

## Cell Biology Techniques: Immunocytochemistry

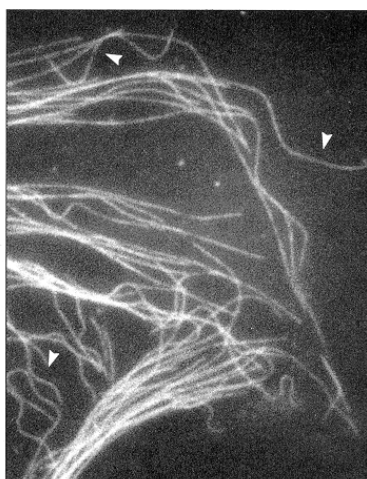
Source: Alberts, B. *et al.* 1994. *Molecular Biology of the Cell*. Garland Publishing, New York, pages 186-188.

### Antibodies Can Be Used to Detect and Isolate Specific Molecules

Antibodies are proteins produced by the vertebrate immune system as a defense against infection (see Chapter 23). They are unique among proteins because they are made in billions of different forms, each with a different binding site that recognizes a specific target molecule (or *antigen*). The precise antigen specificity of antibodies makes them powerful tools for the cell biologist. Labeled with fluorescent dyes, they are invaluable for locating specific molecules in cells by fluorescence microscopy (Figure 4-62); labeled with electron-dense particles such as colloidal gold spheres, they are used for similar purposes in the electron microscope (Figure 4-63). As biochemical tools, they are used to detect and quantify molecules in cell extracts and to identify specific proteins after they have been fractionated by electrophoresis in polyacrylamide gels (see Figure 4-46). When coupled to an inert matrix to produce an affinity column, antibodies can be used either to purify a specific molecule from a crude cell extract or, if the molecule is on the cell surface, to pick out specific types of living cells from a heterogeneous population.



(A)



(B)

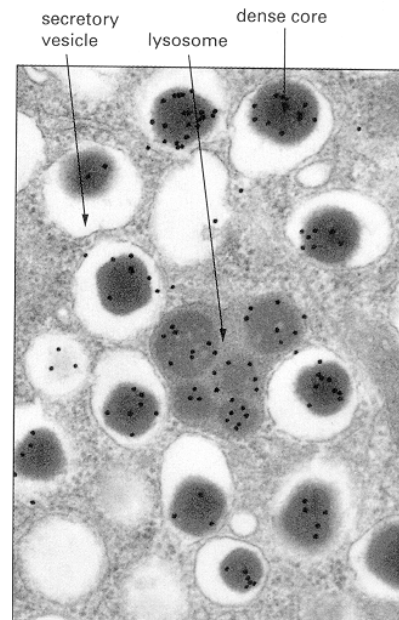
10  $\mu$ m

Figure 4-62. Immunofluorescence. (A) An electron micrograph of the periphery of a cultured epithelial cell showing the distribution of microtubules and other filaments. (B) The same area stained with fluorescent antibodies to tubulin, the protein subunit of microtubules, using the technique of indirect immunocytochemistry (see Figure 4-64). Arrows indicate individual microtubules that are readily recognizable in the two figures. (From M. Osborn, R. Webster, and K. Weber, *J. Cell Biol.* 77:R27-R34, 1978, by copyright permission of the Rockefeller University Press.)

The sensitivity of antibodies as probes for detecting and assaying specific molecules in cells and tissues is frequently enhanced by a signal-amplification method. For example, although a marker molecule such as a fluorescent dye can be linked directly to an antibody used for specific recognition (the *primary* antibody) a stronger signal is achieved by using an unlabeled primary antibody and then detecting it with a group of labeled *secondary antibodies* that bind to it (Figure 4-64).

The most sensitive and versatile amplification

Figure 4-63. Immunogold electron microscopy. Electron micrographs of an insulin-secreting cell in which the insulin molecules have been labeled with anti-insulin antibodies bound to tiny colloidal gold spheres (each seen as a black dot). Most of the insulin is stored in the dense cores of secretory vesicles; in addition, some cores are being degraded in lysosomes. (From L. Orci, *Diabetologia* 28:528-546, 1985.)

1  $\mu$ m

methods use an enzyme as a *marker* molecule attached to the secondary antibody. The enzyme alkaline phosphatase, for example, in the presence of appropriate chemicals, produces inorganic phosphate and leads to the local formation of a colored precipitate. This reveals the location of the secondary antibody that is coupled to the enzyme and hence the location of the antibody-antigen complex to which the secondary antibody is bound. Since each enzyme molecule acts catalytically to generate thousands of molecules of product, even tiny amounts of antigen can be detected. Enzyme-linked immunosorbent assays (ELISA) based on this principle are frequently used in medicine as a sensitive test for pregnancy or for various types of infections, for example.

