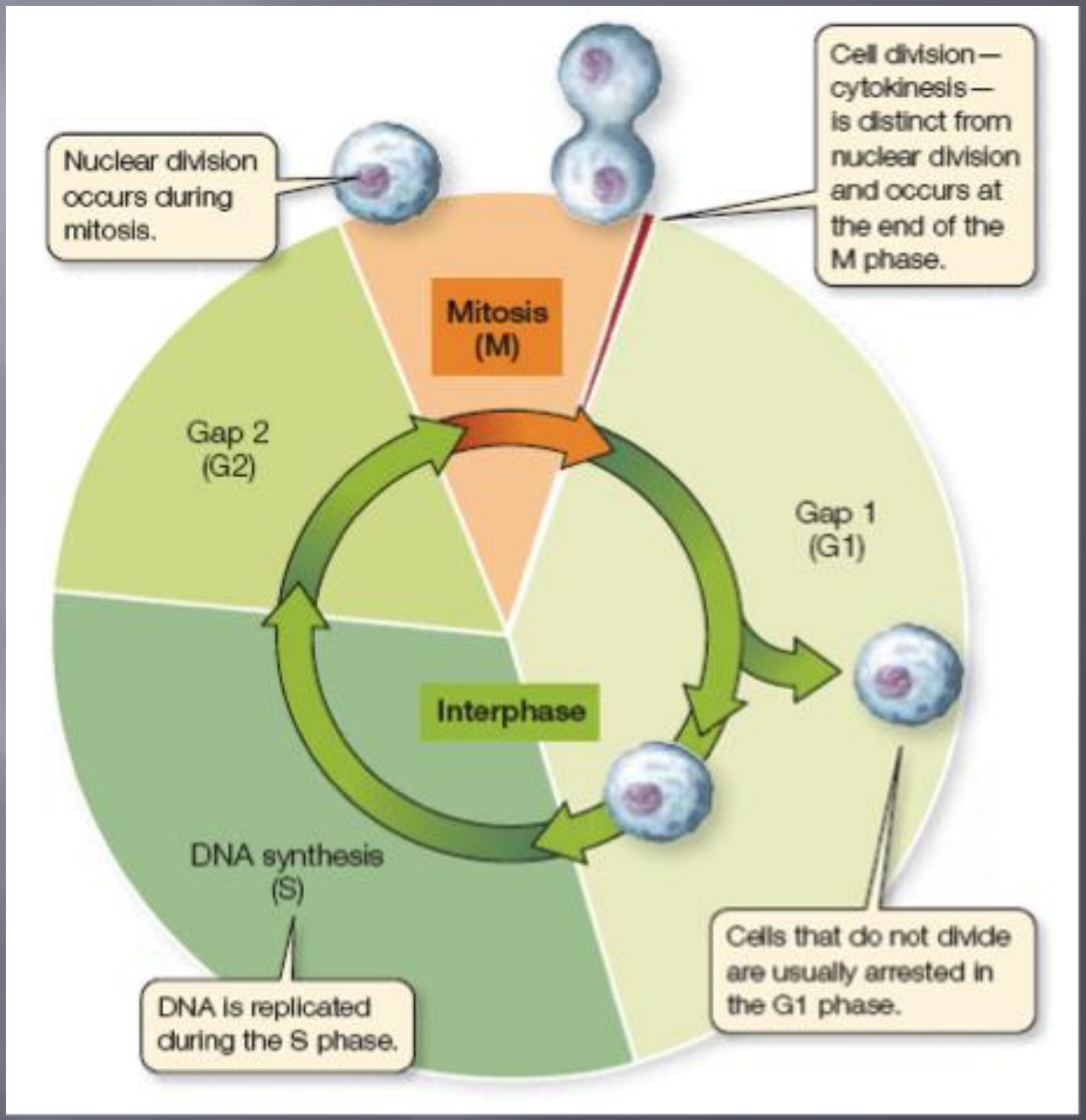
- In this the doubling and division of cytoplasmic components takes place.
- During cytokinesis, the cell as a whole is divided into two.
- Usually, the karyokinesis is followed by cytokinesis, but sometimes the cytokinesis do not occur resulting in the multi nucleate cells. E.g. cleavage of egg in *Drosophila.*



Clip art image source:www.wpclipart.com

3. Centrosome cycle:

- Both the above processes require that the centrosome be inherited reliably and duplicated precisely in order to form the two poles of the mitotic spindle in animal cells.
- Howard and Pele(1953) have divided the eukaryotic cell cycle into four non overlapping phases or stages: G1, S, G2 and M phases.
- The discrete events of the chromosome cycle(DNA synthesis and mitosis) occur during the S phase and the M phase respectively.


Nuclear division occurs during mitosis.
Cell division—cytokinesis—is distinct from nuclear division and occurs at the end of the M phase.
Mitosis (M)
Gap 2 (G2)
Gap 1 (G1)
Interphase
DNA synthesis (S)
DNA is replicated during the S phase.
Cells that do not divide are usually arrested in the G1 phase.

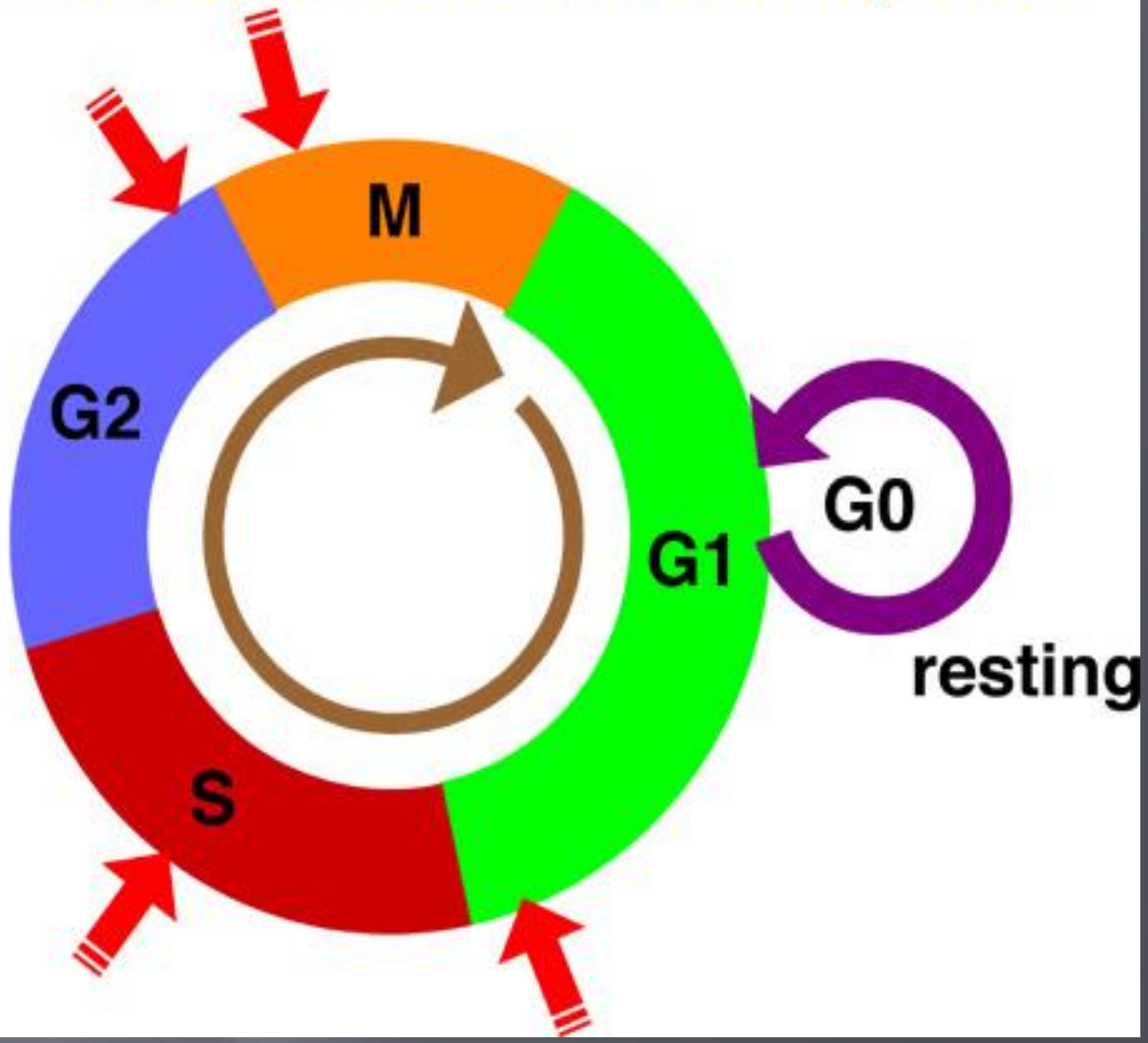
- These land marks are separated by G1 and G2 gap phases, during which mRNA s and proteins accumulate continuously.
- Mitosis is a dramatic event that involves visible recognition of cell structure whereas the rest of the cell cycle(G1,S and G2) is unnoticeable to the eye and is termed as the inter phase.
- Variations may occur in the cell cycle, where one or both gap phases are omitted or where either the S phase or M phase is omitted leading to the halving or doubling of the DNA content respectively.

- Two rounds of DNA synthesis without cell division(no M phase) occur in Drosophila secretary tissues to produce polytene chromosomes.
- On the contrary, two rounds of division without intervening DNA synthesis(no S phase) occur during meiosis.

Cell cycle check points:

- The primary function of the cell cycle is to duplicate the genome and divide it equally between two daughter cells.
- For this reason, it is important that the events of the cell cycle proceed in a correct order and that each stage of the cell cycle is completed before the next commences.
- Thus, if mitosis is blocked , the cell arrests at the M phase until the block is removed.
- Similarly, if DNA replication is prevented, the cell does not attempt to undergo mitosis as it is dependent upon the completion of DNA replication.

The Cell Cycle and the Checkpoints



- The cell possesses a number of regulatory systems which can sense the progress of the cell cycle and inhibit subsequent stages in the event of failure.
- These regulatory mechanisms are termed as cell cycle check points.
- They represent intrinsic signaling systems of cell cycle control.
- The numerous check points present in the cell cycle can be grouped into two major categories- those occuring at G1(regulating entry into the S phase) and those occuring at G2(regulating entry into M phase).

G1 check point:

- The G1 check point is predominant in the budding yeast Saccharomyces cerevisiae(where it is called START) and in animal cells(where it is called the restriction point or commitment point).
- The yeast cells assess nutrient availability and the presence of mating pheromones during G1, whereas animal cells respond to the presence of growth factors.
- Cells of both kingdoms(plants and animals) will arrest at this check point if the environment is unsuitable for growth.

- But once, the cells pass this stage, they are committed to a round of DNA replication and mitosis regardless of their environment.
- It is still not known that what is the G1 checkpoint.
- However it has been reported that passing the G1 check point, results in the accumulation in the cytoplasm of a substance that makes possible entry into the S stage.
- This is called as S phase promoting factor or activator and switches on DNA synthesis.
- The presence of this substance has been demonstrated by cell fusion and nuclear transplantation experiments.

- When a nucleus was transplanted from a cell in the G1 stage into a cell in the S phase(G1 x S), the G1 nucleus enters the S stage at once and both nuclei replicate. This is due to presence of S phase promoting factor in S nucleus.
- When cell fusion experiment was carried between G1 and G2 cells, (G1 x G2) both nuclei failed to undergo replication or mitosis, because both S phase and M phase activators are present only for a short period.

G2 check point:

- A second check point exists in the G1 stage.
- Cells that are arrested at the G2 transition will be tetraploid- that is having passed the S phase they will have twice the usual amount of DNA.
- Triploid nuclei are occasionally found in various tissues; they are common in the liver cells and they are present as a rule in the heart muscle cells of adult humans.
- The critical event at the G2 checkpoint is concerned with the condensation of chromatin, which necessarily preceeds mitosis.

- Chromatin condensation is associated with phosphorylation of H1 Histone proteins.
- Histone H1, like the other histones is synthesized at the same time as the DNA to which it binds.
- However, the enzymes capable of phosphorylation H1 have a peak activity late in G2 stage.
- Thus, G2 check point is somehow associated with inter relationship between H1 phosphorylation and the chromatin condensation.

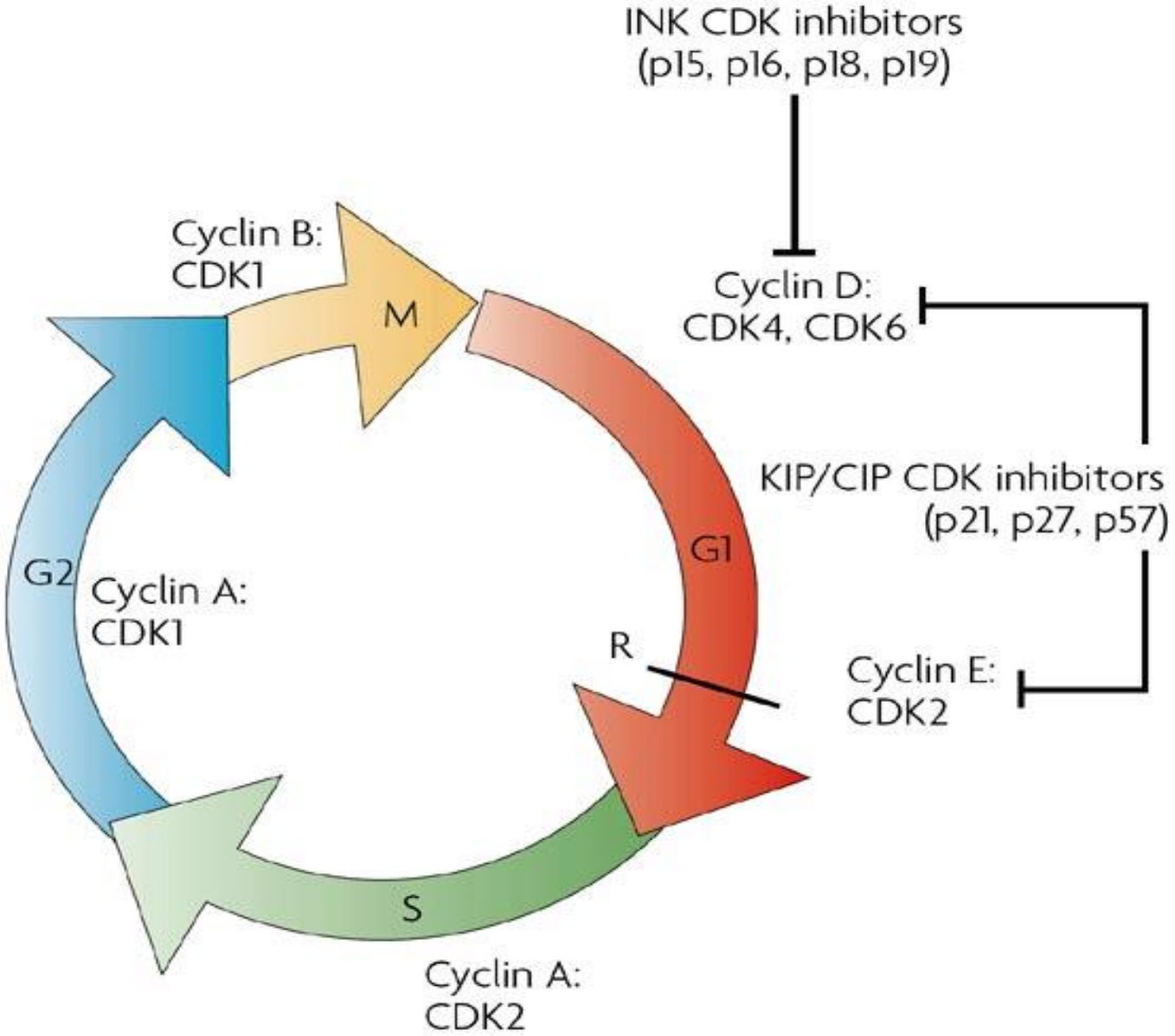
G_0 cells:

- The term G_0 refers to cells that have completed mitosis but are not proceeding towards another one.
- These are quiescent cells where both growth and division are repressed. Ex. Small lymphocytes, Liver cell, Skeletal muscle cells, Neurons etc.,
- The transition from G_0 to G1 requires not just the passage of time, but a specific stimulus i.e. a mitogenic stimulus or one that induces mitosis.
- Mitogenic stimulus is accompanied by biochemical changes in the cell that include increased transcription of DNA and increased level of non histone chromosomal proteins.

Molecular basis of cell cycle regulation:

- The sequential stages of the cell cycle reflect alternate states of Phosphorylation for key proteins(cyclins) which mediate the different cell cycle events.
- The cell cycle transitions represent switches in those phosphorylation sites.
- The G1-S transition involves the phosphorylation of proteins required for DNA replication while the G2-M transition involve the phosphorylation of proteins required for mitosis.
- Kinases, thus coordinate the different activities required for each transition.

- The involvement of protein kinases in cycle control was revealed when analysis of Saccharomyces cerevisiae CDC mutants (CDC-cell division cycle) blocked at START(G1 checkpoint) identified the product of the CDC28 gene, a 34 KD protein kinase, as the principal regulator of the G1-S transition.
- The CDC2 gene, which played an equally important role in the G2-M transition in S.pombe, was found to encode a homologous protein kinase.
- The kinases were found to be present constitutively in the nucleus.
- But to control cell cycle transitions, their activity oscillated.


INK CDK inhibitors
(p15, p16, p18, p19)
Cyclin B:
CDK1
M
Cyclin D:
CDK4, CDK6
KIP/CIP CDK inhibitors
(p21, p27, p57)
G1
G2
Cyclin A:
CDK1
R
Cyclin E:
CDK2
S
Cyclin A:
CDK2

- These molecules were termed as Cyclins.
- A number of cyclin dependent kinases (CDKs) have been isolated from yeast cells.
- But only CDC28 in S.cerevisiae and CDC2 in S. pombe are directly involved in the cell cycle and are required for the G1-S and G2-S transitions in both species.
- Different types of cyclins are synthesized at different stages of the cell cycle in both animals and yeast.
- There are generally three types of cyclins which regulate cell cycle in all organisms: the G1 cyclins which regulate the G1-S transition, the S phase cyclins which are required for DNA replication, and the M phase cyclins which are required for mitosis.

THANK YOU

EVALUATION OF CRUDE DRUGS

- Introduction:

(1) Drug evaluation may be defined as the determination of identity, purity and quality of a drug.

(2) Identity – identification of biological source of the drug.

(3) Quality – the quantity of the active constituents present.

(4) Purity – the extent of foreign organic material present in a crude drug.

- Methods of Drug Evaluation:

✓ The evaluation of a drug is drug done by studying its various properties.

✓ The various properties are

(1) Organoleptic property,

(2) Microscopic property,

(3) Biological property,

(4) Chemical property,

(5) Physical property.

I. Organoleptic (Morphological) Evaluation:

- This refers to drug evaluation by means of our organs of sense and includes other sensory organs like odour, colour, taste and texture.

- It includes the study of morphology and other sensory characters.

(a) Study of Gross Morphology:

- It includes the visual examination of drug.
- These drugs are classified into the following groups.

- ✓ Barks
- ✓ Underground structures
- ✓ Leaves
- ✓ Flowers
- ✓ Fruits
- ✓ Seeds
- ✓ Herbs

✓ Barks:

- It includes all the tissues in a woody stem outside the interfascicular cambium which constitutes to the drug.
- Barks are collected from the trunk or branches of the trees a narrow strips.
- Example: Cinnamon, Cinchona, Ashoka, Kurchi.

- During drying the drug, it undergoes unequal contractions and assumes different shapes.

✓ Underground Structures:

- Rhizomes, Roots, Bulbs, Corm and Tubers are the underground structures of the plant.
- They are swollen due to the storage of food material like carbohydrates and other chemicals.
- Examples: Ginger, Turmeric, Jatamansi.

- Underground storage roots used as drugs are

Ginger

Turmeric

✓ Leaves:

- The shape, margin, base, apex and venation of leaves help in the identification of the drugs.

Senna leaves

Tulsi leaves

✓ Flowers:

- These are the reproductive organs of a plant and possess different shapes, size and colour.

Saffron extracted from the flowers which is used as an essence in the food.

✓ Fruits:

- Fruits arise from the ovary and contain seeds.

- They be globular, oblong or ellipsoidal in shape.

- Examples: Almond, Amla.

✓ Seeds:

- Seeds are developed form the ovules in the carples of the flowers. They are characterised by the hilium, micropyle etc.

- Examples: Linseed, Vomica.

(b) Study of Sensory Characters:

- Colour, Texture, Odour and Taste are useful in the evaluation of drugs.

✓ Colour
✓ Odour
✓ Taste
✓ Texture

✓ Colour:

- Some drugs are green in colour when dried in shade.
- But they become pale and bleached when dried in sunlight.

Terminalia chebula - Fresh and Dried

✓ Odour:

- The odour of the drug may be either distinct or indistinct.

- The terms used for the drugs are aromatic, balsamic, spicy etc.

- Mentha, clove are some of the examples for the drugs which have a distinct odour.

✓ Taste:

- The drugs may be evaluated by drugs also.
- The taste may be saline, sour, salty, sweet, bitter, alkaline etc.
- The substances without taste are regarded as tasteless.
- Examples: Ginger, Capsicum.

✓Texture:

- Sometimes drugs can be examinated by their consistency, texture and nature of fracture.

- Example:

- Colocynth can be compressed easily since its parenchyma is loose.

II. Microscopic or Anatomical Evaluation:

- This method allows a more detailed examination of a drug and it can be used to identify organised drugs by their known histological characters.
- Before examination through a microscope the material must be suitably prepared.
- This can be done by powdering, cutting thin sections of the drug or preparing a macerate.

- Microscope can also be used for a quantitative evaluation of drugs and adulterated powders.
- This is done by counting a specific histological feature such as,

✓ Stomatal Number

✓ Stomatal Index

✓ Vein-islet Number

✓ Palisade Ratio

✓ Quantitative Microscopy

✓ Refractive Index

✓ Stomatal Number:

- The average number of stomata present per square millimeter of the epidermis is known as stomatal number.

- Stomatal number is relatively a constant for a perticular species of same age and hence, it is taken into consideration as a diagnostic character for identification of a leaf drug.

- Example: Datura – 141 (upper epidermis)

✓ Stomatal Index:

- It is the percentafe proportion of the number of stomata to the total number of epidermal cells.

- Stomatal number varies considerably with the age of the leaf but stomatal index is relatively constant for a given species.

- Example: Atropa – 20.0-23.0 (lower epidermis)

✓ Vein-islet Number:

- The term “vein-islet” is used for the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands.
- Vein-islet number is defined as the number of vein-islets per sq.mm. of leaf surface.
- It is constant for a given species of the plant. It is irrespective with the age factor.

- Example: *Cassia senna* (26).

✓ Palisade ratio:

- It represents the average number of palisade cells beneath one epidermal cell, using four continuous epidermal cells for the count.
- It is determined from powdered drugs with the help of camera lucida.

- Example: *Atropa belladona* – 06-10

✓ Quantitative Microscopy:

- It is an important analytical technique for powdered drug, especially when chemical and other methods of evaluation of crude drug fail as accurate measure of quality.

- Example: Lycopodium- spores are very characteristic in shape and appearance.

III. Physical Evaluation:

- Physical contents such as elasticity in fibres, viscosity of drugs containing gums, selling factor for mucilage containing materials, froth number of saponin drugs, congealing point of volatile and fixed oils, melting and boiling points and water contents are some important parameters used in the evaluation of drugs.
- Ultraviolet light is also used for determing the fluorescence of extracts of some drugs.

- Physical constants are extensively applied to the active principles of drugs, such as alkaloids, volatile oils, fixed oils etc.

- A few of them are:-

✓ Moisture Content
✓ Viscosity
✓ Melting point
✓ Optical Ratation
✓ Refractive Index
✓ Ash Content
✓ Extractive values
✓ Volatile oil Content
✓ Rf Values

✓ Moisture Content:

- Presence of moisture in a crude drug can lead to its deterioration due to either activation of certain enzymes or growth of microbes.

- Moisture content can be determined by heating the drug at $150^{o}C$ in an oven to a constant weight and calculating the loss of weight.

✓ Viscosity:

- Viscosity of a liquid is constant at a given temperature and is an index of its composition.
- Hence, it is used as a means of standardising liquid drugs.

- Example:

  Liquid paraffin – less than 64 centistokes.

✓ Melting Point:

- It is one of the parameters to judge the purity of crude drugs containing lipids as constituents.
- They may of animal or plant origin and contain fixed oils, fats and waxes.
- The purity of the following crude drugs can be ascertained by determining their melting points in the range shown against each of them
- Example: Coca butter (30° - 33^oC)

✓ Optical Rotation:

- Many substances of biological origin, having a chiral centre, can rotate the plane of polarised light either to right or to the left.

- The extent of rotation is expressed in degrees, plus(+) indicating rotation to the right and minus(-) indication rotation in the left.

- Such compound are optically active and hence called optical rotation.

✓ Refractive Index:

- When a ray of light passes from one medium to another medium of different density, it is bent from its original path.
- Thus, the ration of velocity of light in vaccum to its velocity in the substance is said to the Refractive index of the second medium.
- It is measured by means of refractometer.

- Example: Arachis oil - 1.4678-1.4698

✓ Ash Content:

- The residue remaining after incineration of a known quantity of the air dried crude drug, is known as the ash content of the drug.
- Ash simply represents the inorganic salts naturally occuring in drug or adhering to it or deliberately added to it as a form of adulteration.
- Example: Ashoka – 11.00

  Ginger – 6.00

✓ Extractive values:

- In crude drugs, sometimes the active chemical constitutes cannot be determined by normal procedures.
- In such cases, water, alcohol or ether soluble extractive values are determined for evaluation of such drugs.

- Example: Water soluble extracts like *Aloe vera*

  Alcohol soluble like Ginger

✓ Volatile oil content:

- Efficiency of several drugs is due to their odorous principle (volatile oils).
- Such crude drugs are standardised on the basis of their volatile oil contents.
- Weighed quantity of the drug is boiled with water in a round bottomed flask fitted with clevenger apparatus. The distillate collected is graquated into volatile oil.
- The amount thus obtained is recorded from the tube.

✓ Rf Values:

- Thin layer chromatography(TLC), has become increasingly popular for both qualitative and quantitative evaluation of drugs.
- Rf values refers to the ration of distance travelled by the solute to the distance moved by the solvent on a thin layer adsorbent.

$$Rf = \frac{\text{Distance travelled by the compound(solute)}}{\text{Distance travelled by the solvent}}$$

IV. Chemical Evaluation:

- Determination of the active constituent in a drug by chemical tests is referred to as chemical evaluation.
- The following are various methods of chemical evaluation:

✓ Instrumental methods

✓ Chemical Constants

✓ Individual chemical tests

✓ Micro chemical tests

✓ Instrumental methods:

- They make use of various instruments for evaluation like colorimetry, flourimetry spectrophotometry etc.

✓ Chemical constants tests:

- These are like acid value, iodine value and ester value etc are used for the identification of fixed oils and fats.

✓ Individual chemical tests:

- These are the tests which are used for identifying particular drugs.
- Examples: Halpher's test for cotton seed oil.

✓ Microchemical tests:

- These are the tests which are carried on slides.
- Example: Euginol in clove oil is precipitated as potassium euginate crystals.

V. Biological Evaluation:

- It is employed when the drug cannot be evaluated satisfactorily by chemical and physical methods.

- In this method, the response produced by the test drug on a living system is compared with that of the stranded preparation.

- Such an activity is represented in units as International Units (I.U).

✓ Indication of Biological Evaluation:

- When the chemical nature of the drug is not known but is has an biological action.
- When chemical methods are not available.
- When the quantity of the drug is small and so it cannot be evaluated chemically.
- Drugs which have different chemical composition but same biological activity.
- Example: Cardiac glycosides arte evaluated by this method on cats, frogs or pigeons.

Thank You

LICHENS

INTRODUCTION

- Lichens are a small group of curious plants, made up of algal and fungal components, living together in an intimate symbiotic relationship.
- The algal component is known as Phycobiont(Gr. phykos=sea weed or alga, bios=life).
- The fungal component is known as Mycobiont(Gr. Mykes= fungus, bios=life).
- The plant body of lichens neither resembles algae nor fungi.
- Thus, lichen is an association of fungus and an algal photosynthetic symbiont resulting in a stable thallus of specific structure.

- The term “lichen” was first used by Theophrastus(371-284 B.C) to denote a superficial growth on the bark of olive trees.

Lichens show the following characteristics:

- Lichens have a composite thalloid structure, formed by the association of algae and fungi.
- On the basis of the structure of thallus, lichens have been classified into three broad types:

I. Crustose lichens
II. Foliose lichens
III. Fruticose lichens.

- On the basis of the fungal component, lichens are divided into two types:

I. Ascolichens

II. Basidiolichens

- Lichens reproduce vegetatively by Fragmentation, Isidia, Soredia and other vegetative propagules.
- Only fungal component of lichens is involved in sexual reproduction.
- The female sex organ is known as Carpogonium which is differentiated into a basal ascogonium and an elongated multicellular filament called the trichogyne.

- The male sex organs are flask-shaped spermogonia, found on the upper surface of the thallus. They produce non motile spermatia.
- The fruit bodies of lichens are cup-shaped apothecia or flask shaped perithecia.
- Each ascus produces 8 ascospores. The hypha produced by the germination of ascospores, when comes into contact with a suitable alga, forms a new lichen thallus.

- The growth of lichens is very slow. Direct light, moderate or cold temperature, constant moisture and pure atmosphere favours their growth.
- Polluted, smoky atmosphere as found in industrial area is not favourable for their growth.

OCCURRENCE:

- Lichens are world wide in distribution.
- These are found growing in a variety of habitats from Arctic to the Antarctic and all the regions in between.
- Some lichens grow on exposed rocks in desert; infact, these are the primary colonizers in many xeroseres.
- They usually grow on the barks of trees, dry logs of wood, bare rocks, humus rich soil and other similar situations.

- In damp forests of the tropical and sub-tropical regions, they often hang in long festoon from trees.
- In arctic zones and alpine regions, lichens grow so profusely and abundant, that these are harvested as fodder for animals.

  Ex. *Cladonia rangifera*
- In India, the lichens are much more common in Eastern Himalayas as compared to that in the Western Himalayas.
- Darjeeling, Gangtok and places upto 10,000ft in Sikkim are ideal for lichen collection and systematic study.

- Based on the type of habitat colonised, the lichens are grouped as:

Corticolous-grow on the bark of trees.

Ex. *Usnea*, *Graphis*, *Parmelia*.

Saxicolous-grow on rocks.

Ex. *Dermatocarpon*, *Verrucaria*.

Lignicolous- grow on wood.

Ex. *Calicium*, *Chaenotheca*

Terricolous- grow on the ground.

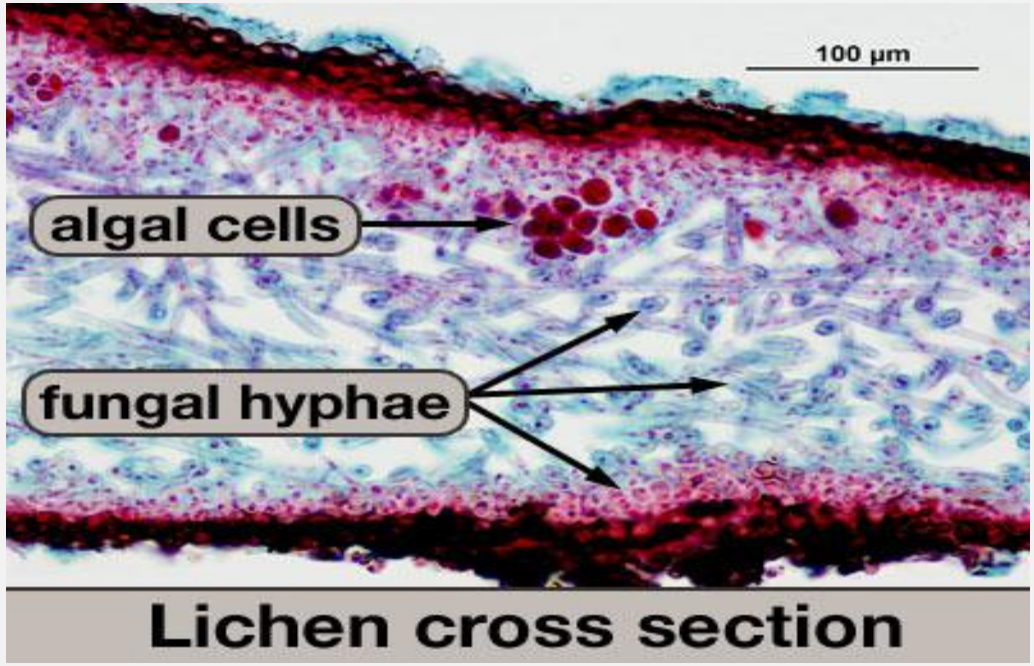
Ex. *Lecidea*, *Cladonia*

COMPONENTS OF LICHEN

- A lichen is made up of two components- the Phycobiont and the Mycobiont.
- The algal component belongs to the class Chlorophyceae or Myxophyceae (Cyanobacteria).
- The fungal component belongs to Basidiomycotina or Ascomycotina.
- The lichen forming green algae are *Protococcus*, *Cystococcus*, *Parmelia*, *Coccomyxa*, *Trebouxa* and *Trentipholia*.

- The lichen forming blue green algae are *Anabaena*, *Nostoc*, *Scytonema and Calothrix*.
- Due to the presence of prokaryotic cell structure, the blue green algae are treated as Cyanobacteria, but not as algae.
- So, Hawks worth and Hill(1984) named the photosynthesizing partner as Phycobiont, which includes the Phycobiont(green algae) and cyanobiont (Cyanobacteria).
- The Mycobiont mostly belongs to Ascomycotina and rarely to Basidiomycotina.

- Among the Ascomycotina, majority are Discomycetes producing abundant apothecia.
- Others are Pyrenomycetes or Loculoascomycetes producing perithecia and pseudothecia respectively.



CLASSIFICATION OF LICHENS

- On the basis of the characters of the thallus, the nature of the photobiont, and the type of spores produced, Zahilbruckner(1926) classified lichens into the following groups.
- CLASS- LICHENS

1. Sub class – Ascolichens: The fungal component of these lichens is a member of the class Ascomycetes. These lichens are divided into two series on the basis of the structure of fruit body.

Series-1. Pyrenocarpae: The fruiting body is a flask-shaped perithecium. These lichens are also known as Pyrenolichens.(e.g. *Dermatocarpon*)

Series-2. Gymnocarpae: The fruiting body is disc-like apothecium. These lichens are also known as discolichens.(e.g. *Parmelia*).

2. Sub class- Hymeno lichens: The fungal component of these lichens is a member of the class Basidiomycetes. Genera like *Corella* and *Dictyonema* belong to this group.

However Alexopoulos and Mims(1979) have classified lichens into 3 main groups on the basis of the component fungal partner.

I. EXTERNAL MORPHOLOGY:

The lichens are broadly grouped into three types, on the basis of their morphology.

1. Crustose lichens:

- The thallus is thin, flat and crust like. The thalli are appressed to the substratum forming thin flat crusts.
- The thalli are partly or wholly embedded in the substratum and cannot be removed from the substratum without injuring the thallus.

- Sometimes only the fruit bodies are visible above the surface of the substratum.

e.g. *Graphis*, *Verrucaria*, *Haematomma and Lecanor*

2.Foliose lichens:

These lichens are flat with leaf like and lobed thallus. They are attached to the substratum with the help of rhizoid-like rhizines. E.g. *Parmelia*, *Physcia*, *Peltigera*, *Gyrophora* and *Cetraria*.

3. **Fruticose lichens**:

These are bush like having cylindrical or strap-shaped branched thallus.

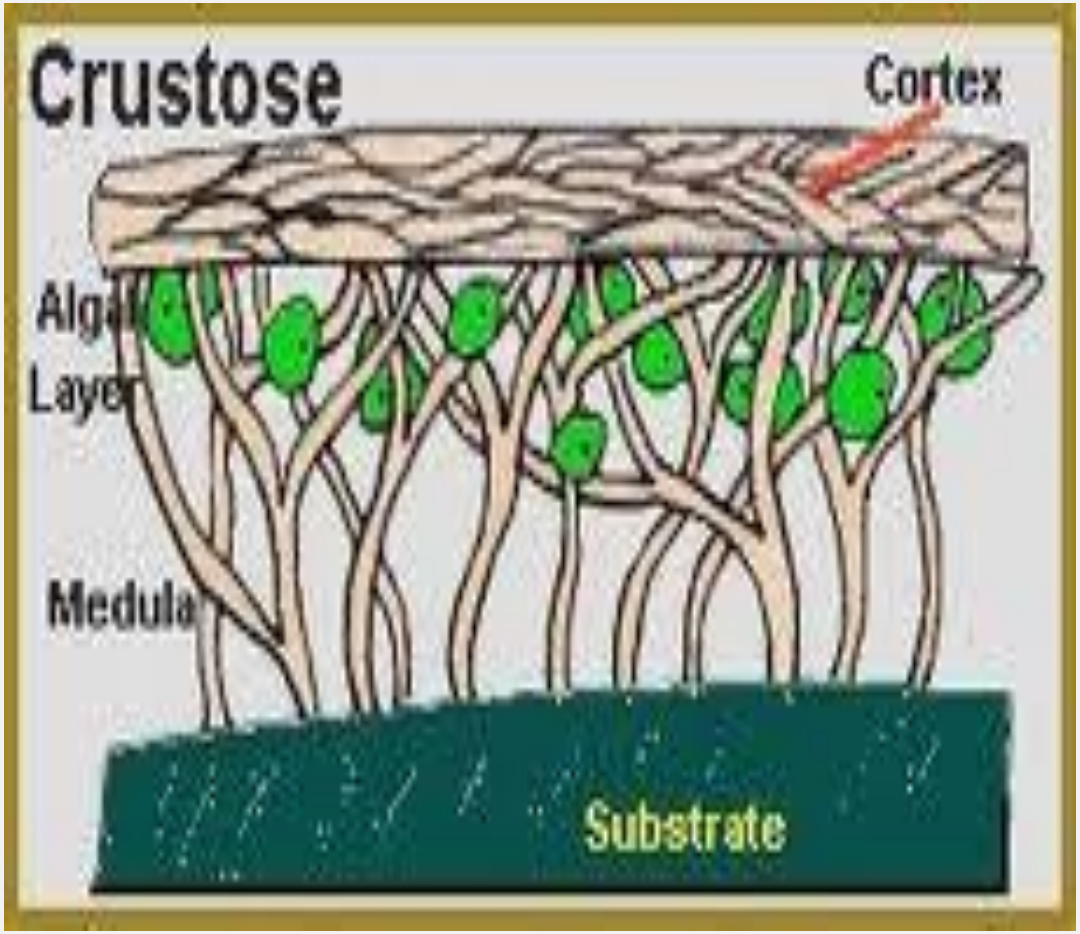
The branches may grow erect or hang from the substratum. The plant body is attached to the substratum with the help of a basal mucilage disc. E.g. *Alectonia*, *Cladonia*, *Usnea*, *Ramalina*.

II. **Internal Structure**:

1. **Crustose lichens**:

- Anatomically, the crustose lichens do not show much differentiation.
- In transverse section, the lichen thallus shows cortex, an algal layer, and medulla.
- The cortex is made up of fungal hyphae, beneath which is the algal layer composed of algae and fungi in close association.
- Below the algal layer is the medulla, made up of a loose tissue of branching hyphae.

•The lower cortex is not distinguishable.
E.g. *Graphis*, *Caloplaca*, *Rhizocarpon*



2. Foliose lichens:

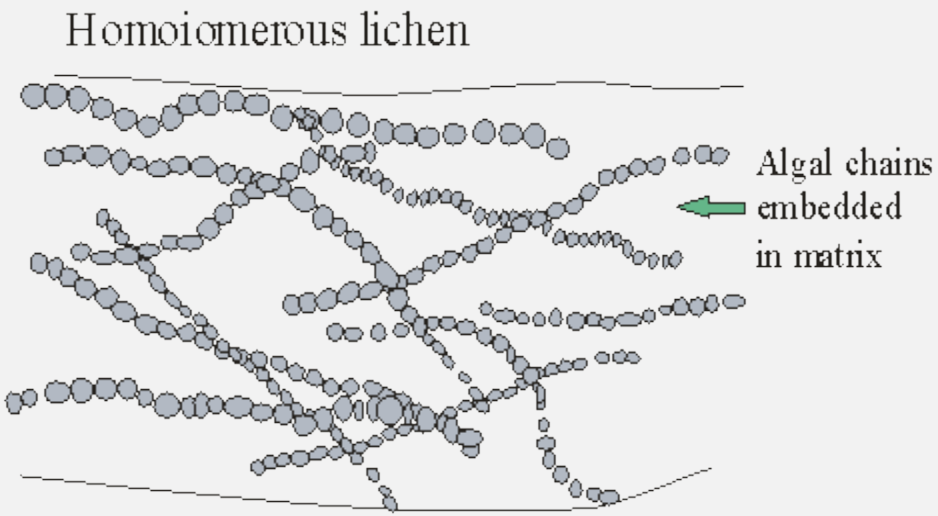
On the basis of distribution of algal cells among the fungal tissue, two types of foliose thalli are recognized. They are:

(i) Homoiomerous:

- The algae are more or less uniformly distributed throughout the thallus.
- Such forms are only few.
- The algae is usually gelatinous and belongs to Cyanobacteria.

The outer protective layer of the thallus is formed by the fungi .

e.g. *Collema*, *Leptogium*.



(ii) Heteromerous type: The algal cells form a distinct layer within the thallus. Bulk of the thallus is made up of fungal hyphae.

Thallus is differentiated into four distinct regions viz., upper cortex, algal layer, medulla and lower cortex.

Upper cortex: It is the outermost thick and protective zone of the thallus. It is made up of compactly interwoven fungal hyphae. The hyphae are arranged at right angle to the surface of the thallus. There are usually no intercellular spaces between the hyphae, and if present, are filled with gelatinous material.

In some lichens(e.g. Parmelia), the upper cortex is interrupted at intervals by pores which are meant for aeration and are known as breathing pores.

Algal layer: Beneath the upper cortex, is the algal layer, also known as gonidial layer.

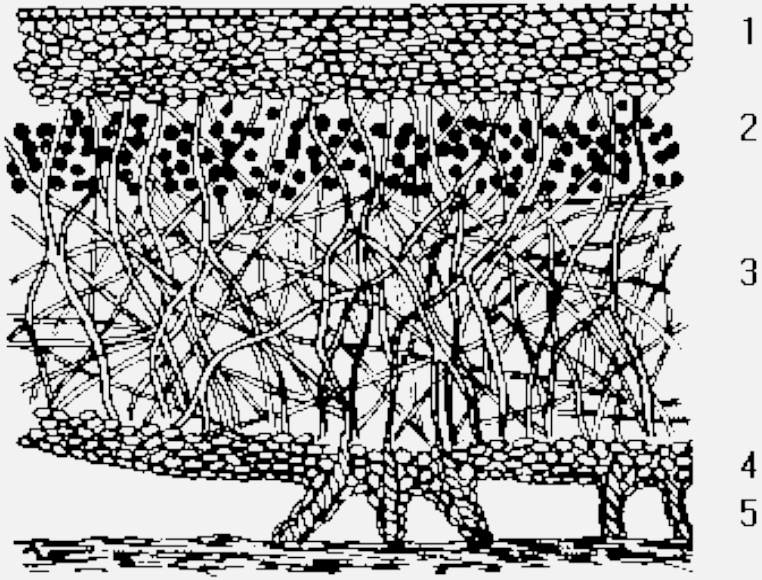
It consists of loosely interwoven fungal hyphae, intermingled with algal cells of a green alga(usually *Chlorella*, *Pleurococcus*, *Cystococcus*) or Myxophyceae(usually *Gloecocapsa*, *Nostoc*, *Rivularia*).

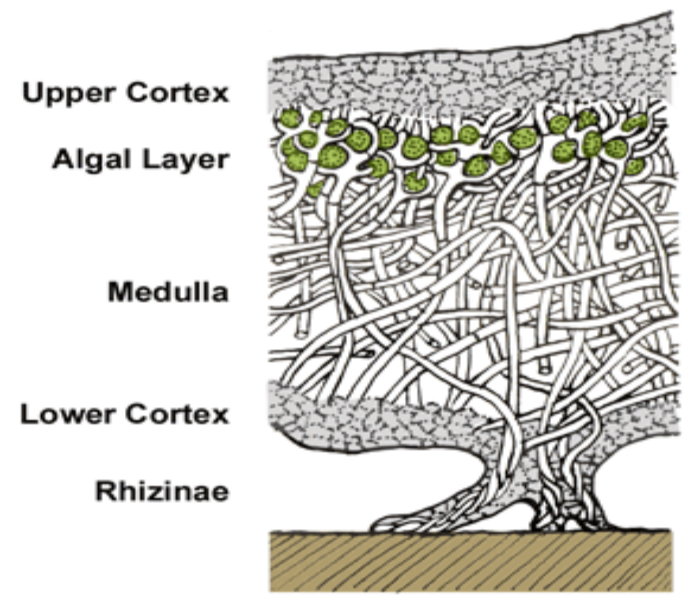
Medulla: It is the central part of the thallus, comprised of loosely interwoven fungal hyphae with large spaces between them. The hyphae are thick walled, oriented in different directions.

Lower cortex: Below the medulla, is the lower cortex consisting of densely compacted hyphae. Some of these hyphae become specialized and extend downward from the lower surface of the thallus and help in the attachment of the thallus to the substratum.

These specialised hyphae are known as Rhizines. Rhizines may be simple or branched.

Foliose lichens- Heteromerous lichens

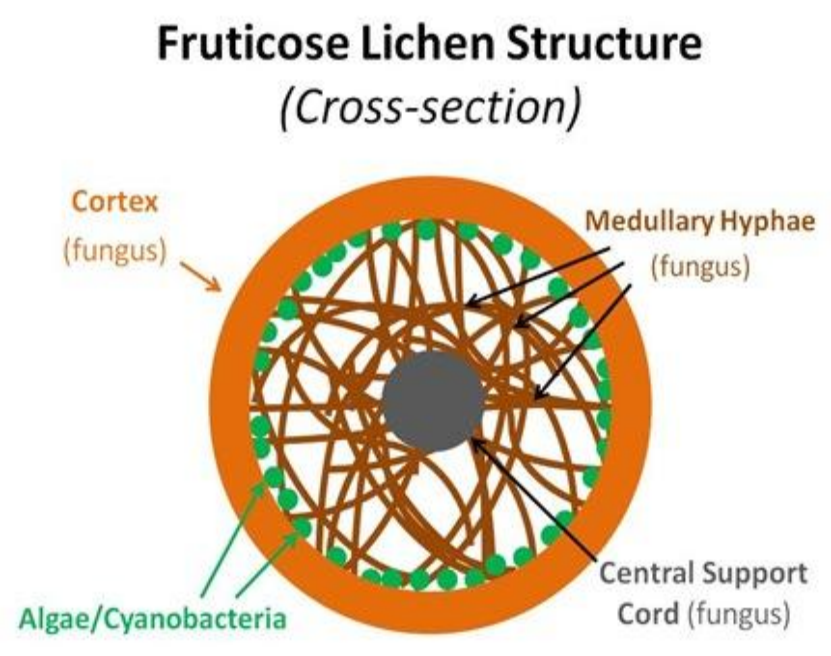


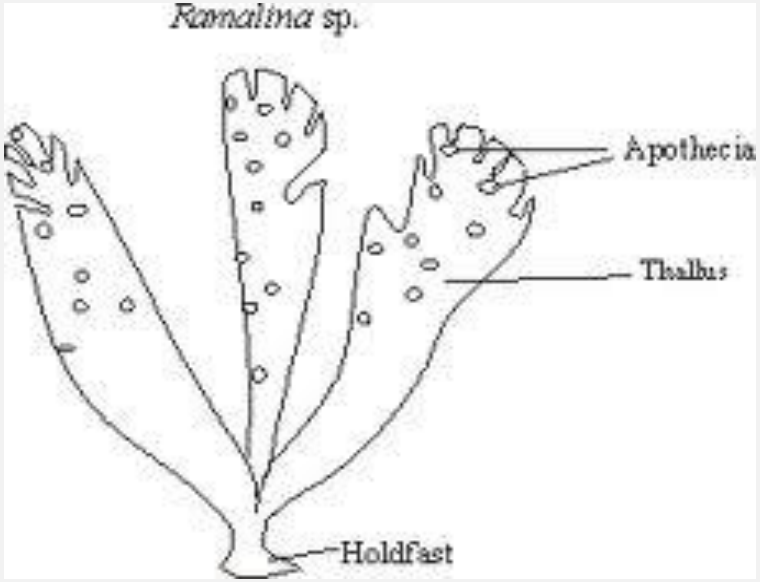


(3) **Fruticose lichens**:

- The lower cortex does not occur in Fruticose lichens due to their cylindrical structure and medulla forms the central part of the axis.
- The external layer of a lichen thallus, or surface is termed the cortex.
- Beneath it is a layer of fungus- enmeshed algal cells called the algal layer.

• Below the algal layer is a region of cottony, loosely woven fungal hyphae free from algal cells, the medulla.





REPRODUCTION:

Lichens reproduce both by vegetative, asexual and sexual methods.

(I) Vegetative reproduction:

Vegetative reproduction is of common occurrence in lichens. Some common methods are:

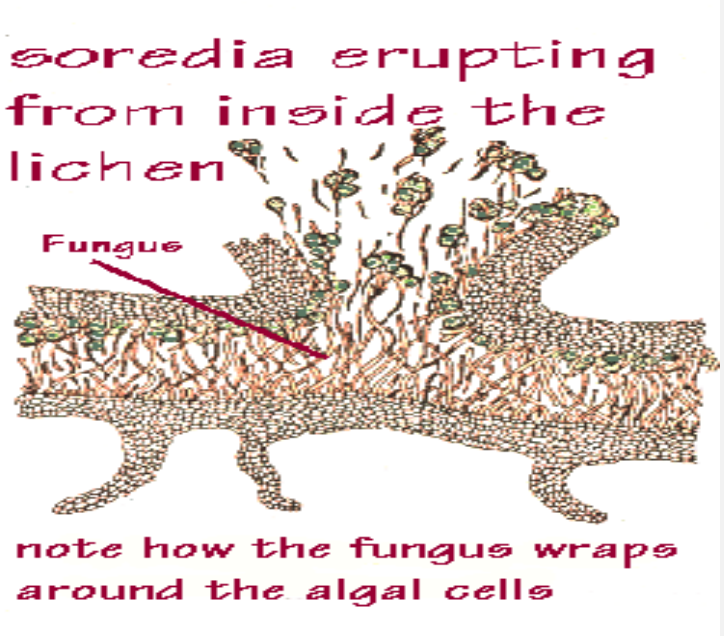
(1) Fragmentation:

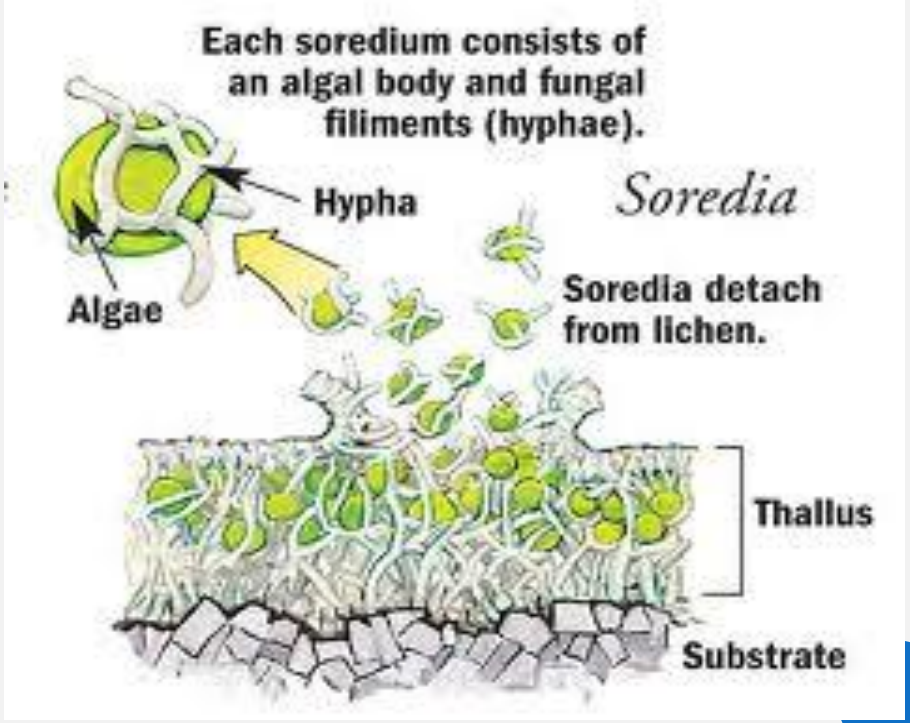
- Small fragments of thallus are formed by accidental breaking or due to death and decay of older parts.
- Each fragment develops into a new thallus, provided the fragment contains both algal and fungal components.

