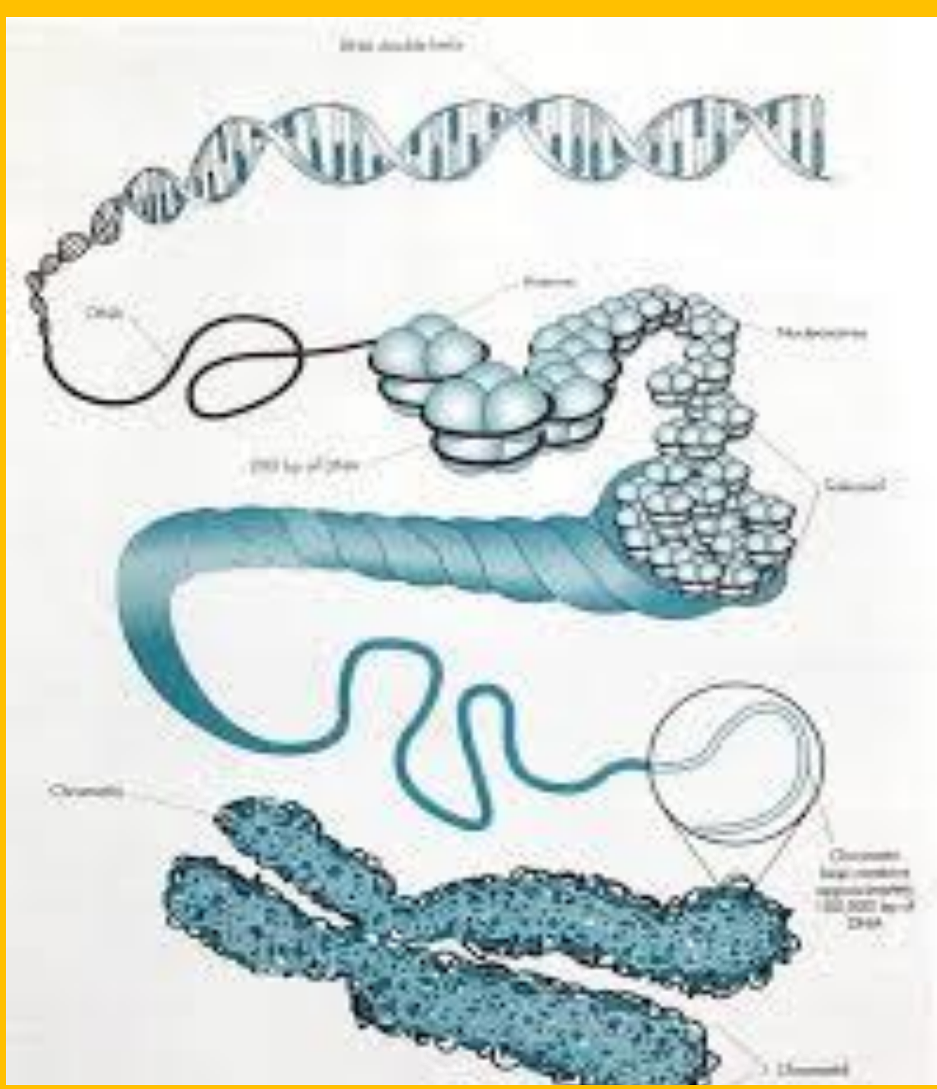
MSCZO-510(L)

M. Sc. II Semester

PRACTICAL BOOK



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General lab Rules for Cytogenetical Experiment

1. Great care should be taken with the microscopes. Once the light source has been properly centred (see Appendix1) there should be very little adjustment needed. Take the time to ask your demonstrator for assistance if you are having difficulties maintaining a clear field of view. Immersion oil should only be used on the drawing and photo microscopes to keep the general use microscopes clean. If you find an eye piece or objective has debris on it, it can be gently cleaned with a Kimwipe or cotton swab. Make sure you turn off your microscope and cover before leaving the lab.Never use alcohol or acetone to clean the microscopes as it can destroy the lens cement.
2. Immersion oil can be cleaned off of objectives or slides using a cotton swab moistened with petroleum ether.
3. Focus in an upward direction when using the coarse adjustment and use the fine focus when focussing downward. Preventing contact between the objective and your slide will avoid costly damage to your microscope.
4. The petroleum ether should be kept at the sink and away from any open sources of flame.
5. Extinguish alcohol burners immediately after use.
6. Any slides that a student prepares are for their personal use. Slides are considered individual work; slide sharing will not be tolerated.
7. If for some reason you cannot attend the lab contact the demonstrator by email or by phone.

UNIT: 01: CYTOLOGY EXPERIMENT

1.1 Objectives

1.2 Introduction

1.3 Study of different stages of mitosis with the help of onion root tip.

1.4 Study of different stages of meiosis with the help og Grasshopper testis.

1.5 Laboratory preparation of following modeles using beads and wire.

1.5.1 Adenosine triphosphate (ATP)

1.5.2 DNA and RNA bases

1.5.3 Nucleosides

1.5.4 Nucleotides

1.1 OBJECTIVES :

To study the Meiosis and describe the chromosomal makeup of a cell using the terms chromosome, sister chromatid, homologous chromosome, diploid, haploid, and tetrad and also recognize the function and products of mitosis and meiosis . Different stages of mitosis with the help of onion root tip and different stage of meiosis with the help of grasshopper testis.Laboratory preparation of following modes using beads and wire.Adenosine triphosphate (ATP). DNA and RNA bases. Nucleosides and Nucleotides.

1.2 INTRODUCATION

Mitosis is the part of the cell cycle when replicated chromosomes are separated into two new nuclei. In general, mitosis (division of the nucleus) is preceded by the S stage of interphase (during which the DNA is replicated) and is often accompanied or followed by cytokinesis, which divides the cytoplasm, organelles and cell membrane into two new cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the mitotic (M) phase of an animal cell cycle (the division of the mother cell into two daughter cells genetically identical to each other). The process of mitosis is divided into stages corresponding to the completion of one set of activities and the start of the next. These stages are prophase, pro-metaphase, metaphase, anaphase, and telophase.

Meiosis is a specialized type of cell division that reduces the chromosome number by half, creating four haploid cells, each genetically distinct from the parent cell that gave rise to them. This process occurs in all sexually reproducing single-celled and multicellular eukaryotes, including animals, plants, and fungi. Errors in meiosis resulting in aneuploidy are the leading known cause of miscarriage and the most frequent genetic cause of developmental disabilities. In meiosis, DNA replication is followed by two rounds of cell division to produce four daughter cells, each with half the number of chromosomes as the original parent cell. The two meiotic divisions are known as Meiosis I and Meiosis II. Before meiosis begins, during S phase of the cell cycle, the DNA of each chromosome is replicated so that it consists of two identical sister chromatids, which remain held together through

sister chromatid cohesion. This S-phase can be referred to as “premeiotic S-phase” or “meiotic S-phase”. Immediately following DNA replication, meiotic cells enter a prolonged G2-like stage known as meiotic prophase. During this time, homologous chromosomes pair with each other and undergo genetic recombination, a programmed process in which DNA is cut and then repaired, which allows them to exchange some of their genetic information. A subset of recombination events results in crossovers, which create physical links known as chiasmata (singular: chiasma, for the Greek letter Chi (X)) between the homologous chromosomes. In most organisms, these links are essential to direct each pair of homologous chromosomes to segregate away from each other during Meiosis I, resulting in two haploid cells that have half the number of chromosomes as the parent cell. During Meiosis II, the cohesion between sister chromatids is released and they segregate from one another, as during mitosis. In some cases, all four of the meiotic products form gametes such as sperm, spores, or pollen. In female animals, three of the four meiotic products are typically eliminated by extrusion into polar bodies, and only one cell develops to produce an ovum. Because the number of chromosomes is halved during meiosis, gametes can fuse (i.e. fertilization) to form a diploid zygote that contains two copies of each chromosome, one from each parent. Thus, alternating cycles of meiosis and fertilization enable sexual reproduction, with successive generations maintaining the same number of chromosomes. For example, diploid human cells contain 23 pairs of chromosomes including 1 pair of sex chromosomes (46 total), half of maternal origin and half of paternal origin. Meiosis produces haploid gametes (ova or sperm) that contain one set of 23 chromosomes. When two gametes (an egg and a sperm) fuse, the resulting zygote is once again diploid, with the mother and father each contributing 23 chromosomes. This same pattern, but not the same number of chromosomes, occurs in all organisms that utilize meiosis.

DIFFERENCES BETWEEN MITOSIS AND MEIOSIS

Stage	Mitosis	Meiosis
Interphase	Each chromosome replicates. The result is two genetically identical sister chromatids (However, do note that interphase is technically not a part of mitosis because it takes place between one mitotic phase and the next)	Chromosomes not yet visible but DNA has been duplicated or replicated
Prophase	Prophase –Each of the duplicated chromosomes appears as two identical or equal sister chromatids, The mitotic spindle begins to form. Chromosomes condense and thicken	Prophase I – crossing-over recombination – Homologous chromosomes (each consists of two sister chromatids) appear together as pairs. Tetrad is the structure that is formed. Segments of chromosomes are exchanged between non-sister chromatids at crossover points known as chiasmata (crossing-over)
Metaphase	Metaphase -The chromosomes assemble at the equator at the metaphase plate	Metaphase I Chromosomes adjust on the metaphase plate. Chromosomes are still intact and arranged as pairs of homologues
Anaphase	Anaphase – The spindle fibres begin to contract. This starts to pull the sister chromatids apart. At the end of anaphase, a complete set of daughter chromosomes is found each pole	Anaphase I Sister chromatids stay intact. However, homologous chromosomes drift to the opposite or reverse poles
Mode of Reproduction	Asexual Reproduction	Sexual Reproduction
Occurrence	All the cells	Reproductive cells
Function	General growth and repair, Cell reproduction	Genetic diversity through sexual reproduction
Cytokinesis	Occurs in Telophase	Occurs in Telophase I and in Telophase II
Discovered by	Walther Flemming	Oscar Hertwig

1.3 STUDY OF DIFFERENT STAGES OF MITOSIS WITH THE HELP OF ONION ROOT TIP

Aim. To understand the process and different stages of mitosis and to visualize different phases of mitosis.

Principle: The genetic information of all organisms resides in the individual DNA molecules or chromosomes. An onion cell possesses eight chromosomes lwhereas human cells possess forty six chromosomes.

In 1842, C. Nägeli first saw chromosomes and in 1888 W. Waldeyer named them. Walther Flemming studied and named the process of cell division as mitosis. Cell division occurs rapidly in growing root tips of sprouting seeds or bulbs.

An onion root tip is a rapidly growing part of the onion and thus many cells will be in different stages of mitosis. The onion root tips can be prepared and squashed in a way that allows them to be flattened on a microscopic slide, so that the chromosomes of individual cells can be observed easily. The super coiled chromosomes during different stages of mitosis present in the onion root tip cells can be visualized by treating with DNA specific stains, like Feulgen stain and Acetocarmine stain .

Materials required: Onion plant with root, acetocarmine stain, 1 N HCl, Scissors, Forceps, Razor blade, Microscopic slides and cover slips, Water bath, LightMicroscope.

Procedure

1. Cut the tip 5 to 8 mm from the tip of the freshly sprouted root. Discard the rest of the root.
2. Wash them in water on a clean microscope slide.
3. Place one drop of 1N HCL on the root tip and add 2-3 drops of acetocarmine stain to the slide.
4. Warm the slide gently over the alcohol lamp for about one minute. (Do not allow the slide to get hot to the touch; you don't want to cook either your fingers or the root. Do not let the root dry out).
5. Carefully blot the excess stain with a blotting paper.
6. After (10 to 20 seconds) put one or two drops of water and blot them carefully using blotting paper.
7. Again put a drop of water on the root tip and mount a cover slip on it avoiding air bubbles.
8. Squash the slide with your thumb using a firm and even pressure. (Avoid squashing with such force that the cover slip breaks or slides).

9. Observe it under a compound microscope in 10x objective. Scan and narrow down to a region containing dividing cells and switch to 40x for a better view.

The process of Mitosis is divided into four stages: Prophase, Metaphase, Anaphase and Telophase

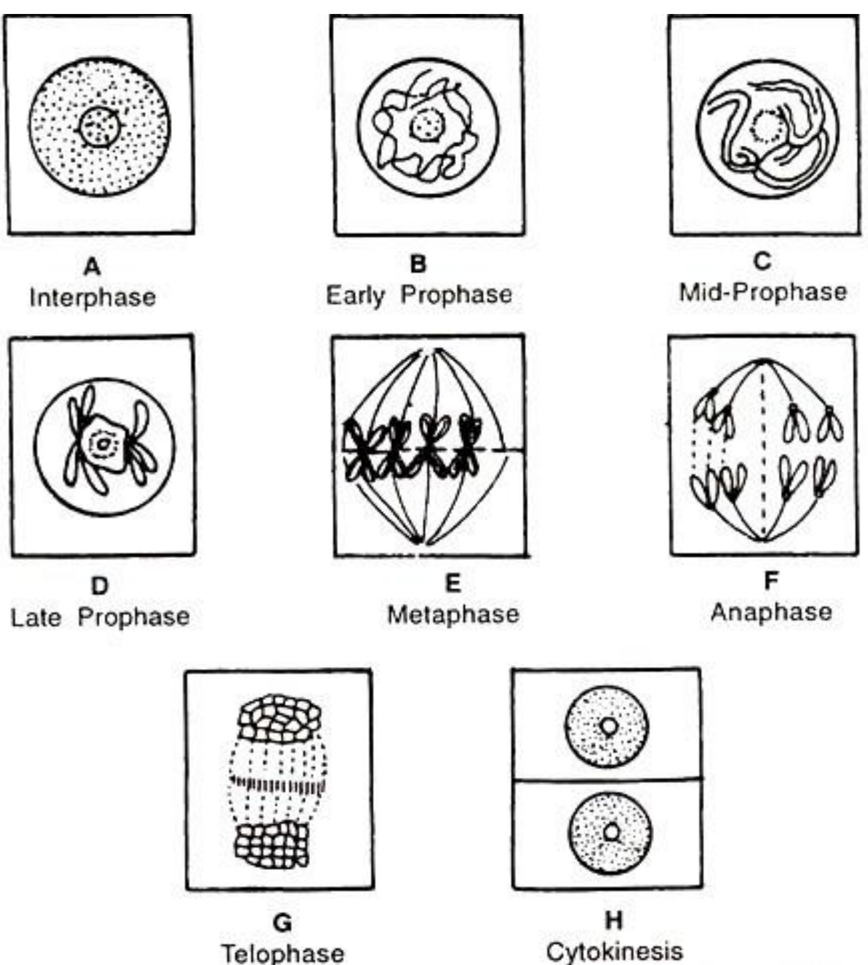
Prophase: During this stage, the chromosomes super coil, condense and become visible for first time during the cell cycle. The spindle fibers start forming. The nuclear membrane starts disintegrating.

Metaphase: During this stage, the spindle fibers reach and attach to centromere of each sister chromatids. The chromosomes align along the center plane of the cell. The nuclear membrane disintegrates completely.

Anaphase: During this stage, the centromeres start splitting and the sister chromatids begin to migrating towards the opposite poles of the cell.

Telophase: During this stage, the chromosomes are clustered on the either end of the cell. The nuclear membrane starts reforming.

The cell plate (new cell wall) starts to form between the two daughter nuclei. This will be followed by cytokinesis.



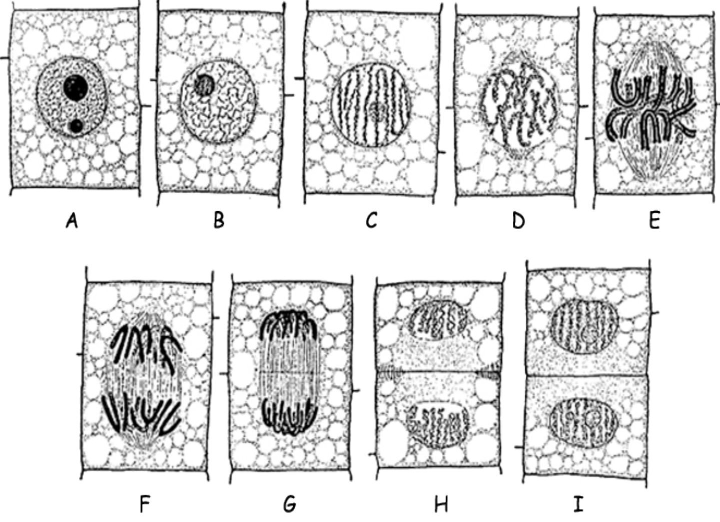


fig : 1.1 onion root tip all stage

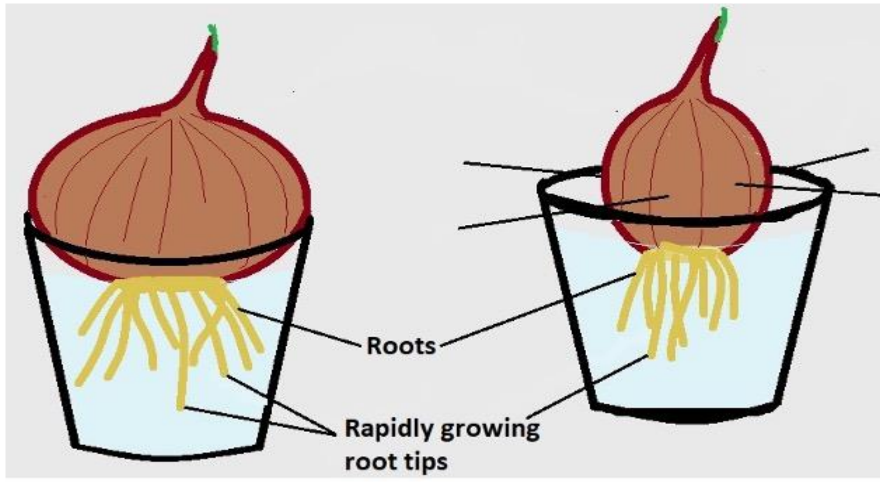


fig : 1.2 onion root tip

fig : 1.3 onion root tip

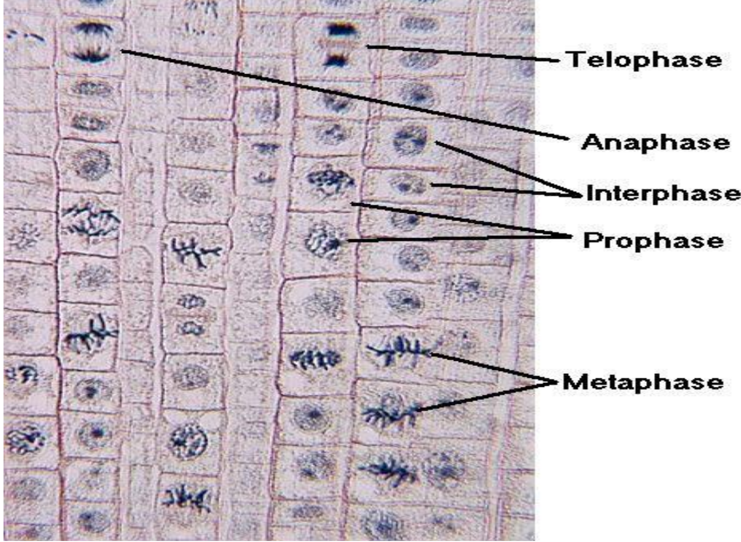


Fig: 1.4 Plant mitosis onion root tip stage

1.4: STUDY OF DIFFERENT STAGES OF MEIOSIS IN GRASSHOPPER TESTIS CELLS

Grasshopper testis is an ideal material for studying various stages of meiosis. Grasshopper is of good choice because it is easily available in lawns and fields, males can be easily distinguished from female and testis is easy to dissect. In addition, it has fewer number of chromosomes (locally available species contain 17 or 19 or 21 chromosomes in males; odd number of chromosomes due to XX/XO sex chromosome system) and all chromosomes are of one type, i.e., acrocentric, facilitating unambiguous identification of division stages.