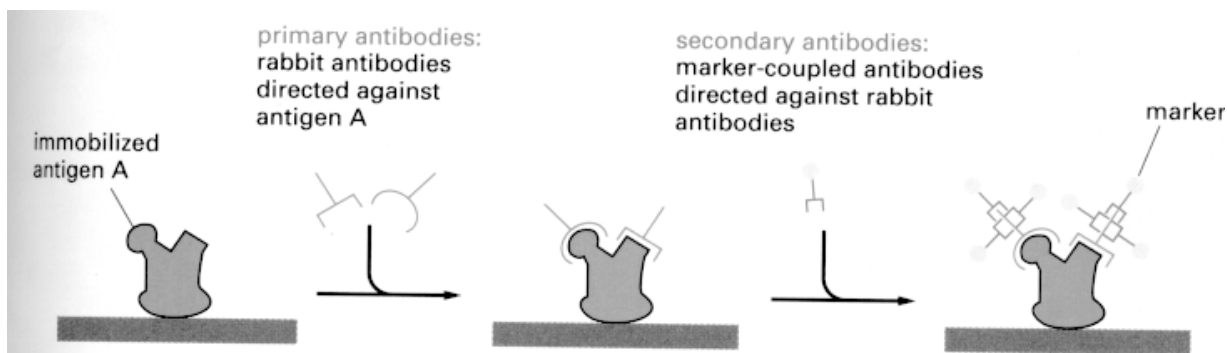


Figure 4-64. Indirect immunocytochemistry. The method is very sensitive because the primary antibody is itself recognized by many molecules of the secondary antibody. The secondary antibody is covalently coupled to a marker molecule that makes it readily detectable. Commonly used marker molecules include fluorescein or rhodamine dyes (for fluorescence microscopy), the enzyme horseradish peroxidase (for either conventional light microscopy or electron microscopy), the iron-containing protein ferritin or colloidal gold spheres (for electron microscopy), and the enzymes alkaline phosphatase or peroxidase (for biochemical detection).

Antibodies are made most simply by injecting a sample of the antigen several times into an animal such as a rabbit or a goat and then collecting the antibody-rich serum. This *antiserum* contains a heterogeneous mixture of antibodies, each produced by a different antibody-secreting cell (a B lymphocyte). The different antibodies recognize various parts of the antigen molecule as well as impurities in the antigen preparation. The specificity of an antiserum for a particular antigen sometimes can be sharpened by removing the unwanted antibody molecules that bind to other molecules; an antiserum produced against protein X, for example, can be passed through an affinity column of antigens Y and Z to remove any contaminating anti-Y and anti-Z antibodies. Even so, the heterogeneity of such antisera sometimes limits their usefulness.

### Hybridoma Cell Lines Provide a Permanent Source of Monoclonal Antibodies

In 1976 the problem of antiserum heterogeneity was overcome by the development of a technique that revolutionized the use of antibodies as tools in cell biology. The principle is to propagate a clone of cells from a single antibody-secreting B lymphocyte so that a homogeneous preparation of antibodies can be obtained in large quantities. The practical problem, however, is that B lymphocytes normally have a limited life-span in culture. To overcome this limitation, individual antibody-producing B lymphocytes from an immunized mouse or rat "re fused" with cells derived from an "immortal" B lymphocyte tumor. From the resulting heterogeneous mixture of hybrid cells, those hybrids that have both the ability to make a particular antibody and the ability to multiply indefinitely in culture are selected. These hybridomas are propagated as individual clones, each of which provides a permanent and stable source of a single type of monoclonal antibody (Figure 4-65). This antibody will recognize a single type of antigenic site—for example, a particular cluster of five or six amino acid side chains on the surface of a protein. Their uniform specificity makes monoclonal antibodies much more useful for most purposes than

conventional antisera, which usually contain a mixture of antibodies that recognize a variety of different antigenic sites, even a small macromolecule.

But the most important advantage of the hybridoma technique is that monoclonal antibodies can be made against molecules that constitute only a minor component of a complex mixture. In an ordinary antiserum made against such a mixture, the proportion of antibody molecules that recognize the minor component would be too small to be useful. But if the B lymphocytes that produce the various components of this antiserum are made into hybridomas, it becomes possible to screen individual hybridoma clones from the large mixture to select one that produces the desired type of monoclonal antibody and to propagate the selected hybridoma indefinitely so as to produce that antibody in unlimited quantities. In principle, therefore, a monoclonal antibody can be made against any protein in a biological sample. Once the antibody is made, it can be used as a specific probe-both to track down and localize the protein that induced its formation and to purify the protein in order to study its structure and function. Since fewer than 5% of the estimated 10,000 proteins in a typical mammalian cell have thus far been

