ORGANOGENESIS

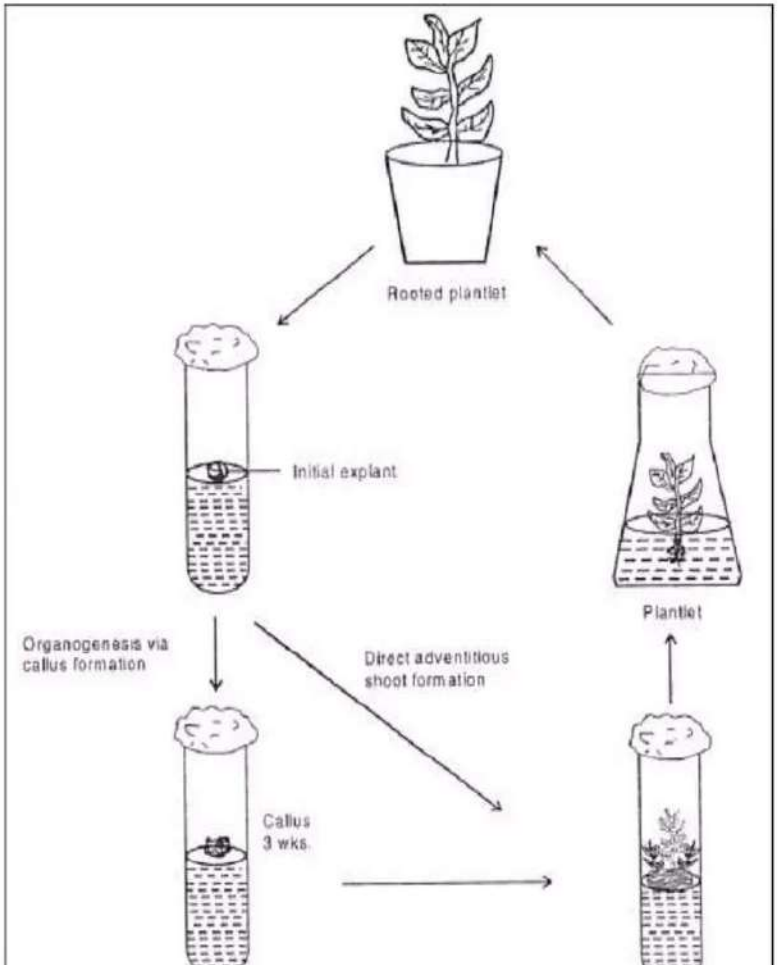
Presentation by Shruti S. Jitkar
For Class- B.Sc. III Biotechnology Optional

PLANT TISSUE CULTURE

- **Plant tissue culture** is a collection of techniques used to maintain or grow in plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition
- Plant tissue culture is widely used to produce clones of a plant in a method known as **Micropropagation**
- Three common pathways of plant tissue culture regeneration are:
  a. **Propagation from preexisting meristems (shoot culture or nodal culture)**
  b. **Organogenesis**

ORGANOGENESIS

- A plant contains many organs like meristem, cortex, phloem, epidermis are consist of structural unit called **Cell**
- An cell have to nature of create whole plant like any organ or tissue of plant also show same nature mean they also create to whole plant in *in-vitro* condition.
- If plant organs used in *in-vitro*



DEFINITION OF ORGANOGENESIS

The ability of non-meristematic plant tissue to form various organs de-novo is termed as Organogenesis

Or

The development of adventitious organs or primordial from undifferentiated cell mass in tissue by the process of differentiation is called Organogenesis

Or

From cell of tissue culture various organs such as roots, stem, leaves or flower may be initiated, is called Organogenesis

Or

The formation of roots, shoots or flower buds from the cells in culture in manner similar to adventitious root or shoot formation in cuttings is called Organogenesis

ORGANOGENESIS

- **Caulogenesis**: Adventitious shoot bud initiation takes place in callus tissue for organogenesis culturing

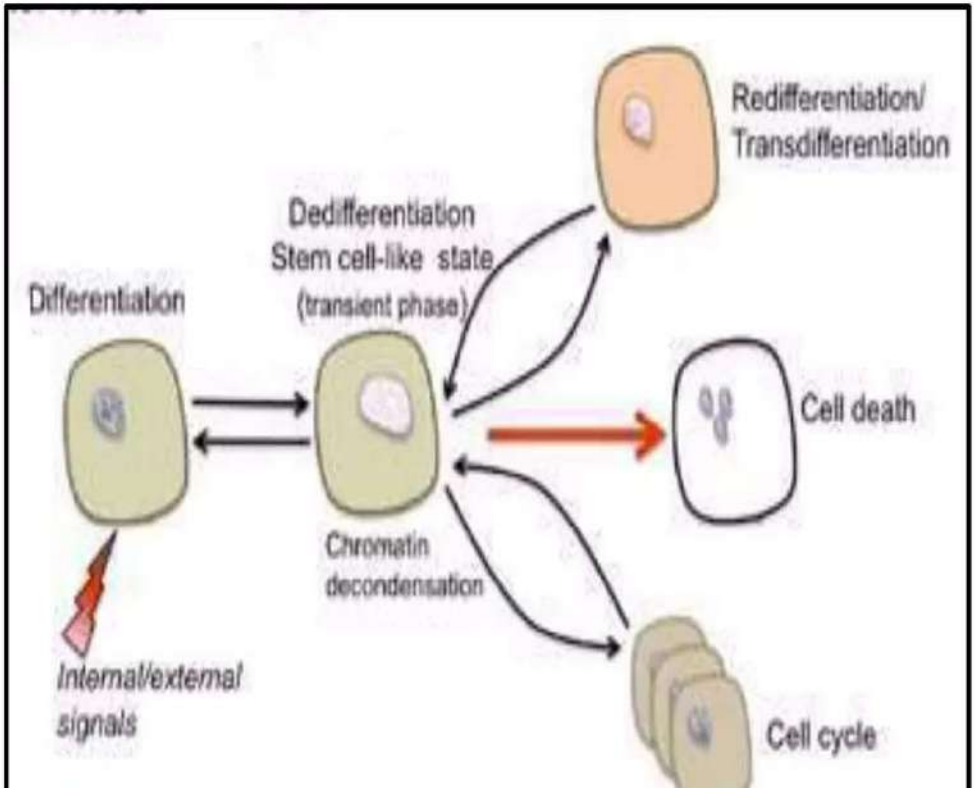
- **Rhizogenesis**: Adventitious root formation takes place in callus tissue for organogenesis culturing

↑ Auxin : ↓ Cytokinins = Increased ROOT growth

↓ Auxin : ↑ Cytokinins = Increased SHOOT growth

STAGES OF ORGANOGENESIS

- Organs are formed in two stages:
  - Dedifferentiation
  - Redifferentiation



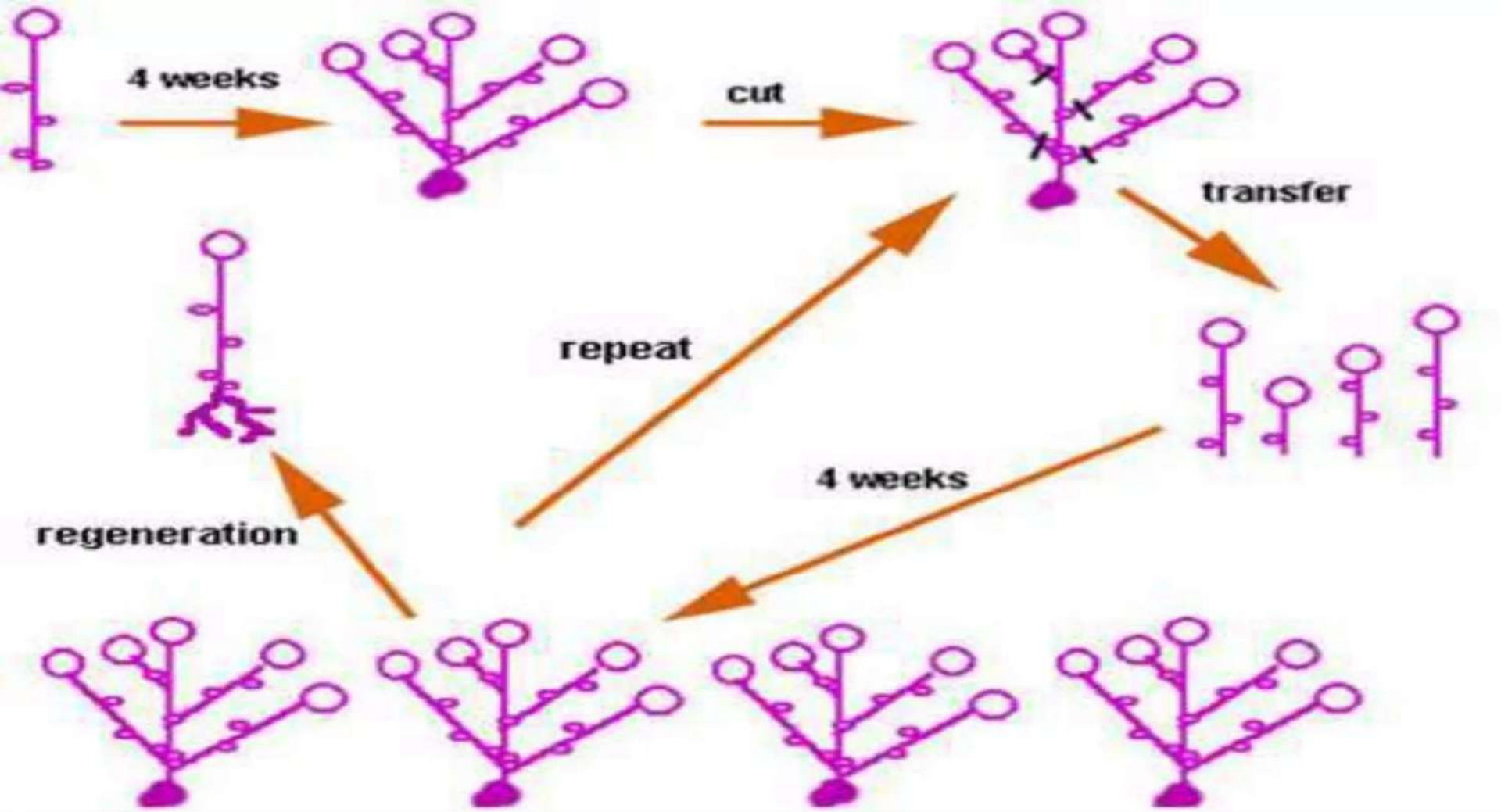
TYPES OF ORGANOGENESIS

- In plant tissue culture, undifferentiated tissue is referred to as callus although a callus can contain meristematic nodules that may not be obvious to the naked eye but which never develop further unless suitable conditions are supplied.