MATERIALS REQUIRED

i. Male grasshopper,

ii. Insect saline (0.67% NaCl),

iii. 1:3 aceto-ethanol fixative, 70% and 90% ethanol,

iv. 2% acetoorcein stain

v. 45% acetic acid,

vi. Slide, Cover glass,

vii. Sealing wax or nail polish

Meiosis occurs in the life cycle of each and every living being whether a plant or an animal, but its period of occurrence varies in different groups. In majority of cases it

occurs prior to gamete formation. The cells undergoing meiosis are known as meiocytes. In animals, the meiocytes are the primary spermatocytes and primary oocytes while in plants these are represented by sporocytes. The relative amounts of RNA and DNA are supposed to initiate meiosis in some way. If the ratio of RNA to DNA is high, the cell will undergo meiosis but if reverse is the case it will lead to mitosis.

PROCEDUR

1. Dissect out testes from male grasshopper. 2. Keeping the testes in normal saline, remove the yellow fat. 3. Fix the testis in fixative in a centrifuge tube for 30 min. 4. Remove fixative and add about 0.5 ml of 60% acetic acid, leave for 2-3 min till the testis appears nearly dissolved. 5. Add 5-6 ml of fixative to the tube without removing the acetic acid. 6. Centrifuge at 1,200 rpm for 5 min. 7. Gradually remove the supernatant and add a few drops (~0.2 ml) of fresh fixative and make a suspension. 8. Drop a few drops of cell suspension on a slide and flame dry. 9. Stain the slides with Giemsa stain, rinse in water, dry and observeunder a microscope. 10. The slide can be mounted with DPX before observing in oil immersion lens.

FIXATION OF GRASSHOPPER TESTES:

1. Hold a male grasshopper in hand, give a small incision with scissors at the junction of thorax and abdomen and press the abdomen gently. The testes covered in yellow fat bodies will pop out. Dissect them out and put in insect saline. Remove yellow fat with the help of forceps as much as possible. A pair of testes (each having a bunch of white tubules) will be seen.
2. Transfer the tubules in a tube and fix in aceto-ethanol fixative, close the tube and leave for 20 minutes.
3. Remove fixative and add 90% ethanol, leave for 2hr.
4. Decant 90% ethanol and add 70% ethanol. The testes can be stored in 70% ethanol for a long period of time if the tube is tightly closed. Storing at 4oC is even better.

STAINING AND MAKING SQUASH PREPARATION:

1. Stain the fixed testis in aceto-orceine for 30 min.

2. Take a drop of 45% acetic acid on slide, place a few tubules of testis in the drop, leave for 1-2 min. If acetic acid drop becomes coloured, it can be decanted and a fresh 45% acetic acid drop can be added.

3. Place a cover glass on the tubules and squash using a rubber-end pencil under the folds of a blotting paper.

4. Seal the edges of the cover glass with molten wax or with nail polish immediately to prevent drying of acetic acid film and entry of air bubbles.

5. The slide is ready for observation under a microscope.

Result: The cells of testis lobes are spread out and became distinct. Carefully observe different stages of meiosis under microscope and draw them in practical notebook.

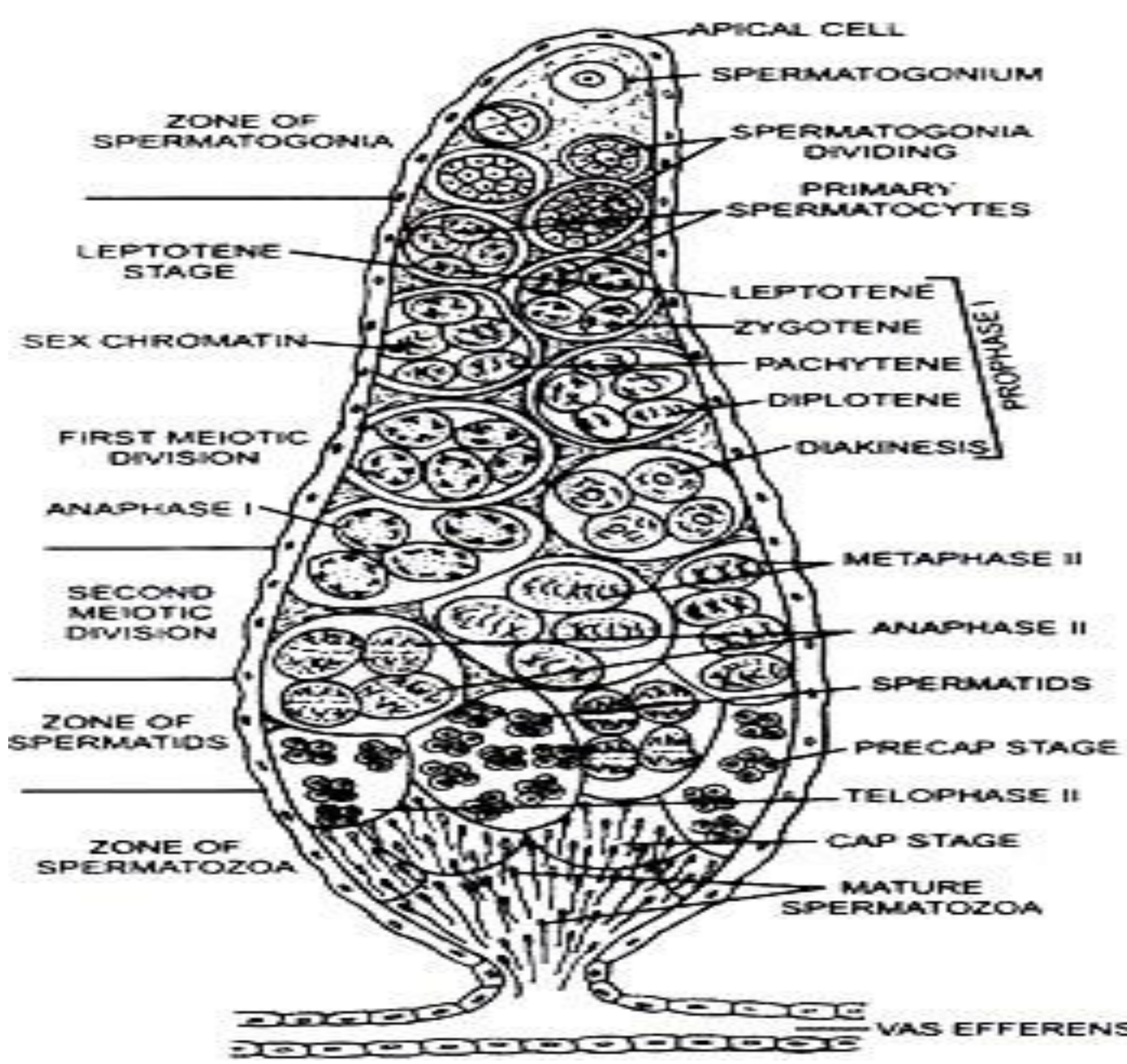


Fig1. 5: T.S one follicle of testis of Grasshopper to show the stages of meiosis.

1.5 LABORATORY PREPARATION OF FOLLOWING MODELS USING BEADS AND WIRE:

BODY NEURON DIRECTIONS

Objective: To identify the anatomy of a typical multipolar neuron. Directions: Get out those beads and make a neuron! You will need 4 different colors. This neuron with seven dendrites requires 65 beads - 42 beads for the dendrites 10 beads for the cell body or soma 12 beads for the axon 1 bead for the synaptic terminal 1. Decide which colors you would like to assign to each of the parts. 2. Get a precut piece of flexible wire / string. 3. String the beads using the pattern in the diagrams below in order. The first part you will construct will be one of the dendrites. You will need 6 beads for each dendrite.

Loop one bead (of the color chosen for the dendrites) through to the mid point of your piece of wire/string. You now have two pieces of free wire/string. Hold the two pieces together and string 5 beads of the same color through the two pieces together as shown on the diagram.

Separate the two pieces of wire/string. Loop two beads (of the color chosen for the soma), through one piece of string. Loop the second piece of string through both beads in the opposite direction. Pull the strings through in opposite directions and adjust to look like the figure to the left. You now have two separate pieces of string.

Using one piece of string, loop 6 beads of the color chosen for dendrites through to create the 2 nd dendrite. Then, loop the same string back through all beads, skipping the last one as shown in the diagram. Repeat this process on the other piece of string to make the 3 rd dendrite

To build on to the soma, arrange three beads as shown on the diagram. Loop one string through all beads in one direction. Then, loop the second string through the opposite direction. (The strings will cross inside the beads).*Repeat Steps 3 and 4 TWICE to make the remaining dendrites and complete the soma. Note that the last part of the soma only has two beads.

With the beads you have assigned as the axon, position one directly underneath and between the last two beads of the soma. Loop one string through the right hole and the other through the left hole (string criss-crosses in the bead). Repeat with the remaining 11 axon beads, completing the length of the axon. At the end of the axon, add the final bead to indicate the synaptic terminal. Tie the string in a knot to finish your neuron

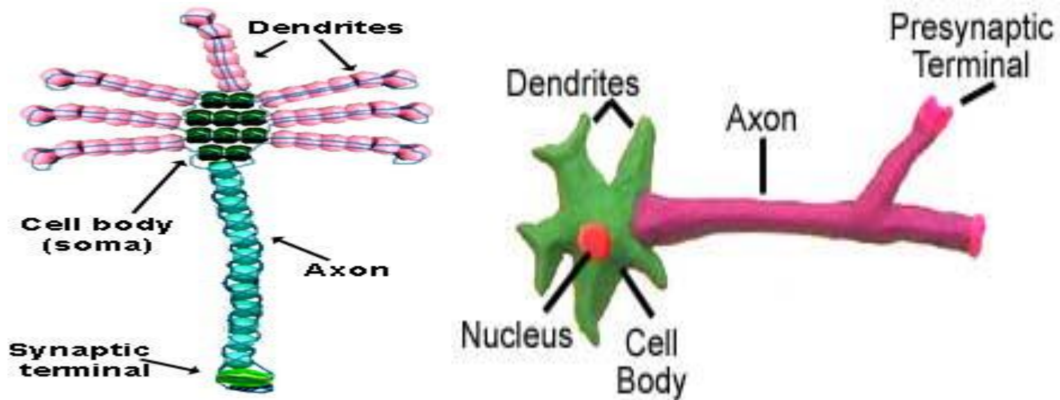
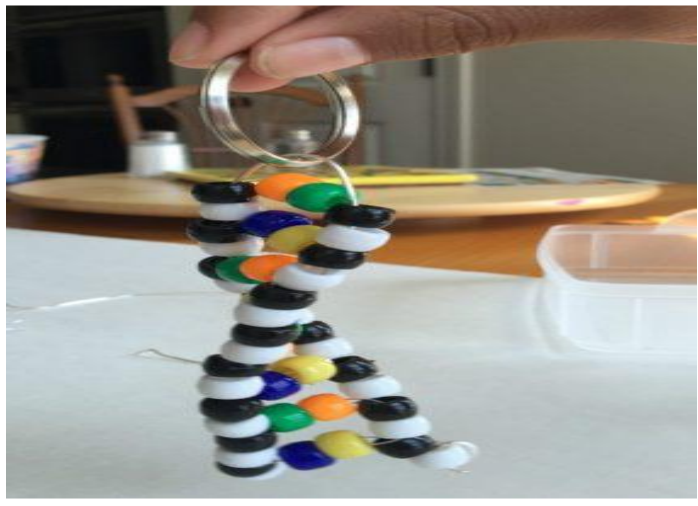


Fig:1.6 Body Neuron beads

HOW TO MAKE A DNA MODEL :ur DNA models are going to be on keychains. Students **love the product** of this activity, but they may need quite a bit of help at first. I have typically done this activity with **freshman and sophmores** in high schoool, but our K-6 grade group didn't have much problem (with a little extra help).Once they understand the pattern it is pretty easy. **Adult supervision is needed** for finishing off the ends with the wire cutters and pliers. The kids loving having something cool to attach to their backpack.Other teachers would stop by to tell me how the kids have all been **showing off their DNA**. You can purchase DNA models from Amazon that range in price from $15 and up, but I love this hands-on version.



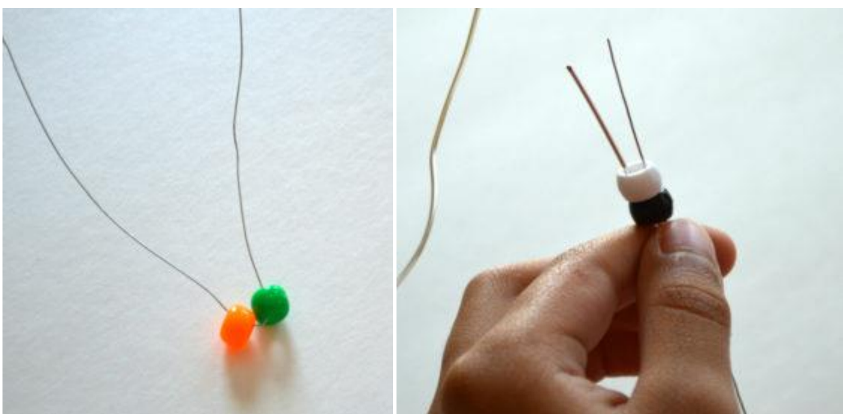
1.7 Steps to Make a DNA Keychain

Precut the Wires: Cut an 18 inch long piece of the heavier gauge wire.Cut a 24 inch long piece of the lighter gauge wire

Pick out Beads: Two colors to be the phosphate and sugar on the sides of the DNA (I)almost always pick black and white, but it is up to you)Four colors in two groupings to be the bases. (Adenine-Thymine) & (Guanine-Cytosine)

Bend the Wires and Start Building

- Take the thinner wire and bend into a u shape (around the middle).
- Add two beads from a bead pair



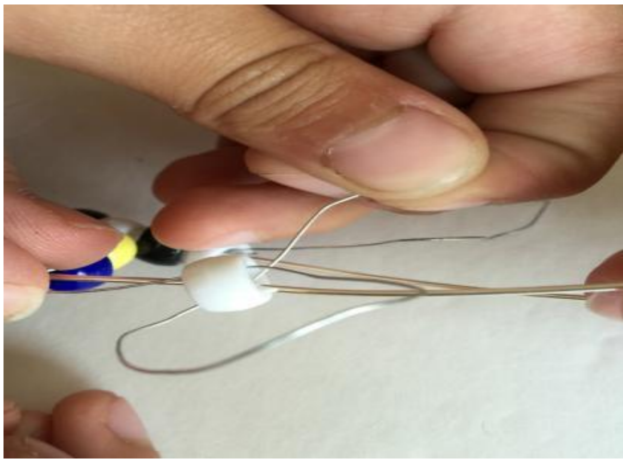
- Take the thicker wire and the thinner wire and add the phosphate and sugar beads to both wires.

Add the First Base Pair

- Now add another base pair on one side of the thin wire.

The Phosphate and Sugar: The thin wire should now be aligned with the thick wire. Another phosphate and sugar molecule go onto both wires

Finish the DNA keychain:End with phosphate and sugar beads.Wrap the wires around the end bead.



Snip the wire as close to the bead as possible to avoid sharp edges. Flip the top base pairs to the inside of the thicker wire.

Fig 1.8 .DNA Models preparation

- Attach key ring.You can lightly twist to represent the shape of DNA in the alpha helix.

1.5.1 ADENOSINE TRIPHOSPHATE (ATP)

ATP – Adenosine triphosphate is called the energy currency of the cell. It is the organic compound composed of the phosphate groups, adenine, and the sugar ribose. These molecules provide energy for various biochemical processes in the body. Therefore, it is called "Energy Currency of the Cell". These ATP molecules are synthesized by Mitochondria, therefore it is called powerhouse of the cell.

ATP Structure and Function

Fig 1.8. *ATP (adenosine triphosphate) has three phosphate groups that can be removed by hydrolysis to form ADP (adenosine diphosphate) or AMP (adenosine monophosphate).The negative charges on the phosphate group naturally repel each other, requiring energy to bond them together and releasing energy when these bonds are broken.*

At the heart of ATP is a molecule of adenosine monophosphate (AMP), which is composed of an adenine molecule bonded to a ribose molecule and to a single phosphate group (Figure 1). Ribose is a five-carbon sugar found in RNA, and AMP is one of the nucleotides in RNA. The addition of a second phosphate group to this core molecule results in the formation of adenosine diphosphate (ADP); the addition of a third phosphate group forms adenosine triphosphate (ATP).

Energy from ATP Hydrolysis is the process of breaking complex macromolecules apart. During hydrolysis, water is split, or lysed, and the resulting hydrogen atom (H^+) and a hydroxyl group (OH^-) are added to the larger molecule. The hydrolysis of ATP produces ADP, together with an inorganic phosphate ion (Pi), and the release of free energy. To carry out life processes, ATP is continuously broken down into ADP, and like a rechargeable battery, ADP is continuously regenerated into ATP by the reattachment of a third phosphate group. Water, which was broken down into its hydrogen atom and hydroxyl group during ATP hydrolysis, is regenerated when a third phosphate is added to the ADP molecule, reforming ATP. Obviously, energy must be infused into the system to regenerate ATP. Where does this energy come from? In nearly every living thing on earth, the energy comes from the metabolism of glucose. In this way, ATP is a direct link between the limited set of exergonic

pathways of glucose catabolism and the multitude of endergonic pathways that power living cells.

Functions of ATP: The ATP is used for various cellular functions, including transportation of different molecules across cell membranes. Other functions of ATP include supplying the energy required for the muscle contraction, circulation of blood, locomotion and various body movements. A significant role of ATP apart from energy production includes: synthesizing the multi-thousand types of macromolecules that the cell requires for their survival. ATP molecule is also used as a switch to control chemical reactions and to send messages.

IMPORTANCE OF ATP MOLECULE IN METABOLISM

1. These ATP molecules can be recycled after every reaction.
2. ATP molecule provides energy for both the exergonic and endergonic processes.
3. ATP serves as an extracellular signalling molecule and acts as a neurotransmitter in both central and peripheral nervous systems. 4. It is the only energy, which can be directly used for different metabolic process. Other forms of chemical energy need to be converted into ATP before they can be used. 5. It plays an important role in the Metabolism – A life-sustaining chemical reactions including cellular division, fermentation, photosynthesis, photophosphorylation, aerobic respiration, protein synthesis, exocytosis, endocytosis and motility.

