chapter 3
The Technologies

"We must, as far as **we** can, isolate physiological occurrences outside the organism by means of experimental procedures. This isolation allows us to see and understand better the deepest associations of the phenomenon, so that their vital role maybe followed later in the organism. "

—CIaude Bernard
1813-1878

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The Technologies

Progress in the scientific techniques of biotechnology clearly has affected society on many levels–medical, social, economic, legal, and ethical. Most of the technologies used to transform undeveloped human tissues and cells mentioned in this report can be categorized into three broad areas: tissue and cell culture technology, hybridoma technology, and recombinant DNA technology. Advances in these technologies have increased our capability to identify and produce important human therapeutic agents. These fundamental scientific techniques are having profound, practical impacts on our society. Thus, it is important to understand the nature of the basic

techniques and how they can be used to manipulate tissues and cells into useful products in order to appreciate the novel legal, economic, and ethical issues raised in this report.

The following brief review outlines the principal tenets of the three main techniques; the large-scale commercial applications of these technologies are discussed in another OTA report (23). While each technology is reviewed individually, keep in mind that it is the marriage of technologies that is the norm—no single technology is the central element in the development or commercialization of human biological material.

TISSUE AND CELL CULTURE TECHNOLOGY

Cells are the basic unit of all living organisms. They are the smallest components of plants and animals that are capable of carrying on all essential life processes, A single cell is a complex collection of molecules with many different activities all integrated to forma functional, self-assembling, self-regulating entity. Higher organisms and plants are multicellular, with certain cells performing specialized (i.e., differentiated) functions,

There are two broad classes of cells: prokaryotic and eukaryotic. The classes are basically defined by the manner in which the genetic material is housed. Prokaryotes, generally considered the simpler of the two classes, include bacteria. Their genetic material is not housed in a separate structure (called a nucleus), and the majority of prokaryotic organisms are unicellular. Eukaryotes, on the other hand, are usually multicellular organisms. They contain their genetic material within a nucleus, and have other specialized structures within their cell confines to coordinate different cellular functions. The genetic material of eukaryotic organisms is a structure called a chromosome—a DNA and protein complex that is usually visible to the eve with standard light microscopy. Humans are eukaryotes. Table 3 compares some of the features that distinguish microbial cells

Table 3.-Comparison of Microbial and Mammalian Cells

Characteristic	Microbial cells	Mammalian cells (in culture)
Size (diameter)	1 to 10 microns	10 to 100 microns
Metabolic regulation ...	Internal	Internal and hormonal
Nutritional spectrum	Wide range of substrates	Fastidious
Doubling time	Typically 0.4 to 2.0 hours	Typically 12 to 60 hours
Environment	Wide range of tolerance	Narrow range of tolerance

SOURCE: Office of Technology Assessment, 1987

(prokaryotes) from cultured mammalian cells (eukaryotes).

Multicellular eukaryotes are complex and difficult, if not impossible, to examine in vivo at the organismal level. Thus, scientists at the turn of the century began studying these organisms using a reductionist approach, They dissected the many biological processes in vitro by examining cells isolated and maintained independently of a whole organism. This approach, called tissue and cell culture, has been refined considerably over the years and the following section discusses this technology as it applies to human cells. A separate sec-

tion is devoted to a special application of cell culture technology-making hybridomas.

Culturing Human Cells

The first experiments using tissue and cell culture technology were conducted in 1907 when a scientist successfully grew frog nerve cells in culture (7). The technology was originally considered a "model system"—a way for scientists to examine physiological events outside an intact organism. The approach was initially criticized as myopic and artifactual, but tissue and cell culture are now seen as fundamental scientific tools. These techniques are no longer only used as model systems, but are widely exploited techniques used in biomedical research.

As a practical matter, the distinction between tissue culture and cell culture is often blurred so the terms are frequently used interchangeably. Strictly speaking, in cell culture technology samples are removed from an organism and in vitro manipulation has destroyed the original integrity of the sample. In time, a sample isolated and established in the laboratory maybe called a cell line, In tissue culture, isolated pieces of tissue are maintained with their various cell types arranged much as they existed in the whole organism and their functions remain largely intact, Tissue cultures presumably have more of their native identity, but are much more difficult to maintain than cell cultures.

Although many advances have occurred since 1907, establishing a human cell culture directly from human tissue--called a primary ceil culture--is still a relatively difficult enterprise. The probability of establishing a cell line from a given sample is low. Success can be undermined by contamination during collection and storage, and is also dependent on how much damage the tissue suffered during collection of the sample. The success rate also depends on the type of human tissue being used. Some cells are easy to culture—human skin fibroblasts and human glial cells can be successfully established nearly 100 percent of the times attempted (14,19). Others, however can be very difficult to establish. Some human tumors can be cultured with about a 10 percent success rate (13).

While it is significantly less difficult to cultivate human cell lines than it is to establish them, working with human materials is still much more problematic than working with simpler organisms such as bacteria or yeast. Nevertheless, scientists are continuing to make progress in developing optimal growth conditions and cell culture equipment.

The food required to sustain human cells in culture is a liquid called growth medium. Different types of human cells require different growth media. Growth media are complex, and until recently animal serum-containing many unidentified, but vital components—was a necessary ingredient of all media. However, media with the identity and quantities of all components defined have been successful in sustaining long-term growth of human cells (8)20).

In addition to the many nutrient requirements of human cells in culture, strict temperature conditions must be maintained. Variation in temperature exceeding 20 C from the optimum usually is not tolerated; higher temperatures in particular are quickly lethal. Buffers are added to growth media to prevent drastic shifts in acidity, and the media must be sterilized. Contamination of samples during the early stages of culturing is a particular concern, and rigorous care must be taken to keep the culture free of contaminants such as yeast, fungi, bacteria, and viruses. Antibiotics and fungicides may be added to further discourage infestation. Table 4 lists some of the requirements for successful cultivation of human cells in the laboratory.