- Development of organized structure can follow in the three pathways:

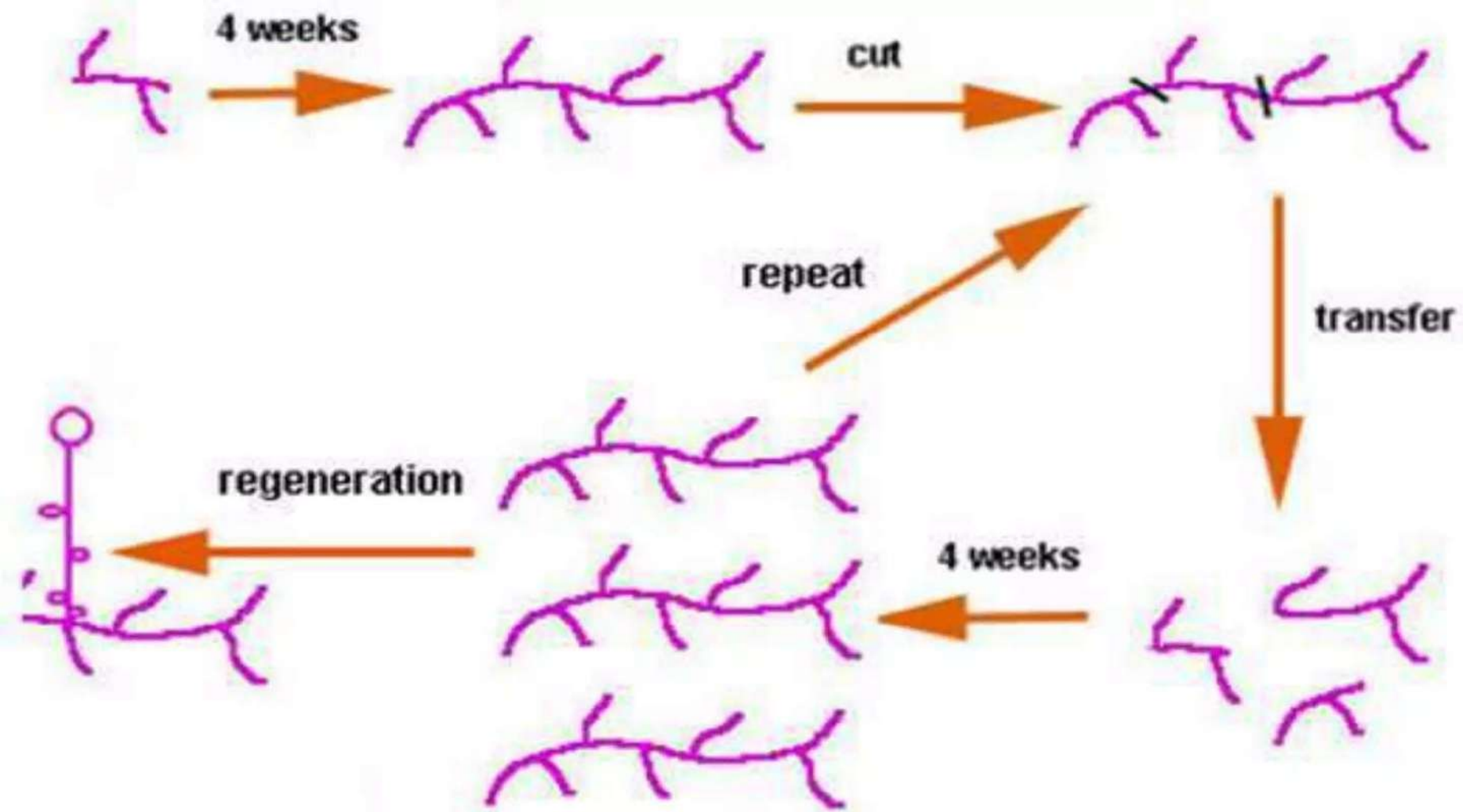
  a. **Shoot regeneration**: Based on a unipolar structure with a shoot apical meristem

  b. **Root regeneration**: Based on a unipolar structure with a root apical meristem



ORGANOGENESIS *VIA* SHOOT CULTURE

Formation of shoot organ



ORGANOGENESIS *VIA* ROOT CULTURE

Formation of root organ

TYPES OF ORGANOGENESIS

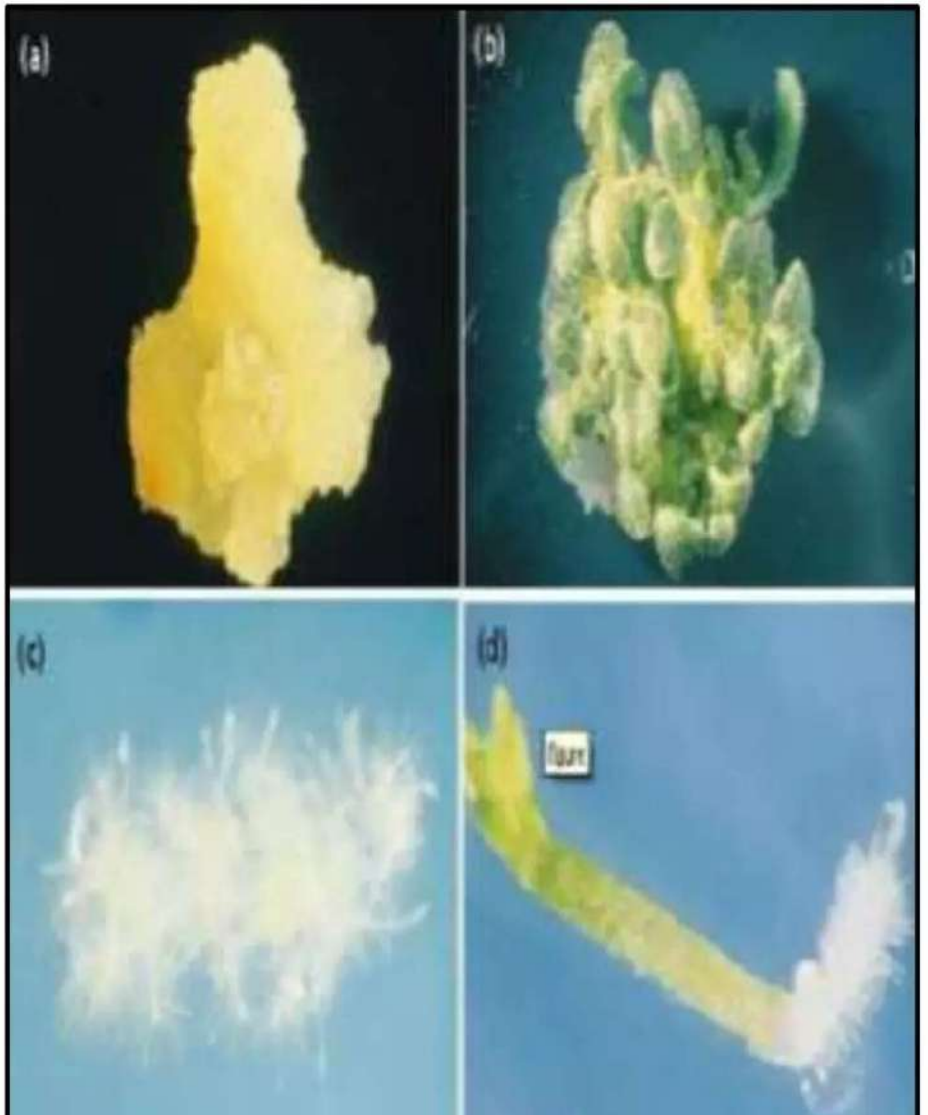
Types of Organogenesis:

(a.) Disorganized callus

(b.) Shoot regeneration

(c.) Root regeneration

(d.) A single somatic embryo



TYPES OF ORGANOGENESIS

In-vitro culturing method of organogenesis are as follows:

a. Indirect organogenesis
b. Direct organogenesis

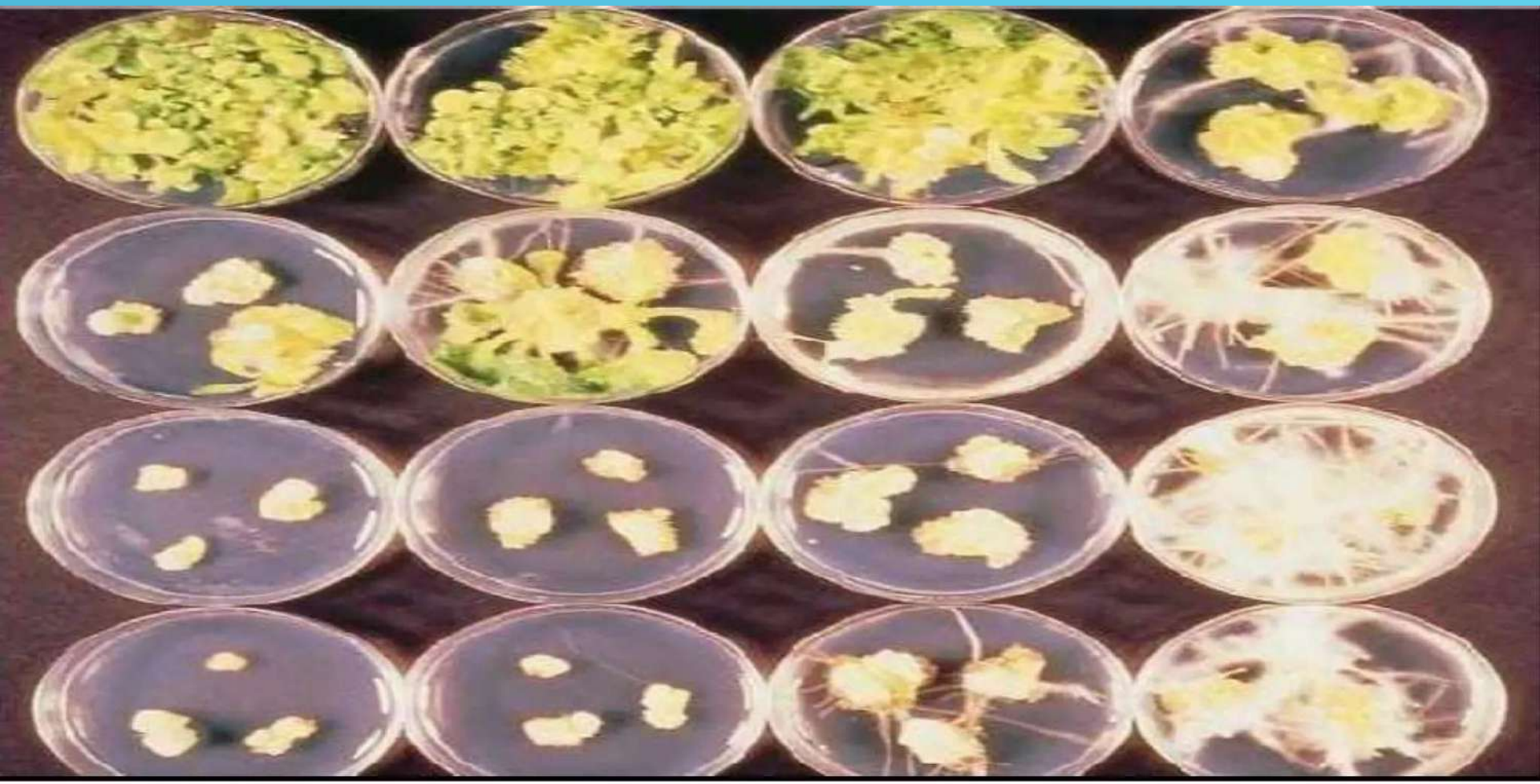
INDIRECT ORGANOGENESIS

- Plant organ formation on callus tissue derived from explants plant growth regulators and differentiation

  Explant -----→ Callus -------→ Meristemoid -------→ Primordium

Skoog and Miller, the direction of differentiation could be influenced by the ratio of the exogenously supplied growth regulators Auxin and cytokinins

- They observed in tobacco stem pith cultures that a high ratio of Auxin to cytokinins led to initiation of roots whereas a low ratio led to development of shoots
- Although there are many species for which this simple manipulation will not work.



INDIRECT ORGANOGENESIS: TOBACCO LEAF EXPLANT CULTURE

Tobacco leaf explants cultured on media with varying concentrations of an Auxin [NAA] and a

DIRECT ORGANOGENESIS

- Formation of organs directly on the surface of cultured intact explants, this process is known as **Direct organogenesis**

Explant -------→ Meristemoid -------→ Primordium

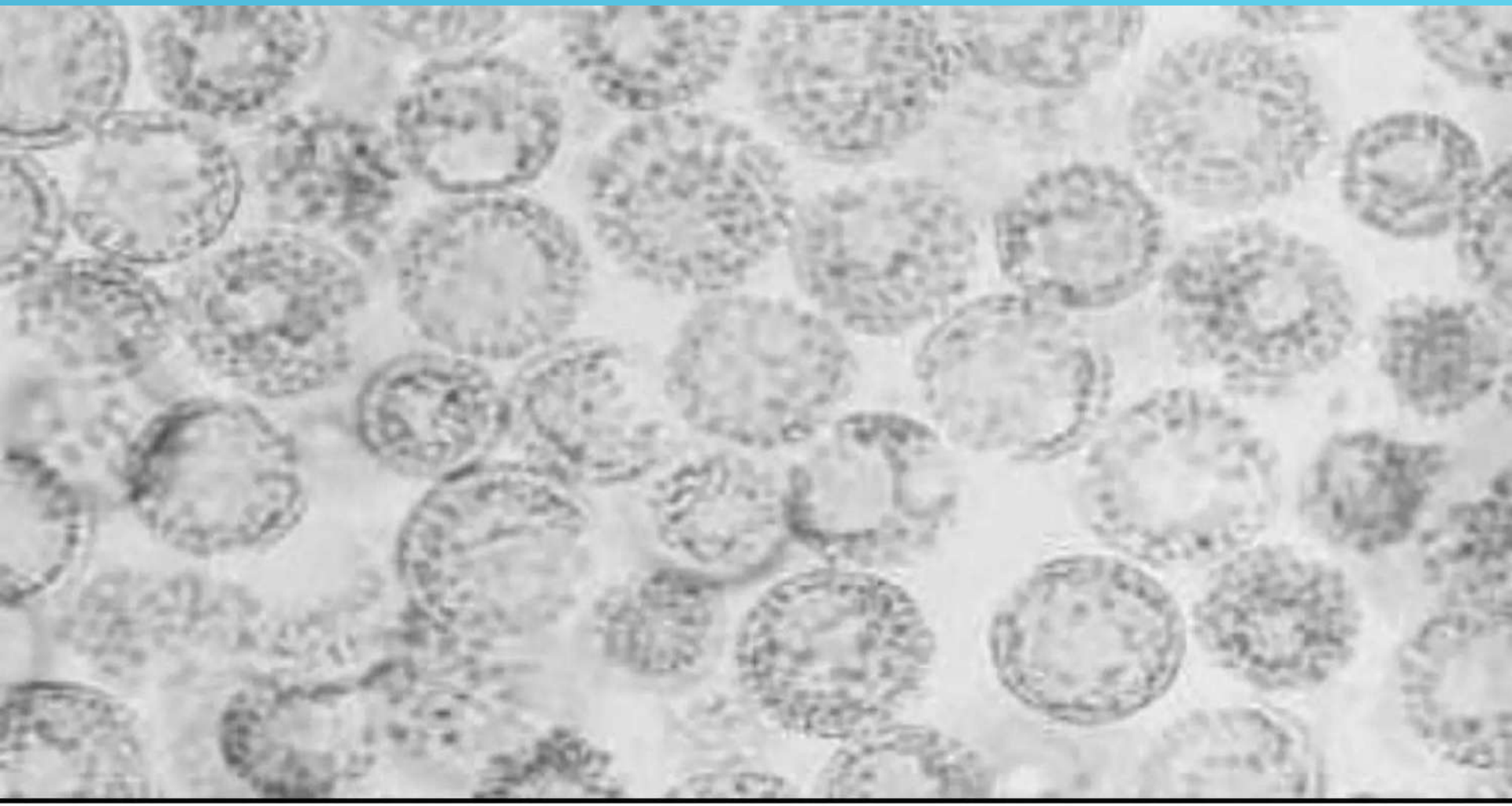
- This process does not involve callus formation

Role of growth regulators:

- Direct organogenesis bypasses the need for a callus phase

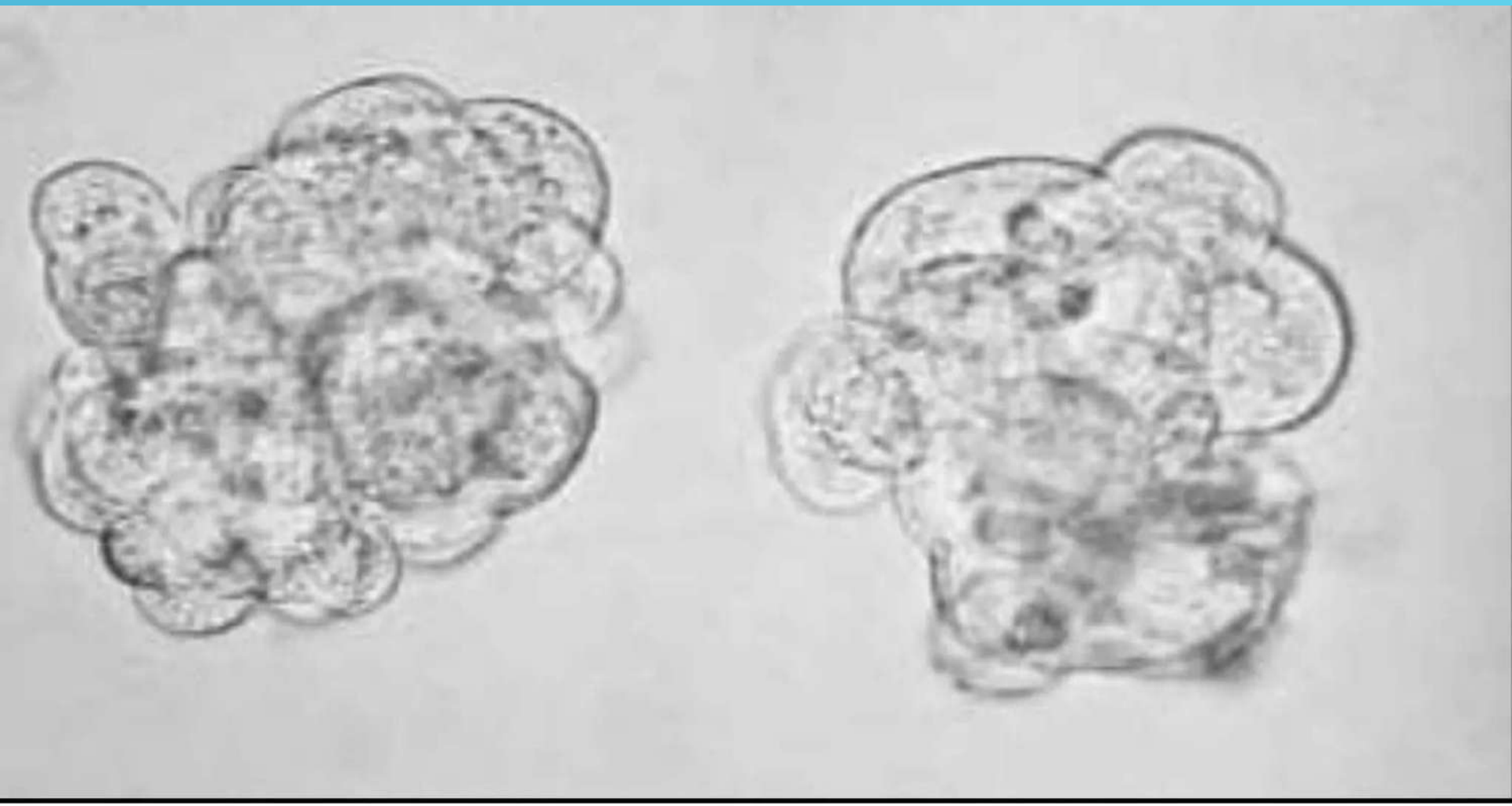
Eg.: Somatic embryogenesis (Formation of somatic embryo)

Most evidence suggests, direct embryogenesis proceeds from cells which were



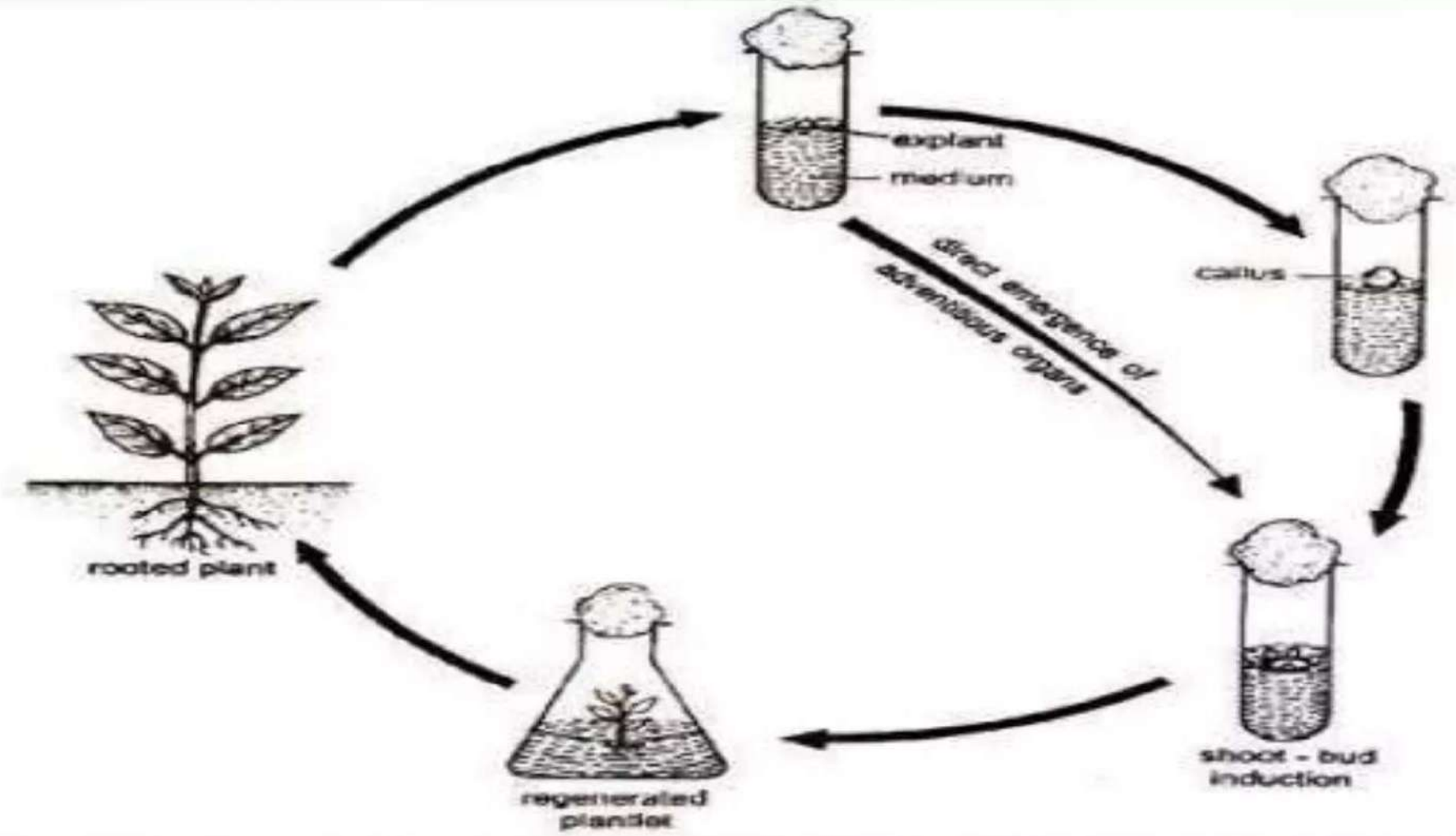
DIRECT ORGANOGENESIS: *TRITICUM AESTIVUM* PROTOPLAST

Isolated Protoplasts of *Triticum aestivum* from leaf mesophyll cell



DIRECT ORGANOGENESIS: *HYOSCYAMUS MUTICUS* PROTOPLAST

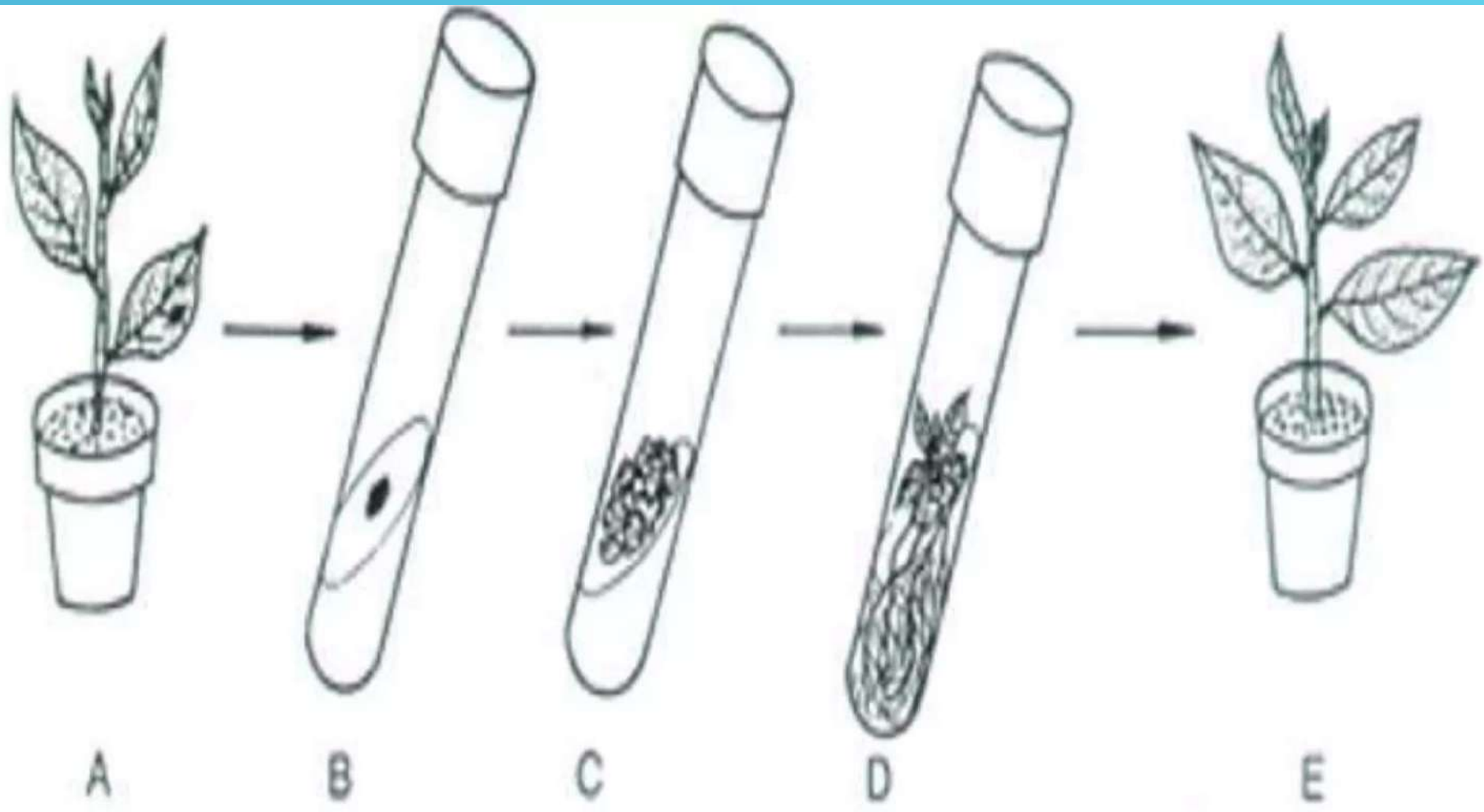
Cell divison in two properties of *Hyoscyamus muticus* has led to formation of two cells