(2) Soredium:

- These are small bud like out growths which develop on the upper surface of the thallus.
- A Soredium contains one or few algal cells closely enveloped by a weft of fungal hyphae.
- Both the algal and fungal components are the same as in the parent thallus.
- The Soredium form a granular layer of grayish-white colour on the surface of the thallus.
- They are detached from the thallus by the impact of wind or rain.

•Sometimes, the Soredia develop in an organized manner in special pustule like areas.
•Then, they are known as Soralia. Ex. *Parmelia*, *Physcia*
•The Soredia germinate on a suitable substratum and form new thalli.





(3)ISIDIUM: These are small coral –like outgrowths which develop on the surface of the thallus. Each isidium has an outer cortical layer of fungal hyphae , enclosing the algal cells.

- The Isidia vary in shape from rod-like(Ex. *Parmelia sexatilis*), coralloid(Ex. Peltigera), Cigar shaped(Ex. *Usnea compasia*) or scale like(Ex. *Collema crispum*).
- The Isidia are primarily meant for increasing the photosynthetic area of the lichen thallus. However, we get detached from the thallus, they behave as reproductive structures.

- The isidium germinates under favourable conditions and forms new thallus.

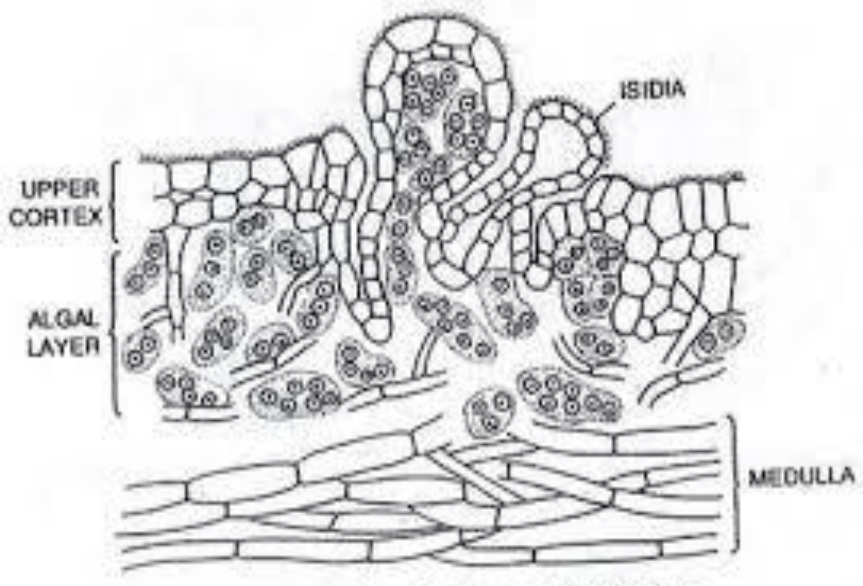
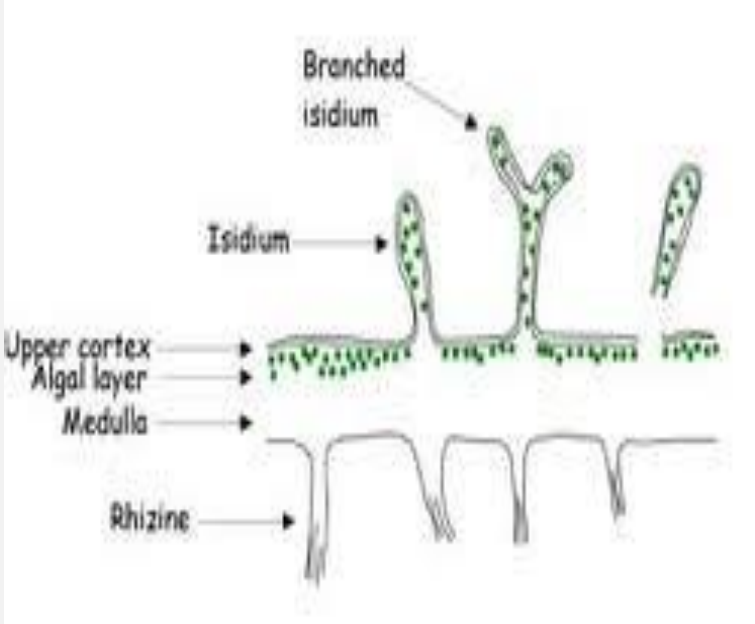


Fig. 18.12. Isidia (*Peltigera* sp.) V.S. thallus.



(4) CEPHALODIUM:

Some lichens are diphycophilous and show three membered symbiosis – two algae + one fungus.

- In such lichens, one of the algal partner is segregated into special external or internal swellings called cephalodia.
- The cephalodia are small, hard, dark coloured, gall like swellings and contain always a different algal component than the lichen thallus.
- For example, in *Peltigera aphthosa*, the cephalodium contains a blue green alga, but the algal component in the thallus is a green algae.

•Thus one of the Phycobiont is confined to cephalodium while the other one is present in the thallus.

•There is no organic connection between them.

E.g. *Lobaria*, *Solarina*, *Peltigera*

(II) Asexual Reproduction:

- Some lichens develop flask-shaped structures called pycnidia immersed in the thallus.
- The opening of the pycnidium is called ostiole.
- The interior of the pycnidium is lined by a number of hyphae, which act as conidiophores or pycnidiophores.
- The conidiophores cuts of a chain of small cells called conidia.
- The conidia on being dispersed, germinate under favourable conditions by sending hyphae in all directions.

•These hyphae, when come in contact with a suitable alga, develop into a new lichen body.

•There is remote possibility of the conidia getting the right algal partner. So their role in the formation of new thalli is negligible

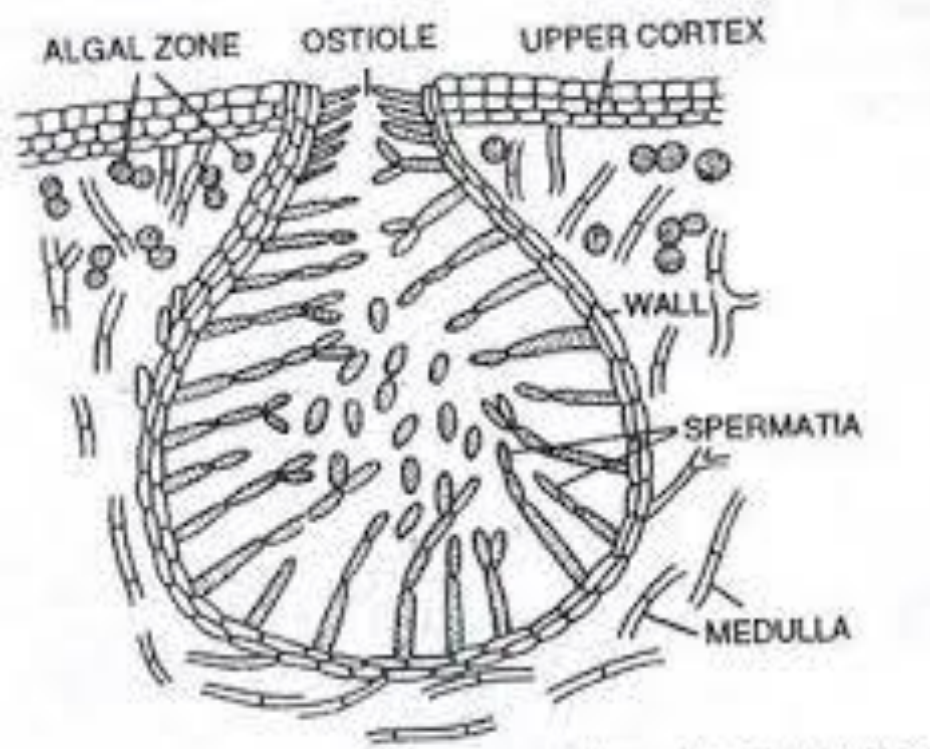


Fig. 18.13. Diagrammatic representation of spermagonium (pycnium) of *Physcia*.

(III) Sexual Reproduction:

In lichens, sexual reproduction is exhibited exclusively by the mycobiont.

The photobiont has no contribution to the sexual process.

The mode of sexual reproduction in Ascolichens is similar to that of Ascomycotina, while that in Basidiolichens is similar to that of Basidiomycotina.

The process sexual reproduction in Ascolichens is described below.

The female sex organs are known as carpogonia. A Carpogonium is differentiated into a basal coiled ascogonium and an elongated multicellular hypha called trichogyne.

The ascogonium remains embedded within the algal layer of the thallus, whereas the trichogyne projects over the surface of the thallus.

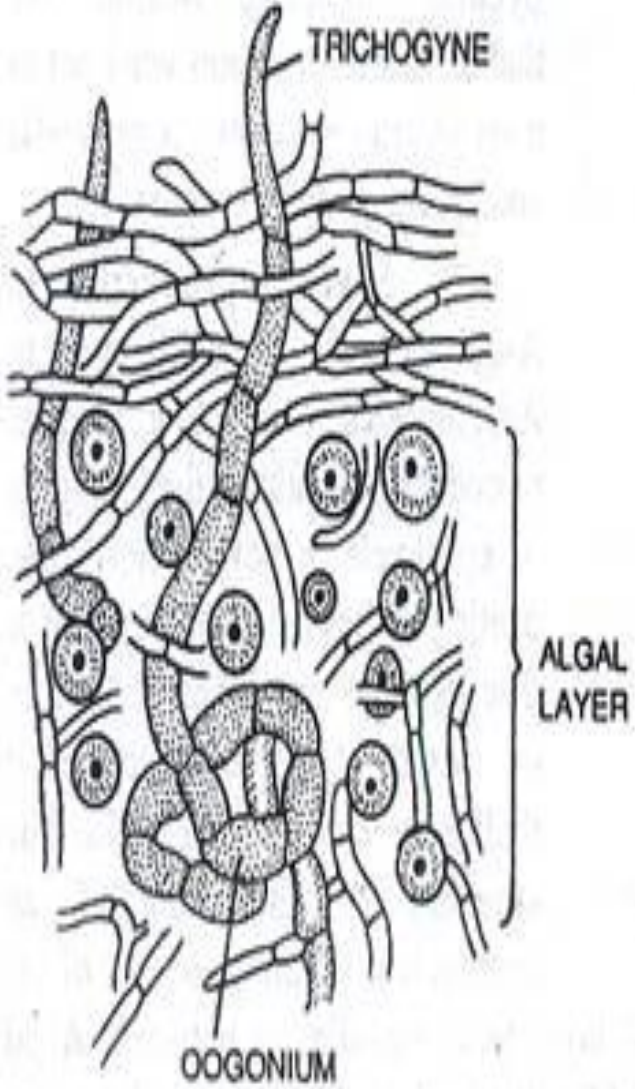


Fig. 18.15. V.S. thallus (*Physcia*).

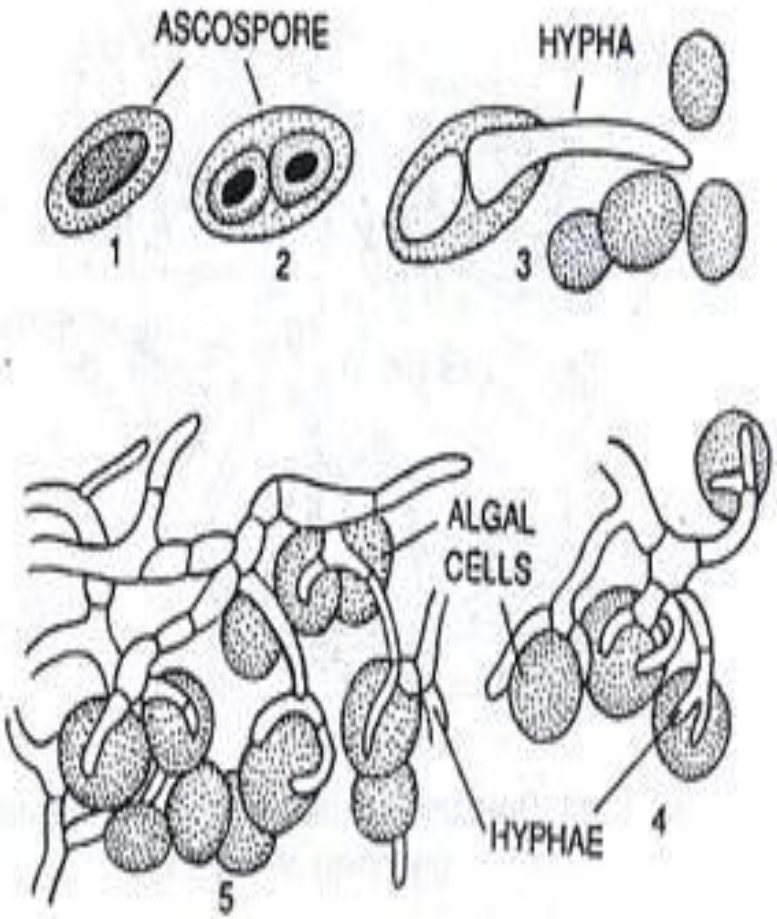


Fig. 18.16. Germination of ascospores and its association with algal cells to form lichen.

The male sex organs are flask shaped spermogonia.

- They form spermatia at the tips of spermatiophores.
- Spermatia act as male gametes.
- They come out of the spermogonium along with gummy fluid. They are deposited on the trichogyne of the ascogonium.
- On dissolution of the walls between the spermatium and trichogyne, the nucleus of spermatium migrates into the carpagonium through trichogyne.
- The male nucleus fuses with the female nucleus.

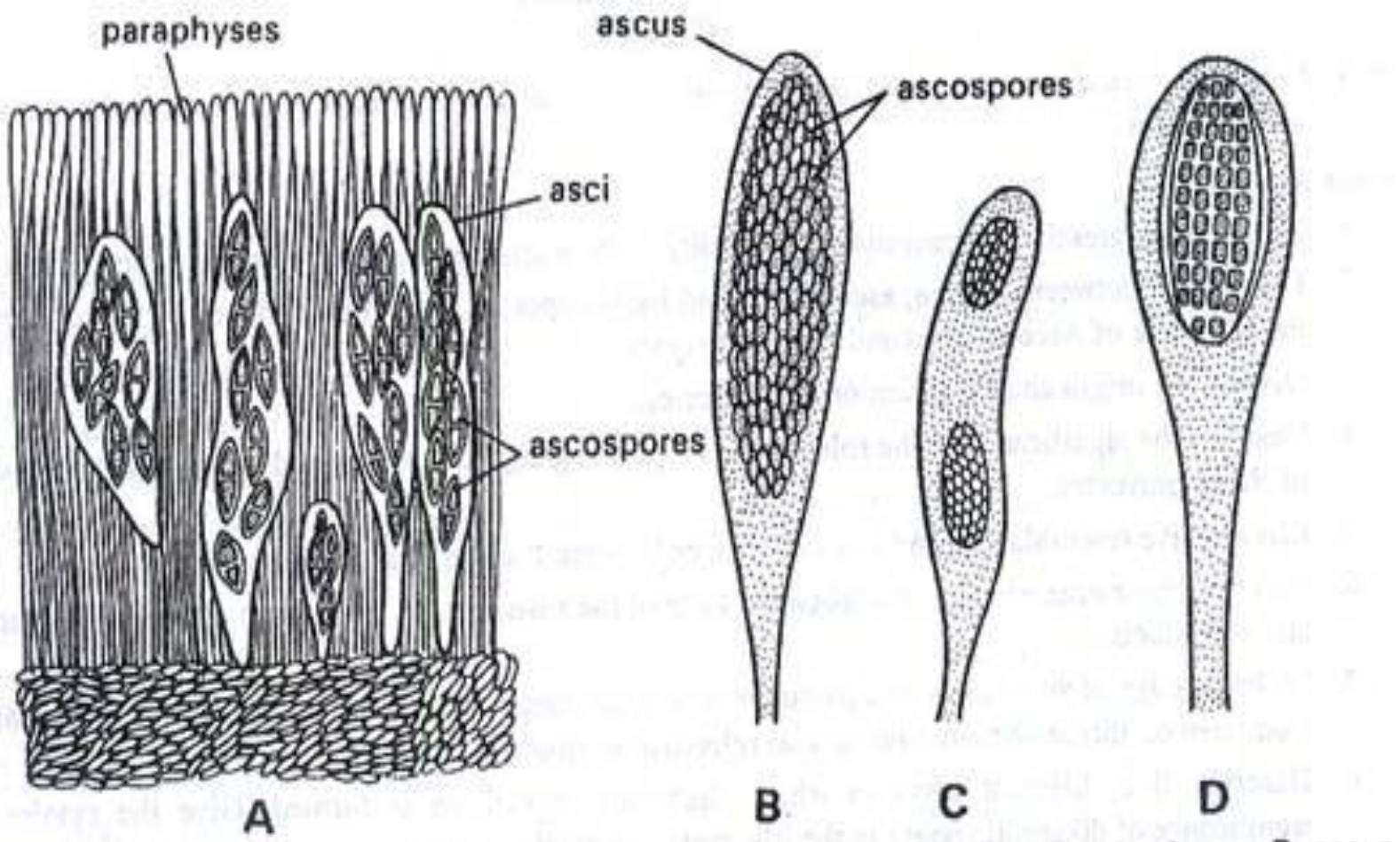


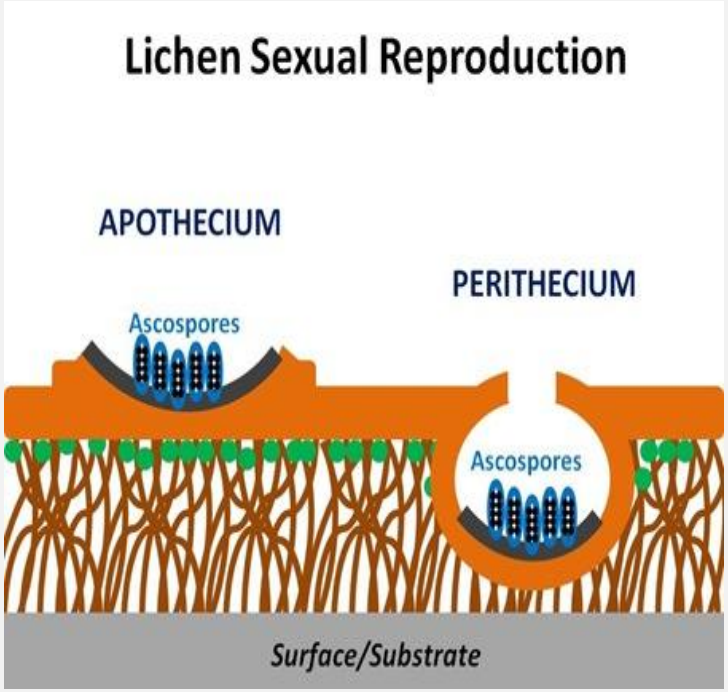
Fig. 18.18. Lichens. A, V.S. of *Physcia* thallus showing asci, ascospores and paraphyses; B, ascus with numerous ascospores; C, bi-spored ascus; D, single spored ascus.

After fertilization, many ascogenous hyphae develop from the basal portion of ascogonium.

This is followed by Crozier formation, and the formation of asci and the ascospores.

The asci bearing fruit body is called ascomata (ascocarp).

The ascocarp may be either an apothecium(e.g. *Parmelia*, *Anaptyahia*) or a perithecium(e.g. *Dermatocarpon*, *Verrucaria*, *Peltigera*).



Ochrolechia africana. Photo by Hugh Nourse.

apothecia

Rhizoplaca chyrsoleuca. Photo by Rick Demmer.

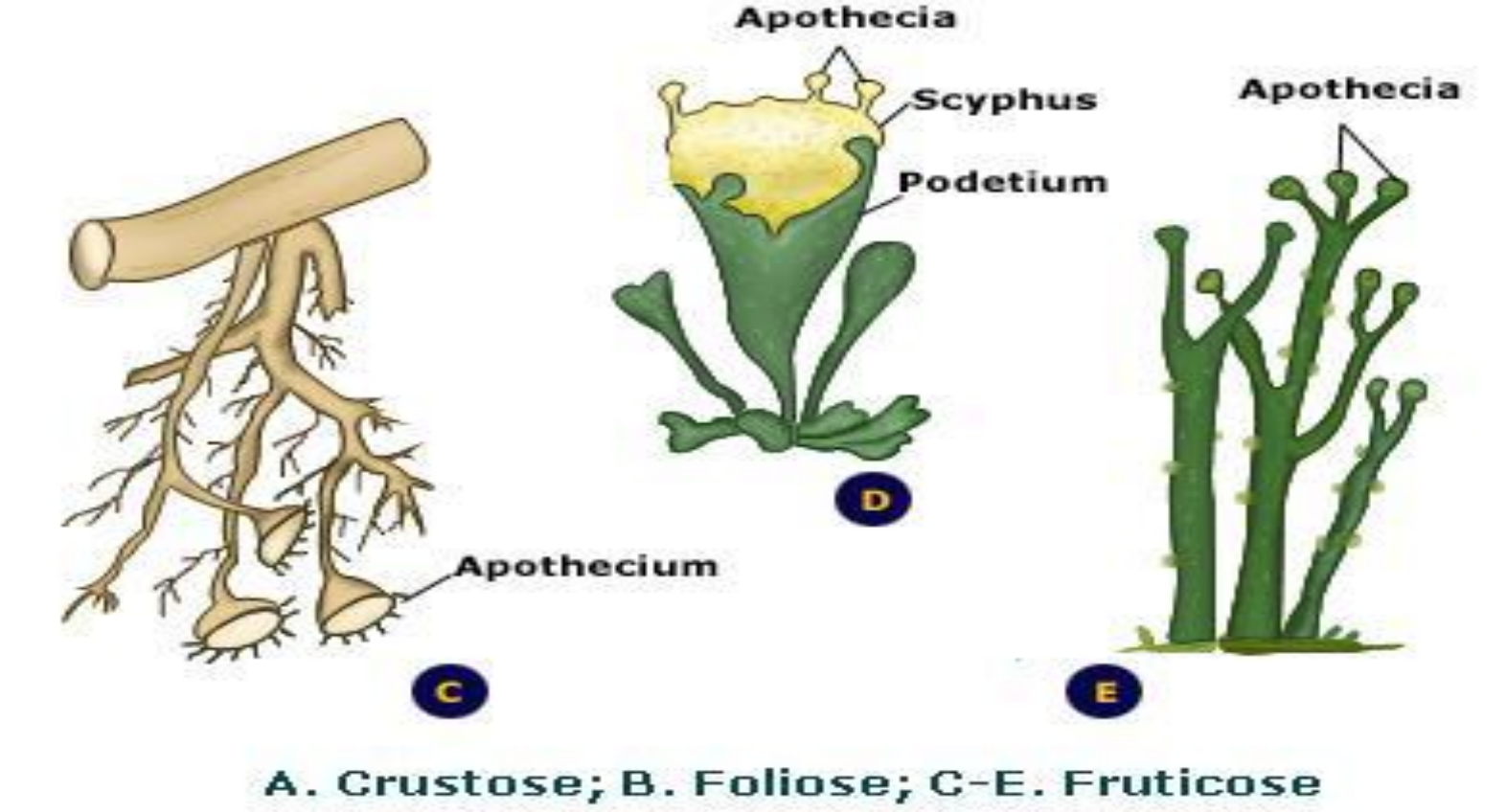
Structure of Apothecium: The apothecia are small, elevated, cup-shaped or disc-shaped fruiting bodies found in many lichens.

They vary in colour from reddish, reddish brown, yellow to black depending on the species.

The bottom of the cup is lined by hymenium that consists of a number of upright asci interspersed with slender paraphysis, containing a reddish oily food-substance.

The wall of the apothecium is composed of the vegetative part of the thallus.

STRUCTURE OF APOTHECIUM



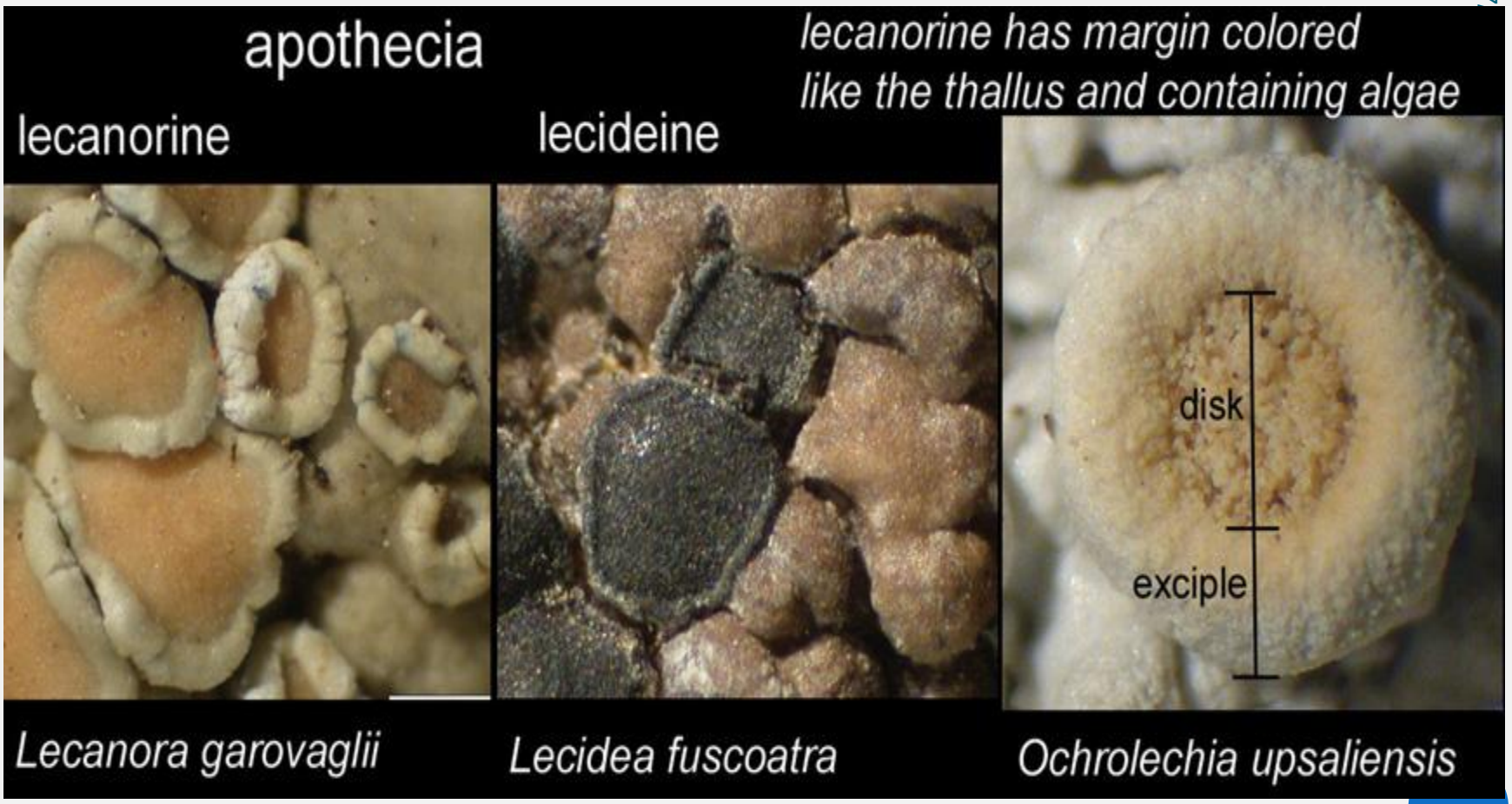
If the vegetative part consists of both the algal and fungal components it is called Lecanorine type.

E.g. *Lecanora*, *Parmelia*, *Physcia*.

If the apothecium contains only the fungal component, it is called Lecideine type.

E.g. *Lecidea*, *Cladonia and Gyrophora*.

Lecanorine type and Lecideine type



ECONOMIC IMPORTANCE

1. Pioneers of vegetation:

- Lichens are the first plants to colonise bare rocks. The acids produced by them disintegrate the rock surface.
- The death and decay of lichens provide nutritive ground for the growth of mosses.
- They play an important role in soil formation.

2. Food:

Some lichens are useful as food to human beings. In Iceland, Cetraria icelandica is boiled and powdered and then made as cakes.

In Japan, *Umbillicaria esculentus* is sold as vegetable. In India Parmelia is used as curry leaf in Bellary District.

Lecanora commonly called as ‘manna lichen’ is used as food by Africans during drought.

Lichens contain a polysaccharide lichenin, but lack true starch and cellulose.

Evernia prunastri is used by Egyptians for making breads.

3.Fodder:

Cladonia rangiferina (reindeer moss) in tundras, *Cetraria icelandica* (Iceland moss), and *Lecanora* are used as fodder.

4.Medicine:

Lichens possess antibiotic properties. A preparation from *Peltigera* is useful against hydrophobia.

Usnae is used as an expectorant.

Lobaria pulmonera for lung troubles, *Xantharia* for jaundice, and *Cetraria icelandica* are some lichens of medicinal value.

Erythrin obtained from *Roccella montagnei* is used in angina, a type of heart disease.

Antiseptic creams such as Usno and Evosin are used for tumour inhibition and spasmolytic activities. Some lichens yield protolicheserinic acid used in anticancer drugs.

Species of *Usnea* and *Evensia furfuracea* are used as astringents in haemorrhages.

:

The aromatic substances present in the lichen thalli are commercially used in the preparation of cosmetics and perfumes.

Several French perfumes considered to be the best are made from *Evernia*(Oak moss) and *Lobaria*.

6.Dyes: Many lichens find use in producing dyes for colouring fabrics.

Orchil obtained from *Roccella tinctoria* is used in dying woollen and silk fabrics.

Brown dye is obtained from *Parmelia*.

Litmus used as acid base indicator in laboratories is obtained from *Roccella montaingnei*.

7. Tanning and distilleries:

The astringent chemicals obtained from Cetraria icelandica, *Laboria pulmonaria* are used in tanning of leather.

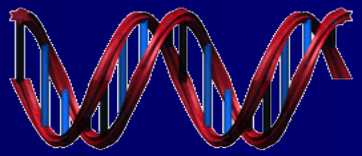
Lobaria finds use in brewing of beer; while *Usnea* and *Ramalina* are used in preparation of alcohol.

8. Harmful aspects:

A few lichens like *Usnea*(popularly, known as mans beard), are the cause of forest fire as they are highly inflammable during summers.


Thank You

Polymerase Chain Reaction (PCR)



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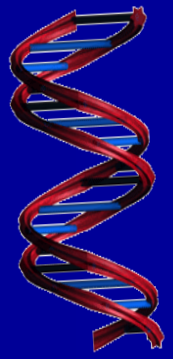
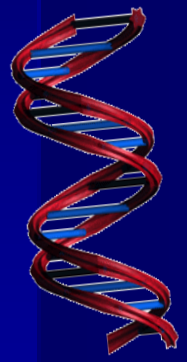
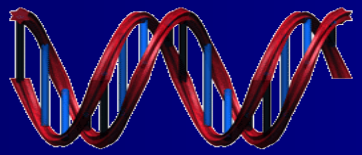
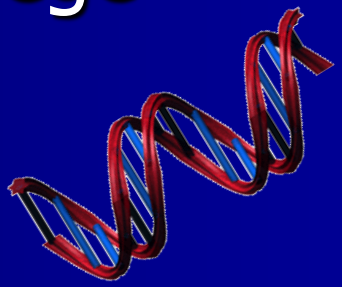
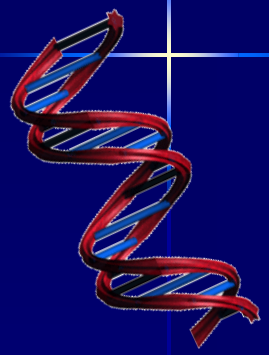




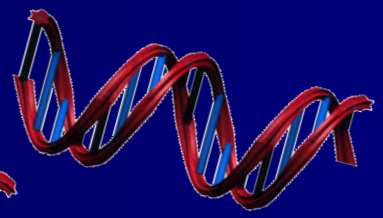




Polymerase Chain Reaction



- Polymerase: DNA polymerase
  - DNA polymerase duplicates DNA
  - Before a cell divides, its DNA must be duplicated
  - Chain Reaction: The product of a reaction is used to amplify the same reaction
  - Results in rapid increase in the product

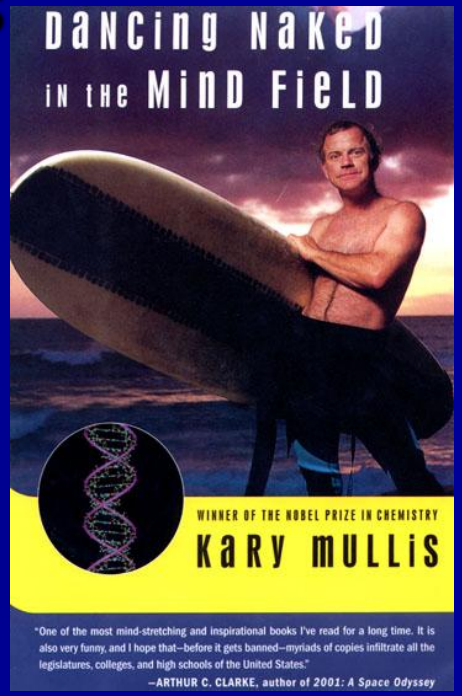


Polymerase Chain Reaction (PCR)

- PCR performs the chemistry of DNA duplication *in vitro*
- Numerous PCR applications make this process a staple in most biology laboratories
- Understanding properties of DNA polymerases helps understanding PCR

Discovery

- PCR was discovered by Kary Mullis
  - On a long motorcycle drive
  - Mentally visualized the process
- Nobel Prize in Chemistry
  - 1993



DNA polymerase

- Duplicates DNA
- Necessary for reproduction of new cells
- More than one DNA polymerases exist in different organisms

Properties of DNA polymearse

- Needs a pre-existing DNA to duplicate
  - Cannot assemble a new strand from components
  - Called template DNA
- Can only extend an existing piece of DNA
  - Called primers

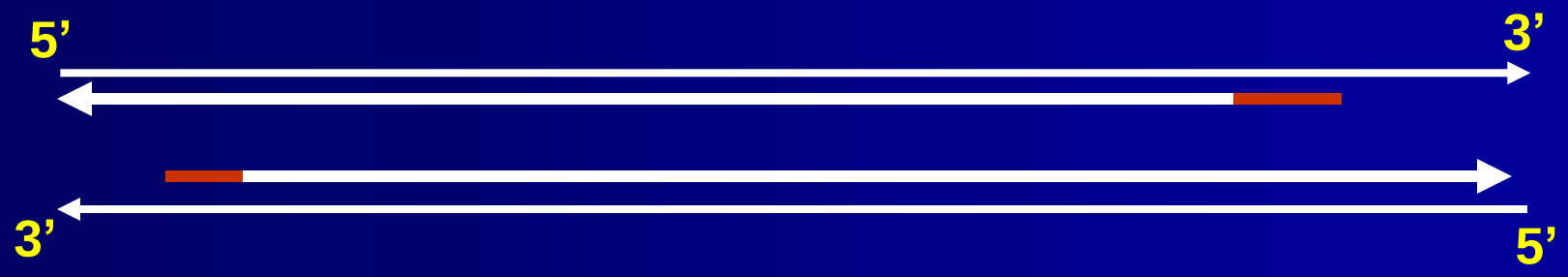
Properties of DNA polymearse

- DNA polymerase needs Mg^{++} as cofactor
- Each DNA polymerase works best under optimal temperature, pH and salt concentration
- PCR buffer provides optimal pH and salt condition

Properties of DNA polymearse

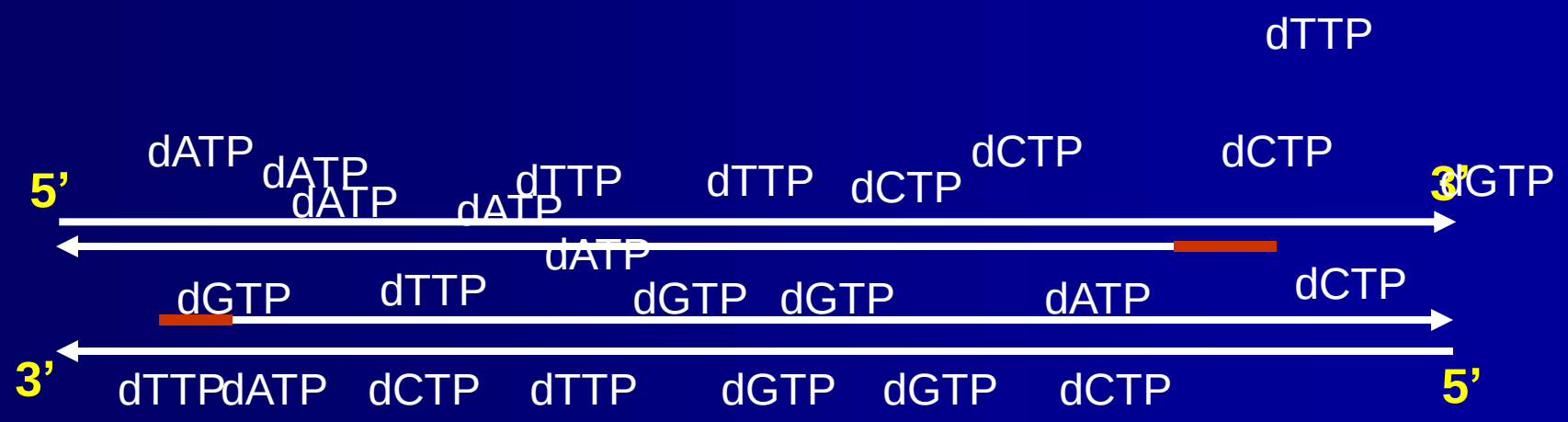
- DNA strands are anti-parallel
  - One strand goes in 5′ → 3′
  - The complementary strand is opposite

- DNA polymerase always moves in one direction (from 5′ → 3′)



Properties of DNA polymearse

- DNA polymerase incorporates the four nucleotides (A, T, G, C) to the growing chain
- dNTP follow standard base pairing rule

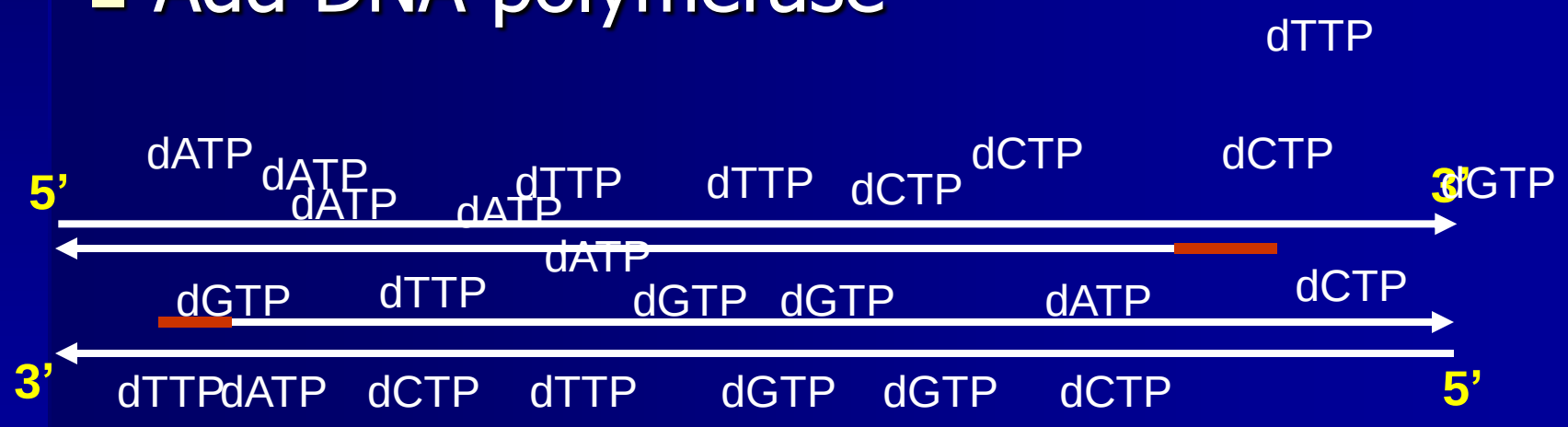


Properties of DNA polymearse

- The newly generated DNA strands serve as template DNA for the next cycle
- PCR is very sensitive
- Widely used

Setting up a PCR Reaction

- Add template DNA and primers
- Add dNTPs
- Add DNA polymerase



Taq DNA polymerase

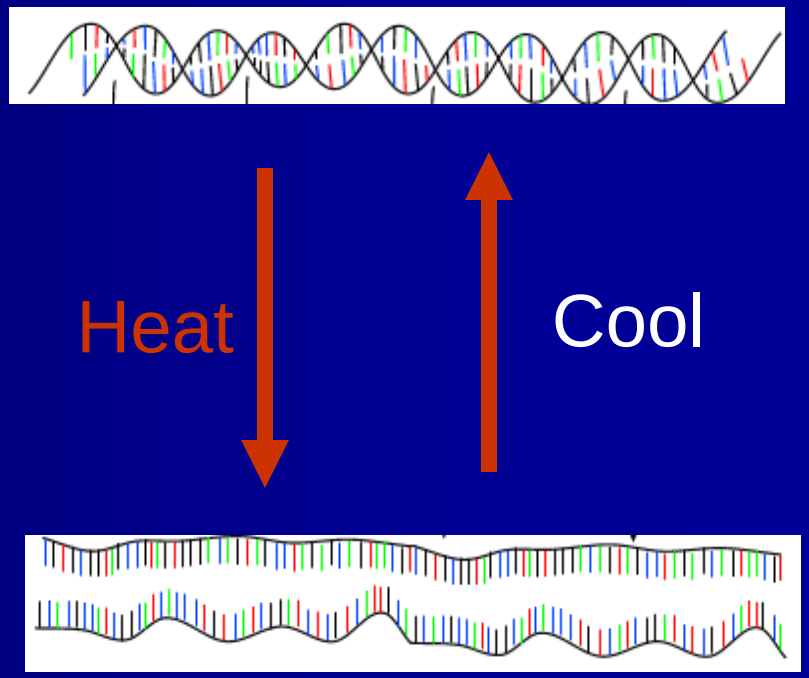
- Derived from *Thermus aquaticus*
- Heat stable DNA polymerase
- Ideal temperature 72C

Thermal Cycling

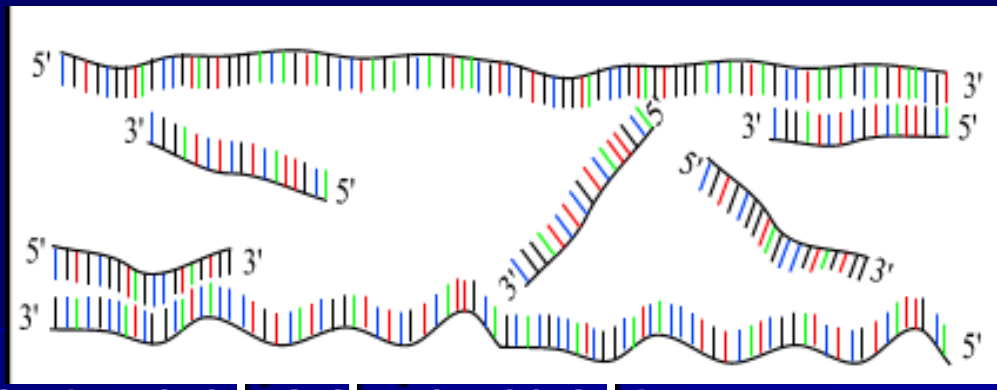
- A PCR machine controls temperature
- Typical PCR go through three steps
  - Denaturation
  - Annealing
  - Extension

Denaturation

- Heating separates the double stranded DNA
  - Denaturation
- Slow cooling anneals the two strands
  - Renaturation



Annealing

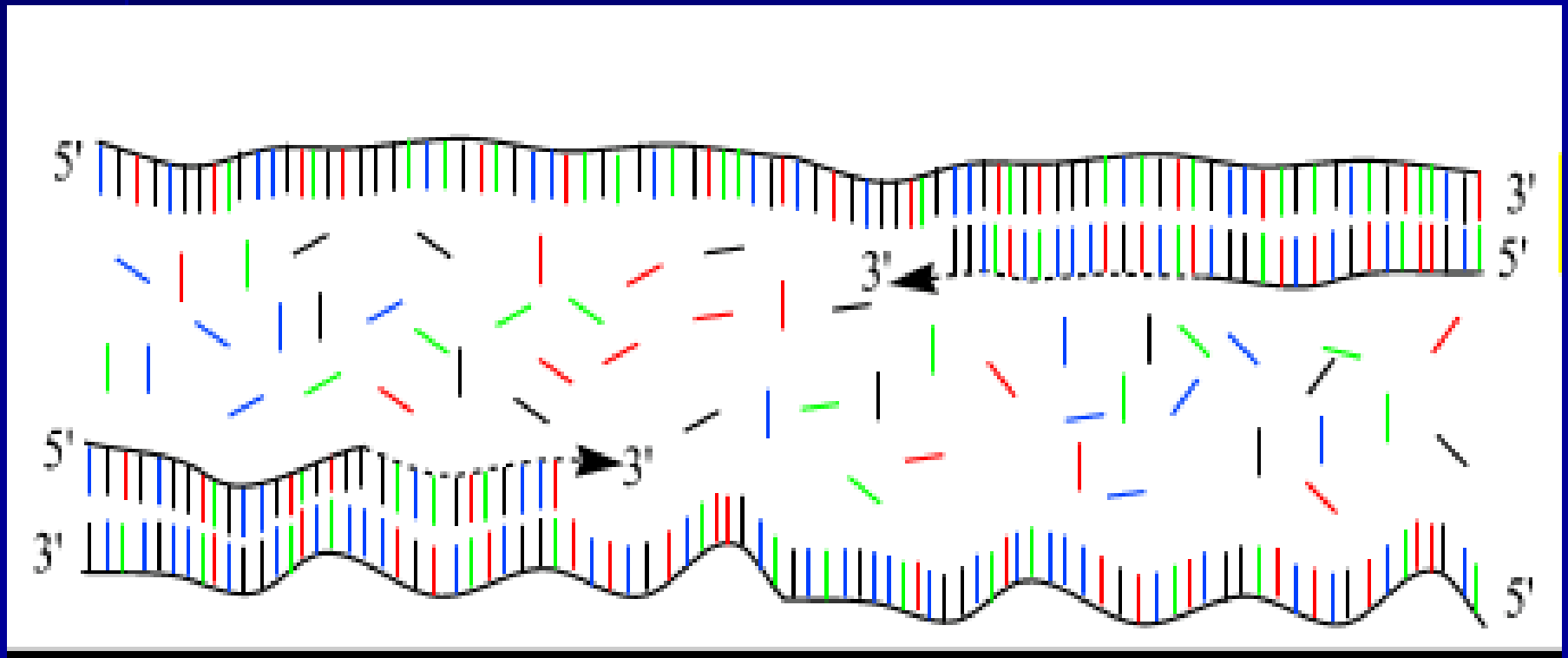


- Two primers are supplied in molar excess
- They bind to the complementary region
- As the DNA cools, they wedge between two template strands
- Optimal temperature varies based on primer length etc.

Typical temperature from 40 to 60 C

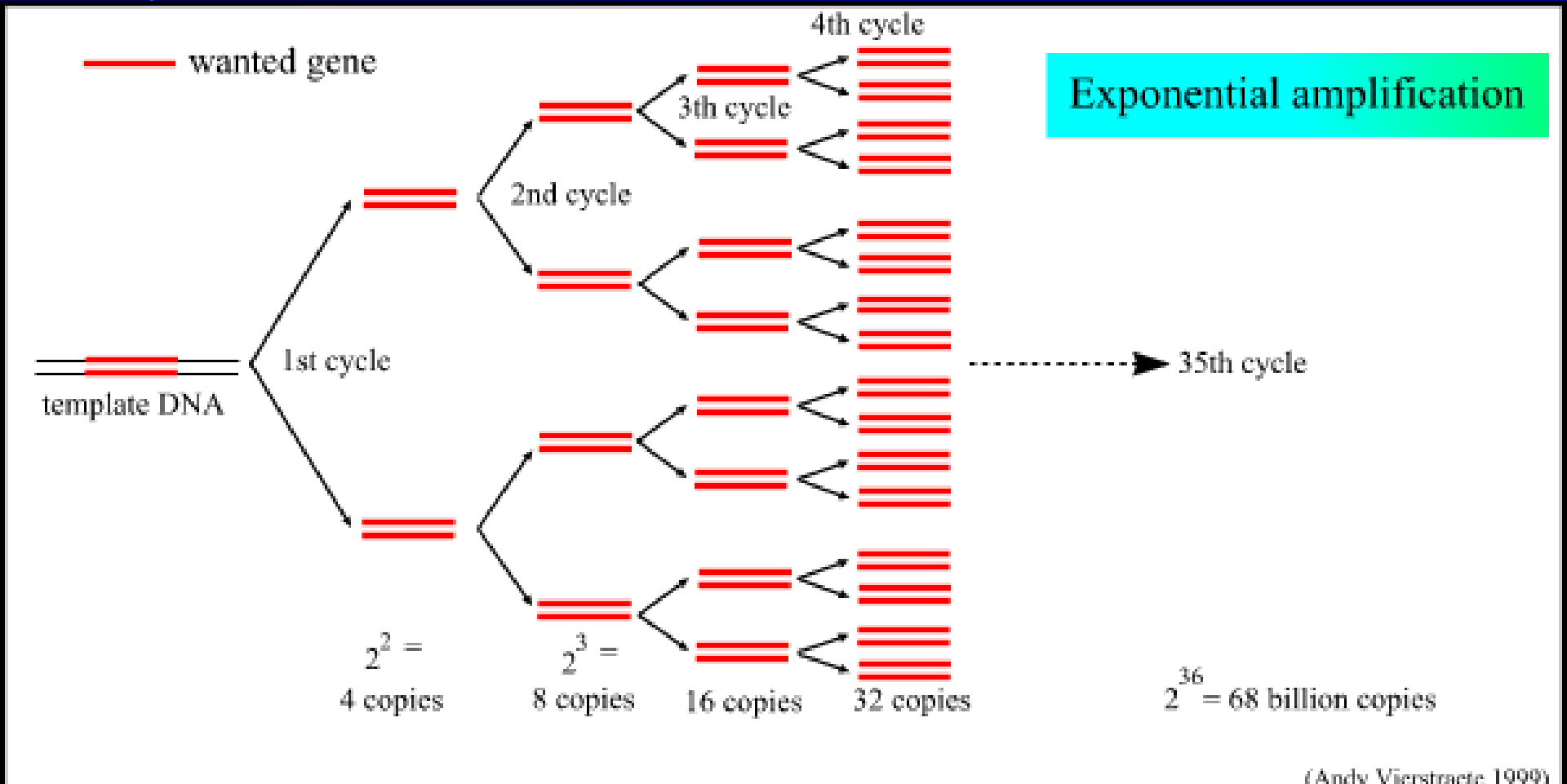
Extension

- DNA polymerase duplicats DNA
- Optimal temperature 72C



PCR Amplification

Exponential Amplification of template DNA



Typical PCR mix

In a thin wall Eppendorf tube assemble the following

PCR components	Amount
Template DNA (5-200 ng)	variable
1 mM dNTPs (200 uM final)	10 uL
10 X PCR buffer	5 uL
25 mM MgCl2 (1.5 mM final)	3 uL
20 uM forward primer (20 pmoles final)	1 uL
20 uM reverse primer (20 pmoles final)	1 uL
5 units/uL Taq DNA polymerase (1.5 units)	0.3 uL
Water	Variable
Final Volume	50 uL

Applications

- Ubiquitous applications
- Revolutionized how we study biology
  - Research
  - Diagnostics
  - Forensics

REPLICATION OF DNA

Introduction:

- The process by which a DNA molecule produces its identical copies is described as DNA replication.
- It is a type of self duplication or self production of DNA, where two daughter molecules are formed from a single DNA molecule.

