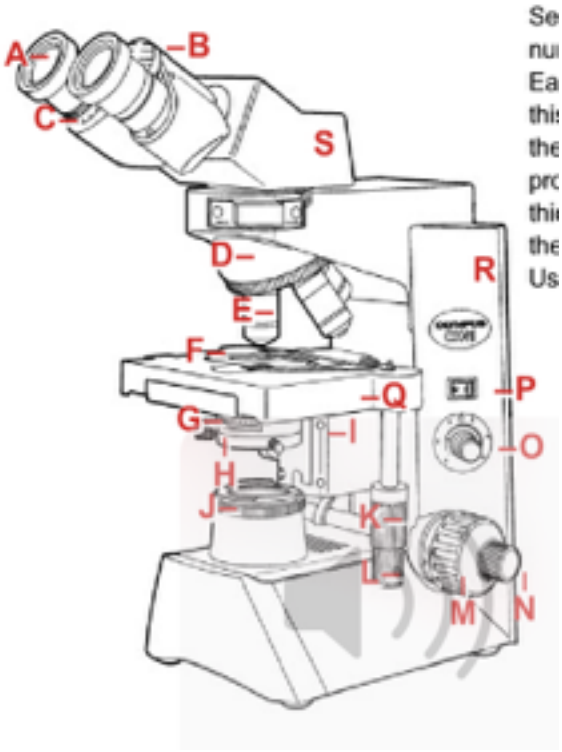
Monday, April 6, 2015

BIO LAB EXAM NOTES

LAB 1- Introduction to Microscopy & Observation of Prokaryotic and Eukaryotic Cells

Compound microscope



A- Eyepieces (oculars)- Magnifying element (usually 10x). Look through the oculars to see the image. Parafocal- when an object is in focus with one objective, the focus will not be completely lost when changing to the next objective.

B- Interpupilary adjustment- compensates for the distance between pupils of each eye and adjusts

C- Diopter adjustment- Adjusts the focus in ones eye, since the focus in both eyes in hardly the same

D- Revolving nose piece- Different objectives are housed on the revolving nosepiece and as it rotates they are brought into the light path of the microscope. -Supports the various objectives- You will only use the 4X, 10X, and 40X objectives.

E- Objective- Objectives and eyepiece lenses determine the visual magnification of a specimen. There is a series of numbers on the objective. The magnification is shown as 4X, 10X, 40X, and 100X. The 100X objective a special objective used with oil immersion.

F- Specimen holder- Position the microscope slide against the left and back sides of the specimen holder and gently release the right arm so that it is against the right front corner of the microscope slide.

G- Aperture iris diaphragm- Used to reduce glare from unwanted light by adjusting angle of the cone of light that comes from condenser. Iris controls resolution by matching numerical aperture of condenser to that objective.

H- Condenser centering screw- The cone of lighting coming from the condenser needs to be perfectly aligned with the opening of the objective (E).

I- Condenser- Focuses light from the lamp into a tightly, focussed cone passing through the specimen on the stage and the objective lenses above. * Does not magnify*

K- Y axis knob- Moves the specimen on the stage towards and away from you

L- X axis knob- Moves the specimen on the stage left and the right

M- Coarse focus knob-Permits rapid change in distance between the specimen and the objective thereby allowing for rough focussing.

N- Fine focus knob- Permits small changes in distance between the specimen and the objective and thereby allows for final focussing of the image.

Q- Stage -Supports the specimen being observed. A system of knobs on the side of the stage allows for you to move the specimen under the objective on the X and Y axes. Has an opening so that light can pass from the condenser(I) underneath, through the specimen and into the objective (E) above.

MOST IMPORTANT PART OF A MICROSCOPE= OBJECTIVE !

Objective- The magnifying element which is closest to the specimen.

Resolving Power- Ability to see 2 objects that are close together and still be able to recognize that they are separate. Human eye sees 2 objects less than 100um apart as being in CONTACT with each other.