DIRECT VS INDIRECT ORGANOGENESIS

Organogenesis *via* Dedifferentiation and Redifferentiation

Direct Organogenesis	Indirect Organogenesis
No intermediate / induction stage	Intermediate/induction stage present
No callus formation	Callus formation
Few plants have Somatic embryogenesis process	Mostly plants have Somatic embryogenesis process
Examples: Peanut	*Examples: Asparagus, Coffee leaf*

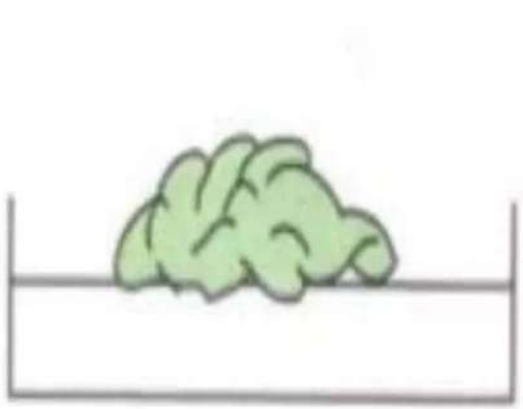


ORGANOGENESIS IN TOBACCO CALLUS

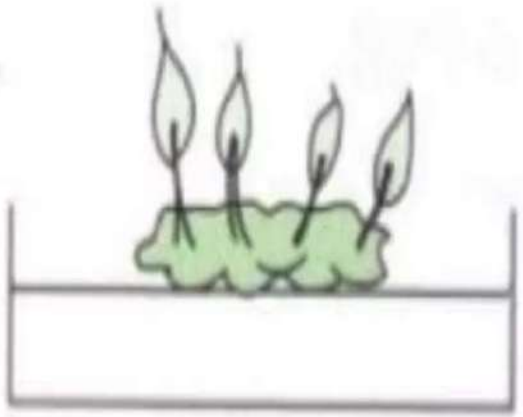
In-vitro regenerated of plants through tissue culture; A. Source of explant; B. Inoculate explant on culture medium; C. Callus formation;

ROLE OF PHYTOHORMONES IN ORGANOGENESIS

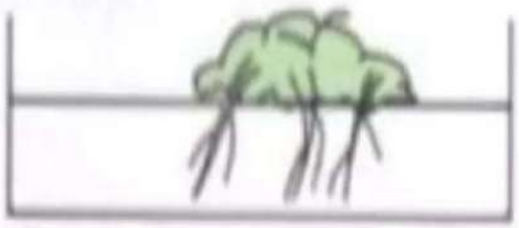
- **Morphogens**: Primary morphogens – Agent forming structure
- For developing changes, Cells treated with chemicals
- Adopt specific developmental pathway
- PO4 and tyrosine induce bud formation
- Polyamine, Zeatin induce shoot bud initiation
- **Auxin and cytokinins**: Helps to regenerate dicot plants
- High 2,4-D concentration in monocot cause changes in development of cultured tissue
- ABA and Gibberellic acid has specific role



Intermediate ratio (callus formation)



Low auxin to cytokinin ratio (shoot formation)



High auxin to cytokinin ratio (root formation)

RATIO OF AUXIN TO CYTOKININ IN CALLUS FORMATION

Ratio of Auxin and Cytokinins in formation of

FACTORS AFFECTING ORGANOGENESIS

Factors of organogenesis which is carried out in *In-vitro* organogenesis are as follows:

- **Size of explant**: *Organogenesis is generally dependent upon size of explant.*

Eg.: Large explant consisting parenchyma, vascular tissues and cambium have greater regenerative ability than the smaller explant

- **Source of explant**: The most suitable part of the plant for starting culture will depend on species.

- **Age of the explant**: Physiological age of explant is important for *in-vitro* organogenesis.

Eg.: In Nicotiana species, regeneration of adventitious shoot is only noted if the leaf explant is collected from vegetative stage i.e., before flowering

- **Seasonal variation**: Regenerated bulblets freely *in-vitro* when explant is taken during spring and autuma period of growth but same explants collected from summer or winter season does not produce any bulbets.

Eg.: Bulb scales of Lilium speciosum regenerate bulblets *explant*

- **Oxygen gradient**: In some cultures, shoot bud formation takes place when the gradient of available oxygen inside the culture vessel is reduce. But rooting requires a high oxygen gradient.

- **Quality and Intensity of light**: Blue region of spectrum promotes shoot formation and red light induce rooting

- **Temperature**: Most tissue culture are grown successfully at temp. around 25°C. In number of bulbous species optimum temperature may be much lower of about 15-18°C.

Eg.: Many Bulbous species

- **Culture medium:** Medium solidified with agar favors bud formation although there are some reports about the development of leaf shoot buds on culture grown in a liquid medium.

- **pH of medium**: pH of the culture medium is generally adjusted between 5.6-5.8 sterilization. The pH may have a determining role in organogenesis.

- **Ploidy level**: Variation in chromosome number i.e., aneuploidy, polyploidy etc, of plant cell in culture has been well documented. With the increase in chromosome instability there is a general decline in morphogenetic potentially of callus tissue.

APPLICATIONS OF ORGANOGENESIS

APPLICATION OF ORGANOGENESIS

- Plant tissue culture or organogenesis now has direct commercial applications as well as value in basic research into cell biology, genetics and biochemistry
- Micropropagation using meristem and shoot culture to produce large numbers of identical individuals
- Screening programmes of cells, rather than plants for advantageous characters
- Large scale growth of plant cells in liquid culture as a source of secondary products

ADVANTAGES AND DISADVANTAGES OF ORGANOGENESIS

Advantages of Organogenesis	Disadvantages of Organogenesis
Single or few cells have ability to produce whole plant	Neither always desirable identical plant nor always needed
Production of secondary metabolites in large scale	Often not clear function in plants
When useful plants are infected, organogenesis process commercially valuable	Limited for some plant species
When species not formed naturally, artificial seed production which rapidly grow in nature	More time consume
Anther culture in haploid plant species	Expertise are needed not done by farmers

CONCLUSION

- Organogenesis is development pathway in which shoot or root have been induced to differentiation from a cell or cell clusters

- *In-vitro* plant regeneration by organogenesis usually involves induction and development of shoot from the explants tissue

THANK YOU

A Biodiction (A Unit of Dr. Divya Sharma)