DNA replication:

- Theoretically, three possible modes of DNA replication are possible. They are:

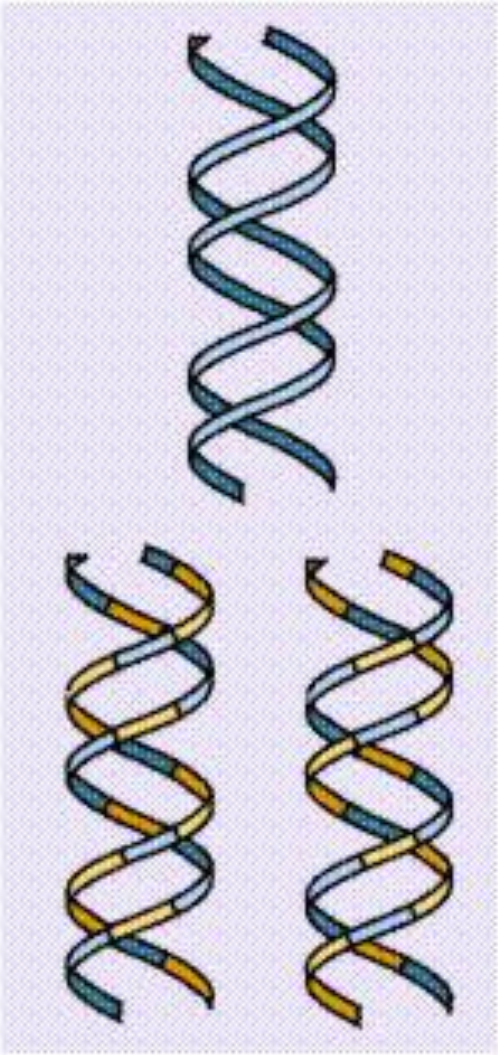
1. Dispersive replication:

- The two strands of parent DNA break randomly and produce several pieces.

Dispersive replication

Original DNA double helix

DNA molecules after one round of replication



- These pieces replicate and reunite to form new daughter DNA molecules.
- These new DNA molecules contain a mixture of old and new nucleotides scattered along, the chains.
- The daughter molecules can be described as hybrids.
- This mechanism is neither accepted nor proved experimentally.

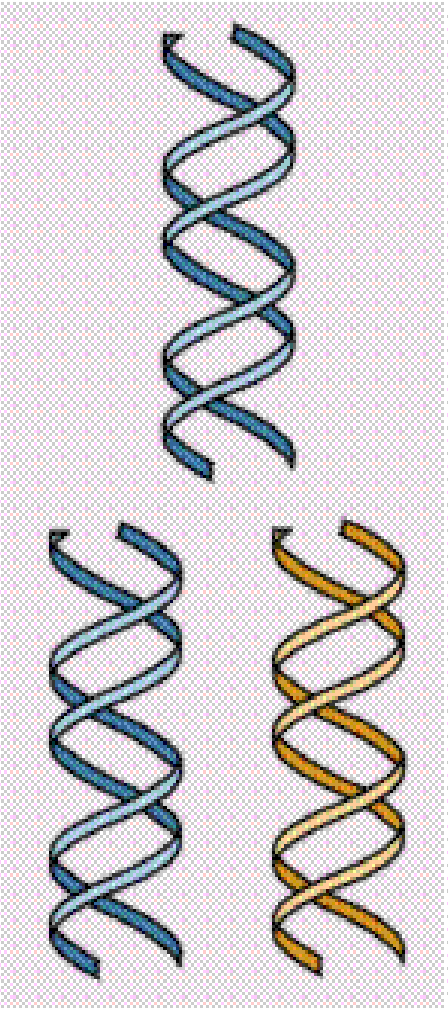
2. Conservative replication:

- After replication, one daughter DNA contains the original two strands of the parent molecule.
- While the other daughter molecule contains two newly synthesized strands.
- This method is also not accepted.

Conservative replication

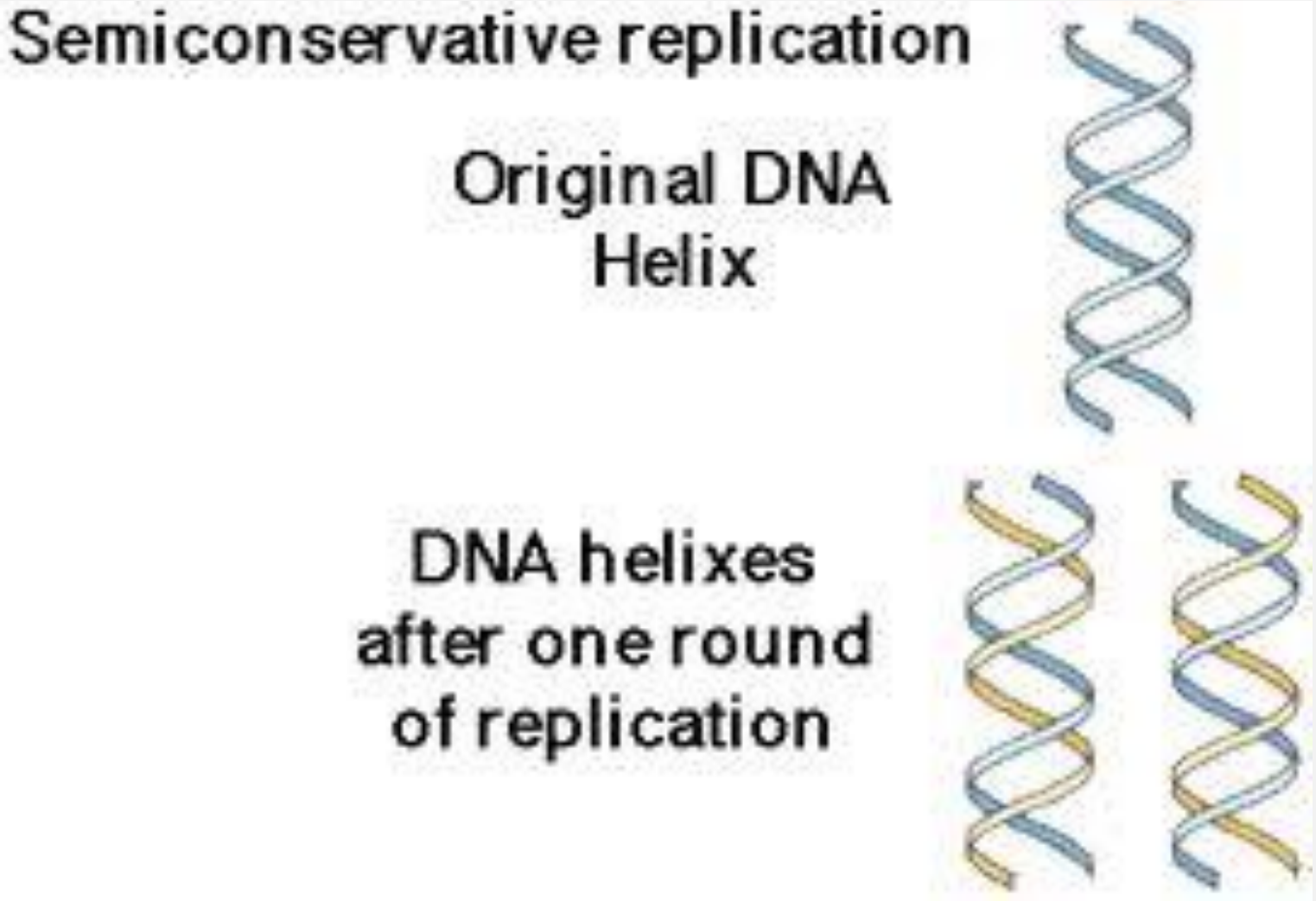
Original DNA double helix

DNA molecules after one round of replication



3. Semi Conservative replication:

- This method of DNA replication was proposed by Watson and Crick.
- Because of the specificity of base pairing, the sequence of bases along one chain automatically determines the base sequence along the other.
- Thus, each chain of the double helix can serve as a template for the synthesis of the complementary strand.
- More precisely semi-conservative means half of the DNA is conserved i.e; only one strand is synthesized and the other half of the original DNA is retained.


Semiconservative replication
Original DNA Helix
DNA helixes after one round of replication

- The main features of this model of DNA replication are as follows:

1. Progressive separation of the two strands of DNA molecule undergoing replication.
2. Complementary base-pairing of the bases located in the single-stranded region with the appropriate free deoxyribonucleotides, and
3. Formation of phosphodiester linkages between the neighbouring deoxyribonucleotides, there by producing the new strand.
4. This ensures that the base sequences of the new strands are strictly complementary to those of the old strands.

5. Each DNA molecule produced by replication has one ‘old’ and one ‘new’ strand.

- Since, each of the two double helices conserves only one of the parent polynucleotide strands, the process is said to be semi-conservative.

Evidences for semi conservative replication:

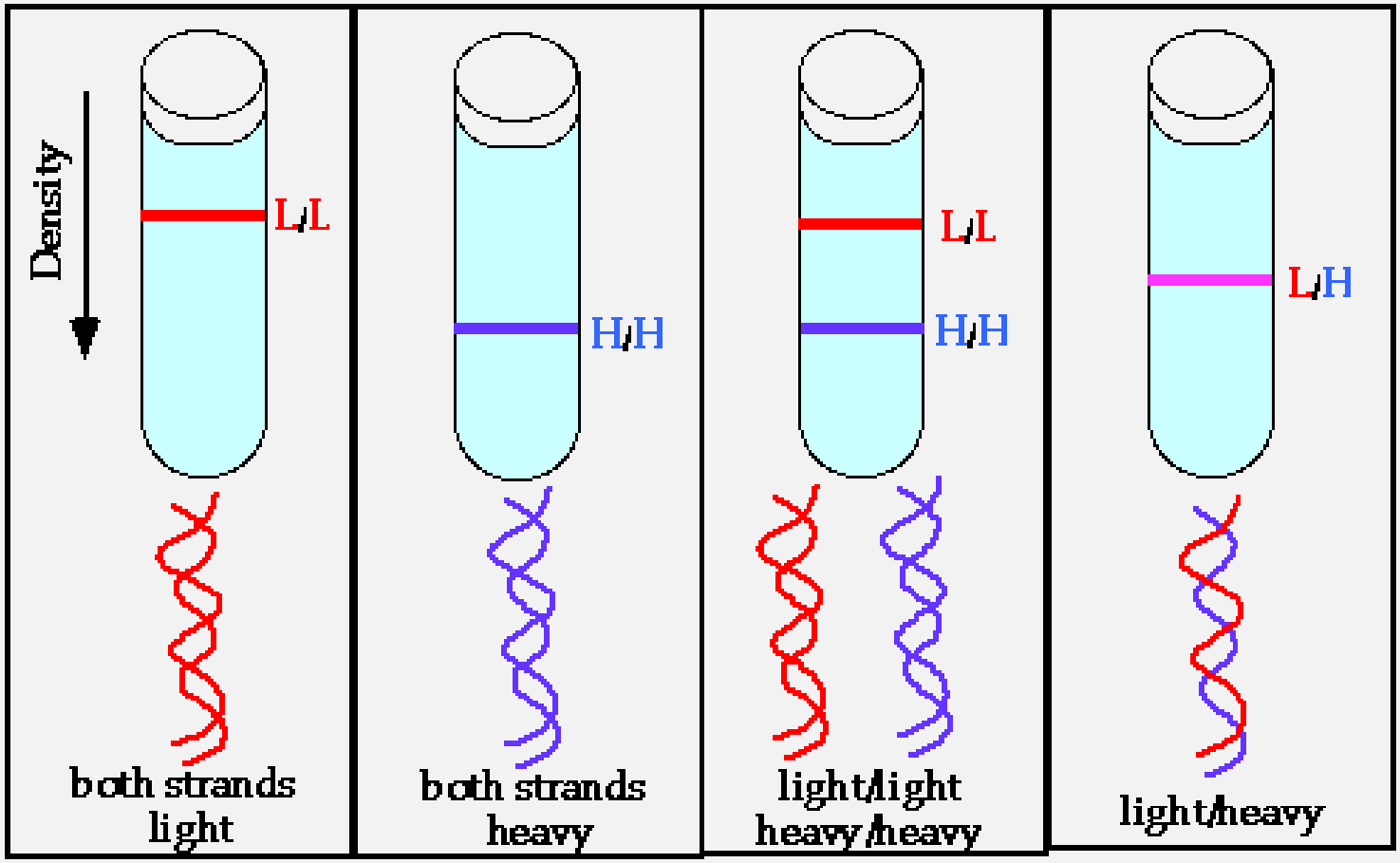
- The evidences for semi conservative replication of DNA molecules were provided by Meselson and Stahl(1958), Cairns(1963).

(A) Meselson-Stahl’s experiments:

- The first evidence for semi conservative replication of DNA was given by Meselson and Stahl in 1958.

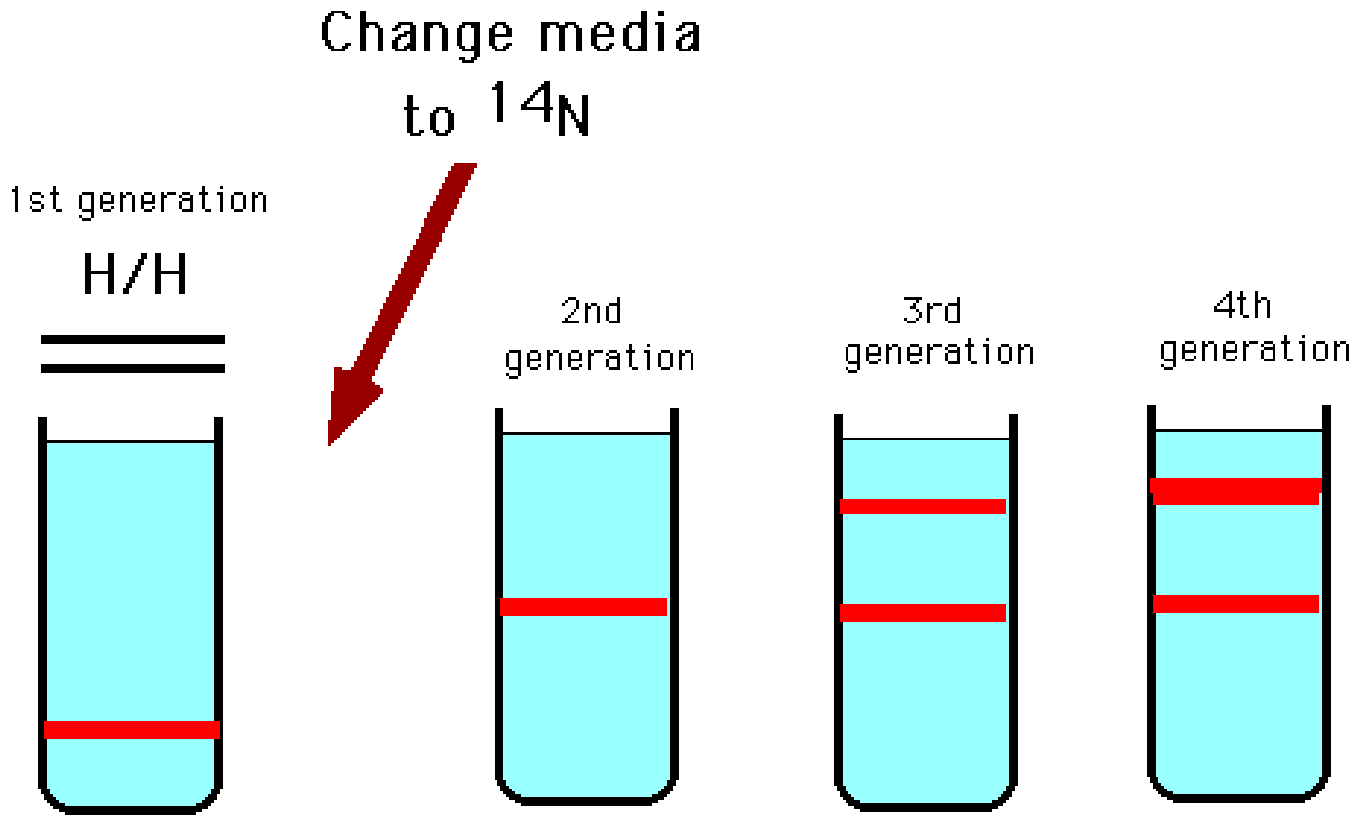
- They grew E.coli on culture medium containing 15N(a heavy isotope of 14N) for several generations, so that the nitrogen present in DNA bases of these cells was 15N.
- DNA having 15N has a detectable higher density than that having 14N.
- Therefore they are called heavy and light DNA respectively.
- Heavy and light DNA molecules can be readily separated through equilibrium density gradient centrifugation.
- They form distinct bands in the centrifuse.

- In density gradient centrifugation, a heavy salt(e.g. $CsCl_2$) is centrifused at high speed(30,000-50,000 rpm) for 40-72 hr.
- This leads to the formation of a linear gradient of increasing density from the top to the bottom of the centrifuse tube.
- When molecules with small differences in density are centrifused in this solution, they form separate bonds.
- Meselson and Stahl transformed the E.coli cells grown on 15N medium to a medium containing normal 14N.


Density
L/L
both strands
light
H/H
both strands
heavy
L/L
H/H
light/light
heavy/heavy
L/H
light/heavy

- They withdrew samples from these E.coli cells after approximately one, two and three cell generations.
- After one cell generation, the DNA formed a single band ‘intermediate’ between heavy(15N) and light(14N) DNAs.
- The DNA obtained after two cell generations formed two bands.
- One of the bands was ‘intermediate’ while the other was ‘light’ in density.
- The same two bands were recovered in DNA isolated after three cell generations.
- These findings can be readily explained on the basis of semi conservative replication of DNA.

What Meselson and Stahl observed

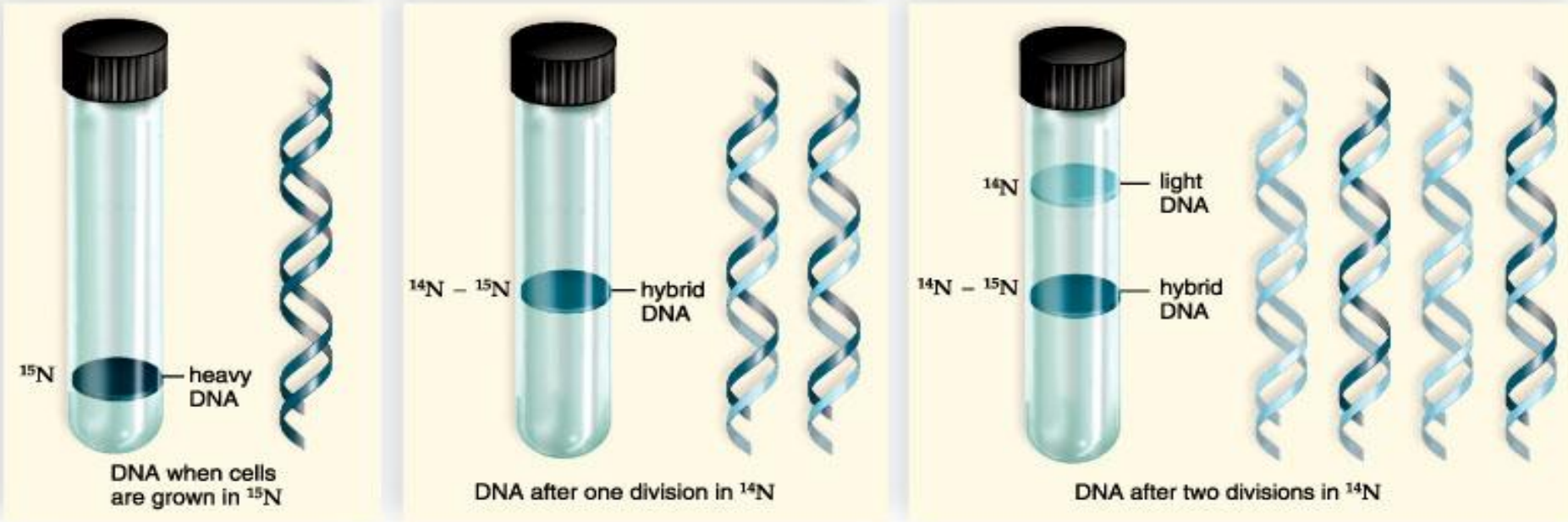


- The DNA from E.coli grown on 15N had 15N in both the strands, therefore it was heavier than the normal DNA having 14N.
- When these E.coli cells were allowed one semi conservative replication of their DNA on 14N medium, each of the DNA molecules would have one heavy(15N) and one light(14N) strand.
- Therefore, these DNA molecules would have ‘intermediate’ density.
- One more semi conservative replication of these DNA molecules(15N/14N) in the 14N medium would generate two types of DNA molecules.

1. Half of the molecules would have one ‘heavy’(15N) and one ‘light’(14N) strand(intermediate density) while
2. The remaining half would have both light density strands(14N/14N).

- These molecules would obviously form one ‘intermediate’ and one ‘light’ band.
- Following the third round of replication of 14N medium the ‘intermediate’ density DNA molecules would yield half, ‘intermediate’ and half ‘light’ molecules.
- The ‘light’ density molecules would yield only ‘light’ density molecules.

a. Possible results when DNA is centrifuged in CsCl



b. Steps in Meselson and Stahl experiment

- This is the reason for the lower intensity of the ‘intermediate’ band after three cell generations.

(B) Cairn’s Auto radiography Experiment:

- J.Cairns(1963) demonstrated the semi-conservative mode of replication of bacterial chromosome using autoradiography technique.
- In this method, tritiated trymidine(H3-TdR heavy redioactive) is used to label or tag DNA of E.coli.
- The normal thymidine is replaced by H3 TdR after replication.
- Then the cells are broken to release the intact bacterial chromosome on slides.

- These slides are covered by photographic emulsion or films and stored in dark.
- The tritiated thymidine emits particles in dark due to its radio active decay.
- These particles expose the photographic films.
- These films are developed and analysed.
- This photograph will then show the regions of the presence of tritium and thus indirectly the presence of labelled DNA.
- Using this above technique, Cairns carried out his auto radiogarphic experiments on E.coli and obtained results as expected, which confirmed the semi conservative mode or DNA replication.ss

- E.coli has a circular DNA.
- The replication starts at an unique site and proceeds simultaneously in the two opposite directions.
- At the replication fork, one DNA molecule appears to be branched into two molecules.
- Such a fork is formed due to a progressive separation of the two strands of a replicating DNA, followed by the synthesis of new complementary strands on these ‘old’ strands.
- So the chromosomes exist as θ shaped structure during replication.

Mechanism of DNA replication:

- DNA replication is a complex event and includes the following 3 major phases:

1. Initiation
2. Elongation and
3. Termination.

- Replication occurs inside the chromosomes during interphase.
- The parent DNA strands serve as templates for the synthesis of new.
- DNA strands DNA replication occurs by semi conservative method.
- It is catalysed by the enzyme DNA polymerase.

1. Initiation:

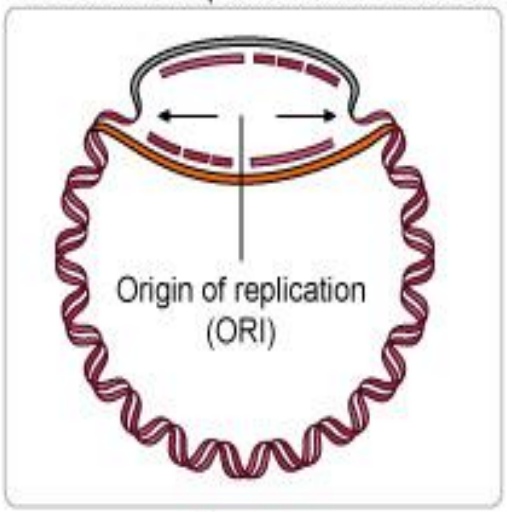
(i) DNA replication begins at certain unique and fixed points called “Origin”(ori).

(ii) Two enzymes DNA gyrase and DNA helicase, bind to the origin points and induce the unwinding and separation of complementary strands of DNA double helix. This separation is known as DNA melting.

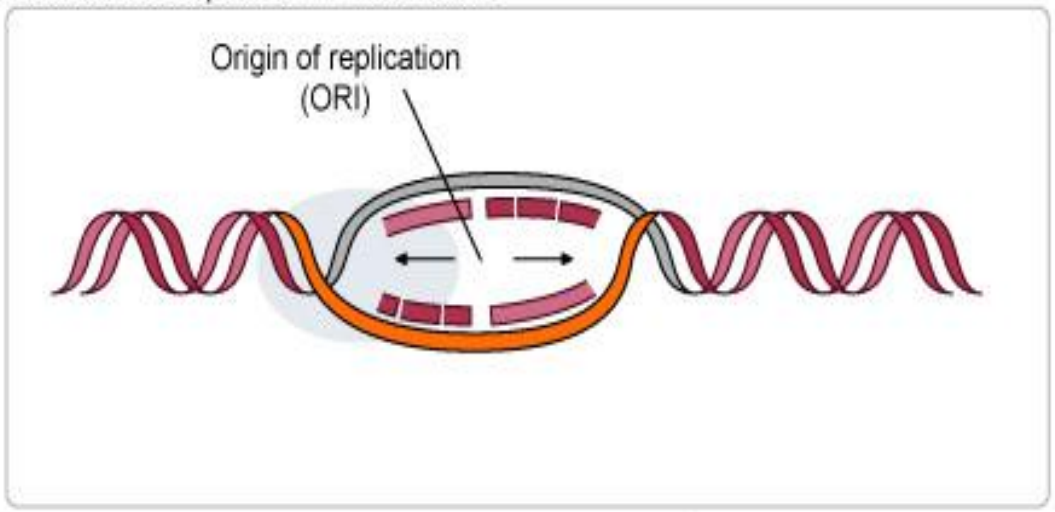
(iii) Melting of DNA produces two Y-shaped forks at origin, one fork is located at each end of the origin. When replication begins, these forks become replication forks.

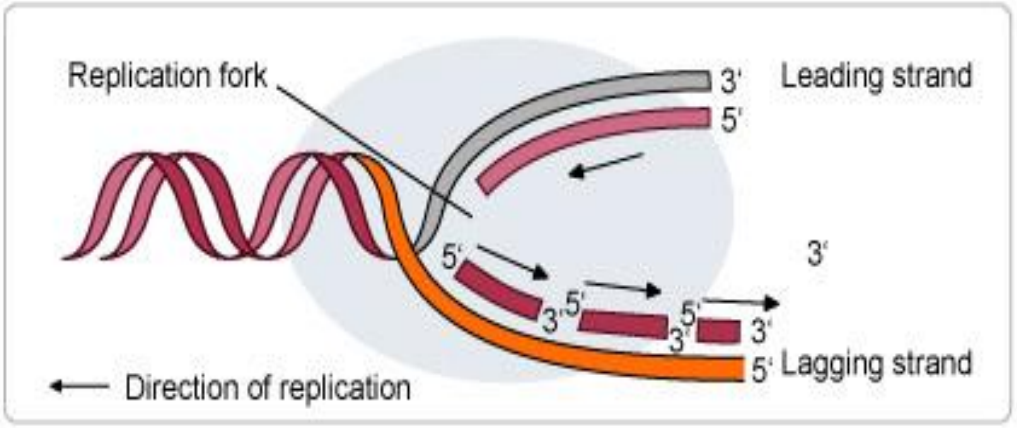
Origins of Replication

Bidirectional replication in circular DNA



Bidirectional replication in linear DNA





(iv) As the two strands separate, the bases are exposed to enzymes. An enzyme called RNA polymerase or primase initiates transcription of the strand(3'→5') and generates a 10-60 nucleotide long primer RNA(transcribed in 5'→3' direction).

2. Elongation:

(v) The free 3'-OH of this primer RNA provides the initiation point for the synthesis of new DNA strand. Deoxyribonucleotides are added to the 3'-OH group of the last ribonucleotide of the RNA primer. This is mediated by DNA polymerase III.

This enzyme requires the fee 3'-OH of a pre-existing polynucleotide for the initiation of DNA replication.

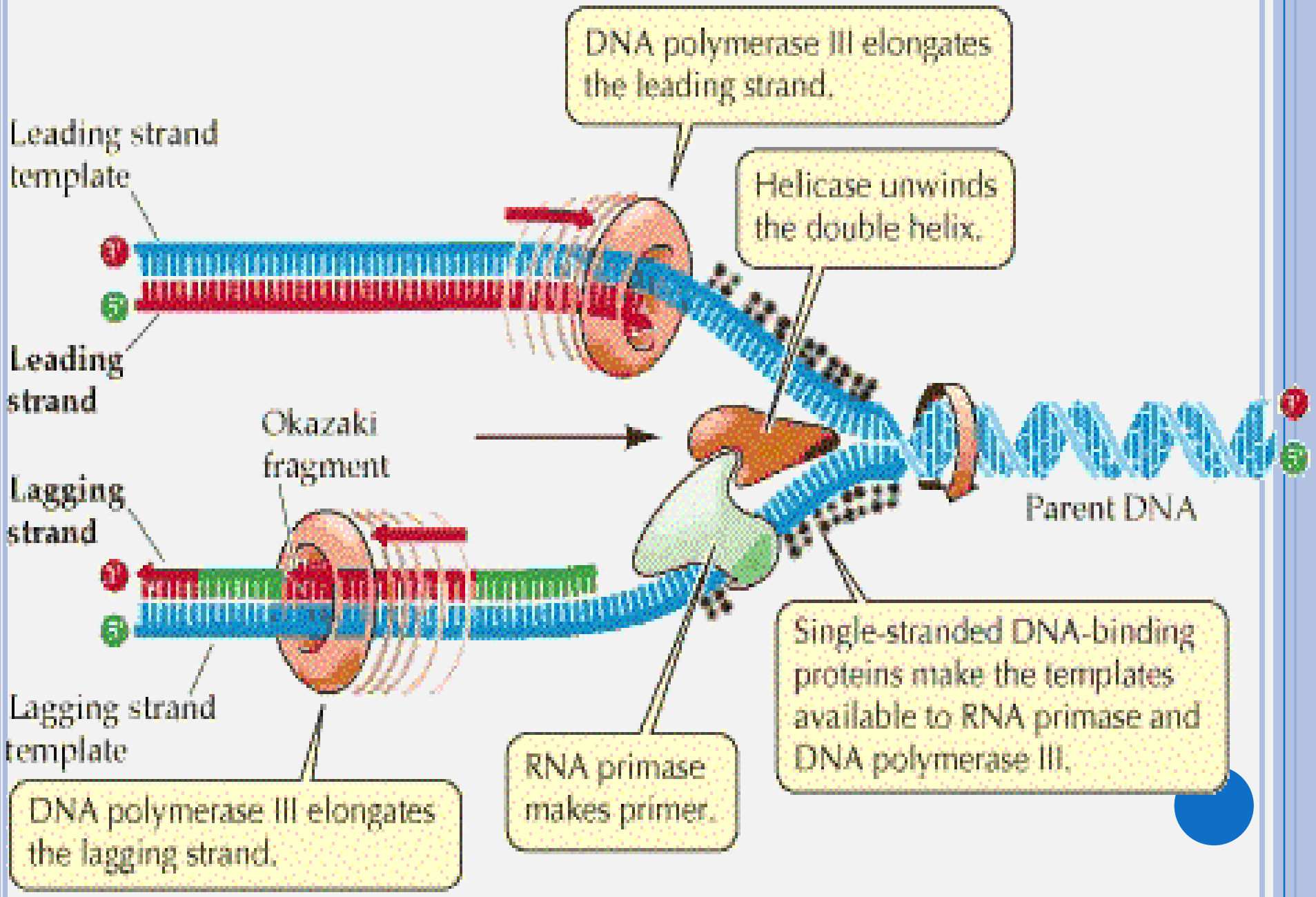
(vi) DNA polymerase progressively adds deoxyribonucleotides to the free 3'-OH of this growing polynucleotide chain. Consequently, the replication of 3'→5' strand of a DNA molecule proceeds continuously.

(vii) The replication of second strand(5'→3' strand) of the DNA molecule is discontinuous. It begins some what later than that of the 3'→5' strand. Therefore the 3'→5' strand of DNA molecule in known as the leading strand, while the 5'→3' strand is termed as the lagging strand.

(viii) The helicase enzyme progressively unwinds the duplex and the replication fork moves along like a bubbe.

(ix) When replication of the 3'→5' strand has progressed for sometime, primase initiates the synthesis of RNA primer on the 5'→3' strand close to the replication fork(away from the origin site). The primer synthesis begins close to the replication fork and progresses towards the origin. The 3'→OH of this primer RNA provides the initiation point for DNA polymerase to catalyse replication of the 'lagging strand'. Obviously, the replication of lagging strand proceeds from the replication fork towards the origin, i.e. its direction is opposite to that of the leading strand.

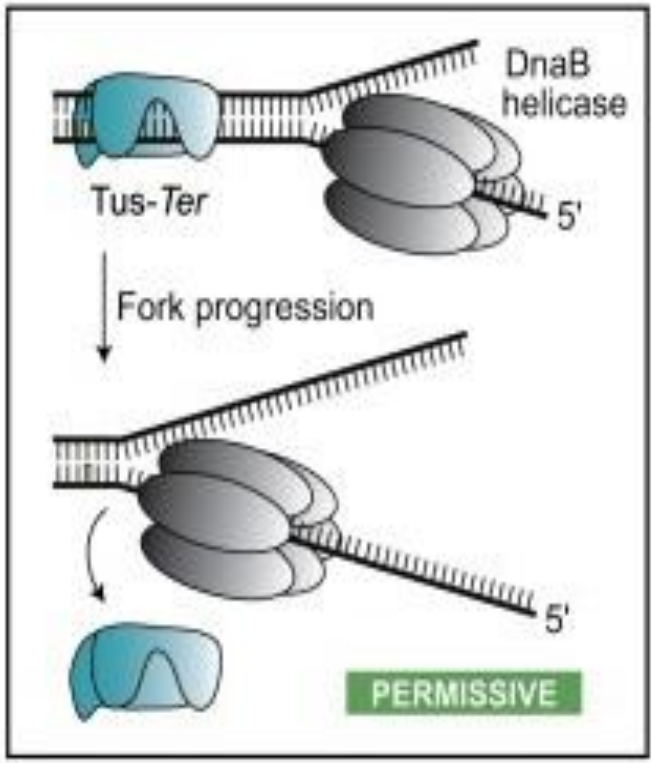
(x) The replication of the lagging strand(5'→3') generates small polynucleotide fragments called Okazaki fragments.

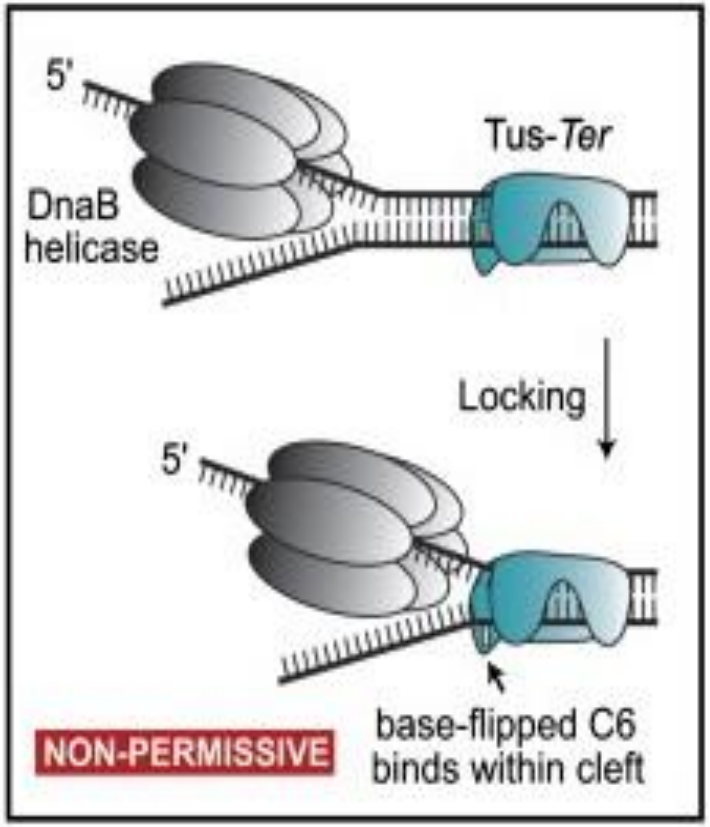

DNA polymerase III elongates the leading strand.
Leading strand template
Helicase unwinds the double helix.
Leading strand
Okazaki fragment
Lagging strand
Parent DNA
Single-stranded DNA-binding proteins make the templates available to RNA primase and DNA polymerase III.
Lagging strand template
RNA primase makes primer.
DNA polymerase III elongates the lagging strand.

(xi) The RNA primer associated with the newly synthesized okazaki fragments is digested by DNA polymerase 1 in prokaryotes. This enzyme also catalyzes the filling of gaps so generated in the new strands. The okazaki fragments are joined together by the enzyme Polynucleotide ligase to form a long polynucleotide chain.

3. Termination:

(xii) In E.coli, the termination is signalled by specific sequences called ter elements. They serve as binding site for protein Tus. The Tus protein binds to ter element and stops helicase enzyme from unwinding DNA. This stops the movement of the replication fork. The leading strand is replicated upto the ter elements. While the lagging strand replication is stopped 50-100 bp before the ter element.


DnaB
helicase
Tus-*Ter*
5'
Fork progression
5'
PERMISSIVE


5'
Tus-*Ter*
DnaB
helicase
Locking
5'
NON-PERMISSIVE
base-flipped C6
binds within cleft

Enzymes involved in DNA Replication:

(1) DNA Polymerase:

- DNA polymerase is the chief enzyme of DNA replication.
- Its activity was discovered by Kornberg in 1956.
- There are atleast three types of DNA polymerases in E.coli. They are: DNA polymerase I(Pol I), II(Pol II), III(Pol III).
- All the DNA polymerases require the following:

(i) A template DNA strand.

(ii) A short primer(either RNA or DNA), and

(iii) A free 3'-OH in the primer.

- They add one nucleotide at a time to the free 3'OH of the primer, and extend the primer chain in 5'→3' direction.

(A) DNA polymerase I:

- This enzyme was first purified by Kornberg in 1956.
- Hence it is also called Kornberg enzyme.
- This enzyme has three activities, which appear to be located in different parts of the molecule.

(i) A polymerase activity, which catalyses chain growth in the 5'→3' direction.

(ii) A 3'→5' exonuclease activity, which removes mismatched bases(DNA proof reading).

(iii) A 5’→3’ exonuclear activity, which degrades double-stranded DNA(excision repair). An exonuclease digests nucleic acids from one end(it does not cut DNA internally).

- DNA polymerase I is encoded by gene pol A, and has a single polypeptide chain.
- This can initiate DNA replication in vitro at a nick in a DNA duplex.

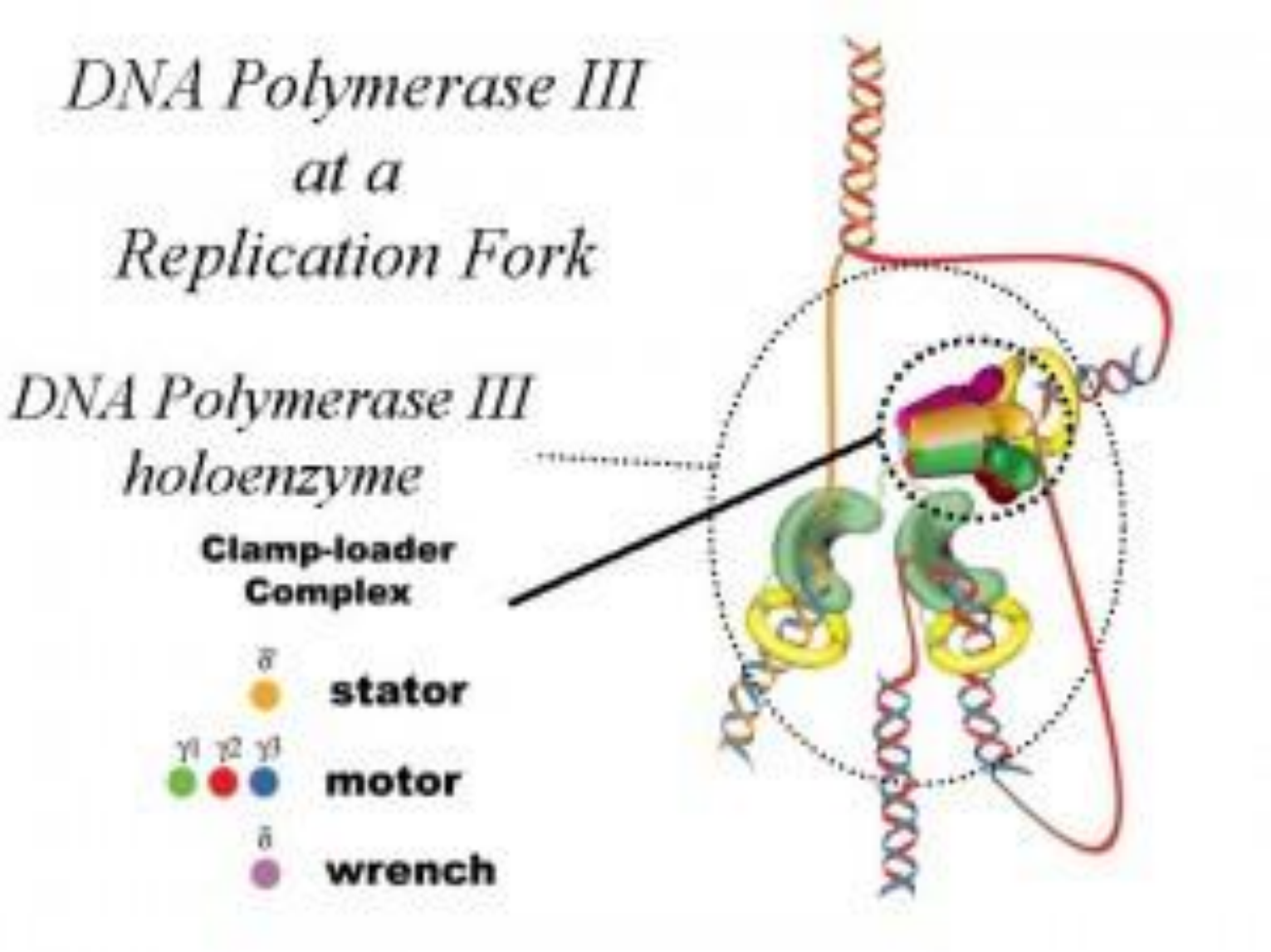
(B) DNA polymerase II:

- This enzyme repairs the damaged DNA.
- It has 5’→3’ polymerase and 3’→5’ exonuclease activities.

(C) DNA polymerase III:

- This enzyme is responsible for DNA replication in vivo.
- It has 5'→3' polymerase and 3'→5' exonuclease activities.
- It is composed of several sub units: α_2, θ_2, ε_2, r, x, ψ, δ, δ', T_2 and β_4 .
- Both leading and lagging strands are elongated by DNA polymerase III holoenzyme.
- This multi subunit complex is a dimer, one half synthesizing the leading strand and the other the lagging strand.

- Having two polymerases in a single complex ensures both strands are synthesized at the same rate.
- Both halves of the dinner contain a α sub unit, the actual polymerase and an ε sub unit which is a 3'→5' proof reading exonuclease.
- The β sub units clamp the polymerase to the DNA.
- The remaining subunits in each half are different and may allow the holoenzyme to synthesize short and long structures of DNA on the lagging and leading strands, respectively.


DNA Polymerase III
at a
Replication Fork
DNA Polymerase III
holoenzyme
Clamp-loader
Complex
δ'
stator
γ1 γ2 γ3
motor
δ
wrench

- Once the lagging strand have been elongated by DNA Polymere III, they are removed and the gaps are filled by DNA polymerase I.
- In vivo, the DNA polymerase III holoenzyme dimer, the primasome and the DNA helicases are believed to be physically associated in a large complex called a replisome which synthesises DNA at a rate of 900 bp per sec.
- The enzyme is assembled at the replication fork as follows:

(i) First, the γ-δ complex(subunits γ δ δ'χ ψ) and a pairs of subunit of β recognize the primed template and binds to it.

(ii) They now attach to the catalytic cored-(αθε subunits).

(iii) Subunit τ now joins the complex. It brings two more β subunits and another catalytic core to the complex . This generates a DNA polymerase III holoenzyme.

2. DNA helicases:

- These are ATP-dependent unwinding enzymes which promote separation of the two parental strands and establish replication fork.

3. DNA gyrases (Topiosomerases):

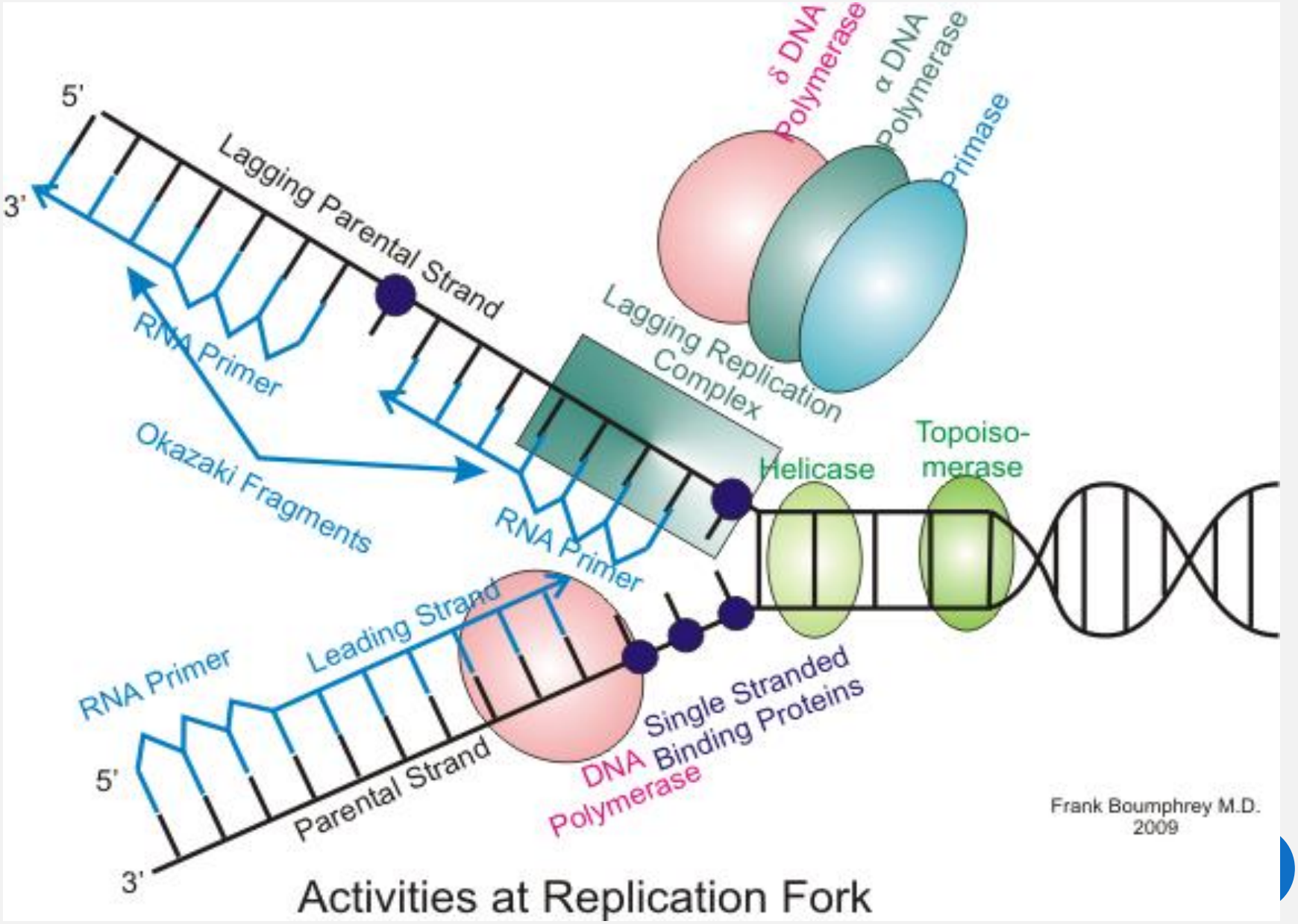
- The action of a helicase introduces a positive super coil into the duplex DNA, ahead of the replication fork.
- DNA gyrases relax the super coil by attaching to the transiently super coil duplex, nicking(cutting) one of the strands and rotating it through the unbroken strand.

DNA replication in Eukaryotes:

- The chromosomes in eukaryotes have much complex structure than that of prokaryotic chromosomes.

- The duplication of the chromosomes of eukaryotes involves not only the replication of their gaint DNA molecules, but also the synthesis of the associated histones and non-histone proteins.
- However, at the molecular level, the replication of DNA in eukaryote is quite similar to that of prokaryotes regardless of its complexity.
- It appears to involve the same chromosomes and mechanism as in prokaryotes.
- Replication, takes place due to the participation of a series of proteins like unwinding proteins, ss DNA binding proteins, topoisomerase, primase, DNA polymerase and ligase.

- Eukaryotic DNA replication is semi conservative and semi- discontinuous.
- Eukaryotic replication begins at different sites of origin.
- Replication takes place simultaneously at many sites along the entire length of chromosome.
- The DNA replicated in smaller units called replicons.
- The sizes of the replicons in eukaryotic chromosomes are generally between 15 and 100μm in length(50 to 300 kilobases).
- Each replicon has its own origin of replication from where the replicating forks move bidirectionally.



Activities at Replication Fork

- The replication fork travels away from each other until they meet a neighbouring fork.
- The chromosome of a yeast cell contains approximately 400 origins of replication scattered throughout their DNA.
- In higher organisms, each chromosome set has nearly 30,000 replication origin points and these are initiated during ‘S’ phase which lasts for several hours.
- Like prokaryotic DNA polymerases there are five different types of DNA polymerases which have been isolated from eukaryotic cells.