Table 4.—Some Nutrient and Growth Condition Requirements for Culturing Human Cells

Water
Salts
Sugars
Vitamins
Amino acids
Hormones
Fats
Buffers (to maintain proper pH—i.e., prevent drastic shifts in acidity)
Gases (oxygen, nitrogen, carbon dioxide)
Temperature (usually 98.6° F [37° C] for optimal growth)
Sterilization
Antibiotics and fungicides (optional)

SOURCE: Office of Technology Assessment, 1987.

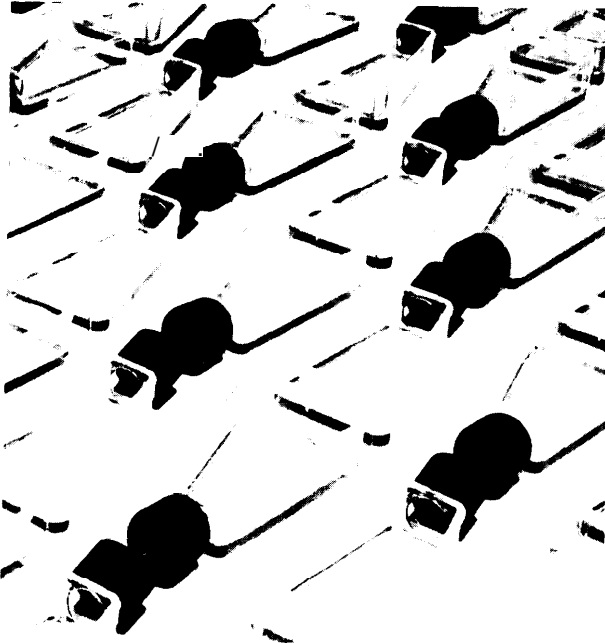**Figure 3.—Plastic Monolayer Cell Culture Flasks**

Photo credit: Ventrex Laboratories, Inc.

Cultured human cells grow as a suspension in solution, or attached to specially treated glass or plastic and submerged in growth medium (figure 3). Human cells typically double in number in 18 to 36 hours, compared to approximately 20 minutes for the bacterium *Escherichia coli.* Samples of human cells can be stored frozen in liquid nitrogen (– 1960 F) for future use. Certain types of cells are more fragile than others, but with modern freezing techniques most samples can be thawed and recovered decades later—often with a greater than 95 percent survivor rate.

Primary cell cultures are derived directly from solid human tissue or blood. In the case of cultures isolated from solid samples, extensive mincing or enzyme treatment maybe necessary to disperse the tissue. **Since the earliest days of tissue and cell culture, it has been clear that not all the cells that are isolated from tissue and put into culture will survive. Thus, as soon as a sample is cultured it may not be representative of the total specimen used, and the longer the sample is in culture, the less it is like the** **original specimen** (2,4). For some liver cells, the fraction of cells resulting in viable outgrowth for any given sample is between only 1/1,000 to 1/100)000 (0.01 to 0.10 percent) (10).

Primary human cell cultures typically maintain the normal diploid number of human chromosomes—46. They may also exhibit the functions and properties indicative of their differentiated origin: liver cultures may produce certain liver-specific proteins or white blood cell cultures may express their own specialized characteristics.

Cell cultures isolated from nontumor tissue have a finite lifespan in vitro (i.e., most cultures die after a limited number of population doubling.) These cultures will almost always age unless pushed into immortality by outside intervention involving viruses or chemicals. This aging phenomenon, called senescence, does not occur en masse, but is a gradual deterioration and death of the cell population. The type of tissue involved and culture conditions are important variables in determining cell lifespan. However, the age of the human tissue source is also a component, and thus primary cell cultures can be studied as models of human aging.

Human Cell Lines

Long-term adaptation and growth of human tissues and cells in culture is difficult—often considered an art—but it has been accomplished and many established human cell lines (cells capable of continuous and indefinite growth in culture) exist. A primary culture that has been transformed into an immortal cell line usually has undergone a "crisis" period. Most established cell cultures have been derived from malignant tissue samples (figure 4). It is important to point out, however, that immortalization does not occur in all samples isolated from tumors. As was mentioned earlier, certain types of tumors seem more likely to establish continuous cultures. Figure 5 illustrates the evolution of cultured cells.

It is not known precisely why a given sample gives rise to a continuous cell culture. It is possible that a small number of cells in the original sample become the immortal cell line. On the other

Technician storing human cell lines in liquid nitrogen refrigerators.

hand, one or a few cells may undergo a transformation event during the "crisis" period to give rise to the immortal cell line. Evidence indicates that the latter explanation is more probable, but the possibility that there is a subpopulation of the original sample with a predisposition to undergo the transformation event cannot be discounted (4).

Established cell lines are usually aneuploid, which means that the number of chromosomes deviates from the normal number of 46 for humans. The first human tumor cell line, HeLa, was isolated in 1951 (5). Derived from a cervical carcinoma, this widely used cell line has a chromosome number that varies from about 50 to 80, depending on the particular isolate.

In addition to having aberrant numbers of chromosomes, established cell lines may not display differentiated functions. Both of these properties may be a result of the nature of the tumor used to establish the cell line, or they may be the result of changes the cells have undergone in order to achieve continuous, long-term culture. After initial immortalization, established lines are usually isolated and expanded from a single cell—a process referred to as cloning. This means that the entire population of cells has resulted after continual growth starting from a single cell.

Cells that have adapted to continuous culture can not be considered entirely representative of the total population of the original isolate and they may continue to change with time (4). Cloning is performed, therefore, to provide a uniform population of cells so that uniformity and accuracy in experimental results can be improved. But, continuous growth of cells is a dynamic process— subpopulations of cells may suddenly accelerate their growth rate, shut down production of or

Figure 4.— Human Tumor Cells in Culture

Photo credit: Robyn Nlshimi

begin to overproduce compounds, or alter their chromosome number. So in order to reproduce earlier experimental results, repeated subcloning of cultured cells may be required.

