Exercise 6

Aim: Preparation and study of mitosis in onion root tips

Principle: Somatic growth in plants and animals takes place by the increase in the number of cells. A cell divides mitotically to form two daughter cells wherein the number of chromosomes remains the same (i.e., unchanged) as in the mother cell. In plants, such divisions rapidly take place in meristematic tissues of root and shoot apices, where the stages of mitosis can be easily observed. In animals, mitotically dividing cells can be easily viewed in the bone marrow tissue of a vertebrate, epithelial cells from gills in fishes and the tail of growing tadpole larvae of frog.

Requirement: Onion bulbs, wide mouth glass tubes/jar/bottle, glacial acetic acid, ethanol 2-4% acetocarmine/acetoorcein stain, N/10 HCl, spirit lamp/hot plate, slide, cover slips, blotting paper, molten wax/nail polish and compound microscope

Procedure

Growing of root tips

Select a few medium-sized onion bulbs. Carefully remove the dry roots present. Grow root tips by placing the bulbs on glass tubes (of about 3–4 cm. diameter) filled with water. Care should be taken so that the stem portion of the bulb (basal part) just touches the water. A few drops of water may be added periodically to compensate evaporation losses. New roots may take 3–6 days to grow. Cut 2–3 cm long freshly grown roots and transfer them to freshly prepared fixative, i.e., aceto-alcohol (1:3:: glacial acetic acid : ethanol). Keep the root tips in the fixative for 24 hours and then transfer them to 70% ethanol (for preservation and use in future). Onion root-tip cells have a cell cycle of approximately 24-hour duration, i.e., they divide once in 24 hours, and this division usually takes place about two hours after sunrise. Therefore, roots grown on water should be cut only at that time to score maximum number of dividing cells.

Preparation of slide

Take one or two preserved roots, wash them in water on a clean and grease-free slide. Place one drop of N/10 HCl on the root tip followed by 2–3 drops of aceto-carmine or aceto-orcein stain on it. Leave the slide for 5–10 minutes

on a hot plate (or warm it slightly on spirit lamp). Care should be taken that the stain is not dried up. Carefully blot the excess stain using blotting paper. Now cut the comparatively more stained (2–3 mm) tip portion of the root and retain it on the slide and discard the remaining portion. After (10–20 seconds) put one or two drops of water and blot them carefully using blotting paper. Again put a drop of water on the root tip and mount a cover slip on it avoiding air bubbles. Place the slide in between the folds of blotting paper using the fingers in such a way that the cover slip mounted on the slide is properly held. Now slowly tap the cover slip using the blunt end of a pencil so that the meristematic tissue of the root tip below the cover slip is properly squashed and spread as a thin layer of cells. Carefully seal the margins of the cover slip using molten paraffin wax or nail polish. This preparation of onion root tips cells is now ready for the study of mitosis.

Study of slide

Place the slide on the stage of a good quality compound microscope. First observe it under the lower magnification (10 X objective) to search for the area having a few dividing cells. Examine the dividing cells under higher magnification of the microscope to observe the detailed features of mitosis.

Observation

The stages of mitosis can be broadly categorised into two parts: **karyokinesis** (division of nucleus) followed by **cytokinesis** (division of cytoplasm, and ultimately of the cell). Those cells, which are not in the phases of cell division are considered to be in **interphase**. You may observe that most of the cells in a microscope field are in interphase

Interphase

The cells are mostly rectangular, oval or even circular in shape, with almost centrally situated densely stained nucleus. The chromatic (coloured) material of the nucleus is homogeneous and looks granular. The boundary of the nucleus is distinct. One or few nucleoli (sing: nucleolus) can also be observed inside the nucleus (Fig. 6.1a).

Stages of Mitosis

(a) Prophase

Intact nuclear outline is seen. The chromatin (seen as a homogeneous material in the nucleus at interphase) appears as a network of fine threads (chromosomes). Nucleoli may or may not be visible (Fig. 6.1b).