1.5.2. DNA AND RNA BASES:

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are perhaps the most important molecules in cell biology, responsible for the storage and reading of genetic information that underpins all life. They are both linear polymers, consisting of sugars, phosphates and bases, but there are some key differences which separate the two[1]. These distinctions enable the two molecules to work together and fulfil their essential roles. Here, we look at 5 key differences between DNA and RNA. Before we delve into the differences, we take a look at these two nucleic acids side-by-side.

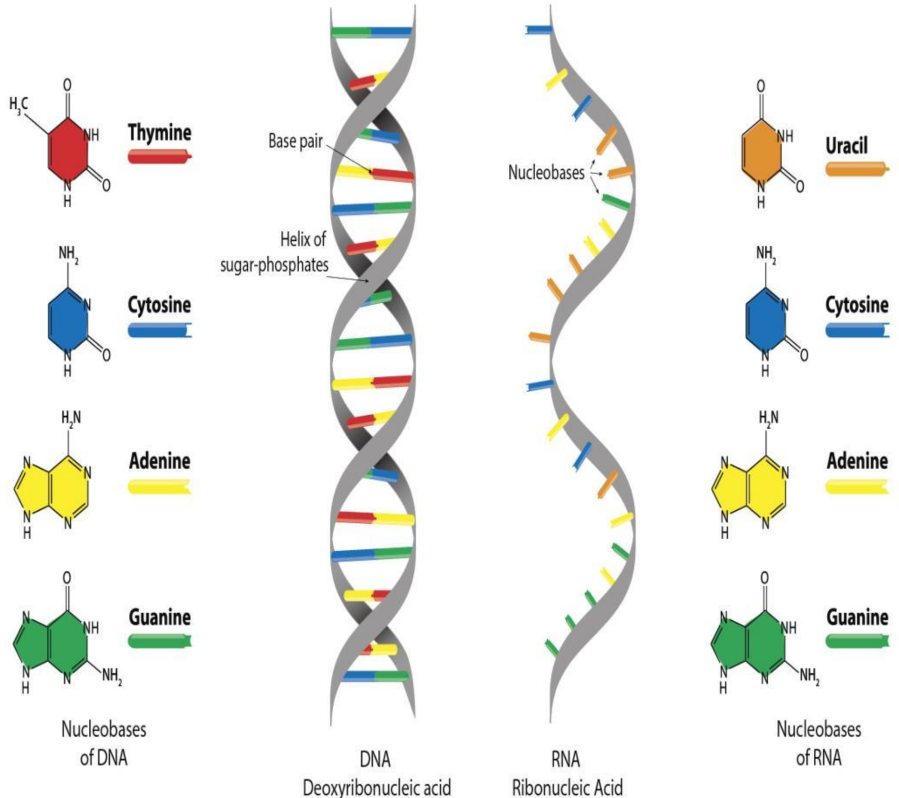


Fig 1.9 : A comparison of the helix and base structure of RNA and DNA

DNA vs. RNA – A comparison chart

Comparison	DNA	RNA
Full Name	Deoxyribonucleic Acid	Ribonucleic Acid
Function	DNA replicates and stores genetic information. It is a blueprint for all genetic information contained within an organism.	RNA converts the genetic information contained within DNA to a format used to build proteins, and then moves it to ribosomal protein factories.

Structure	DNA consists of two strands, arranged in a double helix. These strands are made up of subunits called nucleotides. Each nucleotide contains a phosphate, a 5-carbon sugar molecule and a nitrogenous base.	RNA only has one strand, but like DNA, is made up of nucleotides. RNA strands are shorter than DNA strands. RNA sometimes forms a secondary double helix structure, but only intermittently.
Length	DNA is a much longer polymer than RNA. A chromosome, for example, is a single, long DNA molecule, which would be several centimetres in length when unravelled.	RNA molecules are variable in length, but much shorter than long DNA polymers. A large RNA molecule might only be a few thousand base pairs long.
Sugar	The sugar in DNA is deoxyribose, which contains one less hydroxyl group than RNA's ribose.	RNA contains ribose sugar molecules, without the hydroxyl modifications of deoxyribose.
Bases	The bases in DNA are Adenine ('A'), Thymine ('T'), Guanine ('G') and Cytosine ('C').	RNA shares Adenine ('A'), Guanine ('G') and Cytosine ('C') with DNA, but contains Uracil ('U') rather than Thymine.
Base Pairs	Adenine and Thymine pair (A-T) Cytosine and Guanine pair (C-G)	Adenine and Uracil pair (A-U) Cytosine and Guanine pair (C-G)
Location	DNA is found in the nucleus, with a small amount of DNA also present in mitochondria.	RNA forms in the nucleolus, and then moves to specialised regions of the cytoplasm depending on the type of RNA formed.

Reactivity	Due to its deoxyribose sugar, which contains one less oxygen-containing hydroxyl group, DNA is a more stable molecule than RNA, which is useful for a molecule which has the task of keeping genetic information safe.	RNA, containing a ribose sugar, is more reactive than DNA and is not stable in alkaline conditions. RNA's larger helical grooves mean it is more easily subject to attack by enzymes.
Ultraviolet (UV) Sensitivity	DNA is vulnerable to damage by ultraviolet light.	RNA is more resistant to damage from UV light than DNA.

What are the key differences between DNA and RNA?

We can identify five key categories where DNA and RNA differ:

- Function
- Sugar
- Bases
- Structure
- Location

Function

DNA encodes all genetic information, and is the blueprint from which all biological life is created. And that's only in the short-term. In the long-term,DNA is a storage device, a biological flash drive that allows the blueprint of life to be passed between generations2. RNA functions as the reader that decodes this flash drive. This reading process is multi-step and there are specialized RNAs for each of these steps. Below, we look in more detail at the three most important types of RNA.

What are the three types of RNA?

- Messenger RNA (**mRNA**) copies portions of genetic code, a process called transcription, and transports these copies to ribosomes, which are the cellular factories that facilitate the production of proteins from this code.

- Transfer RNA (**tRNA**) is responsible for bringing amino acids, basic protein building blocks, to these protein factories, in response to the coded instructions introduced by the mRNA. This protein-building process is called translation.
- Finally, Ribosomal RNA (**rRNA**) is a component of the ribosome factory itself without which protein production would not occur1.

Sugar

Both DNA and RNA are built with a sugar backbone, but whereas the sugar in DNA is called deoxyribose (left in image), the sugar in RNA is called simply ribose (right in image). The 'deoxy' prefix denotes that, whilst RNA has two hydroxyl (-OH) groups attached to its carbon backbone, DNA has only one, and has a lone hydrogen atom attached instead. RNA's extra hydroxyl group proves useful in the process of converting genetic code into mRNAs that can be made into proteins, whilst the deoxyribose sugar gives DNA more stability3.

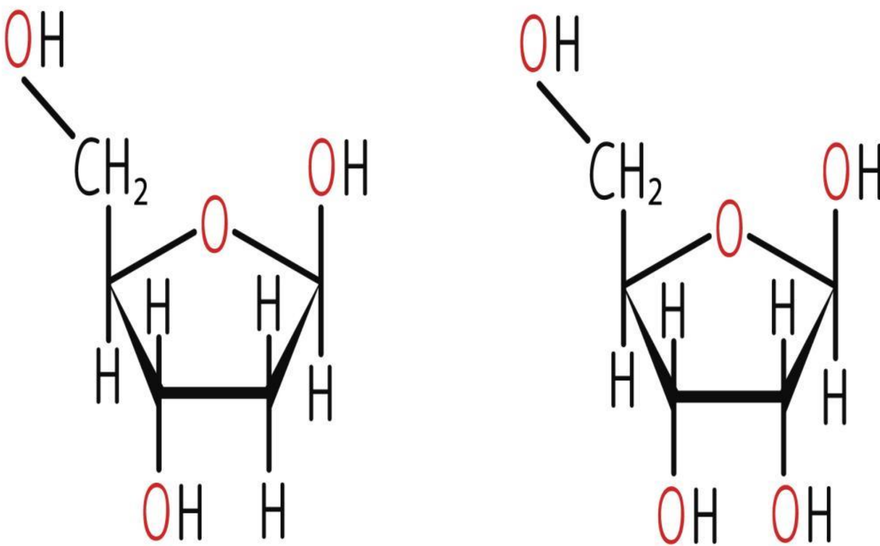


Fig 1.9 : The chemical structures of deoxyribose (left) and ribose (right) sugars

Bases

The nitrogen bases in DNA are the basic units of genetic code, and their correct ordering and pairing is essential to biological function. The four bases that make up this code are adenine (A), thymine (T), guanine (G) and cytosine (C). Bases pair off together in a double helix structure, these pairs being A and T, and C and G. RNA doesn't contain thymine bases, replacing them with uracil bases (U), which pair to adenine1.

Structure

While the ubiquity of Francis Crick and James Watson's (or should that be Rosalind Franklin's?) DNA double helix means that the two-stranded structure of DNA structure is common knowledge, RNA's single stranded format is not as well known. RNA can form into double-stranded structures, such as during translation, when mRNA and tRNA molecules pair. DNA polymers are also much longer than RNA polymers; the 2.3m long human genome consists of 46 chromosomes, each of which is a single, long DNA molecule. RNA molecules, by comparison, are much shorter3.

Location

Eukaryotic cells, including all animal and plant cells, house the great majority of their DNA in the nucleus, where it exists in a tightly compressed form, called a chromosome4. This squeezed format means the DNA can be easily stored and transferred. In addition to nuclear DNA, some DNA is present in energy-producing mitochondria, small organelles found free-floating in the cytoplasm, the area of the cell outside the nucleus.

The three types of RNA are found in different locations. mRNA is made in the nucleus, with each mRNA fragment copied from its relative piece of DNA, before leaving the nucleus and entering the cytoplasm. The fragments are then shuttled around the cell as needed, moved along by the cell's internal transport system, the cytoskeleton. tRNA, like mRNA, is a free-roaming molecule that moves around the cytoplasm. If it receives the correct signal from the ribosome, it will then hunt down amino acid subunits in the cytoplasm and bring them to the ribosome to be built into proteins5. rRNA, as previously mentioned, is found as part of ribosomes. Ribosomes are formed in an area of the nucleus called the nucleolus, before being exported to the cytoplasm, where some ribosomes float freely. Other cytoplasmic ribosomes are bound to the endoplasmic reticulum, a membranous structure that helps process proteins and export them from the cell5.

Unusual types of DNA and RNA

The structure we have described in this article is certainly the most common form of DNA, but it isn't the whole story. Other forms of both DNA and RNA exist that subvert the classical structures of these nucleic acids.

Z-DNA

While the structure of DNA you will see above – and in any biology textbook you might care to open – has a right-handed helix, DNA molecules with left-handed helices also exist. These are known as Z-DNA. Canonical, "classic" DNA is called B-DNA**.**

Z-DNA MOLECULES ARE:

Thinner (18 A wide as opposed to 20 A wide B-DNA) .Have a different repeating unit (two base pairs as opposed to one)Have different twist angles between basesZ-DNA is thought to play a role in regulating gene expression and may be produced in the wake of DNA processing enzymes, like DNA polymerase.

A-DNA

Identified at the same time as B-DNA by Rosalind Franklin, A-DNA is an alternative DNA structure that often appears when the molecule is dehydrated. Many crystal structures of DNA are in an A-DNA form. It has a shorter structure, with different numbers of base pairs per turn and tilt than B-DNA. A-DNA's biological relevance has been greatly expanded on in recent years, and it is now recognized that A-DNA is involved in many roles, such as:

- Binding to DNA enzymes, such as polymerases – this transition may enable specific atoms to be exposed for enzymatic action.
- Protection from damage – A-DNA is far less susceptible to ultraviolet ray damage, and spore-forming bacteria have been shown to adopt an A-DNA conformation, which may be a protective change.

Triplex DNA

A triple-helix DNA structure can form when certain nucleobases – pyrimidine or purine – occupy the major grooves in conventional B-DNA. This can happen naturally or as part of intentional DNA-modifying strategies for research purposes.

Triplex-forming oligonucleotides (TFOs) can bind conventional two-stranded DNA, which can help guide agents that are used to modify DNA to specific genomic locations. H-DNA is an endogenous, triple-stranded DNA molecule that encourages mutation of the genome.

dsRNA

Double-stranded RNA (dsRNA) is most commonly found as the genomic basis of many plant, animal and human viruses. These include *Reoviridae* and the rotaviruses, which are responsible for diseases like gastroenteritis. dsRNA molecules are potent immunogens – they activate the immune system, which then cuts the dsDNA as a protective mechanism. The discovery of the protein machinery that permits this reaction led to the development of gene silencing RNAi technology, which won the 2006 Nobel Prize for Physiology or Medicine.

1.5.3. NUCLEOSIDES:

A nucleoside is a molecule with a pentose sugar linked to a nitrogenous base or glycosylamines. A nucleoside can also be defined as a nucleotide without a phosphate group attached to it.The nucleosides present in DNA contain a 2` – deoxy – D- ribose sugar and nucleosides in RNA contain D-ribose sugar. The main difference is seen at the second position of the pentose structure, in the case of 2` – deoxy -ribose there is an absence of an alcohol group/ oxy group/ -OH group at the second position, hence the name.In the case of D – ribose pentose the –OH group is present at the second position.In both types, the pentoses are present in their β-furanose form which is a close five-membered ring structure.

Type of Nucleosides : The nucleosides can be divided into two types based on the presence of the nitrogen base of the compound.

1. Purine nucleosides
2. Pyrimidine nucleosides

Purine nucleosides

These nucleosides are composed of two nitrogenous bases known as Adenine and Guanine.

In the case of RNA, the nucleosides are – Adenine and Guanine

In the case of DNA, the nucleosides are – Deoxyadenosine and Deoxyguanosine

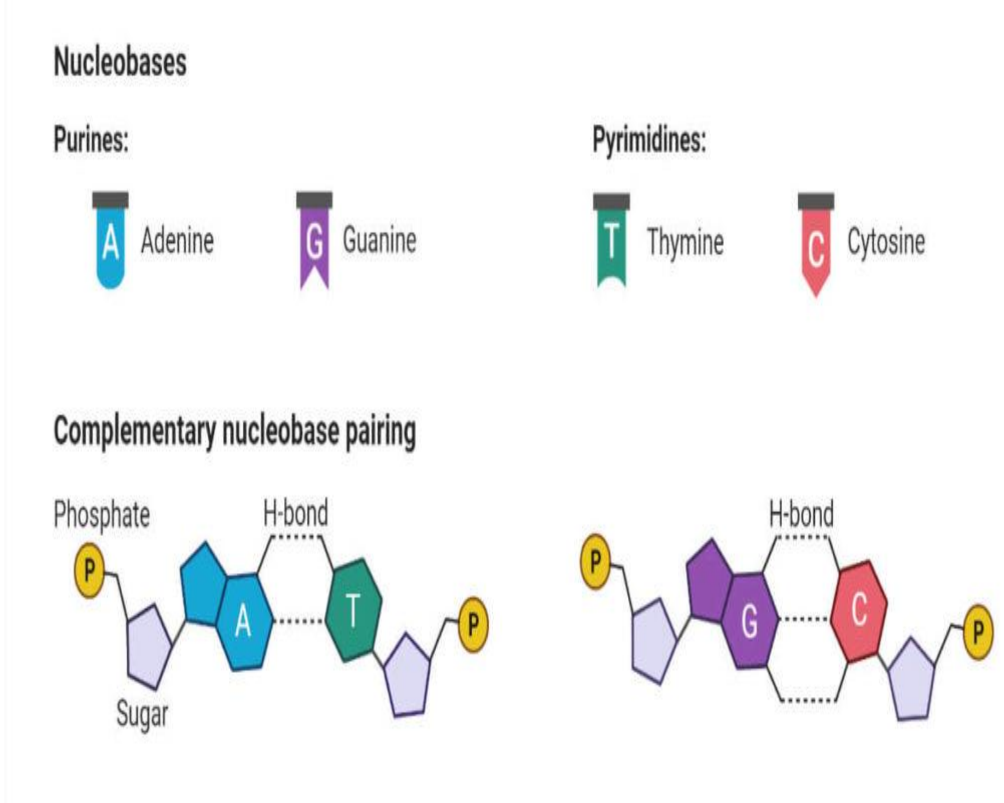


Fig:1.10 Purine, and Pyrimidine. Created with biorender.com

Pyrimidine nucleosides

These nucleotides are composed of three nitrogenous bases known as Thymine, Cytosine, and Uracil.

In the case of RNA, the nucleosides are – Cytidine and Uridine

In the case of DNA, the nucleosides are – Deoxycytidine and Thymidine or Deoxythymidine

Structure of Nucleosides

The nucleosides consist of two main heterocyclic components –

Pentose sugar

It is a five-membered ring structure generally described as a puckered conformation.

- In the case of DNA, the nucleosides lack –OH group at the second position of pentose ring and hence is called as 2` – deoxy – D – ribose.
- In RNA it is a D – ribose pentose ring structure.
- The pentoses are present in β- furanose form in both types of sugars.
- The pentose sugar is attached to the nitrogenous base at the primary carbon atom (1`).
- It is linked by a glycosidic bond (N-β-glycosyl bond).

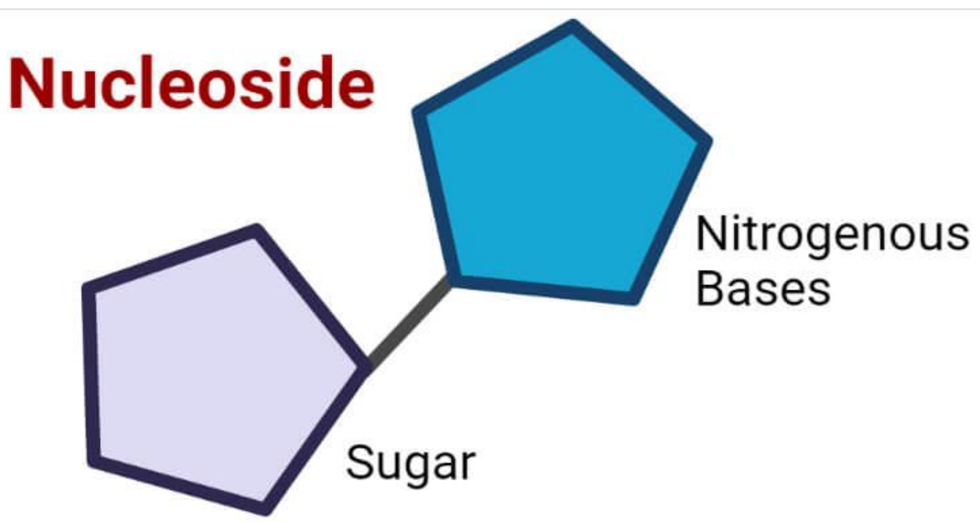


Figure- 1.11 Nucleoside. Created with biorender.com

Nitrogenous base

It is a cyclic carbon structure containing nitrogen and has the properties of a base.

- The bases are derivatives of two major parent compounds – Purines and Pyrimidines.
- In RNA the nitrogenous bases are – Adenine, Uracil, Cytosine, and Guanine.
- In DNA the nitrogenous bases are – Adenine, Thymine, Cytosine, and Guanine.
- The bases are joined covalently to the pentose sugar by N-β-glycosyl bond.
- In the case of purines the N-9 atom bonds to the pentose sugar.
- In the case of pyrimidines the N-1 atom bonds to the pentose sugar.
- Although the majority of the bases are derived from purines and pyrimidines, some minor bases are also present in the DNA.
- Mostly these minor bases occur in methylated forms of purines and pyrimidines.

