COURSE GUIDE

BIO 303
GENERAL CYTOLOGY

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CONTENTS **PAGE**

INTRODUCTION

BIO 303: General Cytology is a first semester, 2-credit unit, 300 level course offered to undergraduate students.

The course guide tells you briefly what the course is all about, what course materials you will be using and how you can work your way through these materials. It guides you on your tutor-marked assignments.

There are self-assessment exercises within the body of the units and/or at the end of each unit. The exercises are an overview of the unit to help you assess yourself at the end of every unit.

WHAT YOU WILL LEARN FROM THIS COURSE

This course contains 13 units which cover various topics in general cytology, ranging from microscopy, cell division and cell cycle control, cell growth and differentiation, to introductory cytogenetics.

The cell is the simplest living organism capable of carrying out all the major characteristics of all living organisms. At the end of this course, you would have acquainted yourself with the general principles of cytology.

COURSE AIMS

This course introduces you to the general concepts of cytology and other related fields.

COURSE OBJECTIVES

In addition to the aim of this course, the course sets an overall objective which must be achieved. In addition to the course objectives, each of the units has its own specific objectives. You are advised to read properly the specific objectives for each unit at the beginning of that unit. This will help you to ensure that you achieve the objectives. As you go through each unit, you should from time to time go back to these objectives to ascertain the level at which you have progressed.

By the time you have finished going through this course, you should be able to discuss:

- the history of general cytology
- microscopy: from light to electron microscopy
- general cytogenetics

- cell cycle control
- cell division, growth and differentiation
- developmental cell biology and reproduction.

WORKING THROUGH THIS COURSE

In this course, you are advised to devote your time in reading through the course materials. You would be required to do all that has been stipulated in the course: study the course units, read the recommended reference textbooks and do all the unit(s) self-assessment exercise(s) and at some point, you are required to submit your tutor-marked assignments (TMAs). You should therefore avail yourself of the opportunity of being present during the tutorial sessions so that you would be able to compare knowledge with your colleagues.

COURSE MATERIALS

You are to be provided with the two major course materials. These are:

1. Course Guide
2. Study Units

The course comes with a list of recommended textbooks. These textbooks are supplement to the course materials so that you can avail yourself of reading further. Therefore, it is advisable you source for these textbooks and read them to broaden your scope of understanding.

STUDY UNITS

This course is divided into 3 modules with a total of 13 units, as follows:

Module 1

Unit 1 Light Microscope
Unit 2 Phase Contrast and Dark Field Microscope
Unit 3 Fluorescence Microscope
Unit 4 Autoradiography
Unit 5 Electron Microscope

Module 2

Unit 1 History and Common Trends in Cell Biology
Unit 2 Introductory Cytogenetics
Unit 3 Molecular Basis of Cell Structure
Unit 4 Proteins and Nucleic Acids

Module 3

Unit 1 Cell Cycle Control
Unit 2 Reproduction and Cell Division
Unit 3 Cell Growth and Differentiation
Unit 4 Developmental Cell Biology

TEXTBOOKS AND REFERENCES

Abramowitz, M. (1993). *Fluorescence Microscopy*. New York: Olympus-America.

Bourne, G.H. (1952). "Autoradiography". *Biological Review*. 27(1): 108-131.

Bruce, A., *et al*. (n.d). *Molecular Biology of the Cell*. (3rd ed.). New York: Graland Publishing Inc.

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Klug, W.S. (2009). *Concept of Genetics*. San Francisco, USA: Pearson-Benjamin Cummings.

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Schul-Schaeffer, J. (1980). *Cytogenetics: Plants, Animals, Humans*. New York: Springer-Verlag.

Swanson, C.P. (1957). *Cytology and Cytogenetics*. USA: Prentice-Hall Inc.

Taylor, D.J., Green, N.P.O. & Stout, G.W.(nd). *Biological Science*. (3rd ed.). Cambridge University Press.

Tymoczko, J.L. & Stryer, L. (2002). New York: W. H. Freeman.

ASSESSMENT

There are two components of the assessment for this course:

1. The Tutor-Marked Assignments (TMAs)
2. The End of Course Examination

TUTOR-MARKED ASSIGNMENT (TMA)

The TMA is the continuous assessment component of your course. It accounts for 30 percent of the total score you will obtain in this course.

FINAL EXAMINATION AND GRADING

The course is to be concluded by the final examination. The final examination constitutes 70 percent of the whole course. You will be adequately informed of the time of the examination. The examination will consist of questions which reflect all the basic concepts you would have learnt through the duration of the course.

SELF-ASSESSMENT EXERCISES (SAEs)

There are also self-assessment exercises (SAEs) within each unit. These are meant to probe your understanding of the concepts in the unit. It is not-graded and as such does not add up to your grade in the course.

SUMMARY

This is intended for you to have an underlying knowledge of the principles of General Cytology. By the time you complete this course, you should be able to answer conveniently questions on the following:

- Microscopy: from light to electron microscope
- History of general cytology
- General cytogenetics
- Cell cycle control
- Cell division, growth and differentiation.

Best wishes as you study this course.

MAIN COURSE

CONTENTS **PAGE**

MODULE 1

UNIT 1 LIGHT MICROSCOPE

CONTENTS

1.0 INTRODUCTION

The microscope is generally one of the most important tools in the study of Biology - especially cell biology because it allows you to visualise cells which are too small to be seen by the naked eye. The use of microscope dates back to the 16th century and Robert Hook, an English physicist used a simple light microscope to visualise a piece of cork in 1665. Furthermore, in the 1670s Antony van Leeuwenhoek observed a variety of cells using the simple microscope. Light microscopes are able to magnify objects up to a thousand times making it possible to observe larger sub-cellular organelles, e.g. chloroplasts, nuclei and mitochondria since most cells are between 1 and 100 μm in diameter.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain how microscope was discovered
- identify the different parts of the simple light microscope and state their functions
- state the terms and definitions applicable in light microscope
- state the use of light microscope.

3.0 MAIN CONTENT

3.1 Discovery of the Microscope

The discovery of the microscope was preceded by a period during which transparent crystals, thicker at the center than the edges were observed to make objects appear bigger when looked through. These magnifiers or lenses were so called because they looked like lentil seeds.

Zaccharias Janssen and Hans, his son observed that close objects appeared bigger when viewed through several lenses fixed to a tube. Galileo regarded as the father of modern physics and astronomy using the results of these early experiments provided the principle of lenses and subsequently made a better instrument having a good focus device.

3.2 Parts of a Light Microscope

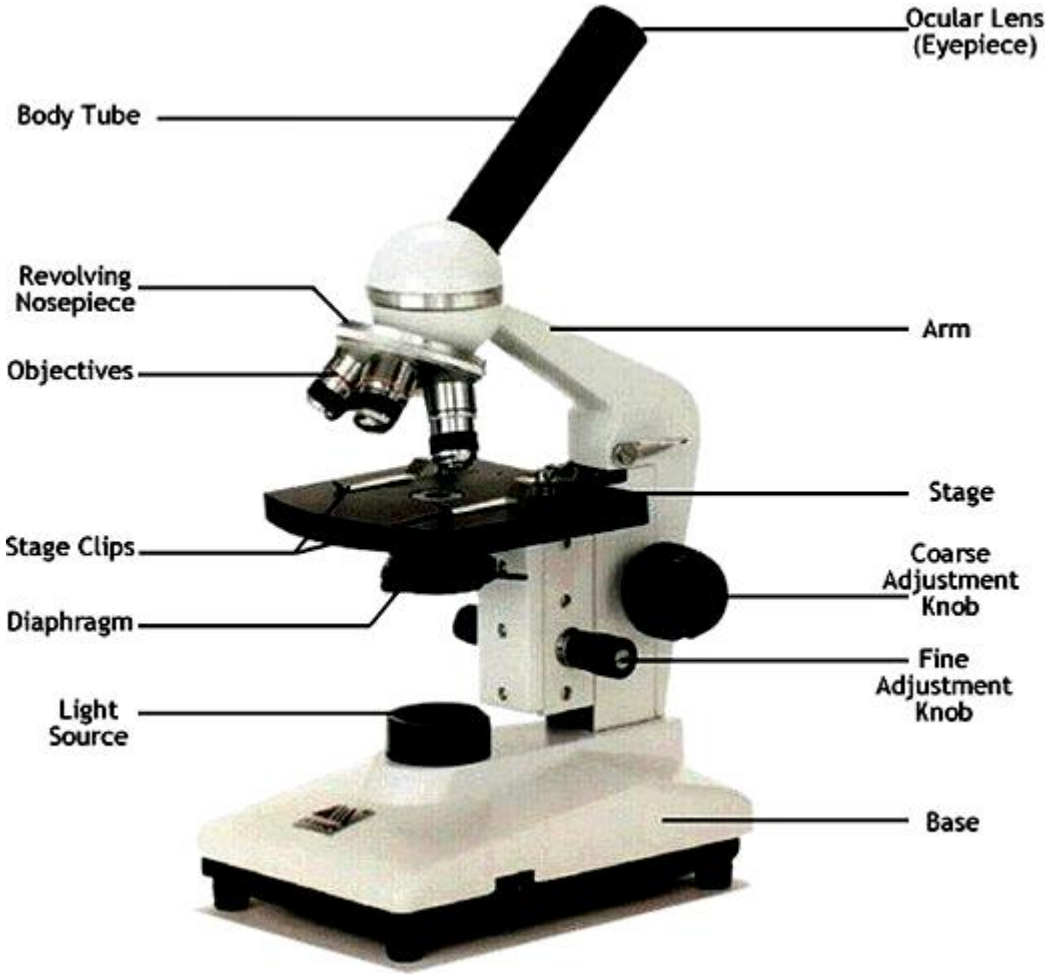

Fig. 1. 1: A Modern Day Light Microscope

Ocular lens or eyepiece: This is the lens at the topmost part the microscope and acts as a magnifying glass.

Body tube: It helps to reflect light to enable the viewer observe the specimen.

Revolving nosepiece: It helps for quick change of objectives.

Arm or neck*:* For safety in transporting the microscope.

Objectives: These are lenses used to observe the slide. The first objective or lens normally used in microscopic work is X 10, Medium objective X 20 and high power objective X 40.

Stage: Slides are placed here for viewing.

Stage clips: Assists to keep the slide being viewed in proper position.

Coarse adjustment knob: Large changes in focus are made here. However, it is important that this should not be used when using high power objectives.

Diaphragm: It controls the amount of light passing through the slide. It is better to reduce the amount of light passing through the slide.

Fine adjustment knob: To make small adjustments to the focus.

Light source: It directs light up through the diaphragm and through the slide to be visualised.

Base: To safely transport the microscope.

3.3 Principle of Operation

The light microscope is the basic tool that is used to observe objects too small to be seen with the unaided eye. Objects or specimens are magnified up to about one hundred times and as such could be observed using light microscope.

The ability to distinguish between two objects as separate entities is referred to as resolution and the greater the ability to distinguish between two close adjacent objects, the greater the clarity of the image produced. A relationship can be obtained for the lower limit of resolution by this equation:

$$r = 0.61\lambda / n \sin\alpha \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{equation 1}$$

where r refers to the resolving power of the microscope and is the smallest distance between two objects, λ is the wavelength of light used

to observe the object. The refractive index of the medium n is 1.0 for air but can be increased to a maximum of approx. 1.5 when oil of immersion lens is used to observe the specimen through a drop of oil and sin α is the sin of half the angle between the object and the objective lens. However, sinα is referred to as the numerical aperature (NA) which may be seen as the size of the cone of light that enters that enters the microscope lens after passing through the object or specimen. The maximum value of α is 90^o meaning that sinα cannot exceed 1.
Equation 1 could be written as follows:

$$r = 0.61\lambda / NA$$

Only a small fraction of variables affect the resolving power of the microscope and λ is between 0.4 to 0.7 μm and so λ is fixed at 0.5 μm for the light microscope. In an optical system that uses oil of immersion lens the maximum numerical aperture obtainable is 1.5 X 1= 1.5.

When white light is used in a microscope having an average wavelength of 550 nm, the resolving power is 550/ 1.5 or about 220nm. Therefore, specimens closer to one another or smaller than 220nm cannot be distinguished. A resolution of 220nm is adequate to view subcellular structures but organelles such as cell membranes, ribosomes, cytoskeleton, etc. cannot be resolved at this level. Living cells could be visualised under the light microscope but it is not easy to observe intact cells due to its thickness and opacity which create problems of resolution and contrast.

4.0 CONCLUSION

Light microscope has been greatly enhanced by the use of video cameras and computers which analyse and process the images produced. Such electronic devices enhance the visualisation of the contrast images obtained with the light microscope.

5.0 SUMMARY

In this unit, you have learnt about the general principle mechanism of light microscope. You also learnt the functions of the different parts of the compound light microscope.

6.0 TUTOR-MARKED ASSIGNMENT

1. Draw and label a compound light microscope.
2. State the functions of five named parts of the microscope drawn in question 1.

7.0 REFERENCE/FURTHER READING

De Robertis, E.D.P. *et al.* (1965). *Cell Biology of General Cytology*. (4th ed.). London: W.B Saunders Company.

UNIT 2 PHASE CONTRAST AND DARKFIELD MICROSCOPE

CONTENTS

1.0 INTRODUCTION

The eye detects variations in wavelength (colour) and in intensity of visible light. The majority of cell components are essentially transparent to the visible region of the spectrum except for pigments (more frequent in plant cells) that absorb light at certain wavelengths (coloured substances) the low light absorption of the living cell is caused largely by its high water content, but even after drying, cell components show little contrast.

In recent years, remarkable advances have been made in the study of living cells by the development of special optics techniques such as phase contrast. The technique is based on the fact that although biological structures are highly transparent to visible light, they cause phase changes in transmitted radiations. These phase differences, which result from small differences in the refractive index and thickness of different parts of the object, can now be made more clearly detectable.
Dark field microscope, also called ultra-microscope is another method used for the study of living cell. It is based on the fact that light is scattered at boundaries between phases having different refractive indexes.

2.0 OBJECTIVES

At the end of the unit, you should be able to:

- discuss the principle of phase contrast and dark field microscope
- discuss the advantages and disadvantages of phase contrast and darkfield microscope.

3.0 MAIN CONTENT

3.1 Principle of Phase Contrast Microscope

In phase contrast microscope, originally developed by Zernike as a method for testing telescope mirrors, the small phase differences are amplified so that they are detected by the eye or the photolithographic plate.

Living cells are made up of organelles and inclusion substances suspended in the cytsol. These materials are transparent and in unstained preparations are colourless. Therefore, they are not seen under the light microscope. But, advantage is made of the fact that each organelle retards or propagates light waves differently because they have different refractive indices. Therefore, they diffract light rays differently. This difference in refractive index is converted to difference in amplitude (degree of brightness) ranging from light to dark. Another property of light, which is also used in phase contrast microscope, is interference. If two rays with the same wavelength and amplitude emerge from a source and hit a screen at the same point there will be an increase in intensity of light. But, if one of the rays passes through a transparent material sufficient to reduce the wavelength by ½, there will be no light on the screen. This is because total interference has taken place between the two rays. If it is such that the ray is not reduced by exactly ½ there will be partial extinction of light. Organelles, in cells behave like these transparent materials and retard light rays sufficiently to reduce the wavelength in relation to rays passing through other parts of the cell thereby causing interference in the final image.

The phase contrast microscope consists of an annulus placed under the substage condenser and a phase plate placed above the objective of the microscope. Light rays passing through the annulus will produce an image of the annulus at the back focal plane of the objective. If a transparent object is placed on the object plane, three images will be seen at the back focal plane of the objective, two object images and the annular image. These object images are ¼ wavelength out of phase with the direct rays of light. A phase plate placed at the back focal plane of the objective enables a further retardation of ¼ wavelength to take place bringing the total to ½ wavelength. So, interference will take place and a picture will be produced.

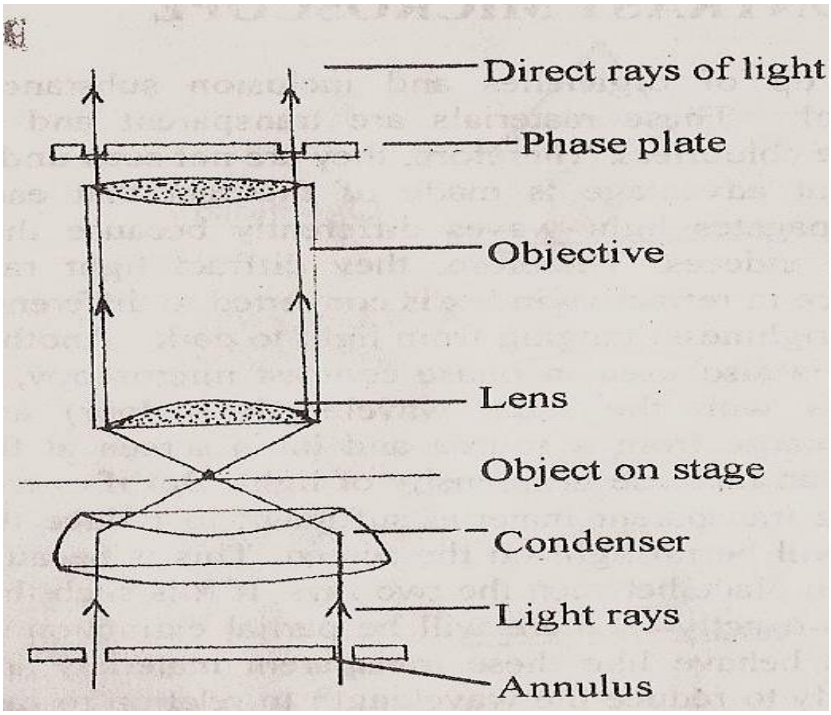

Fig. 2.1: Schematic Illustration of Light Rays in Phase Contrast Microscope

Advantages of phase contrast microscope

1. It is used to study the effect of different chemical and physical agents on the living cell. and to examine the artifact introduced by different methods of fixation and staining.
2. It is used routinely to observe living cells and tissues.
3. It is particularly valuable in the observation of cells cultured in vivo.

Disadvantage of phase contrast microscope

It is not useful for the examination of fixed and stained smears and sections because the refractive indexes of the organelles are altered during the process of fixation.

3.2 Principle of Darkfield Microscope

The instrument is a microscope in which, the ordinary condenser is replaced by one that illuminates the object obliquely. With this darkfield condenser, no direct light enters the objective, therefore, the object appears bright owing to the scattered light, and the background remains dark. In a living cell in a tissue culture, for example, the nucleolus, nuclear membrane, mitochondria and lipid droplets appear bright and the background of cytoplasm is dark.

In the darkfield microscope, objects smaller than those seen with the ordinary light microscope can be detected but not resolved.

Advantage of the darkfield microscope
One advantage of the darkfield microscope is that with it, it is possible to see cells in action and to study the movements involved in such processes as mitosis and cell migration.