- They are designated as alpha(α), beta(β), gamma(γ), delta(δ) and epsilon(ε).
- Polymerase α is tightly associated with the primase, which initiates synthesis of primers at the 5' and of each Okazaki fragment as the polymerase primase complex moves along the lagging strand template.
- Polymerase β plays a role in DNA repair mechanism.
- Polymerase γ replicates mitochondrial DNA.
- Most of the fragments on the lagging strand are assembled by polymerase δ.
- Polymerase ε appears to play some role in replication of nuclear DNA.

- All the eukaryotic DNA polymerases elongate DNA strands in the 5'→3' direction by the addition of nucleotides to a 3'OH group.
- None of them is capable of initiation of a daughter DNA strand without a RNA primer.

Experimental evidence:

- In 1957, J.H.Taylor and P. Wood provided experimental evidence in support of semi conservative replication in eukaryotes by using auto radiography technique and light microscopy in dividing root tip cells of the bean, Vicia faba.

- They labelled V.faba chromosomes by growing root tips for 18 hours in medium containing radioactive 3H thymidine (tritiated).
- The root tips were then removed from the medium, washed and transferred to non-radioactive medium containing colchicine that arrests separation of metaphase chromosomes.
- The distribution of radio active DNA at the first and second metaphase were determined by auto radiography.

- It was found that in the first generation of duplication, both chromatids of chromosome were labelled.
- However, at the second metaphase, only one of the chromatids of each pair was radioactive.
- These results indicated that the replication of DNA in eukaryotes is semi conservative.

THANK YOU

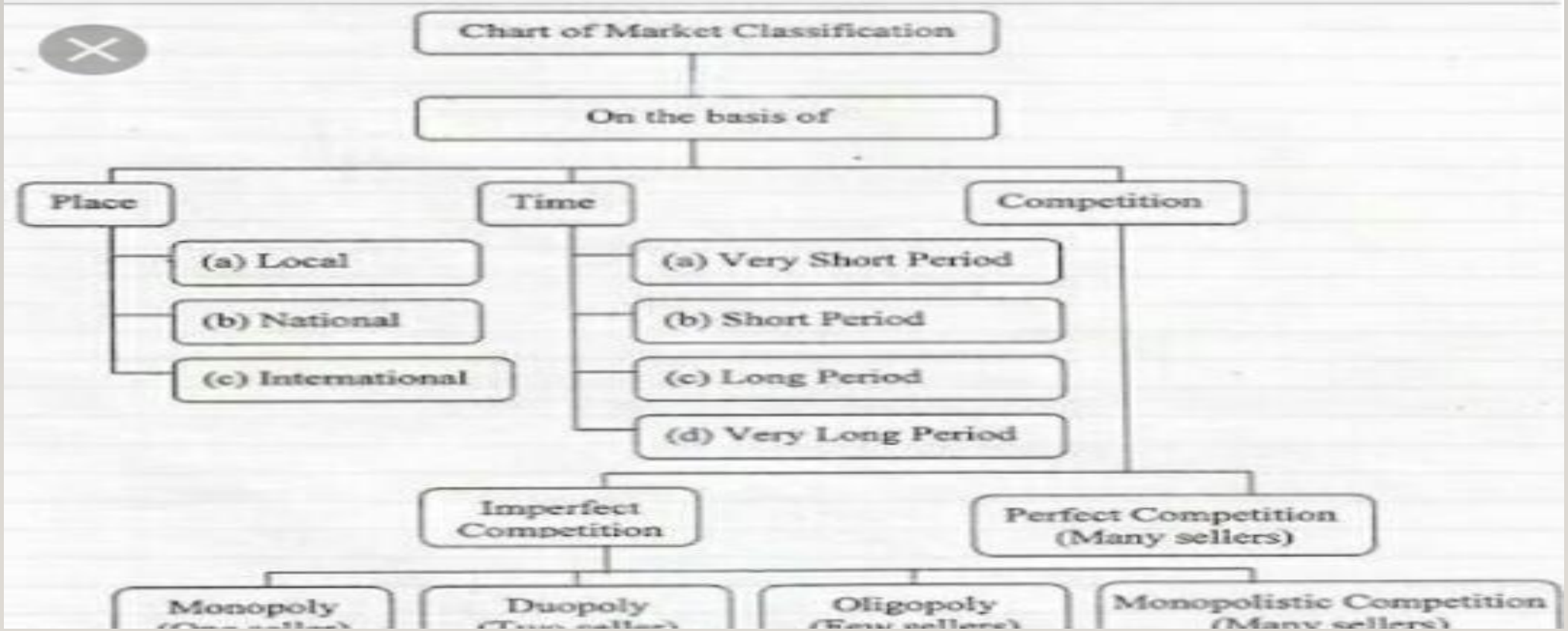
Classification of Market

INTRODUCTION :

MARKET :-

- Market is defined as a place or point at which buyers and sellers negotiate for exchange of well-defined products or services.
- Traditionally, market was referred to a public place in a village or town where provisions and other objects were brought for sale.
- Based on location, markets are classified as rural, urban, national or world markets.
- Market is said to exist wherever there is a potential for trade

CLASSIFICATION OF MARKET :



AREA BASED MARKET

One of the most common questions we get is, *what is placed-based marketing?* The simple answer is that it is marketing placed in a specific location. Unfortunately, that definition is circular and confusing. Our hope is that after reading this, you'll not only be able to answer the question, but understand how you can utilize place-based marketing to fit your needs.

TIME BASED MARKET

"**DEFINITION** of '**Market** Timing' **Market** timing is the act of moving in and out of the **market** or switching between asset classes **based** on using predictive methods such as technical indicators or economic data."

COMPETITION :

"**Competition**: Refers to the most important basis of **classification of market**. On the basis of **competition**, **markets** are **classified** as perfect **market** and imperfect **market**. In a perfect **market**, buyers and sellers are fully aware about the prices of products prevailing in the **market**."

Imperfect Competition

Perfect Competition

1.PARFECT COMPETITION COMPETITION

- Perfect competition is a model used as the starting point to explain how firms operate.
- It is a theoretical model based upon some very precise assumptions.
- It is very important, because once we understand the theoretical assumptions it makes it easy to move towards models of markets that are more realistic.

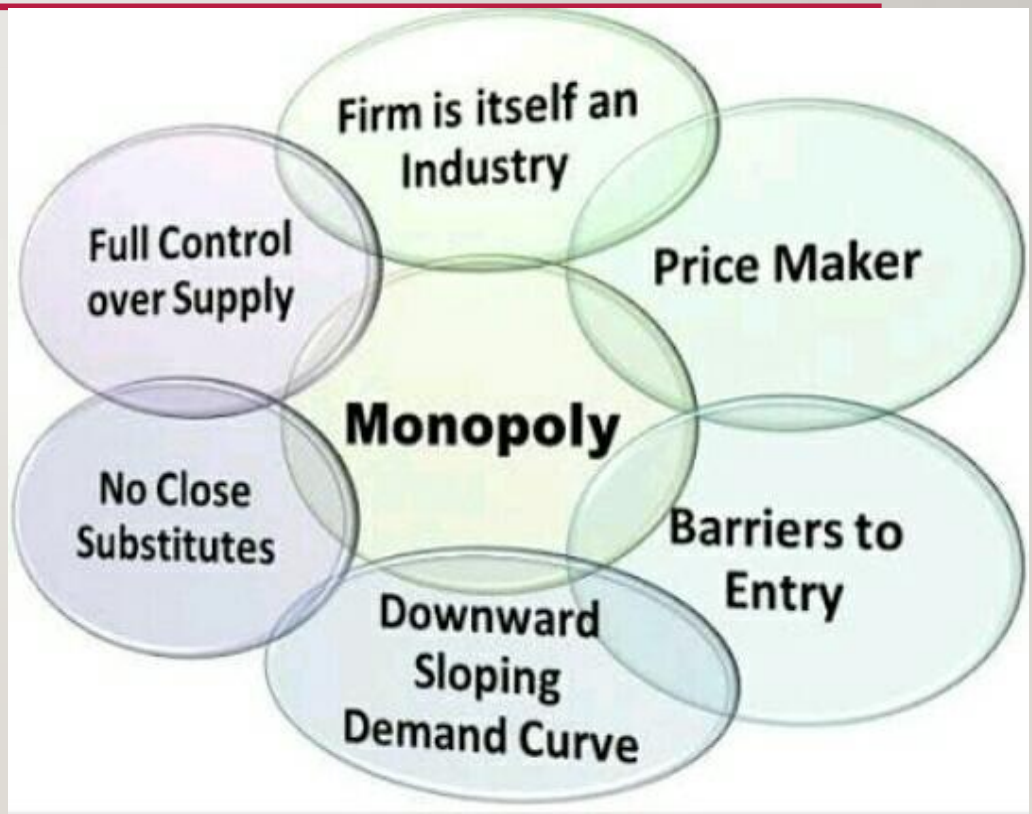
2.IMPERFECT

- Market structures that lack one of the conditions needed for perfect competition are examples of imperfect competition
  - **This means there are only a few sellers and/or products are not standardized**
    - Examples: corn and beef markets

MONOPOLY

- Monopoly is defined as that market form in which a single producer controls the whole supply of a single commodity which has no close substitute. Three points should be noted in regard to this definition.

1. There must be a single producer
2. There must be only one commodity produced by the firm for which there is no substitute.
3. There are strong barriers for the firms to enter into the industry.



DUOPOLY

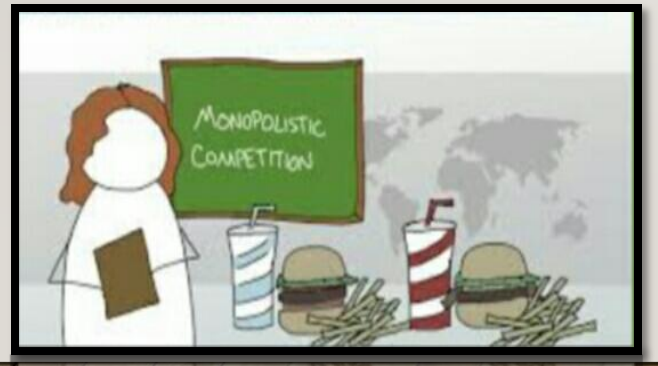
- Has the following characteristics
  - Market is dominated by two large producers
  - Have considerable influence on price
  - Produce differentiated products, with the use of non-price competition
  - Strong barriers to entry of new firms

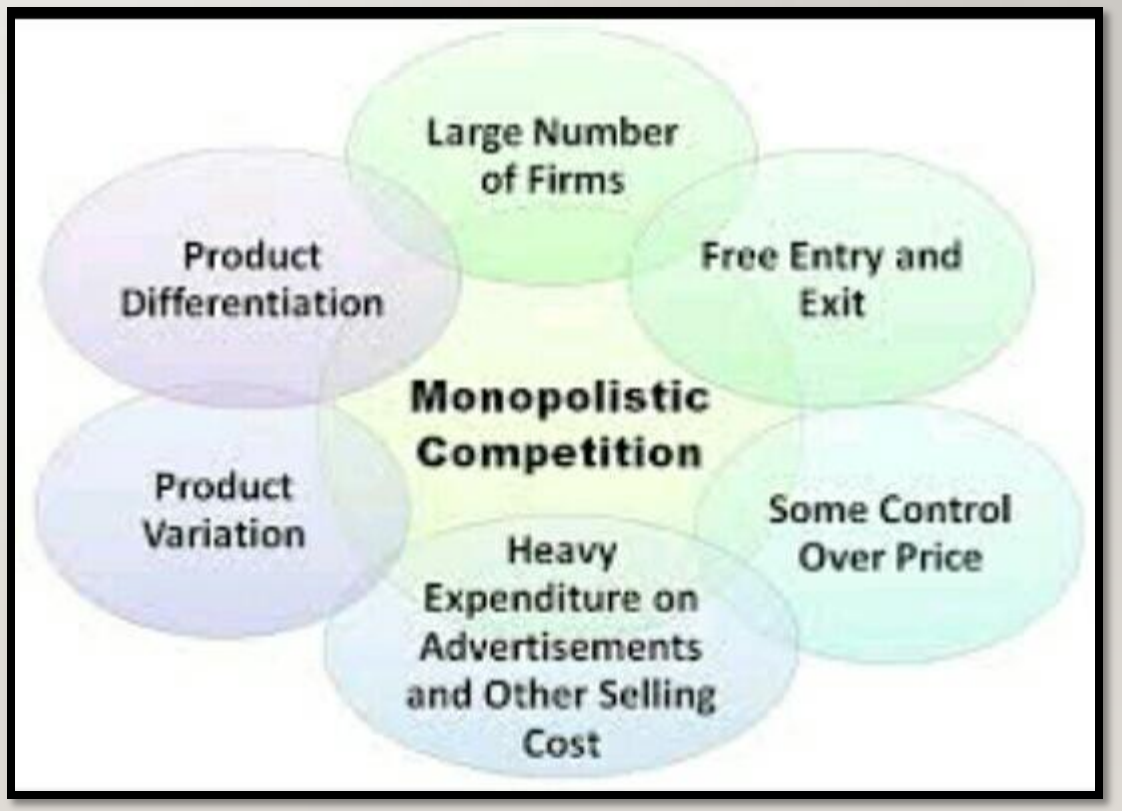
OLIGOPOLY

- Oligopoly is best defined by the **market conduct** (behaviour) of firms
- A market **dominated by a few large firms** I.e. "Competition amongst the few"
- High level of **market concentration**
  - Concentration ratio is the market share of the leading firms
- Each firm tends to produce **branded / differentiated products**

MONOPOLISTIC COMPETITION

"**Monopolistic competition** is a type of imperfect **competition** such that many producers sell products that are differentiated from one another (e.g. by branding or quality) and hence are not perfect substitutes."





NATIONAL INCOME AND IT'S CONCEPTS

GNP,GDP,GVA,NNP,PERSONAL INCOME,PERCAPITA INCOME,DISPOSABLE INCOME.

NATIONAL INCOME

- *The total income of the nation is called National Income.*
- *National income is the flow of goods and services produced in an economy in a particular period that is one year..*

CONCEPTS OF NATIONAL INCOME

- GNP(Gross National Product)

GNP defined as total market value of all the final goods and services produced in an year

. $GNP=C+I+G+(X-M)$

CONDITIONS

1. *Only value of the final goods and services is to be considered*
2. *Pure monitory or financial transactions which are non-productive in nature should be taken into account.*
3. *Services rendered Free of cost are not to be included.*
4. *Profits earned or losses sustain as a result of fluctuations in the market prices of a fixed assets are not to be included.*

- *NNP(Net National Product)*

The net money value at current prices of all goods and services produced during a year in a country.

- *Depriciation:The decrease in the value of the capital goods used in production is known as depriciation.*
- *NI known as National income at market prices.*

NNP=GNP-Depriciation

- *GDP(Gross Domestic Product)*

The value of output produced in a country during a year from its own national resources.

GDP=C+I+G

- *NDP(Net Domestic Product).*
- *The value of net output produced in a country during a year from its own national resources*

. *NDP=NNP-Net income from abroad.*

- *NNI at factor cost.*

The sum total of the remunerations paid to or the total of the income received by the factors of production is known as NI at factor cost.

NI at factor cost tells the value of the production in terms of costs.

- *NI at factor cost=NI at market prices- indirect tax.*
- *Sometimes the market prices of a commodity may also be less than its cost of production.ex subsidy case.*

NI at factor cost=NI at market price-indirect tax+subsidy+ undistributed profits.

- *PERSONAL INCOME.*

It is the sum total of the income actually received by all the individuals or households of a country during an year.

Personal income is less than NI.

- *ALL the incomes earned by them may not actually received by them.*

PI=NNI – Social security contribution – CT – Undistributed profits + TP

- *DISPOSABLE INCOME.*

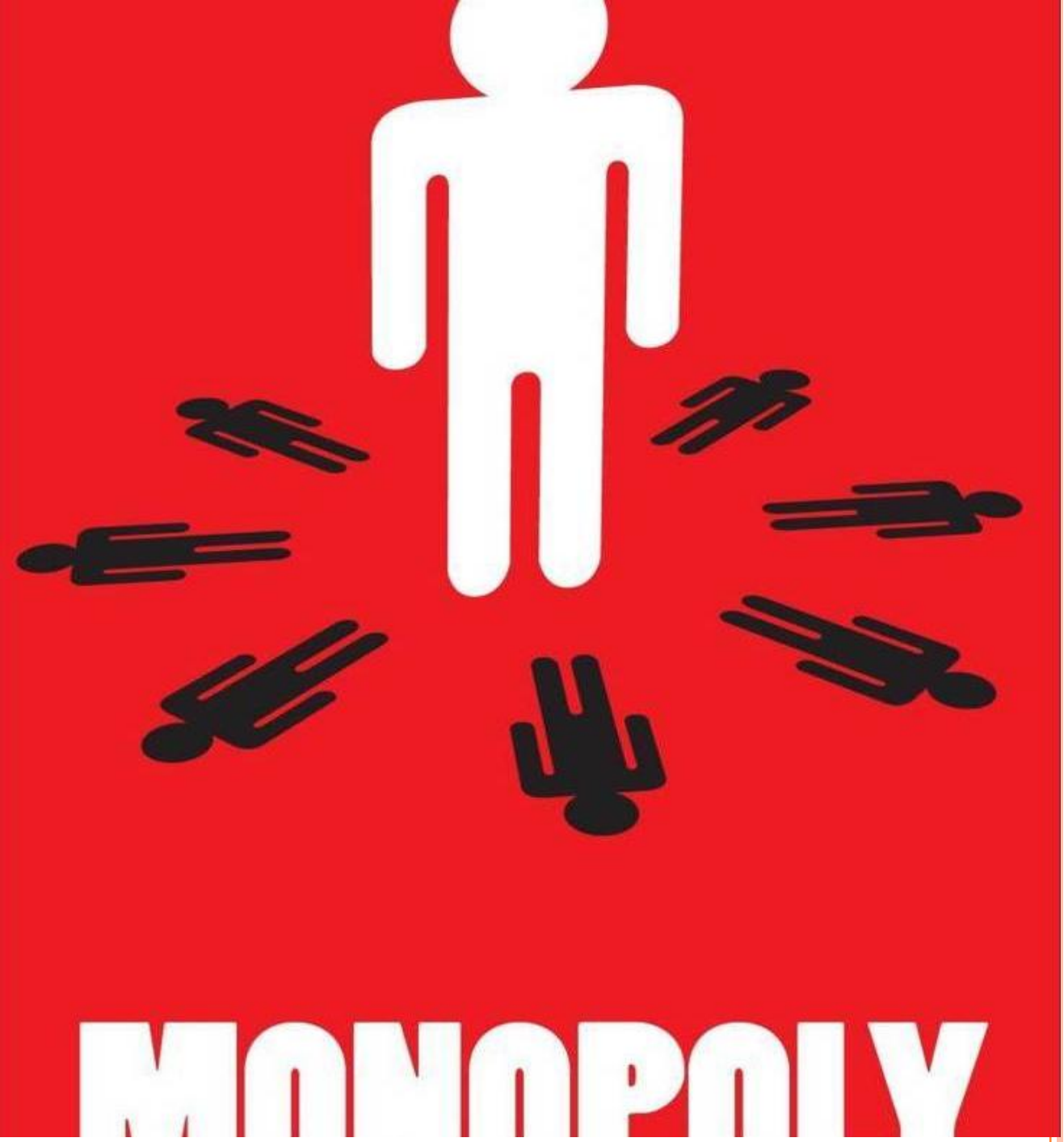
DI is the net income which the people can actually spend as they like.It is less than the PI because of Taxes paid to the governmet.

DI= PI – PERSONAL TAXES

GROSS VALUE ADDED.

The difference between the value of material outputs and inputs at each state of production is value added. If all such differences are added up for all industries in the economy we arrive at GDP

Monopoly



MONOPOLY

- Monopoly exist when a specific person or an enterprise is the only supplier of an particular commodity..!!

Monopoly meaning

- Monopoly is a well-defined market structure where there is only one seller who controls the entire market supply...!!!
- This sole seller in the market is called monopolist..!!!


Firm is itself an Industry
Full Control over Supply
Price Maker
Monopoly
No Close Substitutes
Barriers to Entry
Downward Sloping Demand Curve

MONOPOLY FEATURES

Features

- Monopoly firm itself being the industry
- A monopolist is a price-market and not a price-taker
- Monopolist has a complete control over the market supply
- Only single firm
- Monopoly is a complete negation competition

Monopoly types

Types

- Pure monopoly and imperfect monopoly
- Legal ,natural ,technological and joint monopolies
- Simple monopoly and discrimination monopoly
- Private monopoly and public monopoly

Economics

Concept of Multiplier

John Maynard Keynes, 1919 and 1945.

CONCEPT OF MULTIPLIER

THE AGGREGATE DEMAND IS COMPOSED OF :

1. CONSUMPTION DEMAND

2. INVESTMENT DEMAND

FROM THE CONCEPT OF MULTIPLIER IT IS KNOWN HOW MUCH OR HOW MANY TIMES INCOME INCREASES AS INVESTMENT IS DONE.

- *AS INVESTMENT INCREASES NATIONAL INCOME INCREASES PROPORTIONATELY MUCH MORE.*
- *HOW MANY TIMES IT INCREASES DEPENDS ON MPC.*
- *HIGHER THE MPC THE NATIONAL INCOME WILL BE GREATER DUE TO INVESTMENT.*

MULTIPLIER

CHANGE IN INVESTMENT LEADS TO CHANGE IN NATIONAL INCOME.

- CHANGE IN NATIONAL INCOME IS A MULTIPLE OF CHANGE IN INVESTMENT.
- THIS MULTIPLE IS TERMED AS MULTIPLIER OR INVESTMENT MULTIPLIER.
- INVESTMENT MULTIPLIER IS A MEASURE OF CHANGE IN NATIONAL INCOME AS A RESULT OF CHANGE IN INVESTMENT.

ASSUMPTIONS OF MULTIPLIER

- THERE IS AUTONOMOUS INVESTMENT IN ECONOMY.
- MARGINAL PROPENSITY TO CONSUME REMAINS CONSTANT.
- CONSUMPTION IS THE FUNCTION OF CURRENT INCOME.
- NO TIME LAG BETWEEN RECIEPT OF INCOME AND ITS DISPOSAL IN FORM OF CONSUMPTION.
- NET INCREASE IN INVESTMENT .
- SUPPLY OF CONSUMER GOODS IS ALWAYS IN ECONOMY.

FEATURES OF MULTIPLIER

- IT IS ASSOCIATED WITH CHANGE IN INVESTMENT.
- SIZE OF MULTIPLIER DEPENDS UPON SIZE OF MPC.
- MULTIPLIER WORKS IN BOTH FORWARD AND BACKWARD DIRECTION.
- VALUE OF MULTIPLIER VARIES FROM UNITY TO INFINITY.

SIZE OF MULTIPLIER

- HIGHER THE MPC –LARGER THE MULTIPLIER SIZE.
- LARGEST POSSIBLE MPC IS UNITY.
- IF MPC IS ZERO MULTIPLIER IS UNITY.

K=1/1-MPC THAT IS RECIPROCAL OF MARGINAL PROPENSITY TO SAVE.

USES OF MULTIPLIER

- TOOL OF ANALYSYING GROWTH, PLANNING, PROJECTING, INVESTMENT REQUIREMENT.
- TOOL FOR ACHIEVING TARGETED GROWTH RATE, IF MPC IS GIVEN.
- TOOL FOR ANALYSING THE FLUCTUATIONS IN THE ECONOMY.
- IMPORTANT TOOL FOR ANALYSING IMPACT OF TAXATION, FOREIGN TRADE ON THE ECONOMY.

LIMITATIONS OF A MULTIPLIER

- MULTIPLIER DEPENDS ON A LARGE NUMBER OF FACTORS ALONG WITH MPC.
- EFFICIENCY OF PRODUCTION.
- REGULAR INVESTMENT.
- MULTIPLIER PERIOD.
- FULL EMPLOYMENT CEILING.
- ASSUMPTION THAT GOODS AND SERVICES ARE AVAILABLE IN ADEQUATE SUPPLY.
- GOODS AND SERVICES CANNOT BE PRODUCED IN EXCESS OF THEIR FULL EMPLOYMENT LEVEL.

IMPORTANCE OF MULTIPLIER

- USEFUL TO ANALYZE PUBLIC INVESTMENT.
- REMOVES DEPRESSION THROUGH GOVERNMENT INVESTMENT.
- ACHIEVING FULL EMPLOYMENT.
- MARGINAL EFFICIENCY OF CAPITAL EMPLOYMENT RISES.
- PRIVATE INVESTMENT ENCOURAGED.

Thank you

OLIGOPOLY

- MARKET

Definition: The **Oligopoly Market** characterized by few sellers, selling the homogeneous or differentiated products. In other words, the Oligopoly market structure lies between the pure monopoly and monopolistic competition, where few sellers dominate the market and have control over the price of the product.

Under the Oligopoly market, a firm either produces:

- **Homogeneous product:** The firms producing the homogeneous products are called as Pure or Perfect Oligopoly. It is found in the producers of industrial products such as aluminum, copper, steel, zinc, iron, etc.

- **Heterogeneous Product:** The firms producing the heterogeneous products are called as Imperfect or Differentiated Oligopoly. Such type of Oligopoly is found in the producers of consumer goods such as automobiles, soaps, detergents, television, refrigerators, etc.

Features of Oligopoly Market

- **Few Sellers:** Under the Oligopoly market, the sellers are few, and the customers are many. Few firms dominating the market enjoys a considerable control over the price of the product.
- **Interdependence:** it is one of the most important features of an Oligopoly market, wherein, the seller has to be cautious with respect to any action taken by the competing firms. Since there are few sellers in the market, if any firm makes the change in the price or promotional scheme, all other firms in the industry have to comply with it, to remain in the competition.

- **Advertising:** Under Oligopoly market, every firm advertises their products on a frequent basis, with the intention to reach more and more customers and increase their customer base.This is due to the advertising that makes the competition intense.If any firm does a lot of advertisement while the other remained silent, then he will observe that his customers are going to that firm who is continuously promoting its product. Thus, in order to be in the race, each firm spends lots of money on advertisement activities.
- **Competition:** It is genuine that with a few players in the market, there will be an intense competition among the sellers. Any move taken by the firm will have a considerable impact on its rivals. Thus, every seller keeps an eye over its rival and be ready with the counterattack

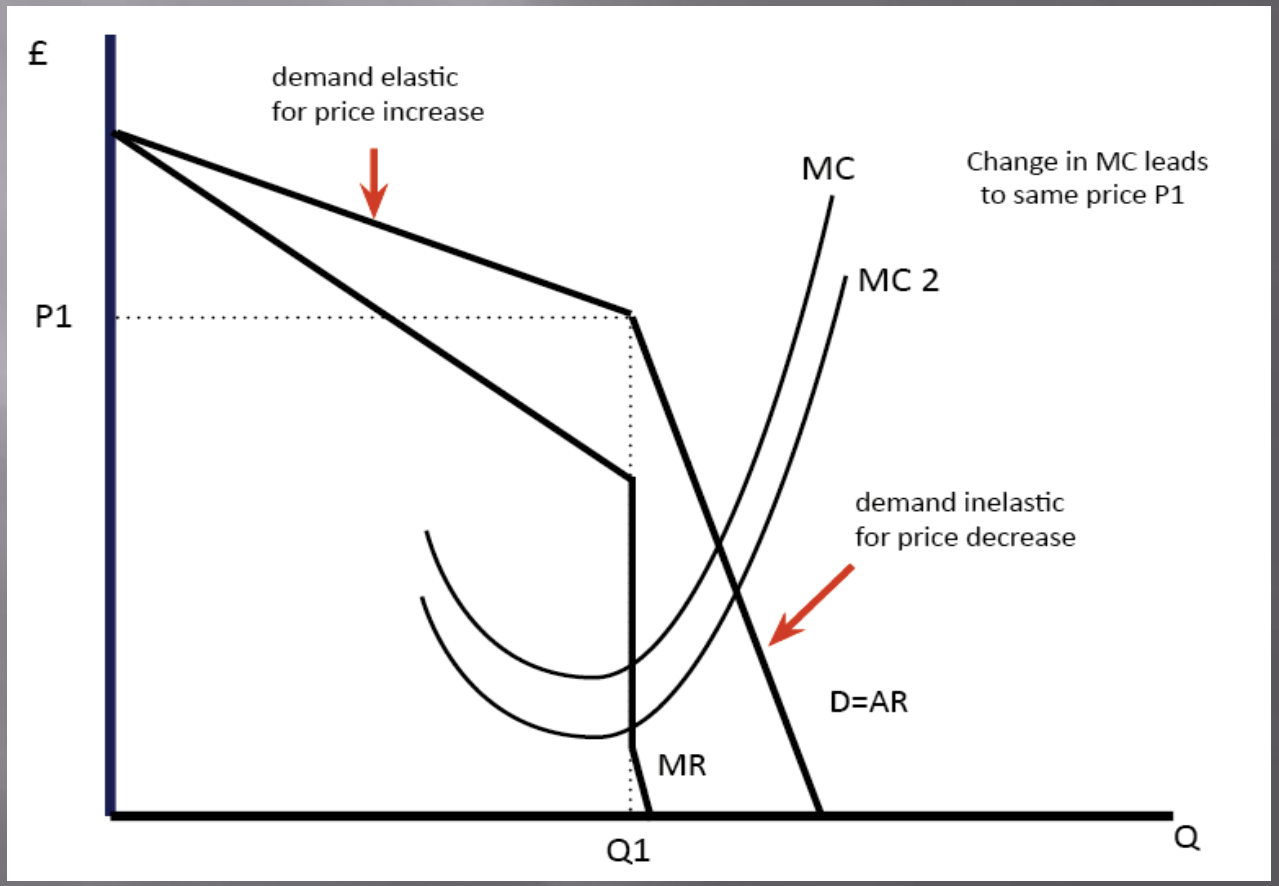
- **Entry and Exit Barriers:** The firms can easily exit the industry whenever it wants, but has to face certain barriers to entering into it. These barriers could be Government license, Patent, large firm's economies of scale, high capital requirement, complex technology, etc. Also, sometimes the government regulations favor the existing large firms, thereby acting as a barrier for the new entrants
- **Lack of Uniformity:** There is a lack of uniformity among the firms in terms of their size, some are big, and some are small.

Since there are less number of firms, any action taken by one firm has a considerable effect on the other. Thus, every firm must keep a close eye on its counterpart and plan the promotional activities accordingly.

OLIGOPOLY DIAGRAM

There are different diagrams that you can use to explain Oligopoly markets. It is important to bear in mind, there are different possible ways that firms in Oligopoly can behave.

Kinked Demand Curve Diagram



The firm maximises profits at Q1, P1 where MR=MC. Thus a change in MC, may not change the market price.

The kinked demand curve makes certain assumptions

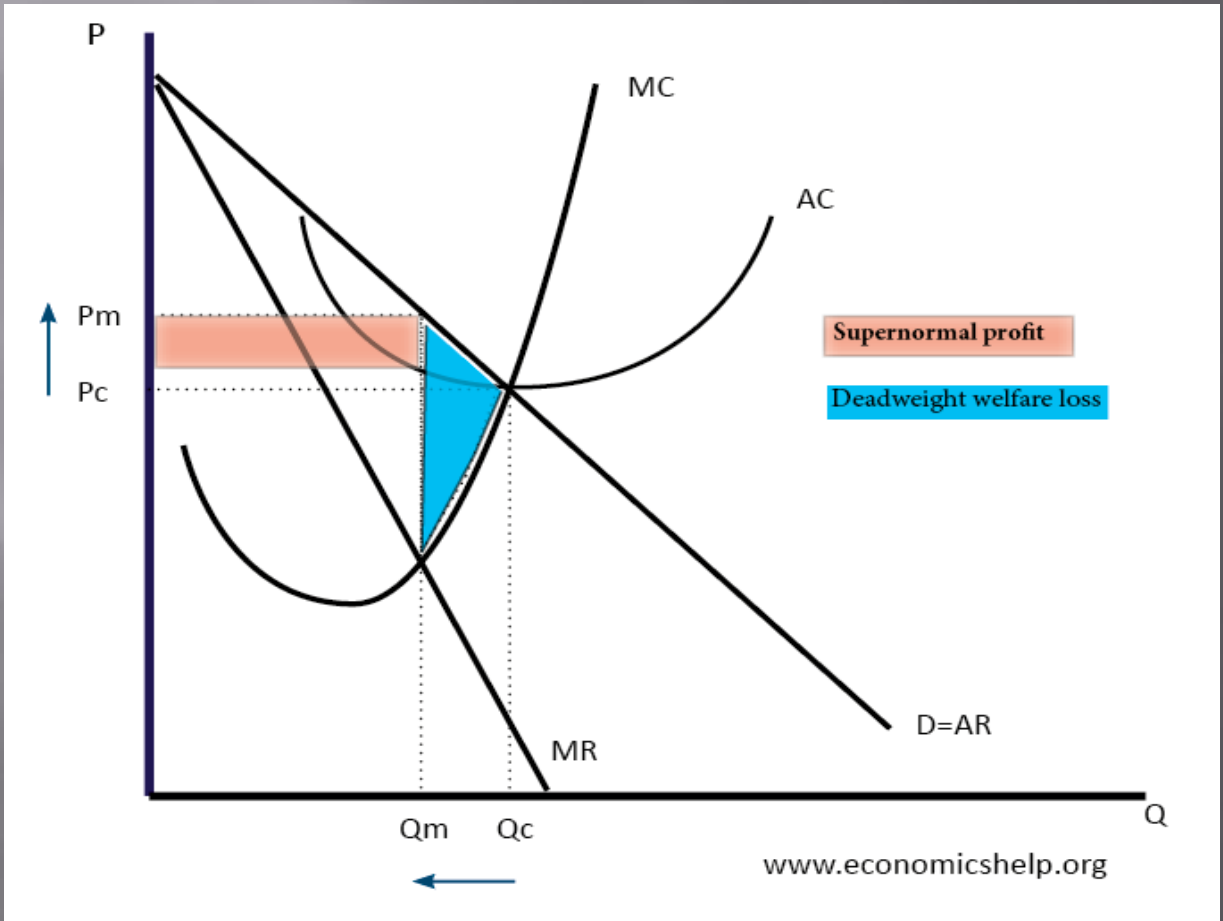
- Firms are profit maximisers.
- If one firm increases the price, other firms won't follow suit. Therefore, for a price increase, demand is price elastic.
- If one firm cuts price, other firms will follow suit because they don't want to lose market share. Therefore, for a price cut, demand is price inelastic.
- This is how we get the 'kinked demand curve

However, the kinked demand curve has some limitations.

- It doesn't explain how the price was arrived at in the first place.
- Firms may engage in price competition

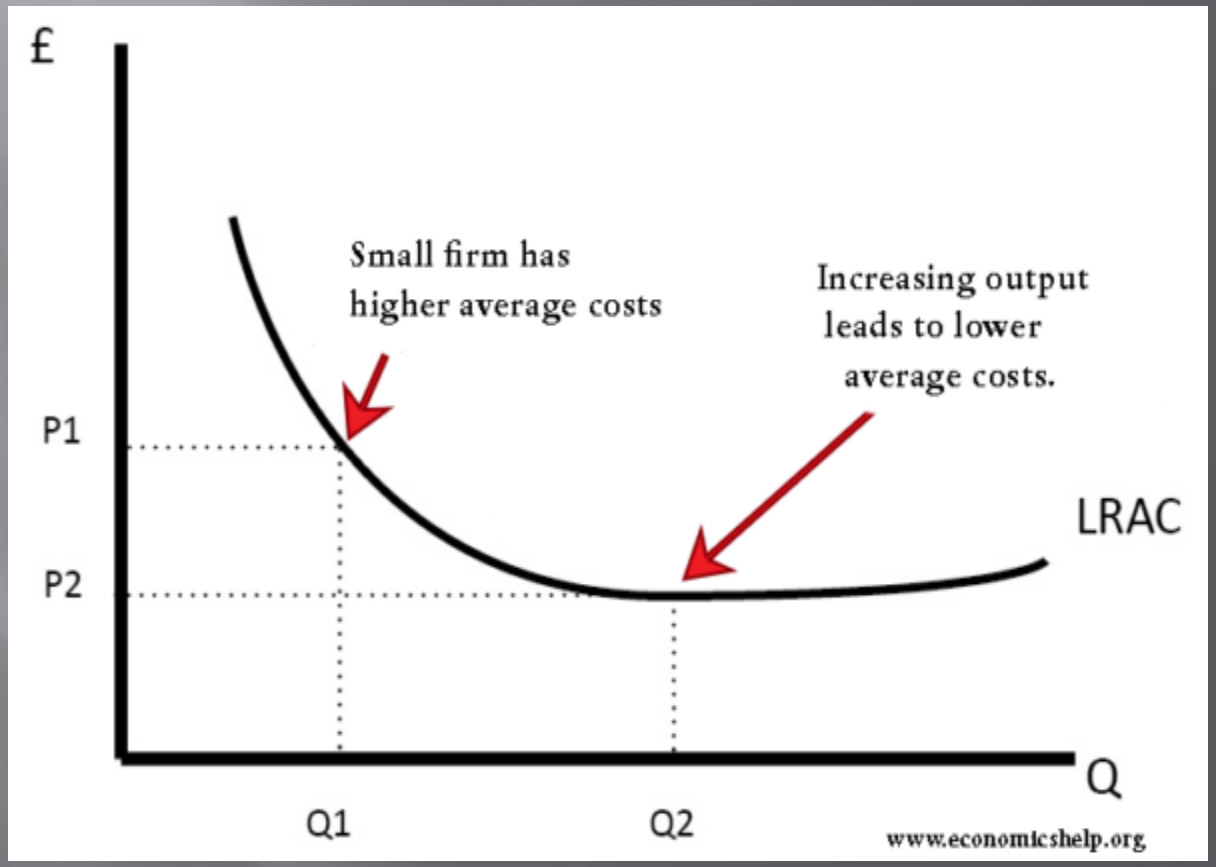
Collusive Oligopoly

If firms in oligopoly collude and form a cartel, then they will try and fix the price at the level which maximises profits for the industry. They will then set quotas to keep output at the profit maximising level.



The price and output in oligopoly will reflect the price and output of a monopoly. The Quantity Qm will be split between the firms in the cartel.

Economies of scale for Oligopolies



Oligopolies may benefit from economies of scale. This enables lower average costs with increased output. FIrms in oligopoly producing at Q1 achieve lower prices of AC1.

EFFICIENCY OF FIRMS IN OLIGOPOLY

- Larger firms can benefit from economies of scale – lower average costs – which might outweigh other inefficiencies.
- Allocative efficiency? Not clear but firms operating under kinked demand curve may end up setting price higher than marginal cost. Also firms able to successfully collude will set prices higher than MC. If oliogopolies are competitive then prices will be lower and more allocative efficient
- Dynamic efficiency? Firms in oligopoly have profits they can use for investment in new products. Also, competitive pressures encourage them to innovate.

CONCLUSION

- Oligopolies can end up looking like monopolies or like competitive markets, depending on the number of firms and how cooperative they are.
- The prisoners' dilemma shows how difficult it is for firms to maintain cooperation, even when doing so is in their best interest.
- Policymakers use the antitrust laws to regulate oligopolists' behavior. The proper scope of these laws is the subject of ongoing controversy.

Lecture 4: Enhanced Entity-Relationship Modeling

- Semantic Data Modeling
- Superclass/Subclass Relationship
- Specialization/Generalization
- Type Inheritance/Constraints/Updates
- Categorization
- Higher-Degree Relationships
- Knowledge Representation
- Aggregation

Enhanced E-R Modeling

- Semantic Data Modeling
  - More complex requirements (class relationship)
  - Requires more complex data modeling (subclass, superclass, inheritance, category)
  - Enhance (extend) the simple E-R model
- Enhanced Entity Relationship (EER) Model
  - E-R model + semantic data modeling concepts
  - EER Diagram

EER Model Concepts

- Subclasses
  - Subgroupings of the entities of an entity type
  - An entity type as a superclass of subclasses
  - Examples
    - EMPLOYEE ⇒ {SALARIED_EMPLOYEE, HOURLY_EMPLOYEE}
    - PATIENT ⇒ {OUTPATIENT, INPATIENT}
    - STUDENT ⇒ {FULL-TIME, PART-TIME}

Superclass/Subclass

- "IS-A" relationship
  - Members of a subclass must be members of the superclass
    - a FULL-TIME STUDENT <u>is a</u> STUDENT
  - Not every entity in a superclass be a member of a subclass
- Subclasses
  - Should have meaningful subgroupings
  - Should be related to database applications

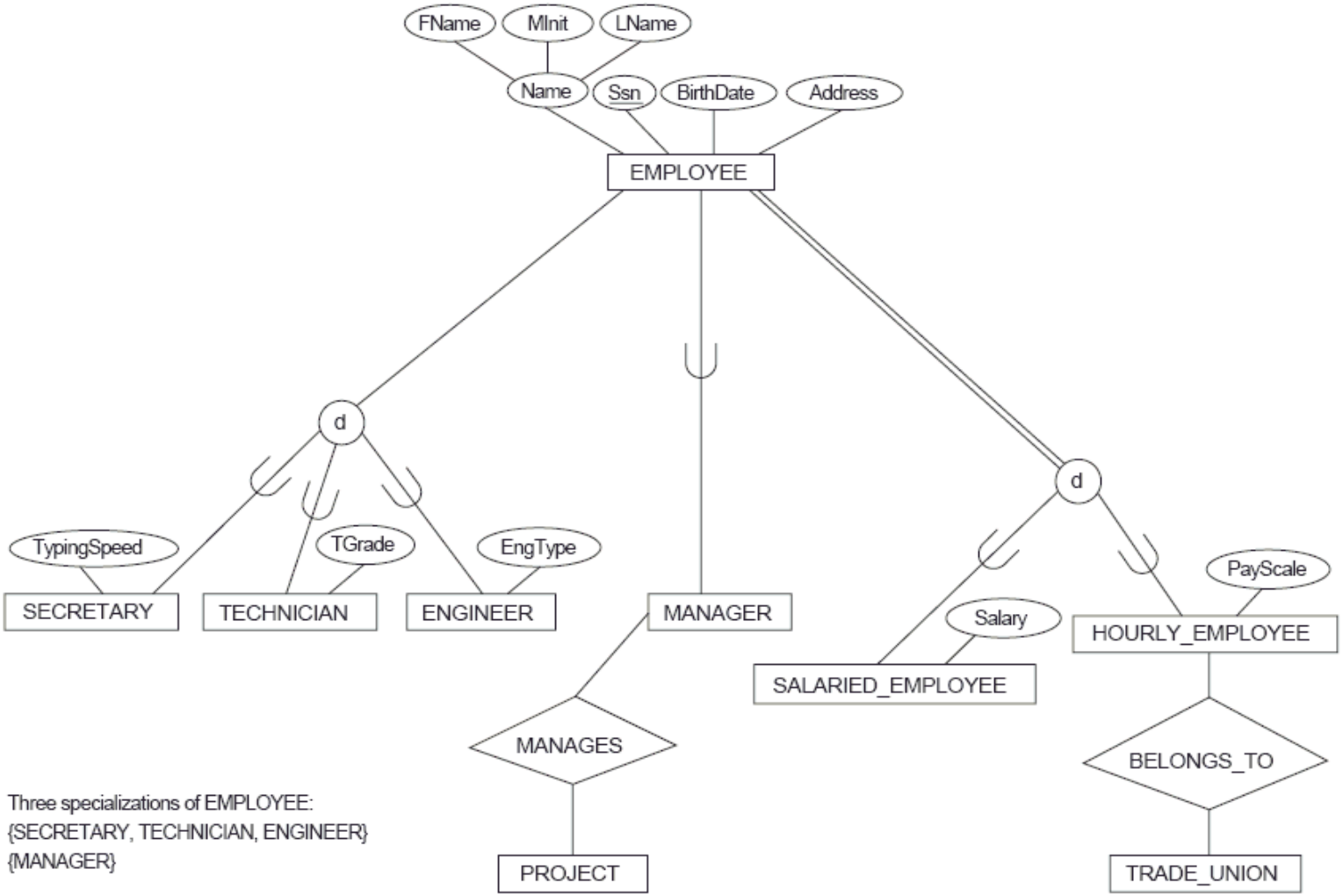
Entity Type Inheritance

- Type Inheritance among Classes
- Inherited Attributes
  - a subclass inherits the attributes of the superclass
  - HOURLY_EMPLOYEE (Name, SSN, Address)
- Local (Specific) Attributes
  - a subclass may have its own attributes
  - HOURLY_EMPLOYEE (Hourly_rate)
  - SALARIED_EMPLOYEE (Annual_salary)

Relationship Type Inheritance

- Inherited Relationships
  - A subclass participates in the relationship type(s) of the superclass indirectly
  - cared_by (INPATIENT, PHYSICIAN)
- Local (Specific) Relationships
  - A subclass may have its own direct relationships
  - assigned_to (INPATIENT, BED)

Figure 4.1 EER diagram notation for representing specialization and subclasses.



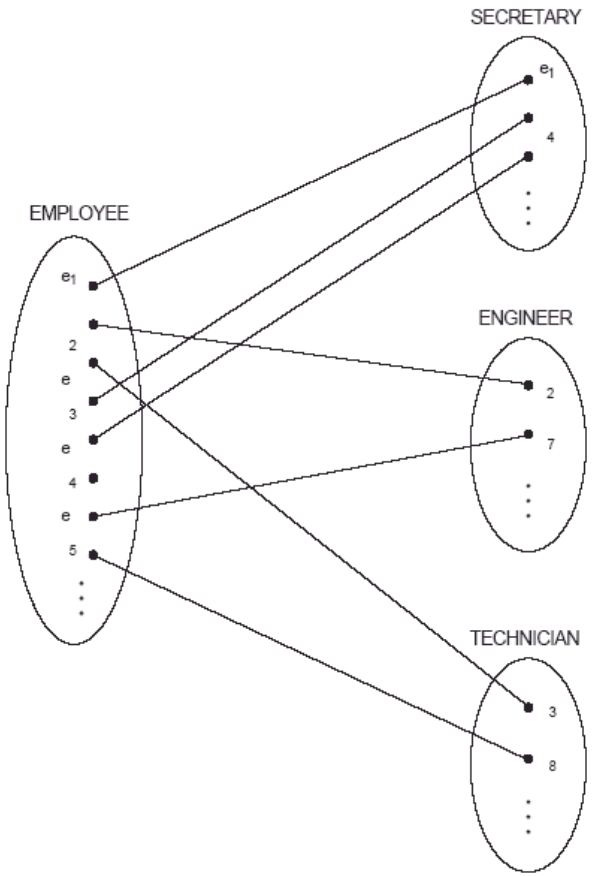
Three specializations of EMPLOYEE:
{SECRETARY, TECHNICIAN, ENGINEER}
{MANAGER}

Specialization and Generalization

- Specialization
  - Process of defining subclasses of an entity type (superclass
  - Defined on the basis of some distinguishing characteristics of the entities in the superclass.
  - A (super)class ⇒ a set of subclasses
    - Method of pay {SALARIED_EMPLOYEE, HOURLY_EMPLOYEE}
    - Job type {SECRETARY, ENGINEER, TECHICIAN}
  - Why specialization
    - Attributes applied to some but not all entities
    - Only members of the subclass participate in a specific relationship type

Specialization and Generalization

Figure 4.2 Some instances of the specialization of EMPLOYEE into the {SECRETARY, ENGINEER, TECHNICIAN] set of subclasses.

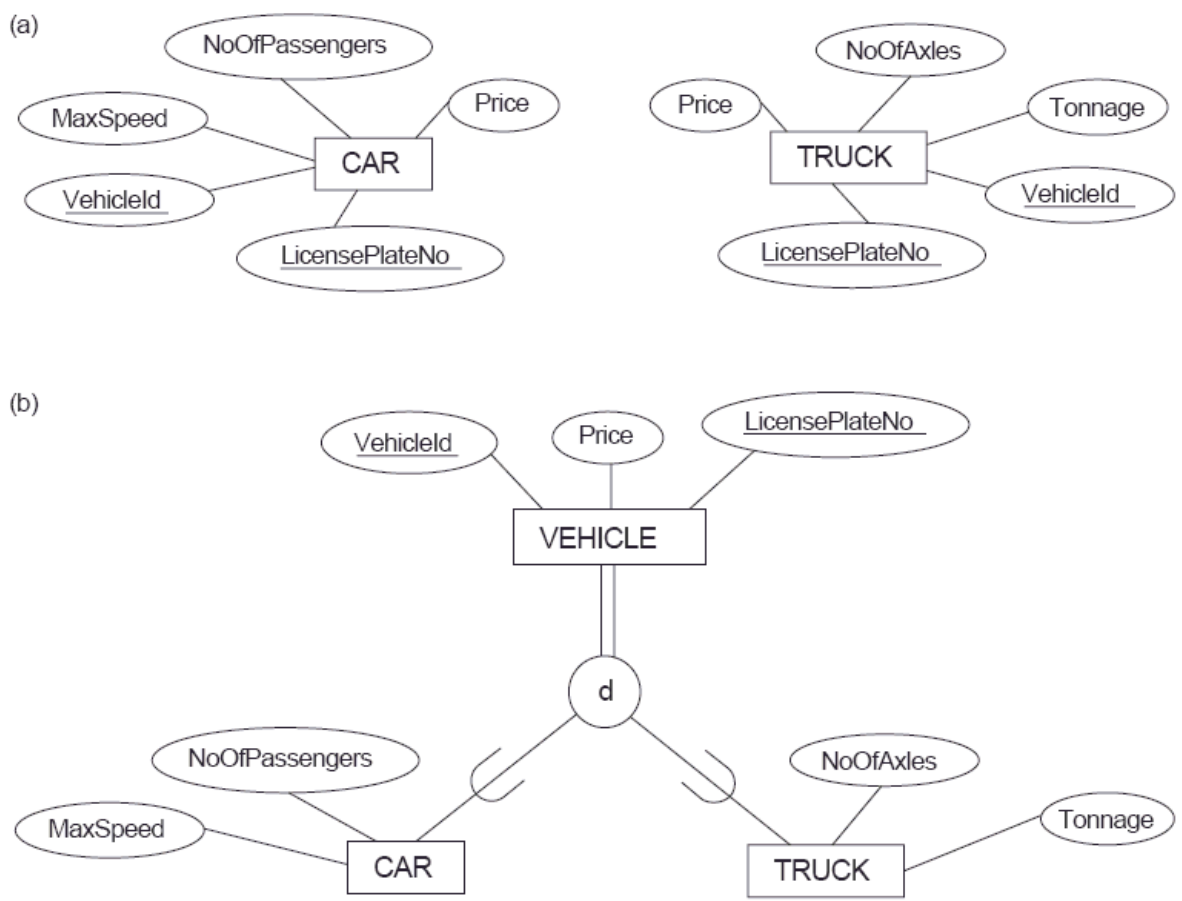


Specialization and Generalization

- Generalization
  - Process of defining generalized entity type from giving entity types by identifying common features (attributes)
  - A set of (sub)classes ⇒ a generalized superclass
  - Reverse process of abstraction
  - {CAR, TRUCK} ⇒ VEHICLE : fig4.3

Specialization and Generalization

Figure 4.3 Examples of generalization. (a) Two entity types CAR and TRUCK. (b) Generalizing car and TRUCK into VEHICLE.