- In the case of some viral DNA, the bases might be hydroxyl-methylated or they might be glycosylated.
- Some of the unusual bases found in DNA are 5-Methlycytidine, N6-Methlyadenosine, 7-Methlyguanosine, 4-Thiouridine.
- **Fucation of Nucleosides**

1. Nucleosides are an integral part of nucleotides. They are precursors for nucleotides. When a phosphate group is attached to the nucleoside it forms the nucleotide which is the backbone of DNA.
2. Nucleoside molecules also function as signaling molecules.
3. Minor bases or altered nitrogen bases in nucleosides have a role in regulating or protecting genetic information.
4. Many nucleoside analogs have been used in the treatment of malignancies, tumors, and viral infections. This is achieved by some modifications in the purine and pyrimidine bases.
5. Cytarabine (cytosine arabinoside) was the first drug approved by the US FDA for treatment against acute myeloid leukemia.
6. Some nucleoside analogs are also used in treatments of Human Immunodeficiency Virus (HIV) disease. Lamivudine is being used in this treatment.
7. Nucleoside transporters play a vital role in the transportation of nucleosides across membranes.
8. There are two types of nucleoside transporters – Concentrative and Equilibrative. These transporters play an important role in the transport of antiviral and anticancer drugs across membranes.

1.5.4. NUCLEOTIDE:

A nucleotide is a pentose sugar linked to a nitrogenous base and a phosphate molecule. Nucleotides are the building blocks of DNA.

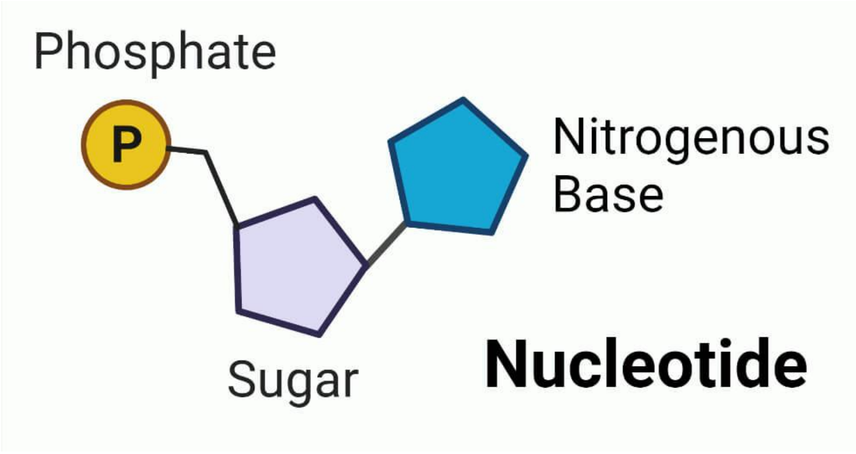


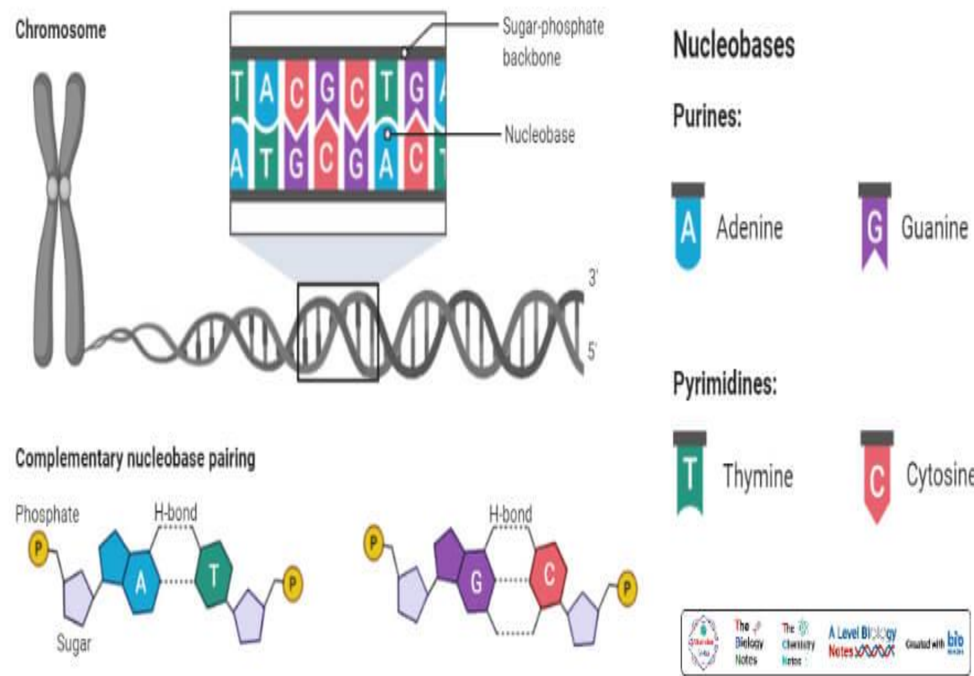
Fig: 1.12 Nucleotide

The nitrogenous bases are derived from two-parent compounds – purines and pyrimidines.The nucleotides present in DNA contain a 2` – deoxy – D- ribose sugar and nucleotides in RNA contain D-ribose sugar.The main difference is seen at the second position of the pentose structure, in the case of 2` – deoxyribose there is an absence of an alcohol group/ oxy group/ -OH group at the second position, hence the name.In the case of D – ribose pentose the –OH group is present at the second position.In both types, the pentoses are present in their β-furanose form which is a close five-membered ring structure.

Characteristics of Nucleotide

1. The nitrogenous base is linked covalently to pentose sugar by an N- glycosidic bond (N-1 in the case of pyrimidines and N-9 in the case of purines is linked to the C-1 carbon atom of the pentoses.)
2. The major purine bases present in DNA and RNA are Adenosine (A) and Guanine (G). The major pyrimidine bases in DNA are Thymine (T) and Cytosine (C), in RNA Thymine is replaced by Uracil (U) as a major pyrimidine base.The

consecutive nucleotides in DNA and RNA are linked by phospho-diester linkages, the 5` phosphate group of one nucleotide is linked with the 3` hydroxyl group of another nucleotide and the complementary nucleotides are linked by hydrogen bonds.Adenosine and Thymine are bonded by two hydrogen bonds and Guanine and Cytosine are bonded by three hydrogen bonds. The backbone of the DNA is composed of the chain of nucleotides linked to each other.



Biosynthesis of Nucleotides

The nucleotides are synthesized by two pathways:

1. De novo synthesis
2. Salvage pathway

A. De novo synthesis of nucleotides

In this pathway, complex nucleotides are synthesized using simple molecules such as sugars and amino acids. The pathway for synthesis of Purines and Pyrimidines is different and requires different precursors.

Synthesis of Pyrimidine nucleotides

The synthesis involves two major steps

1. Formation of a pyrimidine ring which is a 6 membered ring structure
2. Attachment of the pentose sugar (ribose – 5 – phosphate) to the ring structure

Formation of the pyrimidine ring involves Aspartate, PRPP (5-phosphoribosyl-1-pyrophosphate) and, Carbamoyl Phosphate.Uracil and Cytosine are the nucleotides formed in pyrimidine synthesis. These nucleotides are formed in different stages.The enzymes involved in this process are:Aspartate transcarbamoylase (ATCase)Carbamoyl phosphate synthetase (Type II)Dihydroorotate dehydrogenase Orotate phosphoribosyltransferase

Orotidine -5-phosphate carboxylase Glutamine, Aspartate, ATP and, CO_2 are required for the synthesis of pyrimidines.Carbamoyl phosphate required in the biosynthesis of pyrimidine is formed in the cytosol by the enzyme carbamoyl phosphate synthetase II.The first step of pyrimidine synthesis is the reaction between carbamoyl and aspartate to form N-carbamoyl aspartate. This reaction is catalyzed by the enzyme Aspartate transcarbamoylase.One H_2O molecule is removed from the N-carbamoyl aspartate to form l-dihydroorotate and this reaction is carried out by the dihydroorotase enzyme.

This L-dihydroorotate in presence of dihydroorotate dehydrogenase forms orotate. Orotate is the most important compound to which the ribose-5-phosphate sugar is added further. Orotate in presence of enzyme orotate phosphoribosyltransferase forms Orotidylate. In this step the sugar, ribose-5-phosphate is provided by Phosphoribosyl pyrophosphate (PRPP). The sugar attaches to the orotate to form orotidylate. This Orotidylate further undergoes decarboxylation in presence of orotidylate decarboxylase to form Uridylate (UMP) also known as Uridine 5`-monophosphate.

This uridylate is phosphorylated in further steps to form Uridine 5`- triphosphate (UTP).Cytidine 5`- triphosphate (CTP) is formed by the enzyme cytidine synthetase from UTP. In this conversion the nitrogen donor is glutamine.In this way, the biosynthesis of pyrimidines takes place.

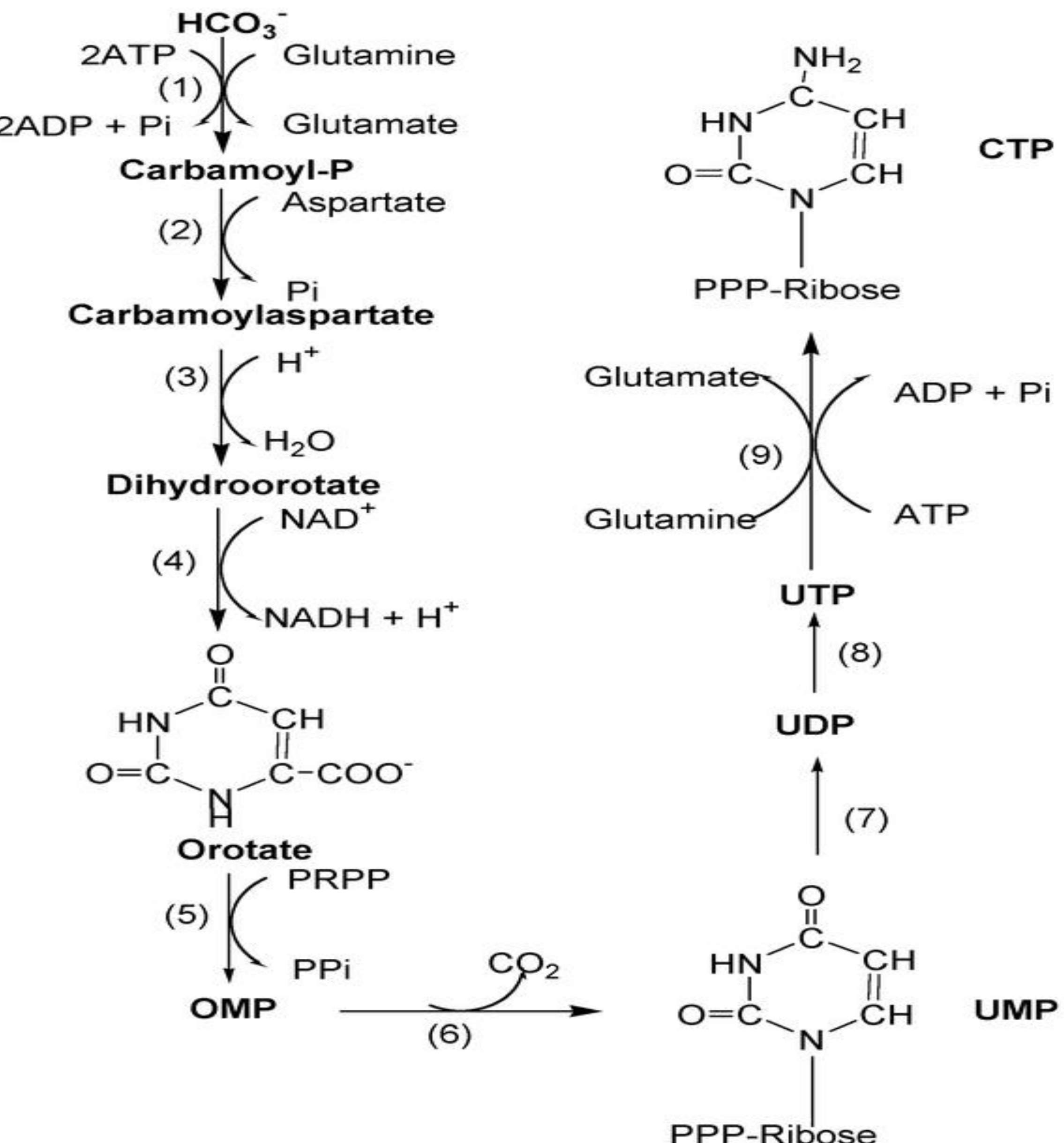


Fig:1.12 De novo biosynthetic pathway of pyrimidine nucleotides in plants. Image Source: Barbara A. Moffatt and Hiroshi Ashihara 2002.

Synthesis of Purine nucleotides

- Unlike in biosynthesis of pyrimidine nucleotides, purine synthesis initiates with PRPP and then the purine ring is built around the sugar.
- The enzymes involved in this process are:
  - Glutamine phosphoribosylpyrophosphate amidotransferase (Glutamine-PRPP)
  - Glycinamide ribonucleotide synthase (GAR synthase)
  - Gylcinamide ribotide transforamylase (GAR transformylase)
  - Formylglycinamide synthase
  - Aminoimidazole ribotide synthase
  - Aminoimidazole ribotide carboxylase
  - Succinylaminoimidazolecarboxamide ribotide synthase
  - Adenylosuccinate lyase
  - Aminoimidazole carboxamide ribotide transformylase
  - IMP cyclohydrolase
- The first process involves the formation of Inosinate (IMP) ad further from the IMP ring the purine monophosphates are synthesized.

1. Phosphoribosyl pyrophosphate (PRPP) in presence of enzyme Glutamine-PRPP forms Phosphoribosyl amine (PRA). The amino group donated by Glutamine is attached to the C1 of the PRPP complex.

2. GAR synthase acts upon PRA to form glycinamide ribonucleotide (GAR). Here three atoms from the glycine molecule are added to the nitrogen atom.

3. GAR forms Formylglyacinamide ribonucleotide (FGAR) in presence of the enzyme GAR transformylase. The added glycine molecule is formylated by N10 – Formyl tetrahydrofolate.

4. FGAR forms formyl glycinamidine (FGAM) in presence of the enzyme FGAM synthase.

5. FGAM is catalyzed by enzyme AIM synthase to form Aminoimidazole ribonucleotide, which is further converted to Carboxyaminoimidalzole ribonucleotide (CAIR) by enzyme AIR carboxylase.

6. CAIR in presence of SAICAR synthase forms complex succinylaminoimidazole ribonucleotide. Here Aspartate donates its amino group.

7. SAICAR is converted to aminoimidazole carboxamide ribonucleotide (AICAR) in presence of the enzyme Adenylosuccinate lyase.

8. AICAR is converted to FAICAR in presence of enzyme AICAR transfromylase. The final carbon for the second ring of purine is contributed by N10 – formyl tetrahydrofolate.

9. Finally the second ring is closed to form the complete purine ring in presence of enzyme IMP cyclohydrolase to form Inosinate (IMP).

10. Now from the IMP AMP and GMP are synthesized using enzymes Adenylosuccinate synthetase and Adenylosuccinate lyase (for AMP) and IMP dehydrogenase and GMP synthetase (for GMP).

B. Salvage pathway of Purine nucleotides

- Adenine is catalyzed by the enzyme adenosine phosphoribosyltransferase (APRT) from purine bases ad purine nucleosides.
- Guanine and hypoxanthine are salvaged by the enzyme Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) through the process of phosphoribosylation.
- Through other processes such as deamination, adenosine deaminase can be converted to adenosine which can further be converted to hypoxanthine.

Salvage pathway of Pyrimidine nucleotides (Thymine)

- The enzyme thymidine kinase can salvage the dTTP (deoxythymidine triphosphate) from thymidine or deoxyuridine.

Pyrimidine biosynthesis regulation

- The Biosynthesis of pyrimidines is regulated at different levels. This is majorly controlled by the enzyme ATCase (Aspartate transcarbamoylase).
- ATCase is a multi-subunit allosteric enzyme with 6 active sites. It plays an important role in biosynthesis of pyrimidines. ATP acts as an activator and CTP acts as an inhibitor of ATCase.
- This enzyme brings about the conversion of aspartate to carbamoyl aspartate. Hence, when the biosynthesis of pyrimidines is to be regulated CTP binds to ATCase to stop the synthesis. This is known as feedback inhibition, where the last product of the pathway inhibits the first enzyme to regulate the synthesis. This is achieved when high levels of CTP are present in the cell.

- Biosynthesis of pyrimidines can also be regulated at PRPP (Phosphoribosyl pyrophosphate) formation. High levels of PRPP indicate high levels of pyrimidine synthesis. Thus CTP synthetase enzyme, which converts UTP to CTP is feedback inhibited by CTP. This enzyme can be activated by GTP.
- These mechanisms regulated the biosynthesis of pyrimidines.

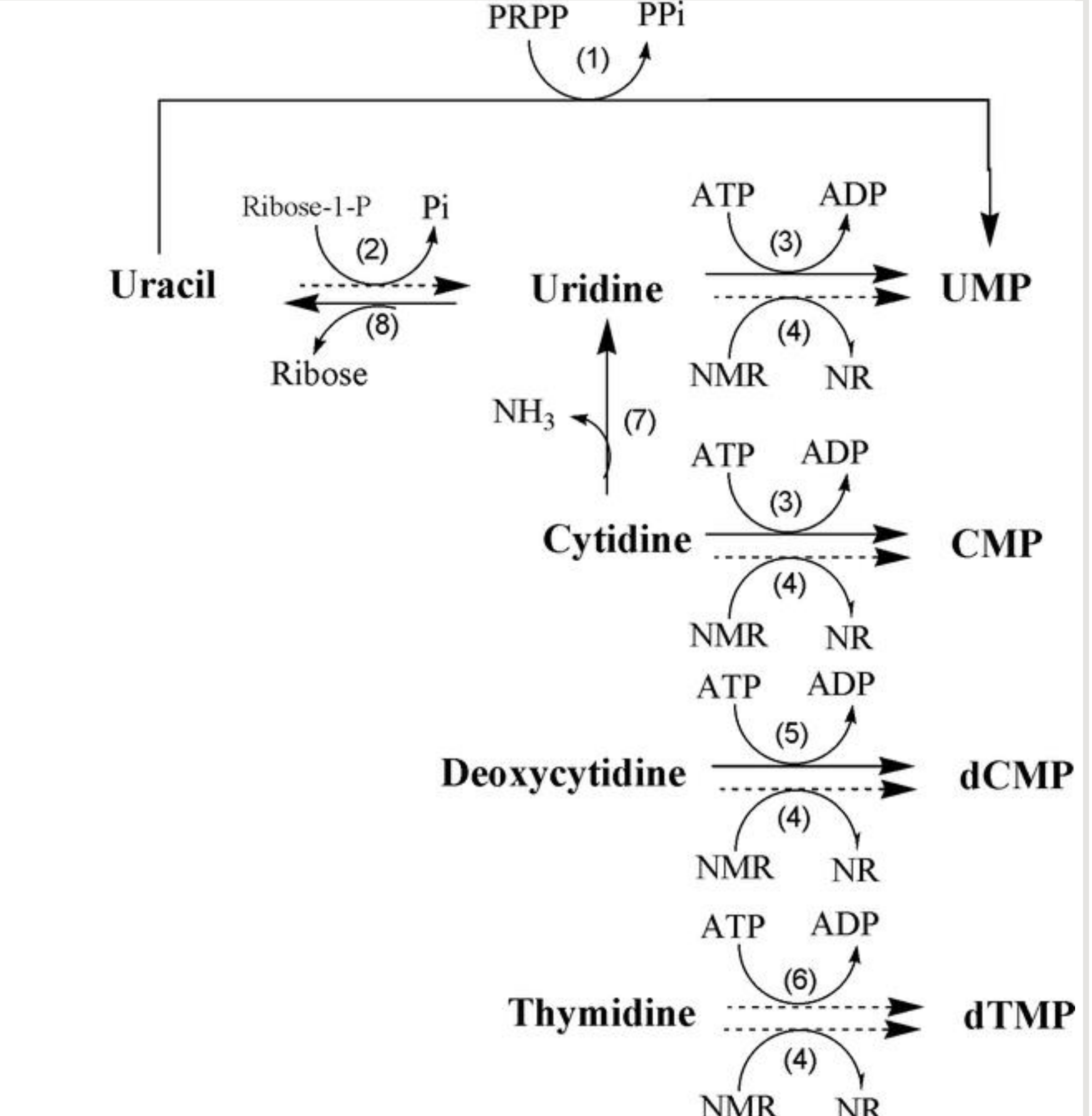


Figure:1.13 Pyrimidine salvage and related pathways in plants. Image Source: Barbara A. Moffatt and Hiroshi Ashihara 2002.