4.0 CONCLUSION

With the help of phase contrast microscope, the ability to view the organelles of a cell and some other components of the microscope was achieved. Darkfield microscope helps to view living cell as they carry out their respective functions.

5.0 SUMMARY

In this unit, you have learnt about the basic principle by which phase contrast and darkfield microscopes work. You also learnt the advantages and disadvantages of the phase contrast and darkfield microscopes.

6.0 TUTOR-MARKED ASSIGNMENT

1. Compare the principle of phase contrast microscope to darkfield microscope.
2. What are the advantages of phase contrast microscope and darkfield microscope?

7.0 REFERENCES/FURTHER READING

Bruce, A. *et al.* (n.d).. *Molecular Biology of the Cell*. (3rd ed.). London: Graland Publishing Inc.

De Robertis, *et al*. (1965). *Cell Biology of General Cytology*. (4th ed.). London: W.B. Saunders Company.

UNIT 3 FLUORESCENCE MICROSCOPE

CONTENTS

1.0 INTRODUCTION

Stokes coined the term ‘‘fluorescence’’ in the middle of the 19th century when he observed that the mineral fluorspar emitted red light when it was illuminated by ultraviolet (UV) excitation. Stokes noted that the fluorescence emission always occurred at a longer wavelength than that of the excitation light. Early investigations showed that many specimens (minerals, crystals, resins, crude drugs, butter, chlorophyll, vitamins, inorganic compounds, etc.) fluoresce when irradiated with UV light. In the 1930s, the use of fluorochromes began in biology to stain tissue components, bacteria, or other pathogens. Some of these stains were highly specific and they stimulated the development of the fluorescence microscope.

Fluorescence microscope has become an essential tool in biology as well as in materials science as it has attributes that are not readily available in other optical microscope techniques. The use of an array of fluorochromes has made it possible to identify cells and submicroscopic cellular components and entities with a high degree of specificity amid nonfluorescing material. The fluorescence microscope can reveal the presence of a single fluorescing molecule. In a sample, through the use of multiple staining, different probes can simultaneously identify several target molecules. Although the fluorescence microscope cannot provide spatial resolution below the diffraction limit of the respective objects, the detection of fluorescing molecules below such limits is readily achieved.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- state the principle of fluorescence microscope
- list the advantages and disadvantages of fluorescence microscope.

3.0 MAIN CONTENT

3.1 Principle of Fluorescence Microscope

To understand how fluorescence microscope works and why it has become so important to modern biology, one must understand what the term fluorescence means. Fluorescence is the luminescence of a substance when it is excited by radiation.

In the fluorescence microscope, objects give off light against a dark background. The major differences between a light microscope and fluorescence microscope are the presence of ultraviolet light source, exciter filter and barrier filter in the fluorescence microscope. The light source, which may be a mercury vapour is, a water-cooled mercury lamp or a carbon arc lamp, produces invisible ultraviolet light and visible light that are transmitted to the exciter filter.

The exciter filter blocks the visible light and allows only ultraviolet light to pass through it. The ultraviolet light which has a short wavelength (180nm-380nm) hits the object on the microscope stage. As it does so, electrons in the object acquire energy and are displaced. When the displaced electrons are returning to their original ground state, they emit energy in form of visible light. The visible light (fluorescence) that has a longer wavelength and the unused ultraviolet light are transmitted to the objective. But, because ultraviolet light is harmful to the eyes, it is removed by a barrier filter so that only visible light produced by interaction between object and ultraviolet light reaches the eye lens and it is seen as fluorescence.

Fluorescence of a substance is seen when the molecule is exposed to a specific wavelength of light (excitation wavelength or spectrum) and the light it emits (the emission wavelength or spectrum) is always of a higher wavelength. To view this fluorescence in the microscope, several light filtering components are needed. Specific filters are needed to isolate the excitation and emission wavelengths of fluorochromes. A bright light source with proper wavelengths for excitation is also needed. If the light produced persists even when the light source has been removed, it is called phosphorescence. If the light produced does not

persist but diminishes within a few seconds after removal of ultraviolet light, it is called fluorescence.

One other component is required which is dichroic beam splitter or partial mirror which reflects lower wavelengths of light and allows higher wavelength to pass. A beam splitter is required because the objectives acts as a condenser lens for the excitation wavelength as well as the objective lens for emission. One only wishes to see the light emitted from the fluorochromes and not any of the excitation light, and the beam splitter isolates the emitted light from the excitation wavelength. This epi-illumination type of light path is required to create a dark background so that the fluorescence can be easily seen. The wavelength at which a beam splitter allows the higher wavelengths to pass must be set between the excitation and emission wavelengths of any given fluorochromes so that excitation light is reflected and emission light is allowed to pass through it.

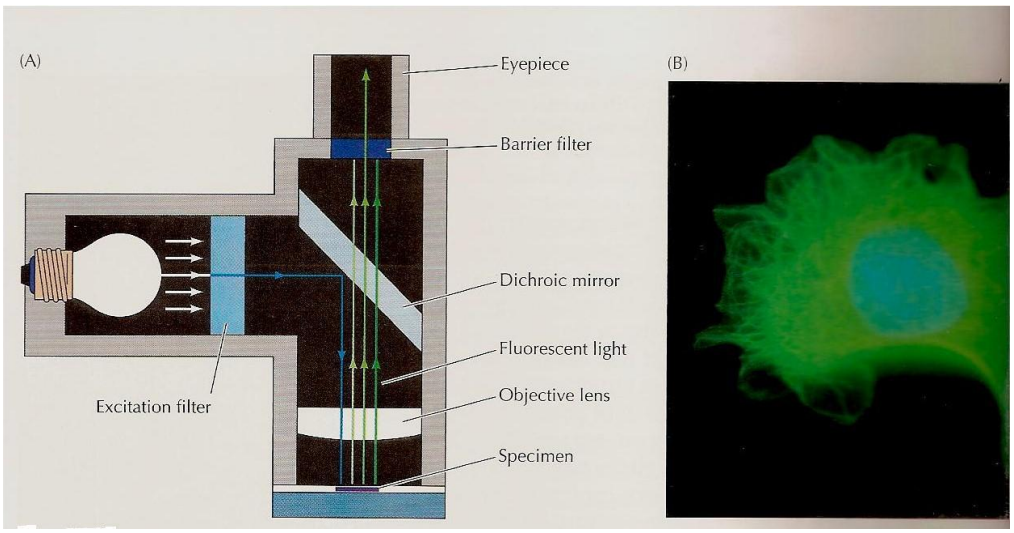

Fig.3.1A **Fig. 3.1B**

Fig. 3.1A: Light passes through an excitation filter to select light of the wavelength (e.g., blue) that excites the fluorescent dye. A dichroic mirror then deflects the excitation light down to the specimen. The fluorescent light emitted by the specimen (e.g., green) then passes through the dichroic mirror and a second filter (the barrier filter) to select light of the wavelength emitted by the dye.

Fig. 3.1B: Fluorescence micrograph of a newt lung cell in which DNA is stained blue and microtubules in the cytoplasm are stained green.

3.2 Advantages and Disadvantages of Fluorescence Microscope

Advantages of Fluorescence Microscope

- Fluorescence microscope can be used for the detection of acid fast bacilli, RNA in carcinoma cells, fungi, amyloid and mucins.
- It is also used in fluorescent antibody techniques.
- It is a rapid expanding technique, both in the medical and biological sciences. The technique has made it possible to identify cells and cellular components with a high degree of specificity. For example, certain antibodies and disease conditions or impurities in inorganic material can be studied with the fluorescence microscope.
- In microscope, fluorescence is used as a means of preparing specific biological probes.

Disadvantage of Fluorescence Microscope

- Fluorescent dyes are expensive and at time not easily accessible.

4.0 CONCLUSION

The modern light microscope combines the power of high performance optical components with computerised control of the instrument and digital image acquisition to achieve a level of sophistication that far exceeds that of simple observation by the human eye. The fluorescence microscope depends heavily on electronic imaging to rapidly acquire information at low light levels or at visually undetectable wavelengths.

5.0 SUMMARY

In this unit, you have learnt about the basic principle by which fluorescent microscope works. You also learnt the advantages of the use of fluorescent microscope.

6.0 TUTOR-MARKED ASSIGNMENT

1. Discuss the principle by which fluorescence microscope operates.
2. Highlight the differences between light microscope and fluorescence microscope.
3. What are the advantages of fluorescence microscope?

7.0 REFERENCES/FURTHER READING

Abramowitz, M. (1993). *Fluorescence Microscopy — The Essentials*. New York: Olympus-America.

Cooper, G.M. (2000). *The Cell: A Molecular Approach*. (2nd ed.). Washington DC: ASM Press.

UNIT 4 AUTORADIOGRAPHY

CONTENTS

1.0 INTRODUCTION

Autoradiography is a histochemical technique whereby a specific chemical compound is localised in an organ or tissue by a natural or induced radioactivity. The radioactivity of the chemical compound is used to produce an image on a photographic film or plate.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss the principle of autoradiography
- state what radioisotopes are
- describe the autoradiography method
- state the advantages and disadvantages of using autoradioisotopes.

3.0 MAIN CONTENT

3.1 Brief History of Autoradiography

The first autoradiography was obtained accidently around 1867 when a blackening was produced on emulsions of silver chloride and iodide by uranium salts. Such studies and the work of the Curies in 1898 demonstrated autoradiography before, and contributed directly to, the discovery of radioactivity. The development of autoradiography as a biological technique really started to happen after World War II with the development of photographic emulsions and then stripping film (see

Rogers, 1979) made of silver halide. Radioactivity is now no longer the property of a few rare elements of minor biological interest (such as radium, thorium or uranium) as now any biological compound can be labelled with radioactive isotopes opening up many possibilities in the study of living systems.

3.2 What are Radioisotopes?

The mass of the atomic nuclei can vary slightly (=isotopes) for a particular element although the number of electrons remains constant and all the isotopes have the same chemical properties. The nuclei of radioactive isotopes are unstable and they disintegrate to produce new atoms and, at the same time, give off radiations such as electrons (b rays) or radiations (g rays). Naturally occurring radioisotopes are rare because of their instability, but radioactive atom can be produced in nuclear reactors by bombardment of stable atoms with high-energy particles. The disintegrations can be detected in three ways. These detection methods are extremely sensitive and every radioactive atom that disintegrates can be detected.

Methods of detection

(i) Electrical
This depends on the production of ion pairs by the emitted radiation to give an electrical signal that can be amplified and registered: used in Geiger counter, ionisation counter and gas flow counter.

(ii) Scintillation
Some materials have the property of absorbing energy from the radiation and re-emitting this in the form of visible light. In a *scintillation counter* these small flashes of light are converted into electrical impulses. Both of these techniques count the pulses of the disintegrating atoms. They are fast and quantitative.

(iii) Autoradiography
It differs from the pulse-counting techniques in several ways. Each crystal of silver halide in the photographic emulsion is an independent detector, insulated from the rest of the emulsion by a capsule of gelatin. Each crystal responds to the charged particle by the formation of a latent (hidden) image that is made permanent by the process of development. The record provided by the photographic emulsion is cumulative and spatially accurate. It provides information on the localisation and distribution of radioactivity within a sample (i & ii do not do this). Thus, there is little point on doing autoradiography on a specimen that is homogeneously labelled. Although it can be quantitative, autoradiography is a much slower and more difficult approach.

Nuclear emulsions have a very high efficiency for b particles (electrons of nuclear origin), particularly those with low energies. Many of the isotopes of interest to biologists have suitable isotopes, e.g. tritium (= hydrogen-3), carbon-14, sulphur-35 and iodine-125. The effective volume of the detector emulsion in the immediate vicinity of the source may be as little as 100 cubic microns.

3.3 Principle of Operation of Autoradiography

The localisation and recording of a radiolabel within a solid specimen is known as autoradiography and involves the production of an image in a photographic emulsion. Such emulsions consist of silver halide crystals suspended in a clear phase composed mainly of gelatin. When a b-particle or g-ray from a radionuclide passes through the emulsion, the silver ions are converted to silver atoms. This results in a latent image being produced, which is converted to a visible image when the image is developed. Development is a system of amplification in which the silver atoms cause the entire silver halide crystal to be reduced to metallic silver. Unexposed crystals are removed by dissolution in fixer, giving an autoradiographic image which represents the distribution of radiolabel in the original sample.

In direct autoradiography, the sample is placed in intimate contact with the film and the radioactive emissions produce black areas on the developed autoradiograph. It is best suited to detection of weak to medium-strength b-emitting radionuclides (H^3, C^{14}, and S^{35}). Direct autoradiography is not suited to the detection of highly energetic b-particles, such as those from P^{32}, or for g-rays emitted from isotopes like I^{125}. These emissions pass through and beyond the film, with the majority of the energy being wasted. Both P^{32} and I^{125} are best detected by indirect autoradiography.

Indirect autoradiography describes the technique by which emitted energy is converted to light by means of a scintillator, using fluorography or intensifying screens. In fluorography the sample is impregnated with a liquid scintillator. The radioactive emissions transfer their energy to the scintillator molecules, which then emit photons which expose the photographic emulsion. Fluorography is mostly used to improve the detection of weak b-emitters. Intensifying screens are sheets of a solid inorganic scintillator which are placed behind the film. Any emissions passing through the photographic emulsion are absorbed by the screen and converted to light, effectively superimposing a photographic image upon the direct autoradiographic image. The gain in sensitivity which is achieved by use of indirect autoradiography is offset by non-linearity of film response. A single hit by a b-particle or g-ray can produce hundreds of silver atoms, but a single hit by a photon of

light produces only a single silver atom. Although two or more silver atoms in a silver halide crystal are stable, a single silver atom is unstable and reverts to a silver ion very rapidly. This means that the probability of a second photon being captured before the first silver atom has reverted is greater for large amounts of radioactivity than for small amounts. Hence small amounts of radioactivity are under-represented with the use of fluorography and intensifying screens. This problem can be overcome by a combination of pre-exposing a film to an instantaneous flash of light (pre-flashing) and exposing the autoradiograph at −70°C. Pre-flashing provides many of the silver halide crystals of the film with a stable pair of silver atoms. Lowering the temperature to −70°C increases the stability of a single silver atom, increasing the time available to capture a second photon.

3.4 Autoradiography Method

Autoradiography method involves the following:

- living cells are briefly exposed to a ‘pulse’ of a specific radioactive compound
- the tissue is left for a variable time
- samples are taken, fixed, and processed for light or electron microscope
- sections are cut and overlaid with a thin film of photographic emulsion
- left in the dark for days or weeks (while the radioisotope decays). This exposure time depends on the activity of the isotope, the temperature and the background radiation (this will produce with time a contaminating increase in ‘background’ silver grains in the film)
- the photographic emulsion is developed (as for conventional photography)
- counterstaining, e.g. with toluidine blue, shows the histological details of the tissue. The staining must be able to penetrate, but not have an adverse affect on the emulsion
- alternatively, pre-staining of the entire block of tissue can be done (e.g. with Osmium on plastic sections coated with stripping film [or dipping emulsion] as in papers by McGeachie and Grounds) before exposure to the photographic emulsion. This avoids the need for individually (post-) staining each slide
- it is not necessary to coverslip these slides
- the position of the silver grains in the sample is observed by light or electron microscope Note: the grains are in a different plane of focus in the emulsion overlying the tissue section. Often oil with x100 objective is used for detailed observation with the light microscope

- these autoradiographs provide a permanent record
- full details on the batch of emulsion used, dates, exposure time and conditions should be kept for each experiment.

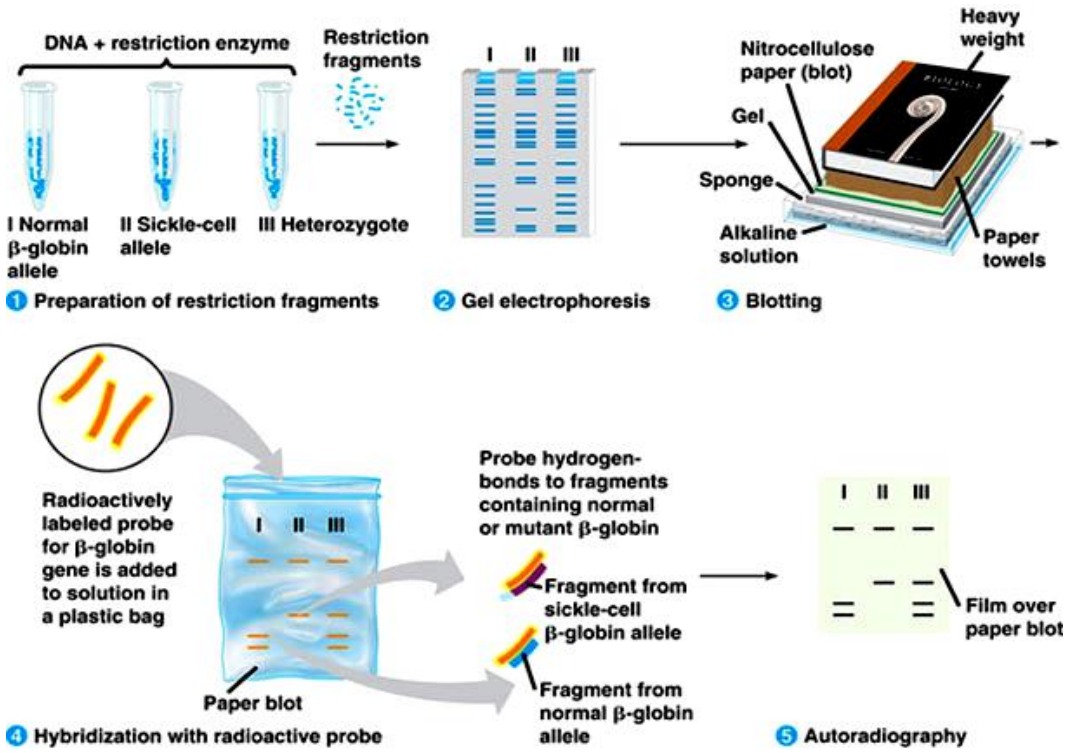

Fig. 4.1: Schematic Diagram showing the Technique of Autoradiography

3.5 Advantages and Disadvantages of Autoradiography

Advantages

- Radioactive isotopes are also used to track the distribution and retention of ingested materials.
- In molecular biology experiments, S ^{35} P ^{32} (and I ^{125}), are widely used to label nucleic acid probes to detect mRNA by *in situ* hybridisation on tissue sections and also for quantitation by Northern analysis on gels.
- Radioisotopes are used to trace molecules in cells and organisms.
- Radioisotope labeling has great sensitivity.

Disadvantages

- Radioisotopes are dangerous because they've been known to cause cancer (especially $I^{125)}$.
- Autoradiography can be expensive and tedious in that each time hybridisation is performed, the probe has to be labeled with fresh radioisotope (since it decays rapidly).

- In using autoradiography, only radioactively labeled molecules of the tissues are viewed while other parts cannot be viewed.

