

LABORATORY 3 - THE CELL, continued - NUCLEUS

OBJECTIVES: **LIGHT MICROSCOPY:** Recognize interphase nucleus and distinguish nuclear membrane (envelope) heterochromatin, euchromatin and nucleolus. Know functions of each region. Nucleus during mitosis and cytokinesis - recognize different stages of mitosis and cytokinesis. Understand the process and significance of mitosis and cell division. Learn to recognize mitotic figures in sections of tissue. Study human karyotype.

ELECTRON MICROSCOPY: Recognize specific regions of interphase nucleus in electron micrographs and correlate them with LM structures and functions including nuclear envelope, heterochromatin, euchromatin and nucleolus.

ASSIGNMENT FOR TODAY'S LABORATORY

GLASS SLIDES

[SL 103](#) (liver) interphase nucleus

[SL 29](#) (whitefish embryo) mitosis

[SL 96](#) (Pharynx epithelium) mitosis in tissue section

[SL 160](#) (lymphocytes) human karyotype (Slide in even-numbered desks and some odd numbered desks.)

ELECTRON MICROGRAPHS (in envelope)

EMs 7-6, 8-5, 1-8, 7-1, 8-1, 1-11, 7-3 to 5 interphase nucleus

POSTED MICROGRAPHS

1B Human karyotype

1C Mitotic figures

[Lab 3 Posted EMs](#)

HISTOLOGY IMAGE REVIEW - available on computers in HSL

Chapter 3, Cytology, Nucleus

Frames: 127-140

SUPPLEMENTARY ELECTRON MICROGRAPHS

Rhodin, J. A.G., [An Atlas of Histology](#)

Copies of this text are on reserve in the HSL.

Nucleus pp. 26-32

NUCLEUS

1. INTERPHASE NUCLEUS

- A. Light Microscope (J. Fig. 3-1; R. Plate 66) - [SL 103](#) - (Liver) Observe the numerous liver cell nuclei (circular areas darkly-stained with hematoxylin) ([med](#), [oil](#)). Most of the cells have a single nucleus. Try to find a cell with two nuclei. Identify the boundary of a nucleus (a dark line). Remember that the nuclear membrane is actually the nuclear envelope consisting of two adjacent membranes that are penetrated by many nuclear pores. The irregularly shaped basophilic masses within the nuclei are heterochromatin. Unstained regions are euchromatin (J. Fig. 3-1). What is the functional difference? As you study the nuclei of several cells, you will find that many nuclei contain a small, distinct, round body in addition to the dispersed chromatin. This structure is the nucleolus, ([red arrows](#)). What is its composition?
- B. Electron Microscope - (J. Figs. 3-2, 3-5, 3-6, 3-13; R. Figs. 3.1, 3.4, 3.5, 3.6, 3.7). Observe the nucleus and nuclear envelope in [EM 7-6](#) and nuclear pores in [EM 8-5](#). Note the arrangement of chromatin into heterochromatin [EM 1-8](#), [7-1](#), [8-1](#), and euchromatin [1-11](#), [7-2](#), [8-2](#). The nucleolus (J. Fig. 3-12; R. Fig. 3.4) is demonstrated in [EM 1-9](#), [3-2](#). Further detail is shown in [EM 7-3](#) (perinucleolar chromatin), [7-4](#) (fibrillar portion) and [7-5](#) (granular portion). These components are also seen in [EM 8-3](#), [8-4](#), [8-6](#). What is the function of the nucleus and nucleolus? What is the significance of a cell with abundant heterochromatin in the nucleus? What is the significance of prominent or multiple nucleoli?

2. MITOSIS (J. Figs. 3-14 to 3-17; R. Figs. 3.12 to 3.16) [SL 29](#). First scan the slide under low power. You will find several sections of a whitefish embryo. The individual cells are large and clearly delineated. Many of the cells are in interphase; each of these cells displays a well-defined, darkly staining nucleus. If you examine the sections carefully, you will be able to identify cells in various stages of mitotic division. These will be examined in more detail with the 40X objective and then with the 100X oil immersion objective. Many of these cells also contain some yolk material that is eosinophilic and appears as large round droplets. Switch to 40X objective and focus. Scan the sections for the following:

- (a) Interphase nuclei: Note that the nuclear material (chromatin) is bounded by a nuclear membrane (envelope).
- (b) Prophase (Prophase): This stage is somewhat difficult to discern. The nuclear membrane (envelope) is becoming disrupted and less distinct in late prophase and may no longer be apparent. The chromatin is undergoing reorganization and should contain multiple fibrillar strands, which will condense further to become identifiable chromosomes.
- (c) Metaphase (Metaphase): In metaphase there is no evidence at all of a nuclear membrane. The chromosomes are condensed and centrally aligned. The mitotic spindle is clearly evident. The microtubules of the spindle converge at a site that contains a pair of centrioles. The centrioles are not visible at this magnification. This region is called the Microtubule Organizing Center (MTOC) or the centrosome. The microtubules in this region are 1) astral microtubules that radiate out from the centrosome and participate in the orientation of the mitotic spindle; 2) mitotic spindle microtubules that extend across the spindle and 3) microtubules that are attached to the chromosomes. Remember that you will encounter many different orientations, some metaphase cells will be viewed from above, others from the side.