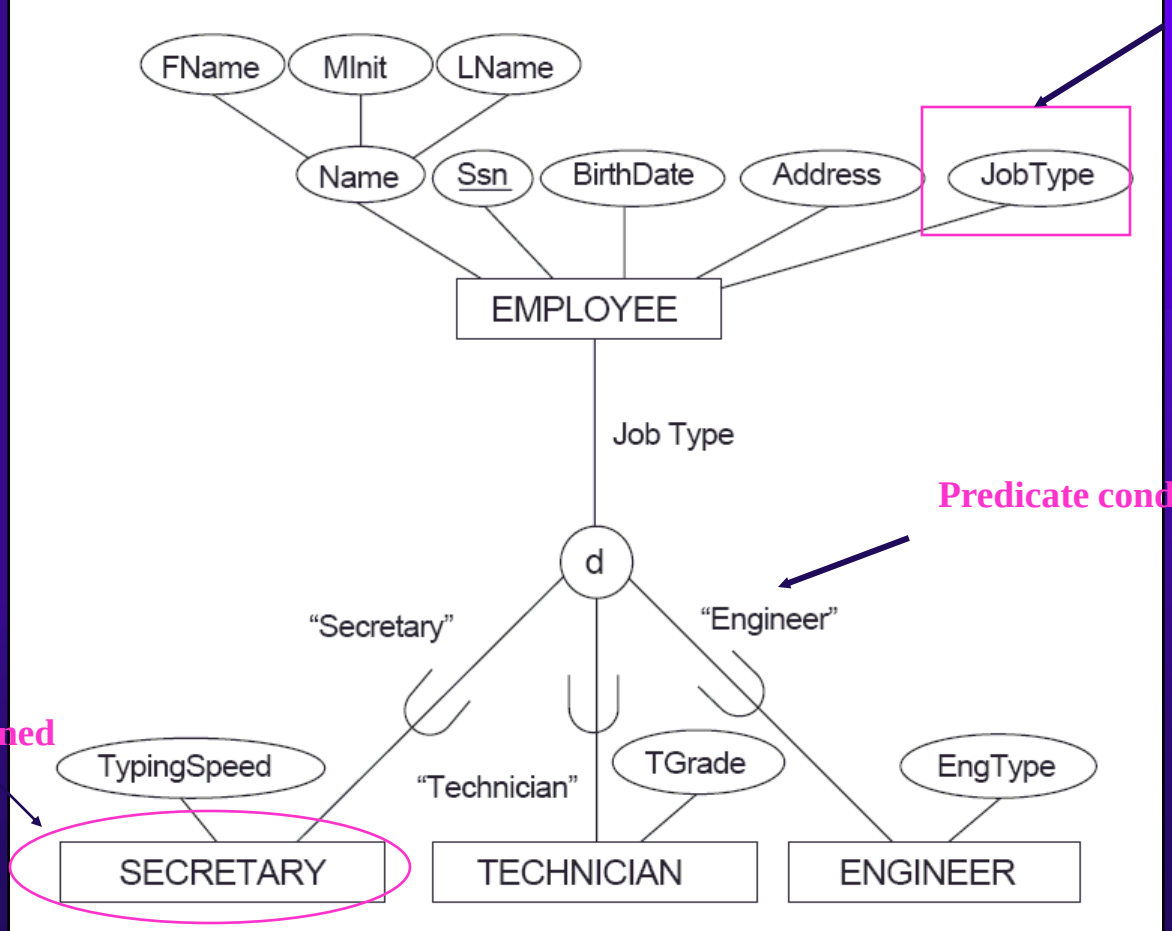


Constraints for Specialization and Generalization

- Attribute-defined specialization
  - Base on values of a superclass attribute (defining attribute)
  - All subclasses have their member condition on th same attribute of the superclass
  - Predicate-defined (condition defined) subclass
  - JobType = ‘Engineer’ => defining predicate
- User-defined Subclass
  - Each membership is determined by the user

Constraints for Specialization and Generalization

Figure 4.4 An attribute-defined specialization on the JobType attribute of EMPLOYEE.

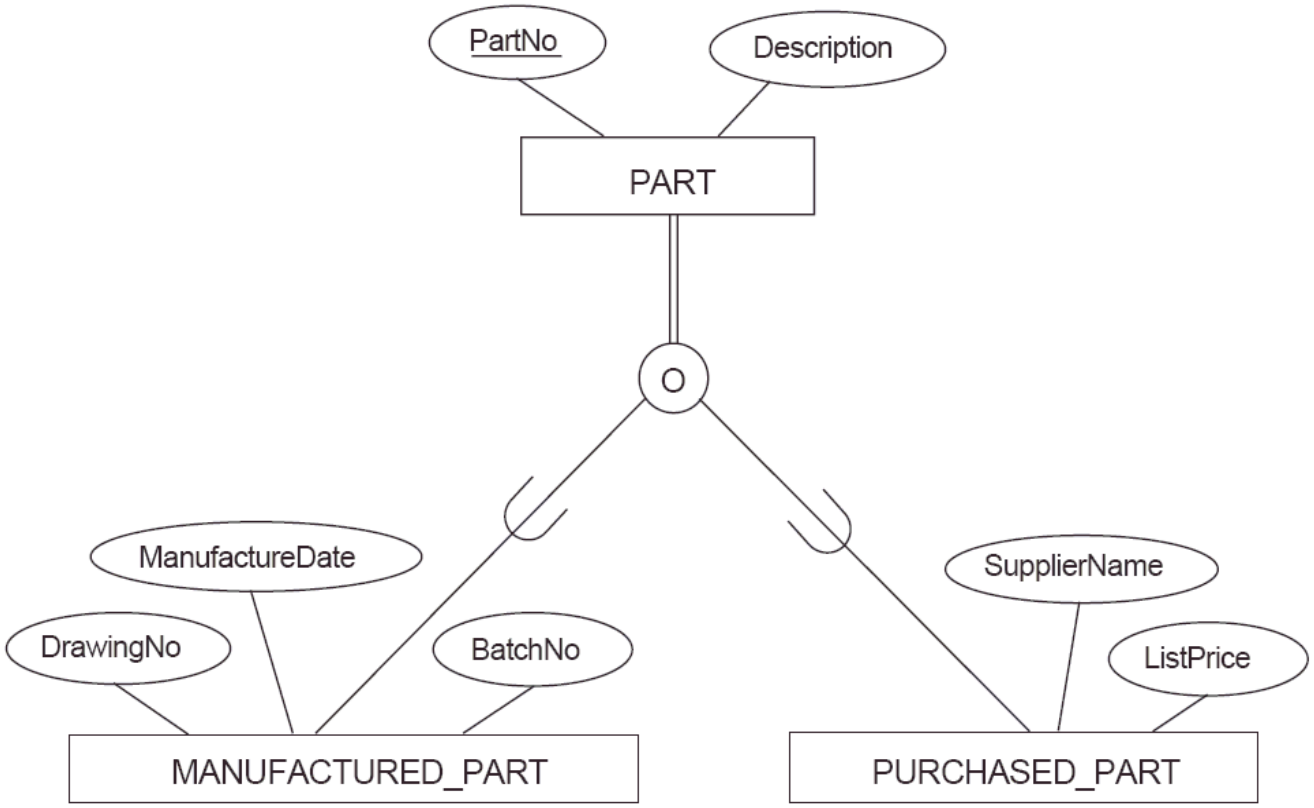


Disjointness constraints

- Disjoint
  - The subclasses of the specialization must be disjoint
  - No entity can be in more than one subclass
  - d notation
- Overlap
  - Entities may belong to several subclasses
  - PERSON ⇒ {EMPLOYEE, ALUMNUS, STUDENT}
  - PARTS=>{MANUFACTURED, PURCHASED} (fig4.5)

Disjointness constraints (cont')

Figure 4.5 Notation for specialization with overlapping (nondisjoint) subclasses.

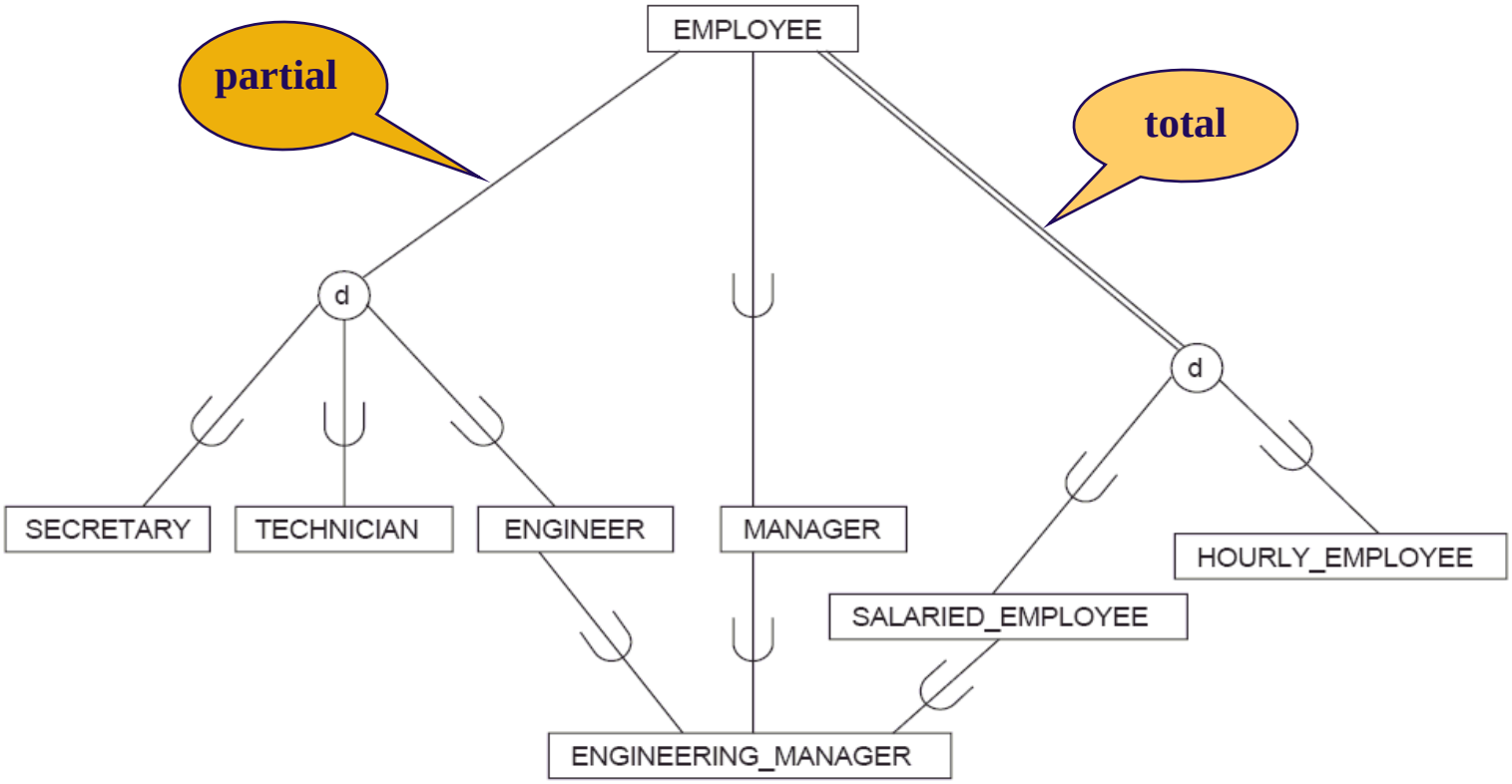


Completeness Constraints

- Partial
  - An entity in a superclass may not belong to any of the subclasses (fig4.6)
  - {SECRETARY, ENGINEER, TECHNICIAN}
- Total
  - every entity in a superclass must belong to at least one of the subclasses
  - {SALARIED_EMPLOYEE, HOURLY_EMPLOYEE}

Completeness Constraints

Figure 4.6 A specialization lattice with the shared subclass ENGINEERING_MANAGER.



Disjointness and completemess

- Independent
- Possible constraints
  - Disjoint, total
  - Disjoint, partial
  - Overlapping, total
  - Overlapping, partial
- Determined by real-world meaning

Update rules for Specialization

- Deletion cascades to subclasses
  - deleting an entity in a superclass leads to the deletion of the entity in the subclasses
- Insertion cascades to subclasses
  - Automatic insertion of an entity in the subclass(es)
  - For predicate-defined subclasses
  - For total specialization

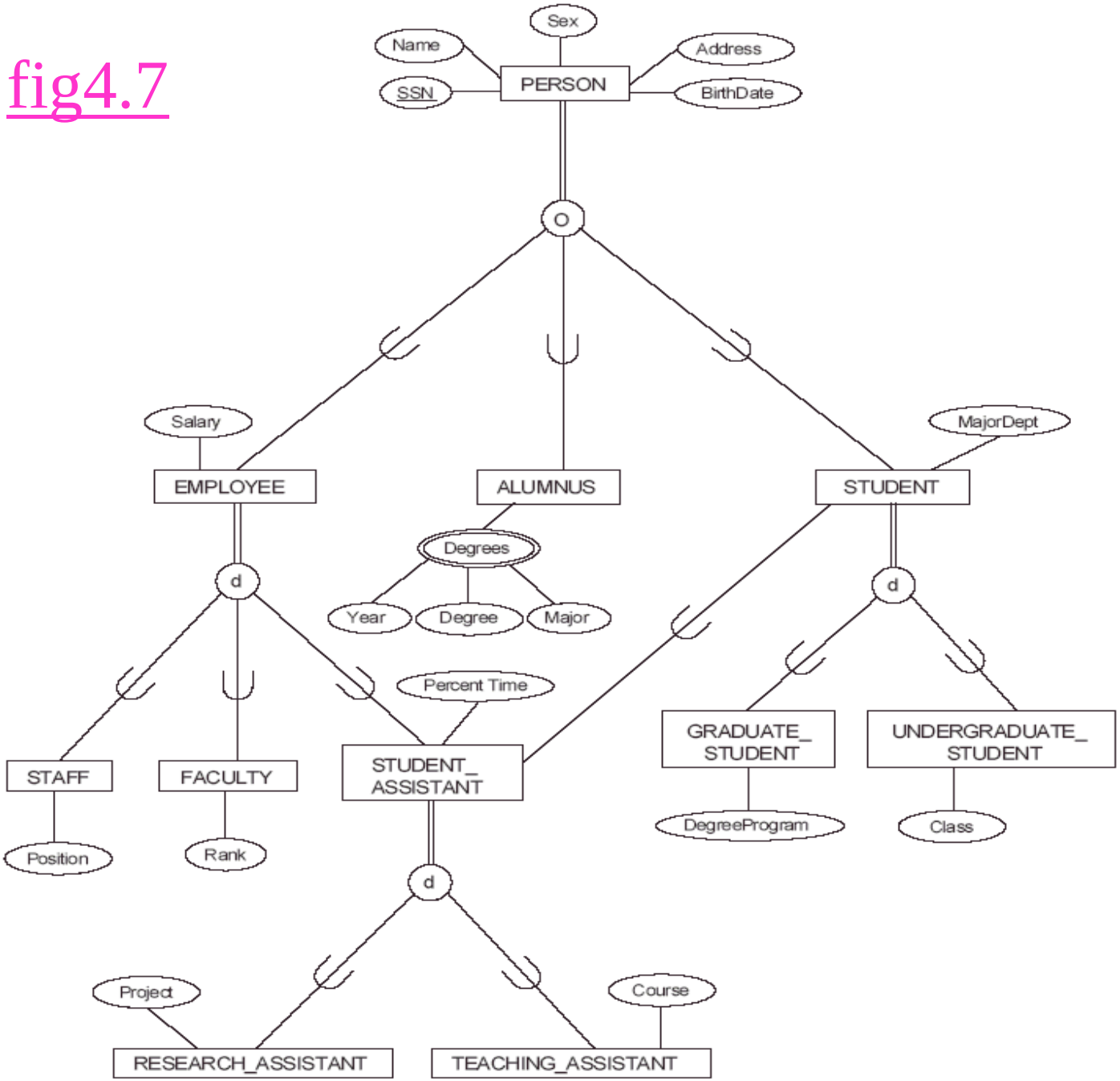
Specialization Hierarchy and Lattices

- Specialization Hierarchy
  - a subclass may have further subclasses
  - every subclass has ONLY ONE superclass
  - a subclass inherits the attributes/relationships not only of its immediate superclass but also of all indirect superclasses in the hierarchy
  - the key of a subclass is the key of the most general superclass

Specialization Hierarchy and Lattices

- Shared Subclass (fig4.6, fig4.7)
  - a subclass with more than one superclass
  - STUDENT ASST ⇐ (EMPLOYEE and STUDENT)
  - Existence of shared subclass leads to lattices
- Multiple Inheritance
  - a shared subclass inherits attributes and relationships from multiple superclasses
  - combinations of subclasses (EMPLOYEE, ALUMNUS, STUDENT): EA, ES, AS, EAS

fig4.7



Refine Conceptual Schema Design

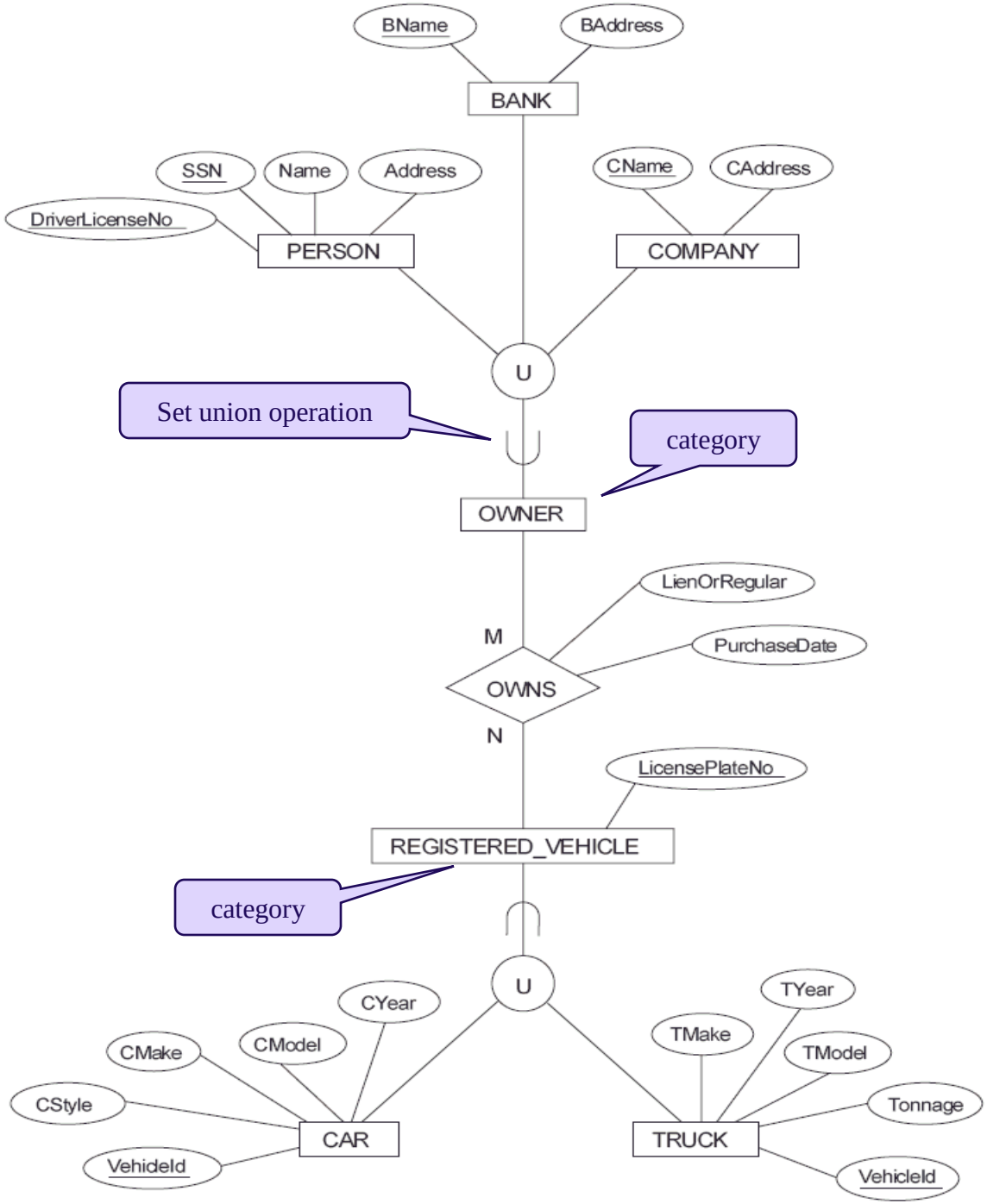
- Specialization and generalization
- Top-Down Conceptual Refinement
  - Start with one entity type
  - Successive specializations from a class into subclasses
- Bottom-up Conceptual Synthesis
  - successive generalizations by forming a superclass from several classes

Categorization by UNION

- Category (Union Type)
  - a subclass represent a collection of objects that is a subset of the UNION of distinct entity types. (notation, $\cup$)
    - (CAR $\cup$ TRUCK) $\Rightarrow$ REGISTERED_VEHICLE
  - a category is a <u>subset</u> of the *union* of its superclasses
  - a category has two or more superclasses
  - other superclass/subclass relationships always have a single superclass

Categorization by UNION

- Attributes Inheritance
  - a category inherits attributes only from the single superclass it belongs to (fig4.8)
- Category vs. Generalized Superclass
  - (CAR $\cup$ TRUCK) $\Rightarrow$ REGISTERED_VEHICLE
  - {CAR, TRUCK} $\Leftarrow$ VEHICLE (fig4.3)
  - VEHICLE may include another type (e.g., MOTORCYCLE) if partial participation



Categorization by UNION

- Category vs. Shared Subclass
  - (CAR $\cup$ TRUCK) $\Rightarrow$ REGISTERED_VEHICLE
  - (GASOLINE $\cap$ ELECTRIC) $\Rightarrow$ HYBRID
  - a shared subclass is a subclass of <u>each of</u> the superclasses
  - a shared subclass is a subset of the *intersection* of the superclasses
  - a category is a subset of the *union* of its superclasses

Mathematical Notation

- Super/Subclass Relationship
  - subclass: **S**, superclass: **C**
  - $\mathbf{S} \subseteq \mathbf{C}$ (**S** is a subset of **C**)
  - IS-A relationship: an entity in **S** is also in **C**
- Specialization/Generalization
  - a set of subclasses: $\mathbf{Z} = \{\mathbf{S_1}, \mathbf{S_2}, .. , \mathbf{S_n}\}$
  - a superclass: **G**
  - $\cup (\mathbf{S_i}) \subseteq \mathbf{G}$

Mathematical Notation

- Constraints
  - Total: $\cup (\mathbf{S_i}) = \mathbf{G}$
  - Partial: $\cup (\mathbf{S_i}) \subset \mathbf{G}$
  - Disjoint: $\mathbf{S_i} \cap \mathbf{S_j} = \phi$ (empty set) for $i \neq j$
  - Overlap: $\mathbf{S_i} \cap \mathbf{S_j} \neq \phi$ (empty set) for $i \neq j$
- Categorization
  - a set of superclasses: $(\mathbf{D_1}, \mathbf{D_2}, .. , \mathbf{D_n})$
  - $\mathbf{T} \subseteq \cup (\mathbf{D_i})$

EER Schema Example

- Data Requirements (EER Schema: fig4.10)

For each person, the database maintains information on the person's Name, social security number, address, sex, and birth date. Two subclasses of the PERSON entity type were identified: FACULTY and STUDENT. Specific attributes of FACULTY are rank, office, office phone, and salary, and all faculty members are related to the academic department(s) with which they are affiliated (a faculty member can be associated with several departments). Each student is also related to his or her major and minor departments, if known, to the course sections he or she is currently attending, and to the courses completed. Each transcript instance includes the grade the student received in the course section.

EER Schema Example

- Data Requirements (con't)

  GRAD_STUDENT is a subclass of STUDENT, with the defining predicate Class = 5. For each graduate student, we keep a list of previous degrees in a composite, multi-valued attribute. We also relate the graduate student to a faculty advisor and to a thesis committee if one exists.

  An academic department has the attributes name, telephone, and office number and is related to the faculty member who is its chairperson and to the college to which it belongs. Each college has attributes college name, office number, and the name of its dean.

EER Schema Example

- Data Requirements (con't)

  A course has attributes course number, course name, and course description. Several sections of each course are offered, with each section having the attributes section number and the year and quarter in which the section was offered. Section numbers uniquely identify each section. The sections being offered during the current semester are in a subclass CURRENT_SECTION of SECTION, with the defining predicate Qtr = Current_Qtr and Year = Current_Year. Each section is related to the instructor who taught or is teaching it ([TEACH], if that instructor is in the database).

EER Schema Example

- Data Requirements (con't)

  The category INSTRUCTOR_RESEARCHER is a subset of the union of FACULTY and GRAD_STUDENT and includes all faculty, as well as graduate students who are supported by teaching or research. Finally, the entity type GRANT keeps track of research grants and contracts awarded to the university. Each grant has attributes grant title, grant number, the awarding agency, and the starting date. A grant is related to one principal investigator and to all researchers it supports. Each instance of support has as attributes the starting date of support, the ending date of the support (if known), and the percentage of time being spent on the project by the researcher being supported.

Conceptual Object Modeling

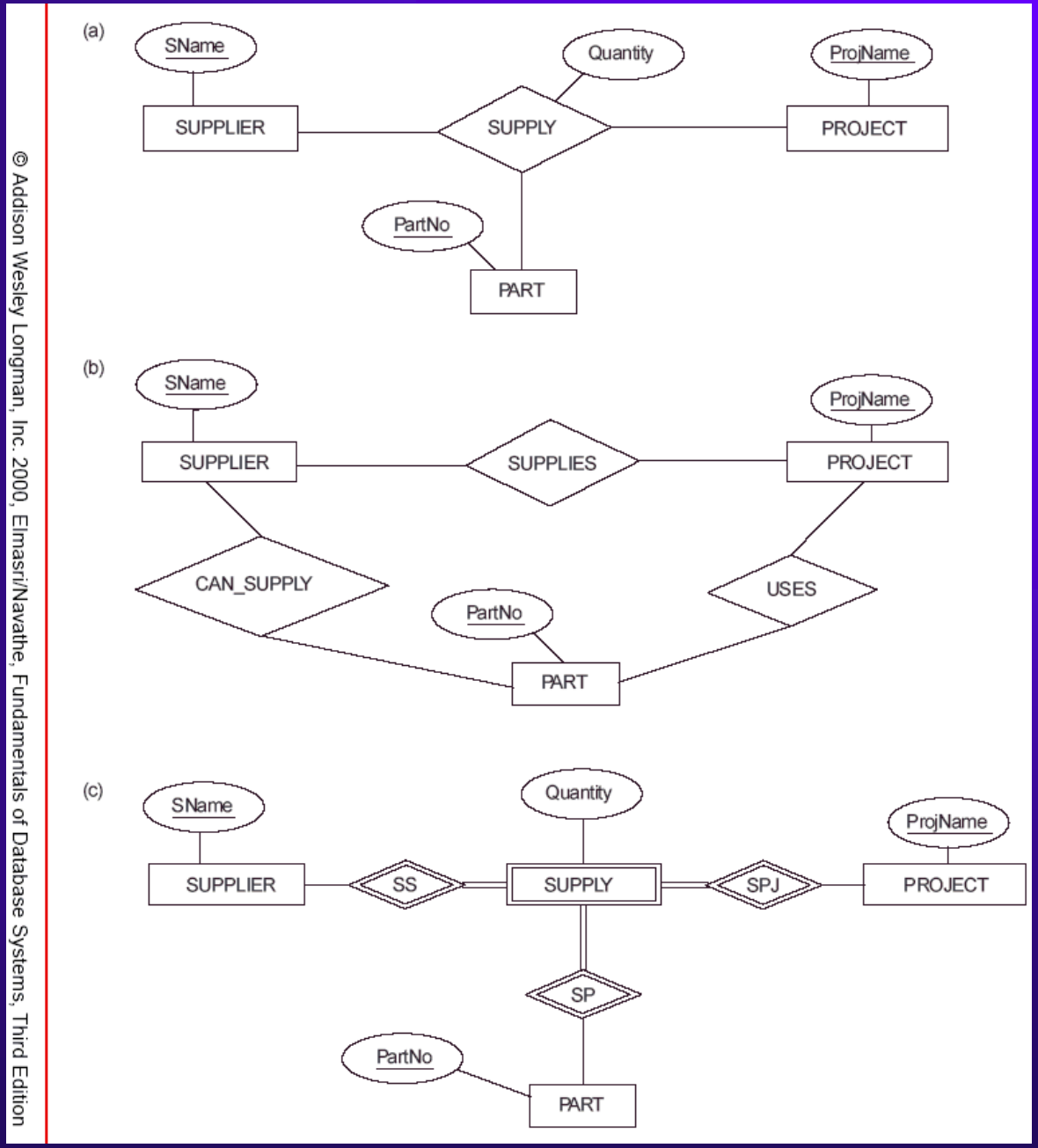
- Object Modeling Methodologies
  - in software engineering: UML, OMT, …
  - specify *operations* applied to objects (entities)
  - class = entity type (attributes) + operations
- Object Modeling Concepts (vs. EER)
  - class: entity type, object: entity
  - association (link): relationship (rel. instance)
  - multiplicity: relationship constraint

UML Conceptual Schema

- UML Class Diagram
  - figure (vs. fig3.15)
  - class: class_name, attributes, operations
  - (min, max) notation, optional value set
  - aggregation: whole-part relationship
- Specialization Notation
  - fig4.10 (vs. fig4.7)
  - disjoint vs. overlapping specialization

Higher-Degree Relationship

- Ternary Relationship Type
  - relates three entity types (fig4.11a, fig3.10)
  - SUPPLY (SUPPLIER:PART:PROJECT)
- Three Binary Relationships
  - meaning is different! (fig4.11b, fig4.14)
  - CAN_SUPPLY (SUPPLIER:PART)
  - SUPPLIES (SUPPLIER:PROJECT)
  - USES (PROJECT:PART)



Higher-Degree Relationship

- Ternary Relationship as Weak Entity Type
  - represents a ternary relationship type as a weak entity type relating to the owner entity types (fig4.11c)
  - includes binary (identifying) relationship types
- As an Identifying Relationship Type
  - a ternary relationship type with a weak entity type and two owner entity types (fig4.13)

Higher-Degree Relationship

- Constraints on Ternary Relationship
  - cardinality ratio, participation

Knowledge Representation

- Knowledge Representation (KR)
  - Concepts for accurately modeling some *domain of discourse* (knowledge) by creating an ontology
  - Similar to conceptual schema, but with more knowledge, rules, and exceptions
  - Include *reasoning mechanism* that deduce additional facts from the stored facts
  - Knowledge-based systems can answer queries that involve *inferences* over the stored data

Knowledge Representation

- KR vs. Semantic Data Modeling (SDM)
  - Both disciplines use an abstraction process to identify common properties and important aspects of objects
  - Both disciplines provide concepts, constraints, operations, and languages for defining and representing knowledge

Abstraction Concepts

- Classification/Instantiation:
  - classification involves assigning similar objects/entities to object classes/entity types
  - instantiation involves generation and examination of distinct objects of a class
- Identification:
  - to identify database classes/objects by means of some identifier
- Specialization/Generalization
- Aggregation/Association

Aggregation

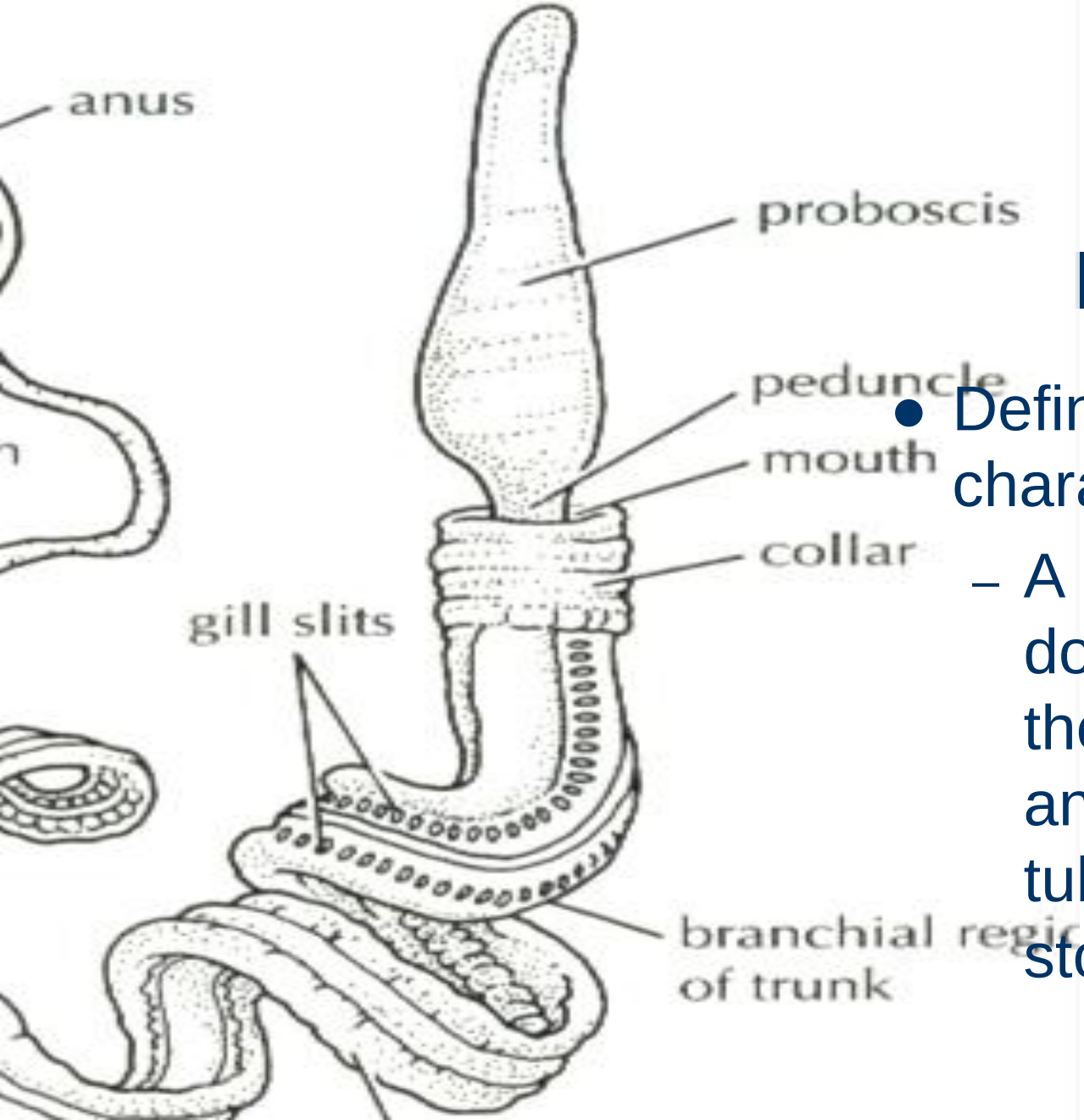
- Aggregation/Association
  - aggregation generates composite objects from their component objects, while association associates objects for several independent classes

Exercise

- Design a EER diagram for a hospital. We want to keep track of patient information. For each patient, we want to keep track of patient ID, her or his name, admission date. There are two kinds of patient, outpatient and resident patient. For outpatient, we want to know the check back date, while for resident patient we want to keep records about discharge date. A patient can not be outpatient and resident patient at the same time. Each resident patient will be assigned with a bed. A bed has a bed ID. Each patient is treated by a responsible physician. A physician may provide one or several treatments for a patient. For each treatment, we want to know the treatment ID, treatment name and patient's reaction. A responsible physician can take care of many patients. For each responsible physician, we want to keep track of her or his name, phone number, physician ID.

Phylum Hemichordata

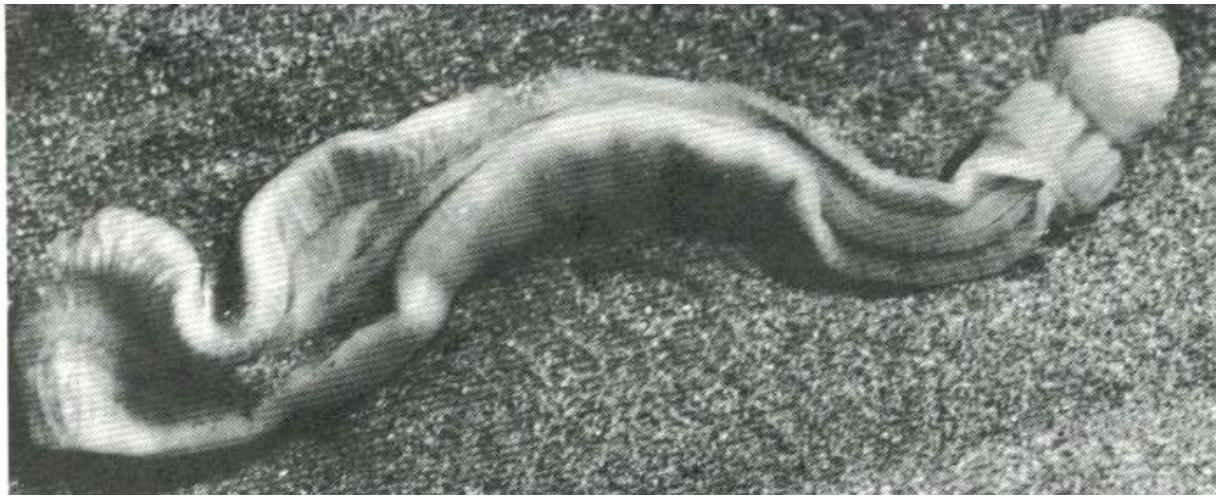
Acorn Worms



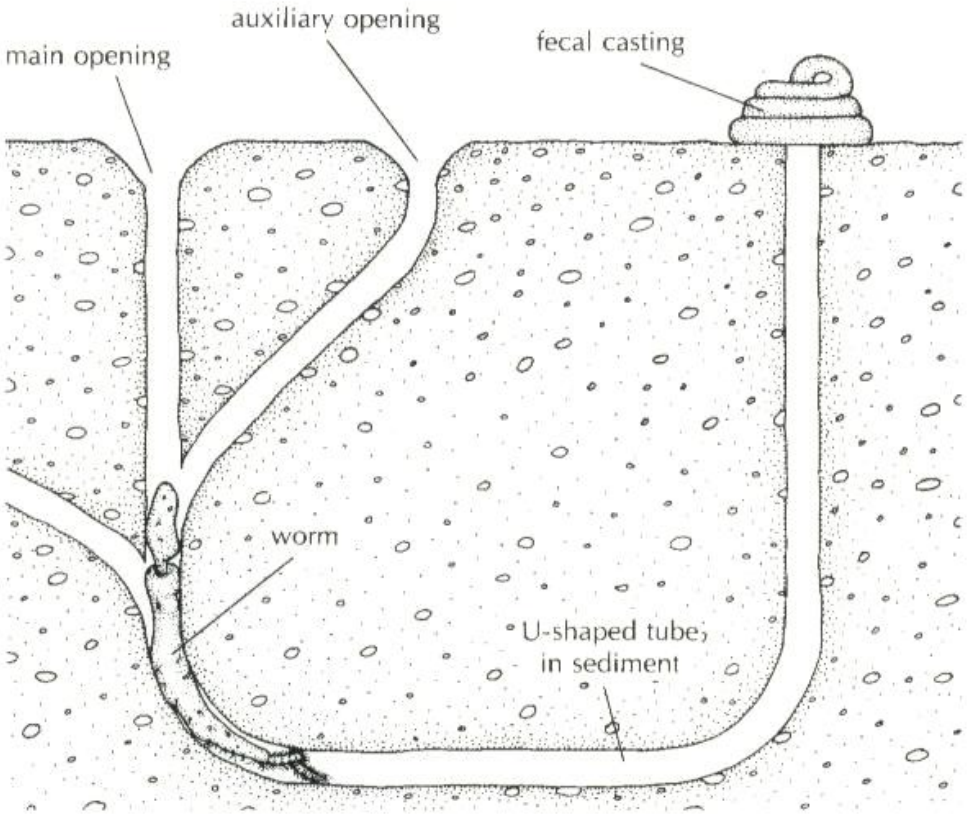
- Defining characteristics
  - A conspicuous dorsal extension of the pharynx forms an anterior buccal tube or stomochord

Acorn Worm Habits

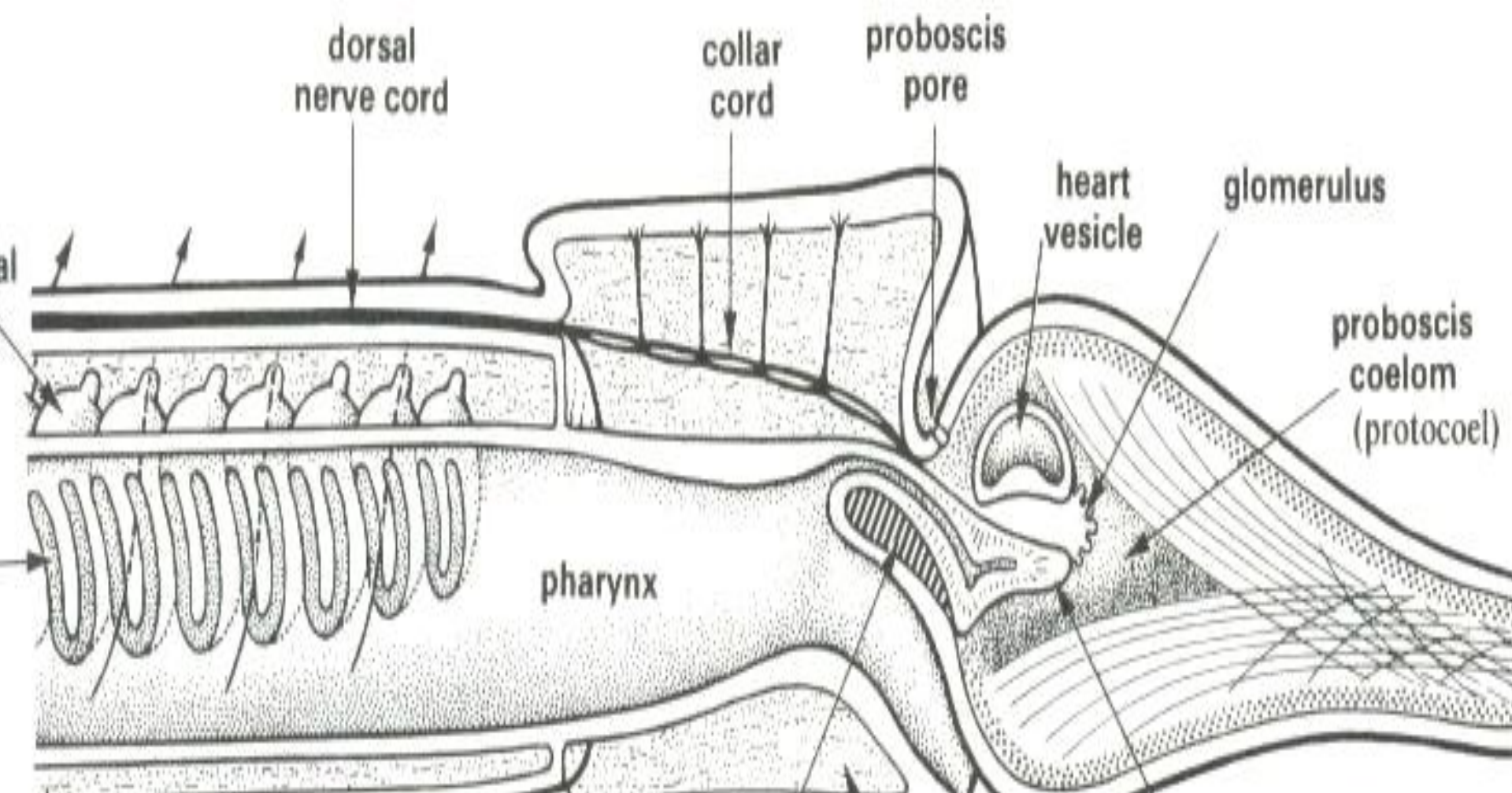
- Rarely found on the surface this group is found in the mud flats of our sounds and creeks
  - Burrow through sediment and ingest it, assimilating what is of value
- Some species remain in mucus lined burrows



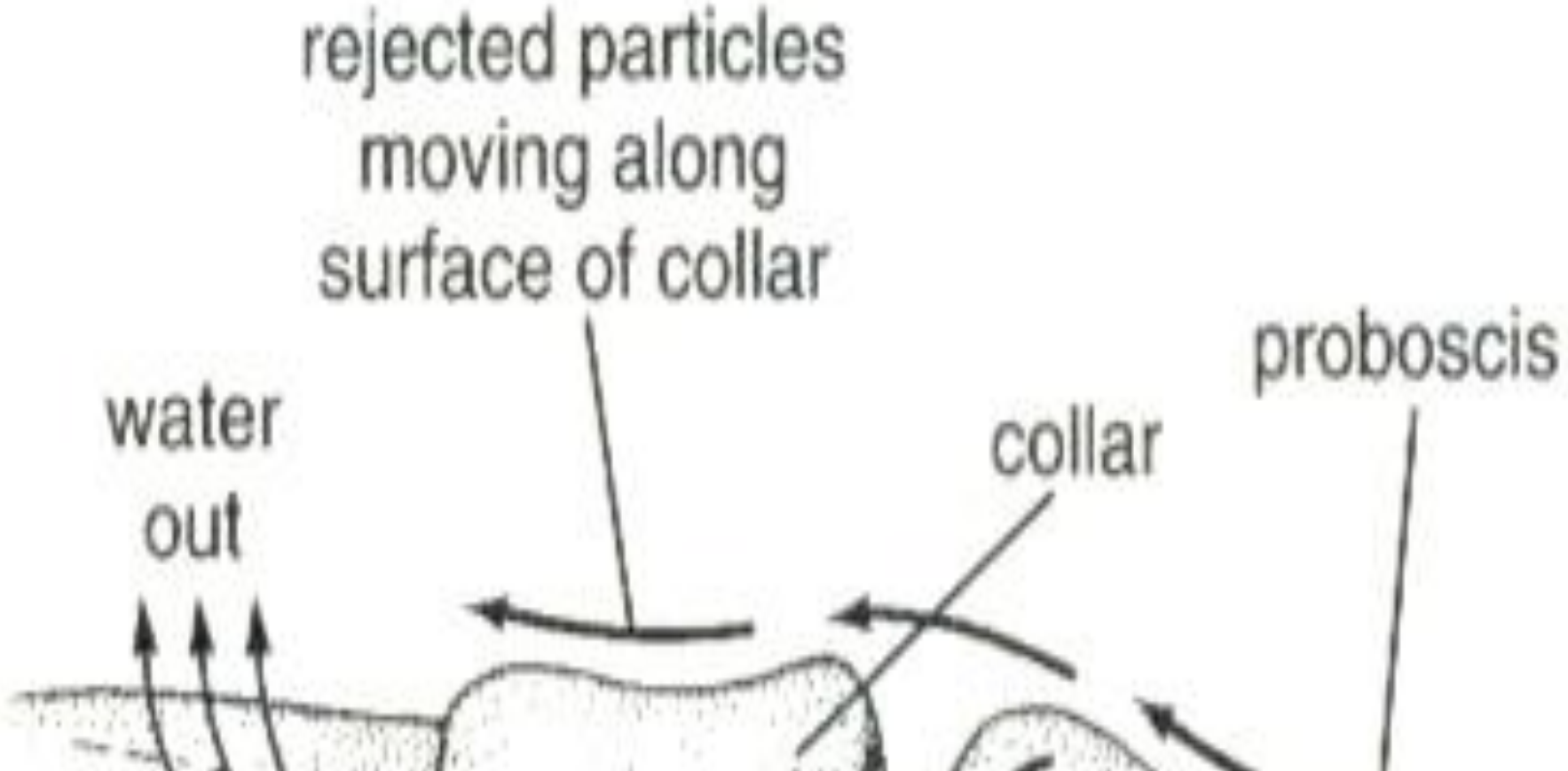
Tube Dweller



Body Structure



Feeding

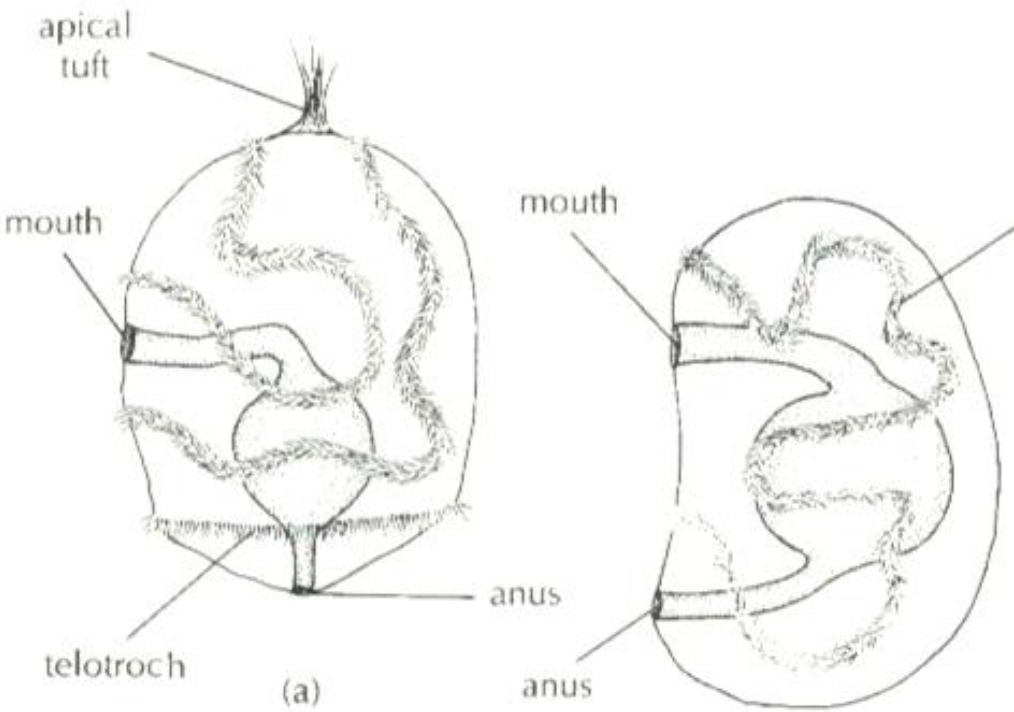

rejected particles
moving along
surface of collar
water
out
collar
proboscis

Internal Transport

- Possess a true circulatory system
  - The blood lacks pigment and runs in dorsal and ventral blood vessels

Reproduction

- Fertilization occurs in the water and forms a feeding planktonic larva
  - **Tornaria**



PLACENTA

- The placenta is an organ that connects the developing fetus to the uterine wall to allow nutrient uptake, waste elimination, and gas exchange via the mother's blood supply.
- Placenta is a characteristic of **eutherian** mammals.
- The placenta functions as a **fetomaternal organ** with two components:

1) The **fetal placenta** (Chorion frondosum), which develops from the same blastocyst that forms the foetus, and

2) The **maternal placenta** (Decidua basalis), which develops from the maternal uterine tissue.

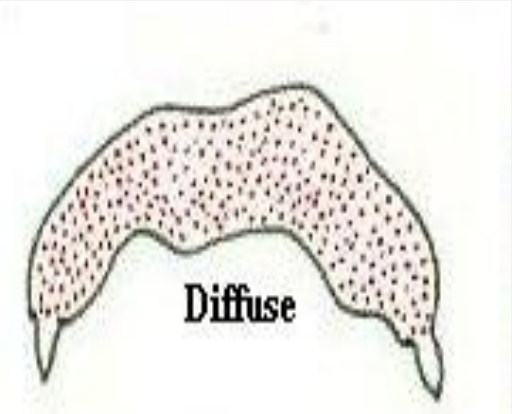
TYPES OF PLACENTA:

- A placenta generally develops due to the fusion of extra embryonic membranes with the endometrium of the uterus of mother.
- Two characteristics are particularly divergent in Eutherian mammals and form basis for classification of placental types:
- The gross shape of the placenta and the distribution of contact sites between foetal membranes and endometrium.
- The number of layers of tissue between maternal and fetal vascular systems.