Using Cell Cultures

The applications of tissue and cell culture technology are wide and varied. At both the basic re- search and commercial levels, cell cultures are used as tools to study basic biological processes. A cell line may be used as a biological factory to produce small or large quantities of a substance. Human proteins may be isolated directly from cultured cells. Cell cultures can also be the source of the genetic material needed to apply recombinant DNA technology in further studying a problem. As will be described later in this chapter, cultured human cells, both primary and established, play an important role in recombinant DNA technology. And finally, companies may use primary and established cultures to test drugs or the toxicity of compounds. The ability to maintain and manipulate many types of human cell lines in a controlled environment has expanded our knowledge of the biological sciences significantly and facilitated biomedical research.

In addition to increasing our knowledge, nearly 50 years after frog nerve cells were first cultured in vitro an important offshoot of growing cells in culture was invented: a technique to fuse cells from different sources. This technique, called cell fusion, has elucidated much of what is currently known about:

- the structure and function of the human genome,
- the expression and mechanism of heritable conditions,
- the regulation of normal biological reactions, and
- the processes of carcinogenesis and many other diseases.

Cell fusion was also central to the development of hybridoma technology.

HYBRIDOMA TECHNOLOGY

Refinements in cell fusion (also called cell hybridization) are responsible for the explosion in hybridoma technology. Hybridomas are special types of hybrid cells and to understand how they were invented and why they are important it is helpful to understand the immune system.

The immune response in higher animals serves to protect the organism against invasion and persistence of foreign substances. It occurs only in vertebrates and is a cooperative effort among several types of cells that results in a complex series of events involving the production of antibodies

Figure 5.— Evolution of a Cell Line
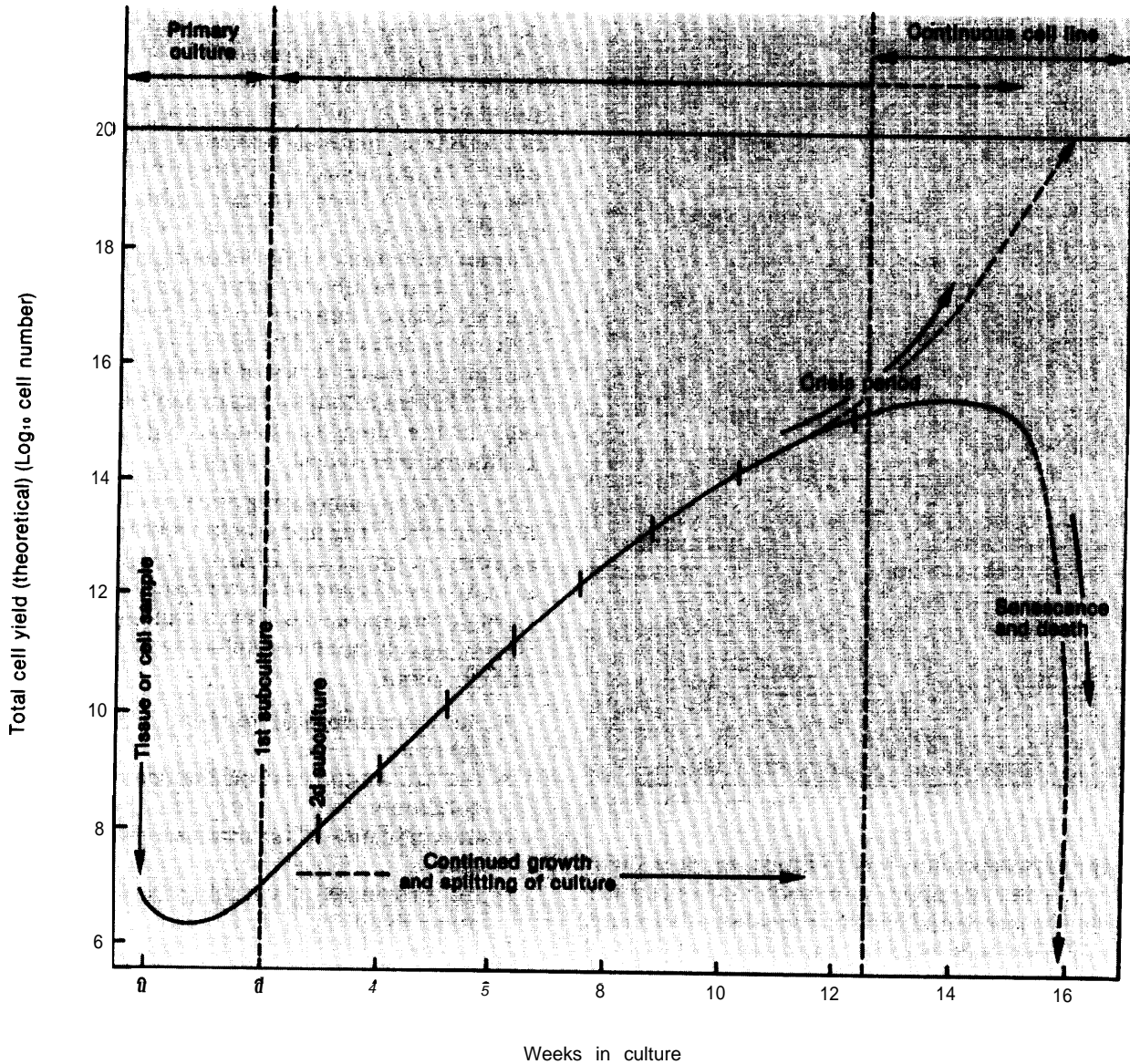
Weeks in culture

The vertical axis represents total cell growth on a log scale and the horizontal axis the number of weeks the hypothetical sam.
ple has been in culture since it has been obtained from a donor. In this example, **a** continuous cell line is depicted as arising
at about 12.5 weeks. Different cultures will give rise to a continuous cell line at different times. In addition, senescence may
occur in a sample at any time, but for human diploid fibroblasts it usually happens between 30 and 60 population doublings
(10 to 20 weeks).

SOURCE: Adapted from R.I. Freshney, Culture *of Animal Cc//s: A Manual of Basic Technique (New* York: Alan R. Liss, Inc., 1983),

and a class of molecules called lymphokines. Antibodies bind to a foreign invader, while lymphokines are necessary for coordinating, enhancing, and amplifying an immune response. Both antibody and lymphokine production operating in concert are necessary for a complete and efficacious response to a foreign challenge.

