

Medical Applications of Biotechnology • part 1

Biotechnology and recombinant DNA

LEARNING OBJECTIVES

- **Describe** the evolution of biotechnology and its medical applications.
- **Describe** the structure and function of cells.
- **Describe** the structure and function of DNA and proteins.
- **Explain** the basic processes behind recombinant DNA technology.
- **Identify** types of pharmaceutical products available through rDNA technology and describe their applications.

This first article in a three-part series provides an introduction to biotechnology. Topics include: the evolution of biotechnology; the structure and function of cells, DNA and proteins; and recombinant DNA technology and its resulting products.

Kimberly A. Bergstrom, Pharm.D., adjunct assistant clinical professor, School of Pharmacy, University of California, San Francisco; Eric T. Herfindal, Pharm.D., M.P.H., professor emeritus, School of Pharmacy, University of California, San Francisco; and Michael Schurr, Ph.D., professor, Tulane University Medical Center, School of Medicine, department of microbiology and immunology, New Orleans, served as consultants for this article for the Certified Medical Representatives Institute Inc.

B iotechnology is not a new science. Since ancient times, humans have used microorganisms in various ways. They have used yeasts to make bread rise, bacteria to process linen and leather, and both bacteria and yeasts to make cheese

and yogurt. Throughout the ages, man has manipulated genetic material by breeding the finest animals or saving the best seeds for the next year's crop.

As researchers have learned more about microorganisms, they have identified increasing applications for microorganisms and for the chemical substances that microorganisms produce. The development of antibiotics to fight infection and the use of bacteria to create efficient sewage treatment plants are two examples of the important roles that microorganisms play in modern life. Recent advances in our understanding of DNA and genetics in general have enabled researchers to manipulate living organisms to produce specific products.

Biotechnology may be defined as the application of scientific knowledge to transfer beneficial genetic traits from one species to another to enhance or protect an organism. In the field of medicine, these tasks have ranged from the production of proteins, such as insulin, to selective targeting and binding of specific cell types.

Biotechnological processes with medical applications include:

- Recombinant DNA technology, which is used to produce pharmaceutical products such as insulin, human growth factor, tissue plasminogen activator (or t-PA) and numerous cytokines.
- Genetic research from the Human Genome Project, which is expected to increase the ability to detect genetic disorders for diagnosis and to create a variety of new drugs.
- Human gene therapy, which holds prom-

ise as a treatment approach in numerous genetic diseases.

- Stem cell therapy, which may produce significant therapeutic advances for a variety of chronic and costly disorders ranging from spinal cord injuries to severe burns to diabetes.
- Cloning, which may be used to develop organs and pharmaceutical products in animals, and may have applications in infertility treatment.
- Monoclonal antibodies (MAbs), which historically have been used in diagnostics and imaging, and more recently are used in therapeutics for a variety of conditions ranging from cancer to gastrointestinal disease to cardiovascular disease.

Structure and function of human cells

The human cell is composed of several major structures:

- Cell membrane – the permeable boundary of the cell that selectively controls the flow of substances into and out of the cell.
- Cytoplasm – the structure of a cell, containing numerous organelles that perform vital functions (listed next).
- Mitochondria – structures that produce energy for the cell's work.
- Endoplasmic reticulum – a network of membrane-enclosed spaces.
- Ribosomes – tiny circular structures involved in protein production.
- Nucleus – a self-contained body that controls all the functions of the cell and contains the cell's DNA. (The cells of simple organisms do not have nuclei; cells that do

REPS: RECEIVE CREDIT AND RECOGNITION

Continuing Education in **Pharmaceutical Representative** aims to provide reps with information to help them meet the needs of the people they serve and to contribute to reps' personal and professional development. Every third issue includes a self-assessment quiz covering the previous three Continuing Education articles. The quiz for this article appears in the March issue. Reps who correctly complete the quiz and return it to the CMR® Institute with a nominal handling fee will receive a completion recognition form showing that they have successfully completed the three-part educational series and earned .25 CEU (2.5 contact hours). The CMR Institute also will send a letter of recognition to reps' managers upon completion of a quarterly series. A Certificate of Achievement will be awarded to any representative who successfully completes four quizzes or has obtained 1.0 CEU.

Note: The Continuing Education quizzes are not part of CMR Institute's certification program and do not count as credits toward the CMR® designation.

About the CMR Institute:

The Certified Medical Representatives Institute is an independent non-profit educational organization established in 1966 to provide a source of professional development and certification for pharmaceutical representatives. The institute provides an up-to-date, approved continuing education curriculum designed to expand and enhance internal company training and development in a cost-effective manner. The curriculum concentrates on providing a general knowledge base and avoids such areas as selling skills and specific product education.

© 2004 The Certified Medical Representatives Institute Inc., Roanoke, VA 24014. All rights reserved. No part of this article may be reproduced by any method or in any form without written permission from the CMR Institute. Reprints of this article are available from the CMR Institute. Request Continuing Education article MB-1.

contain nuclei are called eukaryotic cells.)

The cells of living creatures contain large quantities of DNA that carry the genes essential for survival and reproduction. Higher life forms, such as humans, have two sets of genes, one contributed by each parent.