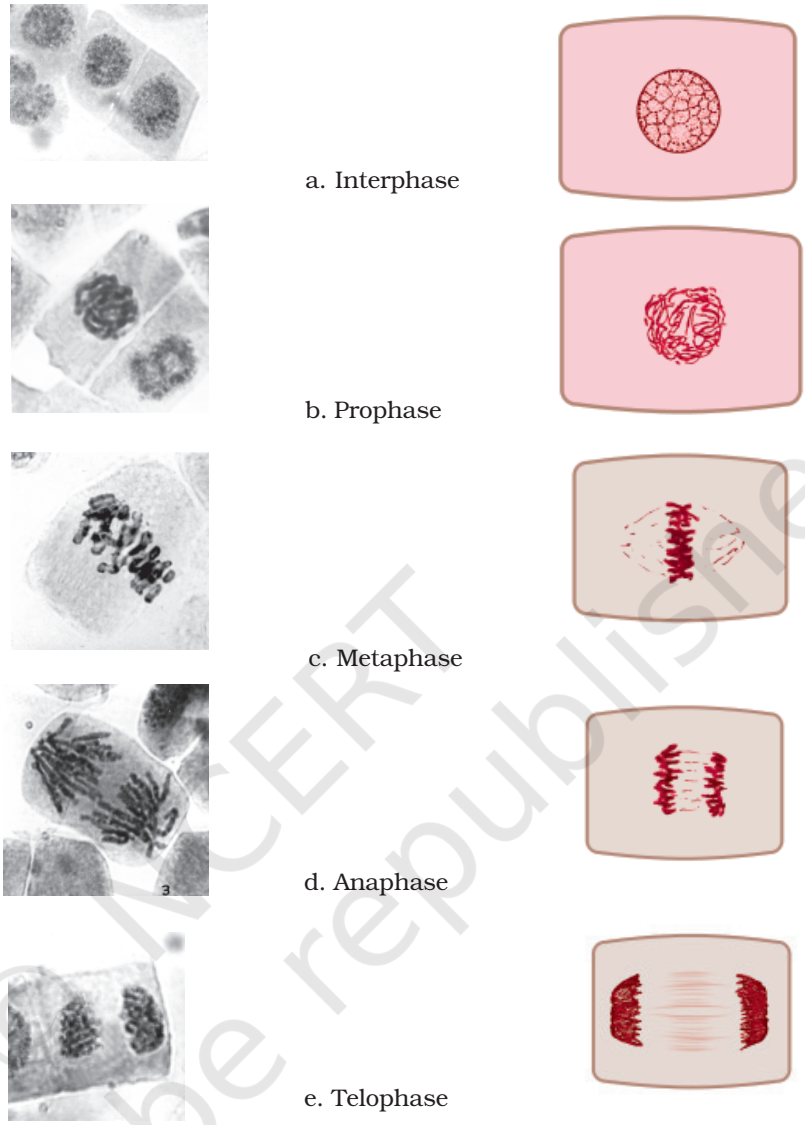

Fig.6.1 Interphase (a) and stages of mitosis (b - e) – actual microscopic view on left side and its diagrammatic representation on the right hand side

If the cell under observation is in the early stage of prophase then the chromatin fibres (chromosomes) are very thin. However, in the cells at late prophase, comparatively thicker chromatin fibres would be visible. Besides this, in the late prophase the nuclear membrane may not be noticed.

(b) Metaphase

The nuclear membrane disappears. Chromosomes are thick and are seen arranged at the equatorial plane of the cell (Fig. 6.1c). Each chromosome at

this stage has two chromatids joined together at the centromere, which can be seen by changing the resolution of the microscope. Nucleolus is not observed during metaphase.

(c) Anaphase

This stage shows the separation of the chromatids of each chromosome. The chromatids separate due to the splitting of the centromere. Each chromatid now represents a separate chromosome as it has its own centromere. The chromosomes are found as if they have moved towards the two poles of the cell. The chromosomes at this stage may look like the shape of alphabets 'V', 'J' or 'I' depending upon the position of centromere in them. Different anaphase cells show different stages of movement of chromosomes to opposite poles, and they are designated to represent early, mid and late anaphase (Fig. 6.1d).

(d) Telophase

Chromosomes reach the opposite poles, lose their individuality, and look like a mass of chromatin (Fig. 6.1e). Nuclear membrane appears to form the nuclei of the two future daughter cells.

Cytokinesis

In plants, a cell plate is formed in the middle after telophase. The plate can be seen to extend outwards to ultimately reach the margin of the cell and divide the cell into two. Such cell plates are characteristic of plant cells (Fig. 6.2). However, in an animal cell, the two sides of the cell show inpushings or constrictions formed from the peripheral region in the middle of the cell, which grow inward and meet to divide the cell into two daughter cells.

Draw labelled diagrams of all the phases of mitosis.

Fig. 6.2 Cytokinesis

Discussion

Mitotic index (MI) is defined as a ratio of the total number of dividing cells (n) and the total number of cells (N) in a particular focus chosen randomly under the microscope and is calculated as MI = $\frac{n}{N} \times 100$. By randomly selecting 5 to 10 such foci, one can estimate the mitotic index for a given type.

The effect of different samples of water (polluted or contaminated) can be assayed on the mitotic-index (an indicative feature of somatic growth rate in them).

Further, the impact of different types of pollutants on different phases of mitosis can also be assayed.

Tabulate your observations in the tabular form given below

Features	Interphase	Karyokinesis				Cytokinesis
		Prophase	Metaphase	Anaphase	Telophase	
1. Cell morphology 2. Nuclear morphology 3. Chromosomes/chromatids						

Questions

1. Suggest names of a few tissues, which are suitable for the study of mitosis.
2. Why is mitosis also known as equational division?
3. What shape would a metacentric and a sub-metacentric chromosome exhibit during the anaphase stage?
4. How does cytokynesis differ in plant and animal cells?