Scientists realized that obtaining a constant and uniform source of a single type of antibody would be essential to understanding the intricacies of the immune response and that such a uniform source of antibodies could provide a powerful, general analytical tool. High concentrations of reliable antibodies and lymphokines also promise rewards in diagnosing and treating human ills, The following two sections describe recently developed technologies that yield pure antibodies and higher concentrations of many lymphokines.

Monoclinal Antibodies

An antibody is a protein molecule with a unique structural organization that enables it to bind to a specific foreign substance, called an antigen. Antibody molecules have binding sites that are specific for and complementary to the structural features of the antigen that stimulated their formation. Antibodies formed by a sheep, for example, in response to injection of human hemoglobin (the antigen) will combine with human hemoglobin and not an unrelated protein such as human growth hormone.

All antibodies are comprised of four protein chains—two identical light chains and two identical heavy chains. These subunits are always linked in a fixed and precise orientation, as illustrated in figure 6. One end of the antibody contains two variable regions, the sites of the molecule that recognize and bind with the specific antigen, To accommodate the many antigens that exist, the variable end of an antibody differs greatly from molecule to molecule. The other end of the antibody is nearly identical among all structures and is known as the constant, or effecter, region. The constant region is not responsible for antibody binding specificity, but has other functions.

other important actors in the immune response are specialized white blood cells called lymphocytes that are present in the spleen, lymph nodes,

Figure 6.—Structure of an Antibody Molecule
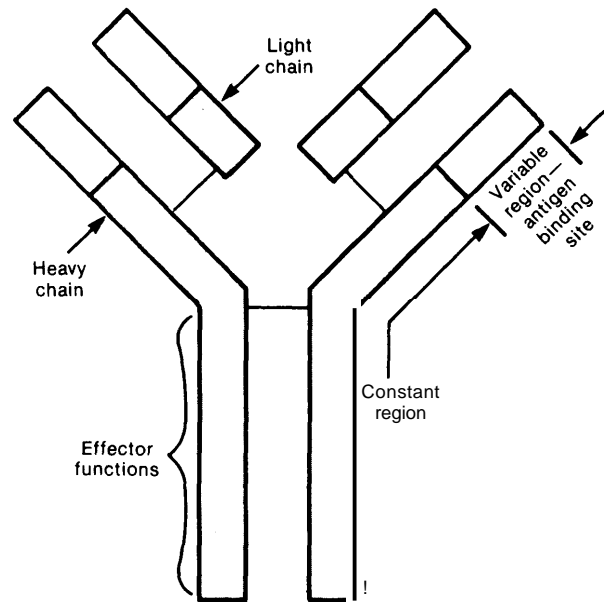
SOURCE: Office of Technology Assessment, 1984

and blood. A particular subclass of lymphocytes, called B lymphocytes or B cells, recognizes antigens as foreign substances and responds by producing antibodies highly specific for a given antigen. Any single B lymphocyte is capable of recognizing and responding to only one antigen. Once a B cell has been activated by an antigen it is committed to producing antibodies that bind to only that one specific antigen.

During an immune response to an invasion by a foreign substance (e.g., a virus), one of the events that occurs within an organism is that many different B cells react and produce antibodies. Different B cells produce antibodies recognizing different parts (called determinants) of the virus, but as mentioned above, an individual B lymphocyte and its progeny produce only one specific kind of antibody. This multiple B cell reaction produces a mixed bag of antibodies with each type of antibody represented in only limited quantities, and is called a polyclonal response. Polyclonal antibodies can be isolated from blood serum, and, until recently, were the principle source of antibodies used by physicians and researchers. While antibodies produced this way were and still are useful tools to scientists and clinicians, a method to

produce a constant and pure source of a single type of antibody was still sought.

The discovery in 1975 of the technique to produce a special hybrid cell known as a hybridoma that produces a specific type of antibody was, in the words of one of the inventors, a "lucky circumstance)" but one with profound effects for biomedical research and commerce. Cesar Milstein and Georges Kohler,[1] working at the Medical Research Council's Laboratory of Molecular Biology in Cambridge, England, used the well-established tissue culture technique of cell fusion to produce a new type of hybrid cell—a hybridoma-capable of indefinitely proliferating and secreting large amounts of one specific antibody (11,12).

Hybridomas are hybrid cells resulting from the fusion of a type of tumor cell called a myeloma with a B lymphocyte freshly isolated from an organism (usually from the spleen or lymph nodes) that had been recently injected with the foreign substance of interest. Due to the recent exposure to the antigen, many of the B cells in such an organism will be producing antibodies specifically complementary to the foreign substance just injected. This enrichment process is a key step in hybridoma technology, since a human, for instance, is capable of producing up to a million different kinds of antibodies.

The hybridoma that results from the fusion of these two types of cells has characteristics of both cells. As is often the case with tumor cells, the myeloma parent cell has the ability to grow and multiply continuously in culture—it contributes this characteristic of "immortality" to the hybridoma. From the B cell, which is incapable of sustained growth and cultivation in vitro, comes the ability to secrete a single, specific type of antibody. Thus, a particular hybridoma clone is a distinct cell line capable of continuously producing one and only one kind of antibody—hence the name monoclinal antibody. The culture conditions and techniques used for hybridomas essentially are those described for tissue and cell culture.