Function and structure of DNA

Deoxyribonucleic acid, or DNA, is a molecule that contains the code for producing all the organism's genetic traits. Deoxyribonucleic acid plays two essential roles in the life of an organism. One is the process of cell replication, whereby the organism can make copies of itself and thus continue on as a life form over time. The other is the encoding of protein sequences. Active proteins are working components of cellular machinery vital to the functioning of all living cells.

The nucleus of a cell contains long strands of DNA called chromosomes. Arranged along the chromosomes are hundreds, even thousands, of regions of DNA known as genes. Human cells contain 23 pairs of chromosomes containing 100,000 to 200,000 genes. These genes contain the code for the production of a particular substance or trait.

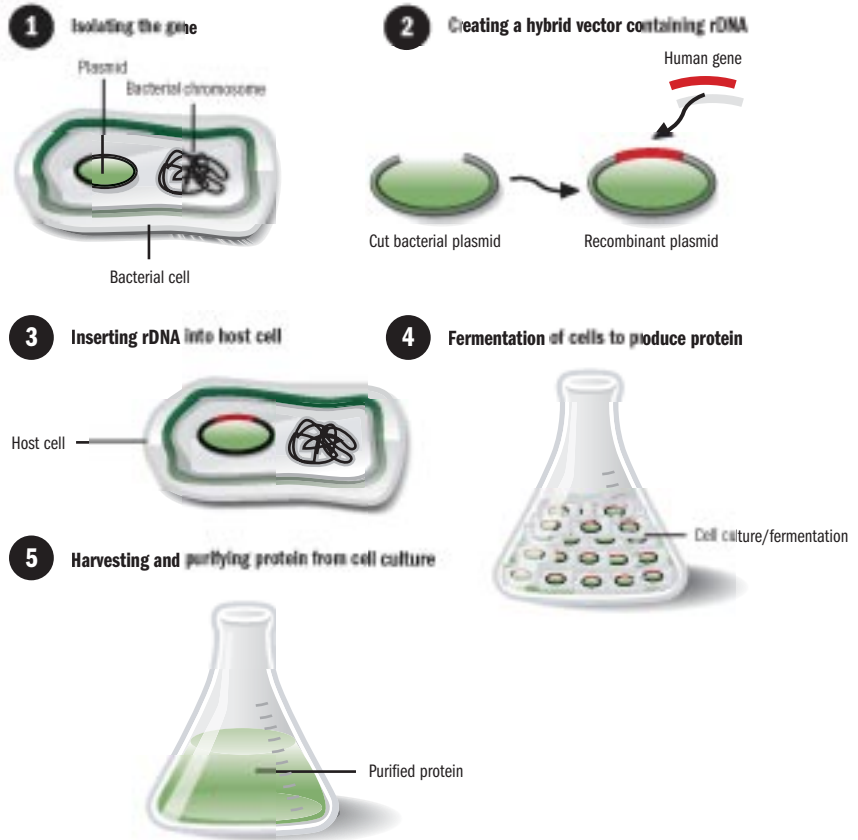
Because all cells in an organism have an identical set of chromosomes, they also all have an identical set of genes. It appears that differentiation of cells is caused not by a loss of genes, but by the selective repression of different genetic functions. Most human cells produce 8,000 to 10,000 different proteins, out of more than 100,000 potential proteins.

The structure of the DNA molecule is commonly described as a double helix. This can be visualized as a flexible ladder that is twisted to resemble a spiral staircase. The sides of the ladder are formed by alternating units of two different chemicals, phosphate and the sugar deoxyribose. The rungs of the ladder are composed of pairs of chemicals known as nitrogen bases.

Structure and function of proteins

Proteins are complex molecules formed from amino acids. They perform a number of roles in the body, including acting as catalysts, structural matter, transport mechanisms and regulatory mechanisms.

Process used in rDNA technology



When a protein is to be synthesized, the DNA double helix unwinds and separates at the site of the gene responsible for producing the desired protein. Once the double helix has separated, messenger ribonucleic acid (mRNA) is synthesized along the length of the single-stranded DNA gene in a process known as transcription.

The mRNA travels out of the nucleus to the ribosomes, where the protein is synthesized from amino acids with the assistance of another type of RNA called transfer RNA, or tRNA. Once the protein chain is assembled, chemical interactions occur to cause it to fold into a specific three-dimensional form.

Recombinant DNA technology

The basic processes used in recombinant DNA (rDNA) technology involve “splicing” a particular segment of DNA into a vehicle (known as a vector), which is then placed into a host cell for massive replication. As the host cell multiplies, so does its

internalized vector with its new genetic code. In this way, the foreign gene is able to produce its specific protein in large enough quantities for the treatment of disease (see illustration above).

Before this can take place, the fragment responsible for producing the desired protein must be isolated from its original chromosome. One process used to accomplish this is called polymerase chain reaction, which allows a gene to be isolated from as little as one cell through the in vitro amplification of specific pieces of DNA.