DNA REPLICATION
B.Sc. II Biotechnology
Optional

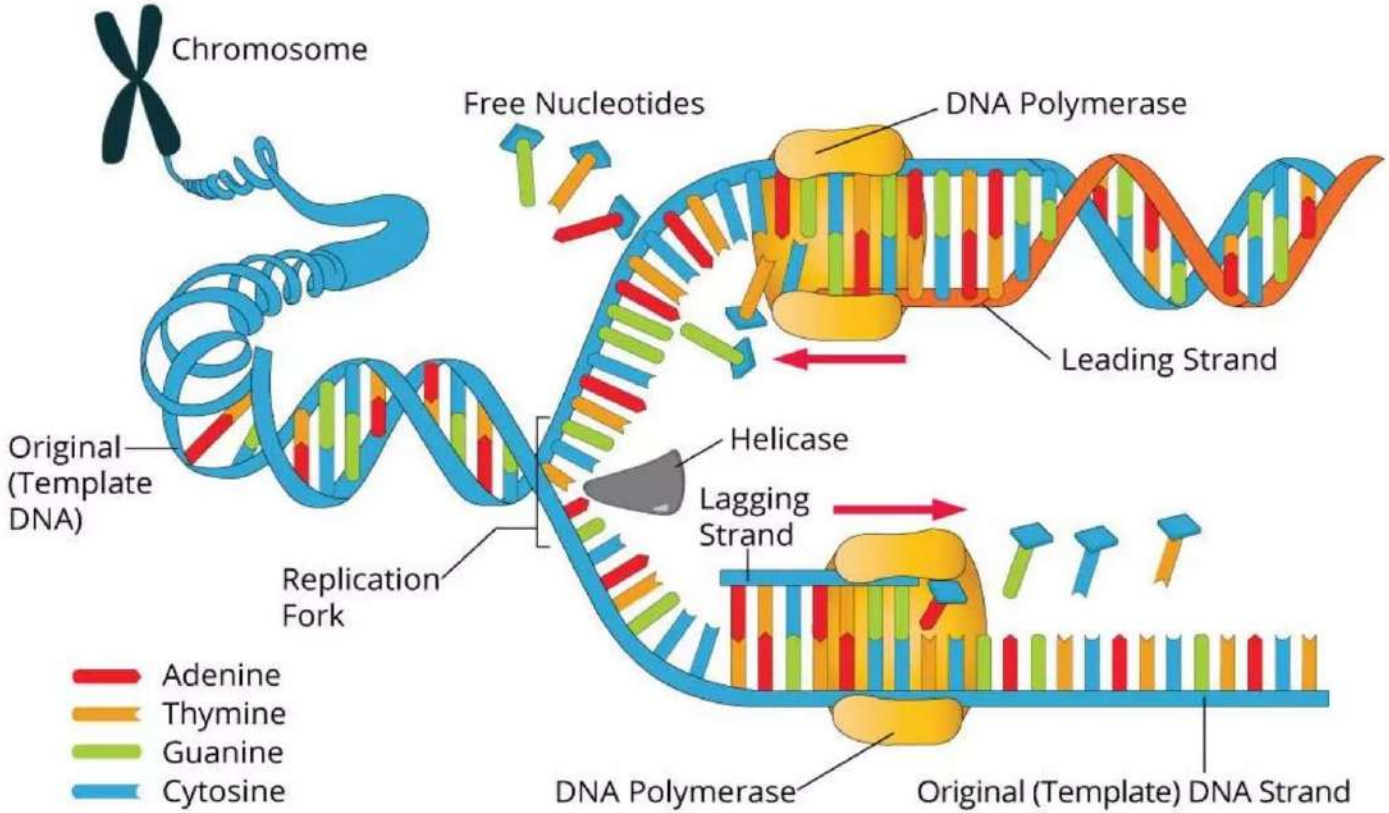
Ms. Shruti S. Jitkar

Introduction

- DNA replication is the biological process of producing two identical replicas of DNA from one original DNA molecule. DNA replication occurs in all living organisms acting as the most essential part for biological inheritance.
- In a cell, DNA replication begins at specific locations or origins of replication in the genome.
- Unwinding of DNA and synthesis of new strands takes place by an enzyme known as helicase which results in the formation of replication forks, growing bi-directionally from the origin.
- Various proteins are associated with the replication fork to help in the initiation and continuation of DNA synthesis.
- Most prominently, DNA polymerase synthesizes the new strands by adding nucleotides that complement each (template) strand.
- DNA replication occurs during the S-stage of Interphase.

Replication process

- DNA replication including all biological polymerization processes in three enzymatically catalyzed and coordinated steps: initiation, elongation and termination.



Initiation

For a cell to divide, it must first replicate its DNA. Once DNA replication begins, it proceeds to completion. Once replication is complete, it does not occur again in the same cell cycle. This is made possible by the division of initiation of the pre-replication complex.

Sequence of events during initiation

- Binding of DnaA protein to oriC in E.coli takes place and forms an initial complex.
- Further DNA helicase is loaded which mediates unpairing of template stands and forms open complex between DnaA and oriC.
- Then primase binds to form primosome.
- Synthesis of RNA primer takes place.
- Initiation of DNA polymerization by DNA polymerase.

Elongation

- DNA polymerases catalyze the step-by-step addition of deoxyribonucleotide units to a DNA chain and it has 5′–3′ activity.
- DNA replication systems require a free 3′ hydroxyl group before synthesis can be initiated (note: the DNA template is read in 3′ to 5′ direction whereas a new strand is synthesized in the 5′ to 3′ direction).
- At each growing fork, on strand called the leading strand is synthesized continuously from a single primer on the leading-strand template and grows in the 5'-3' direction.
- Growth of the leading strand proceeds in the same direction as the movement of the growing fork.

- Synthesis of the lagging strand is more complicated because DNA polymerases can add nucleotides only to the 3' end of a primer or growing DNA strand.
- Movement of the growing fork unveils the template strand for lagging-strand synthesis in the 5'-3' direction.
- After 1000 to 2000 nucleotides of the leading strand have been replicated, the first round of discontinuous strand synthesis on the lagging strand can begin.
- The short pieces of DNA called as Okazaki fragments are repeatedly synthesized on the lagging-strand template.

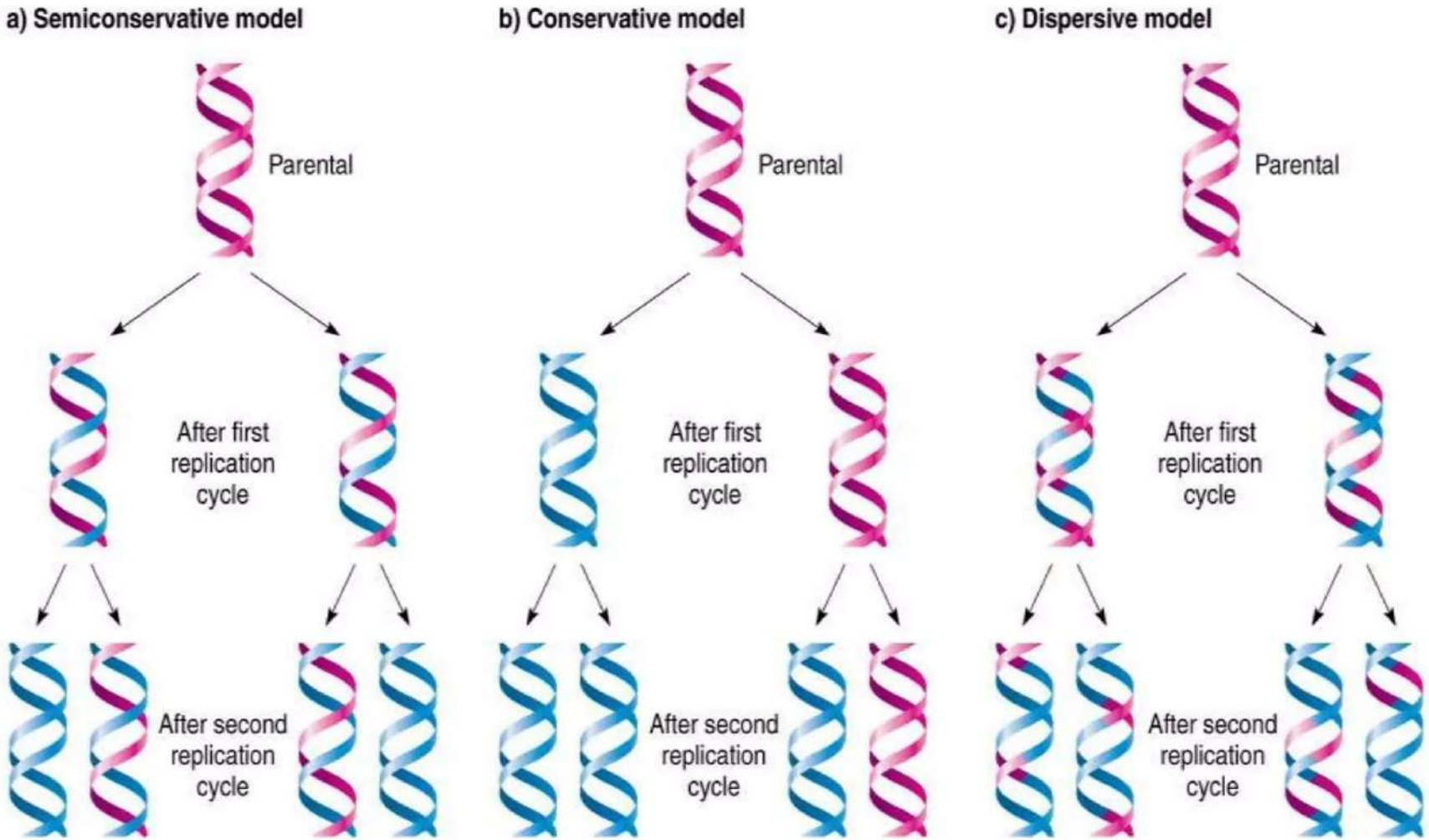
Semiconservative replication

- It is crucial that the genetic material is reproduced accurately. When Watson and Crick worked out the double-helix structure of DNA in 1953, they recognized that the complementary nature of the two strands-A paired with T and G paired with C-might play an important role in its replication. Because the two polynucleotide strands are joined only Genetics by hydrogen bonds, they are able to separate without requiring breakage of covalent bonds.

- If the two strands of a parental double helix of DNA are separated, the base sequence of each parental strand could serve as a template for the synthesis of a new complementary strand, producing two identical progeny double helices. This process is called **semiconservative** replication because the parental double helix is half conserved, each parental single strand remaining intact.

- The alternative methods are *conservative* and *dispersive.*

- In **conservative** replication, the whole original double helix acts as a template for a new one, one daughter molecule would consist of the original parental DNA, and the other daughter would be totally new DNA. In **dispersive** replication, some parts of the original double helix are conserved, and some parts are not. In this model, the parental double helix is broken into double-stranded DNA segments and just like conservative mode of replication acts as templates for the synthesis of new double-stranded DNA segments. The segments then reassemble into complete DNA double helices, each with parental and progeny DNA segments interspersed.