Number of layers between fetus and meternal tissue

Types based on Placental Shape and Contact Points:

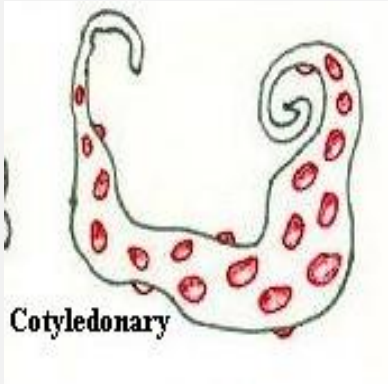
- Diffuse placenta
- Cotyledonary placenta
- Zonary placenta
- Discoid placenta
- **Diffuse**: Almost the **entire surface** of the allantochorion is involved in formation of the placenta.
- Examples: Horses and pigs.

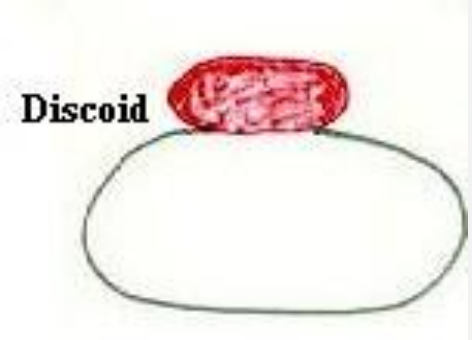


- **Cotyledonary**: Multiple, discrete areas of attachment called cotyledons are formed by **interaction of patches of allantochorion with endometrium**. The fetal portions of this type of placenta are called cotyledons, the maternal contact sites (caruncles), and the cotyledon- caruncle complex a placentome.

  Examples: Ruminants**.**
- **Zonary**: The placenta takes the form of a complete or incomplete **band of tissue** surrounding the fetus. Examples: Carnivores like dogs and cats, seals, bears, and elephants.
- **Discoid**: A single placenta is formed and is **discoid** in shape.

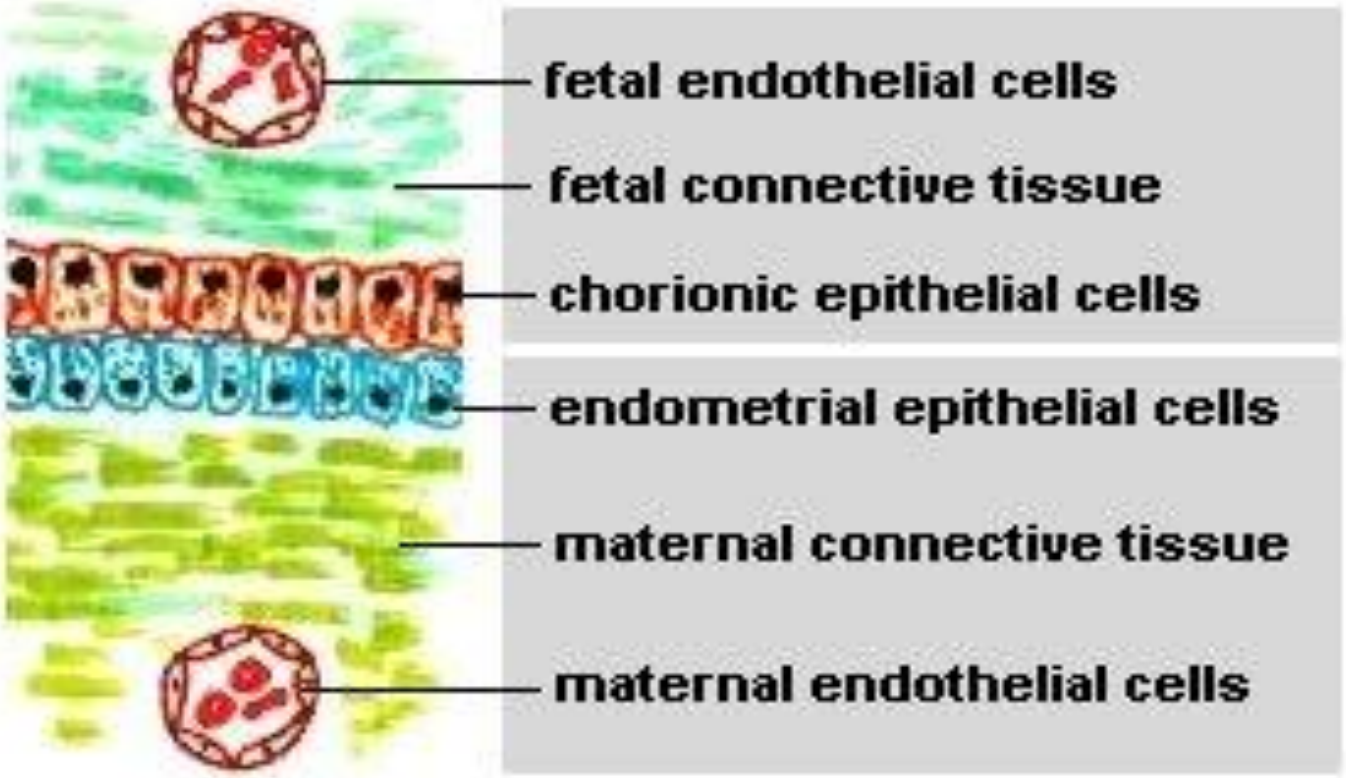
  Examples: Primates and rodents.





Types of placenta based on histology:

1. Epitheliochorial placenta
2. Syndesmochorial placenta
3. Endotheliochorial placenta
4. Haemochorial placenta
5. Haemoendothelial placenta


fetal endothelial cells
fetal connective tissue
chorionic epithelial cells
endometrial epithelial cells
maternal connective tissue
maternal endothelial cells

1) **Epitheliochorial placenta:**

- **It is the primitive type of placenta.**
- **The order of the six layers present between mother and foetus are Endothelium of maternal blood vessel, Endometrial connective tissue (mesenchyme), Uterine epithelium, Ectoderm of the Chorion, Chorionic connective tissue and Endothelium of foetal blood vessel.**
- **The immediate contact of the two halves of placenta involves chorionic epithelium and uterine epithelium. So, it is epithelio chori**
- **Ex. Marsupials, Pig, Hors**

2) **Syndesmochorial placenta**:

- Only **five layers** of tissues are present between mother and the foetus.
- In ruminants of ungulates(cattle, sheep), the foetal and maternal components are fused thereby destructing the uterine epithelium.
- Hence, only five barriers exist between the mother and foetus.
- Ex. Cattle, Sheep.

3) **Endotheliochorial placenta**:

- It is made up of **four layers** of tissues only.
- Uterine mucosa is reduced in this type of placenta.
- The Chorionic epithelium comes in contact with the endothelial walls of the maternal blood vessels.
- Hence, there are only four barriers between foetal and maternal blood streams.
- Ex. Carnivores like dogs, cats, bears etc.,

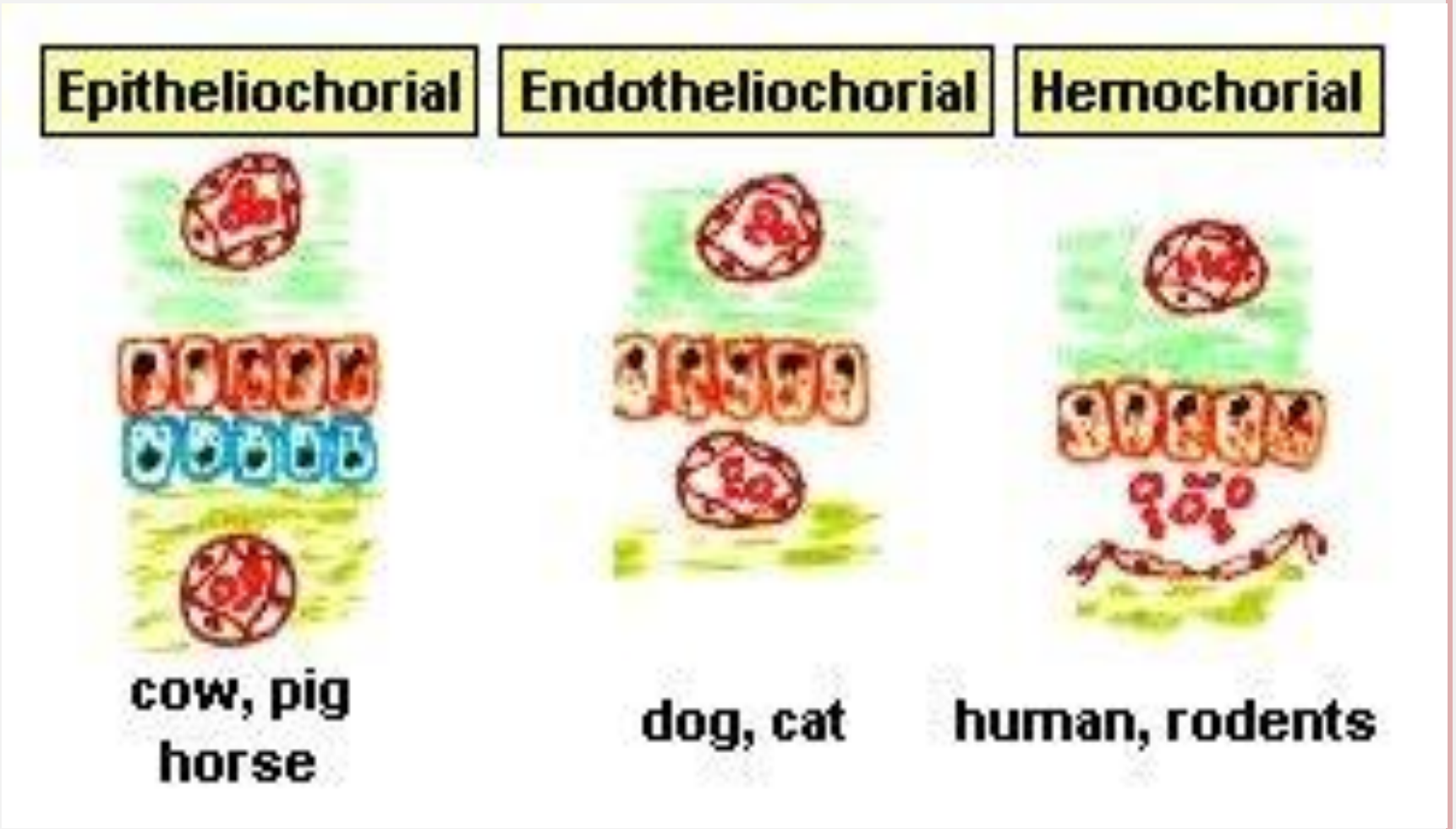
4) **Haemochorial placenta**:

- It is made up of **three layers** of tissues.
- Endothelial walls of maternal blood vessels also disappear and the chorionic epithelium directly comes in contact with the maternal blood.
- Ex. Primates, Insectivores(moles, shrews) and Chiropterans(bats).

5) **Haemoendothelial placenta**:

- It is made up of only **two layers** of tissues.
- The endothelial linings alone separate the foetal blood from maternal sinuses.
- So, the barriers between maternal and foetal blood is reduced to two.
- Ex. Higher rodents like rat, guinea pig, rabbit.


fetal endothelial cells
fetal connective tissue
chorionic epithelial cells
endometrial epithelial cells
maternal connective tissue
maternal endothelial cells

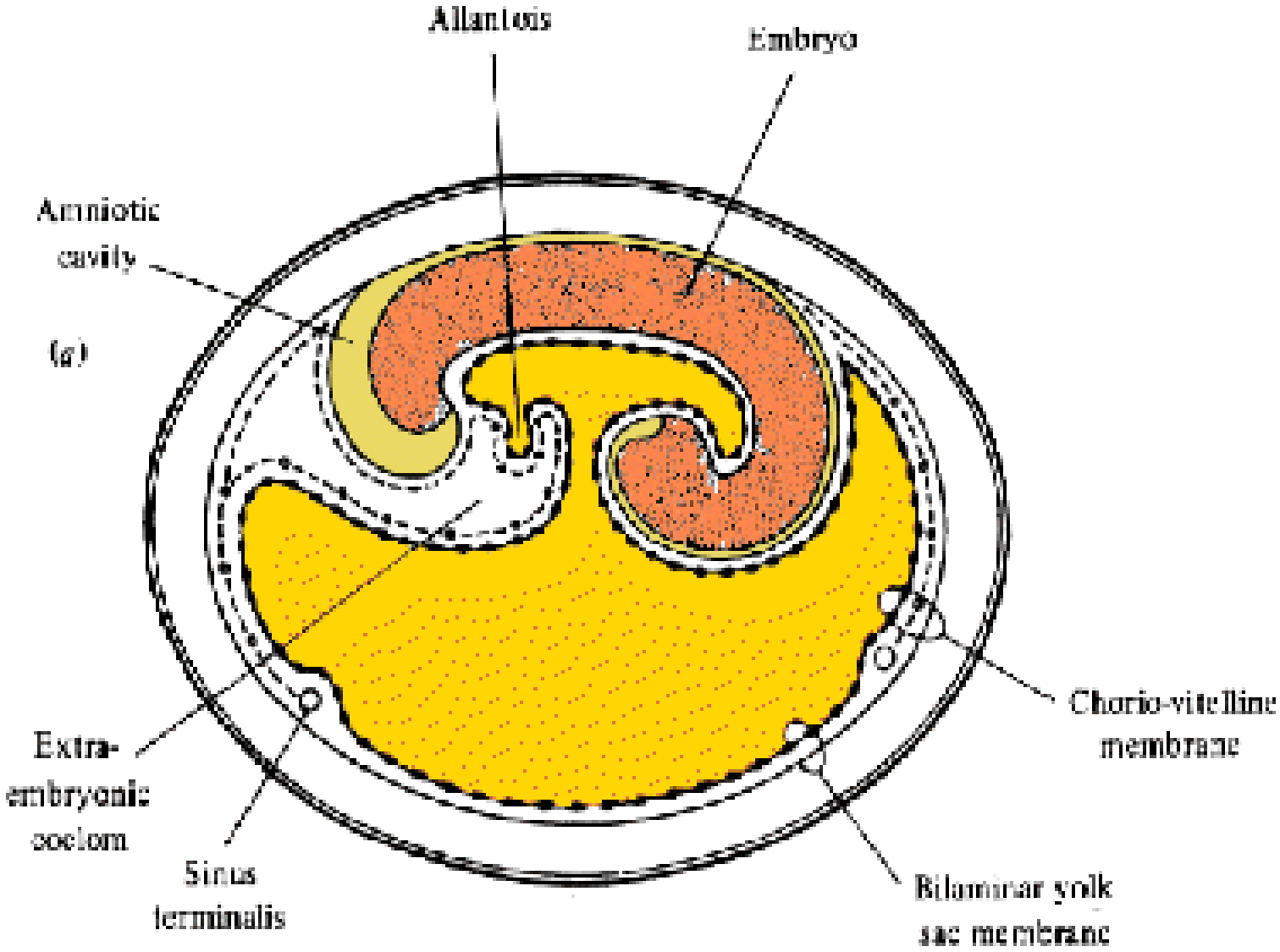

Epitheliochorial
Endotheliochorial
Hemochorial
cow, pig
horse
dog, cat
human, rodents

Based on origin, placenta are of two types:

1) Choriovitelline placenta
2) Chorioallantoic placenta

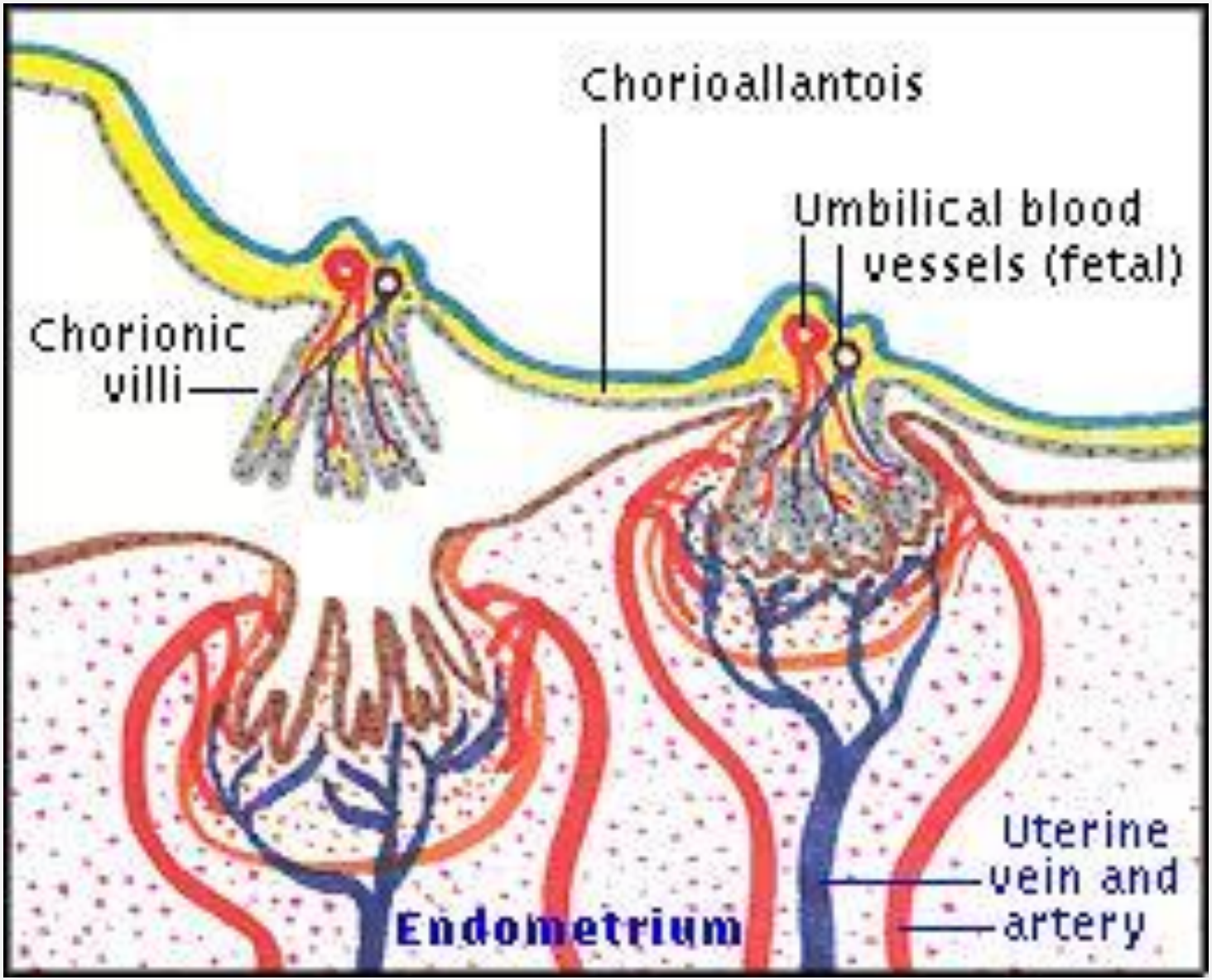
1) **Choriovitelline placenta**:

- In this type of placenta, yolk sac fuses with the Chorion.
- The Chorion receives its blood supply from the network of **vitelline blood vessels** of yolk sac.
- Ex. Marsupials like *Didelphus, Macropus*.


Allantois
Embryo
Amniotic cavity
(a)
Extra-embryonic coelom
Sinus terminalis
Chorio-vitelline membrane
Bilaminar yolk sac membrane

2) **Chorioallantoic placenta**:

- In this type of placenta, the **Allantois fuses with Chorion.**
- Yolk sac remains rudimentary.
- Many villi arises from the Chorion and enters the maternal tissue.
- Ex. Marsupials and all Eutherians.


Chorioallantois
Umbilical blood vessels (fetal)
Chorionic villi
Uterine vein and artery
Endometrium

FUNCTIONS OF PLACENTA:

Nutrition

- The intervillous spaces of the placenta with maternal blood allows the transfer of nutrients and oxygen from the mother to the foetus.
- Nutrient transfer to the foetus occurs via both active and passive transport.

Excretion

- Waste products excreted from the foetus such as urea, uric acid, and creatinine are transferred to the maternal blood by diffusion across the placenta.

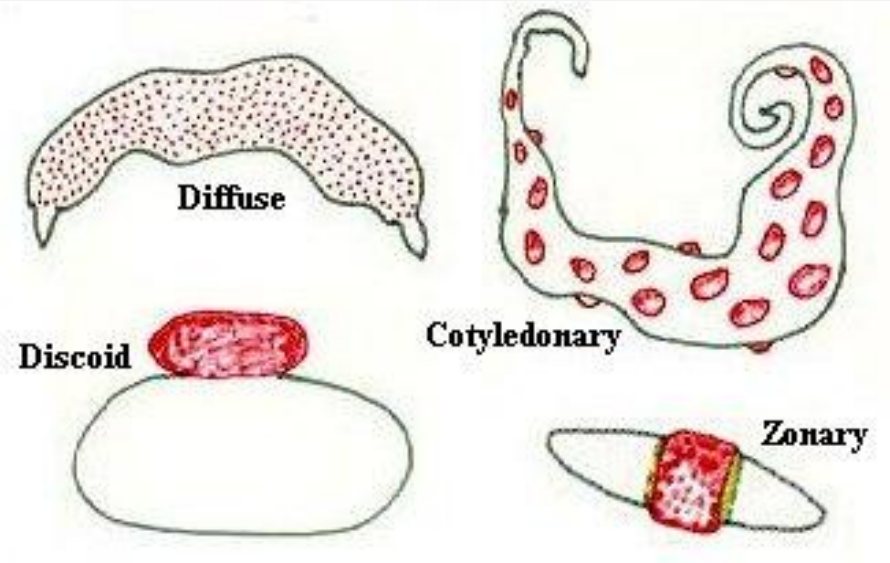
Immunity

- IgG antibodies can pass through the human placenta, thereby providing protection to the foetus.
- The placenta functions as a selective maternal-fetal barrier against transmission of microbes.
- However, insufficiency in this function may still cause mother-to-child transmission of infectious diseases.

Endocrine function

- The placenta secretes many harmones like Human Chorionic Gonadotropin (hCG), Human Placental Lactogen, Oestrogen, Progesterone useful during pregnancy.

THANK YOU


Diffuse
Cotyledonary
Discoid
Zonary

PROTEIN SYNTHESIS

DNA and Genes

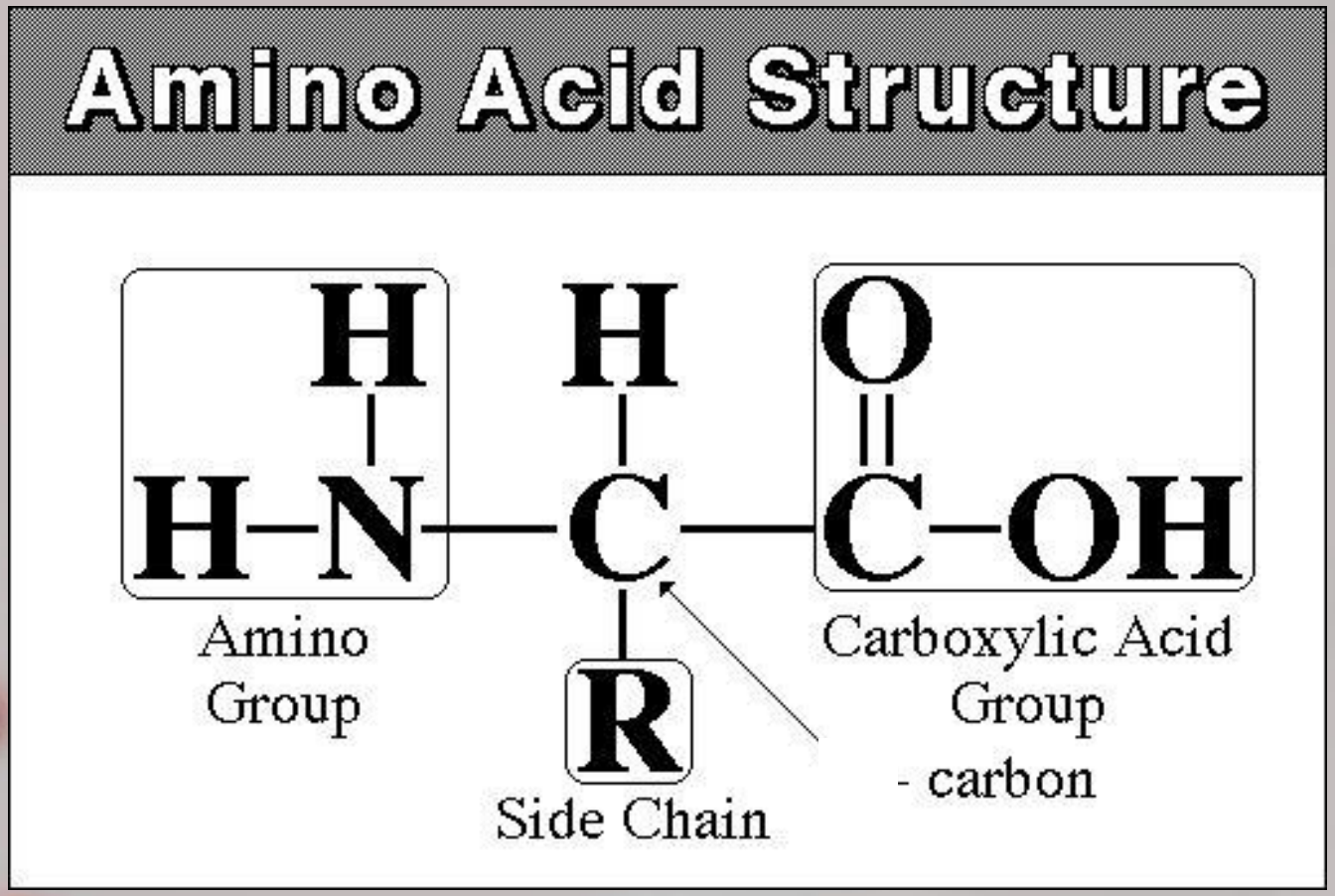
DNA

- DNA contains genes, sequences of nucleotide bases
- These Genes code for polypeptides (proteins)
- Proteins are used to build cells and do much of the work inside cells

Genes & Proteins

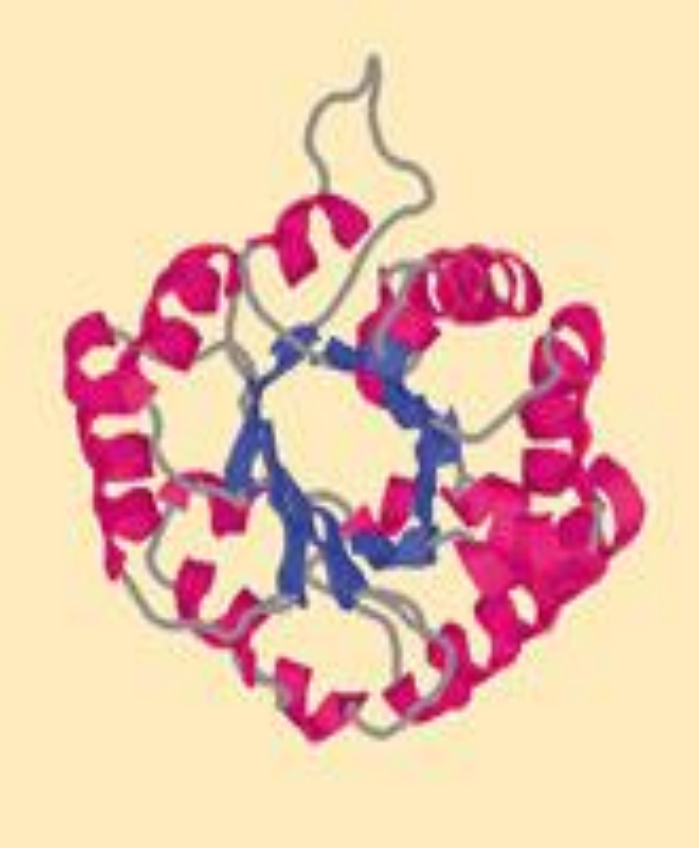
- Proteins are made of amino acids linked together by peptide bonds
- 20 different amino acids exist

Amino Acid Structure



Polypeptides

- Amino acid chains are called polypeptides



DNA Begins the Process

- DNA is found inside the nucleus
- Proteins, however, are made in the cytoplasm of cells by organelles called ribosomes
- Ribosomes may be free in the cytosol or attached to the surface of rough ER

Starting with DNA

- DNA 's code must be copied and taken to the cytosol
- In the cytoplasm, this code must be read so amino acids can be assembled to make polypeptides (proteins)
- This process is called PROTEIN SYNTHESIS

RNA

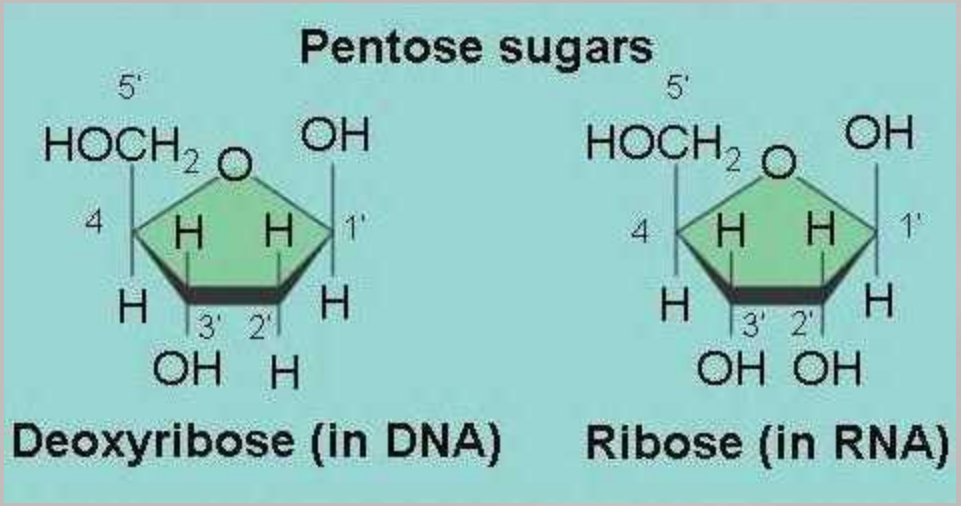
Roles of RNA and DNA

- DNA is the MASTER PLAN

- RNA is the BLUEPRINT of the Master Plan

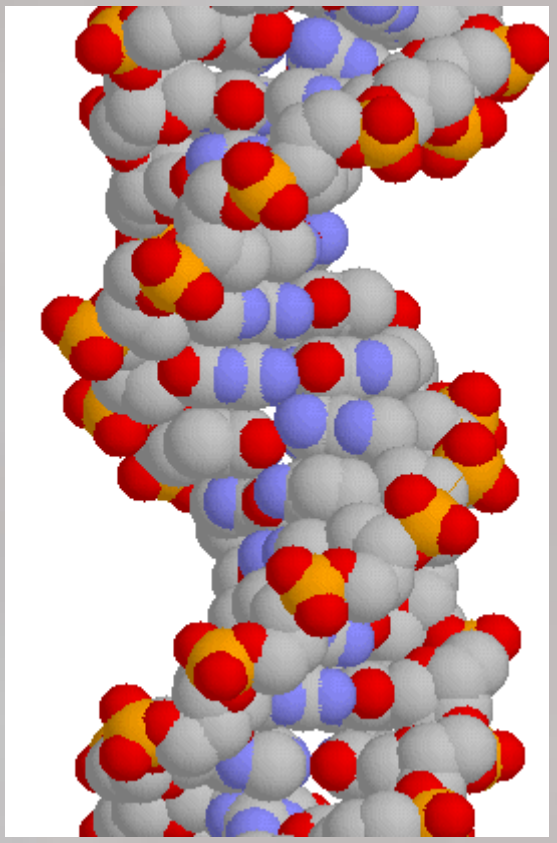
RNA Differs from DNA

- RNA has a sugar ribose
  DNA has a sugar deoxyribose



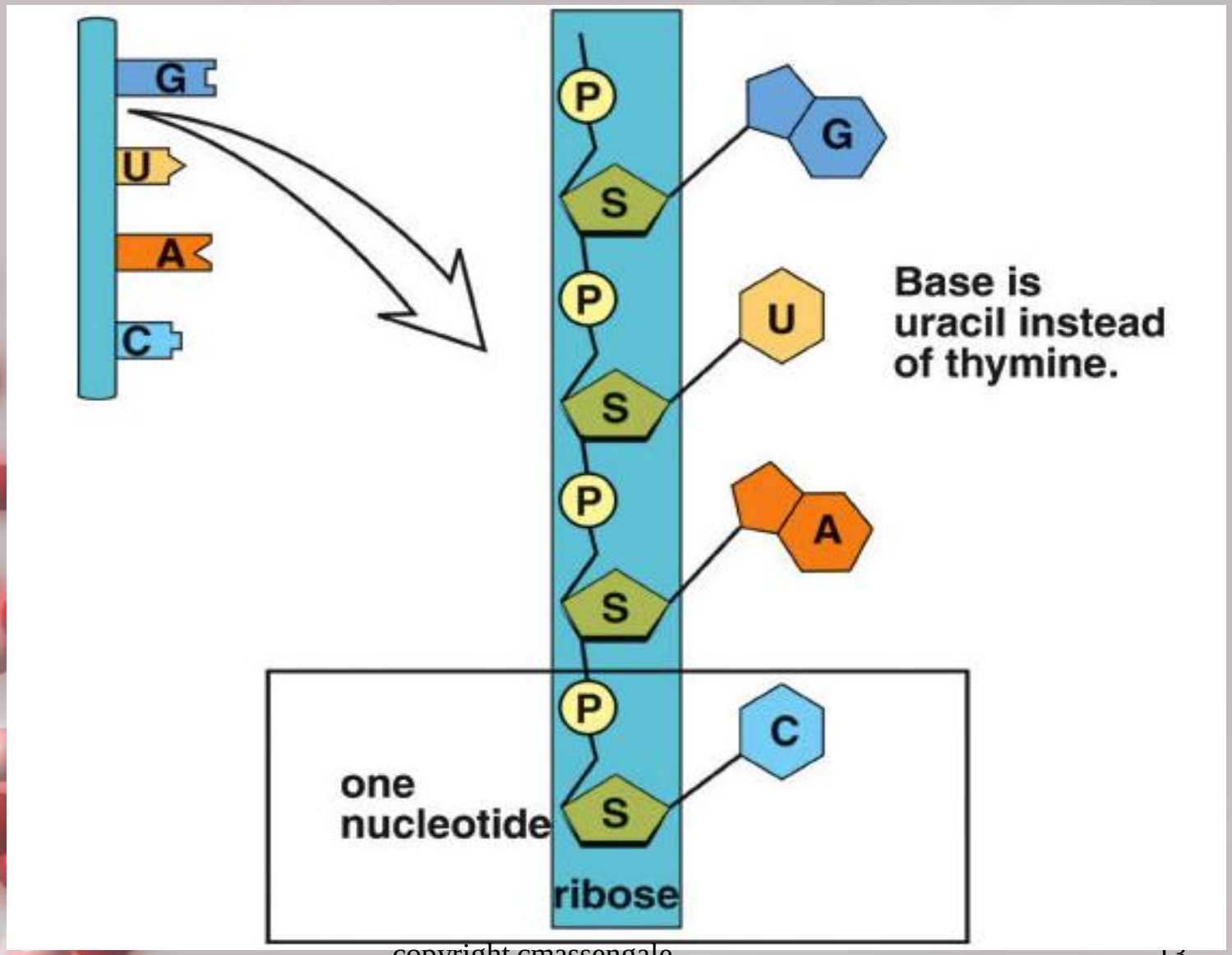
Other Differences

- RNA contains the base uracil (U)
  DNA has thymine (T)
- RNA molecule is single-stranded
  DNA is double-stranded



DNA

Structure of RNA

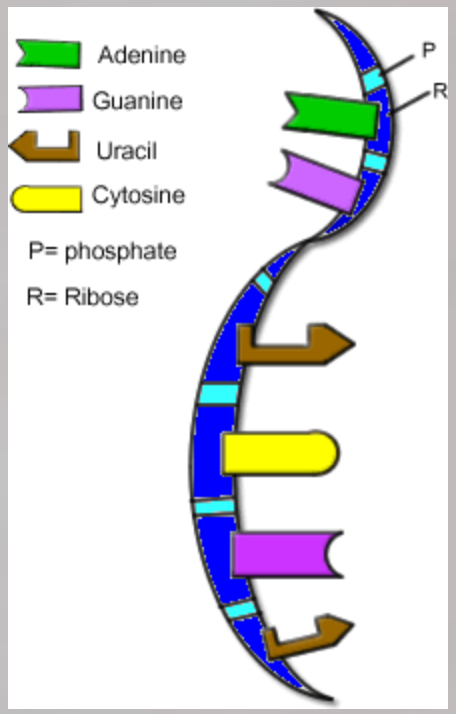


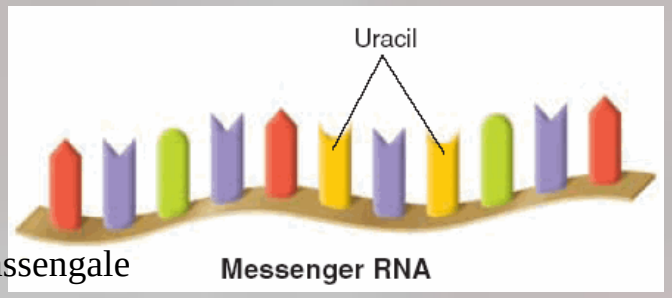
Three Types of RNA

- Messenger RNA (mRNA) copies DNA's code & carries the genetic information to the ribosomes
- Ribosomal RNA (rRNA), along with protein, makes up the ribosomes
- Transfer RNA (tRNA) transfers amino acids to the ribosomes where proteins are synthesized

Messenger RNA

- Long Straight chain of Nucleotides
- Made in the Nucleus
- Copies DNA & leaves through nuclear pores
- Contains the Nitrogen Bases A, G, C, U (no T)



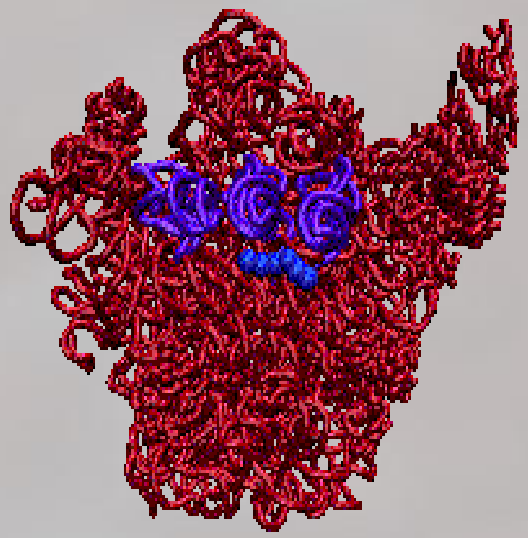


Messenger RNA (mRNA)

- Carries the information for a specific protein
- Made up of 500 to 1000 nucleotides long
- Sequence of 3 bases called codon
- AUG – methionine or start codon
- UAA, UAG, or UGA – stop codons

Ribosomal RNA (rRNA)

- rRNA is a single strand 100 to 3000 nucleotides long
- Globular in shape
- Made inside the nucleus of a cell
- Associates with proteins to form ribosomes
- Site of protein Synthesis

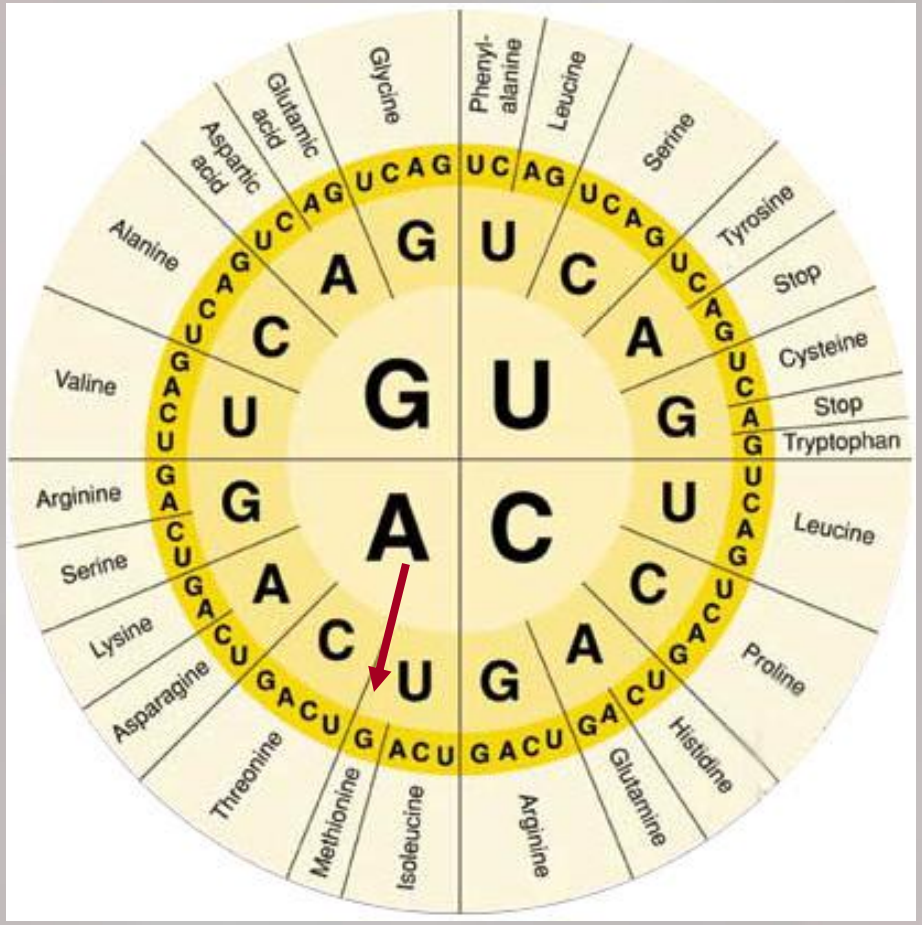


The Genetic Code

- A codon designates an amino acid
- An amino acid may have more than one codon
- There are 20 amino acids, but 64 possible codons
- Some codons tell the ribosome to *stop* translating

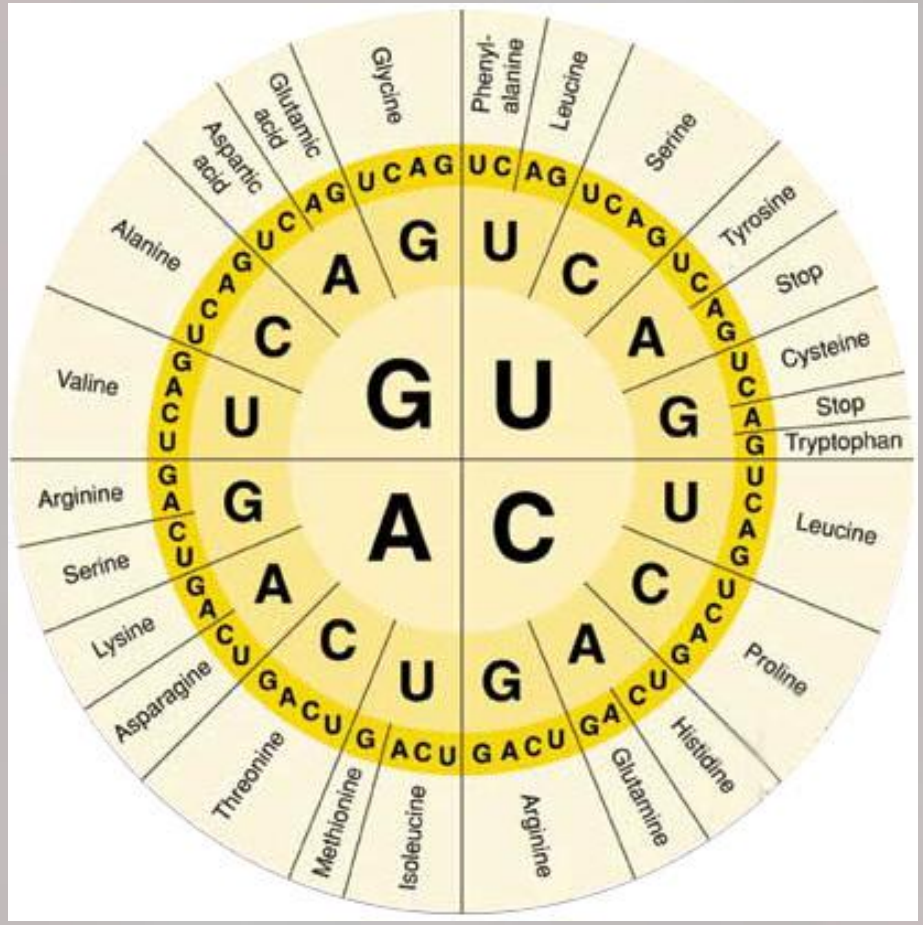
The Genetic Code

- Use the code by reading from the center to the outside
- Example: AUG codes for Methionine



Name the Amino Acids

- GGG?
- UCA?
- CAU?
- GCA?
- AAA?



Remember the Complementary Bases

On DNA:

A-T

C-G

On RNA:

A-U

C-G

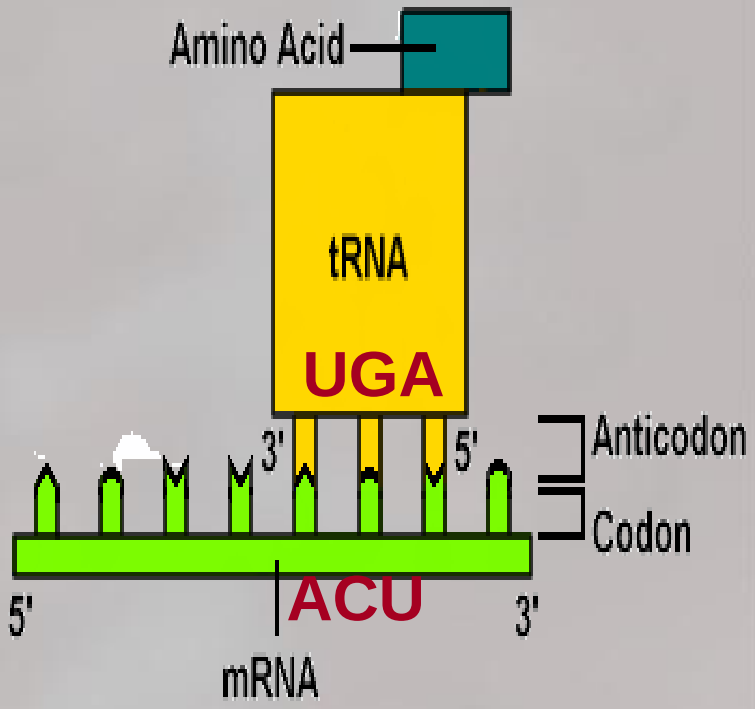
Transfer RNA (tRNA)

- Clover-leaf shape
- Single stranded molecule with attachment site at one end for an amino acid
- Opposite end has three nucleotide bases called the anticodon

Transfer RNA

Codons and Anticodons

- The 3 bases of an anticodon are complementary to the 3 bases of a codon
- Example: Codon ACU
  Anticodon UGA



Transcription and Translation

Pathway to Making a Protein

DNA

↓

mRNA

↓

tRNA (ribosomes)

↓

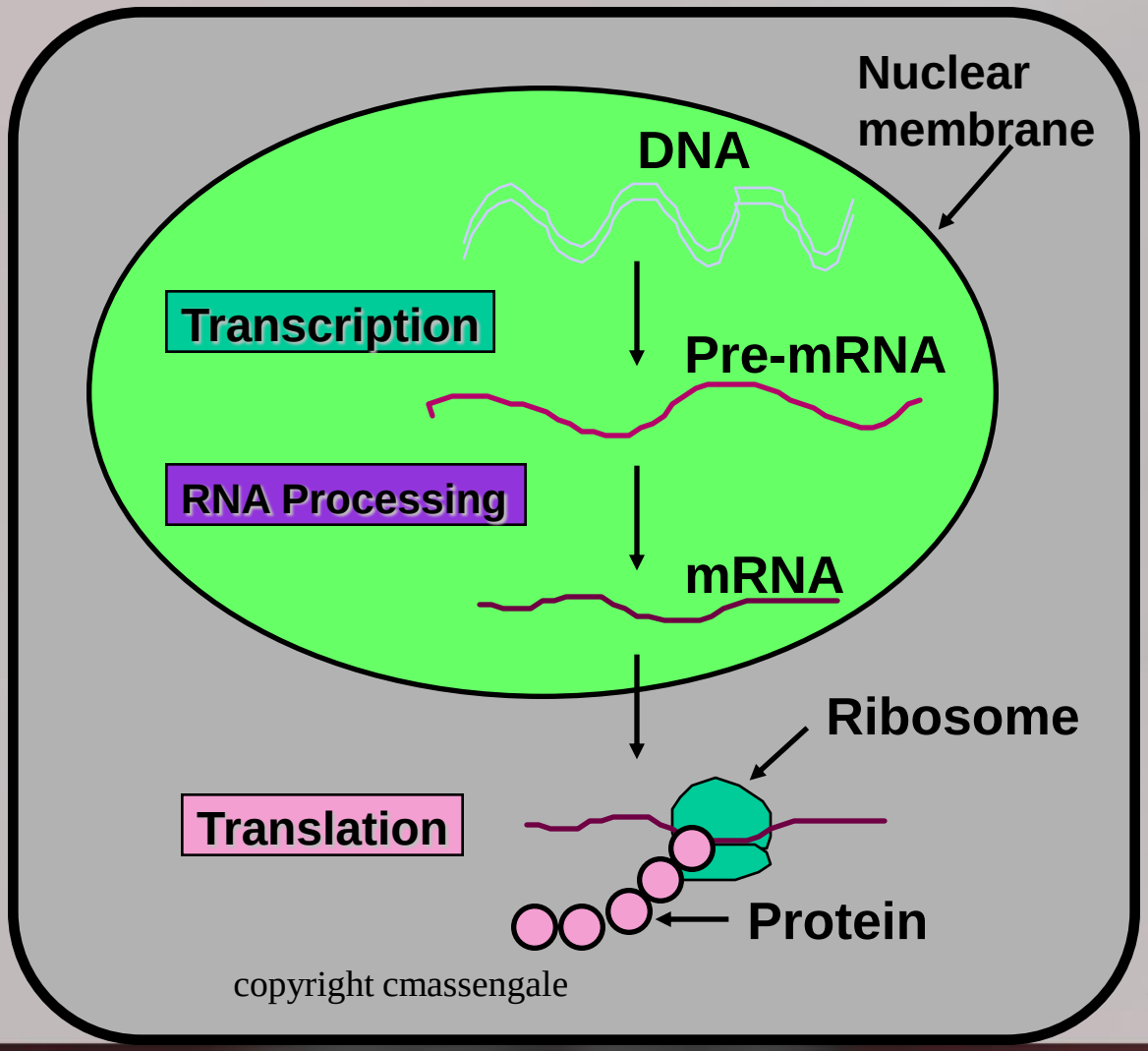
Protein

Protein Synthesis

- The production or synthesis of polypeptide chains (proteins)
- Two phases: Transcription & Translation
- mRNA must be processed before it leaves the nucleus of eukaryotic cells

DNA → RNA → Protein

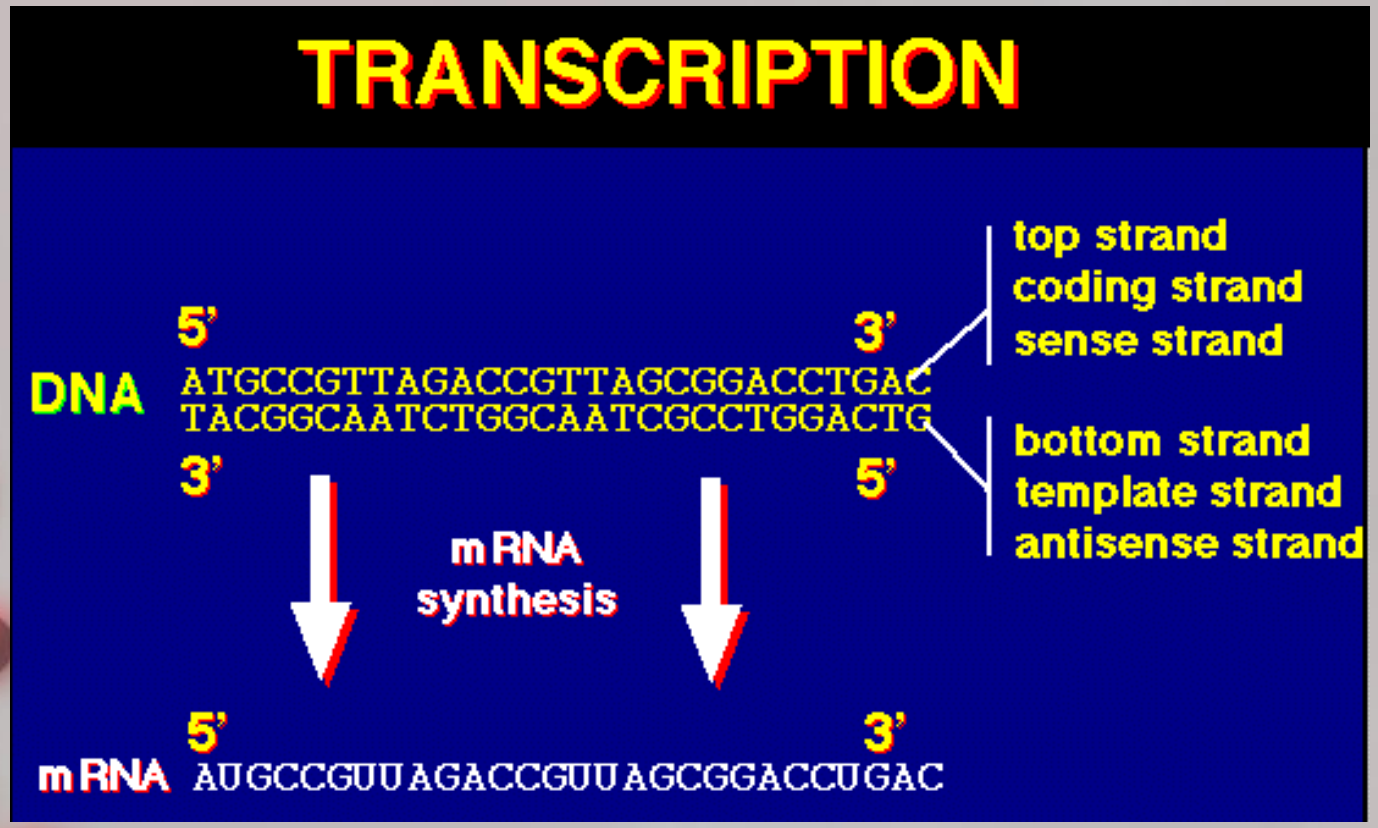
Eukaryotic Cell



Transcription

- The process of copying the sequence of one strand of DNA, the template strand
- mRNA copies the template strand
- Requires the enzyme RNA Polymerase

Template Strand



Question:

- What would be the complementary RNA strand for the following DNA sequence?