Numerical Aperture (NA)- Determines revolving power of the objective

Orientation- If you are using the compound microscope, whatever you view will be flipped down and then opposite. Whatever you move will move in the opposite direction.

Working distance: Distance between the objective lens and the slide. Magnification and working distance have an inverse relationship, meaning that as magnification increases, working distance decreases (so essentially the space between your objective lens and the slide).

Depth of Field (Depth of focus): The number of planes in which an object appears to be in focus. When a microscope is focused on one surface, the other surfaces (higher or lower) will be out of focus. So, as magnification increases, depth of depth of field decreases.

Magnification:

- Objective magnification = 4X, 10X, 40X
- Ocular magnification= 10X
- Light intensity and working distance decrease as magnification increases
- Total magnification would be both values multiple

Stereoscopic microscope (dissecting microscope)

The stereomicroscope also called the dissecting microscope is used to view objects that are too large or too thick to observe under the compound microscope. These microscopes are always equipped with two oculars producing a stereoscopic (3D Image). Unlike the compound microscope, the image is not inverted. These microscopes can be used with reflected or transmitted light. Reflected light is directed unto opaque specimens from above and is reflected to the viewer. Transmitted light is used with translucent specimens and passes through the specimen from beneath the stage into the viewers' eyes.

Prokaryotic and Eukaryotic cells

- Cells can be divided into two general types: prokaryotic and eukaryotic based on the presence of a nucleus and other membrane bound organelles.
- Prokaryotic cells belong to 2 big groups: Archaea and eubacteria. They're usually smaller than eukaryotic cells (typically 1-5 um). Their genetic material is usually composed of one circular chromosome plus other extra chromosomal elements called plasmids.
- Eukaryotic cells are usually much larger. They posses a membrane bound nucleus, their organelles are more complex and numerous, and their genome is larger prokaryotes. Eukaryotic organisms can be uni- or multicellular.

Function of following organelles-

Plasma (cell) membrane- Controls movement of substances both in/out of the cell,barrier between the cell and its environment, maintains homeostasis.

Cell wall- Support, protection, allows for water, oxygen, carbon dioxide to diffuse in and out of the cell

Cytoplasm- Supports and protects cell organelles (creates a suspension for organelles)

Vacuoles- Store food, water, metabolic & toxic wastes. Stores large amounts of food or sugars in plants

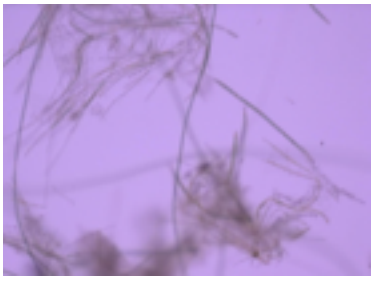
Nucleus- Controls cell activity, contains the hereditary material of the cell

Nucleolus- Organelle within the nucleus, where ribosomal RNA is produced

Chloroplasts- Place where photo synthesis originates

Protoplast- Plant, bacterial, or fungal cell that had its cell wall completely or partially removed using either mechanical or enzymatic means.

Eukaryotic cells: **Elodea-** Made a wet mount, induced plasmolysis with 5% NaCl, and took digital pictures before and after adding NaCl. * the chloroplasts are moving via cytoplasmic streaming*



Prokaryotic cells: Oscillatoria (eubacteria: cyanobacteria) - Wet mount was made using a drop of water, observed the bacteria in an isotonic environment.

Lab #2 Permeability of the Red Blood Cell

Tonicity: Globally, tonicity depicts the ability of a solution to cause a cell to absorb or reject water. Tonicity depends on how well solutes can cross the cell membrane, as well as their concentration.

Hypertonic solution, will cause water to flow from the inside of the cell to the outside. The volume of the cell will decrease and the plasma membrane will start folding.

Hypotonic: A solution which causes water to enter the cell. For a non-penetrating solute, it means its concentration is lower outside the cell compared to inside the cell.

Diffusion- Movement of substances from a region of higher concentration to a region of lower concentration

Electrolyte: compound that dissociates into ions (charged entity) when put in solution.