- (d) [Anaphase \(anaphase\)](#): At this stage the chromosomes are separating towards opposite poles. Again, there is no nuclear membrane. Mitotic spindles and asters (formed of astral microtubules) are clearly apparent.
- (e) [Telophase \(early, late, telophase\)](#): The chromosomes have now completely separated and a new cell membrane should have appeared between the sets of chromosomes to define the limits of the two daughter cells. The chromosomes become more compact, may appear to fuse with one another, and are no longer clearly discernable from one another. Formation of a new nuclear membrane should follow shortly. Sometimes remnants of the mitotic spindle are visible at this stage.
- (f) [SL 96](#) - (Pharynx) [Mitosis in tissue sections](#). In the multiple layers of cells on the surface note mitotic figures as they appear in routine H & E sections (J. Fig. 3-17). Note: Only anaphase, telophase and metaphase stages can be determined with certainty ([low](#), [med](#), [high](#), [red circles](#)).

3. **KARYOTYPE SL 160** - (MAY BE ABSENT FROM SOME DESKS) Human Chromosomes - (J. Fig. 3-11; W. Fig. F3.1.1). Karyotype preparation ([high](#), [oil](#)). This karyotype was obtained by isolating lymphocytes from blood and stimulating them to undergo mitosis by exposure to phytohemagglutinin. Colchicine was added to the preparation to prevent formation of the mitotic spindle and arrest mitosis at metaphase. Only a few cells were in mitosis when the preparation was made, therefore you will have to look through the slide carefully to find a karyotype that is complete.

OBJECTIVES FOR LABORATORY 3: THE CELL III - NUCLEUS

1. Using the light microscope or digital slides, identify:

- Nuclear membrane

- Heterochromatin

- Euchromatin

- Nucleolus

- Stages of the cell cycle (specific stages best seen whitefish embryo)

 - Interphase

 - Prophase

 - Metaphase

 - Anaphase

 - Telophase

- Mitotic spindle

- Interphase nuclei (in tissues)

- Mitotic figures (in tissues it is sometimes difficult to distinguish specific stages of mitosis)

- Karyotype

2. On electron micrographs, identify:

- Nuclear membrane

- Nuclear pore

- Heterochromatin

- Euchromatin

- Nucleolus

 - Perinucleolar chromatin

 - Fibrillar portion

 - Granular portion

REVIEW QUESTIONS ON THE MICROSCOPE AND THE CELL

1. How does the microscope lens system alter the orientation of the observed image compared to observing the same structure with the naked eye?
2. What is the effect of increasing magnification (by changing objectives) on:
 - a. the depth of focus?
 - b. the amount of tissue visible in one field?
3. Define "power of resolution" (not mathematically).
4. Why aren't the following cell components visible with the light microscope using routine H and E preparations?
 - a. neutral fat
 - b. microtubules
 - c. ribosomes
5. The presence of which organelle or inclusion would characterize a cell forming
 - a. cytosolic proteins
 - b. glycoproteins
 - c. steroids
 - d. stored glucose
6. In the slides you observed ([SL 2](#) and [SL 7](#)), what methods were used to specifically identify glycogen and RNA within a section of tissue?
7. Which of the following intracellular components are composed of unit membrane?

Golgi apparatus	smooth endoplasmic reticulum	nucleolus
nucleus	glycogen	mitochondria
lysosomes	secretory vesicles	microtubules
8. When observed in the electron microscope, most cellular membranes appear rather similar to each other. What are some of the basic ways in which they differ?
9. What are some ways of visualizing cells other than bright field light microscopy and thin section transmission electron microscopy?

10. In your reading you will encounter numerous references to the dimensions or size of various cells or cellular components. As an aid, the following table lists the currently accepted nomenclature. Note the term, micron, has been replaced by micrometer and Angstrom is usually replaced by 0.1 nanometer.

1 Kilometer (km)	= 10^3 Meters (m)
1 Centimeter (cm)	= 10^{-2} "
1 Millimeter (mm)	= 10^{-3} "
1 Micrometer (μm) = 1 Micron* (μ)	= 10^{-6} "
1 Nanometer (nm) = 1 Millimicron* ($\text{m}\mu$)	= 10^{-9} "
1 Angstrom* (A) = 0.1 Nanometer	= 10^{-10} "
1 Picometer (pm)	= 10^{-12} "

* older terminology