DNA 5'-GCGTATG-3'

Answer:

- DNA 5'-GCGTATG-3'
- RNA 3'-CGCAUAC-5'

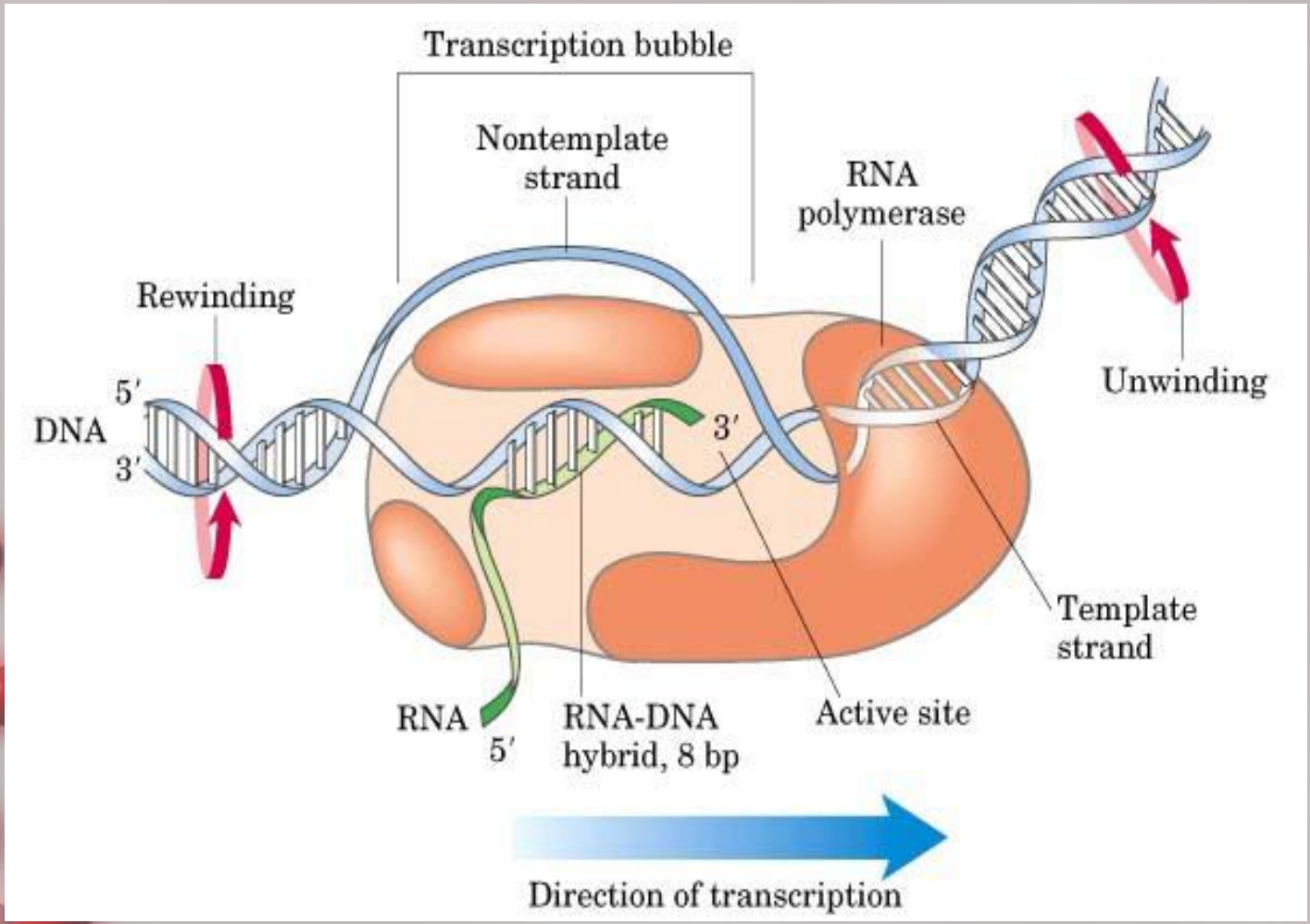
Transcription

- During transcription, **RNA polymerase binds to DNA and separates the DNA strands**
- **RNA Polymerase then uses one strand of DNA as a template to assemble nucleotides into RNA**

Transcription

- Promoters are regions on DNA that show where RNA Polymerase must bind to begin the Transcription of RNA
- Called the TATA box
- Specific base sequences act as signals to stop
- Called the termination signal

RNA Polymerase



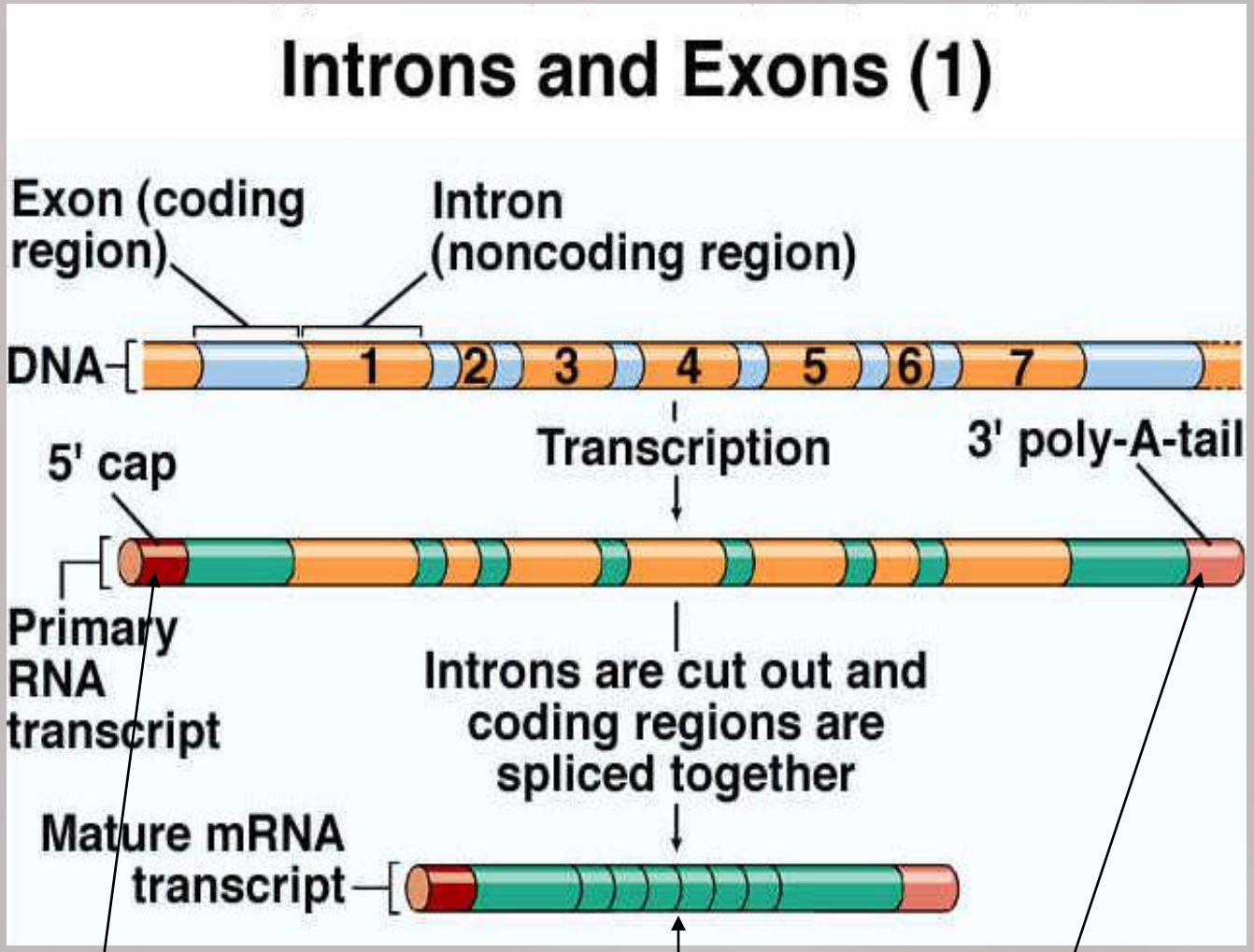
mRNA Processing

- After the DNA is transcribed into RNA, editing must be done to the nucleotide chain to make the RNA functional
- Introns, non-functional segments of DNA are snipped out of the chain

mRNA Editing

- Exons, segments of DNA that code for proteins, are then rejoined by the enzyme ligase
- A guanine triphosphate cap is added to the 5" end of the newly copied mRNA
- A poly A tail is added to the 3' end of the RNA
- The newly processed mRNA can then leave the nucleus

Result of Transcription



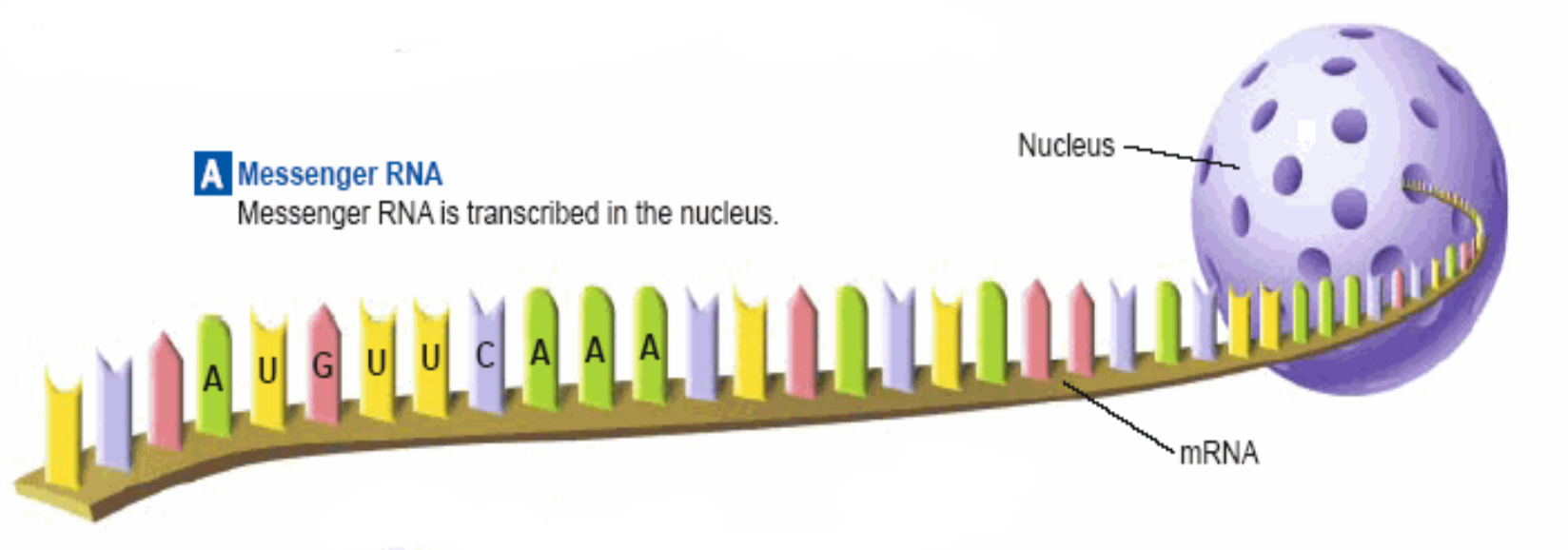
CAP

New Transcript

Tail

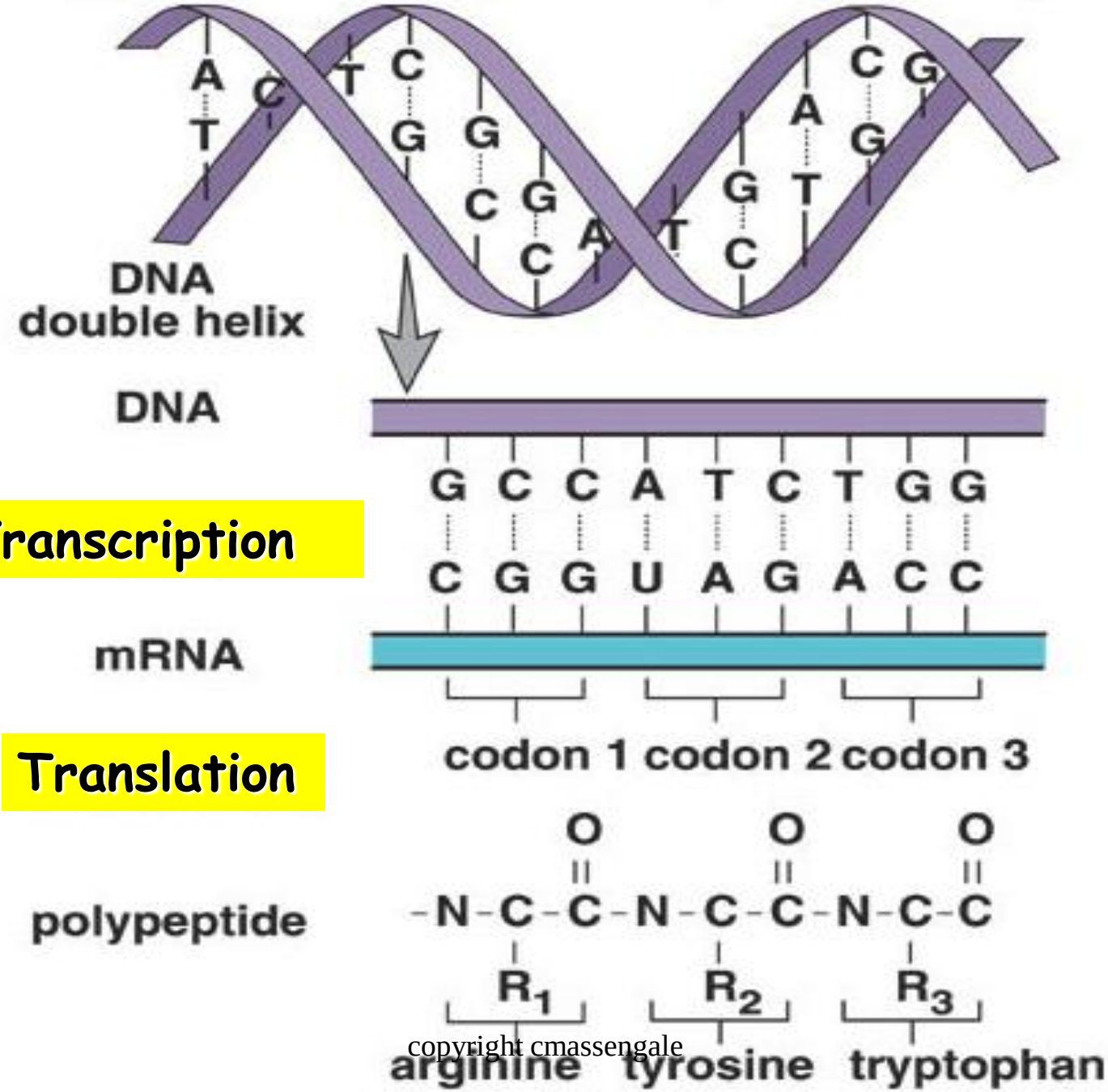
mRNA Transcript

- mRNA leaves the nucleus through its pores and goes to the ribosomes



Translation

- Translation is the process of decoding the mRNA into a polypeptide chain
- Ribosomes read mRNA three bases or 1 codon at a time and construct the proteins



Transcription

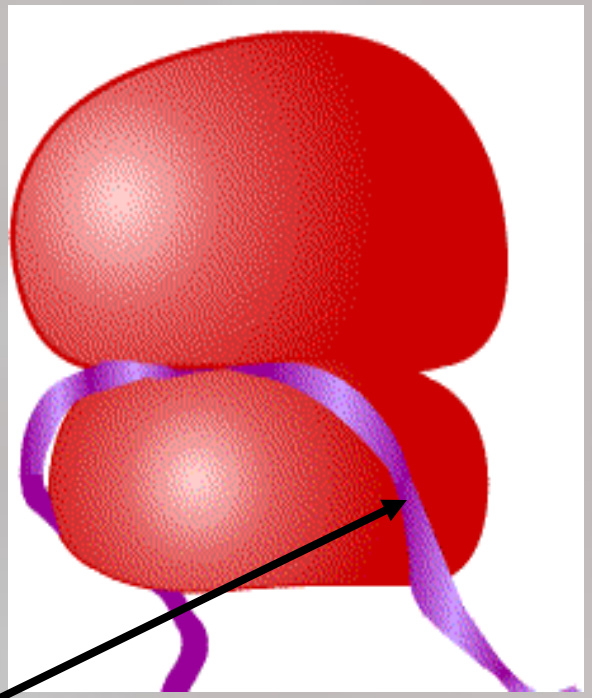
Translation

Ribosomes

- Made of a large and small subunit
- Composed of rRNA (40%) and proteins (60%)
- Have two sites for tRNA attachment --- P and A

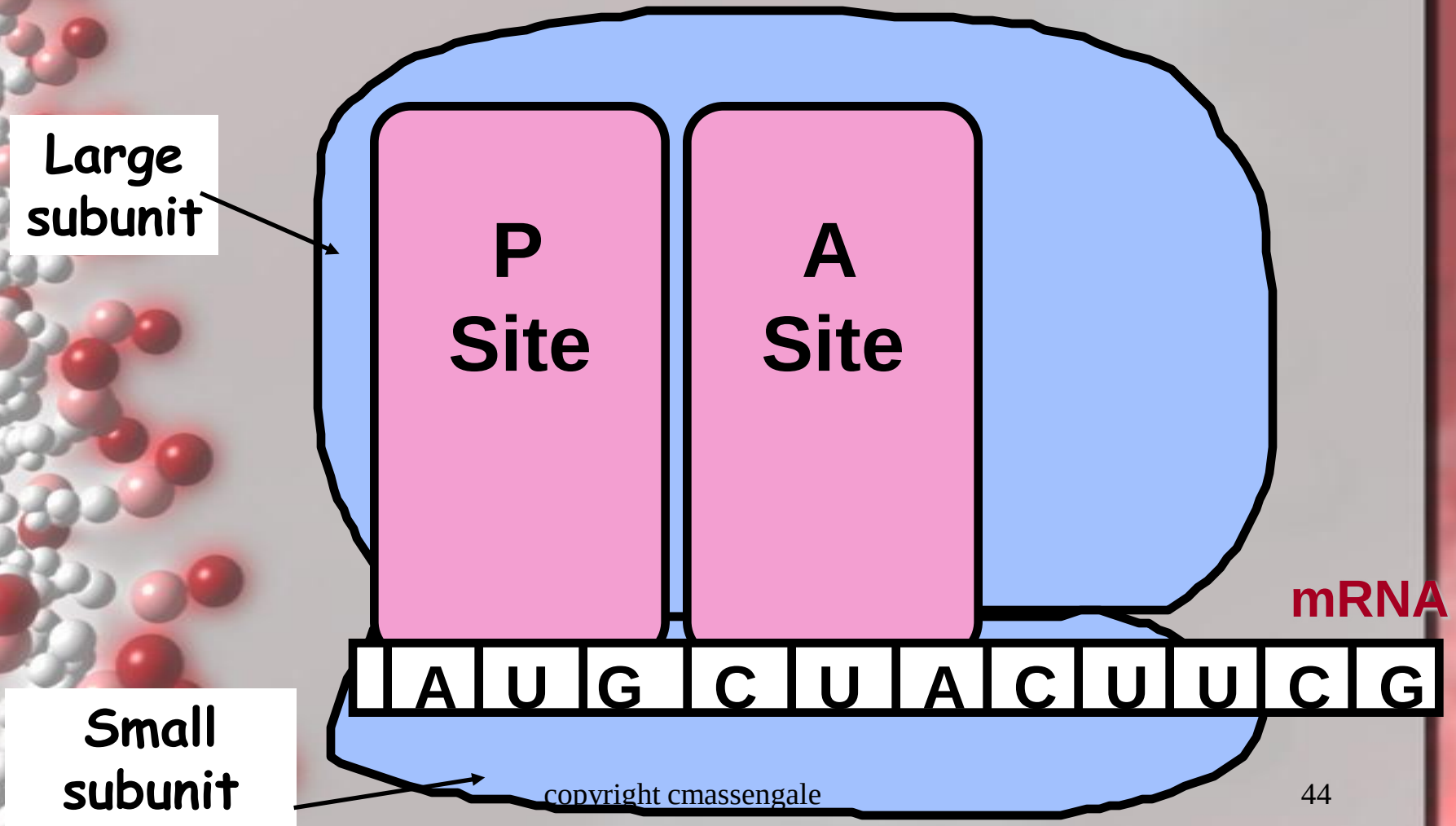
Step 1- Initiation

- mRNA transcript start codon AUG attaches to the small ribosomal subunit
- Small subunit attaches to large ribosomal subunit



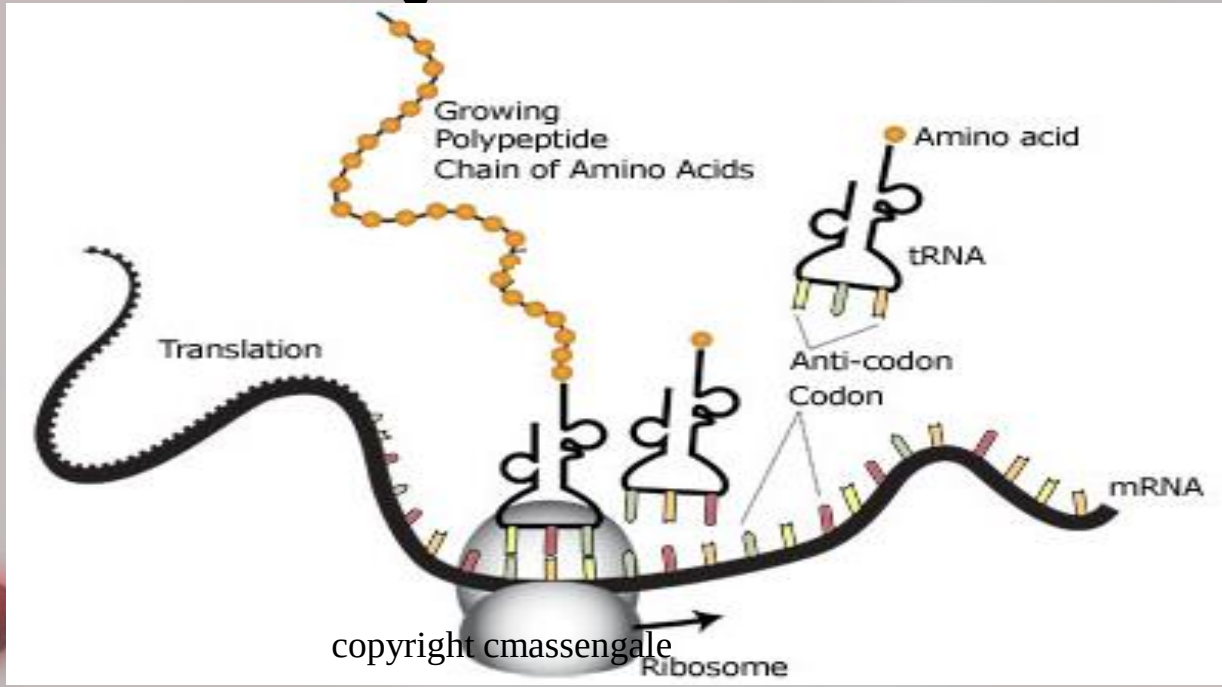
mRNA transcript

Ribosomes

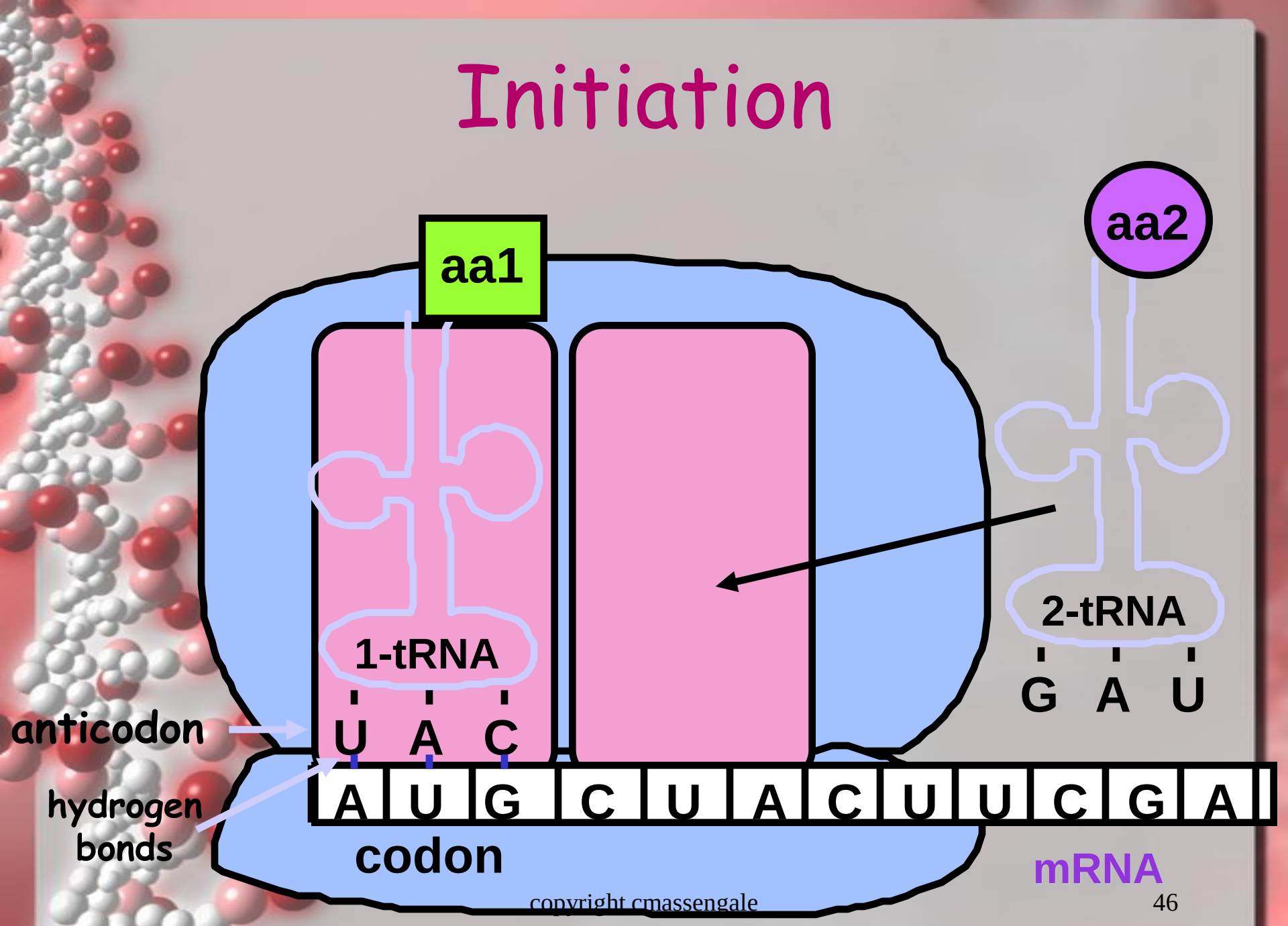


Step 2 - Elongation

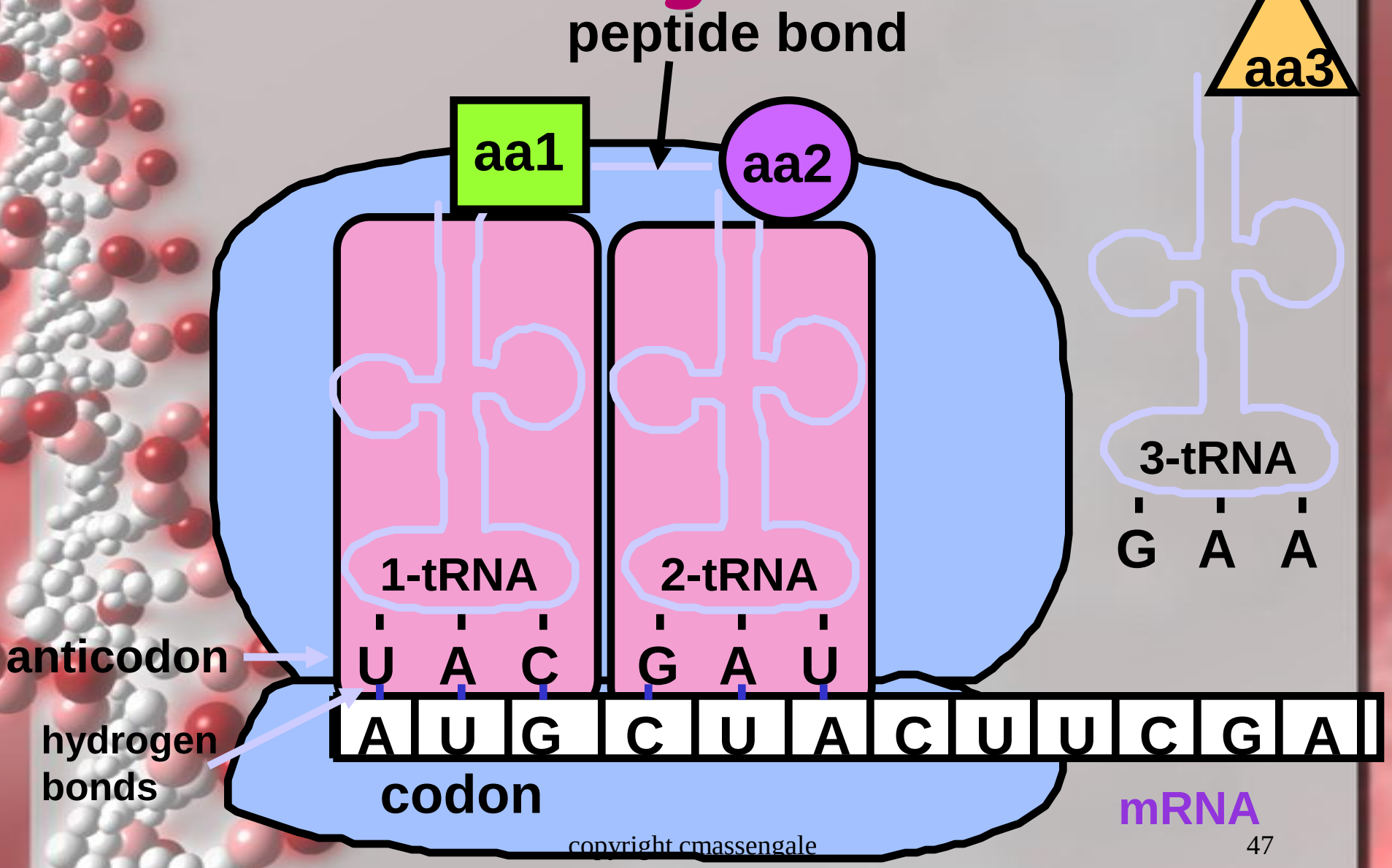
- As ribosome moves, two tRNA with their amino acids move into site A and P of the ribosome
- Peptide bonds join the amino acids

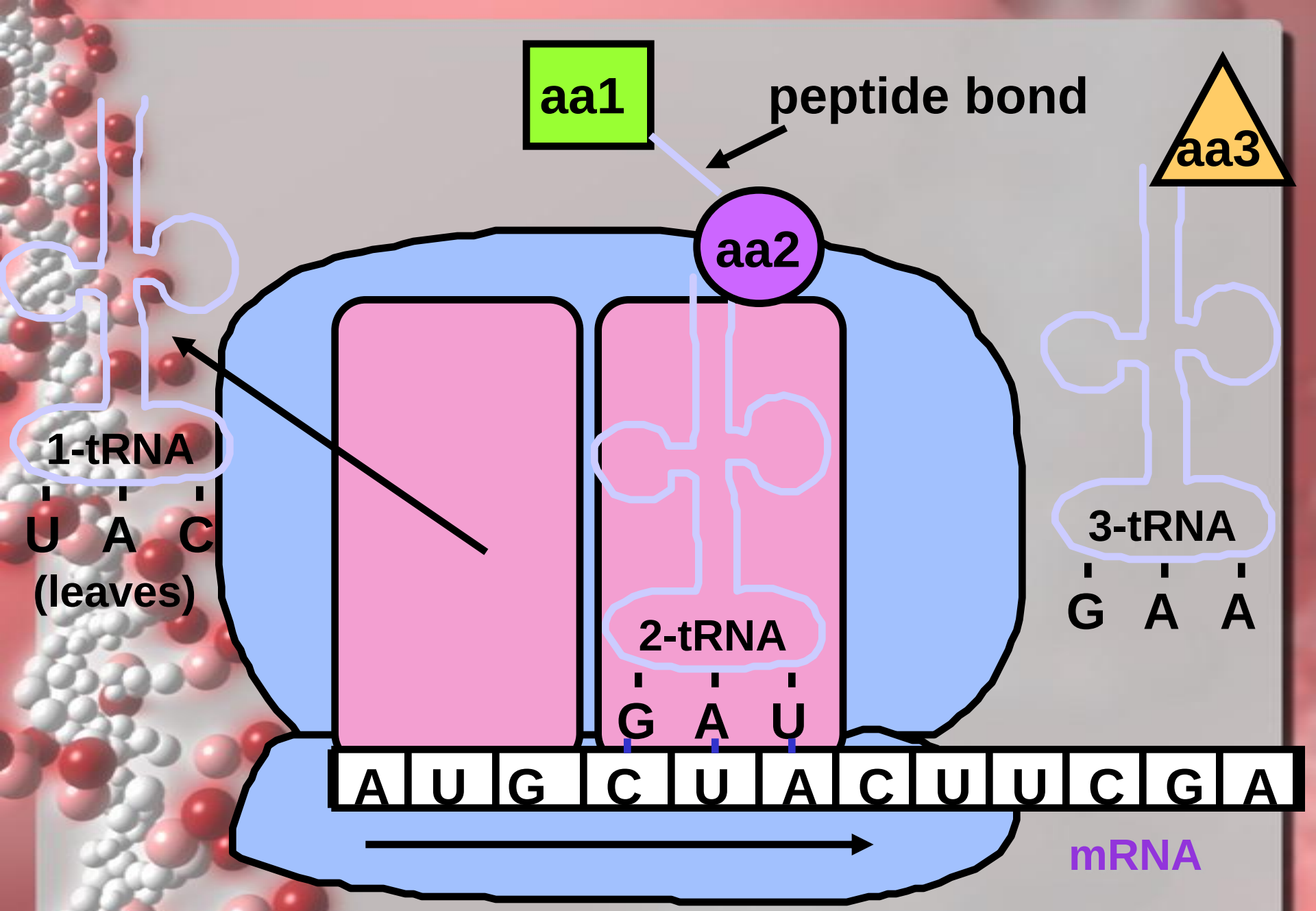


Initiation

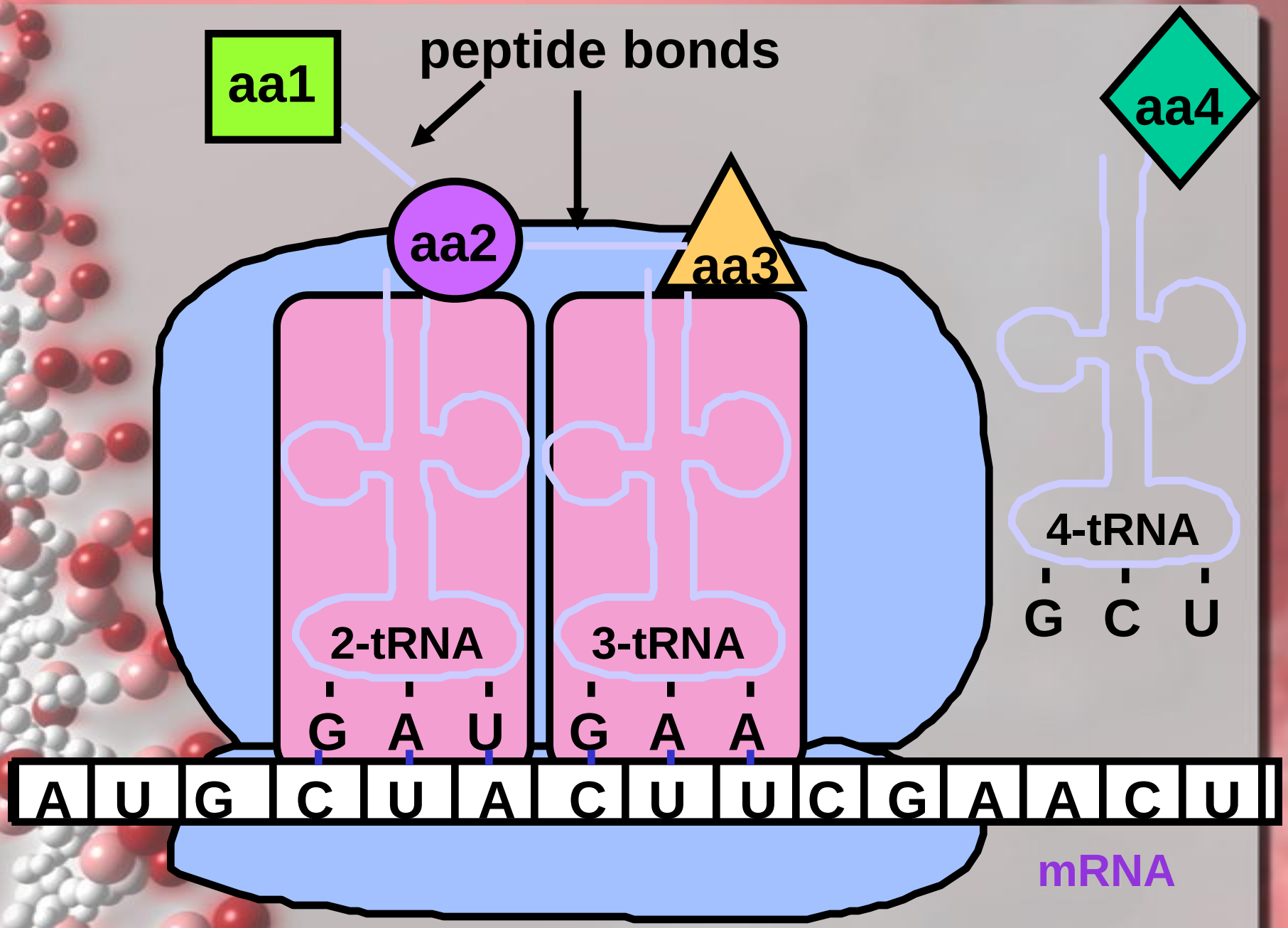


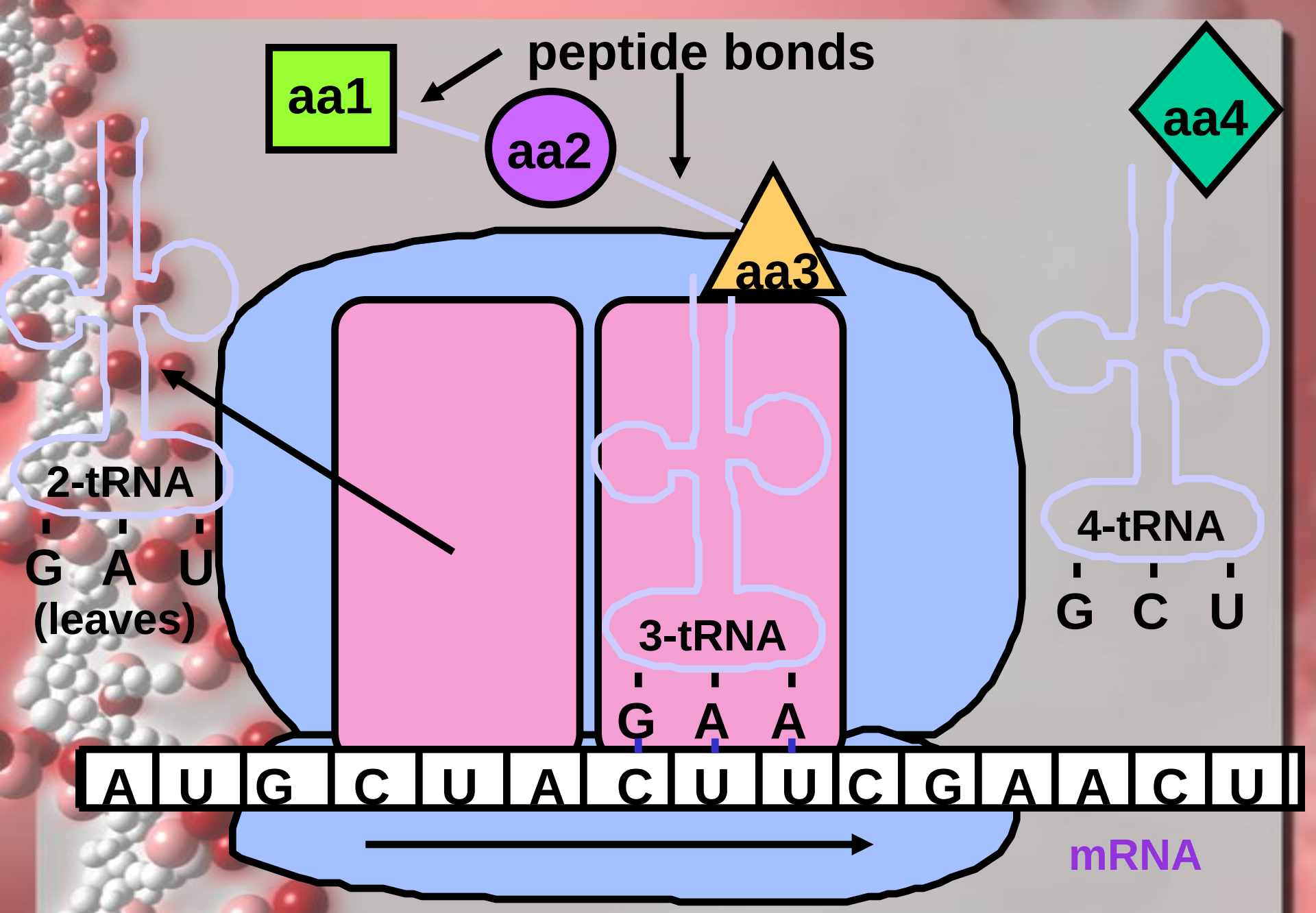
Elongation



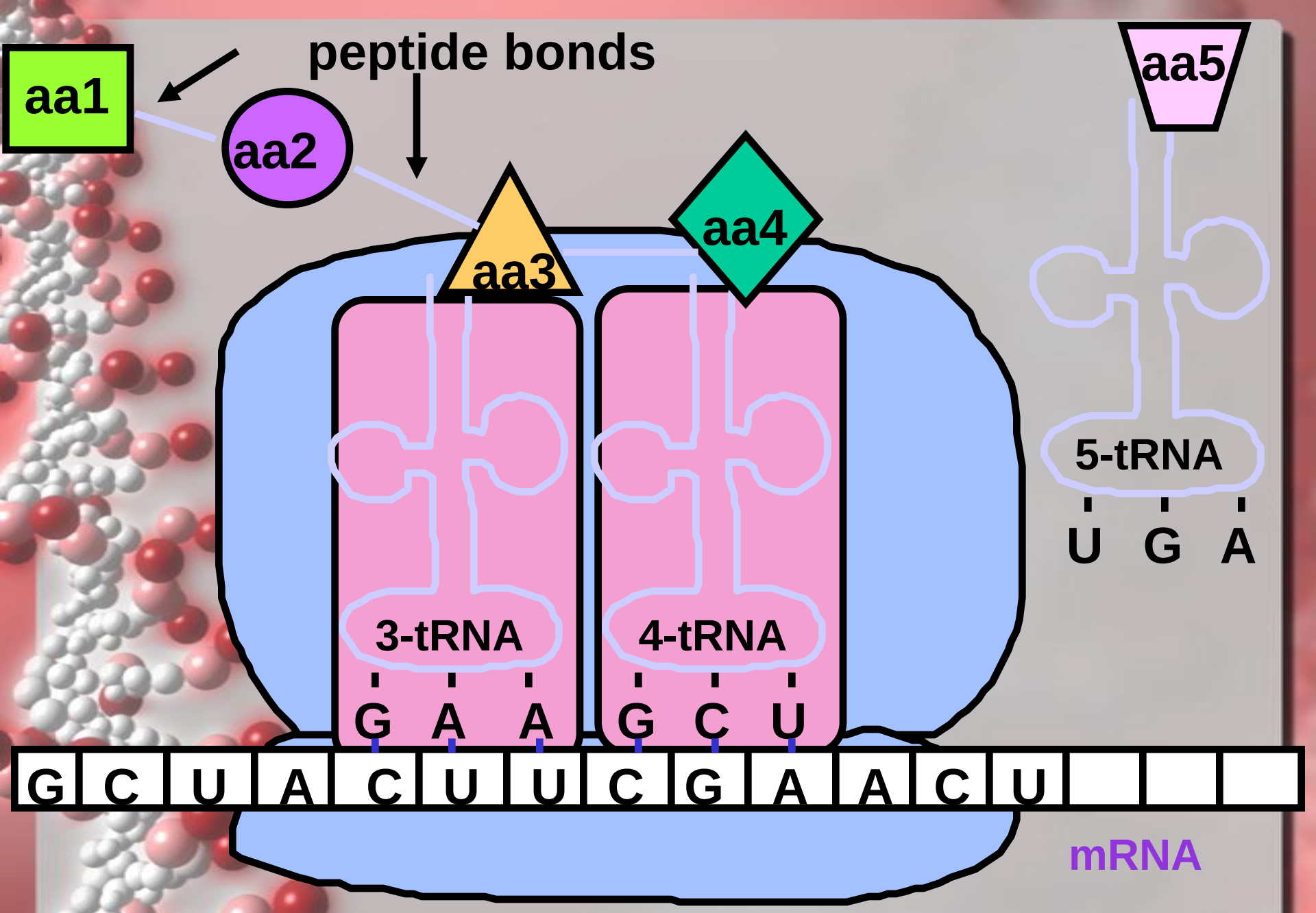


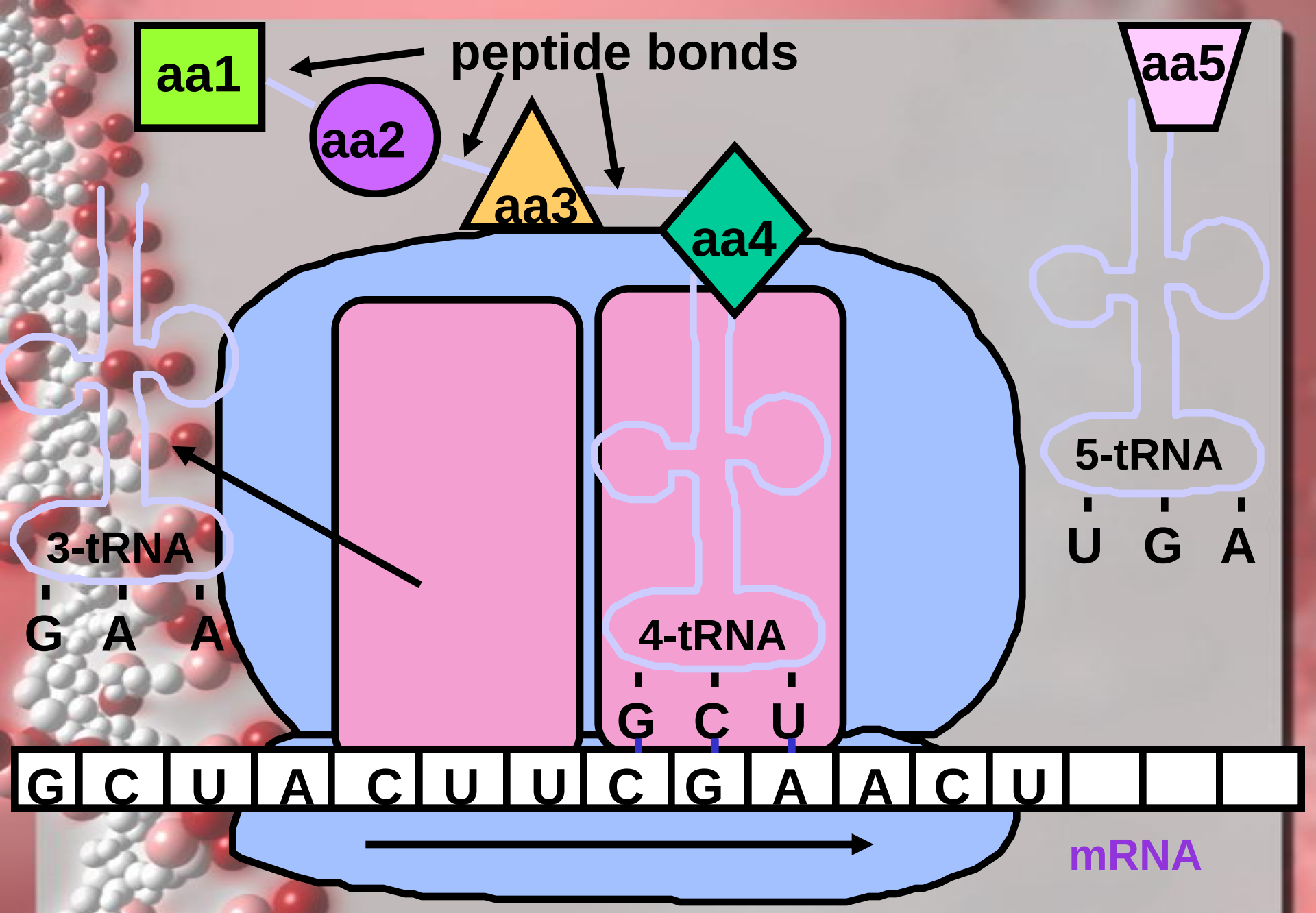
Ribosomes move over one codon


aa1
peptide bonds
aa4
aa2
aa3
4-tRNA
G C U
2-tRNA
3-tRNA
G A U
G A A
A U G C U A C U U C G A A C U
mRNA