Osmosis: Movement of water from a region of lower solute concentration to a region of higher solute concentration, usually across a semi permeable membrane

Osmolarity: concentration of all solutes present in a solution.

Hemolysis- Describes the process of destruction of red blood cells that occurs when too much water flows inside the cell and causes the rupture of the plasma membrane.When erythrocytes are placed in hypotonic environment, water enters the cells and the volume increases.

-Hemolysis can be reached by placing the cells into an isosmotic (same concentration of solutes as the cellular fluid) solution of a penetrating substance.

-Time to hemolyse a red blood cell in a solution of penetrating substances reflects the permeability of the membrane to those substance.

Calculation of mean and standard error of the mean

Y= measurement; n=sample size

Arithmetic Mean : $\bar{Y} = \frac{\sum Y}{n}$ Standard deviation: $s = \sqrt{\frac{\sum Y^2 - \frac{(\sum Y)^2}{n}}{n-1}}$

Standard Error : $SE = \frac{s}{\sqrt{n}}$

- When 75% of the cell population has hemolysed, the suspension of red blood cells becomes suddenly transparent.

<u>Part A- Permeability experiments</u>

5 Solutions- Set A: Distilled water, Glycerol, ethylene glycol, sucrose, urea *(all 0.3M).

5 Solutions- Set B: Distilled water, thriourea, Triton X-100 2%, D- glucose (Dextrose), Ethanol *(all 0.3M)

- If hemolysis occurred instantaneously record <2 as results
- If no hemolysis occurs after 5 minutes examine the test tube at 30 second intervals up to 20 minutes.
- If no hemolysis has occurred at 20 minutes record the value as “>1200” seconds

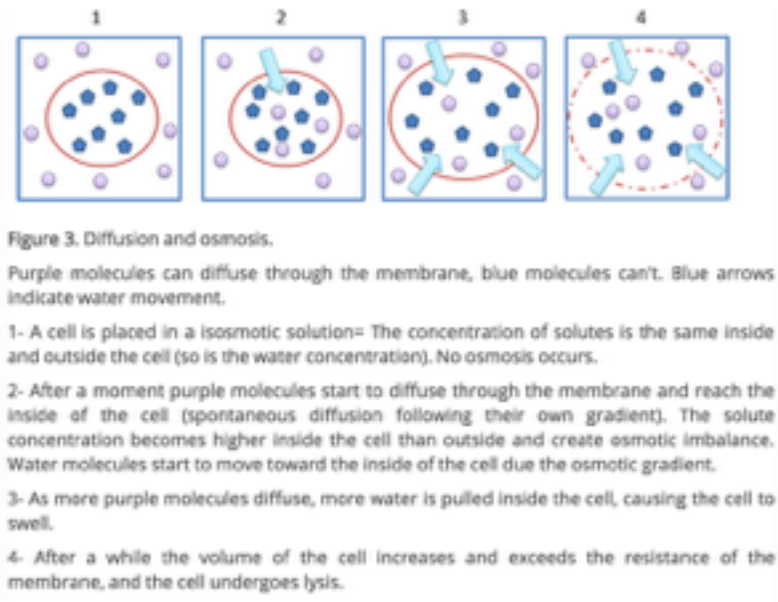


Figure 3. Diffusion and osmosis.

Purple molecules can diffuse through the membrane, blue molecules can't. Blue arrows indicate water movement.

1- A cell is placed in a isosmotic solution= The concentration of solutes is the same inside and outside the cell (so is the water concentration). No osmosis occurs.

2- After a moment purple molecules start to diffuse through the membrane and reach the inside of the cell (spontaneous diffusion following their own gradient). The solute concentration becomes higher inside the cell than outside and create osmotic imbalance. Water molecules start to move toward the inside of the cell due the osmotic gradient.

3- As more purple molecules diffuse, more water is pulled inside the cell, causing the cell to swell.