Purine biosynthesis regulation

- The biosynthesis of purines is regulated at 3 different levels. PRPP synthetase is the first regulatory point in the pathway. Feedback inhibition is exerted by AMP and GMP over PRPP synthetase.
- PRPP amidotransferase is an allosteric enzyme and GTP, ATP, GDP, ADP, GMP and, AMP bind to the enzyme allosterically and regulates the biosynthesis.
- The last regulatory point is the branching point of IMP. Excess amount of ATP accelerated synthesis of GMP and vice versa.

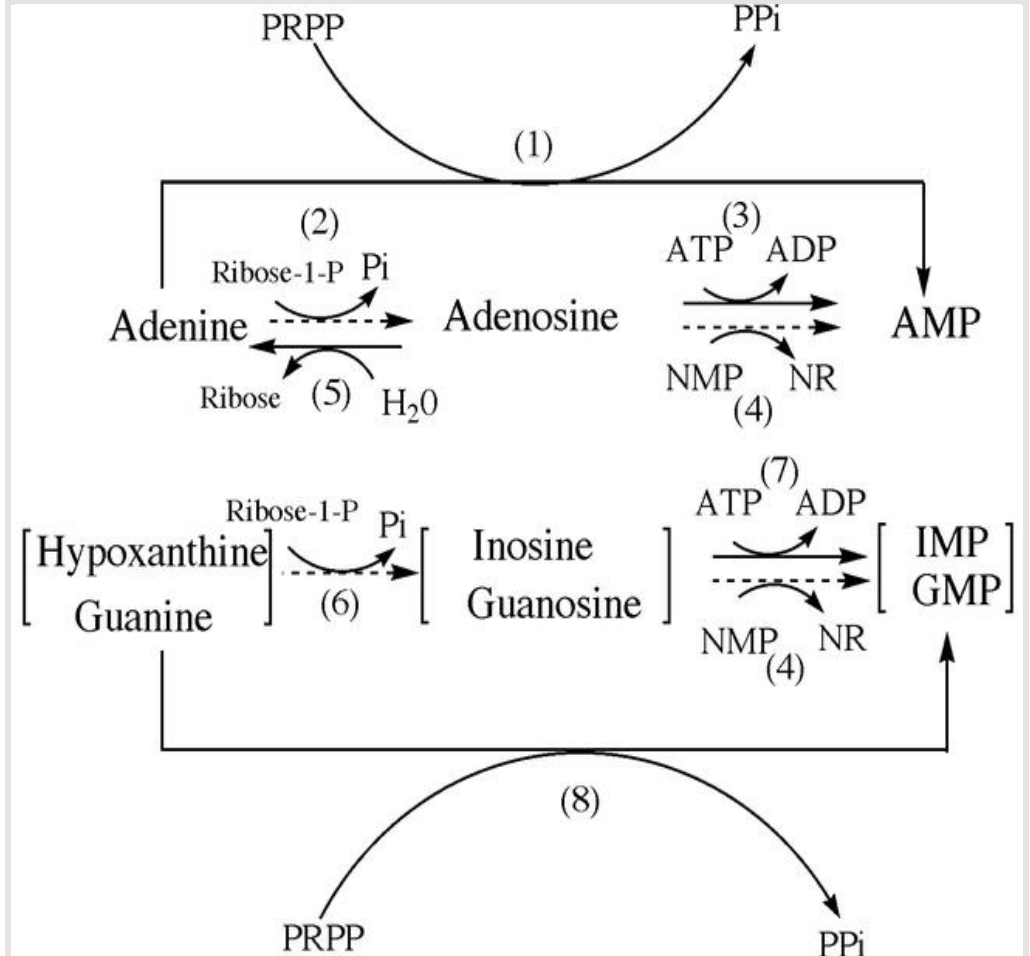


Fig: 1.14 Salvage reactions of purine bases and nucleosides in plants.
Image Source: Barbara A. Moffatt and Hiroshi Ashihara 2002.

Functions of Nucleotide

- Nucleotides act as energy reserves in the body and are involved n many important processes.
- ATP (Adenosine triphosphate) is considered the currency of cell and plays a crucial role in various pathways and acts as a phosphoryl group donor.
- Hydrolysis of ATP yields a large amount of energy which can be utilized by the cell of different functions.
- Nucleotides are a part of many co-factors for many enzymes.

Examples – Acetyl CoA, NAD+ (Nicotinamide adenine dinucleotide), FAD (Flavin adenine dinucleotide)

- cAMP (adenosine cyclic monophosphate) is a common second messenger produced in response to hormones and various chemical signals. cAMP is formed during a reaction of ATP catalyzed by adenylyl cyclase.
- cGMP (guanosine cyclic monophosphate) also has many regulatory functions in the cells.
- ppGpp (guanosine tetraphosphate) function is observed in bacterial cells under amino acids starvation conditions, which is in response to the slowdown of protein production.

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UNIT 2 : EXPERIMENT ON GENETICS

2.1 Objectives

2.2 Introduction

2.3 Morphological study of *Drosophila* for genetic traits

2.4 Preparation of chromosomal maps in *Drosophila* based

On present of crossing over.

2.5 Genetics exercise (Data to be provided)

2.6 Summary

2.7 Terminal question

2.8 Answers

2.1 OBJECTIVES:

Morphological study of Drosophila for genetic traits and preparation of chromosomalmaps in Drosophila based on percent of crossing over. To the data provided makeup of a cell using the terms chromosome, sister chromatid, homologous chromosome, diploid, haploid, and tetrad and also recognize the function and products of mitosis and meiosis. Recognize when cells are diploid vs. haploid and Predict the DNA content of cells in different phases of mitosis and meiosis stage because meiosis is a specialized and rather complicated type of cell division and we have to recall and describe the phases of the cell cycle co-relate the cell cycle stages to changes in DNA content.

2.2. INTRODUCTION:

The gametes, formed as a result of meiosis, possess half the number of chromosomes as found in the parent cells and their chromosome number is represented by n, whereas the zygote formed by the fusion (fertilization) of male and female gametes and the cells derived from it are known as diploid and their chromosome number is symbolized by 2n. The two similar chromosomes of diploid cells are known homologous chromosomes or homologous pair.

The chromosomes of a homologous pair are brought together in the zygote by the union of male and female gametes from the parents. Evolutionary response to selection depend on the amount of genetic variation expressed in the population. Because of this, the effect of environmental changes on the expression of genetic variation in quantitative traits has important evolutionary implications. If genetic variation is inflated in harsh environments, this will result in acceleration of genetic response and, thus, in an increase in the evolutionary rate.

If, on the contrary, genetic variation is decreased in such environments, then the selection response in them will be lower than under normal conditions. In the first case, traits will evolve faster in poor habitats; in the second, in the optimal ones.

2.3 MORPHOLOGICAL STUDY OF DROSOPHILA FOR GENETIC TRAITS:

Model organisms are organisms that are used by scientists to perform experimental studies in an effort to understand their biological processes. The choice of a model organism usually depends on the type of research the scientist intends to conduct.

Drosophila has a number of qualities that make it desirable for scientific studies. Some of its features include:

- Small size (Adults-3mm and eggs-0.5mm in length).
- Easy to handle.
- Sexual dimorphism (different males and females)
- One female can lay about 100 eggs.
- Short generation time (9-10 days)
- 4 pairs of chromosomes and the whole genome is sequenced.
- Low culture and maintenance cost (requires maize food, cultured in small bottles, and re lesser lab space).

Morphology of Drosophila melanogaster

Morphological characteristics	Female	Male
Body size	Long	Short
Abdomen	Long and Pointed	Short and Blunt
Stripes on the abdomen	Thin stripes and more in number	Thick stripes and less in number
Foreleg (appendages)	Not covered with bristles (or sex comb)	Covered with bristles
Image	♀	♂

Fig: 2.1 these features help the scientists to identify the male and female Drosophila in labs

Life Cycle of Drosophila: Drosophila requires 9 to 10 days (at 25 °C) to complete its life cycle; to develop from egg to adult—the development is also temperature-dependent as it takes 19 days to complete the life cycle at 18 °C.

The process of embryogenesis takes 24 hrs followed by 4-5 days in the larval stage—1st, 2nd, and 3rd instar larval stage. After the 5th day, the larvae enter into the pupal stage which lasts around 6-9 days of the development.

At the pupal stage, the tissue progenitor cells start transforming into adult characters (for example eyes, legs, and wings)—the ecdysone hormone is also involved in the transitional development of flies, from the pupal stage to adult.

Then, the adult fly emerges from the pupal case—the process called eclosion—and enters the process of maturation, which takes around 8-12 hours.

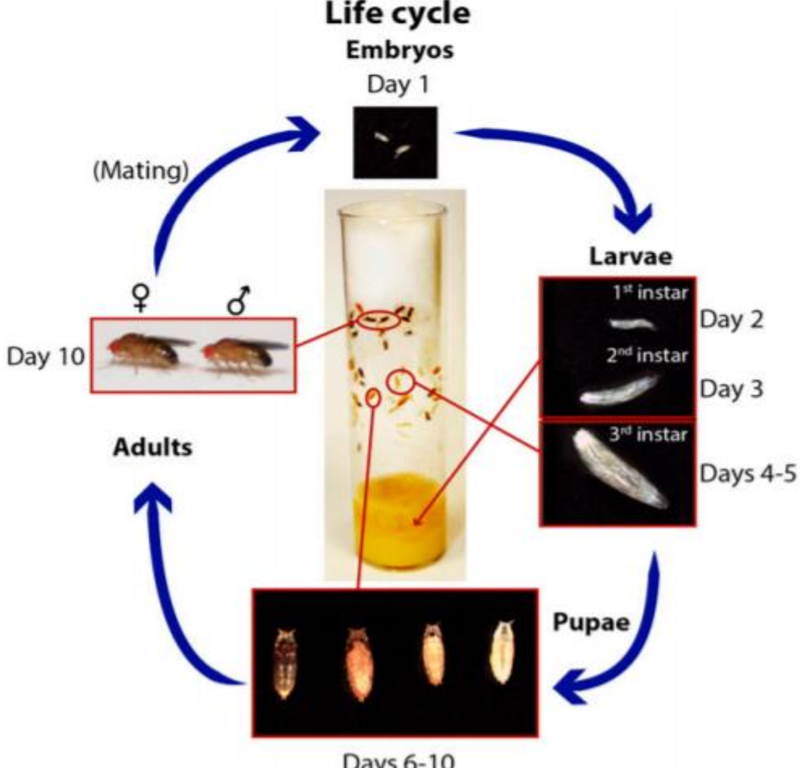

Fig: 2.2 the image shows the complete cycle of Drosophila cultured in a vial in the lab.

Experiments

A lot of information has been decoded about the Drosophila genome. Some genetic characteristics of drosophila are known as "**genetic tools**", which provide essential aid in various researches including genetics, biotechnology, drug discovery, etc.

1. **Classical genetics:** It has been almost 100 years since the use of Drosophila in various researches. These meticulous studies have contributed a lot to the understanding of heredity and gene activity, construction of recombination maps, and the relation between sex linkage and inheritance of sex chromosomes.Mutagenesis studies on Drosophila led to the construction of genetic maps, which was also facilitated by the studies of banding patterns of polytene chromosomes found in salivary glands of *Drosophila* larva.

2. Transposable elements: The P elements carry the gene for transposase activity and are responsible for hybrid dysgenesis—series of mutations as a result of crossing male with P element and a female without P element which leads to sterility and

chromosomal aberration—in Drosophila. Its use in introducing cloned DNA in Drosophila was discovered way back in 1982.

The P elements are now an essential tool in genetics for gene tagging, gene disruption, chromosome engineering, and inducible gene expression. Moreover, the mutation, by the introduction of P elements into a gene, allows the molecular identification of the affected gene.The controlled activity of transposase aids scientists to insert P elements at the desired location and generate a large number of mutations required for their studies.

3. The Drosophila genome: The whole genome of Drosophila was sequenced in 2000. Researchers have found that Drosophila has approximately 70% of orthologous genes associated with human diseases.The availability of a chunk of information about Drosophila genomes aid researchers (as a model organism) for human disease research and drug discovery and it also avoids the labor of performing a number of molecular manipulations of Drosophila DNA.

Experimental methods to study Drosophila in labs

The study of Drosophila in labs serves many purposes which include the understanding of neurons, developmental processes, mutational studies, gene regulation, and signaling. A number of methods are used to study the different processes in different experiments. In this section, you will get a brief of cytogenetic methods used to study different parts/processes of Drosophila and its culturing in labs.Other than the mentioned cytogenetic experiments in this section, the two other, most commonly used bioengineered, techniques used to study the Drosophila are:

- The GAL4/UAS system: Used to study gene expression and function in organisms.
- The FLP/ FRT system: Site-directed recombination technology.

1. Culturing Drosophila in labs

Equipment:

The *Drosophila* culturing does not require standard equipment. The great scientist T. H. Morgan used glass milk bottles to culture *Drosophila* for his experiments! Now, laboratories use bottles and vials to culture *Drosophila*.Bottles are used to maintain a large population and culturing vials are used to maintain a small population and make crosses. Generally, glass bottles are preferred but autoclaved plastic bottles can also work well. Moreover, the size of small vials ranges from 96 mm by 25 mm to larger vials. To cover the bottles, plugs are used which can be cotton or foam plugs. However, cotton plugs are mostly preferred.

Media: The preparation of media involves two ways: cooking the media or using ready-made and dehydrated media. The latter is most preferred, which avoids the labor of cooking the media, and is easier and quicker than the former. However, rehydration is required to use readymade media. To ensure the complete rehydration and culture process, follow the given procedure:

Rehydration Procedure of ready-made media:

1. Add ⅕ to ⅖ volume of dry media to the bottle/vial.
2. Add water to completely moisten the media.
3. Let the vial rest for a few minutes and add water until it seems hydrated.
4. The surface of completely hydrated media looks shiny without any spaces in the media.
5. Allow the media to warm to room temperature. The optimum temperature to grow flies is at 25 °C with 60 % humidity.
6. Add several grains of yeast on the surface of the media after the media is hydrated.
7. Transfer the flies in the vial/bottles and plug it.
8. Flies should be transferred to different clean vials/bottles to maintain the culture.

NOTE: Change the food always after or between 10-14 days.

Fig: 2.3 The image shows Drosophila culture in a bottle with a demonstration of different stages (larva and pupa).

2. Orcein staining to identify Polytene chromosomes

Procedure:

1. **Squash Preparation**

- Select 3rd instar larva from the culture.
- Place them in a container having PBS and 0.8% saline.
- Put the larva on the slide containing a drop of 45% acetic acid and dissect the larva.
- Remove the salivary gland and allow it to sit in 45% acetic acid for 2-3 minutes.
- Stain the gland with the lactic–acetic-orcein stain for 5 minutes.
- Take a clean slide and put a drop of 1: 2: 3 fixatives. Transfer the gland to this slide.
- Put a coverslip over the gland and by using bibulous paper score a zigzag pattern over the coverslip to shear the chromosomes into fragments.
- Seal the edges of the coverslip and observe it in the microscope.

2. **Identifying chromosomes**

- To identify the chromosome, resolve the banding patterns of the telomeric region and look for some landmarks such as puffs, constriction, and banded regions.

3. In-situ hybridization to polytene chromosomes

Procedure:

1. **Polytene Chromosome Preparation**

- Dissect the salivary glands of the Drosophila in a drop of 0.7% NaCl and then transfer to 45 % acetic acid.
- Transfer the glands to the fixative for 3 minutes.
- Spread chromosomes by tapping the coverslip with a pencil in a circular motion.
- Cover the slide in blotting paper and leave overnight for better fixation.
- Freeze the slide in liquid nitrogen and flip off the coverslip.
- Place the slide in 70% ethanol for 5 min and air dry. The chromosomes can be stored desiccated at room temperature.

2. **Denaturation of Chromosome**

- Two methods of denaturation are available: Alkali denaturation (involves the use of freshly prepared NaOH)and Heat denaturation (involves the use of Tris HCl, pH 7.5, and then transfer to 70 % and 96% ethanol).
- Use the denatured slide on the same day.

3. **Preparation of Labeled probes**

- Prepare probes using DNA amplified by the polymerase chain reaction (PCR) in the presence of biotinylated nucleotides.

4. **Hybridization**

- Pipette probe onto the chromosomes and cover it with a siliconized coverslip.

- Overnight incubate the slide in a box lined with moist tissue at 58 °C.
- Remove the slide from the box and put it in 2X SSC. Wash the slide at 58 °C for 1 hour, with three changes of the solution.

5. **Signal Detection**

- Wash the slides in PBS and PBS-TX for 2 minutes.
- Make a 1:250 dilution of streptavidin–horseradish peroxidase conjugate in the buffer and add to the chromosomes.
- Place the slide in a humid box and incubate at 37°C for 30 minutes.
- Wash the slide in PBS and PBS-TX.
- Place the DAB solution onto the chromosomes.
- Incubate at room temperature for 10-15 minutes.
- Rinse the slides in PBS.
- Observe the slide under the Phase Contrast microscope.

4. Immunostaining to study Larval brains

Procedure:

1. **Fixation of Mitotic Chromosomes**

- Transfer the 3rd instar larva in a few drops of physiological solution (0.7% NaCl in distilled water) and dissect out its brain.
- Transfer the brain to a few drops of hypotonic solution (0.5% Trisodium citrate dihydrate in distilled water) for 8 minutes.
- Transfer the brain to a fixative solution (2% formaldehyde, 45% acetic acid in distilled water) for 1-8 minutes.
- Transfer four fixed brains to 4 drops of fixative solution and cover it with a siliconized coverslip.
- Put another clean slide on the coverslip, invert the sandwich, and squash for 1 minute between blotting paper.