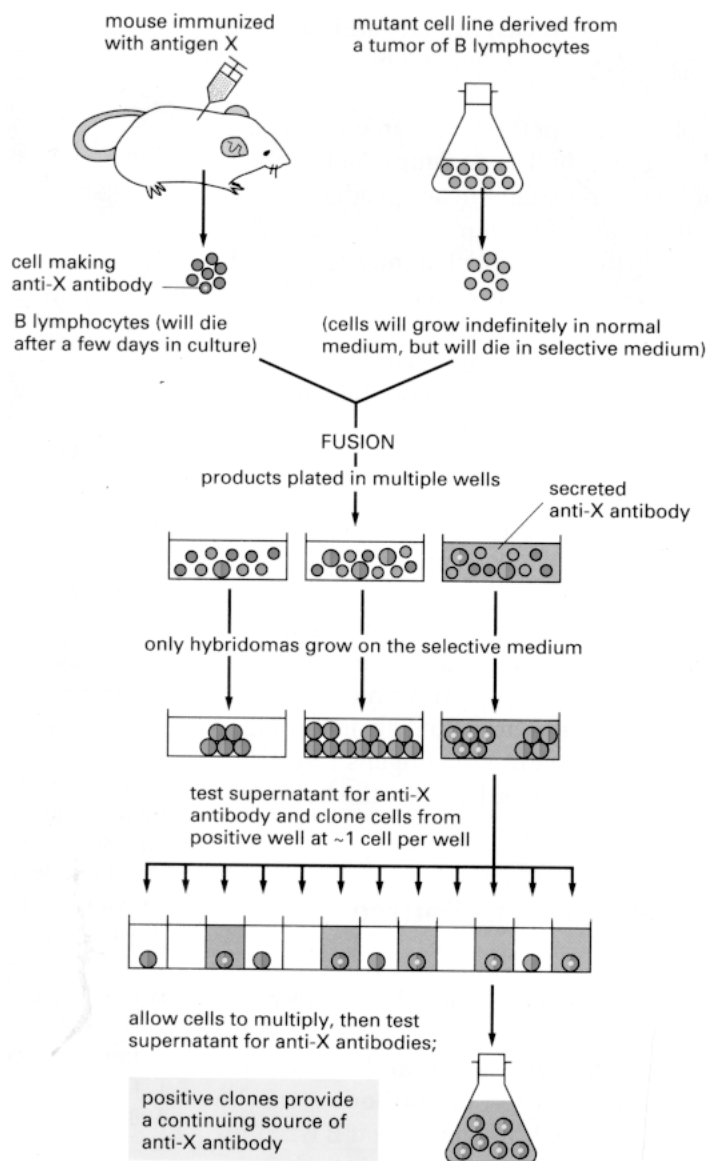


Figure 4-65. Preparation of hybridomas that secrete monoclonal antibodies against a particular antigen (X-). The selective growth medium used contains an inhibitor (aminopterin) that blocks the normal biosynthetic pathways by which nucleotides are made. The cells must therefore use a bypass pathway to synthesize their nucleic acids, and this pathway is defective in the mutant cell line to which the normal B lymphocytes are fused. Because neither cell type used for the initial fusion can grow on its own, only the hybrid cells survive.

isolated, many monoclonal antibodies made against impure protein mixtures in fractionated cell extracts identify new proteins. Using monoclonal antibodies and gene-cloning technology, it is no longer difficult to identify and characterize novel proteins and genes. The problem is to determine their function, and the most powerful way of doing this is often by the use of recombinant DNA technology, as we discuss in Chapter 7.

You will be provided with a simplified immunocytochemistry protocol in class.

**Optional Additional Readings** (available from your instructor or the non-virtual library of your choice):

1. Milstein, C. 1980. Monoclonal antibodies. *Sci. Am.* 24 3(4): 666-71.  
- a synopsis of the development of monoclonal antibodies by one of its Nobel Laureate inventors.
2. Lipsitz, R. (2000) Working knowledge. Pregnancy tests. *Sci. Am.* 283 (5): 110-111.  
- the application of monoclonal antibodies in home pregnancy tests
3. Ezzel, C. 2001. Magic bullets fly again. *Sci. Am.* 285 (4): 34-41.  
- the use of monoclonal antibodies as therapeutic agents and new methods of production

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### **Obituary of Dr. César Milstein (1927 - 2002)**

Source: New York Times Service (2002)

Dr. César Milstein, who shared the 1984 Nobel Prize in Medicine for a revolutionary technique to produce antibodies that latch onto specific proteins, has died in Cambridge, England. He was 74.

In 1975, Milstein and Dr. Georges Koehler invented a method of forcing immune system cells to produce one particular type of antibody.

Usually, the body's defense system generates a profusion of different antibodies to kill invading bacteria and viruses.

Milstein and Koehler's research on pure antibodies, known as monoclonal antibodies, provided an important cornerstone in molecular biology research.

Born in Bahia Blanca, Argentina, César Milstein was educated at the University of Buenos Aires and received his doctorate from Cambridge in 1960. He worked at the National Institute of Microbiology in Buenos Aires from 1961 to 1963 until political turmoil led him to resign and return to Cambridge, where he spent the rest of his career.

Milstein is survived by his wife, Celia.