4- After a while the volume of the cell increases and exceeds the resistance of the membrane, and the cell undergoes lysis.

<u>Part B- Red Blood Cells subjected various osmotic conditions</u>

- Put a very small quantity* of blood on a microscope slide then add one drop of 0.145M NaCl. Cover the preparation with a coverslip. Repeat this procedure with blood 0.145 M NaCl +0.350 M NaCl and then blood + 0.065 M NaCl.

* Remember to include factors from the conclusion of the lab*!

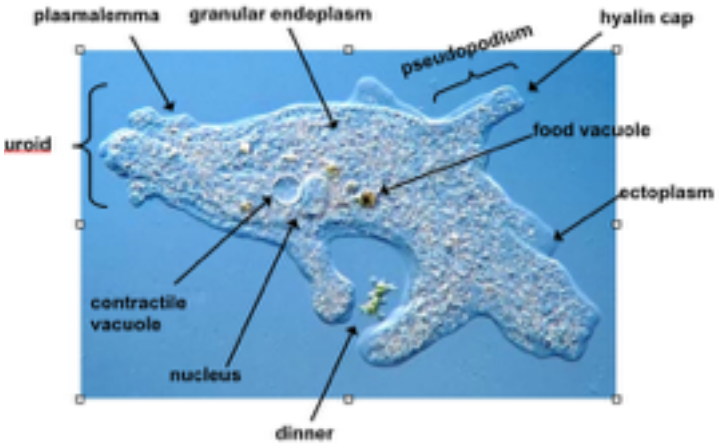
<u>Lab #3 Cellular Processes in *Amoeba proteus*</u>

Amoeba proteus is a protist that belongs to the *Amoebozoa* group. It is a **single-celled eukaryote organism** which is common to the bottoms of freshwater ponds and lakes. It continuously changes shape and is very mobile. It extends its pseudopodia as a way to move and catch food (including preys) that will then be digested in food vacuoles.

In order to conduct prolonged observations and prevent the amoebas from being crushed we will use small chambers made of parafilm melted onto a microscope slide.

Amoebas are sensitive to light of short wavelengths and to heat. Therefore, we should turn off the light of the microscope between observations, and work at a relatively low light intensity. ***Amoeba will die if coverslip on wet mount does not cover specimen***

Amoeba proteus uses membrane protrusions called pseudopodia to achieve movement. Once one of several pseudopodia form the rest of the cell seems to crawl in that direction. Formation of pseudopodia is described and relies on a process shared by all cells: extension of long polymers of **actin.** Actin is present in the cytoskeleton (microfilaments) of eukaryotic cell and is involved in muscle contractions in animals.



When pseudopodium are formed: molecules of free actin (G-action) bound to each other to create a series of long polymer fibres (called F-actin in this form). As these fibres grow they push the plasma membrane in the direction of the extending pseudopodium. Adhesive molecules attach to the newly formed pseudopodium to the substratum, whereas the same adhesive molecules will detach at the “back” of the cell (called uroid). A series of contractions occur at the uroid region helping the cell content to be pushed towards the front of the cell.

Part I: Amoeba anatomy

Pseudopodium- “ False feet” that are used to engulf prey and for movement. *Amoeba proteus* moves by extending their cytoplasm and appears to do so in a slow, gliding fashion. These extensions of their cytoplasm are called pseudopodia. This form of

movement by extension of cytoplasm is called “amoeboid movement” and is a common method of movement in other cells. (region)

Ectoplasm- Clear cytoplasm that is found near the cell membrane, it is a semi-solid substance

Hyaline cap-

Granular endoplasm (gelatinous/fluid)-

Nucleus- The nucleus of A. proteus is a membrane bound organelle which houses most of the cell’s genetic information and controls the actions of the amoeba.

Contractile vacuole- It’s function is to regulate the water content of the cell. It is also a means of excreting its waste from the cell (out through the cell membrane) VIA diffusion. A. proteus regularly moves to the surface of the water, when it needs to discharge wastes and excess water. This is done via osmosis, where there is a semi permeable membrane that lets the flow of materials through the cell. Without the contractile vacuole, the amoeba may burst.