Independently isolated lines of hybridomas, each originating from a single B cell fusing with a single myeloma cell, produce distinctive monoclinal antibodies. Each line is unique to the original contribution of the particular B cell parent. In the case of Milstein and Kohler each different hybridoma cell line isolated is an immortal antibody-producing factory targeted toward a different part of a sheep red blood cell. The method used to produce mouse monoclinal antibodies is illustrated in figure 7.
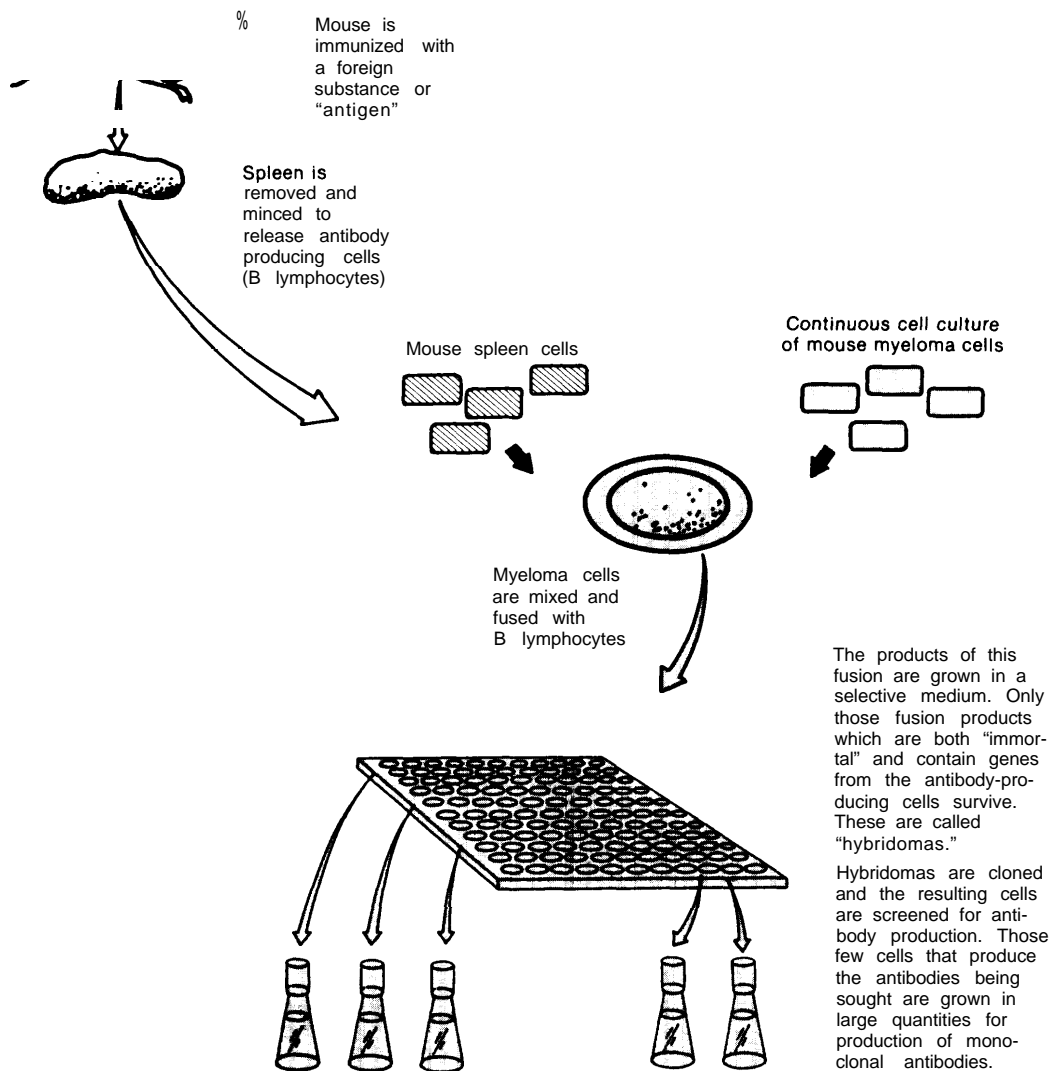
Virtually all of the monoclinal antibodies currently being used in humans as therapeutic agents or imaging tools are rodent antibodies because the production of human hybridomas has been much more difficult than the production of rodent hybridomas. To avoid some of the complications in patients treated with rodent antibodies, refinements in human monoclinal antibody technology will be necessary. Researchers have developed ingenious in vitro methods and successfully isolated suitable immortal parental cell lines, so production of human hybridomas is rapidly progressing (16,17). Recently, researchers have developed a promising new method to produce large quantities of human monoclinal antibodies (1).

The availability of large supplies of monoclinal antibodies is revolutionizing basic research, medicine, and commerce. Researchers have come to value monoclinal antibodies as important tools for dissecting the molecular structure and mechanisms of genes; more often than not, monoclinal antibody technology is combined with recombinant DNA technology. High-volume production of rodent monoclinal antibodies has had a significant impact on the diagnostic industry in particular. Monoclinal antibodies are reagents that are easily standardized and provide reproducible results, These substances have been adapted to clinical and home test kits, such as pregnancy diagnostic kits, with much success. Use of monoclinal antibodies for prophylactic or therapeutic regimens in humans is in an embryonic stage.

Lymphokines

Two other specialized cell types involved in the immune response are T lymphocytes and macrophages. Like B lymphocytes, both of these cell

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[1] In this case, the antibody recognized a particular part of a sheep red blood cell. It is interesting to note that Milstein and Köhler did not apply for a patent on this technique.

Figure 7.— Preparation of Mouse Hybridomas and Monoclinal Antibodies

% Mouse is immunized with a foreign substance or "antigen"

Spleen is removed and minced to release antibody producing cells (B lymphocytes)

Mouse spleen cells

Continuous cell culture of mouse myeloma cells

Myeloma cells are mixed and fused with B lymphocytes

The products of this fusion are grown in a selective medium. Only those fusion products which are both "immortal" and contain genes from the antibody-producing cells survive. These are called "hybridomas."

Hybridomas are cloned and the resulting cells are screened for antibody production. Those few cells that produce the antibodies being sought are grown in large quantities for production of monoclonal antibodies.

SOURCE: Office of Technology Assessment, 1987.

types can detect and respond to the presence of foreign substances. However, rather than producing antibodies, T cells and macrophages produce a variety of protein molecules that regulate the immune response. These molecules serve as messengers that transmit signals between cells to orchestrate a complete and efficient immune response against a foreign invader. The term "lymphokines" was coined in 1969 to describe this group of nonantibody immune response modulators (3). Since that time, more than 90 lymphokine activities have been described.