- Freeze in liquid nitrogen, flip off the coverslip, and immerse the slide in 1X PBS at room temperature.

2. **Fluorescent immunostaining on Fixed chromosomes**

- Put the slide in 1X PBS with dried nonfat milk and incubate in it for 30 minutes.
- Clean the slides in 1X PBS for 3 minutes.
- Put the primary antibody solution on the mitotic preparation and incubate for 1 hour at room temperature.
- Wash the slides in PBS for 5 minutes (three times).
- Put the secondary antibody solution on the mitotic preparation and incubate it for 1 hour at room temperature.
- Wash the slides in 1X PBS (three times) for 5 min in the dark.

3. **DAP1 staining and mounting**

- Stain the slides in the DAP1 staining solution at room temperature for 4 minutes.
- Wash the slides in 1X PBS for 30 seconds.
- Mount the slide in a drop of antifade solution.
- Analyze the slides using an epifluorescence microscope (The slide can also be used to perform the FISH technique).

Note: To grasp the principle and theory of Cytogenetic techniques you can refer to "*Cytogenetics: An advanced technique to color Chromosomes* and *Molecular Cytogenetics: in situ Hybridization-based technology*".

Conclusion: Drosophila studies have unraveled many mysteries of biological processes and today its use in biomedical research cannot be ignored. A number of proven methods are available to study any characteristics/features of Drosophila and understand different processes (neural functions, signaling, development, etc.). The availability of huge information on Drosophila in combination with an array of

genetic tools allows researchers to tackle any problems in biology. Today, it is extensively used in applied and translational research towards human health and it is certainly expected by scientists that it can make a major breakthrough in regenerative medicine.

2.4. PREPARATION OF CHROMOSOMALMAPS IN DROSOPHILA BASED ON PERCENT OF CROSSING OVER.

LINKAGE AND CROSSING-OVER: According to Mendel's principle of independent assortment, a dihybrid cross with unlinked markers ought to produce a 1:1:1:1 ratio. If a significant deviation from this ratio occurs, it may be evidence that for linkage, that is, that the loci are located close to each other on the same chromosome pair. During meiosis, a pair of synapsed chromosomes is made up of four chromatids, called a tetrad. The phenomenon of a cross - over occurs when homologous chromatids in the tetrad (one from each of the two parents) exchange segments of varying length during prophase. The point of crossover is known as a chiasma (pl. chiasmata). A tetrad typically has at least one chiasma along its length. Generally, the longer the chromosome, the greater the number of chiasmata. There are two theories on the physical nature of the process. The classical theory proposes that cross-over and formation of the chiasma occurs first, followed by breakage and reunion with the reciprocal homologues. According to this theory, chiasma formation need not be accompanied by chromosome breakage. Alternatively, according to the chiasmatype theory, breakage occurs first, and the broken strands then reunite. Chiasmata are thus evidence, but not the causes, of cross-overs. Recent molecular evidence favours the latter theory, although neither is a completely satisfactory explanation of all of the evidence.

Gametes produced by a dihybrid heterozygous individual with linked loci

Condition	Meiotic tetrad	Gametes	Combination
No crossover	a + b+ a + b+ a b a b	a + b+ a + b+ a b a b	Parenta P P P
Single crossover: markers in cis a+ b+ //ab	a + b+ a + b+ a ⇕ b a b	a + b+ a + b a b+ a b	P Recombinant Recombinant P

Single crossover: markers in trans a+ b//ab	a + b a + b a b+ a b+	a + b a + b+ a b a b+	P Recombinant Recombinant P

In dihybrid crosses, an arrangement in which the wild-type alleles of both loci are contributed by one parent is referred to as a cis configuration; the alternative arrangement is called a trans configuration. A gamete that shows the same configuration as the parent is referred to as a parental type; where the configuration is altered, the gamete is referred to as a recombinant type. Cis and trans configurations are altered by recombination. Linkage between loci is indicated when the recombinant phenotypes occur less frequently than the parental types. The frequency of crossing over (% recombination) between two loci is directly related to the physical distance between those two loci. Percent recombination in a test cross equals map distance (1 map unit = 1 % recombination).

P1

a+ b//a+ b x ab+ //ab+

F1 a+ b//ab+ x ab//ab (test cross)

F2: a+ b//ab , ab+ //ab 90% -

a+ b+ //ab, ab//ab 10% - recombinant

10% recombinant indicates that loci a and b are 10 map units apart.

Gene Map Ab 10 m. u.

Conversely, if it is known that loci b and c are 16 map units apart, then the expected proportions of parental and recombinant phenotypes in a test cross can be predicted:

eg. P1 b+ c//b+ c x bc+ //bc+
F1 b+ c//bc+ x bc//bc (test cross)

In a test cross

♀ ♂	**8 % b+ c+**	**8% Bc**	**42% b+ c**	**42% bc+**
100 % bc				

In a F1 x F1 cross

♀ ♂	**8% b+ c+**	**8% bc**	**42% b+ c**	**42% bc+**
50% b+ c				
50% bc+				

Multiple Crossovers Analysis of the genetic behaviour of three or more linked loci may show evidence of multiple cross-overs. When three loci are involved, there will be two parental types, four recombinant classes with single cross-overs and two recombinant types showing cross-overs between all three loci (= double cross-over). The two parental types will be most abundant, the four single cross-over (SCO) recombinants will be next while the two double cross-over (DCO) recombinants will be least abundant. Each cross-over situation results in two parental gametes and two recombinant gametes. The maximum recombination between any two loci is 50% (since ½ of the gametes are parental type).

Gametes produced by trihybrid heterozygous individual. a+ b+ c+ / a b c

	Meiotic tetrad	Gametes	Combination
Single Crossover (a-b)	a+ b+ c+ a+ b+ c+ a ⇕b c a b c	a+ b+ c+ a+ b c a b+ c+ a b c	P R R P
Single Crossover (b-c)	a+ b+ c+ a+ b+ c+ a b ⇕c a b c	a+ b+ c+ a+ b+ c a b c+ a b c	P R R P
Double Crossover	a+ b+ c+ a+ b+ c+ a ⇕b⇕ c a b c	a+ b+ c+ a+ b c+ a b+ c a b c	P R R P

MAKING A GENE MAP: The recombination frequency is constant for any pair of linked loci and represents the "genetic" distance between them. Each 1 m.u. is the distance that will generate 1% recombination. It is possible to develop a gene map, showing the order of the loci and the distance between them by observing the number of offspring showing recombinant phenotypes. Example 1: A standard problem in genetics is to determine the order of three loci known to be linked on one pair of the autosomes. Solution of the problem requires (1) a determination of the relative order of loci, and (2) the map distances between loci. A cross is made between homozygous wild-type female Drosophila (a+ a+ b+ b+ c+ c+) and triple-mutant males (aa bb cc) (the order here is arbitrary). The F1 (a+ a b+ b c+ c) females are test crossed back to the triple-mutant males and the F2 phenotypic ratios are as follows:

"a+ b c" 18

"a b+ c" 112

"a b c" 308

"a+ b+ c" 66

"a b c+" 59

"a+ b+ c+" 321

"a+ b c+" 102

"a b+ c+" 15

1000

1. The gene order can be determined by examination of the relative frequencies of the F2 phenotypes.

a. Because linked loci tend to stay together, the non-crossover (NCO) or parental phenotypes should be most frequent (and equal in number). In this case a+ b+ c+ (321) and a b c (308)

b. Because simultaneous crossovers between the outside and middle loci are unlikely, the double-crossover (DCO) genotypes should be the least frequent. We observe a+ b c (18) and a b+ c+ (15)

c. Then, to determined the physical order of loci, compare the parental and doublecrossover phenotypes. The marker that appears to "switch places" is in the middle [technically, this marker is said to be "out of phase"]. Here, the a+ b+ c+ NCO and a b+ c+ DCO phenotypes indicate that the a locus falls between the b andc loci. The correct order of the loci is b a c . [Note that this order is equivalent to c a b , and that the order of the outside markers is arbitrary].

d. The coupling phase of the trihybrid F1 is b+ a+ c+ / b a c

2. The remaining two pairs of phenotypes correspond to single-crossovers (SCO) events in the region between either b and a, or between a and c. a. b+ a c (112) and b a+ c+ (102) phenotypes indicate crossovers between b & a. b. b+ a+ c (66) and b a c+ (59) phenotypes indicate crossovers between c & a.

3. The percent recombination between two markers indicates the map distance between them: 1% recombination = 1 map unit (m.u.). To determine the map distance between a pair of loci, count the number of SCO and DCO events, and use the following formula [the most common error is to neglect the DCO classes].

Map distance = % recombination =

(# in SCO phenotypes + # in DCO phenotypes x 100)

(total # progeny)

(ba) Map distance = 112 + 102 + 18 + 15 x 100 = 24.7% = 24.7 m.u.

1000

(ac) Map distance = 66 + 59 + 18 + 15 x 100 = 15.8% = 15.8 m.u.

1000

(bc) Map distance = 24.7 m.u. + 15.8 m.u. = 40.5 m.u.

4. We can now draw a map segment showing order and distances among loci. Again, note that the orders b-a-c and c-a-b are equivalent and that the left/right the orientation of this map is arbitrary]

5. B ac

24. 7 | 15. 8

40. 5

USING A GENE MAP:

The gene map can be used as a table of probabilities to predict the expected amount ofrecombination between certain loci.In a test cross the male contributes only recessive alleles. Recombination occurs in the formation of the female gametes. Therefore whatever alleles present in the female gamete will be expressed in the phenotype of the offspring. There is a certain probability that a cross-over will form between a and b loci (= map distance between a and b) and another independent probability that a cross-over will occur between b and c loci (= map distance between b and c). The probability of a double cross-over is the product of these two independent probabilities.

Example 2: Given the map segment cn vg sm

| 9. 5 24. 5

In a test cross of cn+ vg+ sm+ // cn vg sm

Expected DCO = (% recomb. cn-vg) (% recomb. vg-sm)

= 0.095 x 0.245 = 2.3%

Therefore we expect to find 2.3% of the female gametes to be the results of double crossovers

1.15% cn+ vg sm+

1.15% cn vg+ sm

Expected SCO (cn-vg)

From the gene map 9.5% of the gametes would be expected to have crossovers between cn and vg, however this includes the 2.3% of double crossovers. Therefore 9.5 -2.3 = 7.2% of the female gametes should have single crossovers:

3.6% cn vg+ sm+ &. 3.6% cn+ vg sm

Expected SCO (vg-sm)

From the gene map 24.5% of the gametes would be expected to have crossovers between vg and sm, this includes the 2.3% of double crossovers. Therefore 22.2% of the female gametes should have single crossovers

11.1% cn+ vg+ sm & 11.1% cn vg sm

Total crossovers = 2.3% + 7.2% + 22.2% = 31.7% Expect 68.3% parental gametes (34.15% of each).

Gametes♀	**♂gametes = 100 % cn vg sm**
34.15 % cn+ vg+ sm+ **34.15 % cn vg sm** **11.1 % cn vg sm+** **11.1 % cn+ vg+ sm** **3.6 % cn vg+ sm+** **3.6 % cn+ vg sm** **1.15% cn vg+ sm** **1.15 % cn+ vg sm+**	

These percentages can then be used to determine an expected ratio.
wild-type : cn vg sm : cn vg : sm : cn : vg sm : vg : cn sm
34.15 : 34.15 : 11.1 : 11.1 : 3.6 : 3.6 : .15: 1.15
29.7 :29.70 : 9.7 : 9.7 : 3.1 : 3.1 : 1: 1

INTERFERENCE and COINCIDENCE:

Crossing over does not occur uniformly along a chromosome. For example, fewer crossovers occur in the area around the centromere than in other areas of the chromosome (making the loci appear closer together than they actually are). Also, the formation of one chiasma typically makes it less likely that a second chiasma will form in the immediate vicinity of the first. This seems to be due may be due to the inability of the chromatids to bend back upon themselves within a certain minimum distance. This lack of independence is called interference and results in the observation of fewer double crossover types than would be expected according to true map distance. Interference varies in different sections of the chromosome and is measured by the Coefficient of Coincidence (C.C.) which is the ratio of observed to expected double crossover types

C. C. = (observed DCO) / (expected DCO)Interference = 1 - C.C.

To calculate expected DCO, actual distances from gene map should be used when available. If C.C. = 0 then interference is complete and no double crossovers are observed. In general, double-crossovers do not occur between loci less than 10 m.u. apart. C.C. values between 0 and 1 indicate partial interference. Generally interference decreases as the distance between the loci increases. If C.C. = 1 then there is no interference and all the expected double crossovers are observed. With loci more than 45 m.u. apart there is little or no interference. In some cases there may be an excess of double crossovers, i.e. negative interference.

In EXAMPLE 1: C.C. = (observed DCO) / (expected DCO)

= (33) / (0.247)(0.158)(1000) = 33 / 39 = 0.846

Seeing 84.6% of the double crossovers expected. Interference = 1 - C.C. = 1 - .846 = 0.154 = 15.4%

The coefficient of coincidence can also be used to modify the number of double crossovers predicted from a map.

In EXAMPLE 2: In the region cn-sm 2.3% double crossover type were expected. However if the C.C. is known to be 70% for this region, then the number of expected double crossovers is modified (.7 x 2.3) = 1.61% and the number of other expected phenotypes are modified accordingly: Source : https://www.csus.edu › indiv › dulaik › exercise7

2.5 GENETICS EXERCISE (DATA TO PROVIDED) :

Solutions for Practice Problems in Genetics

NOTE: When a genotype is listed as, for example, A_, it means that the second allele is unknown. The actual genotype may be AA or Aa

Problems Involving One Gene

1. First assign symbols for the alleles, following the rules and conventions. For example, the letter "L" could be used for this gene. The symbols would thus be L for the dominant short allele and l for the recessive long allele. The problem tells you that the parents are both homozygous (the short-haired one because you are told it is true-breeding, the long haired one because it has the recessive characteristic, which automatically makes it homozygous). So here's your mating

Mating: ll X LL

Gametes: Only l Only L

Offspring: All Ll: All short -

Haired

2. From problem 1 you already know the dominance here. Go ahead and use the same symbols

Mating: ll X ??

Gametes: Only l ?

Short haired: Ll

OffspringLong-haired: ll

Since the known parent is ll, it contributes a recessive allele to all offspring. So the final phenotype of the kittens is actually determined by the unknown parent. Since some of the kittens are short-haired, they must have received the dominant allele from this unknown parent. The kittens who are long-haired must have received the recessive allele from the unknown parent. So that parent must be Ll, and must be short-haired.

3. Again, assign symbols. A good choice here would be W for widow's peak and w for no widow's peak. As both parents here have the dominant trait, they must each have at least one W. Their first child obviously inherited this allele from at least one of them, as he also possesses a widow's peak. The second child does not, and thus must be ww. But note that there is no evidence here that prevents us from assuming that both of these parents could be carrying hidden recessive w alleles, so Mr. Smith is not justified. Here's the Punnett's Square, assuming these two are truly the parents of both children:

	W	w
W	W W	Ww
w	Ww	ww

Genotypic ratio predicted: 1 WW : 2 Ww : 1 ww

Phenotypic ratio predicted: 3 Widow's Peak : 1 No Widow's Peak

4. Once again, we begin with some pedigree analysis to figure out everyone's genotypes. A bit of rumination reveals that this problem describes a nearly classic dihybrid cross. "Pure breeding" is a euphemism for "homozygous," so all of the original parents are homozygous. We mate a bbLL cat to a BBll cat, producing a bunch of BbLl kittens as an F1. The problem then instructs us to create an F2 by mating the kittens to each other. Here's this mating:

Parents: BbLl X BbLl
Gametes: BL BL
Bl Bl
bL bL
bl bl

	BL	Bl	bL	bl
BL	**BBLL**	**BBLl**	**BbLL**	**BbLl**
Bl	**BBLl**	**BBll**	**BbLl**	**Bbll**
bL	**BbLL**	**BbLl**	**bbLL**	**bbLl**
bl	**BbLl**	**Bbll**	**bbLl**	**bbll**

Genotypic Ratio:
1 BBLL , 2 BbLL , 1 BBll , 2 Bbll ,1 bbLL, 2 BBLl, 1 bbll ,2 bbLl, 4 BbLl
Phenotypic Ratio: 9 Black Short : 3 Black Long : 3 Siamese Short : 1 Siamese Long
The actual question is, "what is the chance of producing a Siamese, short kitten?" The answer is 3/16

2.6 SUMMARY:

Morphological study of Drosophila for genetic traits and preparation of chromosomalmaps in Drosophila based on percent of crossing over. To the data provided makeup of a cell using the terms chromosome, sister chromatid, homologous chromosome, diploid, haploid, and tetrad and also recognize the function and products of mitosis and meiosis .The gametes, formed as a result of meiosis, possess half the number of chromosomes as found in the parent cells and their chromosome number is represented by n, whereas the zygote formed by the fusion (fertilization) of male and female gametes and the cells derived from it are known as diploid and their chromosome number is symbolized by 2n.
The two similar chromosomes of diploid cells are known homologous chromosomes or homologous pair."The chromosomes of a homologous pair are brought together in the zygote by the union of male and female gametes from the parents. Evolutionary response to selection depends on the amount of genetic variation expressed in the population. Because of this, the effect of environmental changes on the expression of genetic variation in quantitative traits has important evolutionary implications.

It has been almost 100 years since the use of Drosophila in various researches. These meticulous studies have contributed a lot to the understanding of heredity and gene activity, construction of recombination maps, and the relation between sex linkage

and inheritance of sex chromosomes.Mutagenesis studies on Drosophila led to the construction of genetic maps, which was also facilitated by the studies of banding patterns of polytene chromosomes found in salivary glands of *Drosophila* larva.

Transposable elements: The P elements carry the gene for transposase activity and are responsible for hybrid dysgenesis—series of mutations as a result of crossing male with P element and a female without P element which leads to sterility and chromosomal aberration—in Drosophila. Its use in introducing cloned DNA in Drosophila was discovered way back in 1982.The P elements are now an essential tool in genetics for gene tagging, gene disruption, chromosome engineering, and inducible gene expression. Moreover, the mutation, by the introduction of P elements into a gene, allows the molecular identification of the affected gene.The controlled activity of transposase aids scientists to insert P elements at the desired location and generate a large number of mutations required for their studies.

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The gene map can be used as a table of probabilities to predict the expected amount ofrecombination between certain loci.In a test cross the male contributes only recessive alleles. Recombination occurs in the formation of the female gametes. Therefore whatever alleles present in the female gamete will be expressed in the phenotype of the offspring. There is a certain probability that a cross-over will form between a and b loci (= map distance between a and b) and another independent probability that a cross-over will occur between b and c loci (= map distance between b and c). The probability of a double cross-over is the product of these two independent probabilities.

When a genotype is listed as, for example, A_, it means that the second allele is unknown. The actual genotype may be AA or Aa .