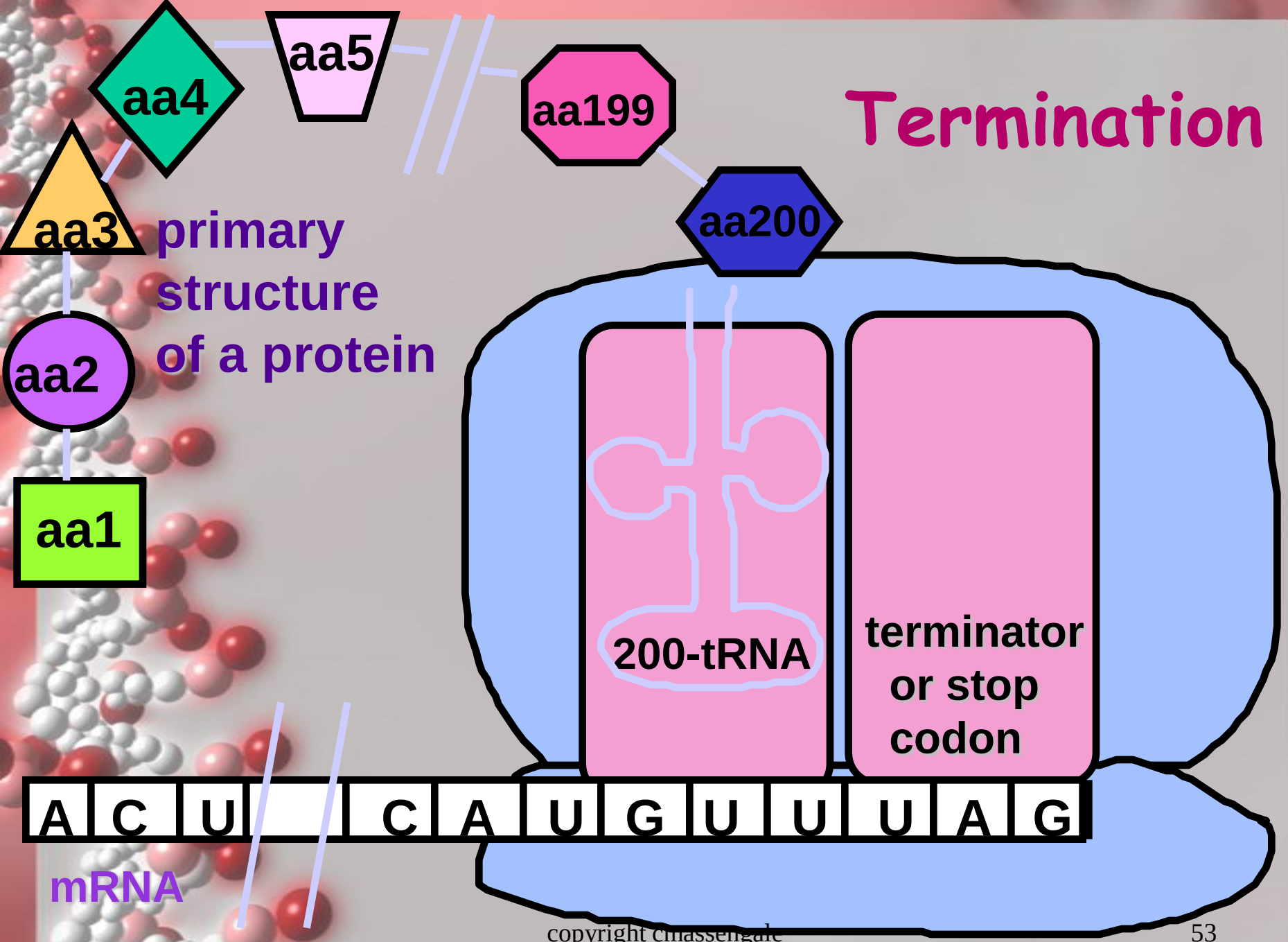
Ribosomes move over one codon


peptide bonds
aa1
aa2
aa3
aa4
aa5
3-tRNA
G A A
4-tRNA
G C U
5-tRNA
U G A
G C U A C U U C G A A C U
mRNA



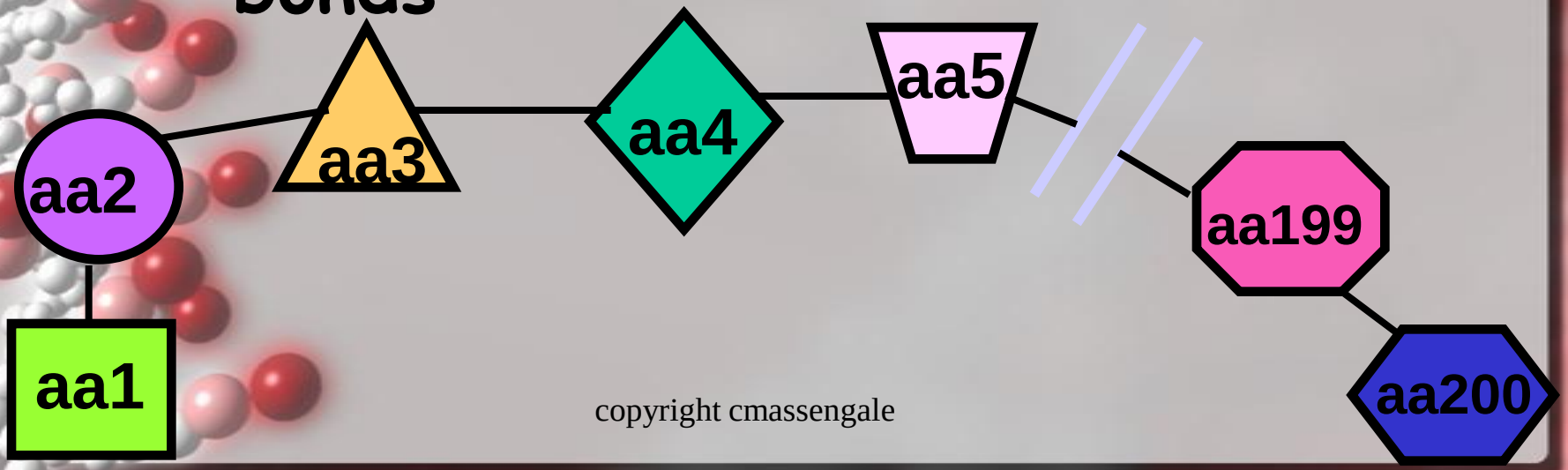
Ribosomes move over one codon

Termination

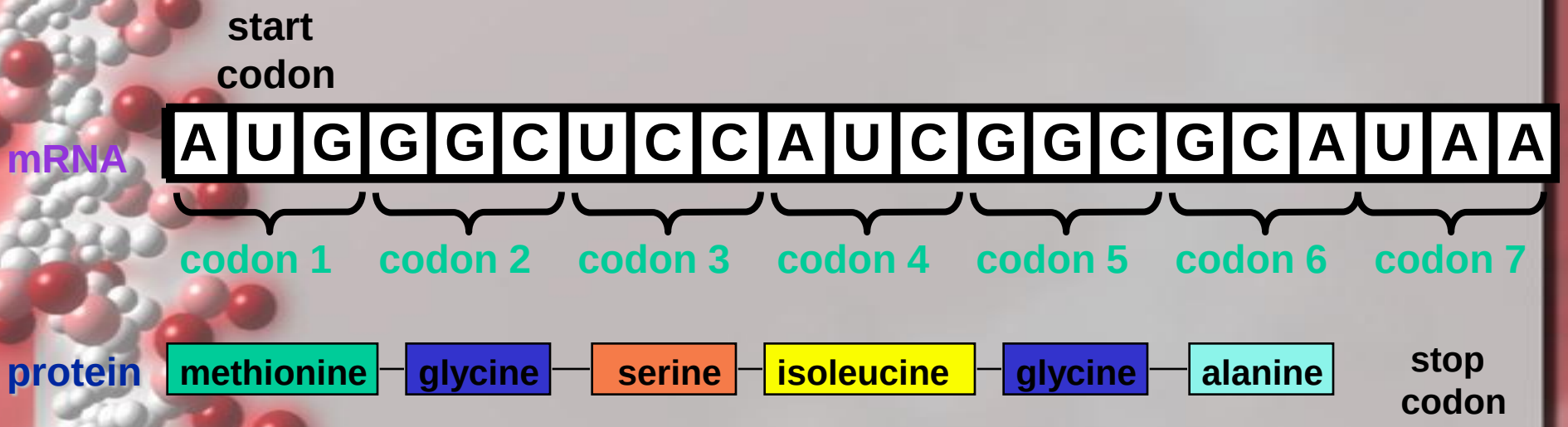


End Product –The Protein!

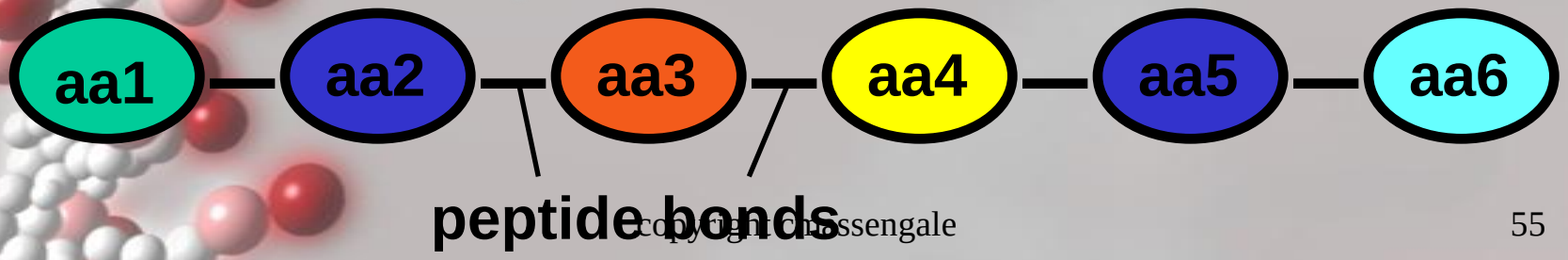
- The end products of protein synthesis is a primary structure of a protein
- A sequence of amino acid bonded together by peptide bonds

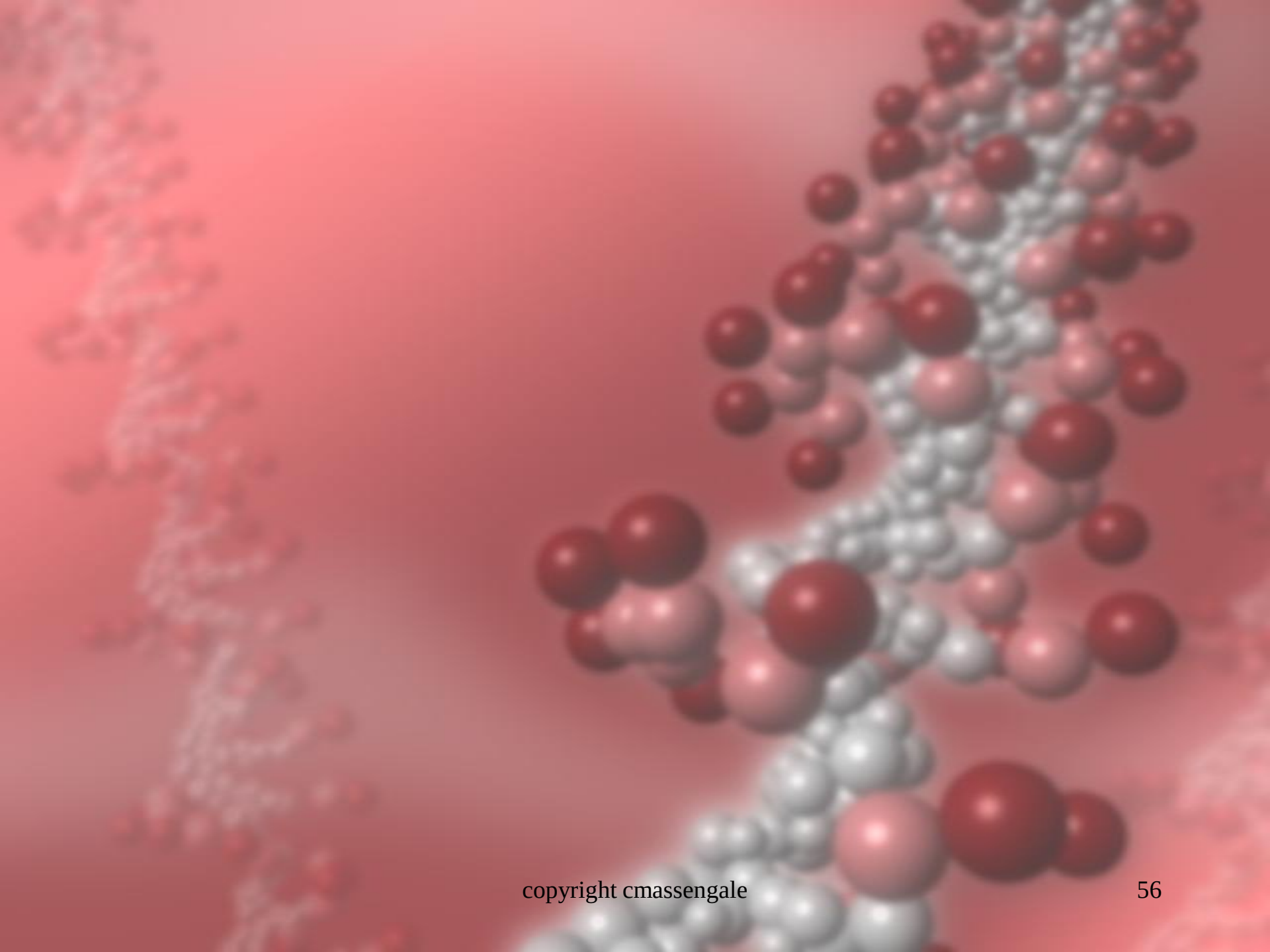


Messenger RNA (mRNA)



Primary structure of a protein

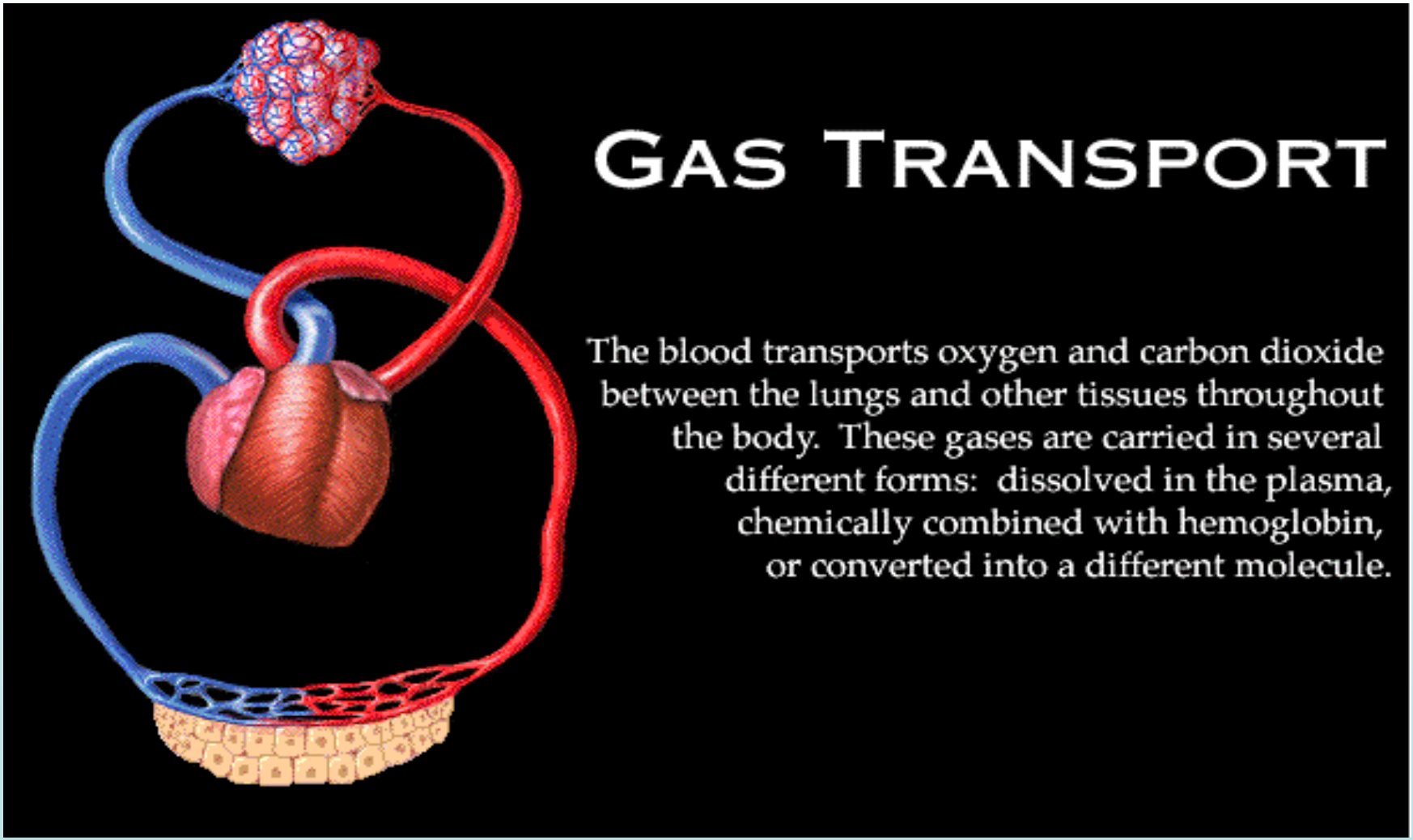




Transport of Oxygen and Carbon Dioxide

How Gases Are Transported

Introduction

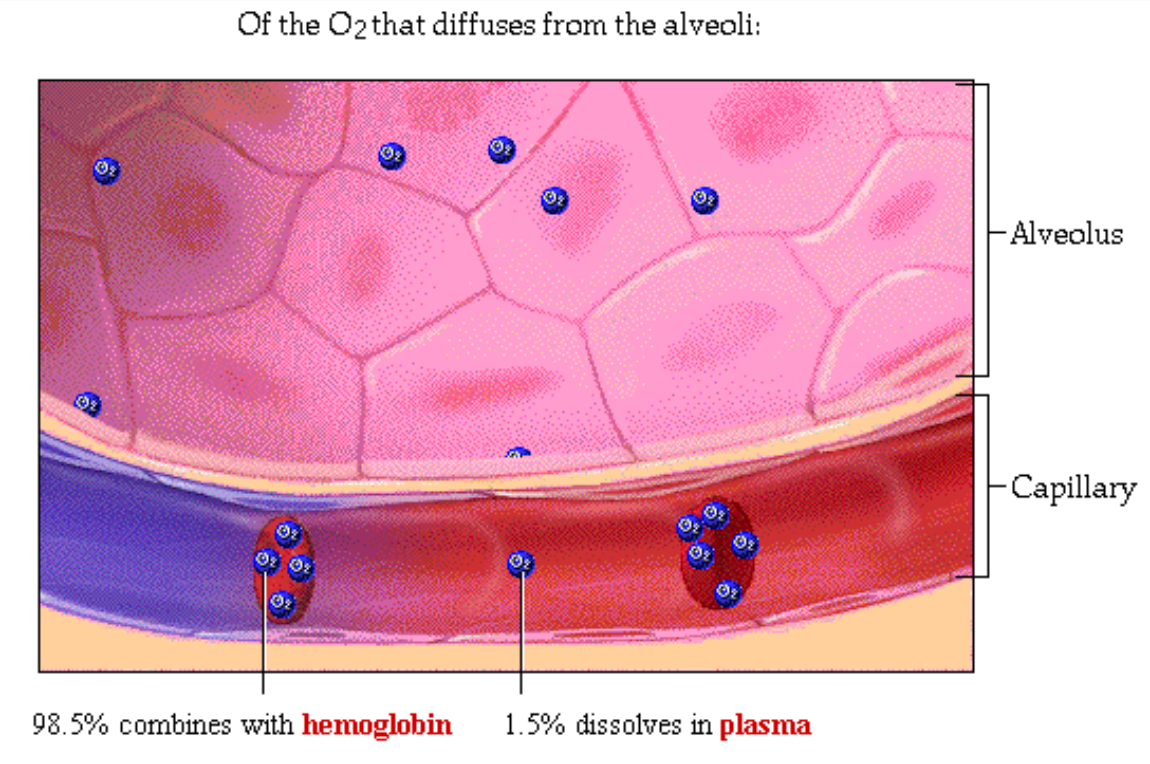


Goals For Learning

- To explore how O2 is transported in the blood.
- To explore how Co2 is transported in the blood.
- This will include understanding the oxygen dissociation curve.

- What you need to know
- Definition of partial pressure
- Processes of external respiration and internal respiration.

Oxygen Transport



- O_2 is transported by the blood either,
  - Combined with haemoglobin (Hb) in the red blood cells (>98%) or,
  - Dissolved in the blood plasma (<2%).

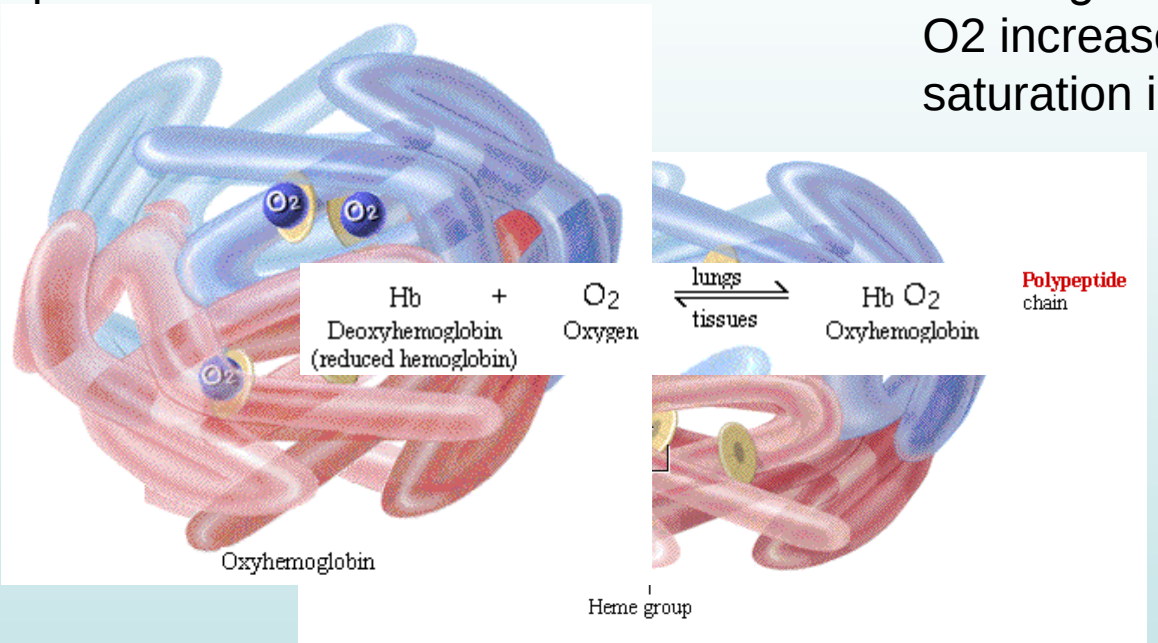
Oxygen Transport

- The resting body requires 250ml of O2 per minute.
- We have four to six billion haemoglobin containing red blood cells.
- The haemoglobin allows nearly 70 times more O_2 than dissolved in plasma.

Haemoglobin

Haemoglobin molecules can transport up to four O2's

Co-operative binding: haemoglobin's affinity for O2 increases as its saturation increases.



When 4 O2's are bound to haemoglobin, it is 100% saturated, with fewer O2's it is partially saturated.

Oxygen binding occurs in response to the high PO2 in the lungs

Lets Now Look at Haemoglobin Saturation

- Haemoglobin saturation is the amount of oxygen bound by each molecule of haemoglobin
- Each molecule of haemoglobin can carry four molecules of O2.
- When oxygen binds to haemoglobin, it forms OXYHAEMOGLOBIN;
- Haemoglobin that is not bound to oxygen is referred to as DEOXYHAEMOGLOBIN.

Haemoglobin Saturation

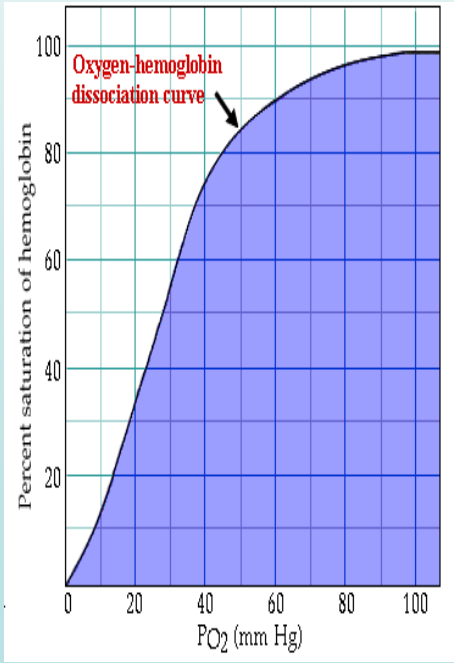
- The binding of O_2 to haemoglobin depends on the PO_2 in the blood and the bonding strength, or affinity, between haemoglobin and oxygen.
- The graph on the following page shows an oxygen dissociation curve, which reveals the amount of haemoglobin saturation at different PO_2 values.

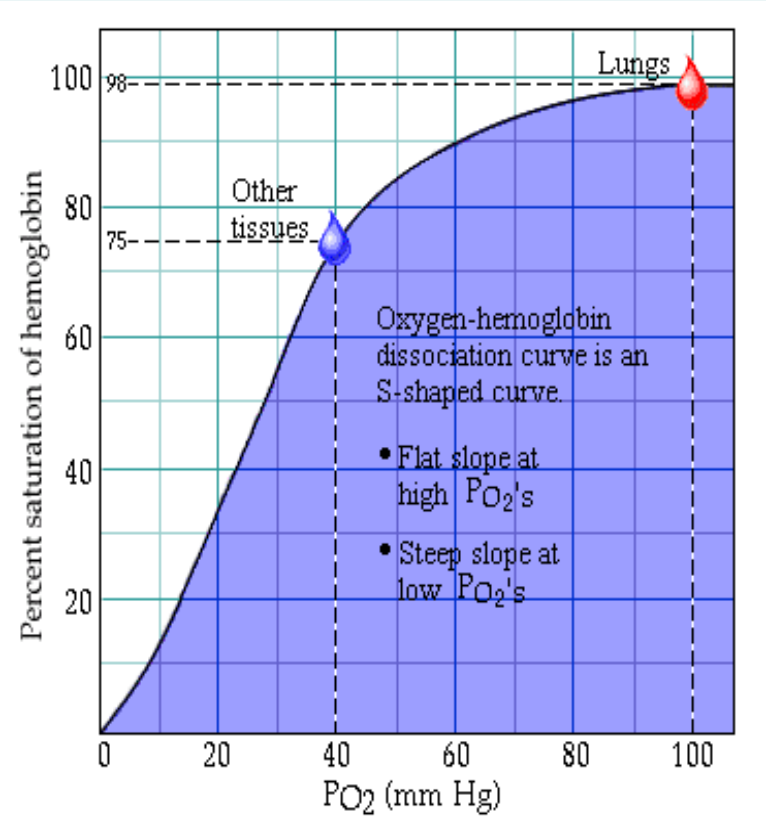
The Oxygen Dissociation Curve

- Reveals the amount of haemoglobin saturation at different PO_2 values.

The Oxygen Disassociation Curve

Haemoglobin saturation is determined by the partial pressure of oxygen. When these values are graphed they produce the Oxygen Disassociation Curve



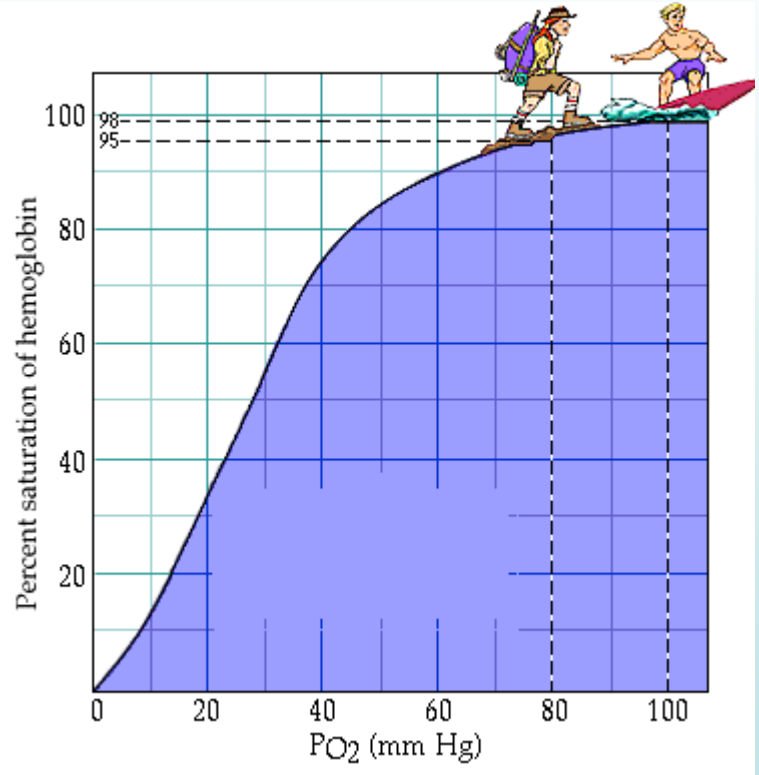


In the lungs the partial pressure is approximately 100mm Hg at this Partial Pressure haemoglobin has a high affinity to 02 and is 98% saturated.

In the tissues of other organs a typical PO2 is 40 mmHg here haemoglobin has a lower affinity for O2 and releases some but not all of its O2 to the tissues. When haemoglobin leaves the tissues it is still 75% saturated.

Haemoglobin Saturation at High Values

Lungs at sea level: PO2 of 100mmHg haemoglobin is 98% SATURATED



Lungs at high elevations: PO2 of 80mmHg, haemoglobin 95 % saturated

When the PO2 in the lungs declines below typical sea level values, haemoglobin still has a high affinity for O2 and remains almost fully saturated.

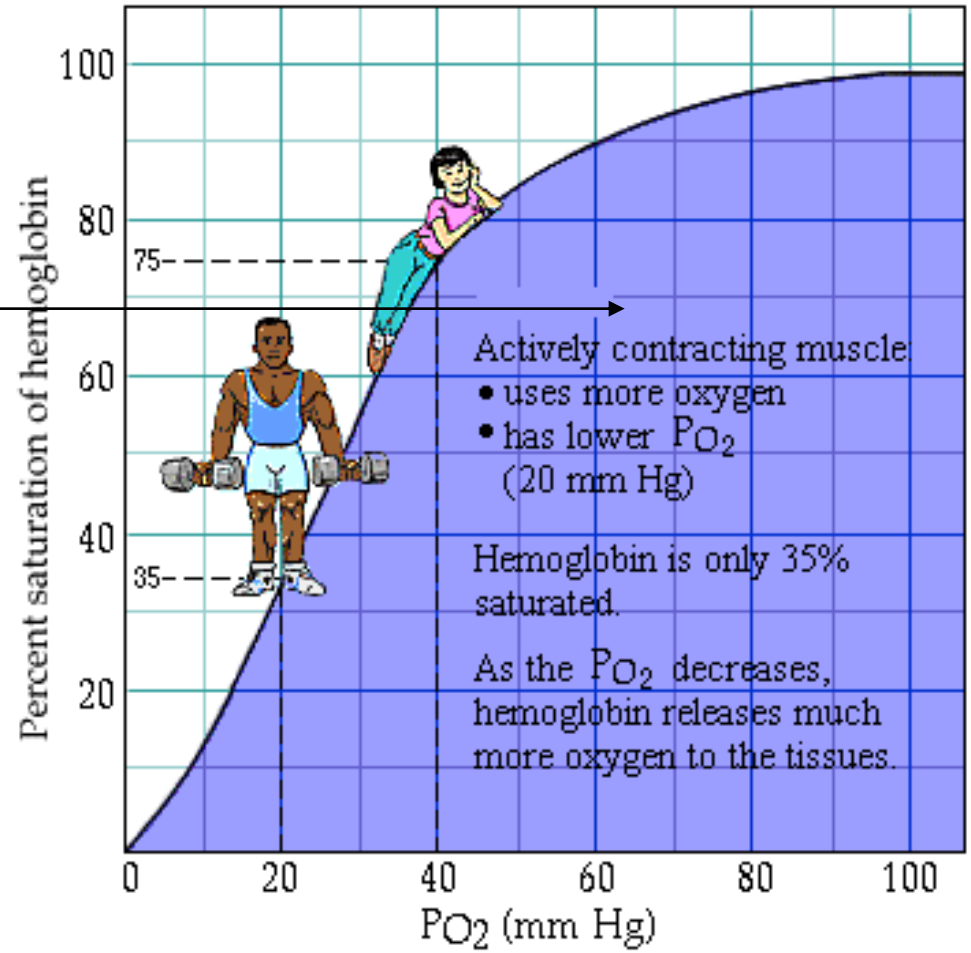
Even though PO2 differs by 20 mmHg there is almost no difference in haemoglobin saturation.

Haemoglobin Saturation at Low Values

Hemoglobin Saturation at Low PO_2's

At a P_{O_2} of 40 mm Hg, hemoglobin has a lower affinity for oxygen and is 75% saturated.

In vigorously contracting muscles, would you expect the P_{O_2} to be lower or higher than in resting muscles?



Factors Altering Haemoglobin Saturation

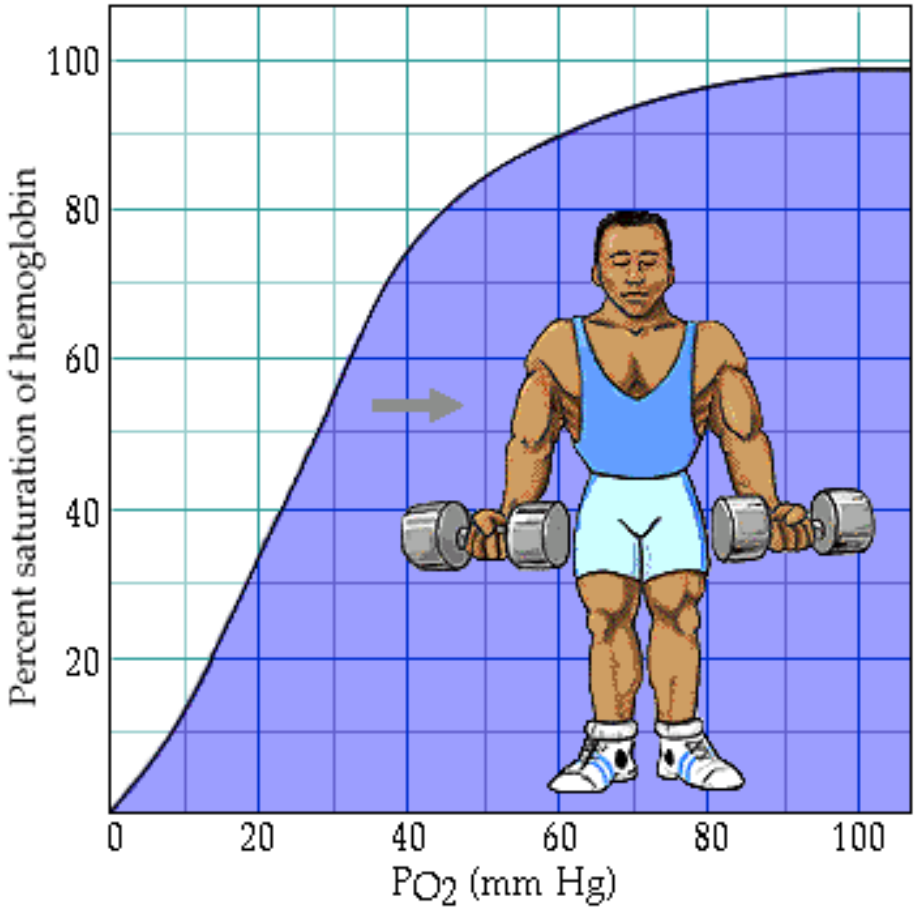
FACTORS ALTERING HEMOGLOBIN SATURATION

In addition to P_{O_2}, hemoglobin saturation is altered by four other factors:

Exercise

- ↓ **pH**
- ↑ Temperature
- ↑ P_{CO_2}

These conditions decrease hemoglobin's affinity for oxygen, releasing more oxygen to the active muscle cells.

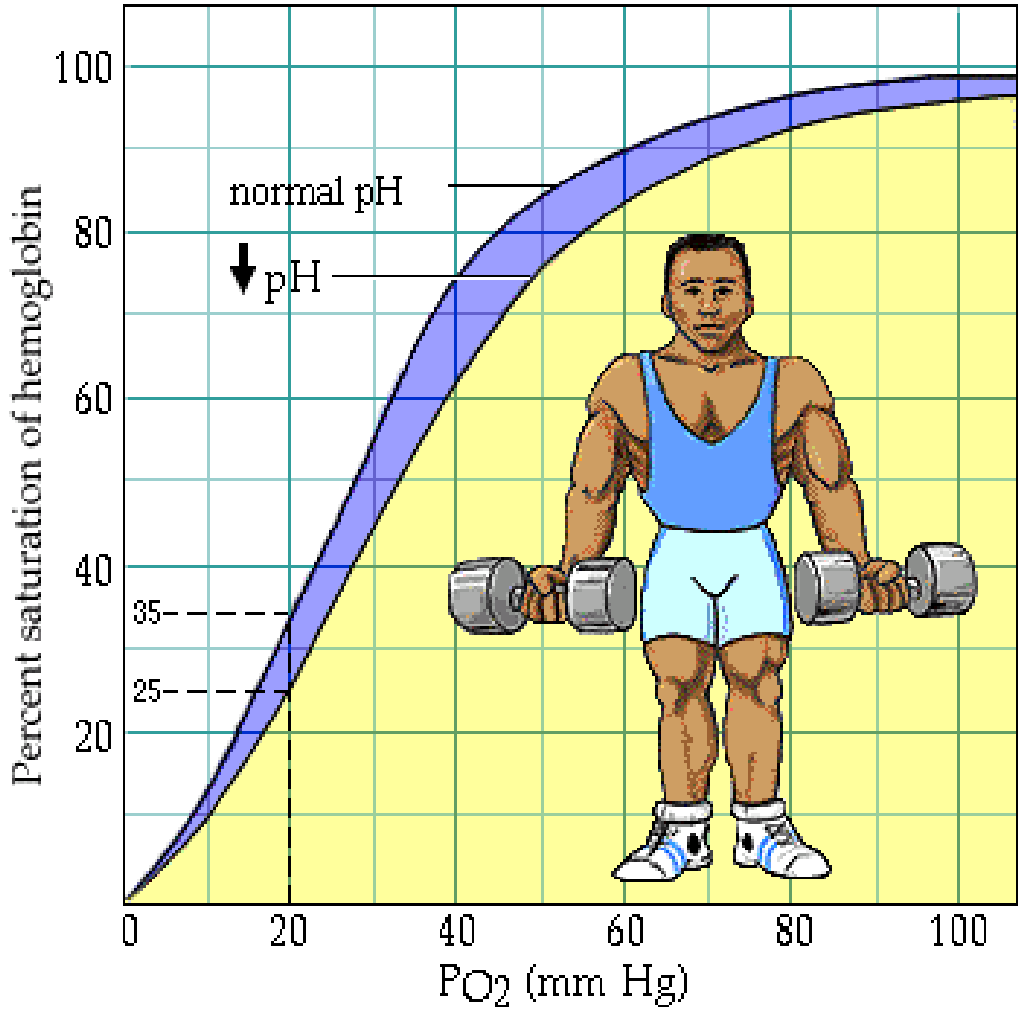


Factors Altering Haemoglobin Saturation (Exercise)

When pH decreases, the curve shifts to the right (increased oxygen unloading).

A similar shift occurs in response to:

- ↑ Temperature
- ↑ P_{CO_2}



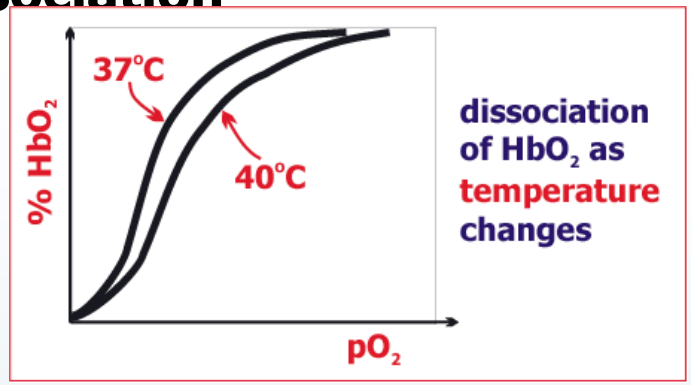
Factors Affecting Haemoglobin Saturation

- Blood acidity…
- Blood temperature…
- Carbon Dioxide concentration

Factors affecting Disassociation

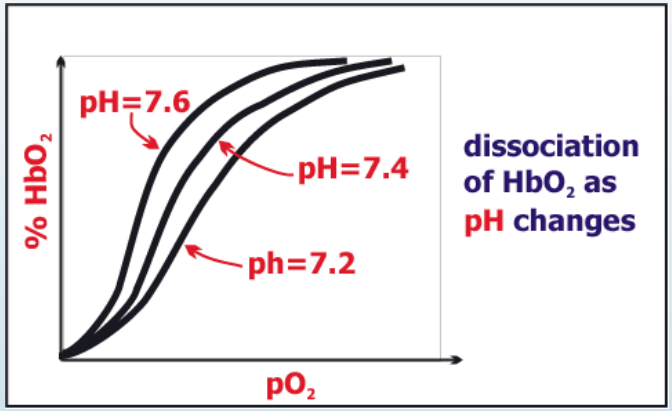
BLOOD TEMPERATURE

- **increased** blood **temperature**
- **reduces haemoglobin affinity for O_2**
- hence more O_2 is delivered to warmed-up tissue



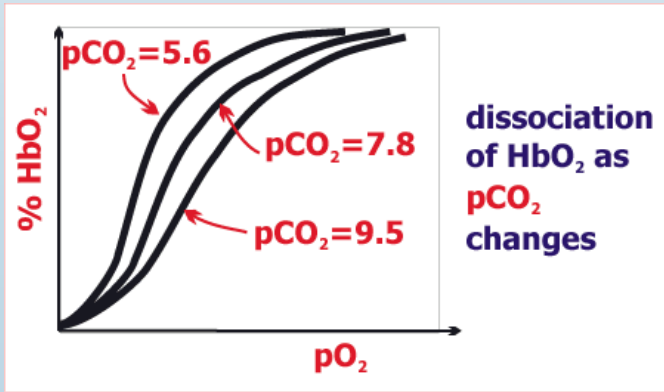
BLOOD Ph

- **lowering** of **blood pH** (making blood more acidic)
- caused by presence of H^+ ions from lactic acid or carbonic acid
- **reduces affinity of Hb for O_2**
- and more O_2 is delivered to acidic sites which are working harder



CARBON DIOXIDE CONCENTRATION

- the **higher CO_2 concentration** in tissue
- the **less the affinity of Hb for O_2**
- so the harder the tissue is working, the more O_2 is released



Key Point

- Increased temperature and hydrogen ion (H^+) (pH) concentration in exercising muscle affect the oxygen dissociation curve, allowing more oxygen to be uploaded to supply the active muscles.

Carbon Dioxide Transport

- Carbon dioxide also relies on the blood fro transportation. Once carbon dioxide is released from the cells, it is carried in the blood primarily in three ways…
- Dissolved in plasma,
- As bicarbonate ions resulting from the dissociation of carbonic acid,
- Bound to haemoglobin.

Dissolved Carbon Dioxide

- Part of the carbon dioxide released from the tissues is dissolved in plasma. But only a small amount, typically just 7 – 10%, is transported this way.
- This dissolved carbon dioxide comes out of solution where the P_{CO_2} is low, such as in the lungs.
- There it diffuses out of the capillaries into the alveoli to be exhaled.

In Review

1) Oxygen is transported in the blood primarily bound to haemoglobin though a small amount is dissolved in blood plasma.
2) Haemoglobin oxygen saturation decreases.
    1) When PO2 decreases.
    2) When pH decreases.
    3) When temperature increases.

In Review

Each of these conditions can reflect increased local oxygen demand. They increase oxygen uploading in the needy area.

3) Haemoglobin is usually about 98% saturated with oxygen. This reflects a much higher oxygen content than our body requires, so the blood's oxygen-carrying capacity seldom limits performance.

In Review

4) Carbon dioxide is transported in the blood primarily as bicarbonate ion. This prevents the formation of carbonic acid, which can cause H+ to accumulate, decreasing the pH. Smaller amounts of carbon dioxide are carried either dissolved in the plasma or bound to haemoglobin

Transport of carbon dioxide

- Transport of carbon dioxide in the blood is considerably more complex. A small portion of carbon dioxide, about 5 percent, remains unchanged and is transported dissolved in blood. The remainder is found in reversible chemical combinations in red blood cells or plasma. Some carbon dioxide binds to blood proteins,

Less than 10 percent of the total quantity of carbon dioxide carried in the blood is eliminated during passage through the lungs. Complete elimination would lead to large changes in acidity between arterial and venous blood. Furthermore, blood normally remains in the pulmonary capillaries less than a second, an insufficient time to eliminate all carbon dioxide.

Transport of CO_2

- principally hemoglobin, to form a compound known as carbamate. About 88 percent of carbon dioxide in the blood is in the form of bicarbonate ion. The distribution of these chemical species between the interior of the red blood cell and the surrounding plasma varies greatly, with the red blood cells containing considerably less bicarbonate and more

Transport of CO_2

- carbamate than the plasma.
- Carbon dioxide enters blood in the tissues because its local partial pressure is greater than its partial pressure in blood flowing through the tissues. As carbon dioxide enters the blood, it combines with water to form carbonic acid (H_2CO_3), a relatively weak acid, which dissociates into

Transport of CO_2

- hydrogen ions (H^+) and bicarbonate ions (HCO_3^-). Blood acidity is minimally affected by the released hydrogen ions because blood proteins, especially hemoglobin, are effective buffering agents. (A buffer solution resists change in acidity by combining with added hydrogen ions and, essentially, inactivating them.) The natural conversion of carbon dioxide to

carbonic acid is a relatively slow process; however,

carbonic anhydrase, enzyme found in red blood cells, gastric mucosa, the enzyme is present only inside the red blood cell, pancreatic cells, and renal tubules that catalyzes the interconversion of carbon dioxide (CO_2) and carbonic acid (H_2CO_3). Carbonic anhydrase plays an important role in respiration by influencing CO_2 transport in the blood

Transport of CO_2

- The capacity of blood to carry carbon dioxide as bicarbonate is enhanced by an ion transport system inside the red blood cell membrane that simultaneously moves a bicarbonate ion out of the cell and into the plasma in exchange for a chloride ion. The simultaneous exchange of these two ions, known as the chloride shift, permits the plasma to be used as a storage site for bicarbonate without

Chloride shift

- **Chloride shift** (also known as the Hamburger's shift or Hamburger's phenomenon, named after Hartog Jakob Hamburger) is a process which occurs in a cardiovascular system and refers to the exchange of bicarbonate (HCO_3^-) and chloride (Cl^-) across the membrane of red blood cells (RBCs

partial pressures. RBCs contain appreciable quantities of carbonic anhydrase, an enzyme which converts CO_2 to carbonic acid and which is not highly expressed in interstitial fluid and plasma. RBC carbonic anhydrase converts dissolved CO_2 and intracellular water to carbonic acid (H_2CO_3), which spontaneously dissociates to form bicarbonate (HCO^-_3) and a hydrogen ion (H^+). In response to the fall of intracellular PCO_2, more CO_2 passively diffuses into the cell.

Red blood cell membranes are impermeable to hydrogen ions but are able to exchange bicarbonate ions for chloride ions using the anion exchanger protein. A rise in intracellular bicarbonate causes chloride intake and bicarbonate export. The term "chloride shift" refers to this exchange. As a result, blood chloride concentration is lower in systemic venous blood than in systemic arterial blood or in pulmonary circulation because the levels of CO_2 and therefore bicarbonate are higher in systemic venous blood, providing less of a driving force for exchange.

The opposite process occurs in the pulmonary capillaries of the lungs when the PO_2 rises and PCO_2 falls, and the Haldane effect occurs (release of CO_2 from hemoglobin during oxygenation). This releases hydrogen ions from hemoglobin, increases H^+ concentration within RBCs, and shifts the equilibrium towards CO_2 and water formation from bicarbonate. The subsequent decrease in intracellular bicarbonate concentration reverses chloride-bicarbonate exchange. Inward movement of bicarbonate via the Band 3 exchanger allows carbonic anhydrase to convert it to CO_2 for expiration.[2]

Transport of CO_2

- changing the electrical charge of either the plasma or the red blood cell. Only 26 percent of the total carbon dioxide content of blood exists as bicarbonate inside the red blood cell, while 62 percent exists as bicarbonate in plasma; however, the bulk of bicarbonate ions is first produced inside the cell, then transported to the plasma. A reverse sequence of reactions occurs when blood reaches the lung, where the partial pressure of carbon dioxide is lower than in the blood.

- Thank you