CYTOPLASMIC ORGANELLES

OBJECTIVES:

After completing this exercise, you should be able to:

1. Recognize features of the cytoplasm in the light microscope.
2. Identify major cellular organelles in electron micrographs.
3. Provide general functions of cellular organelles.

ASSIGNMENT FOR TODAY'S LABORATORY

GLASS SLIDES - https://medmicroscope.uc.edu/

SL 181 (spinal cord) rough endoplasmic reticulum
SL 108 (pancreas) rough endoplasmic reticulum
SL 125 (inflammation) rough endoplasmic reticulum and Golgi apparatus

ELECTRON MICROGRAPHS (Gray envelope)

EM 3-6, 4-5, 12-1, 16 plasma membrane
EM 1-3, 2-1, 2-2, 3-5, 4-1, 10-5, 14-3 rough endoplasmic reticulum
EM 4-2, 10-6, 12-3, 13-7, 14-5 smooth endoplasmic reticulum
EM 5-2 and 5-inset ribosomes
EM 11-1 to 11-4 Golgi apparatus
EM 6-10 to 6-13, 2-3 to 2-5 also 3-4, 1-4, 12-2 and 13-6 mitochondria
EM 3, 4, 16 and 17 Magnification and Resolution

POSTED ELECTRON MICROGRAPHS

1 Organelles
6 Organelles
Lab 2 Posted EMs

SUPPLEMENTAL MATERIAL:

SUPPLEMENTARY ELECTRON MICROGRAPHS

Rhodin, J. A.G., An Atlas of Histology

Plasma membrane	Fig. 2-2; 2-3
Rough ER	Fig 2-26; 2-29; 2-30; 2-31; 2-32
Smooth ER	Fig 2-33; 2-34; 2-35
Ribosomes	Fig 2-27; 2-28; 2-30; 2-31; 2-32
Golgi apparatus	Fig 2-36; 2-37; 2-38
Mitochondria	Fig 2-39; 2-40; 2-41

In the last lab, you were introduced to cells and extracellular matrix, followed by a focus on the nucleus. Today, we will examine the plasma membrane and the major organelles within the cytoplasm (endoplasmic reticulum, Golgi apparatus, mitochondria). The next laboratory will focus on the remainder of the cytoplasmic organelles (e.g. secretory vesicles, lysosomes).

PLASMALEMMA OR PLASMA MEMBRANE - The plasma membrane forms the outer boundary of cells and consists of a trilaminar, unit membrane. Prior to considering other organelles by electron microscopy you should become familiar with the appearance of the plasma membrane.

Electron Microscope - Study diagrammatic representation in text and atlas (J. Figs. 2-1 to 2-8; R. Figs. 2.2 to 2.11). Observe the plasma membrane in EM 3-6, 4-5, 12-1. Note that cells have many membranes; the outermost membrane is the cell membrane or plasma membrane (or plasmalemma). In EM 16, locate the plasmalemma and note its trilaminar (three-layered) appearance (J. Fig. 2-1; R. Fig. 21.2). Remember that *when sufficiently magnified,* all plasma membranes and all membranes in the cell appear trilaminar since they have two dark laminae separated by a central light lamina.

RIBOSOMES

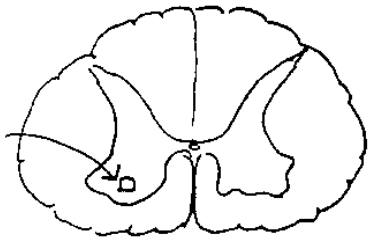
Electron Microscope (J. Fig. 2-9, 2-10a,b; R. Fig. 2.30). Single ribosomes or multiple ribosomes linked together by mRNA to form polysomes (polyribosomes) may be found either free in the cytoplasm or associated with a membrane system, the rough endoplasmic reticulum (see below). EM 5-2 shows cytoplasm that contains ribosomes and polyribosomes. Compare the size of ribosomes to other cell constituents. In EM 5 (inset) observe that polysomes are formed of evenly spaced ribosomes. These ribosomes are linked together by a strand of messenger RNA, which is not visible in the micrograph. What is the function of polysomes?

ENDOPLASMIC RETICULUM. Endoplasmic reticulum is a membranous organelle that is formed of flattened cisternae or irregular tubules. A variety of enzymes may be associated with the ER. There are two distinct types of ER, which are often interconnected: rough ER (RER), which has associated ribosomes, and smooth ER (SER), which lacks ribosomes and in which the organization of membranes is different.

