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CULTURING AND VISUALIZING CELLS

REVIEW THE CONCEPTS

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

2. 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 $D = 0.61\lambda N \sin a$, 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 the 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.

3. 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.

4. 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.

5. 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.

6. 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.

7. 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.

8. FACS (see Figure 9-2), whereby labeled cells pass through a laser light beam and the fluorescent intensity of light emitted is measured, allowing the computer to assign each cell with an electric charge proportional to the fluorescence. Fibroblasts having twice the amount of DNA (G2 phase) compared to the normal diploid cells will emit more fluorescence and therefore have a different electric charge, which allows them to be separated and collected.