2.7 AND 2.8 : TERMINAL QUESTIONS WITH ANSWER S

1. The tendency of an offspring to resemble its parent is known as

1. Variation

2. Heredity

3. Resemblance

4. Inheritance

Also read: Heredity

2. Who is known as the "Father of Genetics"?

1. Morgan
2. Mendel
3. Watson
4. Bateson

Also read: Genetics

3.. The genotypic ratio of a monohybrid cross is

1. 1:2:1
2. 3:1
3. 2:1:1
4. 9:3:3:1

Also read: Monohybrid cross

4. Which of the following statements is true regarding the "law of segregation"?

1. Law of segregation is the law of purity of genes
2. Alleles separate from each other during gametogenesis
3. Segregation of factors is due to the segregation of chromosomes during meiosis
4. All of the above

Also read: Law of segregation

5. An exception to Mendel's law is

1. Independent assortment
2. Linkage
3. Dominance
4. Purity of gametes

Also read: Mendel's Laws of Inheritance

6. Pea plants were used in Mendel's experiments because

1. They were cheap
2. They had contrasting characters
3. They were available easily
4. All of the above

7. The smallest unit of genetic material which produces a phenotypic effect on mutation is

1. Muton
2. Gene
3. Recon
4. Nucleic acid

Also read: Difference between genotype and phenotype

8. Mendel's findings were rediscovered by

1. Correns
2. De Vries
3. Tschermark
4. All

9. Alleles are

1. Alternate forms of genes
2. Linked genes
3. Chromosomes that have crossed over
4. Homologous chromosomes

Also read: Difference between gene and allele

10. When the activity of one gene is suppressed by the activity of a non-allelic gene, it is known as

1. Pseudo-dominance
2. Hypostasis
3. Epistasis
4. Incomplete dominance

Also read: Incomplete Dominance

11. Cystic fibrosis is

1. Sex-linked recessive disorder
2. Autosomal dominant disorder
3. Autosomal recessive disorder
4. Sex-linked dominant disorder

Also read: Genetic Disorders

12. 9:7 ratio in the F2 generation represents

1. Incomplete dominance
2. Co-dominance
3. Epistasis
4. Complementary interaction

13. A small amount of lethal mutation is always present in the population due to

1. Positive selection

2. Negative selection
3. Frequency-dependent selection
4. Mutation-selection balance

14. If a plant with genotype AaBb is self-fertilized, the probability of getting AABB genotype will be (A and B are not linked)

1. ½
2. ¼
3. ⅛
4. 1/16

15. How many phenotypes can occur in the human blood group ABO with alleles IA IB i?

1. 2
2. 3
3. 4
4. 1

Also read: Blood Groups

16. The geometrical device that helps to find out all the possible combinations of male and female gametes is known as

1. Bateson Square
2. Mendel Square
3. Punnett Square
4. Mendel's Cube

17. Which term represents a pair of contrasting characters?

1. Heterozygous
2. Homozygous
3. Codominant genes
4. Allelomorphs

Answer Key

1- 2	**2- 2**	**3- 4**	**4- 1**	**5- 2**
6- 4	**7- 3**	**8- 2**	**9- 2**	**10- 1**
11- 4	**12- 1**	**13- 3**	**14- 3**	**15- 3**
16- 4	**17- 4**	**18- 3**	**19- 3**	**20- 4**

1. A represents the dominant allele and a represents the recessive allele of a pair. If, in 1000 offspring, 500 are aa and 500 are of some other genotype, which of the following are most probably the genotypes of the parents?

a. Aa and Aa (answers)

b. Aa and aa

c. AA and Aa

d. AA and aa

e. aa and aa

2. Which of the following best describes the parents in a testcross?

a. One individual has the dominant phenotype and the other has the recessive phenotype. (answers)

b. Both individuals are heterozygous.

c. . Both individuals have the dominant phenotype.

d. Both individuals have the recessive phenotype.

e. Both individuals have an unknown phenotype

3. DNA replication can be described as

a. Semiconservative (answers)

b. conservative

c. degenerative

d. Dispersive

e. Radica

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UNIT 3: EXPERIMENTS ON ECOLOGY: CALCULATION OF THE FOLLOWING SHALL BE DONE ON THE BASIS OF GIVEN DATA

3.1 Objectives

3.2 Introduction

3.3 Calculation of similarity index between different communities

3.4 Calculation of concentration of dominance for different communities

3.5 Calculation of Shannon Weiner Index of diversity in different communities

3.6 Determination of dissolved oxygen

3.7 Determination of free CO2 in water sample

3.1 OBJECTIVES

1. Calculation of similarity index between different communities
2. Calculation of concentration of dominance for different communities
3. Calculation of Shannon Wiener Index of diversity in different communities
4. Determination of dissolved oxygen
5. Determination of free CO2 in water sample

3.2 INTRODUCTION

Ecology is a branch of science, including human science, population, community, ecosystem and biosphere. Ecology is the study of organisms, the environment and how the organisms interact with each other and their environment. It is studied at various levels, such as organism, population, community, biosphere and ecosystem. **Community ecology**, study of the organization and functioning of communities, which are assemblages of interacting populations of the species living within a particular area or habitat.

As populations of species interact with one another, they form biological communities. The number of interacting species in these communities and the complexity of their relationships exemplify what is meant by the term “biodiversity.” Structures arise within communities as species interact, and food chains, food webs, guilds, and other interactive webs are created. These relationships change over evolutionary time as species reciprocally adapt to one another through the process of co-evolution. The overall structure of biological communities, the organization of

inters specific interactions, and the effects the co-evolutionary process has on the biological community are described below.

An ecological community can be regarded as an assemblage of species populations that has the potential of interaction. More precisely, a community may be defined as an interactive assemblage of species occurring together within a particular geographical area, a set of species whose ecological function and dynamics are in some way interdependent.

Some examples of this interaction include:

1. Overt and critically determining competitive interaction and feeding relationship.
2. Subtle interactions, such as reliance of plants on animals for pollination and seed dispersal or of animals on plants for meeting habitat requirements.

Communities are invariable discrete units. Commonly their boundaries merge imperceptibly along environmental gradients, forming an ever changing complex which is defined as an ecocline.

Species Richness, Evenness and Diversity:

Species richness is the number of species in a community. Each species is not likely however, to have the same number of individuals. One species may be represented by 1000 plant, another by 200 and third only a single plant. The distribution of individuals among the species is called species evenness or species equitability.

Evenness is a maximum when all species have the same number of individuals.

The inverse of evenness is concentration of individuals.

Species diversity is a product of richness and evenness; it is species richness weighted by species evenness. A community shows species diversity, yet a community is described by the major category of growth forms like trees and shrubs. They are called dominant species.

There is another category of species called keystone species. A keystone species occupies a critical central role in the community so much so that its activity determines the community structure & dynamics.

The significance of a keystone species in a community is mostly disproportionate to its dominance status in a community.

Whittaker described three types of diversity:

A) Alpha diversity refers to diversity within a particular area of ecosystem, and is usually expressed by the number of species (species richness) in that ecosystem.

B) Beta diversity examines the change in species diversity between two ecosystems.

C) Gamma diversity is a measure of the overall diversity for the different ecosystems within a region.

3.3 AIM: CALCULATION OF SIMILARITY INDEX BETWEEN DIFFERENT COMMUNITIES

Similarity index is a comparison of the current vegetation (in terms of kinds, proportions, and amounts) on an ecological site to what the site is capable of producing at its reference state.

Simpson's Index:

$D = 1- \sum(p_1)^2$

Where D is the index number; S the total number of species and the proportion of all individuals in the sample which belongs to species i

The Simpson index is a dominance index because it gives more weight to common or dominant species. In this case, a few rare species with only a few representatives will not affect the diversity.

In the Simpson index, p is the proportion (n/N) of individuals of one particular

Species found (n) divided by the total number of individuals found (N), Σ is still the sum

of the calculations, and s is the number of species

community similarity

A) Calculating community similarities (what the communities have in common in terms of species) helps us determine if we are comparing apples to apples and oranges to oranges.

B) There are many indices that do this, we will use Sorenson's coefficient.

C) Sorenson's coefficient gives a value between 0 and 1, the closer the value is to 1, the more the communities have in common.

D) The equation is: Sorenson's Coefficient (CC) = 21

. A Complete community overlap is equal to 1; complete community dissimilarity is equal to 0.

3.4 AIM: CALCULATION OF SHANNON WIENER INDEX OF DIVERSITY IN DIFFERENT COMMUNITIES.

Diversity Indices:

A) A diversity index is a mathematical measure of species diversity in a given community.

B) Based on the species richness (the number of species present) and species abundance (the number of individuals per species).

C) The more species you have, the more diverse the area, right?

D) However, there are two types of indices, dominance indices and information statistic indices.

Shannon Diversity Index: The Shannon Diversity Index is a way to measure the diversity of species in a community.

The Shannon index is an information statistic index, which means it assumes all species are represented in a sample and that they are randomly sampled.

In the Shannon index, p is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), ln is the natural log, Σ is the sum of the calculations, and s is the number of species.

Denoted as *H*, this index is calculated as:

***H* = -Σ*p*i * *ln* (*p*i)**

Where, **Σ:** A Greek symbol that means "sum"

***ln*:** Natural log

pi = proportion of total sample represented by species

i. Divide no. of individuals of species i by total number of samples.

S = number of species, = species richness

Hmax = ln(S) = Maximum diversity possible

E = Evenness = HHmax

$H = 1 - \sum n_1/n$

Where H is the diversity index in bits/individual, n_1 the individual density of one species and n the density of all the species.

The higher the value of *H*, the higher the diversity of species in a particular community. The lower the value of *H*, the lower the diversity.

A value of $H = 0$ indicates a community that only has one species.

The minimum value the Shannon diversity index can take is 0. Such a number would tell us that there's no diversity - only one species is found in that habitat.

There's no upper limit to the index. The maximum value occurs when all species have the same number of individuals. It equals log (k), where k is the number of species.

In real-world ecological data, the Shannon diversity index's range of values is usually 1.5- 3.5.

The Shannon diversity index **tells you how diverse the species in a given community are**. It rises with the number of species and the evenness of their abundance.

The higher the index, the more diverse the species are in the habitat. If the index equals 0, only one species is present in the community.

It may be easier to interpret the result if you calculate the evenness: $E = H / \ln(k)$, where k is the number of species. Evenness gives you a value between 0 and 1 (so you can think of it as a percentage). Remember: the lower the evenness, the higher the diversity.

The Shannon diversity index (a.k.a. the Shannon–Wiener diversity index) is a popular metric used in ecology. The index takes into account the number of species living in a habitat (richness) and their relative abundance (evenness).

Use the Shannon diversity index formula:

$H = -\sum [(pi) * \log(pi)]$,

Where: H - Shannon diversity index;

pi - Proportion of individuals of i-th species in a whole community:

$pi = n / N$,

Where: n - individuals of a given type/species; and

N - total number of individuals in a community,

$\sum$ = Sum symbol; and

Imagine we need to assess the species diversity in a part of a rain forest. We know that 5 scarlet macaws, 13 blue morpho butterflies, 2 capybaras, 5 three-toed sloths, and 1 jaguar live in the region we are interested in.

Species	Number of individuals (n)	Proportion pi = n / N	ln(pi)	Pi * ln(pi)
Scarlet Macaw	5	0.2	-1.609	-0.322
Blue Morpho Butterfy	12	0.48	-0.734	-0.352
Capybara	2	0.08	-2.526	-0.202
Three-Toed Sloth	5	0.2	-1.609	-0.322
Jaguar	1	0.04	-3.219	-0.129

To calculate diversity, we will use the Shannon diversity index formula:

H = -∑[(pi) * ln(pi)]

1. First of all, we need the total number of individuals:
   N=5+12+2+5+1=25.
2. For each species, calculate its proportion in the whole community (third column of the table).
3. Multiply ln (pi) by pi and round the answer to three decimal places (fourth column).
4. Sum all the results from the final column according to the Shannon-Wiener diversity index equation. Since we were going to multiply them by -1, we can do it straight away and ignore the minus signs:
   H = 0.322 + 0.352 + 0.202 + 0.322 + 0.129 = 1.327
5. Here's our rounded Shannon-Wiener diversity index: H ≈ 1.3.

3.5 AIM: CALCULATION OF CONCENTRATION OF DOMINANCE FOR DIFFERENT COMMUNITIES

Dominance: Usually a community has one or more species that occur in large numbers. Such species are called dominants. Sometimes only one species may be dominant e.g. a community may have more trees of Pinus. It is called Pine forest or community. Sometimes more than one type of plants are dominant e.g. Quercus – Rhododendron forests.

3.6 AIM: DETERMINE DISSOLVED OXYGEN (DO) OF GIVEN WATER SAMPLE.

Introduction

Dissolved oxygen (DO) levels in environmental water depend on the physiochemical and biochemical activities in water body and it is an important useful in pollution and waste treatment process control.

Two methods are commonly used to determine DO concentration:

(1) The iodometric method which is a titration-based method and depends on oxidizing property of DO and

(2) The membrane electrode procedure, which works based on the rate of diffusion of molecular oxygen across a membrane.

In the Iodometric method, divalent manganese solution is added to the solution, followed by addition of strong alkali in a glass-stopper bottle. DO rapidly oxidize an equivalent amount of the dispersed divalent manganese hydroxide precipitates to hydroxides of higher valence states. In the presence of iodide ions in an acidic solution, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent of the original DO content. The iodine is then titrated with a stranded solution of thiosulfate.

The titration end point can be detected visually with a starch indicator.

The Winkler Method is a technique used to measure dissolved oxygen in freshwater systems. Dissolved oxygen is used as an indicator of the health of a water body, where higher dissolved oxygen concentrations are correlated with high productivity and little pollution. This test is performed on-site, as delays between sample collections and testing may result in an alteration in oxygen content.

The Winkler Method uses titration to determine dissolved oxygen in the water sample. A sample bottle is filled completely with water (no air is left to skew the results). The

dissolved oxygen in the sample is then "fixed" by adding a series of reagents that form an acid compound that is then titrated with a neutralizing compound that results in a color change. The point of color change is called the "endpoint," which coincides with the dissolved oxygen concentration in the sample. Dissolved oxygen analysis is best done in the field, as the sample will be less altered by atmospheric equilibration.

Dissolved oxygen analysis can be used to determine:

The health or cleanliness of a lake or stream,

The amount and type of biomass a freshwater system can support,

The amount of decomposition occurring in the lake or stream.

Dissolved oxygen should be measured as quickly and carefully as possible. Ideally, samples should be measured in the field immediately after collection. The following protocol is adapted from Washington State.

Reagent List:

- 2ml Manganese sulfate
- 2ml alkali-iodide-azide
- 2ml concentrated sulfuric acid
- 2ml starch solution
- Sodium thiosulfate

Principle

Dissolved oxygen (DO) determination measures the amount of dissolved (or free) oxygen present in water or wastewater. Aerobic bacteria and aquatic life such as fish need dissolved oxygen to survive. If the amount of free or DO present in the wastewater process is too low, the aerobic bacteria that normally treat the sewage will die. DO is determined by the titrimetric method developed by Winkler.

1. Dissolved molecular oxygen in water is not capable of reacting with KI, therefore an oxygen carrier such as manganese hydroxide is used. $Mn(OH)_2$ is produced by the action of KOH on $MnSO_4$.
2. Mn $(OH)_2$ so obtained reacts with dissolved molecular oxygen to form a brown precipitate of basic manganic oxide, $MnO(OH)_2$.
3. $MnO(OH)_2$ then reacts with concentrated sulphuric acid to liberate nascent oxygen.
4. Nascent oxygen results in oxidation of KI to I_2.
5. This liberated iodine is then titrated against standard sodium thiosulphate solution using starch as an indicator.

6. Thiosulphate reduces iodine to iodide ions and itself gets oxidized to tetrathionate ion.

Calculations

1000 mL of 1N Na2S2O 3 = 8 g oxygen

\ V mL of 0.025 N EDTA = × 0.025 × 8/1000 V g oxygen per 100 mL water sample

= V × 0.025 × 8 mg oxygen per 100 mL water sample

= V × 0.025 × 8 × 10 mg oxygen per 1000 mL water sample

Result

The given water sample has ________________ ppm of dissolved oxygen.

Precautions:

1. Do not allow air to trap while sampling water during BOD analysis.
2. Dip the tip of the pipette just at the bottom of the BOD bottle and gently release the reagents.
3. Take care that the chemicals do not flow out from the bottle while shaking/swirling.
4. Observe the colour changes during the BOD reaction, if any.

3.7 AIM: ESTIMATION OF DISSOLVED CARBON DIOXIDE (DCO2) IN THE WATER SAMPLES

INTRODUCTION:

Dissolved carbon dioxide (DCO_2) is the amount of carbon dioxide that is present in water. Water bodies receive carbon dioxide from the atmosphere and from the respiratory activity of aquatic organisms. It is also released by the process of decomposition in the aquatic ecosystem. A small fraction of the CO_2 that dissolves in water reacts rapidly to form carbonic acid. This, in turn, partially dissociates to form hydrogen, bicarbonate and carbonate ions. CO_2 will continue to dissolve until equilibrium is reached.

Carbon Dioxide is present in water in the form of a dissolved gas. Surface waters normally contain less than 10 ppm free carbon dioxide, while some ground waters may easily exceed that concentration. Carbon dioxide is readily soluble in water. Over the ordinary temperature range (0-30o C) the solubility is about 200 times that of oxygen. Calcium and magnesium combine with carbon dioxide to form carbonates and bicarbonates. Aquatic plant life depends upon carbon dioxide and bicarbonates in

water for growth. Microscopic plant life suspended in the water, phytoplankton, as well as large rooted plants, utilize carbon dioxide in the photosynthesis of plant materials; starches, sugars, oils, proteins. The carbon in all these materials comes from the carbon dioxide in water. When the oxygen concentration in waters containing organic matter is reduced, the carbon dioxide concentration rises. The rise in carbon dioxide makes it more difficult for fish to use the limited amount of oxygen present. To take on fresh oxygen, fish must first discharge the carbon dioxide in their blood streams and this is a much slower process when there are high concentration of carbon dioxide in the water itself. Source of CO2 Almost all natural waters contain some carbon dioxide which they gain in several ways. Carbon dioxide gas (CO,) is present in the air to the extent of 0.03 percent by volume and 0.05 percent by weight. As rain falls through the air, it absorbs some of this gas. On reaching the earth, the rainwater is slightly acid, so, it will absorb additional amounts of carbon dioxide if it flows through decaying vegetation. At the same time, the carbon dioxide reacts with waterand becomes carbonic acid. If, these water passes through limestone formations, its carbonic acid content will react with the limestone to form soluble calcium bicarbonate. In this process the carbonic acid is partially neutralized. On the other hand, if water passes through rock formations, such as granite, no such reaction occurs. The carbonic acid is not neutralized. It continues as carbonic acid until drawn to the surface where it can then cause corrosion if not neutralized. If nature or chemical agents do not neutralize carbonic acid, it will cause Corrosion of both copper and galvanized plumbing systems. It can leads to serious damaging of plumbing equipment.

PRINCIPLE:

Free Carbon dioxide is determined by titrated the water sample against a strong alkali to pH 8.3 Reagent: 1. 0.05 N NaOH: Dissolve 2 gm NaOH in 1litre of distilled water. 2. Phenolphthalein indicator: 0.25% solution in 60% ethyl alcohol.