Digestive and food vacuoles- They are not concrete meaning that they appear and disappear. They are a result of phagocytosis – the process by which A. proteus engulfs its prey. A food vacuole is basically a storage unit of food for the amoeba and is formed only when the amoeba has engulfed its prey completely – then digestive enzymes are released into the vacuole.

Triuret crystals-

Contractile vacuole- The amoeba proteus has only one contractile vacuole

Time zero- corresponds to the time when the amoeba empties the content of its contractile vacuole to the outside (systole).

Endocytosis- Bulk-phase (or nonspecific) endocytosis may be induced by a large variety of substances: proteins, amino acids, salts (particularly cations), basic stains, etc… A solution of 0.05% Alcian blue at pH 4.4, containing 1% bovine plasma albumin to induce pinocytosis.

- Place some amoebas in a watch glass with minimum liquid, then add one drop of the inducing agent. After 30 seconds prepare a wet mount using the slide in which you observed the ameboid movement.
- Once the amoeba is in contact with the inducing agent, the cell will stop moving and its shape will become “rounder”. The endoplasm flow ill also stop and endoplasm will

become restricted to the central region of the cell. Small protrusions like mini pseudopodia will appear). Endocytosis canals can be observed more easily in these structures.

Lab #4 Mitosis

In eukaryotes there are two types of cell division:

1- Cell division that will rise to two daughter cells identical to the original mother cell. Mitosis belongs to this type of division and it designates the process where the genetic material is evenly separated in two.

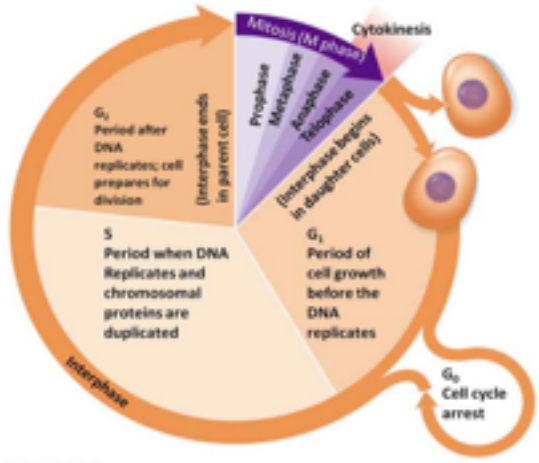
2- Cell division that will produce gametes (meiosis). Cells produced by meiosis are different from the original cell and contain a different genetic content. These cells will be used for sexual reproduction.

The cell cycle

The cell cycle can be broken down into different phases-

Cell cycle= M phase (Mitosis + cytokinesis) + Interphase (G1(+/- G0) + S + G2

Cell division (or M phase) as seen in this equation is composed of both nuclear (mitosis) and cytoplasmic (cytokinesis) divisions. Interphase is the phase separating two cell divisions, in which cells enter a period of growth and potentially preparation to the next M-phase. A cell spends most of its time in interphase and it is during that time when cellular processes are carried out.



Interphase can be divided into substages: G1(first gap) stage is a period of growth and active synthesis of all groups of macromolecules (Including RNAs and proteins). It is also during G1 that cytoplasmic organelles are replicated.

The S (synthesis) phase follows with precise replication of all DNA and synthesis of DNA- associated proteins (such as histones in eukaryotic cells) or microtubule-associated proteins (such as the centrioles in animal cells). At the end of the S phase,

each duplicated chromosome consists of two **chromatids** joined together by protein complexes called cohesins.

Once the S phase stops, the G2 (gap 2) phase begins and it involves protein synthesis and production of structures needed for mitosis, like spindle fibres.