4.0 CONCLUSION

Autoradiography utilises the photographic action of ionising radiation for locating radioactive material in a specimen. However, this technique has its own drawbacks. In direct autoradiography, the sample is placed in intimate contact with the film and the radioactive emissions produce black areas on the developed autoradiograph. It is best suited to detection of weak to medium-strength b-emitting radionuclides (H^3, C^{14}, and S^{35}). Direct autoradiography is not suited to the detection of highly energetic b-particles, such as those from P^{32}, or for g-rays emitted from isotopes like I^{125}. These emissions pass through and beyond the film, with the majority of the energy being wasted. Both P^{32} and I^{125} are best detected by indirect autoradiography.

5.0 SUMMARY

In this unit, you have learnt about the history of autoradiography and what radioisotopes used by autoradiography are. Furthermore, you also learnt the basic principle involved in autoradiography. The advantages and disadvantages of autoradiograph were also discussed.

6.0 TUTOR-MARKED ASSIGNMENT

1. Briefly discuss the principle by which autoradiography works.
2. What are the advantages and disadvantages of using autoradiography?

7.0 REFERENCES/FURTHER READING

Rogers, A. (1979). *Techniques of Autoradiography*. (3rd ed). North Holland: Elsevier.

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UNIT 5 ELECTRON MICROSCOPE

CONTENTS

1.0 INTRODUCTION

By the early 1900s progress on understanding cell structure was limited by the fact that no matter how good the quality of a light microscope its maximum magnification is limited to about x1500. The development of electron microscopes started in the 1930s and they came into regular use in 1950s. A considerable increase in resolving power has been made possible by the development of the electron microscope. We have noted that the wavelength of the light with which objects are illuminated sets an absolute lower limit for discriminatory vision. With this limitation in mind, scientists deliberately set out to invent a different kind of microscope, one which used radiation of a much shorter wavelength. In electron microscope, radiation is used as electrons. Under certain circumstances they can behave as waves. They have two great advantages over light. Firstly, they have extremely short wavelengths, about the same as X-rays. Secondly, because they are negatively charged, a beam of electrons can easily be focused through a specimen using electromagnets.

With the electron microscope, magnification up to x25, 000 are commonly obtained with biological material.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- briefly discuss the history of electron microscope
- discuss the principle of electron microscope

- discuss the differences between resolution and magnification
- state some types of electron microscope
- list the advantages and disadvantages of electron microscope.

3.0 MAIN CONTENT

3.1 Brief History of Electron Microscope and its Application to Cell Biology

Dates	Contributions
1897	**J.J Thomson** announced the existence of negatively charged particles later termed electrons.
1924	**De Broglie** proposed that a moving electron has wavelike properties.
1926	**Busch** proved that it was possible to focus a beam of electrons with a cylindrical magnetic lens, laying the foundations of electron optics.
1939	**Siemens** produced the first commercial transmission electron microscope.
1945	**Porter, Claude, and Fullam** used the electron microscope to examine cells in tissue culture after fixing and staining them with OsO_4.
1959	**Brenner and Horne** developed the negative staining technique, invented four years previously by Hall, into a generally useful technique for visualising viruses, bacteria, and protein filaments.
1963	**Sabatini, Bensch, and Barnett** introduced glutaraldehyde (usually followed by OsO_4) as a fixative for electron microscope.
1965	**Cambridge Instruments** produced the first commercial scanning electron microscope.
1968	**De Rosier and Klug** described techniques for the reconstruction of three-dimensional structures from electron micrographs
1979	**Heuser, Reese**, and colleagues developed a high-resolution, deep-etching technique based upon very rapid freezing.

3.2 Principle of Electron Microscope

The electron microscope is like an upside-down light microscope. The radiation enters at top and the specimen is viewed at the bottom. The principle is the same as in a light microscope in that a beam of radiation is focused by condenser lenses through the specimen, and then the image is magnified by further lenses. A high voltage, such as 50,000V, is passed through a tungsten filament, like the filament of a light bulb, at

top of the column. The white hot filament releases a stream of electrons, kicked out of their orbits by the high voltage. Electromagnets focus the beam. The inside of the column has to be kept under a high vacuum, otherwise the electrons would collide with air molecules and be scattered. Only very thin sections of material or very small particles can be observed, because electrons are easily absorbed by larger objects. Those parts of the specimen which are denser absorb electrons and appear blacker in the final picture. Density differences can be made greater by using stains which contain heavy metals such as lead and uranium.

Electrons cannot be seen with the human eye, so the image is made visible by shining the electrons on to a fluorescent screen. This gives a black and white picture. The screen can be lifted out of the way to enable the electrons to pass on to a photographic film so that a permanent record can be obtained of any interesting features. A photograph taken with an electron microscope is called an **electron micrograph**.

Although it has revealed much about the structure of living things, two difficulties limit use of the electron microscope in chromosomal studies. The electron must pass through an evacuated system, which requires that the preparations be thoroughly dried. The weak penetrating power of the electrons demands extraordinarily thin preparations.

3.3 Resolution and Magnification

The ability to distinguish between two separate objects is known as resolution. If two separate objects cannot be resolved, they will be seen as one subject. Resolution is not the same as magnification. Magnification can be increased, but resolution of the photograph stays the same. Resolution is much greater in the electron microscope. The shorter wavelengths of electrons are said to have greater resolving power than those of light.

The resolution of an electron microscope is about 0.5nm in practice, compared with 200nm for the light microscope. This does not mean that electron microscopes are better. They are used for different jobs.

3.4 Types of Electron Microscope

Transmission electron microscope
The transmission electron microscope is one in which the electron beam is transmitted through the specimen before viewing and was the first type to be developed. In its overall design, the transmission electron microscope (TEM) is not unlike a light microscope, only that it is larger

and upside down. The source of illumination is a filament or cathode that emits electrons at the top of a cylindrical column about two meters high. If a linear electron beam is to be formed, air must first be pumped out of the column, creating a vacuum. The electrons are then accelerated from the filament by a nearby anode and passed through a tiny hole to form an electron beam that passes down the column. Magnetic coils placed at intervals along the column focus the electron beam, just as glass lenses focus the light in a light microscope. The specimen is put into vacuum through an air lock and is then exposed to the focused beam of electrons. Some electrons passing through the specimen are scattered, according to the local density of the material, and the remainder are focused to form an image, in a manner analogous to that by which an image is formed in a light microscope, either on a photographic plate or on a phosphorescent screen. Because the scattered electrons are lost from the image, the dense regions of the specimen show up as areas of reduced electron flux.

Scanning electron microscope

This is a type of electron microscope in which the electron beam is scanned to and fro across the specimen and electrons that are reflected from the surface are collected. Here, the electron beam is reflected from the surface of the specimen rather than passing through it as happens in the transmission electron microscope. The specimen to be examined is fixed and dried and then coated with a thin layer of heavy metal evaporated onto it in a vacuum. In the scanning electron microscope, the specimen is scanned with a focused beam of electrons, as the beam hits the specimen, secondary electrons are produced from the metallic surface, and these are detected and converted into an image on a television screen. Since, the amount of light scattering depends on the relative angle of the beam to the surface, the image has bright high points and dark shadows that give it a three-dimensional appearance. In most forms of scanning electron microscope, the resolution attainable is not very high; as a result, the technique finds its main use in the size range between intact single cells and small organisms.

3.5 Advantages and Disadvantages of Electron Microscope

Advantages of electron microscope

- It has very high resolution (0.5nm in practice) and its high magnification.
- Due to its high resolution, it has been possible to study object details at the sub-cellular levels so that comparison between structure and function can be made.

Disadvantages of electron microscope

- The electron microscope is very expensive to maintain.
- A special type of microtome, the ultra microtome is required for the cutting of sections.
- Very thin sections are required for electron microscope, because electrons have poor penetrating power.
- The preparation of material is time-consuming and requires expert training.
- Specimen may be damaged as a result of the great heat produced during microscope. The specimen gradually deteriorates in the electron beam.
- Living cells and tissues cannot be examined.
- A very small area of the specimen is examined. This may not give a true representation of the specimen.

4.0 CONCLUSION

A wide range of microscopic techniques is available for studying cells. The electron microscope is used for the examination of extremely small substances which cannot be revealed by light microscope. The transmission electron microscope allows cells to be examined at much higher resolution and shows the arrangement of their organelles, membranes, and protein filaments.

5.0 SUMMARY

In this unit, you have learnt about the brief history of electron microscope and the principles by which electron microscope works. The difference between resolution and magnification was discussed. Also discussed are some types of electron microscope and the principle by which they work.

6.0 TUTOR-MARKED ASSIGNMENT

1. Describe the principle by which scanning and transmission electron microscope works.
2. What is the difference between magnification and resolution?
3. What are the advantages and disadvantages of electron microscope?

7.0 REFERENCES/FURTHER READING

Taylor, D.J., Green, N.P.O. & Stout, G.W.(n.d). *Biological Science*. (3rd ed). Cambridge University Press.

Bruce, A. *et al. Molecular Biology of the Cell*. (3rd ed.). London: Graland Publishing Inc.

MODULE 2

UNIT 1 HISTORY AND PRESENT TRENDS IN CELL BIOLOGY

CONTENTS

1.0 INTRODUCTION

Like all other components of the earth, living organisms are made up of smaller units/components. Man's curiosity about his unit components was pioneered by Robert Hooke in 1665 during his investigation of the texture of cork. Although, man's curiosity dates back to the time of Aristotle, it was Robert Hooke who first observed these units using the microscope and later coined the word "cell" to describe the units.

The cell is thus, the fundamental structural and functional unit of living organisms. Understanding the molecular biology of cells therefore, is essential to cell biologist (cytologist) as it will give a better understanding of the organism as a whole. Since the cell itself can be considered an organism on its own.

Researches in cell biology have been successfully applied in various fields such as agriculture, biotechnology and medicine.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss the study of cell biology/cytology briefly
- discuss briefly on one newer cytology technique.

3.0 MAIN CONTENT

3.1 History of Cell Biology (Cytology)

Cytology is one of the oldest fields of biological sciences. Below are some of the historical events that occurred in the advancement of cell biology.

Year	Scientist	Event
	Aristotle & Paracelsus	"Concluded that all animals & plants, are constituted of few elements which are repeated in each of them"
1665	Robert Hooke	Coined the word "cells"
1674	Anthoin van Leeuwenhoek	Discovered free living cells which he referred to as animalcules (spermatozoas & bacteria)
1831	Robert Brown	Established that the nucleus is a fundamental & constant component of the cell
1839	Theodor Schwann & Matthias Scheilden	Proposed the Cell Theory
1858	Rudolf Virchow	Applied the cell theory in pathology
1882	Walter Flemming	Described mitosis in animals
1884	Edward Strasburger	Described fertilisation in angiosperms

3.2 Newer Tools and Techniques Used in Cell Biology

Most of our current understanding of cell structure and function came from the use of microscope since cells are not visible to the naked eye. As earlier stated Robert Hooke coined the term "cell" from his observations of dead cells of cork. However, since light microscope has limited resolution, improvements made in the use of light microscope, electron microscope became important in order to study the detailed structure of cells. The advent of electron microscope led to the visualisation of intact biological materials such as isolated sub-cellular materials, bacteria, macromolecules, etc. Microscopic studies alone will not provide all the information as to the functions of the cell and its components. It is therefore imperative that additional studies be carried out to understand the cell and its components. Thus, sub-cellular fractionation studies are undertaken and this involves the lysis (breakdown) of cells by disruption of the plasma membrane and the cellular components are separated by series of centrifugations at increasing speeds. The disruption of the membrane could be carried out using different methods such as sonication (using high speed frequency), mechanical by grinding, etc. This procedure allows the cellular

organelles to remain intact, be separated and subjected to biochemical studies.

Cell culture studies

The ability to grow cells outside of the organism enables one to study growth and differentiation as well as being able to manipulate them to help in understanding gene structure and function. The first classic experiment was done by Ross Harrison in 1907, by removing a piece of embryonic nervous tissue of frog and cultured same in a tiny drop of lymphatic fluid. After a few days he observed under the microscope that the nerve cells were still healthy and had processes that entered into the surrounding medium. The use of cultured cells had revolutionised agriculture. It is possible to grow in large numbers by cell culture certain crops or animals. This allows for selection of genetically superior traits such as disease resistance, high yielding plants, colour, etc.

Viruses

These are frequently used in cell biology experiments because of their simplicity and their ability to highjack the host's genome and direct the latter cellular activities. They cannot replicate on their own but reproduce by infecting their host. The fundamental aspects of cell biology were elucidated from the metabolism of infected cells since virus replication depends on it. Therefore, because of the complexity of animal cell genome the use of viruses is more important to the study animal cells than in bacterial cells.

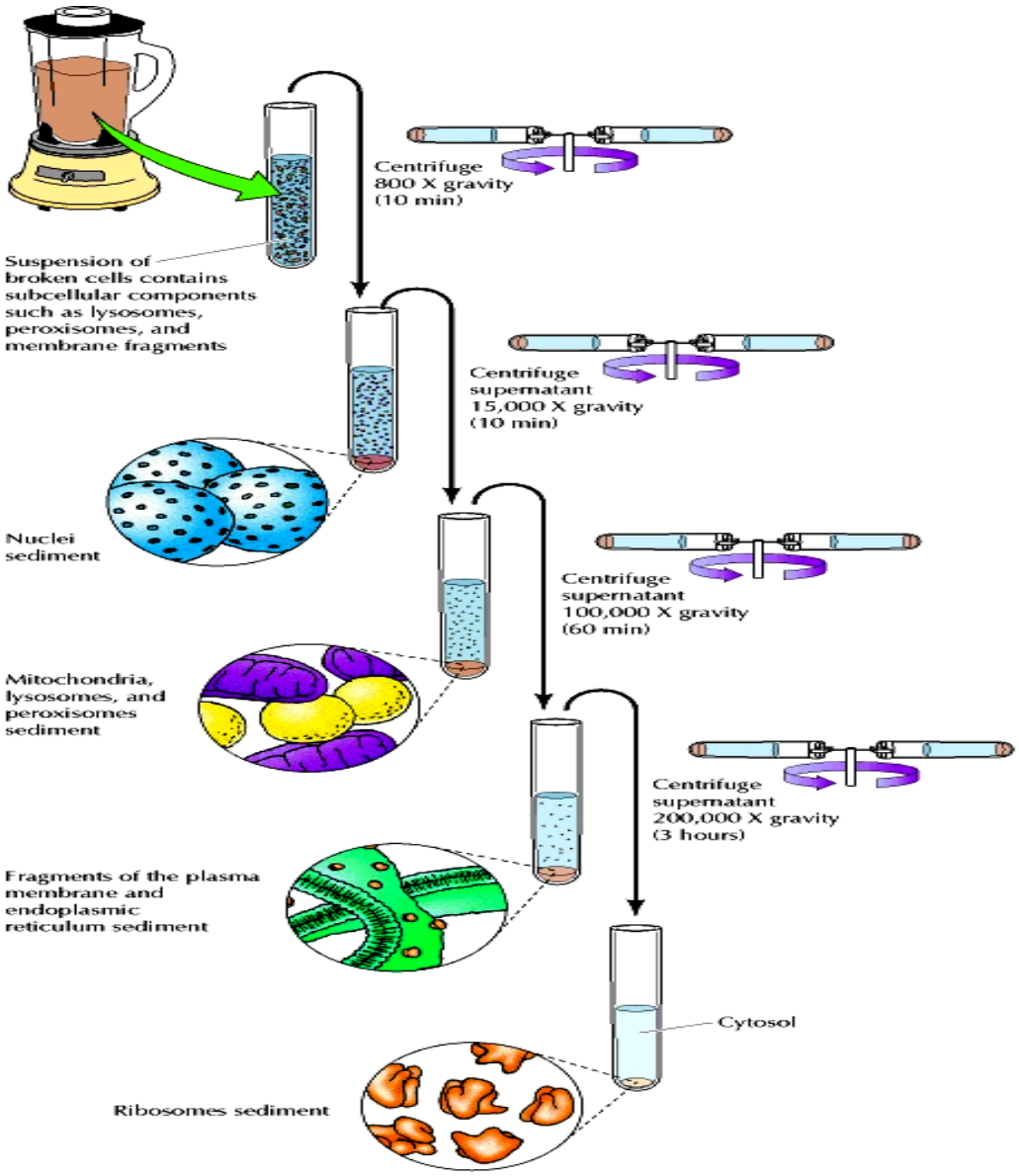

Fig.1.1: General Procedure of Sub-Cellular Fractionation through Centrifugation

4.0 CONCLUSION

Cells are the basic structural and functional unit of all living organisms. It can be considered as an organism on its own. It is the smallest independently functioning unit in the structure of an organism, usually consisting of one or more nuclei surrounded by cytoplasm and enclosed by a membrane. Cells also contain organelles such as mitochondria, lysosomes, and ribosomes.

5.0 SUMMARY

In this unit, you have learnt:

- that cells are the fundamental structural and functional unit of living organisms
- about the history of cell biology.

6.0 TUTOR-MARKED ASSIGNMENT

1. Highlight the events in the history of cytology.
2. Describe the process of sub-cellular fractionation using centrifugation.

7.0 REFERENCE/FURTHER READING

Schul-Schaeffer, J. (1980). *Cytogenetics: Plants, Animals, Humans*. New York: Springer-Verlag.

UNIT 2 INTRODUCTORY CYTOGENETICS

CONTENTS

1.0 INTRODUCTION

Cytogenetics is a branch of science that emerged from cytology and genetics. Cytology deals with information as it pertains to cell structure and function, while genetics is the biology of heredity. Cytogenetics aims to understand how the genetic or hereditary components of the cell and their changes affect the nature of the cell. The genetic components as we know are packaged in sub-cellular structures called chromosomes, which are structural and numerically specific among organisms. Cytogenetics therefore, is the study of how chromosomal behaviour and how alterations in their structure and number affect cellular function and the organism as a whole.

The microscope is an essential tool used by cytogeneticists to study chromosomes, and stains are required to differentiate the changes in chromosomes.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- trace the history of cytogenetics
- discuss at least three chromosomal variations
- describe the general procedures employed in cytogenetic techniques
- discuss at least one cytogenetic technique
- discuss the importance and application cytogenetic techniques.

3.0 MAIN CONTENT

3.1 History of Cytogenetics

Since cytogenetics involves the investigation and analysis of chromosomal behaviour, it is essential to commence the history of cytogenetics with the "discoverers" of the chromosome. Karl Wilhelm von Nägeli, the Swiss botanist in 1842, was the first to observe chromosome behaviour in plant cells while Walter Flemming, the German biologist in 1882, also discussed in detail chromosomal behaviour in the animal cell in is work with the salamander. He discovered that chromosomes split in a longitudinal fashion during cell division, then, he referred to them as "chromatin". The name 'chromosome' was coined by von Waldeyer in 1888. In 1884 van Beneden and Heuser observed that the longitudinal halves of chromosomes are passed on to daughter cells during cell division. These together with some others can be rightly designated as the fathers of cytogenetics.