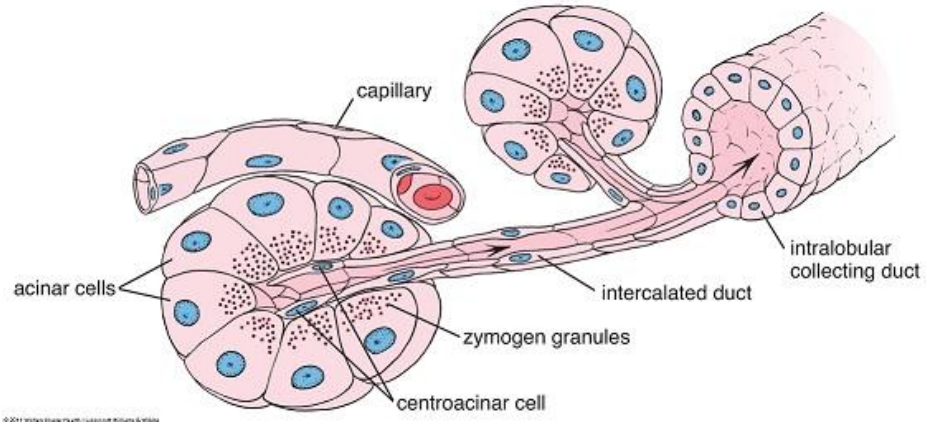
1. ROUGH ENDOPLASMIC RETICULUM

A. Electron Microscope - (J. Figs. 2-10; R. Fig. 2.25, 2.26, 2.30). Observe RER in electron micrographs. EM 1-3, low magnification 2-1 (cisternae), 2-2 (ribosome). Also note in EM 3-5, 4-1, 10-5, and 14-3. What is the function of RER?

B. Light Microscope (SL 181) (scan, low) - Rough surfaced endoplasmic reticulum cannot be seen, as such, with light microscopy. But in cells in which it is abundant its presence can be detected and its intracellular distribution determined by staining for the structural RNA (rRNA) associated with the ribosomes that line the surface of the RER. In these cases it is known as cytoplasmic basophilia. In neurons, extensive RER appears as irregularly shaped masses, which are referred to as Nissl substance (red arrows) (J. Fig. 9-3; R. Plate 31), because they stain robustly with a stain called Nissl stain. See also neurons (SL 181) in the spinal cord, located in the region shown in the rectangle on the left side of the diagram below (a similar region is shown here enclosed by red line).



As mentioned in the previous laboratory, routine H&E-stained slides of tissues with abundant RNA in the cytoplasm also exhibit cytoplasmic basophilia. For example, SL 108 is a section of pancreas stained with H&E. For orientation, examine this slide at low, medium, and high power, and then switch to oil. The cells of the exocrine pancreas are organized into acini (see diagram below), which are circular clusters of 10-15 cells (green outline is an acinus, yellow outline is a single cell). The cells in the acinus surround a space, the lumen (Xs in the labeled image), into which the cells release their digestive enzymes. Note that due to tissue shrinkage, most lumens are not visible on the slide. The side of each cell facing the lumen is the apical side of the cell (pointed region of the yellow outline), and the side of the cell furthest from the lumen is the basal side. (FYI, more details about acini, apical, basal are forthcoming in later sessions.)



Cells in the exocrine portion of the pancreas secrete an enormous amount of digestive enzymes, which are synthesized on the rough endoplasmic reticulum localized to the basal aspects of these cells (see EM 3, the apical aspect of this cell is the upper right). The extensive rough endoplasmic reticulum results in intense cytoplasmic basophilia in the basal aspect of the cell (oil).

2. SMOOTH ENDOPLASMIC RETICULUM

A. Electron Microscope (J. Fig. 20-13; R. Fig. 2.31). Observe series of membranes of SER. EM 12-3, 10-6, 13-7, and 14-5. Note in EM 4-2 the continuity of SER membranes with those of RER. Functions?

B. Light Microscope – Smooth endoplasmic reticulum is not specifically distinguishable by light microscopy.

GOLGI APPARATUS

A. Electron Microscope - (J. Fig. 2-13, 2-14; R. Fig. 2.33). Identify the Golgi apparatus in EM 11; note 11-1, transport vesicles; 11-2 flattened cisternae of the cis (forming) face; 11-3 dilated cisternae of the trans (maturing) face; and 11-4 secretory vesicles. Function?

B. Light Microscope (SL 125) (skin inflammation) – Because rough endoplasmic reticulum and the Golgi apparatus work together in protein synthesis and modification, cells that have a well-developed Golgi apparatus also have abundant rough endoplasmic reticulum. As noted above, the abundant rough ER exhibits cytoplasmic basophilia. Here, note that since the Golgi apparatus does not have ribosomes, the region of the Golgi in a cell will be devoid of cytoplasmic basophilia. Such a pale area surrounded by intense cytoplasmic basophilia is referred to as a Golgi ghost. The upper left region of SL 125 is an area of inflammation that has been infiltrated by numerous white blood cells. Many of the cells in this region have intense cytoplasmic basophilia; these are antibody-secreting plasma cells (plasma cells unlabeled, plasma cells indicated by arrows). If you look very carefully, the center of the cell is slightly paler than the surrounding cytoplasm; this is the Golgi ghost (best seen in plasma cell in the center of the image).