Procedure:

1. Take 10 ml of the water sample in conical flaks.
2. Add 2-3 drop of phenolphthalein indicator. The color change to pink indicates the absence of free CO_2.
3. In Case sample remains colorless, titrate it with 0.05 N NaOH.
4. At the end point a pink color appear.

5. Calculate free CO_2.

The carbon dioxide (CO_2) that is dissolved in water when titrated against sodium bicarbonateforms carbonic acid. The formation of which is indicated by phenolphthalein indicator. CO_2 undergoes chemical reactions as follows:

$CO_2 + H_2O \longrightarrow H_2\,CO_3$
$H^+ + HCO^{-3} \longrightarrow H_2\,CO_3$
$CO^{-3} + H^+ \longrightarrow HCO^{-3}$
$HCO^{-3} \longrightarrow$ CO2 + OH
$H^+ + OH^- \longrightarrow$ H2O

REQUIREMENTS:
0.045 N Na_2CO_2, Phenolphthalein indicator, glassware, water samples, etc.

Observations: The readings of titrant volume for each water sample were recorded as follows:

Table 1: Observation table for water sample A (Pond water).

No. of observations	Initial reading (in ml)	Final reading (in ml)	Volume of titrant
1.			
2.			
3.			

Table 2: Observation table for water sample B (Stream water)

No. of observations	Initial reading (in ml)	Final reading (in ml)	Volume of titrant
1.			
2.			
3.			

Table 3: Observation table for water sample C (Tap water)

No. of observations	Initial reading (in ml)	Final reading (in ml)	Volume of titrant
1.			
2.			
3.			

Calculations:-		
DCO_2 = _____Volume of titrant used/ ______		X 1000
Volume of sample taken for titration		
=………..	mg/L	

RESULT:
Report the value to the nearest of first decimal i.e. 0.1 mg/L.

DISCUSSION:

Discuss your results, why DCO2 is highest in pond water followed by that of tap water and
lowest in stream water

Observation and calculation

1. Water sample takes = 10 ml.
2. Normality of NaOH = 0.05 N
3. mL of NaOH used=R= = __________mL
4. Eq. Wt. of CO2 = 12 + (16 X 2) = 44

Free CO2 as mg/l = R x N
X 1000 x Eq. Wt. of CO2 ml. of sample taken = = _________mg/l

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UNIT 04:CYTOLOGY EXPERIMENT

CONTENTS

4.1 Objective

4.2 Introduction

4.3 Prepared slide study of meiosis and mitosis

4.4 Summary

4.5 Terminal question and answer

4.6 Glossary

4.7 References

4.1 OBJECTIVES

To study the different stages of meiosis and mitosis through permanent cytological experiment.

4.2 INTRODUCTION

The mitosis occurs in the vegetative or somatic cells of the plants and animals. Mitosis is the process of cell division where the chromosomes are duplicated and distributed equally to the daughter cells. The term mitosis is generally used for the entire process of cell division, but more strictly it should include only the division of the nucleus. The mitotic cycle is divided into many phase: prophase, prometaphase, metaphase, anaphase and telophase, the period between two mitotic cycles is called interphase or 'resting stage'.

Meiosis is a specialized type of nuclear division which divides a diploid cell into four haploid daughter cell. The division of cell in which the number of chromosomes is reduced to half that produces haploid sex cells or gametes from diploid cells. The meiosis consists of two cell divisions, meiosis I and meiosis II. Meiosis I a special cell division reduces the cell from diploid to haploid. The meiosis I has the same stages as mitosis. These stages are knows as prophase I, promataphase I, metaphase I, anaphase I and telophase I. Meiosis II is equational division resulting in the formation of four daughter cells. The second mitotic division consists of prophase II, metaphase II, anaphase II and telophase II.

Meiosis I and meiosis II which are further divided into Karyokinesis I and II and Cytokinesis I and Karyokinesis II and Cytokinesis II respectively. The preparatory steps that lead up to meiosis are identical in pattern and name to interphase of the mitotic cell cycle. Interphase can be divided into three phases: (1) The Growth phase (G1) takes place at the end of one cell division. RNA and proteins are synthesized during this period, phase, (s) synthesis phase and (G2) Growth phase. Meiosis I and II are each divided into prophase, metaphase, anaphase and telophase stages.

4.3 PREPARED SLIDE STUDY OF MEIOSIS AND MITOSIS

4.3.1 MITOSIS:

Study different stages of mitosis and their features are as follows:

Prophase: During mitotic prophase the cell becomes spheroid, and there is increase in viscosity and refractivity. The chromosomes shorter and thicker and appears a double structure consisting of two identical threads called chromatids. The two chromatids of each chromosome are united during prophase and attached to each other at a specialized region on each chromatid, known as the centromere.

Metaphase: The chromosomes have reached the central or equatorial portion of the spindle. They are lined up in one place to form the equatorial plate or metaphasic plate. The centrioles lie on the equator of the spindle. Usually the arms of the chromosomes lie on the equator of spindle.

The specific spindle fibers to which the chromosomes are attached are known as chromosomal fibers. The others, lacking attached chromosome, extend from pole to pole and are known as continuous fibres. Spindles are of two types, direct and indirect. In the direct type chromosomal fibres connect the chromosomes directly with the pole. In the indirect type the chromosomes are connected with the continuous fibres.

Anaphase: Metaphase passes into anaphase when the centromeres of all the chromosomes divided and each becomes double structure. The daughter centromeres move apart, the chromatids also separate and begin to move towards the poles. The chromatids, after they become separate are called the daughter chromosomes and move closer to their respective poles. During the latter half of the anaphase, the spindle fibres between the two groups of chromosomes appears to be stretched and a group of fibres, called the interzonal fibres, appears. This may be called the pushing body of the spindle and serve to push the chromosomes to the poles at the end of anaphase.

Telophase: The end of polar migration of two groups of chromosomes marks the beginning of telophase. Nuclear membrane is formed again. The chromosomes lose their state of condensation and the nucleoli reappear.

Cytokinesis: After the division of the nucleus of the nucleus, the cytoplasm of the cell also divides. A constriction appears in the equatorial region and the parent cell into two. The centrosomes now cease their activity and the asters disappear.

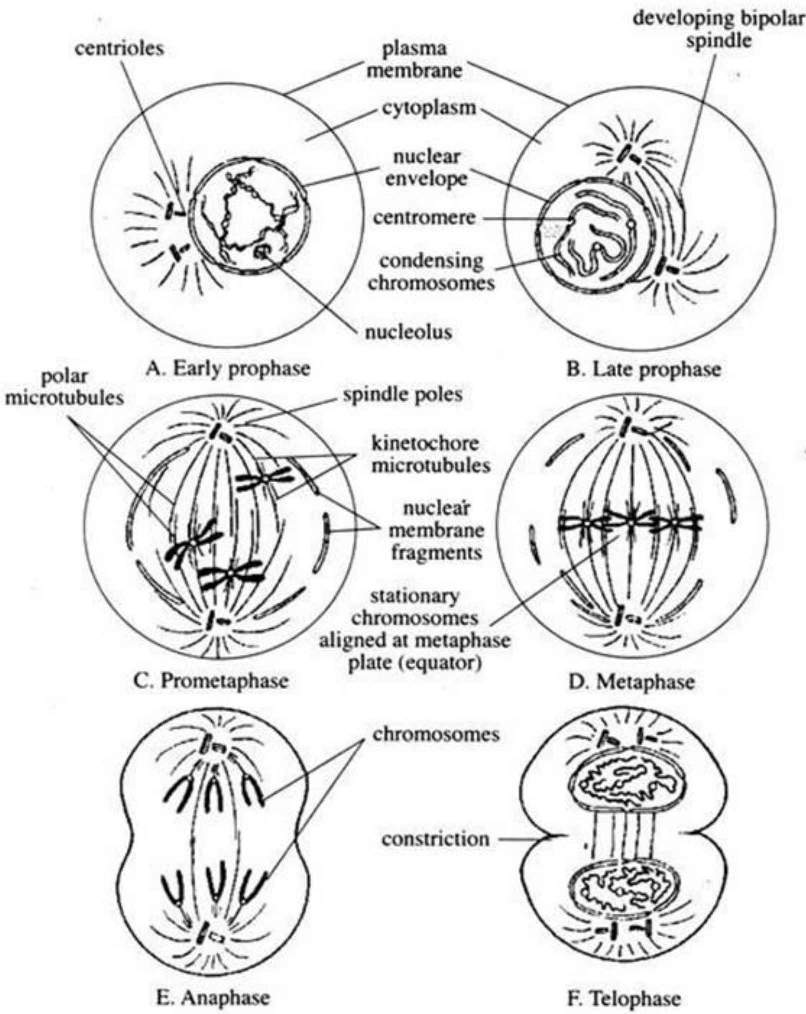

Fig.4.1 Different stages of mitosis
(https://www.notesonzoology.com/cytology/mitosis-and-cell-division-cytology/2209)

4.3.2 MEIOSIS

Study different stages of meiosis and their features are as follows:

A.FIRST MEIOTIC DIVISION (MEIOSIS I)

Prophase I: It is the most important phase of meiosis. Five phases can identify in it.

a) Leptotene (leptos- slender, tene- thread):

During this stage the chromosomes are long slender thread showing numerous chromomeres of different sizes distributed along their whole length. The leptene chromosomes are generally arranged in a bouquet in which all their ends are drawn together in a small area on one side of the nuclear membrane and the remaining parts extending into the nuclear cavity in the form of loops.

b) Zygotene:

Pair of chromosomes having similar genes which control the characters are called as homologous chromosome. One member of a pair of homologous chromosomes comes from male parent and the other from female parent. The homologous chromosomes begin to pair length wise due to force of attraction. The pairing proceeds from one end to another. The pairing of homologous chromosome is known as synapsis or syndesis. Usually pairing takes place not only between homologous chromosomes. Synaptonemal complexes first appear during zygotene.

c) Pachytene (thick thread):

Pachytene is the long stage of prophase I. The chromosomes become shorter and thicker. During pachytene the chromosome are associated in bivalents or tetrads. Each bivalent consists of four chromatids (two pairs). Each chromatid pair is united by a cetromere. The number of bivalents is half the diploid number of chromosomes.

d) Diplotene:

The homologous chromosomes begin to separate repelling each other. The separating chromosomes are held together at one or more points where breaks and fusion had occurred, these points are called chiasmata. A chiasmata formed at the ends of chromosomes is called a terminal chiasma. Chiasmata formed along the lengths of chromosomes are called interstitial chiasmata.

During diplotene the chiasmata begin to be displaced along the length of the chromosome. The terminal chiasma slip off the ends of the chromosomes and its position is taken up by an interstitial chiasma. This displacement of chiadmata is termed as *terminalization.* When terminalization is complete the homologues remain in contract through the terminal chiasma. The degree of terminalization is expressed by the terminlization coefficient (T).

$$T = \frac{\text{Number of terminal chiasmata}}{\text{Total number of bivalents}}$$

The frequency of chiasmata (Fq) is the average number of chiasmata in a bivalent, or bivalent of a nucleus

$$\text{Frequency (Fq)} = \frac{\text{Total number of chiasmata}}{Totalnumberofbivalents}$$

When there is a single chiasma the arms of the bivalent rotate through 180º and form a cross. With more than two chiasmata a series of loops is formed. Rotation is due to the same force of repulsion which brings about terminalization.

e) Diakinesis:

The chromosomes become more contracted and the bivalents are more evenly distributed in the nucleus and migrate towards the periphery. The homologues remain in contact with each other by their terminal chiasmata. At this stage the nucleolus is detached from the chromosome or disappears and the formation of nuclear spindle starts.

Prometaphase I: The nuclear membrane disappears in prometaphase I and chromosome reach their maximum contraction. Spindle formation begins.

Metaphase I: The chromosome now becomes arranged on the equator of the cell and the spindle is formed. Spindle fibres become attached to the centromeres of the two homologous chromosomes. The two centromeres of each bivalents lie on opposite sides of the equatorial plate. The metaphasic chromosomes of meiosis differ from those of mitosis.

Anaphase I: The homologous chromosomes, each consisting of two chromatids united by a centromere, move towards the poles of the cells. In each homologous chromosome one chromatid is unchanged, while the other has undergone mixing of maternal and paternal sections. The division of centromeres, which is characteristic of the anaphase of mitosis, does not take place in meiosis.

Telophase I: The chromosomes reach the poles of the spindle they persist for some time in the condensed state. Later they undergo despiralization and become elongated, though not to the extent as in mitosis. The nuclear membrane is reformed, but the nucleoli do not reappear.

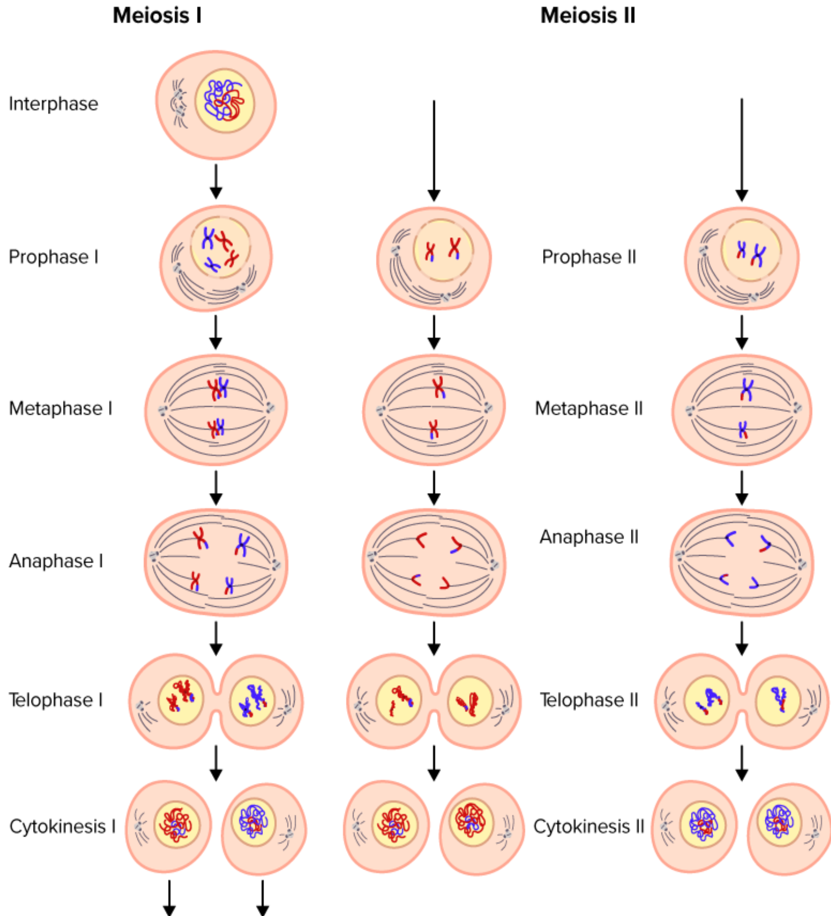

Fig. 4.2 Stages of meiosis
(Source: https://www.ck12.org/c/biology/meiosis/lesson/Meiosis-BIO/)

B. SECOND MEIOTIC DIVISION (MEIOSIS II)

The second meiotic division is essentially similar to mitosis. It differs from mitosis in that DNA does not duplicate, while the centromeres do so. Telophase I is followed by a short period of interkenesis (interphase II). This differs from interphase I and interphase of mitosis in that there is no duplication of DNA.

Prophase II: Prophase II does not show the complex nuclear behavior of prophase I. The chromatids of prophase II have widely separated arms, and in this respect differ from the chromatids of mitosis, which are close together. Spindle formation takes place in prophase II as in mitosis and the nuclear membrane disappears.

Metaphase II: The chromosomes become oriented on the equatorial plate and have the same relationship to the spindle as in mitosis.

Anaphase II: The cetromeres divide and the two chromatids of each chromosome separate and move to the poles. After separation the chromatids are called chromosomes.

Telophase II: The nucleus, centriole and the chromosome return to the interphasic condition. Each nucleus contains the haploid number of chromosomes.

4.4 SUMMARY

The **cell division** is a characteristic feature of the entire cellular organism and is basically similar in all species. Development of an organism from the zygote to the full grown stage takes place by the division of cells. When cell division observed under a microscope, a series of stages seen, are known as cell cycle. During the cell cycle, the phase in which cells divide, is called the mitosis phase (**M phase**), whereas the period between two mitotic cycle is called interphase. Interphase can be divided into three periods. G1 phase S phase and G2 phase.G1 takes place at the end of one cell division. RNA and proteins are synthesized during this period, but there is no synthesis of DNA. During the synthesis phase (S) DNA is formed from purine and pyrimidine nucleotides. During this period the DNA content of the nucleus is doubled. During the phase G2, synthesis of RNA and protein continues, but DNA synthesis stops. The time taken for the S phase, the G2 phase and mitosis is approximately equal. The length of G1 is greater and is also subjected to greater variation. Cell which do not divided frequently have a longer G1 phase, while frequently dividing cells have a shorter phase. The G1 phase can be terminated by various stimuli, and has begun DNA replication; it has usually committed itself to division.

4.5 TERMINAL QUESTIONS

1) What is cell cycle?

2) Name the phase of cell cycle?

3) What is mitosis?

4) What is meiosis?

5) What is Diakinesis?

6) What is the difference between mitosis and meiosis?

4.6 GLOSSARY

Anaphase: Stage in mitosis where chromosomes begin moving to opposite ends (poles) of the cells.

Cell: The smallest structural unit of living organisms that is able to grow and reproduce.

Cell Cycle: The term given to the series of tightly regulated steps that a cell goes through between its creation and its division to form two daughter cells.

Centrioles : Cylindrical structures that are composed of groupings of microtubules arranged in 9+3 pattern.

Chromatid: One oftwo identical copies of a replicated chromosome.

Cromatin: Mass of genetic material composed of DNA and proteins that condense to form chromosomes during eukaryotic cell division.

Chromosome: A long, stringy aggregate of genes that carries heredity information (DNA) and is formed from condensed chromatin.

Cytokinesis: Division of cytoplasm that produces distinct daughter cells.

Daughter cell: A cell resulting from the replication and division of a single parent cell.

Diploid cell: A cell that contains two sets of chromosomes. One set of chromosomes is donated from each parent.

G1 phase: The first gap phase, one of the phases of interphase. It is the period that precedes the synthesis of DNA.

G2 phase: The second gap phase, one of the phases of interphase. It is the period that follows DNA synthesis but occurs before the start of prophase.

Haploid: An individual or cell having a single set of chromosomes.

Metaphase: Stage of mitosis where chromosomes align along the metaphase plate in the center of the cell.

Nucleus: A membrane- bound structure that contains the cell's hereditary information and controls the cell's growth and reproduction.

Nucleotide: Portion of DNA or RNA molecule composed of one deoxyribose phosphate unit
(in DNA) or one ribose phosphate unit (in RNA) plus a purine or a pyrimidine.

Prophase: Stage in mitosis where chromatin condenses into discrete chromosomes.

Purine: Nitrogenous base occurring in DNA and RNA, these are adenine and guanine.

Pyrimidine: Nitrogenous base occurring in DNA (thymine and cytosine) or RNA (uracil and cytosine).

S phase: The synthesis, one of the phase of interphase. It is the phase during which the cell's DNA is synthesized.

Telophase: Stage of mitosis where the nucleus of one cell is divided equally into two nuclei.

4.7REFERENCES

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