Early cytogenetic techniques employed procedures such as fixation, staining and standard optical observation. Advances in the field of microscope therefore lead to the birth of newer and better cytogenetic techniques such as Fluorescent In Situ Hybridisation (FISH), array-comparative genomic hybridisation (array-CGH), Microarrays and so on. These newer techniques make the analysis of variations/changes in the entire genome possible.

3.2 Chromosomal Variations

Chromosomal variation could either be numerical or structural in nature. When chromosomes undergo changes or variations, mutation is believed to have occurred. This mutation may affect the whole chromosome, whole chromosome sets or parts of the chromosome. These mutations often lead to detrimental and sometimes favourable phenotypes in organisms.

3.2.1 Structural Variations

These include deletions, duplications, inversions and translocation.

- **Deletions**

This is when a portion of the chromosome gets lost. Deletions are usually very detrimental especially when they occur in gene coding regions of chromosomes. In humans, most deletions results into syndrome giving rise to more than on phenotype/symptoms.

- **Duplications**

Duplications results when any part of the genome- a locus or a portion of the chromosomes, occurs more once in the genome. Duplications, like deletions lead to observable phenotypes although it could be less detrimental. Duplications could either arise as a result of unequal crossing-over between synapsed chromosomes during meiosis or as a result of replicating error.

- **Inversions**

Inversion occurs when a portion of a chromosome is turned 180^0 within the chromosome. An inversion does not involve loss of genetic material, but rather it leads to the rearrangement of the linear gene sequence in the chromosome. Inversions could either **paracentric** or **pericentric.** When the inverted portion of the chromosome also includes the centromere, the inversion is termed pericentric, but when the centromere is not included, a paracentric inversion has occurred. Inversions usually have less impact on the organism as a whole, but their effects are still very important.

- **Translocation**

This is when a segment of a chromosome moves from one part of the genome to another.

3.2.2 Numerical Variations

This includes the duplication or omission of a whole chromosome, thus affecting the overall number of the chromosome set (ploidy number). Numerical variation could be **Euploidy** or **Aneuploidy**.

Euploidy

This is when the duplication or omission affects an even number of a chromosome set/ compliment or the chromosomes pair. For instance, humans, being diploid organisms are expected to have two copies of each chromosome one from each parent, 46 in total), a euploidy condition will result in the deletion/addition of the two copies of either chromosome 1, 2, or any affected chromosome. Thus, the affected individual will be two chromosomes short, thereby giving rise to an even ploidy number (e.g. 44 instead of 46).

Aneuploidy

This is when the addition or omission affects a member of a chromosome set/compliment. Thus, a member of a chromosome pair is either lost or added. For instance, one of the members of chromosome 1 pair might be lost giving rise to an odd ploidy number (e.g. 45 instead of 46).

3.3 Cytogenetic Techniques

General cytogenetic procedure

The general cytogenetic procedures synonymous to all cytogenetic techniques both old are new are listed below:

Specimen of interest: This depends on the organism of interest, be it plants or animals. While specimens from plant (popularly root tips) can be directly used for cytogenetic analysis, animal specimens are usually cultured first in a culture media prior analysis

Hypotonic treatment: Samples/specimens are pre-treated in hypotonic salt solutions to swell and spread the chromosomes. For an animal cell sample this might lead to the eventual bursting of the cell membrane

C-Methapahase arrest: Dividing cells are arrested at metaphase by using colchicines solution in case of plant samples and colcemid in case of mammalian samples. This enables the optimal visualisation and analysis of chromosomes since at metaphase; chromosomes are at their highest coiled and stable state.

Slide preparation: This is the most variable step of cytogenetic procedures as the specific requirement of each technique diverges here. Generally, squashed (in case of plants) or busted samples are placed on the slide, fixed using methanol/ethanol: glacial acetic acid (3:1) and then heated slightly to dry. Then the respective stains or dyes of each technique is added or incorporated for observation.

Creation of Karyogram: This involves cutting up and alignment of homologous (similar) chromosomes to give a pictorial representation of the chromosomal compliment of the sample. Chromosomal variations, either numerical or structural, will also be detected here.

Karyotyping

Karyotyping is one of the oldest cytogenetic techniques that came about as a result of discovery of the microscope as an optical observation instrument and also because different portions of the chromosomes differentially take up stains thereby creating different **banding pattern**.

Karyotypes are phenotypic appearance of mitotic chromosomes. It includes the number, type, shape, and banding pattern of chromosomes. Karyotype can also be used to study interphase chromosomes, especially in the detection of ***Barr body*** in the determination of the sex of a foetus.
Karyotypes studies are usually done using different staining methods, and based on this, different kinds of banding exist. The popular banding techniques include: C, G, Q, and R. A band is defined as a part of the chromosome that is clearly distinguishable from its adjacent segments by appearing darker or lighter.
The following characteristics can be observed in the chromosome after karyotyping:

- differences in the sizes of chromosomes
- differences in the basic number of chromosomes
- differences in the position of the centromere
- differences in degrees and distribution of heterochromatic and euchromatic regions of the chromosomes. Heterochromatic regions stain darker than euchromatic regions, indicating tighter packing of the chromosomes and genetically inert (inactive) regions.

Newer techniques

Fluorescence In Situ Hybridisation (FISH)

FISH visualises genetic alterations, such as translocations, deletions, duplications or inversions directly on interphase nuclei as well as metaphase chromosomes (as in conventional karyotyping techniques). Fluorescent-labeled DNA probes are utilised for visualisation in this technique. The probes are allowed to hybridise (bind) to the portion of the genome/chromosome which they are complimentary during slide preparation. The slide is then viewed under an ultraviolet (UV) light. Translocated, inverted, deleted and duplicated portions of the chromosome can be detected using FISH.

Array-Comparative Genomic Hybridisation (ARRAY-CGH or a-CGH)

While conventional karyotyping and FISH, permits the detection of chromosomal variation both in the interphase nuclei and metaphase chromosomes, with ARRAY-CGH, simultaneous detection of gene dose imbalances in the whole genome can be achieved. First the test DNA and the reference DNA samples are differently labeled and then the sample is mixed together and added on to the probe area of the array-CGH glass slide. The mixed sample is allowed to hybridise on to the probes of the array for one or two days. After the hybridisation, the glass

slide is washed and scanned. The image is loaded into a computer program measuring the ratio between the two labels in each probe. An uneven ratio between the different labels indicates a loss or gain of genomic material in the test sample (see fig.1 below). Duplications and deletions of parts of the genome can be detected using this technique.

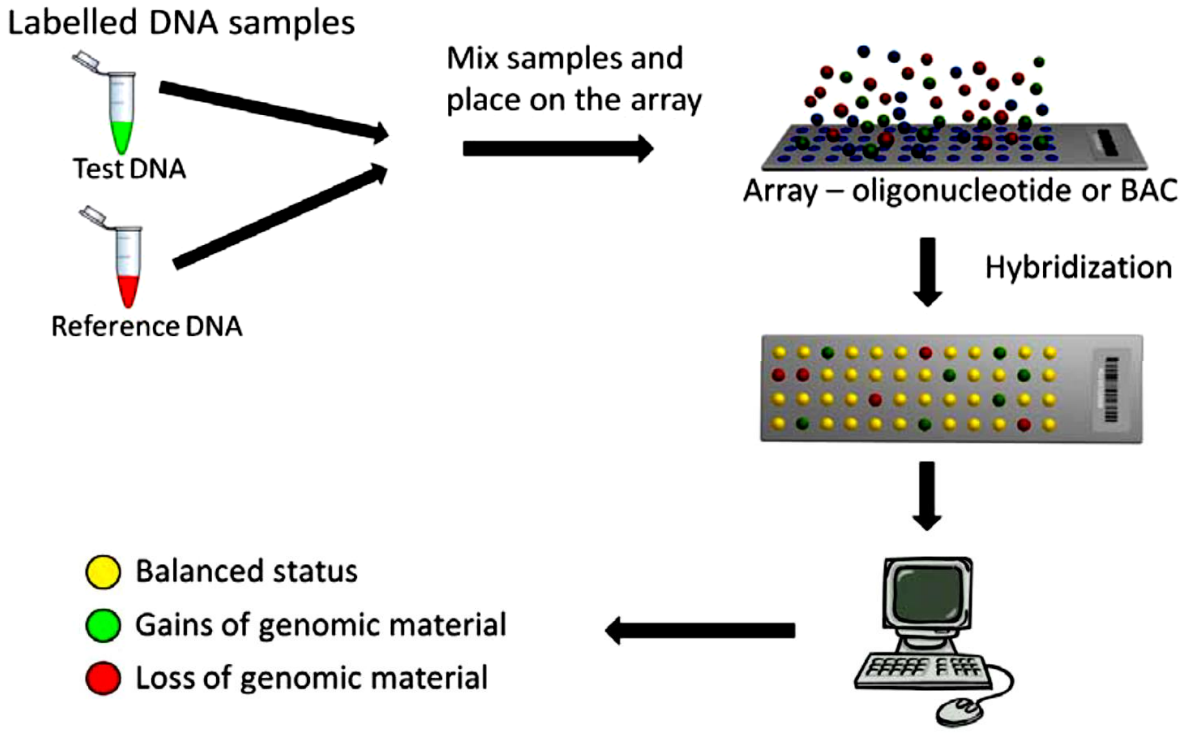

Fig.2.1: Illustration of ARRAY-CGH

3.4 Importance and Applications of Cytogenetics

- **Cytogenetics in sex determination**

Since the human sex chromosomes (X & Y) can be differentiated morphologically, the sexes of controversial individuals (possessing ambiguous genitalia) can be determined through proper karyotyping techniques.

- **Detection of variation**

Chromosomal aberrations such as polyploidy, inversions, deletions, duplications and translocations can be easily detected.

- **Application in medical diagnosis**

Cytogenetics has been successfully applied in medicine, especially in the area of diagnosis. Medical conditions such as Down syndrome, chronic myelogenous leukaemia, and so on, have been successfully

diagnosed using cytogenetic techniques. For instance, relevant cytogenetic techniques such as karyotyping is used to diagnose Down syndrome, a condition often caused by the presence of three copies of chromosome 21 (trisomy 21).

- **In toxicity and susceptibility testing**

ARRAY-CHG can be used for susceptibility testing by screening for dosage imbalances in the genomes of affected and non affected individuals. In this case the test and reference DNA samples will be that of the normal and affected individuals respectively.

4.0 CONCLUSION

Chromosomes are the carriers of genes, thus the need for their study. Cytogenetics, like most fields of science in the 21st century, is rapidly evolving due to advances technology and synergy between various related fields.

5.0 SUMMARY

In this unit, you have learnt about the history of cytogenetics. The different cytogenetic techniques and the applications of cytogenetics were treated.

6.0 TUTOR-MARKED ASSIGNMENT

1. Highlight and discuss three different staining patterns utilised in karyotyping.
2. Discuss, in detail the various structural chromosomal variations.
3. Highlight three applications of cytogenetic techniques.

7.0 REFERENCES/FURTHER READING

Swanson, C.P. (1957). *Cytology and Cytogenetics*. United States: Prentice-Hall Inc.

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UNIT 3 MOLECULAR BASIS OF CELL STRUCTURE

CONTENTS

1.0 INTRODUCTION

In 1943 Erwin Schrödinger, a Viennese physicist provoked life scientists (biologists) to think of the living cells in their physical and chemical nature. To him, there was no major difference between the living and the non-living molecules since they all obey the same laws of physics and chemistry; "*Life was like a matter doing something*" i.e. undergoing metabolism. Consequently, modern cytology seeks to understand cellular processes in terms of chemical and physical reactions.

In its basic units, the cell is composed of water, organic molecules and inorganic ions. Organic macromolecules such as the nucleic acid, proteins, carbohydrates and lipids constitute about 80-90% of the dry weight of most cells, water, constitutes above 70% of total cell mass and inorganic ions constitutes less than 1% of the cell mass. Each of these molecular constituents plays its crucial roles in various cellular metabolic activities from bioenergetics, biosynthesis, cell signaling. But more importantly, their interactions with each other and the aqueous (water) environment of the cell directly influence then of cellular structures such as the cell membrane. Another compound important in cell chemistry is water accounting for up to at least 70% of cell mass. Water is a polar molecule and form hydrogen bonds with each other or other polar molecules and interacts with negatively or positively charged ions. The interactions between polar and non polar molecules and water are important in the formation of cell membranes. However, the organic molecules are very crucial in understanding the structure and function of cells.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss in detail at least three constituents of the cell structure
- discuss the molecular composition of inorganic ions.

3.0 MAIN CONTENT

3.1 Molecular Composition of the Cell

As earlier mentioned, the major components of the cell are water, inorganic ions and organic (carbon containing) molecules just as in non-living matters. Unlike water and the inorganic ions, organic molecules present in the cell are usually made up of hundreds to thousand low-molecular weight precursor units (monomers) joined together to form organic polymers. For instance, amino acids, nucleotides, sugars, and fatty acids are the precursor units of proteins, nucleic acids, carbohydrates and lipids respectively.

3.1.1 Water

Water is the most abundant molecule in the cell. Its interactions with other components of the cell are of utmost importance in biological/life chemistry. Water as a chemical compound is made up of two hydrogen atoms bound to a single oxygen atom in a covalent bond (electrons are shared between hydrogen and oxygen atoms) (fig. 1). The polar (opposite charges in opposite directions) nature of water, with slightly positive hydrogen atoms and slightly negative oxygen atom, enables it to form hydrogen bonds with itself, other polar molecules as well as negatively and positively charged ions.

This property is what makes water the universal solvent ensuring an aqueous environment in the living cell essential for almost all the metabolic activities of the cell. As a result, polar molecules are readily soluble in water and the aqueous environment (hydrophilic) of the cell. This is in contrast to non-polar molecules which tend to minimise contact with water, rather associating more with themselves. They are usually sparingly/poorly soluble in water (hydrophobic).

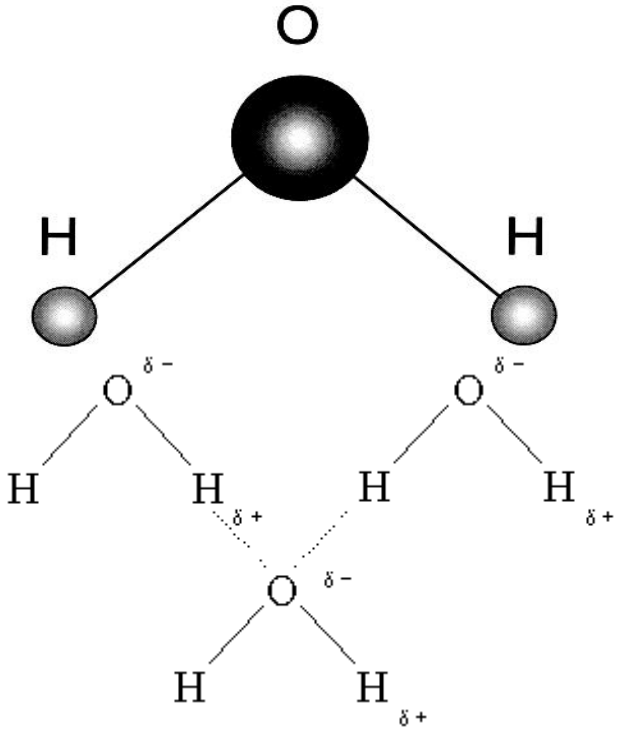


Fig.3. 1: Water is a Polar Molecule with Two Hydrogen Atoms Covalently Bound to an Oxygen Atom

3.1.2 Proteins

Proteins are the most important biomolecule in the cell carrying out a wide range of functions, from transporting and storage of small molecules (e.g. transport of oxygen by haemoglobin), serving as a structural component of the cell (as in transmembrane proteins), providing immunity (antibodies), to cell signaling (protein hormones) and the most important of all, serving as biocatalyst (enzymes) in virtually all the chemical reactions in the cell.

Structurally, proteins are a polymer of amino acids joined together by peptide bonds. Each amino acids consists of a central carbon atom (called the α carbon) bonded to a carboxyl group (-COOH), an amino group ($-NH_2$), a hydrogen atom, and a variable side chain (fig. 2). There are 20 naturally occurring amino acid, and based on the properties of their side chains, they can be divided into four main groups; polar (e.g. serine, tyrosine), non-polar (glycine, alanine), basic (e.g. lysine, arginine), and acidic (aspartic and glutamic acids). Peptide bonds are formed between the α amino group of one amino acid and the α carboxyl group of another (fig. 3a&b), thus, a polymer of proteins contains hundreds to thousands peptide bonds (polypeptide bonds).

Rather than being long chains of polypeptide bonds, proteins adopt a three dimensional shape/structure determined by the interactions between their constituent amino acids. Each protein has a unique amino

acid sequence which helps it determine its 3D- structure. These structures are very important in protein function. The structure of proteins is considered to fall into four levels viz; the primary, secondary, tertiary and quaternary structures (fig. 4). The primary structure reveals the linear amino acid sequences that make up the chain while in the secondary structure, the conformations of the portions of the polypeptide are shown. The tertiary structure describes the entire conformation of the polypeptide and the quaternary structure shows the conformations of subunits if the protein is made up of more than one polypeptide chain (secondary structure). According to Linus Pauling and Robert Carey, proteins exist in two conformations namely the α- and the β-conformation. The greatest portions of biological activities are found in the flexible portions of the polypeptide chain not organised into α- or β-sheets which may consist of turns, loops, fingerlike extensions or hinges.

During the process of folding, hydrophobic are usually found association with themselves within the protein structure while hydrophilic amino acids are on the exterior, interaction with the aqueous environment of the cell cytoplasm.