Mitosis is the part of cell division which refers specifically to the process of nuclear division where the chromosomes are equally distributed between two daughter nuclei. After mitosis and cytokinesis, both daughter cells are identical to each other and to the parental cell. 3 principle cell types are found: **1)** cells that divide continually (e.g. cells of the gut epithelium or germinal layers of the skin), **2)** cells that leave the cell (G0) and do not divide again during the life of the organism and **3)** cells that enter a resting state (G0,G1,G2) but after some stimulus return to the cell cycle and divide.

Summary of Cell phases in plants

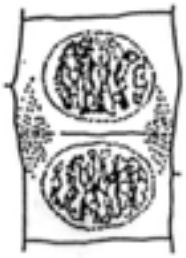
Interphase- Growth, synthesis or macromolecules and assembly of organelles as well as DNA replication occur during this phase. Features of this phase: Clear-cut nucleus, dark staining nucleoli within the nucleus and heterochromatin (tightly packed DNA) in the nucleus.

Prophase- Chromosomes shorten and thicken and now two **chromatids** attached with a centromere is visible. Each chromatid of a chromosome contains identical information and comprises the DNA duplicated during interphase (S-phase). During prophase the **microtubules** of the cytoskeleton disassemble into tubulin subunits that begin to reassemble, thus forming mitotic spindles.The **nucleoli** starts to disappear. The centrosome (including its two centrioles) duplicates just before the S phase on interphases. During prophases in animal cells, the two centrosomes separate and migrate towards the opposite poles of the cell.

Prometaphase- Beginning of prometaphase is marked by the fragmentation or breakdown of the nuclear membrane. Chromosomes will eventually be found at opposite poles of the cell **(spindle poles)**. **Kinetochore** (protein complex) forms on the centromere of each chromatid. The kinetochore of one sister chromatid attaches to microtubules from one pole (polar microtubules) while the kinetochore of the other sister chromatid attaches to polar microtubules of the opposite pole. When chromosomes are slowly pulled toward opposite poles they slowly migrate toward the **metaphase (equatorial) plane,** which is an imaginary plane located midway between each pole of the cell.

Metaphase- Mitotic spindle microtubules are fully formed between the poles. The centromeres of all chromosomes are located at the metaphase plate. Cohesion complexes are cleaved by an enzyme called separate which releases the junctions between sister chromatids. The centromeres then start to separate.

Anaphase- Each chromatid (now called a **daughter chromosome)** moves to the opposite poles of the cell. The movement of chromatids towards the pole is driven by shortening of microtubules, which occurs by depolymerization at the level of kinetochores.

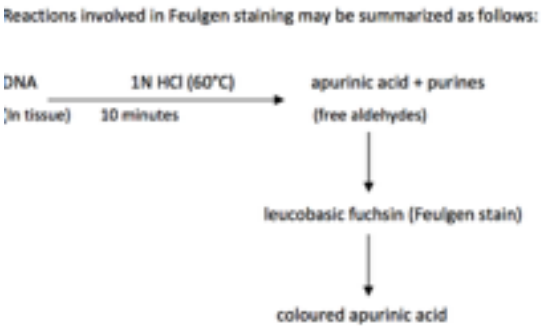


Telophase- Chromosomes now located at the poles decidedness and become longer and thinner. The nuclear membrane reappears and the nucleolus reforms. In plant cells, the **phragmoplast** guides the formation of the new cell wall across the centre of the cell, while in animal cells a **cleavage furrow** pinches the cell in two.

*THESE GROWTH CENTRES OCCUR PRINCIPALLY IN ROOT TIPS AND IN STEM TIPS **(APICAL MERISTEMS)***

-To observe chromosomes a number of stains may be used such as **Feulgen stain.**

How does the Feulgen stain work?



DNA contains deoxyribose sugar molecules chemically linked through aldehyde groups to purine and pyrimidine bases. To form free aldehyde groups in DNA so that it will react with the Feulgen stain. The DNA is hydrolyzed with hot (60 degree) 1N HCl , that removes the purine bases and frees the aldehyde groups of the deoxyribose sugar. The resulting product is called apurnic acid.