Lymphokines may recruit other cells to participate in and augment an immune response. Some lymphokines stimulate B cells to produce antibodies. Other molecules are released that suppress the immune reaction or ensure that the system focuses on the irritant and does not run rampant in a nonspecific attack that would damage host tissue.

Lymphokines are present in human blood in extremely small amounts-on the order of parts per billion. Interferon, for example, has been the most

widely examined Iymphokine to date by virtue of its relatively "high" abundance. It takes approximately 65,000 liters of blood to produce 100 milligrams of interferon (21). A comparable task would be the search for less than one-eighth of a teaspoon of salt in a swimming pool. Thus, scientists knew that to use lymphokines to treat human illness would require a source yielding a high-quality, high-quantity sample.

In addition to the problem of obtaining sufficient quantities of these important biological regulators, different lymphokines with antagonistic functions are often difficult to separate. In the past, such impure preparations of lymphokines have hampered efforts to understand the basic mechanism of how the immune system responds to cancer or an agent of disease. Autoimmune diseases, for instance, are aberrations of the immune system resulting in an organism attacking itself as a foreign substance. The availability of a lymphokine drug to suppress an individual's immune response could alleviate much suffering. Similarly, other lymphokines could be used as therapeutic agents to boost a patient's own immune system to combat a foreign invasion.

Recent progress in obtaining pure lymphokine preparations is a result of advances in cell culture, hybridoma, and recombinant DNA technology. Scientists have now developed cell culture conditions capable of sustaining continuous growth of cell lines producing elevated levels of one or more lymphokines. Some of these lymphokine-producing cell lines are derived from tumor cells that have been adapted to tissue culture conditions. Other cell lines have been isolated from normal cells that have been manipulated in a manner to transform them into immortal lymphokine-producing cultures.

The explosion in hybridoma technology also has influenced the study and development of hybrid T cell lines to produce lymphokines (6). Investigators have had some success producing these hybrid lymphokine factories, often referred to as T cell hybridomas. T cell hybridomas are the prod-

ucts of fusion events between immortal cancer cells and isolated T lymphocytes.

Even though researchers have isolated and identified many types of human cells producing lymphokines, these cell lines are still not capable of generating sufficient quantities of these molecules for widespread use. The human cell lines are very important, however, as rich deposits of source material to clone lymphokine genes. Several different genes have been cloned from human cells that produce measurable amounts of lymphokines (16), and once a particular lymphokine gene has been cloned, large quantities of the protein molecule can be obtained via the methods developed for large-scale production of recombinant DNA products.

Large-scale production of pure lymphokines now enables scientists to examine many aspects of the immune system puzzle by manipulating cells and lymphokines in vitro. The availability of commercial quantities of these pure immune regulators also affords physicians an opportunity to use lymphokines for treating human disease. Human alpha-interferon has been approved by the Food and Drug Administration (FDA) to treat certain medical conditions and interleukin-2 is being used in clinical trials to combat certain types of cancers or viral infections. Table 5 lists some of the lymphokines that have been characterized and have received considerable attention for their possible use as human therapeutic agents.

Table 5.—Some Lymphokines With Therapeutic Potential

Interferon
Interleukin-l (also known as lymphocyte activation factor)
Interleukin-2 (also known as T cell growth factor)
Interleukin-3
Interleukin-4
Colony stimulating factors
B-cell growth factor
Microphage activity factor
T-cell replacing factor
Migration inhibition factor

SOURCE: Adapted from A. Mizrahi, "Biological From Animal Cells in Culture, " *Biotechnology* 4:123-127, *1966.*

RECOMBINANT DNA TECHNOLOGY

History

In 1865, Mendel postulated that discrete biological units were responsible for maintaining characteristics in organisms from one generation to the next. The faithful transmission, or inheritance, of these units—called genes—is common to the entire spectrum of living organisms, It is a result of the remarkable capacity of a living cell to encode, translate, and reproduce a chemical into its ultimate biological fate. The chemical responsible for inherited characteristics is deoxyribonucleic acid, or DNA.

In 1965, a century after Mendel described the concept and principles of inheritance, also called genetics, the term "genetic engineering" was coined (9). The term genetic engineering is now also popularly referred to as "gene cloning" or "recombinant DNA ." These techniques usually involve direct manipulation of the genetic material—the DNA-of a cell. Rather than rely on the appearance of spontaneous mutants or laborious extraction of minute quantities of a valuable substance from tissue, it is now possible to use these techniques to isolate, examine, and develop a wide range of biological compounds quickly. Like the use of cell culture, the use of recombinant DNA techniques is a reductionist approach that has shed further light on the molecular details of regulation of many important biological processes, including arthritis, cancer, and development, The principal advantages of these techniques are speed and ease of application.

Gene Cloning

DNA, which takes the structural form of a double-stranded helix (figure 8), is the information system of living organisms. DNA in all organisms is composed, in part, of four chemical subunits called bases. These four bases—guanine (G), adenine (A), thymine (T), and cytosine (C)—are the coding units of genetic information. These bases normally pair predictably—A with G, and T with C—to form the DNA double helix structure. It is the unique ordering of these bases in the helix that determines the function of a given gene, and

Figure 8.— The Structure of DNA
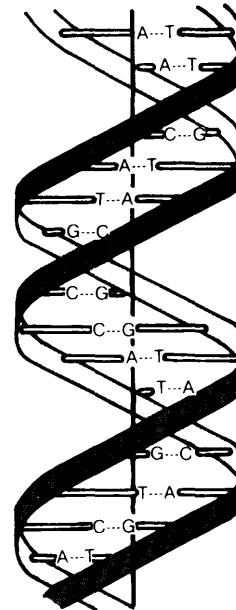
SOURCE" Office of Technology Assessment, 1984

the complete blueprint for an organism is coded within its DNA.

There are two broad categories of genes: structural and regulatory. Structural genes code for products, such as enzymes—proteins that catalyze biological reactions. Regulatory genes function like traffic signals, directing when or how much of a substance is produced. The process whereby the code of DNA is interpreted and a protein synthesized is summarized in figure 9.