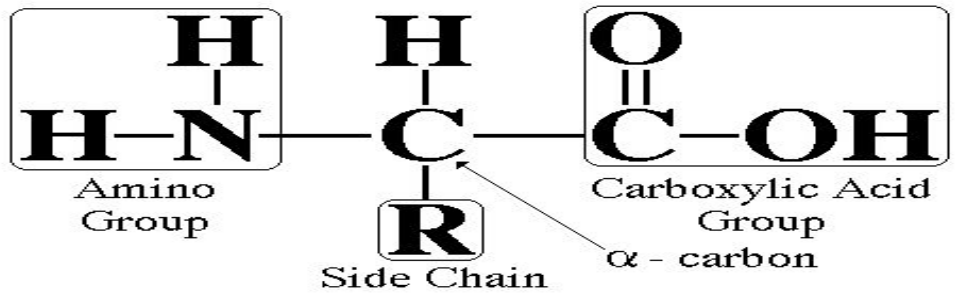


Fig.3. 2: General Structure of Amino Acids, each Amino Acid differs from each other depending on its Side Chain

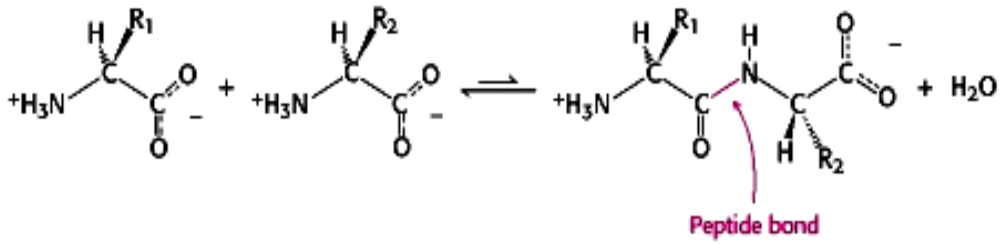


Fig.3. 3a: Peptide Bond Formation

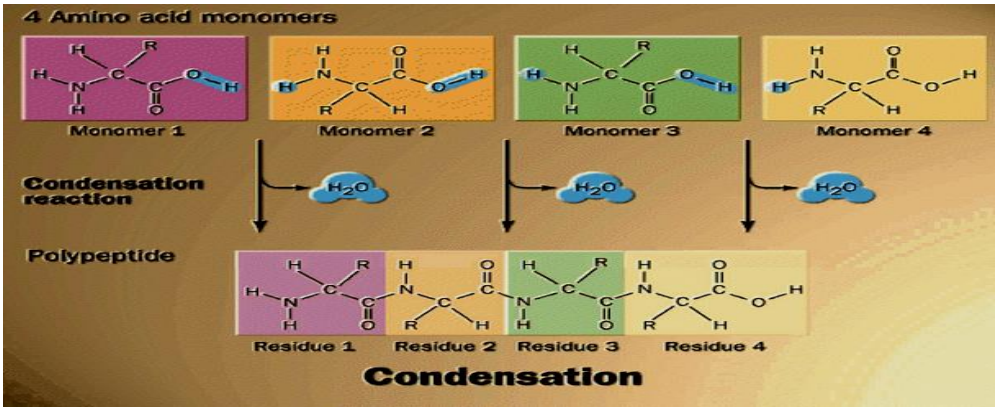


Fig.3. 3b: Polypeptide Bonds Formation via Amino Acid Condensation

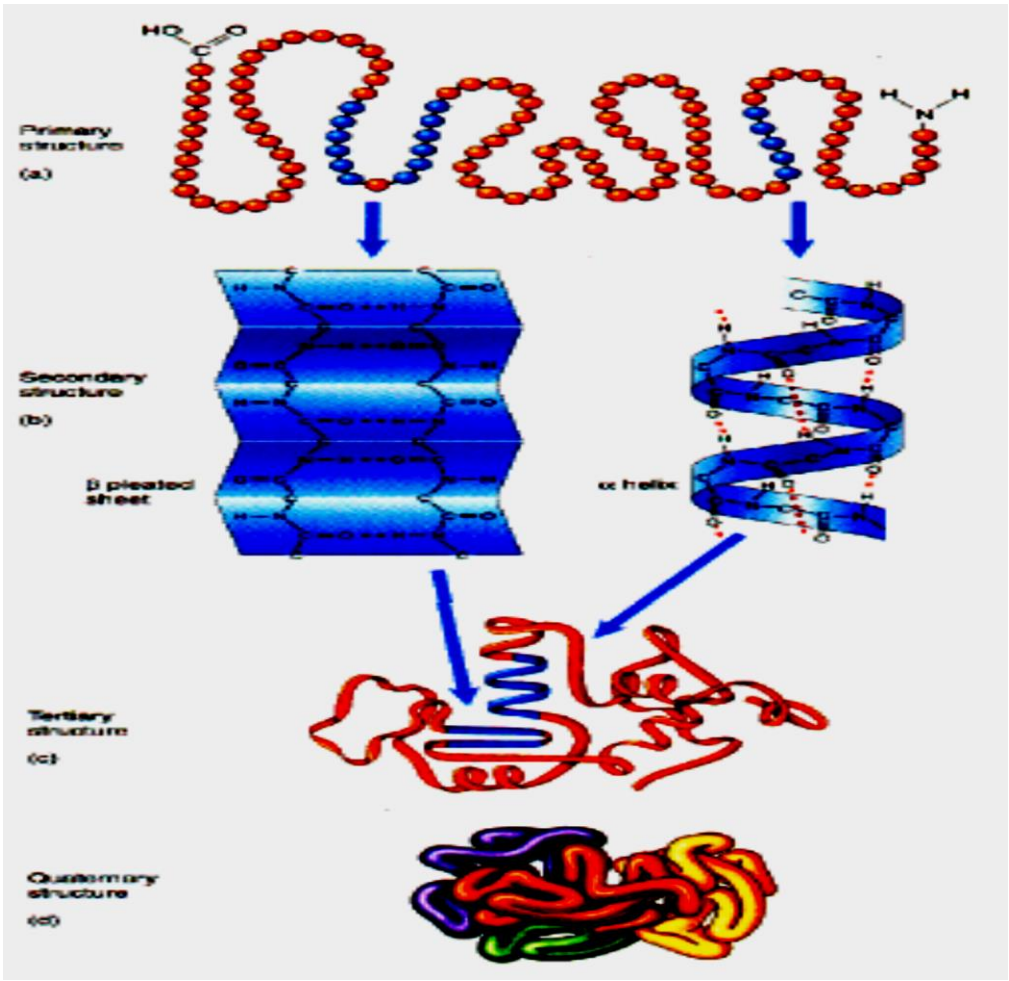


Fig.3. 4: Differtent Protein Structures, 3-Dimensional Folding Proteins

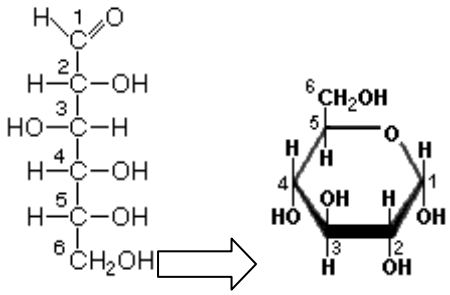
3.1.3 Carbohydrates

Carbohydrates are organic biomolecules made up of polymers of simple sugars. They are often characterised by having the ratio 1:2:1 of carbon to hydrogen to oxygen respectively and a general formula $(CH_2O)n$. Sugars that have three carbon atoms are called the trioses (e.g. glyceraldehydes & dihydroxyacetone), four carbon atoms as

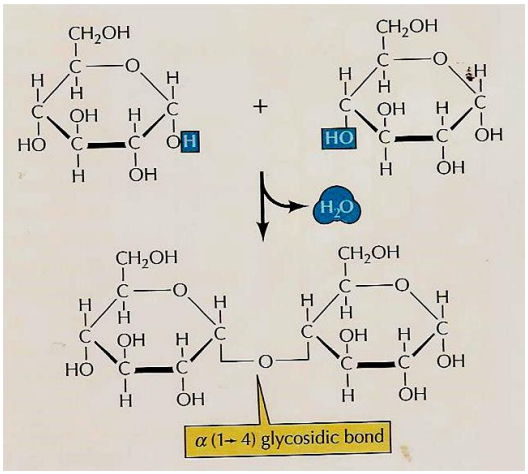
tetroses, five carbon atoms as pentoses (e.g. ribose), six carbon atoms as hexoses (e.g. glucose, sucrose) and seven carbon atoms as heptoses. In cellular metabolism, sugars having carbon atoms ranging from three to seven are of importance. For example, the six-carbon sugar, glucose is essentially important in cells since it provides the principal source of cellular energy. Apart from the six carbon sugars, five carbon sugars, pentoses are the most common. They are major components of the nucleic acids.

There are three types of carbohydrates; monosaccharides, disaccharides, and polysaccharides. Monosaccharides are the simplest sugars. Disaccharides consist of monosaccharides bonded together by a dehydration reaction, through a glycosidic linkage (fig. 5). When more than two sugar molecules are joined together, they yield polymers called oligosaccharides. Polysaccharides are macromolecules that are made up of hundreds to thousands of monosaccharides. These polymers are used for storage or for structure. Starch and glycogen are polysaccharides commonly used for energy storage in plants and animals respectively. In fact they are of nutritional importance because most plants and animals, bank their excess energy in these forms. Both types of polysaccharides are composed of glucose monomers. Starch consists of a mixture of amylase and amylopectin structures of these two compounds vary. Glycogen a branched polymer and starch have their sugars joined by α-1, 4 glycosidic bonds while occasionally the former and amylopectin contain α – 1, 6 linkages Cellulose, another polysaccharide composed mainly of glucose units is the principal structural component of plant cell wall. Other complex polysaccharides include chitin and glycosaminoglycans which are structural polysaccharides and form tough durable structural materials unlike others that are easily digested energy stores. Chitin, found in exoskeletons of arthropods and in cell walls of fungi, is used as a strengthening structure and for protection.

In cell, carbohydrates sometimes interact with lipids and proteins to form glycolipids and glycoproteins respectively. These molecules are important in cellular structure and cell-cell recognition (signaling).



a)



b)

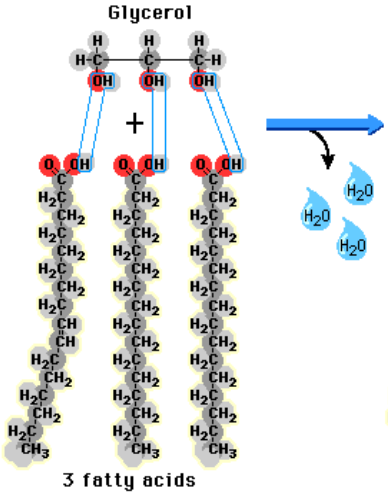
Fig.3. 5a: Glucose **Fig.3. 5b: Glycosidic Bond Formation**

3.1.4 Lipids

Lipids are another group of important biomolecules active in cell structure and function. Lipids form an integral part of the cell membrane, ensuring a controlled regulation of molecules across the membrane while promoting the fluidity of the cell membrane. Lipids are also important for energy storsge (unsued energy molecules are stored as lipids).

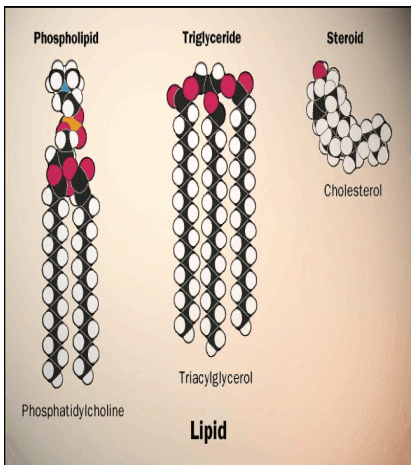
They are generally made up of a group of heterogeneous macromolecules easily characterised by their relative insolubility in water and their solubility in organic solvents such as benzene, petroleum ether and chloroform. They are composed of long aliphatic hydrocarbon chains or benzene rings which are non-polar and hydrophobic (water repelling). Lipids are composed of carbon, hydrogen and oxygen with a carbon to hydrogen ratio of 2:1. Thus, they contain more carbon - hydrogen bonds than carbohydrates, a property that makes them better energy storing molecule. The lipids generally present in biological systems are neutral fats, phospholipids, glycolipids, steroids and terpenes. Fats are composed of glycerol molecules linked by ester bonds to three fatty acids, together called **triacylglycerol** (fig. 6a). Fatty acids which are long unbranched hydrocarbon chains are the simplest lipids having only a single carboxyl group at one end. Triacylglycerol as a result of their insolubility in water form fat droplets in the cytoplasm. The two ends of fatty acid are different thus, they have different properties. The carboxyl group of the fatty acid chain is hydrophilic because it interacts with water molecules while the hydrocarbon chain is hydrophobic.

Fatty acids vary in length of their hydrocarbon chains as well as the presence or absence of double bonds. They could be monoacylglycerol, diacylglycerol or triacylglycerol depending on the number of fatty acids attached to the glycerol. Fatty acids lacking double bonds are said to be saturated while those with double bonds are referred to as unsaturated. Most vegetable oils contain unsaturated fatty acids. Fats that are liquid at room temperature are called oils. Lipids due to the diversity in their structures also exhibit diverse functions; however the unifying feature of all lipids is their hydrophobic nature. The property of hydrophobicity imposes the task of creating barriers between aqueous environments thus, lipids form components of membrane systems of organisms.



a)

Fig.3. 6a: Triglycerol Formation



b)

Fig.3. 6b: Examples of Lipids

Phospholipids which constitute the main components of cell membranes are made of two fatty acids linked to a polar head group (phosphate). The polar head group may be linked to smaller polar groups such as choline, serine, ethanolamine or inositol. Phospholipids are amphipathic molecules by having both hydrophobic tail and hydrophilic head groups within one molecule. Another important phospholipid called sphingophospholipid plays important roles in the structure and function of cellular membranes. In sphingophosphlipids e.g. sphingomyelin, the phosphate group is esterified not to glycerol but a complex amino alcohol called sphingosine. Phospholipids also participate in cell signaling as well as serving as messenger molecules within cells by sending signals from cell surface receptors to intracellular targets.

Glycolipids are made of two hydrocarbon chains joined to polar head groups containing carbohydrates. They are also amphipathic molecules but do not contain phosphate groups therefore they are less soluble in water than the sphingo-phospholipids. They are highly concentrated in central nervous system and also important component of cell membranes.

Steroids: These are characterised by having a four ringed hydrocarbon skeleton called phenanthrene and this makes them structurally distinct from other lipids. Cholesterol, a steroid is a component of animal cell membranes and a precursor for the synthesis of many steroid hormones such as estrogen, testosterone. It is also amphipathic because the hydrocarbon ring structure is strongly hydrophobic whereas the hydroxyl group which is attached to one end of cholesterol is weakly hydrophilic.

3.1.5 Nucleic Acids

The information regarding the structures of proteins in an organism is stored in macromolecules called nucleic acids. Nucleic acids are constructed from repeating units of monomers called nucleotides. They store and transmit genetic information and may also play structural and catalytic roles. There are two types of nucleic acids – DNA and RNA. The DNA plays a vital role as the genetic material and in eukaryotic cell is located within the nucleus. RNA on the other hand takes part in various cellular activities and they are of different types. Messenger RNA (mRNA) is involved in carrying the genetic information contained in DNA to the ribosome where it serves as a template for protein synthesis. There are other forms of RNA such as ribosomal RNA (rRNA), transfer RNA (tRNA) and both participate in protein synthesis. Each nucleotide consists of a five-carbon ribose sugar, a phosphate group and a nitrogenous base. The sugar present in DNA is a deoxyribose sugar and phosphodiester bonds are formed between 5' phosphate of a nucleotide and the 3' hydroxyl of another as nucleotides polymerise to form nucleic acids (fig. 7). An RNA or DNA strand contains four different nitrogenous bases which could be adenine, thymine, cytosine, guanine and uracil. Adenine and guanine are purine bases while thymine, cytosine and uracil are pyrimidine bases. Thymine replaces uracil in DNA and in RNA, uracil is present. Nucleosides are formed when the bases are linked to 2'-deoxyribose sugar in DNA or ribose sugar in RNA but when phosphate groups are linked to 5' carbon of the nucleosides, nucleotides are formed. Nucleotides could be oligo- or polynucleotides depending on the number of nucleotides that are polymerised to form them. Polynucleotides may be composed of thousands or millions of nucleotides and are always synthesised in the 5' to 3' direction and free nucleotide are added to the 3' OH group of a growing chain.

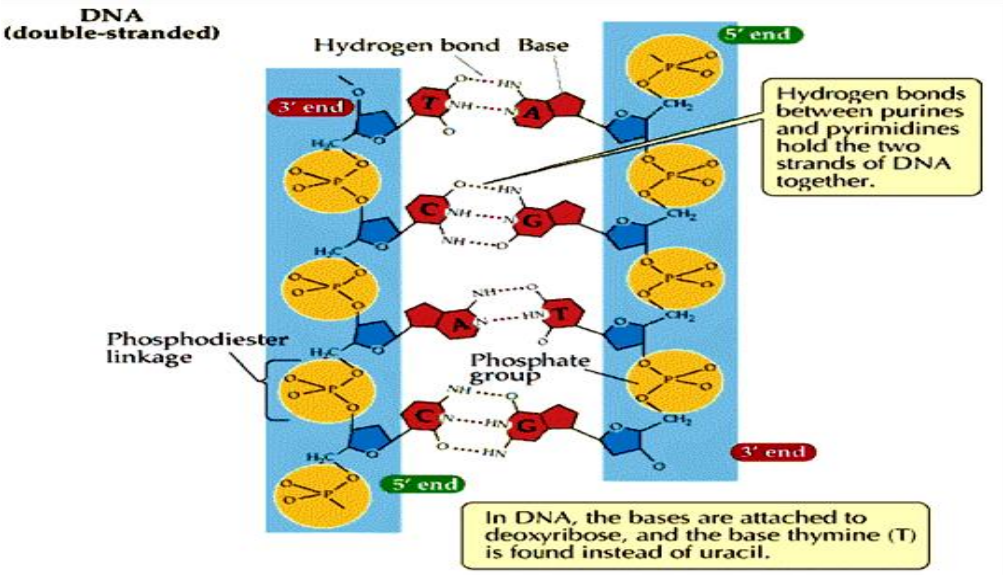


Fig.3. 7: Polynucleotide Formation in Nucleic Acid (DNA)

3.1.6 Inorganic Ions

Inorganic ionic constituents of the cell include ions such as Na^+, K^+, Mg 2+,Cl-, Ca2+,HPO42-, $HCO3^-$. Though they make up about 1% or less of cell mass, these ions are important in proper cell metabolism and functioning.

4.0 CONCLUSION

At the molecular level, cellular structure comprises of organic biomolecules (proteins, lipids, carbohydrates, and nucleic acids), water and some inorganic ions which interact via chemical bonds and reactions to ensure the structural integrity of the cell.

5.0 SUMMARY

In this unit, you have learnt that the cell at the molecular level is made up of proteins, water, lipids, carbohydrates, inorganic ions and nucleic acids. Proteins, nucleic acids, lipids and carbohydrates are polymers of amino acids, nucleotides, fatty acids, simple sugars respectively.

6.0 TUTOR-MARKED ASSIGNMENT

1. Briefly discuss at least three biomolecules and their roles in cell structure.

7.0 REFERENCE/FURTHER READING

Cooper, G.M. (2000). *The Cell: A Molecular Approach*. (2nd ed.). Washington DC: ASM Press.

UNIT 4 PROTEINS AND NUCLEIC ACIDS

CONTENTS

1.0 INTRODUCTION

Proteins and Nucleic Acids (DNA and RNA) are important biomolecules necessary for the structure and proper functioning of the cell. Nucleic acids are mostly found in the core/centre of the cell, where they direct cellular activities and division, while proteins are found everywhere in the cell.