MITOCHONDRIA

Electron Microscope - (J. Fig. 2-19 and 2-20; R. Fig. 2.37). Note the detailed structure of a mitochondrion EM 6-10 (matrix), 6-11 (cristae), 6-12 (outer mitochondrial membrane) and 6-13 (granules); 2-3 (mitochondrial cristae), 2-4 (outer mitochondrial membrane), and 2-5 (inner mitochondrial membrane). Also note mitochondria in EM 1-4, 3-4, 12-2, and 13-6. Review functions of mitochondria and the locations of these functions.

RELATIONSHIP OF MAGNIFICATION AND RESOLUTION IN ELECTRON MICROGRAPHS

The relationship of magnification and resolution in the evaluation of electron micrographs (EM) # 17, 3, 4, 16 (in gray envelope).

EM 17

Notice that the image includes about four "whole" liver cells and parts of adjacent cells. Even if there were no scale bar on the micrograph, it is evident that this is a low magnification image. Cell boundaries are visible because there is a slight separation (extracellular space) between the cells, but, because the magnification is low, a definitive plasma membrane is difficult to see in this image, and intracellular details such as ribosomes and ER are hard to discern. The micrometer marker indicates the magnification in the print is about 5,500 X.

EM 3

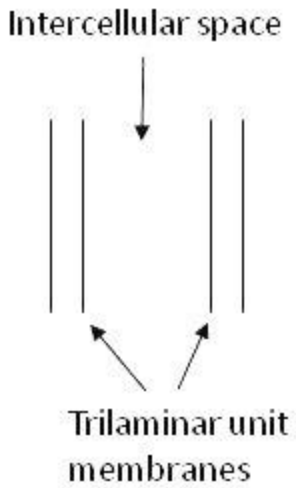
This micrograph is an image of pancreatic acinar cells; one "whole" cell and portions of two adjacent cells are included in the field. If no scale bar were on the micrograph, it would be reasonable to conclude that this image is a higher magnification than #17 because fewer cells are in the field, and intracellular structures are more easily seen in #3. Notice that the item marked 6 and identified, as plasmalemma in the micrograph appears to be a single line, but this single line is actually two closely apposed adjacent plasma membranes that cannot be resolved at this magnification. The cell's basal surface is readily detected because of the presence of a basal lamina (7). The scale bar indicates magnification of the print is about 10,000 X.

EM 4

The identification of this portion of a cell as an intestinal absorptive cell is based on the knowledge of the source of the image, since characteristics typical of an absorptive cell (apical surface with microvilli) are not included in the field. Many other cell types have the same organelles as shown here. Without the scale bar, it is apparent that this image was photographed at a higher magnification because only a small portion of a cell occupies most of the field, with only small portions of adjacent cells (upper left corner, lower right corner). The mitochondria are larger, their double membrane apparent, and ribosomes are easily identified. The parallel plasma membranes of adjacent cells, item 5, are clearly separated from each other by a small intercellular space. The cell membranes in the lower right corner "disappear" in the image in areas where they are not cut exactly transversely by the plane of section; the membranes in the upper left corner are more transversely cut, thus more distinct over a greater distance. The scale bar indicates that this image is magnified about 66,000 X.

EM 16

Even without the scale bar this image would be determined to be very high magnification because only a small portion of the apical surface of two adjacent cells is shown. No mitochondria are present and most other cell organelles are excluded from this limited apical region. The apical surface with microvilli is evident as well as the junctional complex that includes a zonula occludens (1), a zonula adherens (2), and macula adherens (desmosomes) (3). In ideal transverse planes of section through the plasma membranes, the trilaminar unit membrane is visible. The closely parallel plasma membranes can be viewed as two trilaminar membranes (dark-light-dark) on both sides of a narrow (wide at this magnification!) intercellular space. The micrometer marker on this image indicates the magnification is about 120,000 X.



Compare this image in EM 16 with the appearance of two parallel plasma membranes in EM 4 (also dark-light-dark) but not at high enough magnification to reveal the trilaminar unit membranes.

SPECIFIC OBJECTIVES FOR CYTOPLASMIC ORGANELLES

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

   Endoplasmic reticulum – when stained with Nissl substance in neurons, or as cytoplasmic basophilia in H&E sections
   Golgi apparatus – as a Golgi ghost in cells with intense cytoplasmic basophilia

2. On electron micrographs, identify:

   Plasma membrane
   Endoplasmic reticulum
      Smooth
      Rough
         Cisterna
         Ribosome
   Free ribosomes
      Polysomes / polyribosomes
   Golgi apparatus
      Transport vesicles
      Cis face
      Trans face
      Secretory vesicles
   Mitochondria
      Matrix
      Cristae
      Outer mitochondrial membrane
      Inner mitochondrial membrane
      Inter-membrane space