All cells, except egg and sperm cells and some cells of the immune system, contain the total information capacity of the organism. Thus, the DNA present in one human cell is identical to all other cells within the individual and has the capability of directing all possible functions. In individual human cells, however, not all functions operate simultaneously.

The amount of DNA present in each cell of a human being is 3.3 billion base pairs (15). About 50,000 genes make up the complete human master plan, and the average gene contains about 1,000

Figure 9.-The Process of Gene Expression
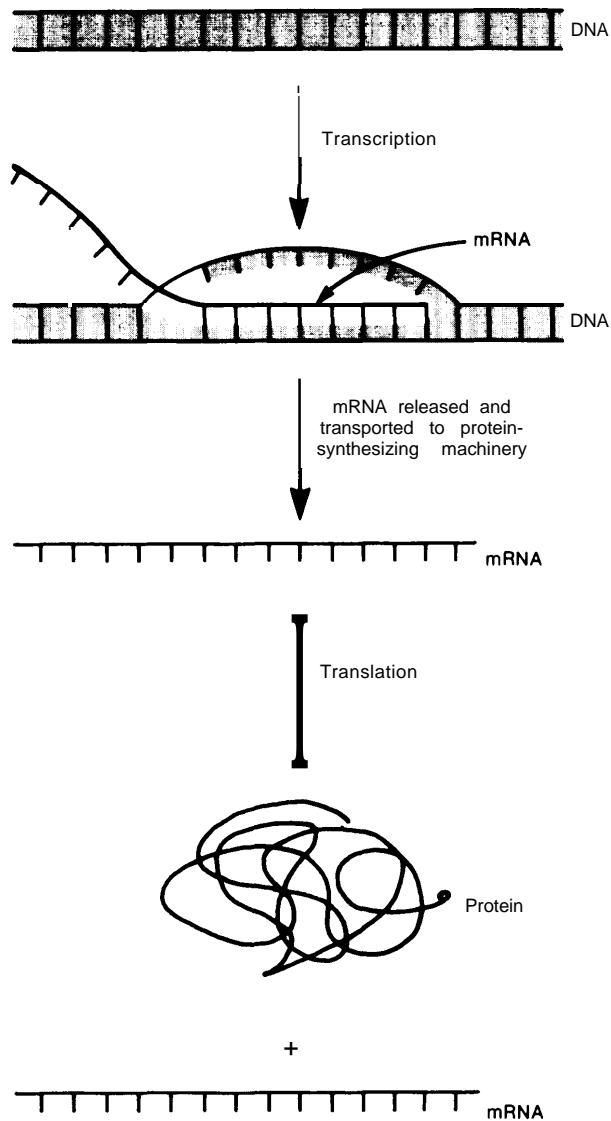
During gene expression, the genetic material of an organism is decoded and processed into the final gene product (usually a protein). In the first step, called transcription, the DNA double helix unwinds in the area near the gene, and a product called messenger ribonucleic acid (mRNA) is synthesized. This piece of mRNA is a single-stranded, linear sequence of nucleotide bases chemically very similar to DNA and it is complementary to the section of the unzipped DNA. The second step of the process is called translation. The mRNA is released from the DNA, becomes associated with the protein-synthesizing machinery of the cell, and is decoded and "translated" into a protein product.

SOURCE: Office of Technology Assessment, 1987.

base pairs. Since this accounts for about only 50 million base pairs, it is apparent that not all of the DNA within a human cell is devoted to modulating or specifying a particular gene product. To date, specific functions have only been assigned to about 50 million of the 3.3 billion base pairs present in humans. There is speculation that some of the unassigned 3.25 billion base pairs may contain some genes, but that much of the "excess" DNA is for architectural or other unknown functions.

Gene cloning refers to a process that uses a variety of procedures to produce multiple copies of a particular piece of DNA. Since the amount of DNA in a human cell is enormous compared to the amount present in an individual gene, the search for any single gene within a cell is like searching for a needle in a haystack. Therefore, a range of tools have been developed that allow investigators to both identify a gene and amplify the number of copies of the gene. As a metal detector allows easier detection of a needle in a haystack, and a photocopy machine reproduces documents, "recombinant DNA technology" is a group of methods that accelerates the investigation or production of genes. The specific details of these methods to join segments of DNA—sometimes from different species—vary from project to project and purpose to purpose. In general, however, all recombinant DNA methods require the following:

- a suitable vector,
- an appropriate host,
- a system to select host cells that have received recombinant DNA, and
- a probe to detect the particular recombinant organism of interest.

Perhaps the hallmark discovery that allowed scientists to clone genes was the isolation of naturally occurring enzymes in bacteria that recognize and cut DNA at specific strings of bases. The string of bases recognized by the enzyme is usually four to six bases in length and depends on the particular bacteria from which the enzyme is isolated. These enzymes, called restriction endonucleases, are used in gene cloning to fragment DNA into discrete, precise segments. Recent reports have described modified restriction en-

Figure 10.— Recombinant DNA: The Technique of Recombining Genes From One Species With Those From Another
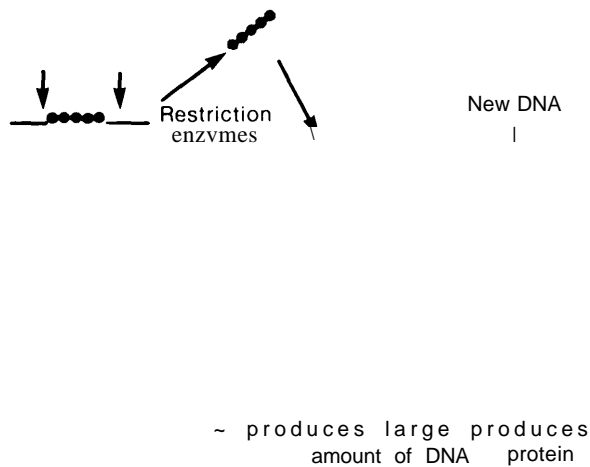
Restriction enzymes recognize sequences along the DNA and can chemically cut the DNA at those sites. This makes it possible to remove selected genes from donor DNA molecules to form the recombinant DNA. The recombinant molecule can then be inserted into a host organism and large amounts of the cloned gene, the protein that is coded for by the DNA, or both, can be produced.