Before the revolutionary discovery of the DNA structure by Watson and Crick (after drawing conclusion from many scientists before them), proteins were thought to be the hereditary material (genes). This is because there are a lot of proteins each made up of a long chain of amino acids folded together to form complex structures, while DNA contains only four repeating units of nucleotides. It therefore, seemed unlikely that the simple nature of the DNA can enable it carry the complex information required to specify the distinct form of each of the infinite kinds of cell that constitute a living being.

Extensive research in cytology and related Genetics field has revealed that while nucleic acids carry the genetic information of the cell, proteins are primarily responsible for the execution of tasks directed by the information. This is represented in the ***central dogma*** equation. DNA is transcribed to mRNA in the nucleus; mRNA is then translated to amino acids in the cytoplasm which later form protein polymers.

DNA ⇨ mRNA ⇨ Protein

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss in detail proteins
- discuss in detail nucleic acids.

3.0 MAIN CONTENT

3.1 Nucleic Acids

Nucleic acids are principal information molecules that store and transmit hereditary information. There are two types of nucleic acids, namely; **DNA**- ***deoxyribonucleic acids*** and **RNA**- ***ribonucleic acid***. DNA plays a unique role in cell replication (through mitosis & meiosis), protein synthesis and in eukaryotic cells it is located in the nucleus. Different types of RNA participate in various cellular activities, messenger RNA (mRNA) information from carries information from DNA to the ribosomes, where it serves as a template a template for protein synthesis. Ribosomal RNA (rRNA) and transfer RNA (tRNA) are involved in protein synthesis.

As earlier mentioned, nucleic acids are polymers of nucleotides which consist of purines and pyrimidines bases linked to phosphorylated sugars. The pyrimidine cytosine pairs with purine guanine and pyrimidine thymine and uracil pair with the purine adenine. Each nucleotides consists of a nitrogen base, a five carbon sugar (pentose sugar), and a phosphate group.

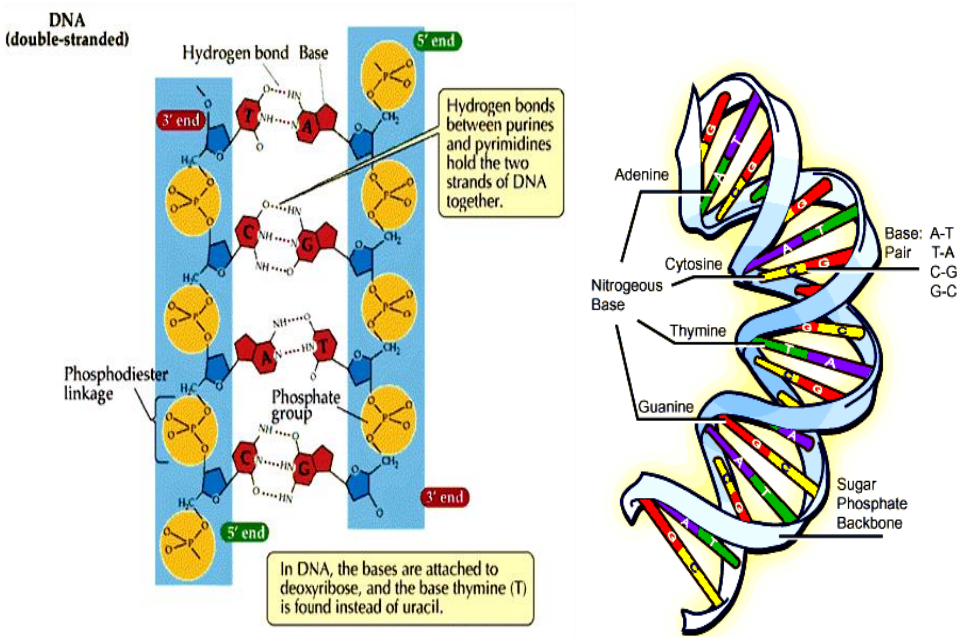


Fig.4. 1: Structure of DNA

3.2 Proteins

Proteins, unlike nucleic acids are composed of 20 different, naturally occurring amino acid monomers in various combinations. They are the major functional macromolecule of living organisms, loyally carrying out the instructions dictated/encoded in Nucleic acids (DNA & RNA). Structurally, proteins are more complex than nucleic acids, since they have higher number of monomers and thus, higher number amino acid sequence combinations. Four major structural levels of proteins exist, viz. primary, secondary, tertiary, and quaternary structural levels (fig. 2).

Primary structures

These consist of the freshly translated amino acid sequences that make a particular protein. It is the polypeptide sequence of proteins.

Secondary structures

These are of two types, the alpha-helices (alpha-helixes) and beta-pleated sheets. Secondary structures are polypeptide chains organised into regular structures held together by hydrogen bonds between one of the lone pairs on the oxygen atom (of the carboxyl group) and the hydrogen atom (of the amino group).

Tertiary structures

These are several secondary structures held together by various chemical bonds between their side chains. These bonds could either be, ionic (common amongst amino acids with –COOH group in their side chain), hydrogen (amongst amino acids with –OH/NH_2), van der Waals forces (amongst amino acid with long hydrocarbon chains), disulphide bonds (between amino acid containing sulphur).

Quaternary structure

These are several tertiary structures bounded together.

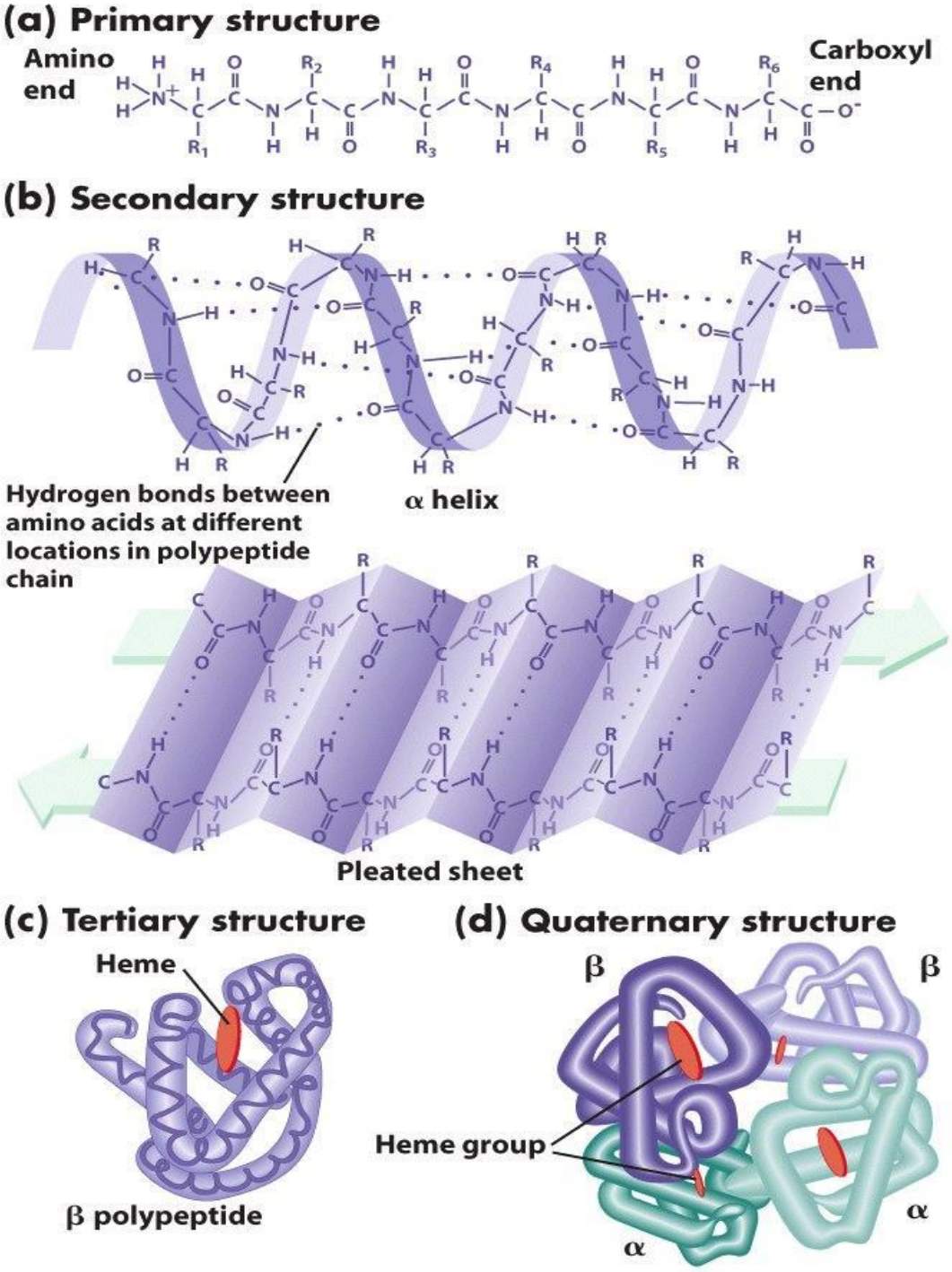


Fig.4. 2: Different Level of Protein Structure

3.3 Differences and Similarities between Nucleic Acids & Proteins

A brief comparison between DNA, RNA and Protein is tabulated below:

Table 4. 1: Differences and Similarities between Nucleic Acids and Proteins

Nucleic Acid	DNA	RNA	Protein
Monomers	Nucleotides (A,G,T,C)	Nucleotides (A,C, G, U)	Amino acids
Structure	Double Stranded Helix	Single stranded	Three dimensional structure
Distribution	Nucleus	Nucleolus, Cytoplasm	Everywhere in the cell
Functions	Cell replication, protein Synthesis	Protein synthesis	Structural & Metabolic activities
Reactivity	Very stable Low Reactivity	Reactive	Most reactive
Replication	Self Replicating	Transcribed from DNA	Translated from RNA

4.0 CONCLUSION

Nucleic acids and proteins are essential biomolecules essential for proper cell structure and functioning.

5.0 SUMMARY

In this unit, you have learnt about nucleic acids and proteins. The similarities and differences between nucleic acids and proteins were treated.

6.0 TUTOR-MARKED ASSIGNMENT

1. Describe the different structural levels of proteins.
2. Give the differences between proteins and nucleic acids.

7.0 REFERENCE/FURTHER READING

Cooper, G.M. (2000). *The Cell: A Molecular Approach*. (2nd ed).. Washington DC: ASM Press.

MODULE 3

UNIT 1 CELL CYCLES

CONTENTS

1.0 INTRODUCTION

Reproduction, an inherent characteristic of all living organisms, ensures the continuity of life through the development of young ones at the organism level and the formation of daughter cells via cell division at the cellular level.

Cellular division through repeated cell cycles ensures the development of a single fertilised egg into the more than 10^{13} cells of the human body and the multiplication of a single bacterium into a colony consisting of millions of daughter cells during an overnight incubation. The cellular events that occur in sequence from one cell division to another are referred to as *cell cycle*. A Cell cycle, either in a simple prokaryotic or complex eukaryotic cell is highly regulated ensuring that parent cells only divide only when the need arise most especially in the presence of certain factors that stimulate proliferation (cell division). These factors range from the availability of nutrient agar, space and the optimum temperature for a simple bacterium to the complex signal transduction pathways between surface receptors of the eukaryotic cells. Many variations exist in the overall duration of cell cycles depending on the cell type and the requirement of the organism. For instance, a rapidly dividing embryonic cell has short cell cycle duration of 30 mins, while

the slowly dividing muscle cell of humans has a cell cycle that could last several days.

In humans, the study of cell cycle and its control is usually closely connected with the study of cancer as it has been observed that the progression of cancer arise as a result of defects in the cell cycle control.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- describe the different phases of cell cycle
- highlight the factors that affect cell cycle
- discuss the process of cell cycle control.

3.0 MAIN CONTENT

3.1 Phases of Cell Cycle

Two major cell cycle types exist based on the two types of cell: i.e. the Prokaryotic and Eukaryotic cell cycle. Cell cycles, whether prokaryotic or eukaryotic can be broadly divided into a growth and division phase comprising mainly of cell growth and DNA replication & division. In this unit more emphasis will be laid on the Eukaryotic cell cycle.

A typical Eukaryotic cell cycle comprises of the following phases:

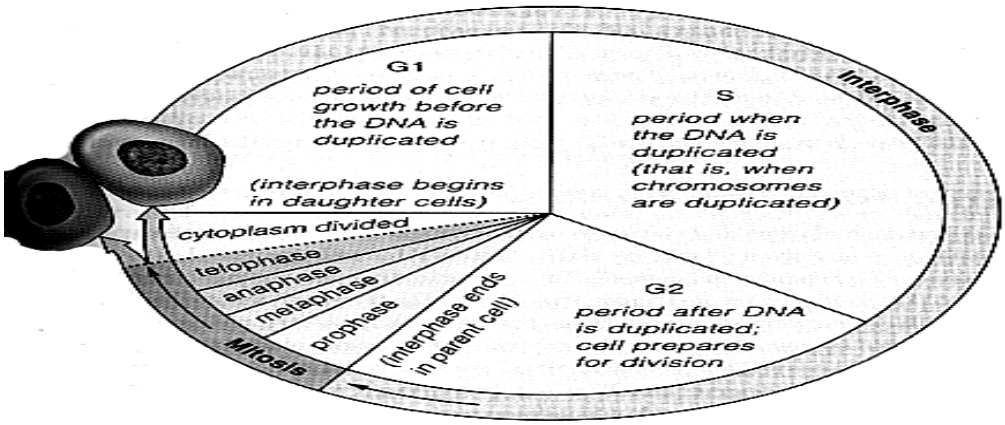


Fig.1. 1: Phases of a Cell Cycle
Source: www.googleimages.com/cellcycle

- **G_0 phase**

This phase is also known as the 'resting phase'. In this phase the cell is described as being quiescent, as it is neither growing nor dividing but metabolically active (although lesser proteins are synthesised as

compare to other phases). Most cells of the internal organs are in the G_0 phase. Some of these cells can be induced to divide by appropriate stimuli. For example, the lymphocytes are induced to divide in the presence of pathogens (foreign particles), the skin fibroblast divide in response to tissue damage or wound, and the liver cells divide following excision or damage of any of its parts.

Not all cells in the G_0 phase can be stimulated to divide, some remain in quiescent for life, examples are the neurons of a developed brain, and the nerve cells. These cells are usually indicators of age related diseases (e.g. dementia).

- **G_1 (gap 1) phase**

This is also known as the growth phase. It is the most variable phase of the cell cycle in terms of length, although some variations also occur in the S & G_2 phases. The length of G_1 phase ranges from being non-existent (as observed in sex and embryonic cells) to being as long as 11hours for a typical cell cycle of 24 hours (a typical cultured human cell). Some cells have short G_1 phases, example, the epithelial cells, in which case their generation tine (time it takes to complete a cycle) would also be short.

In the G_1 phase, the cell progressively grows in size and is metabolically active with profound protein synthesis occurring. Duplication of organelles occurs, proteins (enzymes) required for DNA replication are synthesised and sufficient mitochondria are produced for the energy requirement of the next phase. Eukaryotic cells proceed into the G_1 phase from their resting phase in response to external factors. For instance, in the yeast cell, availability of nutrients is enough to trigger the initiation of the G_1 phase. Whereas in most animal cells, initiation of the G_1 phase is triggered by external growth factors, e.g. the skin fibroblast progresses into the G_1 phase only in response to the platelet-derived growth factor released by blood platelets during clotting at the site of an injury. The gap one phase is the interval between the S and M phases.

- **S phase**

The S phase is also known as the synthesis phase. The cell in this phase is in a low metabolic state and the major activity of the cell centers around the DNA/Genome duplication. The duplication of the DNA is necessary for the distribution of the complete of the DNA to the daughter cells ensuring that the daughter cells have genes exactly similar to the parent cells. 99% of cell that enter into the S phase eventually divide, i.e. go through mitosis. This is because cells in the S phase are

unstable due to their abnormal DNA constitution. For instance, a diploid cell with 2n constitution will have a 4n constitution in S phase. There is need therefore, for the 4n DNA constitution to be divided into the more stable 2n status during cell division (mitosis). The prominent features of the S phase are the intact nuclear membrane required for anchorage of the un-twisting DNA molecule during DNA replication, presence of DNA untwisting enzymes such as helicases, and topoisomerase and an increased mitochondrial content and activity. The progression the G_1 phase to the S phase is a major checkpoint in cell cycle control/regulation.

- **G_2 (Gap 2) phase**

In this phase, cellular growth continues as in G_1. The proteins required for the next phase (M phase) are synthesised. The activities in this phase can be grouped into 3:

i. maturation - spindle forming proteins are assembled, nuclear membrane disappear
ii. repair - damages in the DNA are repaired
iii. packaging - DNA molecules are packaged into the chromosome structure for easy transfer or transportation.

- **M phase**

This is also called Mitosis phase. Cell division through the distribution and transfer of the earlier duplicated chromosomes into daughter cells occurs in this phase. It is usually the shortest and last for about an hour for a typical 24 hours cycle. M phase is usually followed by cytokinesis usually after the movement of the divided chromosomes to the opposite poles by the spindle fibre. Cytokinesis results in the cell undergoing cell cycle to be divided into two daughter cells; it involves the division of the cytoplasm. In some cycles the M phase is not followed by cytokinesis resulting in the attachment of the repeatedly formed daughter cells,

The first three phases of the cell cycle (G_1, S, G_{2s}), usually occur during the interphase of a normal dividing eukaryotic cell, while the prophase, metaphase, anaphase and telophase occurs at the M phase. Although there is no definite start point for the eukaryotic cell cycle, it is usually known to commence when the cell enters into the S phase, since all cycling cells that enter this must divide (go through M phase). Not all cells that emerge from the M phase go through to complete the cycle; some cells never go through to the G_1 phase.

3.2 Cell Cycle Control

The progression of the eukaryotic cell cycle is regulated by a system of control checkpoints and feedback controls that prevent the entry into the next phase of the cycle until events of the preceding phase have been completed. These checkpoints are in turn controlled by both extracellular signals from the environment, as well as internal signals that monitor and coordinate the processes that take place during the different cell cycle phases.

3.2.1 Control Checkpoints

Three major control checkpoints exist during the course of the eukaryotic cell cycle. They occur at certain intercepts between phases.

- **G_1/S phase checkpoint**

This checkpoint occurs during the late G_1 phase, determining the progression of the cycle from the G_1- S phase. The activities of this checkpoint are controlled by external signals. In the budding yeast (*Saccharomyces cerevisiae*), this checkpoint is known as **START** and it is regulated by the availability of nutrients and cell size. In the animal cells, however, it is known as the **RESTRICTION POINT** and it is controlled by external growth factors, such as the epidermal growth factors, platelet-derived growth factors and so on. Cells that pass through this checkpoint will enter into the S phase and go through the rest of the cycles. Cells that do not go through this checkpoint will enter into the quiescent phase (G_0 phase). The presence of sufficient nutrients or extracellular growth factors indicates the need for cell division.