The Meselson-Stahl experiment showed that DNA replicates by a semiconservative mechanism

Experiment

- *E. coli* cells initially were grown in a medium containing ammonium salts prepared with heavy nitrogen ($_{15}N$) until the entire cellular DNA was labeled.
- After the cells were transferred to a medium containing the normal light isotope ($_{14}N$), samples were removed periodically from the cultures and the DNA in each sample was analyzed by equilibrium density-gradient centrifugation.
- This technique can separate heavy-heavy ($_{15}N$-$_{15}N$), light-light ($_{14}N$-$_{14}N$), and heavy-light ($_{15}N$-$_{14}N$) duplexes into distinct bands.
- After one generation of growth, the entire extracted DNA had the density of $_{15}N$-$_{14}N$ DNA.
- After two generations, approximately half the DNA had the density of $_{15}N$-$_{14}N$ DNA; the other half had the density of $_{15}N$-$_{14}N$ DNA.

- With additional generations, a large fraction of the extracted DNA consisted of $_{14}N$-$_{14}N$ duplexes; $_{15}N$-$_{15}N$ duplexes never appeared.

- These results match the predicted pattern for the semi-conservative replication mechanism.

- Meselson and Stahl experimentally demonstrated the semi-conservative replication of DNA in *E. coli* in 1958. If the parental DNA carries a *heavy* density label because the organism has been grown in medium containing a suitable isotope (such as $_{15}N$), its strands can be distinguished from those that are synthesized when the organism is transferred to a medium containing normal *light* isotopes.

Enzymes involved

- **DNA helicase-** Also known as helix destabilizing enzyme. Helicase separates the two strands of DNA at the Replication Fork behind the topoisomerase.

- **DNA polymerase-** The enzyme responsible for catalyzing the addition of nucleotide substrates to DNA in the 5′ to 3′ direction during DNA replication. Also performs proof-reading and error correction. There exist many different types of DNA Polymerase, each of which performs different functions in different types of cells.

- **DNA clamp-** A protein which prevents elongating DNA polymerases from dissociating from the DNA parent strand.

- **Single-strand DNA-binding protein-** Bind to ssDNA and prevent the DNA double helix from re-annealing after DNA helicase unwinds it, thus maintaining the strand separation, and facilitating the synthesis of the nascent strand.

- **Topoisomerase-** Relaxes the DNA from its super-coiled nature.

- **DNA gyrase-** Relieves strain of unwinding by DNA helicase; this is a specific type of topoisomerase

- **DNA ligase-** Re-anneals the semi-conservative strands and joins Okazaki Fragments of the lagging strand.
- **Primase-** Provides a starting point of RNA (or DNA) for DNA polymerase to begin synthesis of the new DNA strand.

- **Telomerase-** Lengthens telomeric DNA by adding repetitive nucleotide sequences to the ends of **eukaryotic chromosomes**. This allows germ cells and stem cells to avoid the Hayflick limit on cell division.

Various model of replication

- **Rolling circle replication**

- **Rolling circle replication** (RCA) is a process of unidirectional nucleic acid replication that can rapidly synthesize multiple copies of circular molecules of DNA or RNA, such as plasmids, the genomes of bacteriophages, and the circular RNA genome of viroids. Some eukaryotic viruses also replicate their DNA or RNA via the rolling circle mechanism.

- As a simplified version of natural rolling circle replication, an isothermal DNA amplification technique, rolling circle amplification was developed. The RCA mechanism is widely used in molecular biology & biomedical nanotechnology, especially in the field of biosensing.

Circular DNA Replication

- Rolling circle DNA replication is initiated by an initiator protein encoded by the plasmid or bacteriophage DNA, which nicks one strand of the double-stranded, circular DNA molecule at a site called the double-strand origin, or DSO.

- The initiator protein remains bound to the 5' phosphate end of the nicked strand, and the free 3' hydroxyl end is released to serve as a primer for DNA synthesis by DNA polymerase III.
- Using the unnicked strand as a template, replication proceeds around the circular DNA molecule, displacing the nicked strand as single-stranded DNA.
- Displacement of the nicked strand is carried out by a host-encoded helicase called PcrA (the abbreviation standing for plasmid copy reduced) in the presence of the plasmid replication initiation protein.
- Continued DNA synthesis can produce multiple single-stranded linear copies of the original DNA in a continuous head-to-tail series called a concatemer.

- These linear copies can be converted to double-stranded circular molecules through the following process:

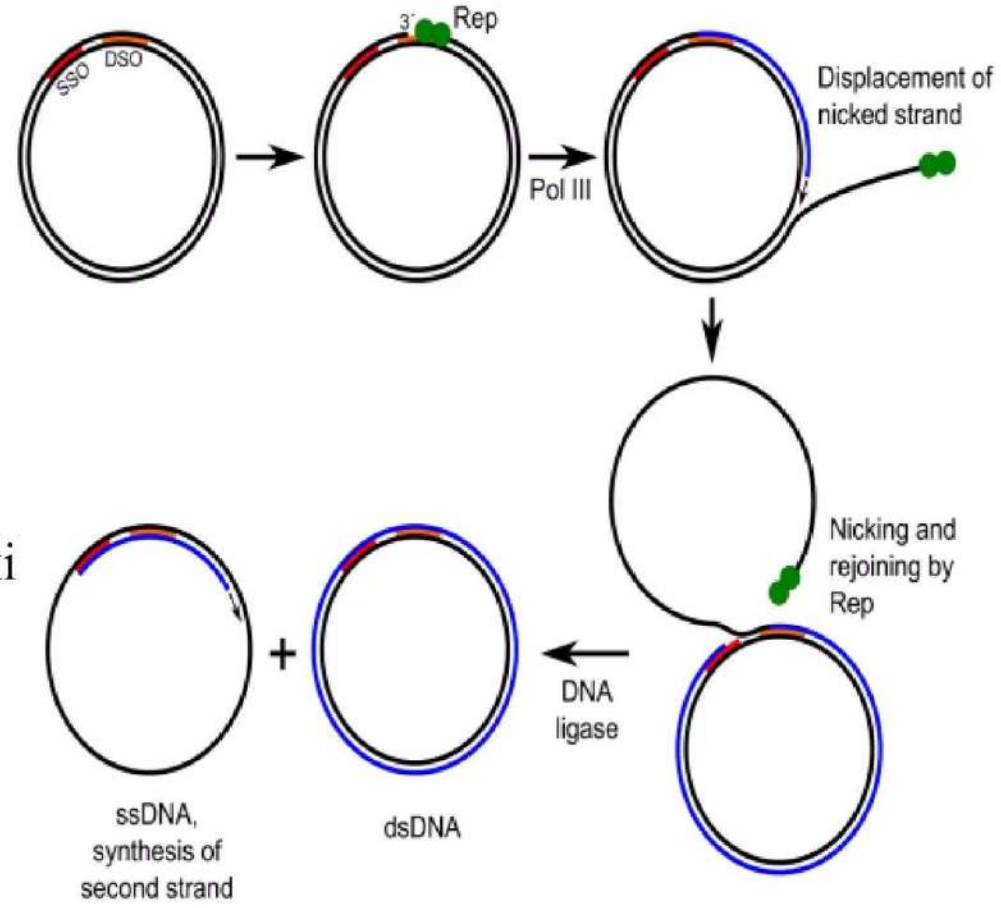
  - First, the initiator protein makes another nick in the DNA to terminate synthesis of the first (leading) strand.

  - RNA polymerase and DNA polymerase III then replicate the single-stranded origin (SSO) DNA to make another double-stranded circle.

  - DNA polymerase I removes the primer, replacing it with DNA, and DNA ligase joins the ends to make another molecule of double-stranded circular DNA.

As a summary, a typical DNA rolling circle replication has five steps:

- Circular dsDNA will be "nicked".
- The 3' end is elongated using "unnicked" DNA as leading strand (template); 5' end is displaced.
- Displaced DNA is a lagging strand and is made double stranded via a series of Okazaki fragments.
- Replication of both "unnicked" and displaced ssDNA.
- Displaced DNA circularizes.

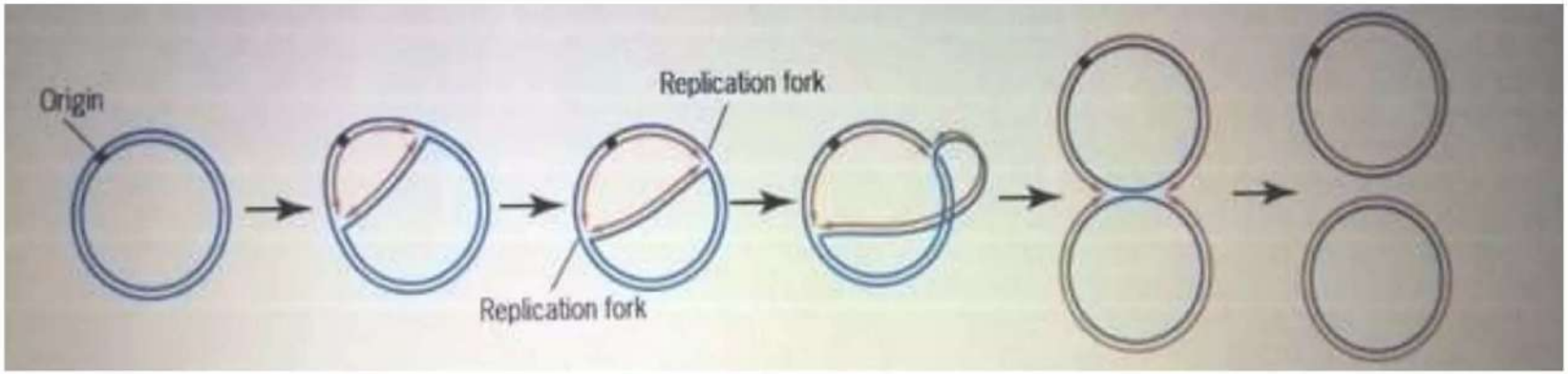


θ (Theta) mode of replication

The theta mode of replication is adapted by the prokaryotes to replicate their genetic material. The circular DNA has only a single origin of replication, unlike the eukaryotic DNA with multiple origins of replication for faster process. The two complementary strands of the parental DNA separate at the origin of replication by the action of helicase enzyme which literally unzips the strands by breaking the bonds. DNA polymerase enzyme then comes into action and starts the process of replication in the 5′ to 3′ direction. Once the replication is done, ligase enzyme glues the loose ends together and two daughter strands are formed. During the breaking of the strands by helicase enzyme, the circular DNA forms the Greek symbol 'θ' like structure, and so the name.