SOURCE: Office of Technology Assessment, 1987

zymes that are now capable of cutting DNA at a sequence of the investigator's choice (18,22). With the aid of restriction enzymes, a particular fragment of DNA-often the gene of interest and some neighboring bases-can be excised away from large, unwieldy pieces of DNA.

Cloning human and other eukaryotic genes is usually more difficult technically than cloning bacterial and viral genes. Refinements in recombinant DNA methods, however, have been invented. Figure 10 illustrates the basic technique for preparing a recombinant DNA molecule. The recombinant molecule can be prepared in a number of ways, but ultimately the process involves linking the DNA sequence of interest to a second piece of DNA known as the vector,

Vectors serve as vehicles for the isolation and high copy reproduction of a particular DNA fragment free from its normal environs. Vectors can be bacterial, viral, phage, or eukaryotic DNA-or they may be combinations of these DNAs. The characteristics of vectors differ from construction to construction. Some are capable of stably maintaining a large piece of foreign DNA, some reproduce rapidly and in high copy number, while others, called shuttle vectors, can reproduce and function in both eukaryotic and prokaryotic cells. A critical consideration in commercial development of a cloned gene is the ability of the vector to achieve high product expression.

The other principal player in a cloning system is the host organism. Once foreign, or donor, DNA has been inserted into the vector, the recombinant molecules must be introduced into an organism that provides an optimal environment for increasing the number of copies of the cloned DNA, producing large amounts of a gene product, or both. The host is often the bacterium *Escherichia coli,* but human cells, yeasts, and other cells can be suitable hosts. Mean generation time, ease of culture, ability to stabilize and adjust to presence of the vector(s), and ability to add sugar groups to a gene product are some important factors to consider in selecting a host.

In general, recombinant DNA technology works in this sequence: first, donor DNA is cut by restriction enzymes into many fragments, one of which contains the sequence of interest. These different fragments are joined with vector DNA to become recombinant DNA molecules. The recombinant molecules are then introduced into the host; for a variety of reasons, only some host cells will take up the recombinant DNA. After this process, the fraction of host cells that received any recombinant DNA must be identified. This initial selection is often accomplished through the use of antibiotics that kill those host cells that did not receive recombinant molecules.

Finally, the small number of recombinant organisms containing the specific donor DNA fragment of interest must be found. This process is completed via a tool that detects the gene or gene product of interest. This tool is called a gene probe. Examples of gene probes include a segment of DNA similar to the gene of interest, but from a

different organism; a synthetic fragment of DNA deduced from the protein sequence of a gene product; a piece of RNA; or an antibody that binds to the product of interest.

Once identification and purification of the genetically engineered (recombinant) organism has been achieved, the host population containing the cloned gene can be expanded and the cloned gene used to identify, isolate, and scrutinize scarce biological compounds.

SUMMARY AND CONCLUSIONS

Technologies grouped under the umbrella term "biotechnology" include tissue and cell culture, hybridoma technology, and recombinant DNA technology. Tissue and cell culture, the oldest of the three technologies, involves converting undeveloped human biological materials into cell lines capable of indefinite growth in a laboratory. Establishing human cultures is still a relatively difficult enterprise, and the human cell line resulting from any single sample has undergone many changes. Continuous cultivation of cell cultures requires stringent control of temperature, nutrient, pH, and sterile conditions. The use of human cell lines in research has contributed much to our knowledge about human genetics and the regulation of normal and abnormal biological processes. Cell lines also have been used for a broad range of commercial purposes.

Hybridoma technology is a spinoff technique from cell culture. Hybridomas are special hybrid cells that are produced by fusing two types of cells: an antibody-producing B lymphocyte and a tumor cell called a myeloma. A hybridoma is capable of multiplying continuously in culture (a property it receives from the myeloma) as well as secreting antibodies with a single specificity (an ability gained from the B lymphocyte). The antibodies produced by hybridomas are called monoclinal antibodies. Not only are monoclinal antibodies important laboratory tools, but some are significant commercial commodities. one specific mouse monoclinal antibody was approved by the FDA in 1986 for use in the treatment of kidney transplant rejection.

Lymphokines are molecules that are secreted by specialized cells called T lymphocytes and macrophages. Many of these substances occur naturally in the human body, but were previously available in minute and usually impure amounts—if at all. Lymphokines, also called bioregulators or biological response modifiers, have significant therapeutic promise in the treatment of a spectrum of diseases because of their exquisite specificity and reduced toxicity. Hybridoma, cell culture, and recombinant DNA technologies permit lymphokines to be isolated in pure form and in quantities facilitating further analysis or use. Human alpha interferon, a lymphokine produced by a combination of the biotechniques, was approved in 1986 by the FDA for use in the treatment of one form of leukemia.

Genes are composed of DNA and they are responsible for the faithful inheritance of characteristics from one generation to the next. Recombinant DNA technology, also called genetic engineering, involves techniques that allow direct manipulation of the genes—the DNA-of a cell.

Courtesy of: *L.Gonich* and *M.Wheelis,* The Cartoon Guide to Genetics

Gene cloning uses a variety of these recombinant DNA techniques to join segments of DNA, sometimes from different species, in a form that allows multiple copies to be made. These multiple copies can then be used to examine the regulation of a biological process, identify and isolate scarce compounds, or produce commercial quantities of an important substance. Three commercial products created through gene cloning—human growth hormone, human insulin, and human alpha interferon–have been approved for use in humans by the FDA.

The ease of application of biotechnology processes has allowed researchers to turn undeveloped human tissues and cells into human biological products with significant therapeutic promise and commercial potential. Yet the ultimate value of these technologies may not be simply their end products; their greater value may be the insights they `provide` about disease processes.

CHAPTER 3 REFERENCES

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