- **G_2/M phase checkpoint**

This checkpoint prevents the initiation of mitosis prior the completion of the S phase, thereby preventing the transfer of incompletely and improperly replicated cellular genome to the daughter cell during mitosis. In response to a damaged or unreplicated DNA, the cell cycle is arrested at the G_2 phase. This arrest allows for the damaged DNA to be repaired and completely replicated. The regulation of this checkpoint also ensures that genome replication occurs once per each cycle. Thus, once the DNA has been replicated; the initiation of another S phase is prevented.

- **Metaphase/anaphase checkpoint**

This checkpoint occurs towards the end of mitosis. It monitors the alignment of the chromosomes to the mitotic spindle fibre, thus ensuring

that a complete set of chromosomes is accurately distributed to the daughter cells.

3.2.2 Regulation by CDK Proteins

Cyclin-dependent Kinases (CDKs) or Maturation Promoting Factors (MPF) are a group of evolutionarily conserved proteins that coordinate the transition between cell cycle phases at checkpoints. MPF was first discovered in the amphibian oocyte by Yoshio Masui and Clement Markert in 1971, and purified in 1988. Each MPF is consists of a dimer of a cyclin-dependent kinase (cdk) coupled to a particular cyclin protein that direct transitions between different phases of the cycle (table 1). For instance, the CDK that controls the initiation of DNA synthesis (S phase) is different from that that controls the onset of mitosis (G_2/M phase checkpoint), thus, the activities of specific cyclins and kinases are at their peaks at the phases or checkpoints they coordinate.

A feedback mechanism that ensures that the activities of one cdk/cyclin complex do not affect that of the others is accomplished by a group of proteins called the cdk inhibitors. These inhibitors monitor the activities of the CDK proteins.

Table 1. 1: CDK/Cyclin Complexes and their Inhibitors

Checkpoints/Cell Cycle Phases	Cyclin-dependent Kinases	Cyclin	CDK Inhibitors
	Budding Yeast		
START	cdc 2	cln 1,2,3	Far 1
S phase	cdc 2	clb 5, 6	Sic 1
G_2/M	cdc2	clb 1,2,3,4	--
	Humans/Animal cells		
Restriction Point	cdk 4,6	cyc D	cip/kip and Ink 4 family
Later G_1- Early S	cdk 2	cyc E	cip/kip family
S	cdk 2	cyc A	cip/kip family
G_2/M	cdk 1	cyc B	--

Source: Cooper, 2000

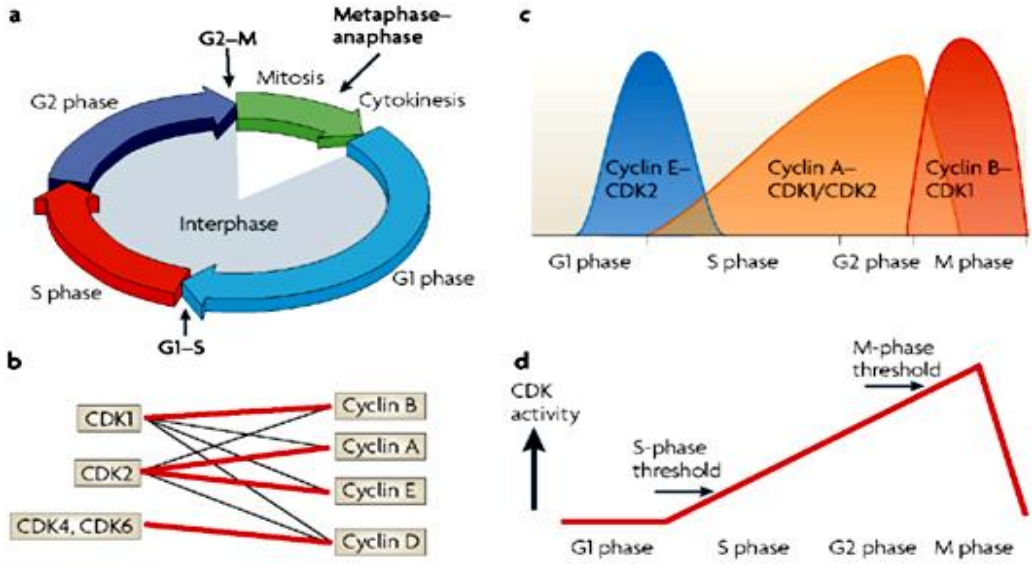


Fig.1. 2: Classical Model of Human Cell Cycle and its Control

Source: Hochegger *et al*.(2008)

4.0 CONCLUSION

The cell, synonymous to an organism, exhibits all the characteristics of a living organism one of which is reproduction. Reproduction of the cell occurs through the mitotic cell division in a series of cell cycles. These cell cycles are highly regulated at specific checkpoints and a defected control of cell cycles often leads to cancer (uncontrollable proliferation).

5.0 SUMMARY

In this unit, you have learnt that cell cycles occur in different phases. You learned that the progression of the cycle from one phase to another is controlled by extracellular and intracellular factors. Cell cycle in the eukaryotic cell is highly regulated at checkpoints. A defective cell cycle regulation is the major feature of cancer.

6.0 TUTOR-MARKED ASSIGNMENT

1. Write briefly on the phases of a typical eukaryotic cell cycle.
2. Highlight the factors (both intrinsic and extrinsic) that influence cell cycle progression between the phases.
3. Discuss cell cycle control.

7.0 REFERENCES/FURTHER READING

Cooper, G.M. (2000). *The Cell: A Molecular Approach*. (2nd ed.). Washington DC: ASM Press.

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www.googleimages.com/cellcycle

UNIT 2 REPRODUCTION AND CELL DIVISION

CONTENTS

1.0 INTRODUCTION

The continuity of life of all living organisms is ensured through the process of reproduction. Genetic continuity between generations of asexually reproducing and sexually reproducing organisms is maintained through the processes of the cell divisions, mitosis and meiosis respectively. These cell divisions ensure that the integrity and characteristics of parents are conserved and transferred meticulously to their progenies. Although the mechanisms of the two divisions are similar in many ways their outcomes are quite different. During the stages of both mitosis and meiosis, the genetic material is condensed into discrete structures called, chromosomes.

Mitosis leads to the production of two daughter cells, each with the same number of chromosomes as the parent cells. It typically converts a diploid cell into two diploid cells, with identical genetic constitution. In contrast meiosis reduces the genetic content of the parent cell by half and distributes it into four daughter cells all with variable genetic constitution. In diploid, sexually reproducing organisms, meiosis prevents the doubling of the genetic material between generations ensuring the maintenance of a balanced genetic constitution from one generation to another. The meiotic cell division leads to the production of gametes.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- state the role of cell division in reproduction
- illustrate in full, the mitotic cell division
- illustrate in full the meiotic cell division

- differentiate between the mitotic and meiotic cell divisions.

3.0 MAIN CONTENT

3.1 Mitotic Cell Division

The mitotic cell division is critical to some single-cell organisms such as protozoan, some fungi and algae as it is the basis for their reproduction (asexual), an in all eukaryotic organisms as it is the basis for their growth. In eukaryotic organisms, it typically occurs in the somatic cells (non-sex cells).

There are five stages in mitosis which are as follows:

Interphase
Interphase consist of three stages; G1, S and G2. Chromosome replication takes place in interphase. During interphase the individual chromosomes are elongated and are difficult to see under the light microscope. The DNA of each chromosome is replicated in the S phase, giving two exact copies called sister chromatids, which are held together by the replicated but unseparated centromeres.

Prophase
During this stage the chromosomes become visible as threads because they condense more. This is followed by progressing coiling and folding. Each prophase chromosome now consists of two adjacent chromosome threads called chromatids. The nucleolus breaks down and disappears. Electron microscopic studies have shown that the component parts of the nucleolus disperse throughout the nucleus during this stage. At the end of prophase, the nuclear envelope breaks down into fragments. This allows the chromosomes to spread over the greater part of the cell and gives them a better chance to separate as chromatids during poleward movement.

Metaphase
At metaphase the chromosomes are at their highest level of coiling and therefore appear to be shorter and thicker than in any other stage. The chromosomes move to the equator of the cell. With the attachment of the spindle fibers and the completion of the spindle itself, the chromosomes move into position in the equatorial plane of the spindle called Metaphase Plate. Alignment of the chromosomes on this plate marks the end of metaphase.

Anaphase
This is a stage of active and rapid movement and is the shortest of all mitotic stages. During this stage, the sister chromatid separate and move

towards the opposite poles on the spindle. The physical separation of the sister chromatids and their movement to opposite poles are two separate activities.

Telophase

At the end of anaphase, the separated sister chromatids have been pulled to opposite poles of the cell. At that time the nuclear envelope reforms around the two daughter nuclei, the nucleoli form at the distinct site of the nuclear organiser chromosomes, and the chromosomes fuse into an indistinguishable mass of chromatin. The uncoiling of the chromatin threads aid in this process of reforming an interphase nucleus where the chromosomes lose their density and stainability.

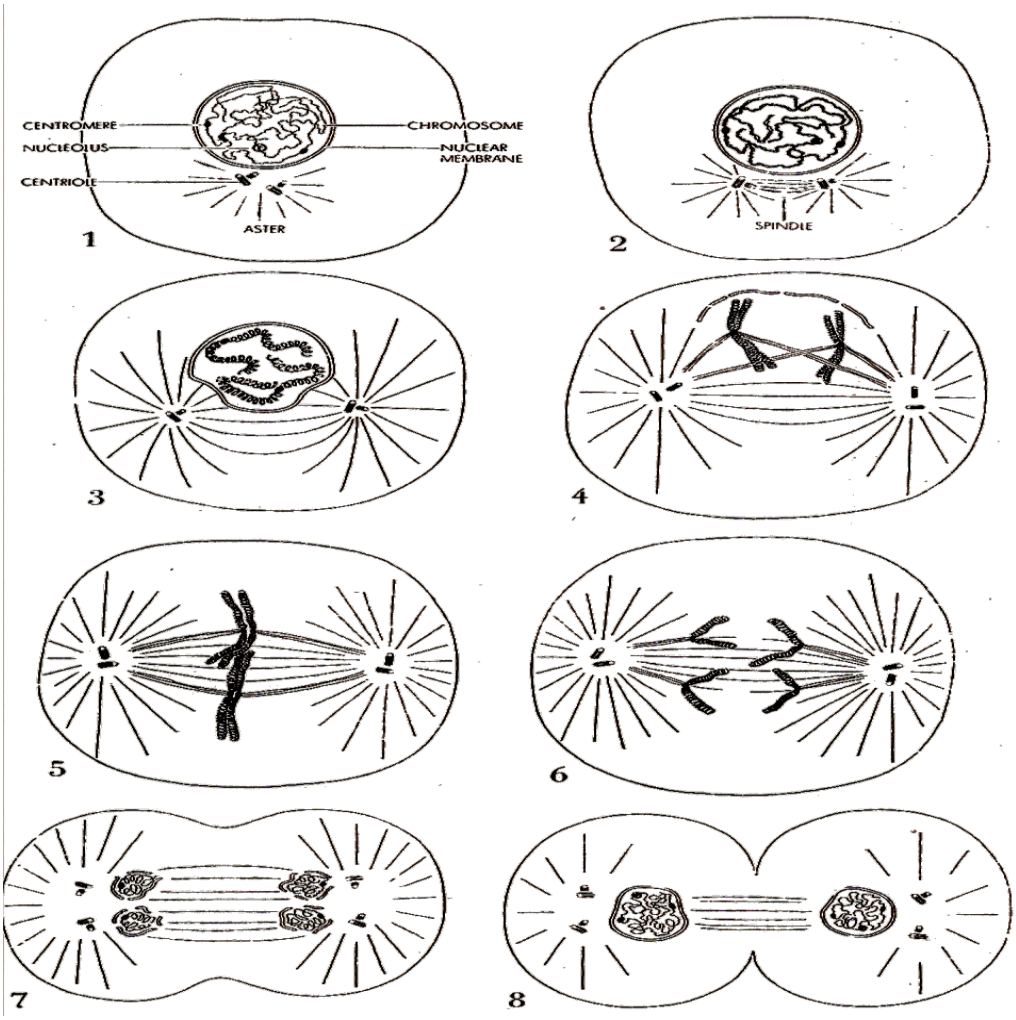

Fig.2. 1: Schematic View of the Mitotic Process in an Ideal Cell containing Two Chromosomes. The Cycles of the Chromosomes and Centrioles are emphasised. 1-3, Prophase, 4, Pro-Metaphase, 5, Metaphase, 6, Anaphase, 7-8, Telophase

3.2 Meiotic Cell Division

Like mitosis, meiosis is a continuous process. Meiosis includes two nuclear divisions that generally succeed each other rapidly and during which the chromosomes divide only once. These two divisions have been called different names according to the different functions carried out during this division by the chromosomes. Names like heterotypic and homeotypic as well as reductional and equational division are some familiar terms. The most commonly used nomenclature for the two divisions are the terms meiosis 1 and meiosis 2. Meiosis like mitosis has been divided into stages and substages. They are called:

Interphase, Prophase 1 (subdivided into 5stages which are Leptotene, Zygotene, Pachytene, Diplotene, Diakinesis), Metaphase 1, Anaphase 1, Telophase 1, Prophase 2, Metaphase 2, Anaphase 2, Telophase 2.

Prophase 1

An important feature of prophase 1 is the great increase in volume of the nucleus. This increase is greater than that during mitosis. It is divided in 5 substages:

Leptotene

Leptotene does not differ very much from early prophase in mitosis, with exception that meiotic prophase cells are larger than mitotic ones. During this stage, the chromatin material begins to condense, and the chromosomes, although still extended become visible. Along each chromosome are chromomeres localised condensations that resemble beads on a string.

Zygotene

The chromosomes are more shorten and thicken during this stage. There is complete pairing of homologous chromosome which started from leptotene. There is formation of a more extensive ultrastructural component called synaptonemal complex between homologs. It is at the completion of zygotene that the paired homologs are referred to as bivalents.

Pachytene

The chromosomes continue to coil and shorten, and further development of the synaptonemal complex occurs between the two members of each bivalent. This is the stage where crossing over takes place between non-sister chromatids; i.e. exchange of genetic material between the

homologous chromosomes. The point where crossing over takes is called chiasma. (pl: Chiasmata).

Diplotene

During this stage the chromosomes further contract and thicken. This is also the stage where the chiasmata become apparent as visible evidence of crossing over. The synaptonemal complex disintegrates and the bivalent is held together by the chiasmata.

Diakinesis

This is the final stage of prophase 1. The chromosomes pull farther apart, but non-sister chromatids remain loosely associated at the chiasmata. As separation proceeds, the chiasmata move toward the ends of the tetrad. The process of terminalisation begins in late diplotene and is completed during diakinesis. During this final stage, the nucleolus and nuclear envelope break down, and the two centromeres of each tetrad attach to the recently formed spindle fibers. By the completion of prophase 1, the centromeres of each tetrad structure are present on the metaphase plate of the cell.

Metaphase 1

The bivalents become arranged around the equator of the spindle, attached by their centromeres.

Anaphase 1

Spindle fibers pull homologous chromosomes, centromeres first, towards opposite poles of the spindle. This separates the chromosomes into two haploid sets, one set at each end of the spindle.

Telophase 1

The arrival of homologous chromosomes at opposite poles marks the end of meiosis 1. Halving of chromosomes number has occurred but the chromosomes are still composed of two chromatids.

Meiosis 2 is Similar to Mitosis

Interphase 2

This stage is present usually in animal cells and varies in length. No further DNA replication occurs.

Prophase 2

This stage is absent if interphase 2 is absent. The nucleoli and nuclear envelopes disperse and the chromatids shorten and thicken. Centrioles, if present, move to opposite poles of the cells and at the end of prophase 2 new spindle fibers appear. They are arranged at right-angles to the spindle of meiosis 1.

Metaphase 2

Chromosomes line up separately around the equator of the spindle.

Anaphase 2

The centromeres divide and the spindle fibers pull the chromatids to opposite poles, centromeres first.

Telophase 2

As telophase in mitosis but four haploid daughter cells are formed. The chromosomes uncoil, lengthen and become very indistinct. The spindle fibers disappear and the centrioles replicate. Nuclear envelopes re-form around each nucleus which now possesses half the number of chromosomes of the original parent cell (haploid).

3.3 Comparison of Mitosis and Meiosis

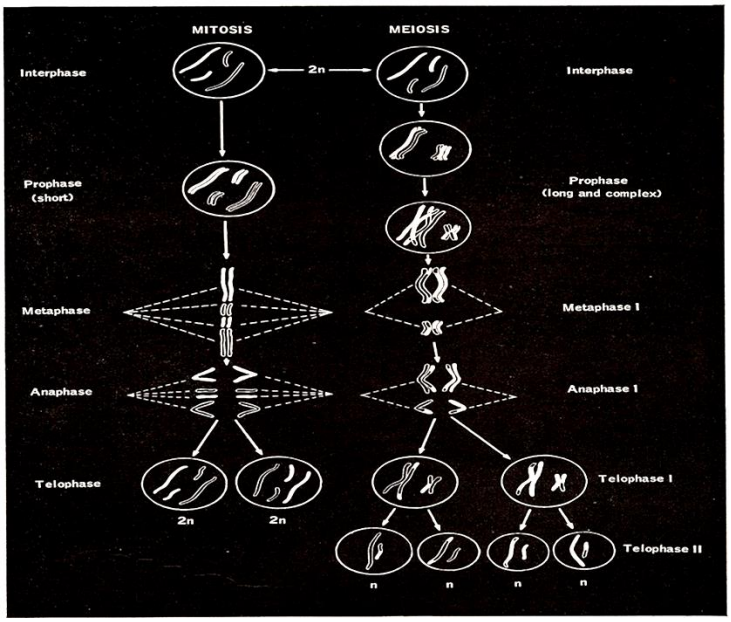

Fig.2. 2: Comparative Diagram of Mitosis and Meiosis

4.0 CONCLUSION

It is noteworthy that reproduction, whether sexual or asexual requires cell division. With the mitotic cell division being an eminent and only feature of the asexually reproducing organism and meiosis a prominent feature of the sexually reproducing organism. It should be noted, however, that both the mitotic and meiotic cell divisions occur in sexually reproducing organism.

5.0 SUMMARY

At the end of this unit, you have learnt that:

- mitotic and meiotic cell divisions are necessary in reproduction
- asexually reproducing organisms employ the mitotic cell division while sexually reproducing organisms employ the meiotic cell division
- at the end of mitosis two daughter cells are produced which are genetically identical to the parent cells
- meiosis ensures the balance of genetic constitution in diploid organisms from generation to generation.

6.0 TUTOR-MARKED ASSIGNMENT

1. Illustrate using diagrams, the mitotic and meiotic cell divisions.
2. Differentiate between the mitotic and meiotic cell divisions.