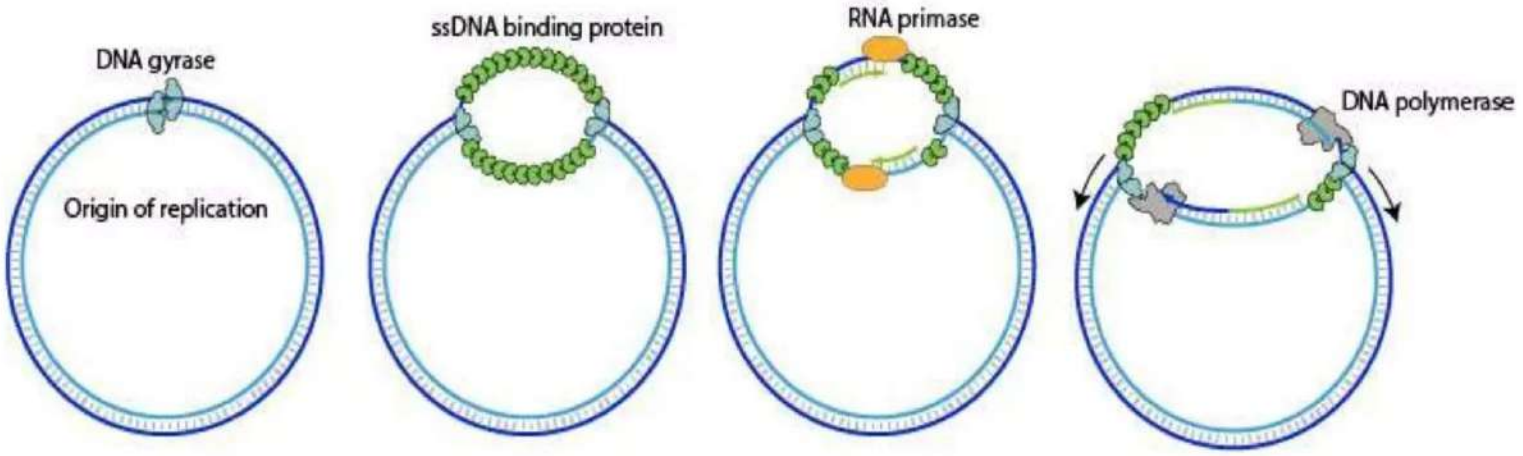
Process of DNA replication (Theta model) –

- Initiation of replication occurs at a specific region called **origin of replication**.
- ds-DNA denatures to form ss-DNA, denatured segment of DNA is called the **replication bubble**.
- DNA unwinds and y-shaped structure is formed known as the **replication fork**.
- In such cases, bidirectional replication occurs.
- The fork is generated by a complex of 7 proteins called **primasome** that includes – Dna G primase, Dna B helicase, Dna C helicase assistant, Dna T, Primase A, B and C.



Replication of linear ds-DNA

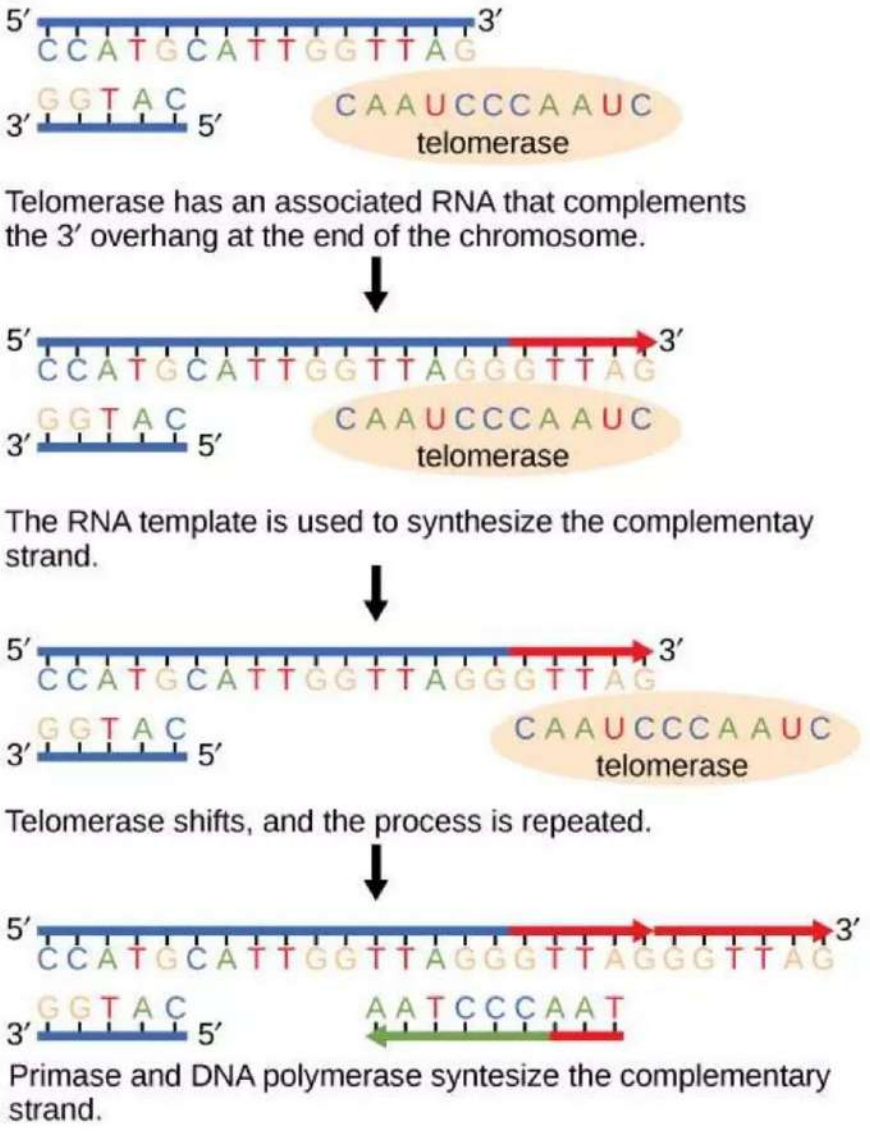
This is related to most nuclear dsDNA viruses, and many phages. Located in host cell nucleus (eukaryots) or cytoplasm (prokaryots). This kind of replication is used by all cellular organisms and some DNA viruses. It is the most classical way of replicating genomic nucleic acid.



- DNA replication begins at specific locations in the genome, called "origins".
- A topoisomerase unwinds the DNA double-strand at the origin of replication.
- ssDNA-binding proteins cover the single strand DNA created in the replication bundle.
- A primase synthesizes short RNA primers that are then used by the DNA polymerase to prime DNA synthesis.
- The DNA polymerase and associated factors begins to elongate the leading strand at the fork. For the lagging strand Okazaki fragments are elongated after sequential RNA primer synthesis by the primase.
- The lagging strand RNA primers are removed and Okazaki fragments ligated.
- The replication forks go on until they reach the end of linear genome or until they meet at the opposite side of a circular genome.
- After synthesis, topoisomerase allows separation of the two strands resulting from the replication.

Replication of the 5’ end of linear chromosome

Linear chromosomes have an end problem. After DNA replication, each newly synthesized DNA strand is shorter at its 5′ end than at the parental DNA strand’s 5′ end. This produces a 3′ overhang at one end of each daughter DNA strand, such that the two daughter DNAs have their 3′ overhangs at opposite ends.



Telomere Replication

- The ends of the linear chromosomes are known as telomeres: repetitive sequences that code for no particular gene and protect the important genes from being deleted as cells divide and as DNA strands shorten during replication.

- In humans, a six base pair sequence (i.e., TTAGGG) is repeated 100 to 1000 times. After each round of DNA replication, some telomeric sequences are lost at the 5′ end of the newly synthesized strand on each daughter DNA, but because these are noncoding sequences, their loss does not adversely affect the cell. But these sequences are not unlimited, therefore, after sufficient rounds of replication all the telomeric repeats are lost and the risks of losing coding sequences of DNA after subsequent rounds.

- The discovery of the enzyme telomerase helped in the understanding of how chromosome ends are maintained. The telomerase enzyme attaches to the end of a chromosome and contains a catalytic part and a built-in RNA template. Telomerase adds complementary RNA bases to the 3′ end of the DNA strand. Once the 3′ end of the lagging strand template is sufficiently elongated, DNA polymerase adds the complementary nucleotides to the ends of the chromosomes; thus, the ends of the chromosomes are replicated.

Thank you