The acid hydrolysis serves to remove the RNA from the tissue so that it cannot react, and it softens the tissue. The timing of the acid hydrolysis is very important (10min): a short hydrolysis will not free all aldehyde groups while over hydrolysis will cause the destruction of the apurnic acid. Both of these cases lead to reduced staining.

<u>Root growth</u>

- Many cells in the root tip undergoing mitosis

Root cap- End of root tip, a mass of irregular dead cells with thicker walls. This protects the apical meristem of the root tip as it is pushed into the soil. It is the site that detects gravity and controls the direction of root growth.

- Right above the root cap is the **region of apical meristem**. It is the zone of cell division or the site of apical growth of the root. At the base of the apical meristem is the **quiescent centre**, it is a relatively inactive region where the cells are arrested in G1 or interphase.

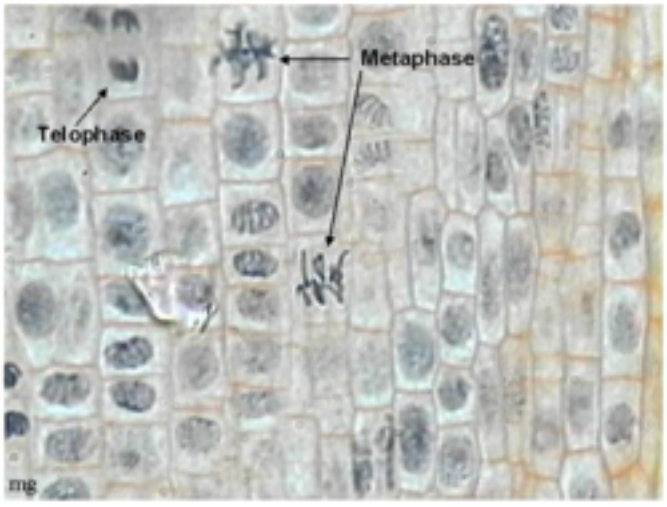
-Above is the **region of elongation**. The elongation of cells in this region results in most of the increase in length of the root.

- Above is the **region of maturation** in which most of the cells of the primary tissues mature. Root hairs are also produced in this region. The **xylem cells** of these bundles transport water and salts from the soil to the rest of the plant. The **phloem cells** transport carbohydrates from the photosynthetic portions of the plant to the roots.

Animal mitosis

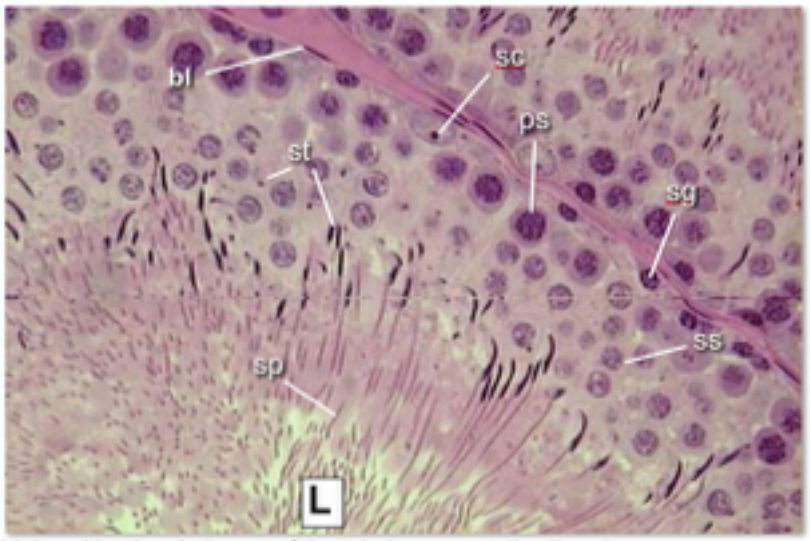
In animal cells there is an **aster,** a semicircle of fibrils around each end of the spindle. There are no **asters** in plant cells.

Onion (*Allium cepa*) root tip (40 min.)