7.0 REFERENCES/FURTHER READING

De Robertis, E.D.P., Nowinski & W.W. & Saez, F.A. (1966). *Cell Biology*. (4th ed.). Philadelphia: W.B. Saunders Company.

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UNIT 3 CELLULAR GROWTHS AND DIFFERENTIATION

CONTENTS

1.0 INTRODUCTION

Cellular growth, through exponential division occurs via mitosis (discussed in previous units). Cellular differentiation on the other hand, is the progressive specialisation in structure and function of the cell. It involves the transformation of a more general and homogeneous structure to a more specialised and heterogeneous one reflected in both physiological and morphological characteristics. In other words, the more specialised a cell is the more it loses the general feature of potency.

The multiplicity of functions in an organism (multicellular) necessitates cellular specialisation/differentiation, thus leading to the morphological modification of certain cell types adapted to specific functions. For example, the nerve cells are structurally modified to adapt to the functions of irritability and conductivity which enables them to react to stimuli and transmit signals from one part of the organism to another.

Cellular differentiation is predominantly cytoplasmic rather than nuclear. This is because every cell present in an organism has the same genomic content in their nucleus, the only occurs in their cytoplasmic content and structure and the type of genes expressed in the cells (gene expression). It is for this reason that cells such as the muscle cells, nerve cells and plant cell wall have different structural morphologies adapted to their function. Cytoplasmic differentiation may be as a result of modifications of either the plasma membrane, cytoskeleton (cytoplasmic matrix) or the cellular organelles. Cellular growth and differentiation occur throughout life however; its peak is observed during embryonic development.

Cellular growth and differentiation is synonymous to both plant and animal cells.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss briefly on cellular growth and differentiation
- discuss growth and differentiation in plant cell wall and embryonic cells.

3.0 MAIN CONTENT

3.1 Growth and Differentiation in Embryonic Cells

As earlier mentioned, the peak of cellular growth and differentiation occurs in the developing foetus through the process of embryogenesis. It is the peak of cellular growth because it is in this process that the single celled fertilised ovum multiplies and differentiates into the various parts of the body (fig. 1). Embryonic development occurs through three main processes; growth, differentiation and metabolism. Growth is an increase of the spatial dimensions and depends on the multiplication of cells (via mitosis). Differentiation is characterised by the increase in complexity and degree of organisation while metabolism includes the chemical changes in the embryo that supply the necessary substrates for synthetic processes and chemical energy for other processes.

Immediately after fertilisation, the ovum undergoes a series of repeated cell divisions (**cleavage**) to form the embryonic structure called the ***blastula***. This process is mainly quantitative, leading only to an increase in the number of cells (growth). During **gastrulation**, the cells of the blastula rearrange themselves to form the three germ layers; the ectoderm, mesoderm and endoderm. The body axis and site of the future organs are also determined, and from this point cellular differentiation proceeds. **Histogenesis**, the formation of various tissues, and **organogenesis**, the separation of these tissues into organs, follows. After the process of organogenesis, cell division of the differentiated tissues continues until the whole organ is formed.

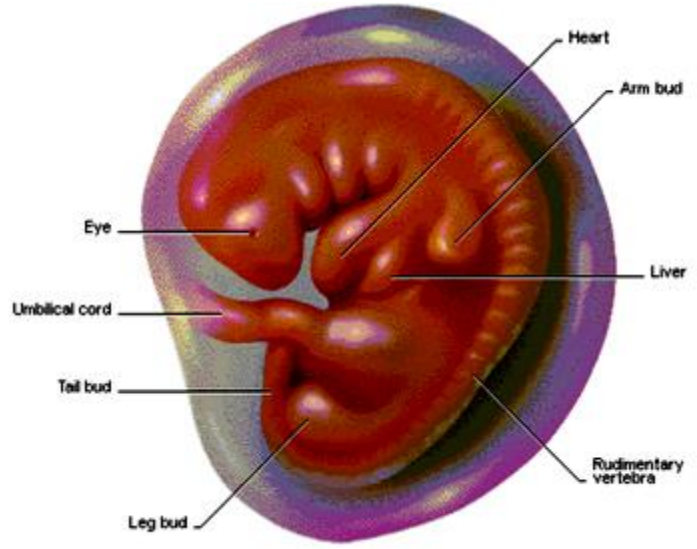

Fig.3. 1: Developing Foetus Showing Locations of Differentiated Organs

Source: googleimages.com

3.2 Growth and Differentiation in Plant Cell Wall

Plant cell walls are complex and highly differentiated. There major characteristic is their rigidity and they function to protect the plasma membrane and its content. Cell walls are made up of regular patterns of framework which provides the mechanical support for plant tissues. It was this framework that Robert Hooke observed microscopically.

Studies have shown that cell walls are made up mainly of cellulose ***microfibrils*** (each fibril containing about 2000 cellulose chain) and some other materials such as hemicellulose, pectin, lignin and mineral deposits. Cellular growth and differentiation occur in time sequence through the successive deposition of several layers of microfibrils leading to differential thickness of the cell wall (apposition). Base on the thickness and direction of the deposition of the microfibrils as well as the incorporation of other materials, cell wall can be divided into ***primary***, ***secondary*** and sometimes ***tertiary*** walls (fig. 2). The primary wall is composed of microfibrils that run in all direction while in the secondary wall the microfibrils run parallel and are more densely packed. In some tissues the tertiary wall is deposited at the interior of the secondary wall and is composed mainly of *xylan*.

The cell wall is the product of the cytoplasm (recall cytoplasmic differentiation). Immediately after nuclear division (during cell division and before cytokinesis), the ***Phragmoplast*** is formed which in turns forms the ***cell plate*** that later becomes the cell wall.

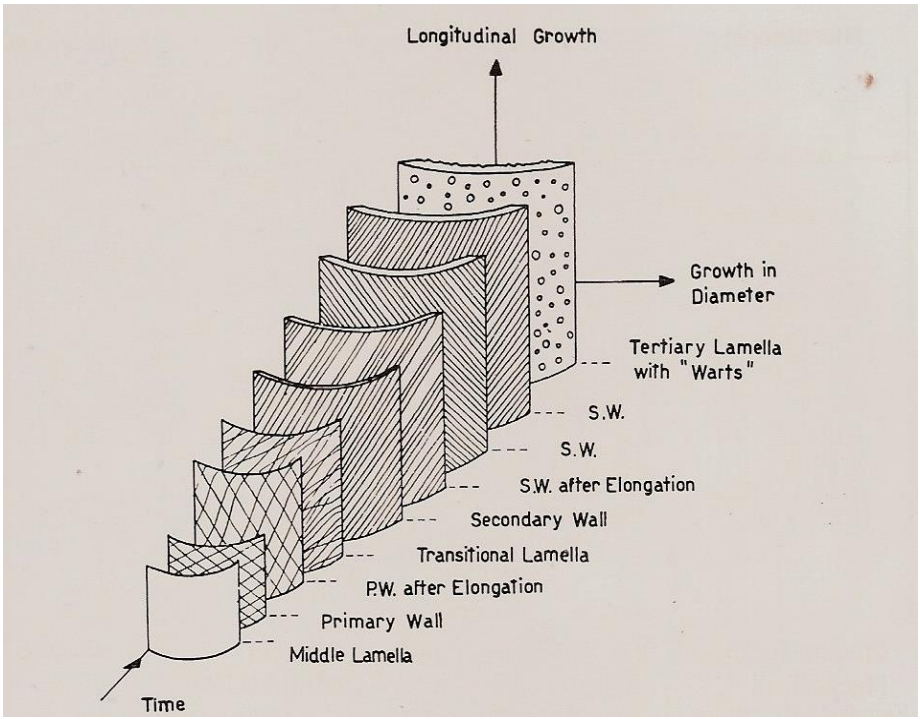

Fig.3. 2: Time Sequence of Formation of the Various Types of Cell Wall Layers in a Tracheid. *P.W.* - Primary Wall; *S.W.* - Secondary Wall. Notice that cellular growth is occurring in both the longitudinal (height) and horizontal (diameter) directions and the arrangement of fibrils and other structures in the different membranes.

Source: Mühlethaler (1961)

4.0 CONCLUSION

Cellular growth and differentiation is synonymous to both plant and animal cells.

5.0 SUMMARY

In this unit, you have learnt:

- that cellular growth and differentiation occurs throughout life of a multicellular organism, although its peak is observed during embryogenesis
- about cellular growth and differentiation in the embryonic cells
- about cellular growth and differentiation in the plant cell wall.

6.0 TUTOR-MARKED ASSIGNMENT

1. Discuss cellular growth and differentiation in embryonic cells.

2. Describe cellular growth and differentiation in plant cell wall.

7.0 REFERENCES/FURTHER READING

De Robertis, E.D.P., Nowinski, W.W. & Saez, F.A. (1966). *Cell Biology*. (4th ed.). Philadelphia: W.B. Saunders Company.

Mühlethaler, K. (1961). "Plant Cell Walls". *The Cell*. 2:85.

UNIT 4 DEVELOPMENTAL CELL BIOLOGY

CONTENTS

1.0 INTRODUCTION

Developmental cell biology is the science that studies the processes involved in the conversion of a single-celled fertilised zygote into a multicellular embryo of organisms. It is often referred to as Embryology, while the processes/stages involved in the development are collectively referred to as Embryogenesis. Embryogenesis can be divided into three main stages, namely: ***cleavage***, ***gastrulation,*** and ***organogenesis***.

Various similarities and differences exist in the embryogenesis of different organisms; for instance, cleavage patterns differ between different organisms but gastrulation is similar in them all.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- highlight and discuss the processes of embryogenesis
- highlight the events that take place during foetal development.

3.0 MAIN CONTENT

3.1 Stages of Embryogenesis

Cleavage
Immediately after fertilisation, the ovum undergoes a series of successive mitotic cell division. The stages of development are: ***Fertilised ovum (zygote) → 2-cell stage → 4-cell stage → 8-cell stage → Morula → Blastula → Early Gastrula → Late Gastrula*** (fig. 1). This is mainly a multiplication process.

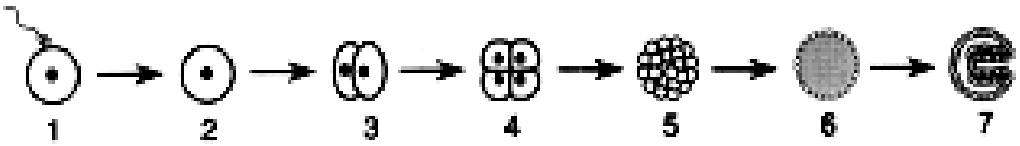

Fig.4. 1: Cleavage;1- Sperm and Ovum, 2- Zygote (fertilised ovum), 3- 2-cell stage, 4- 4-cell stage, 5- Morula, 6- Blastula, 7- Grastula

Gastrulation

Cellular differentiation is initiated at this stage. The three (ectoderm, mesoderm & endoderm) germ layers that are differentiated into different organs are developed. The fate of an organ depends on the part of the germ layers it is found. Gastrulation is similar amongst various phyla of animals.

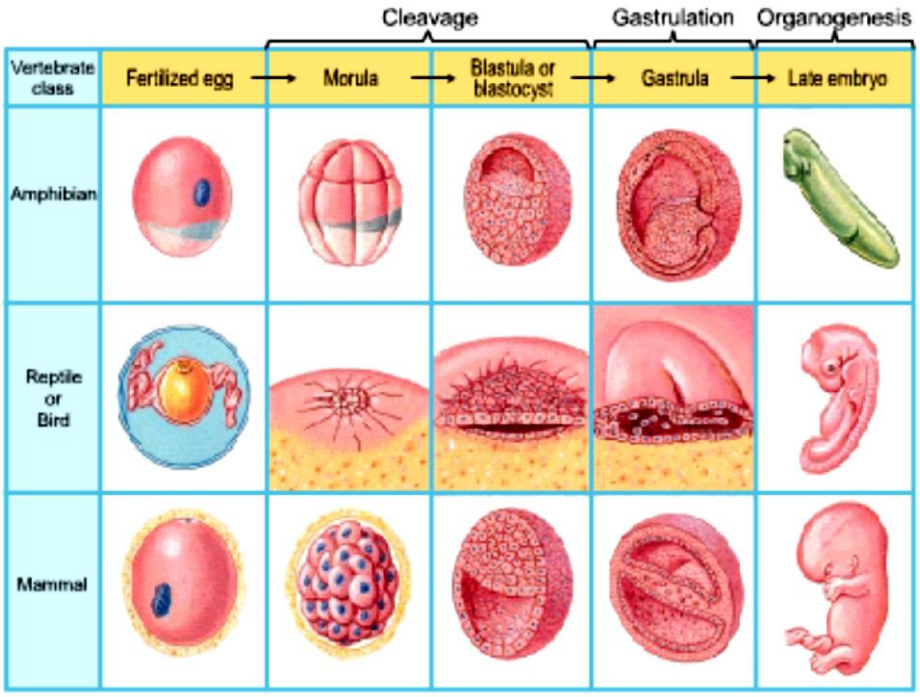

Fig.4. 2: Comparison of Embryogenesis in Amphibians, Reptiles and Mammals (Humans)

Organogenesis

Complete development of organs from various germ layers occurs. Embryos of different phyla can be differentiated, i.e. a baby fish, bird, and human can be differentiated. The different organs that are differentiated from various germ layers are listed below:

Endoderm

- the innermost layer
- goes on to form the gut.

Mesoderm

- in the middle
- goes on to form the muscles, circulatory system, blood and many different organs.

Ectoderm

- the outermost
- goes on to form the skin and nervous system.

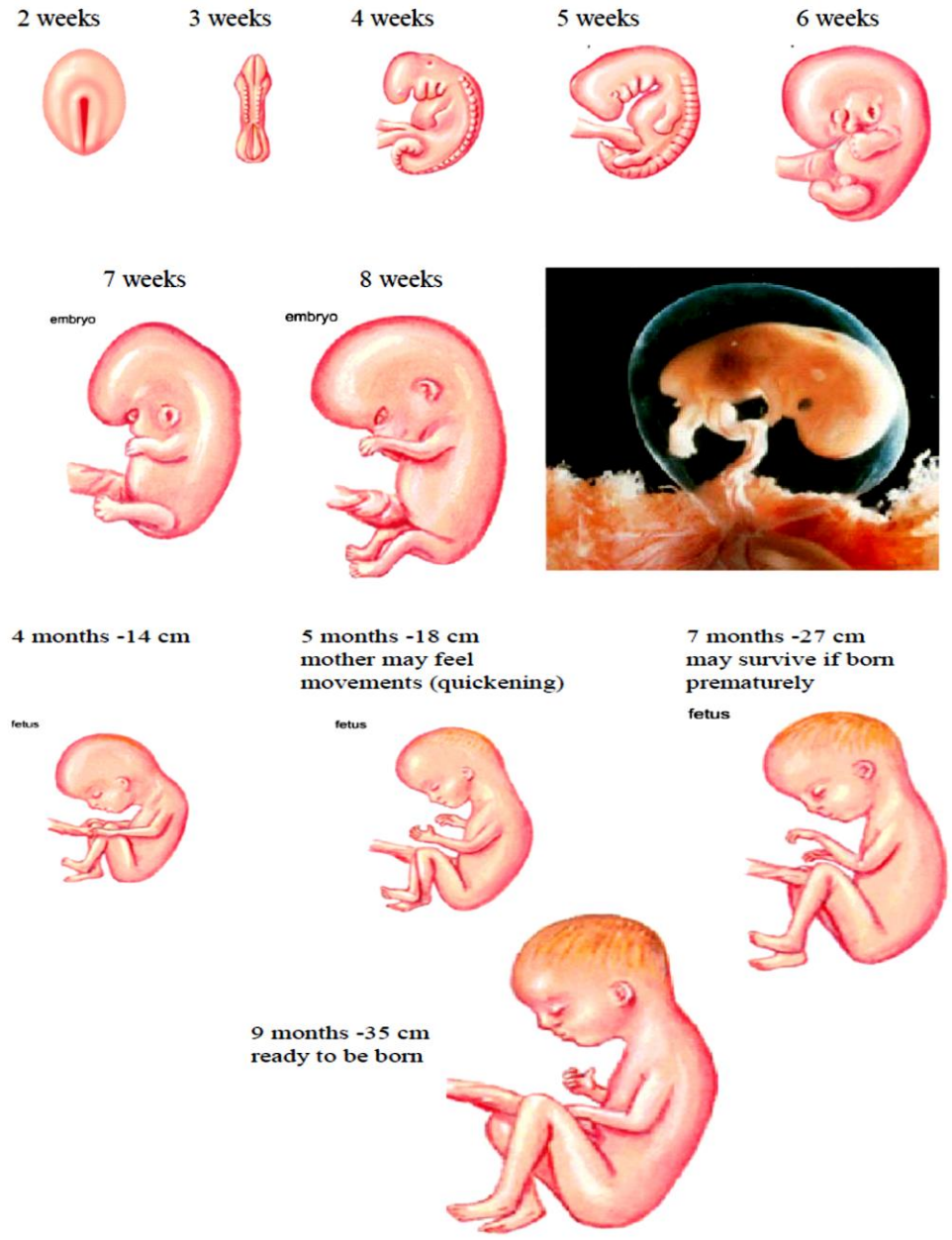

Fig.4. 3: Foetal Development in Human Embryogenesis

Embryogenesis in Humans: the Pregnancy Time-Table

Below are the events that occur in the progression of foetal development in humans.

Week 3: Central nervous system begins to develop, heartbeat is initiated and heartbeat can be felt.

Week 5: Brain is developed into5 components and the lumen of spinal cord is continuous with the brain, allowing free cerebral-spinal fluid flow. Nose and lip formation begins.

Week 8: Major organs begin development, hands and feet are seen, baby is extremely reactive to environment, and male sex hormone (testosterone) is produced by testes initiating masculine development in males. No changes observed in female baby. Embryo weighs about 4 grams.

Week 12: Baby is about an ounce in weight and the unique finger prints are developed.

Week 16: Baby's skeleton starts to harden.

Week 20: Eyebrows and lashes are well developed.

Week 28: Baby can open and close eyes and follow light. Baby can be born prematurely at this stage.

4.0 CONCLUSION

Developmental biology is the science that describes the processes involved in the development of the young ones of organisms. It is also referred to as embryology.

5.0 SUMMARY

In this unit, you learnt about the different stages of embryogenesis and the events that take place during human foetal development.

6.0 TUTOR-MARKED ASSIGNMENT

1. Highlight the organs that originate from the different germ layers.
2. Briefly discuss the stages of embryogenesis.

7.0 REFERENCES/FURTHER READING

De Robertis, E.D.P., Nowinski, W.W. & Saez, F.A. (1966). *Cell Biology*. (4th ed.). Philadelphia: W.B. Saunders Company.

Mühlethaler, K. (1961). "Plant Cell Walls". *The Cell*. 2:85.