The isolated DNA fragment is inserted into a vector, which is a DNA molecule that can replicate independently of the host chromosome in at least one organism, and can have additional DNA inserted into it. The foreign DNA is spliced (or recombined) with the DNA of the vector, forming a hybrid vector, the new rDNA molecule. Two major types of vectors used in rDNA technology are plasmids (circular fragments of DNA

from bacteria and yeasts) and viral vectors.

The hybrid vector is made possible through the use of two specialized enzymes – restriction enzymes, which cut the required piece of DNA, and DNA ligases, which join the new gene to the vector.

After formation, the hybrid vector is placed into a host cell. In time, the host cell replicates into millions of genetically identical cells capable of producing the desired protein. The host cell colony thus becomes a small production “factory” for the desired substance.

There are three major types of host cells used in rDNA technology – bacteria, yeasts and mammalian cell lines. The choice of a host cell is important because it can influence the character, quantity and cost of a final product. While bacteria and yeasts are easy to grow and relatively inexpensive, mammalian cell lines are required to produce more complex proteins.

The host cells go through a fermentation process, during which they are grown in culture and then transferred to large vessels containing culture medium, often thousands of gallons on an industrial scale. The protein is then harvested from the culture medium, purified and formulated into the final commercial product.

Drugs produced using rDNA technology must adhere to specific storage and handling guidelines to ensure safe and efficacious use.

Products of rDNA technology

The first products available through rDNA technology were proteins, such as insulin and human growth hormone. These products were relatively simple to produce using genetically engineered *Escherichia coli* bacteria. They were followed by more complicated proteins which treated conditions such as blood clots, anemia and hemophilia. These more complex proteins required mammalian cell cultures, which are more complicated and costly to produce. In addition, rDNA technology is now being used to produce vaccines.

Cytokines. Cytokines (also called biologic response modifiers) are a key group of proteins being developed through rDNA technology for therapeutic use. Cytokines are naturally occurring proteins that regulate the growth and differentiation of immune system cells and are also messen-

gers inside the cell that influence activities such as inflammation, bone metabolism, immune response and tissue repair. Types of cytokines include:

- Interleukins – two of these are approved for use in treating cancer, while a number of others are under evaluation (also for cancer treatment).
- Colony-stimulating factors (CSFs) – these regulate the growth and development of blood cells; six CSFs have been approved by the Food and Drug Administration for clinical use in the treatment of blood disorders.
- Interferons (IFNs) – these are glycoproteins produced in the body that demonstrate antiviral effects and stimulate hormones, immune response, metabolism and tumor development; they are used in the treatment of cancer, hepatitis, solid tumors, hematologic malignancies, multiple sclerosis and chronic granulomatous disease, an inherited autoimmune disorder.

Vaccines. Biotechnology can be employed to create vaccines that have no pathogenic potential or extraneous material. Vaccines created using rDNA technology introduce plasmid DNA directly into individuals to elicit an immune response.

It appears that even very small amounts of protein are sufficient to induce immune responses following DNA immunization, and thus, this approach to vaccine development appears to be applicable to a broad number of diseases.

While the development of biotechnologically derived vaccines is expensive, there appear to be advantages to this approach to immunization. Because the immune response is induced by a single gene, rather than the entire organism, infection of the vaccinated individual or infection spreading to others is unlikely. Available data suggest that immunity conferred by rDNA vaccines is of longer duration than that seen with conventional vaccinations.

Delivery and administration of vaccines in the form of edible transgenic fruits, vegetables or even grains has become a real possibility and an attractive alternative to traditional injectable vaccines. Edible vaccines will offer advantages such as ease of production, noninvasive application, and low-budget storage and delivery.

The development of these vaccines has

been proposed especially for Third World countries, because besides their other advantages, the less potent edible vaccines are less likely to cause disease in populations suffering from malnourishment or diseases like AIDS.

Potatoes, tomatoes, bananas, soybeans, alfalfa and cereals are the most common foods proposed for edible vaccine delivery.

Drugs derived through biotechnology now are used to treat a wide range of diseases, including: autoimmune disorders, blood disorders, cancer, cardiovascular disease, endocrine disorders, genetic disorders, infectious disease, reproductive disorders, immune function disorders, and hepatitis A and B. PR

The next article in the series will discuss genetic therapies.

ARTICLE SUMMARY

- Since ancient times, humans have used microorganisms in various ways.
- Biotechnology may be defined as the application of scientific knowledge to transfer beneficial genetic traits from one species to another to enhance or protect an organism.
 - Biotechnological processes have numerous medical applications.
- The human cell is composed of several major structures.
- The cells of living creatures contain large quantities of DNA.
 - DNA contains the code for producing all the organism's genetic traits.
 - DNA's essential roles are cell replication and production of proteins.
- Proteins are complex molecules, formed from amino acids, that perform a number of roles in the body.
- rDNA technology is used for massive replication of a desired protein.
- Cytokines are a key group of proteins being developed through rDNA technology for therapeutic use.
- Vaccines created using rDNA technology introduce plasmid DNA directly into individuals to elicit an immune response.
- Delivery and administration of vaccines in the form of edible transgenic fruits, vegetables or even grains has become a real possibility.
- Drugs derived through biotechnology are now used to treat a wide range of diseases.