Lab #5 - Meiosis

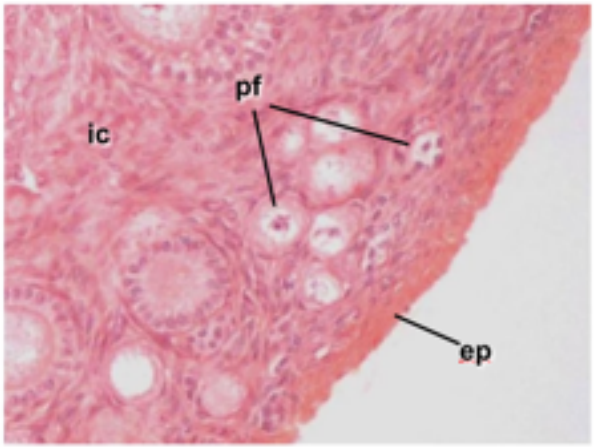
Meiosis assures: 1) that the chromosome number will be stable from generation to generation ,2) that each offspring resulting from sexual reproduction will receive two entire sets of genetic instructions, 3) that genetic diversity is promoted among the product



bl: basal lamina, L: lumen of the tubule, sc: sertoli cell nucleus, sg: spermatogonia, ps: primary spermatocyte, ss: secondary spermatocyte, sp: sperm cells (cross section), st: spermatids

Primordial follicle

Primordial follicle (**pf**) are located underneath the ovary epithelium (**ep**)
Surrounded by one layer of squamous (=flat) follicular cells
ic: interstitial cells



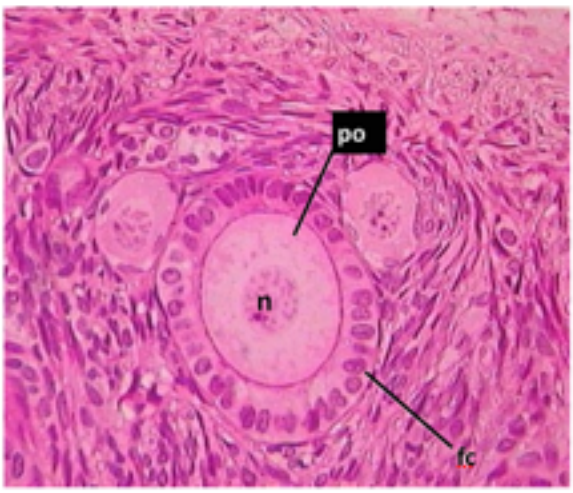
Primary unilaminar follicle

Located deeper in the ovary

Surrounded by one layer of cuboidal follicular cells (**fc**)

Contains a primary oocyte (**po**)

n: oocyte pronucleus



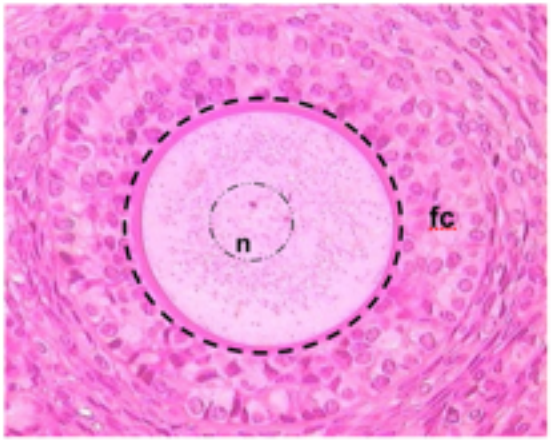
Growing follicle (primary multilaminar):

Slightly deeper in the ovary

Surrounded by several layers of follicular cells (**fc**)

Contain primary oocyte (dash lines).

n: oocyte pronucleus



Mature or Graafian Follicle

- Largest follicle
- Many layers of follicular cells (**fc**)
- One big or several fluid-filled cavities (**fca**)
- Contains secondary oocyte (**so**) stopped in metaphase II = mature oocyte
- Comes near to ovarian epithelium prior to ovulation