9 Visualizing, Fractionating, and Culturing Cells

Review the Concepts

1. The major organelles of the eukaryotic cell are the nucleus, the endoplasmic reticulum, the Golgi complex, mitochondria, chloroplasts (plants), endosomes, and lysosomes (animals) or vacuoles (plants). The nucleus encloses the cell's DNA, contains the machinery for RNA synthesis, and physically separates the process of RNA synthesis from that of protein synthesis. The endoplasmic reticulum is responsible for the synthesis of lipids, membrane proteins, and secreted proteins. The Golgi complex processes and sorts secreted and membrane proteins. Mitochondria are the principal sites of ATP synthesis in aerobic cells. Chloroplasts are the site of photosynthesis, which produces ATP and carbohydrates in plant cells. Endosomes take up soluble material from the extracellular environment and lysosomes (in animal cells) degrade much of the material internalized in endosomes for use in biosynthetic reactions. In plant cells, vacuoles function like lysosomes in that they degrade material, but they also serve as storage sites for water, ions, and nutrients, and generate the turgor pressure that drives plant cell elongation. The cytosol is the unstructured aqueous phase of the cytoplasm excluding organelles, membranes, and insoluble cytoskeletal components. Cellular metabolism, protein synthesis, and signal transduction, all take place in the cytosol.

2. Endocytosis occurs when a portion of the plasma membrane invaginates, forming a coated pit containing the protein or macromolecule. Numerous proteins, including clathrin, cover the cytosolic face of the pit and aid in its invagination and internalization. Eventually the pit pinches itself off into a membrane-bound vesicle, which is delivered to an early endosome where it gets sorted, either back to the plasma membrane, to the late endosome for further sorting, or to the lysosome where the contents are degraded. In the case of phagocytosis, large particles, including cells that have undergone apoptosis or bacteria, are enveloped by the plasma membrane and internalized for degradation in the lysosome.

3. The multiple membranes of mitochondria and chloroplasts act to create additional compartments with specialized functions within these organelles. The polarized stack of compartments that form the Golgi apparatus is associated with the assembly line organization of enzymes that modify many ER-derived products.

4. Specific types of cells in suspension may be isolated by a fluorescence-activated cell sorter (FACS) machine in which cells previously "tagged" with a fluorescent-labeled antibody are separated from cells not recognized by the antibody. The scientist selects an antibody specific for the cell type desired. Specific organelles are generally separated by centrifugation of lysed cells. A series of centrifugations of successive supernatant fractions at increasingly higher speeds and corresponding higher forces serves to separate cellular organelles from one another on the basis of size and mass (larger, heavier cell components pellet at lower speeds). This is often combined with density-gradient separations to purify specific organelles on the basis of their buoyant density.

5. Electron microscopy has better resolution than light microscopy, but many light-microscopy techniques allow observation and manipulation of living cells.

6. The total magnification of an image is described as the product of the magnification of the

individual lenses, where the objective lens magnification immediately above the specimen, is multiplied to that of the projection or eyepiece lens. Being able to clearly distinguish between two closely spaced points at even the highest total magnification is the ultimate goal because if the two objects are already blurred and cannot be discriminated at a lower magnification, simply increasing the magnification will have no effect. In fact, the formula defining the resolution (D) of a lens does not take magnification into account and is written as

$$D = \frac{0.61\,\lambda}{N \sin a}$$

where λ is the wavelength of light used to illuminate the specimen, N is the refractive index of the medium (usually air) between the front face of the objective lens and the specimen, and a is half-angle of the cone of light entering the face of the objective lens. N sin a is often referred to as the lens' numerical aperture, which is physically stamped on the barrel of the objective lens. Since only three of the values can be altered, to achieve the best resolution (the smallest D possible), one has to either decrease the wavelength of light or increase the numerical aperture by gathering more light into the front face of the objective lens. In most circumstances, therefore, the limitations include the use of wavelengths in the visible spectrum and the ability to gather more light to increase the numerical aperture. Increasing the numerical aperture is accomplished by placing a drop of oil or water, which have greater refractive indices (1.5 and 1.3, respectively), relative to that of air (1), between the specimen and the objective lens.

7. Chemical stains are required for visualizing cells and tissues with the basic light microscope because most cellular material does not absorb visible light and therefore cells are essentially invisible in a light microscope. The chemical stains that may be used to absorb light and thereby generate a visible image usually bind to a certain class of molecules rather than a specific molecule within that class. For example, certain stains may reveal where proteins are in a cell but not where a specific protein is located. This limitation can be overcome by fluorescence microscopy, in which a fluorescent molecule may be either directly or indirectly attached to a molecule of interest which is then viewed by an appropriately equipped microscope. Only light emitted by the sample will form an image, so the location of the fluorescence indicates the location of the molecule of interest. Confocal scanning microscopy and deconvolution microscopy build on the ability of fluorescence microscopy by using either optical (confocal scanning) or computational (deconvolution) techniques to remove out-of-focus fluorescence and thereby produce much sharper images. As a result, these techniques facilitate optical sectioning of thick specimens as opposed to physical sectioning and associated techniques that may alter the specimen.

8. Certain electron microscopy methods rely on the use of metal to coat the specimen. The metal coating acts as a replica of the specimen, and the replica rather than the specimen itself is viewed in the electron microscope. Methods that use this approach include metal shadowing, freeze fracturing and freeze etching. Metal shadowing allows visualization of viruses, cytoskeletal fibers, and even individual proteins, while freeze fracturing and freeze etching allow visualization of membrane leaflets and internal cellular structures.

9. A cell strain is a lineage of cells originating from a primary culture taken from an organism. Since these cells are not transformed, they have a limited lifespan in culture. In contrast, a cell line is made of transformed cells and therefore these cells can divide indefinitely in culture. Such cells are said to be immortal. A clone results when a single cell is cultured and gives rise to genetically identical progeny cells.

10. These cultured cells allow one to conduct experiments to determine the roles of individual genes and proteins in the function of fat and muscle cells, for instance the metabolism of

lipids in adipocytes and the contraction of muscle.

11. Normal B lymphocyte cells can produce a single type of antibody molecule. However, such cells have a finite lifespan in culture. Researchers use cell fusion of B lymphocytes and immortalized myeloma cells to create immortalized, antibody-secreting cells. Such cells, called hybridoma cells, retain characteristics of both parent cells, allowing for production of a single type, or monoclonal, antibody.

Analyze the Data

a.

Organelle	Marker molecule	Enriched fraction (no.)
Lysosomes	Acid phosphatase	11
Peroxisomes	Catalase	7
Mitochondria	Cytochrome oxidase	3
Plasma membrane	Amino acid permease	15
Rough endoplasmic reticulum	Ribosomal RNA	5
Smooth endoplasmic reticulum	Cytidylyl transferase	12

b. The rough endoplasmic reticulum is more dense than the smooth endoplasmic reticulum, since it is found in a gradient fraction with a higher sucrose concentration (more dense solution).

c. The plasma membrane represents the least dense fraction because it has been fragmented into small pieces that reseal to form small vesicle-like structures.

d. Addition of a detergent to the homogenate would eliminate the basis for equilibrium density-gradient centrifugation, as each organelle membrane would be solubilized by the detergent. Subjecting a detergent-treated homogenate to equilibrium density-gradient centrifugation would most likely produce a single peak at relatively low percent sucrose that would contain all marker molecules.