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Biotechnology: An Overview

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This issue of *Science* is devoted to a broad sampling of the status of a revolution in applied biology. In applications of recombinant DNA and hybridomas, progress has been rapid. As a result, substantial improvements in human and animal health care will occur in the next few years. Human insulin was released for sale in September 1982. Among the other products that have been created, most of which are under clinical or animal test, are a dozen or more subtypes of interferon, human growth hormone, human calcitonin, human albumin, thymosin alpha-1, tissue-type plasminogen activator, porcine and bovine growth hormones, a vaccine for foot and mouth disease, and a bovine leukocyte interferon. Earlier, many of these products, such as interferons, could be obtained only in tiny amounts.

Other areas of applied biology are in earlier phases of development. Protein engineering to produce superior catalysts is doable and is being tackled with vigor. Progress is being made in tissue culture of plants, and ultimately recombinant DNA techniques will be applied in this area. Advances also have been made in industrial fermentations. Microorganisms are being employed to produce antibiotics and an increasing number of specialty chemicals such as amino acids. Ultimately, they will be used in the production of large volumes of commodity substances such as oxychemicals.

This potentially rich harvest is the result of wise investments in basic biological research by the National Institutes of Health. Other consequences of NIH support have not been emphasized. Irvine Page has been wont to speak of the chemotactic effect of money. NIH funds combined with the attraction of research opportunities in biomedicine have lured many of the most intelligent and creative scientists into fundamental biology. Many of these people are now key members of the new dynamic bioengineering companies. NIH funds also have created a market for instrumentation and special products used in research. The instrumentation available in this country is superb and is constantly being improved. Companies providing items used in research, such as enzymes, follow trends closely and are quick to supply needed materials, cultures, or animals.

Gene Splicing

A technique that is basic to much of the progress in applied biology is gene splicing. In concept it is simple, but in practice it is complex. A major objective of recombinant DNA techniques is to insert a foreign gene for a desired product into an organism under conditions such that the foreign gene will be expressed more abundantly than the native genes. A favorite organism for this purpose is a mutant of *Escherichia coli* that is unable to synthesize the essential amino acid tryptophan. There are circular forms of DNA (plasmids) that contain a gene for making tryptophan. A foreign gene can be spliced into these plasmids near the tryptophan gene in such a manner that the foreign gene and the tryptophan gene will be expressed simultaneously. Placed in a culture of the mutant *E. coli*, some of these plasmids enter the bacterial cells and, by supplying the needed tryptophan, enable them to survive and multiply. Mechanisms within the *E. coli* are such that a new plasmid may be replicated 20 to 40 times within each bacterial cell. Since tryptophan is in great demand, the gene for it and simultaneously the foreign gene are preferentially expressed.

The tryptophan mutant of *E. coli* is not always the best choice for making recombinant DNA products. Other mutants of the organism may be more convenient, or plasmids of other prokaryotes can be used. But *E. coli* will continue to be used for the synthesis of many proteins. A large body of information and know-how has been accumulated about it, and it has been found to make virtually all the proteins now undergoing clinical tests. Thus, when Genentech decided to seek to prepare a vaccine for rabies, they investigated the possibility of preparing it by recombinant DNA procedures involving *E. coli*. That decision was made despite the fact that the coat protein of the virus contains carbohydrates. *Escherichia coli* could synthesize the protein chain, but not the carbohydrates. In this issue, Elizabeth Yelverton and her colleagues provide a detailed account of procedures leading to proteins that conform by biochemical and antigenic criteria to rabies glycoprotein.

In addition to its synthetic limitation, *E. coli* has other drawbacks. Mutant forms tend to revert to wild types. The densities to which cultures can be grown are limited. *Escherichia coli* produces an endotoxin. It generally does not secrete proteins into the medium. In contrast, yeasts, which also have plasmids, can be grown to very high densities, are stable with respect to mutation, produce no toxins, and can secrete proteins. In addition, they are eukaryotes and can synthesize glycoproteins. Because of these attractive features, a number of companies have been using yeast for recombinant DNA work. Hitzeman and colleagues have employed *Saccharomyces cerevisiae*. In this issue they describe the procedures that resulted in the secretion of interferon, a desired product.

Discovery of the mechanisms by which genes are turned off or on in higher organisms, such as humans, is one of the major challenges of biology. Liver and skin process the same DNA, but there are controls on its transcription that make the tissues quite different. Delineating these processes would be of transcending importance in fundamental

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biology. In studying controls on the expression of eukaryote genes, the transcription of simian virus 40 has been used as a model system. Weiher, Konig, and Gruss have identified nucleotide sequences that influence transcriptional activity.

Before recombinant DNA products can be brought to market, they must be subjected to extensive clinical tests. The identity and quality of the product must also be established beyond reasonable doubt. In this issue, Johnson describes some of the laboratory procedures employed in providing the chemical information necessary for the Food and Drug Administeration to release human insulin for general use.

Monoclonal Antibodies

It is now possible to create long-lived cloned cell lines that produce unique antibodies. The cells have been used to prepare antibodies against many viruses, bacteria, fungi, and parasites. The technique is being employed to produce diagnostic aids, more than a score of which have already been released by the Food and Drug Administration. The cell lines are prepared in vitro by chemically mediated fusion of lymphocytes from immunized mice and a mouse tumor (myeloma) cell line. The hybrid cells have antibody-producing capability from the lymphocyte and ability to grow permanently in culture from the myeloma. Nowinski and colleagues have used the techniques to produce antibodies for diagnosis of some diseases that are sexually transmitted in humans.

Diagnosis and treatment of the various diseases known as cancer is a continuing goal. Monoclonal antibodies have been prepared against forms of cancer. It has been found that some cancer cells are considerably more antigenic than corresponding normal cells—that is, antibodies find more sites to occupy on the cancer cell surfaces than on normal cells. In this issue Vitetta, Uhr, and co-workers describe experiments in which antibodies are used as vehicles to bring a toxic chemical selectively to cancer cells.

Protein Engineering

Given a set of instructions furnished by a gene, organisms can synthesize proteins corresponding to the gene. The template may be obtained from another life form, or it can be an artificial gene. We are now in the beginning phase of exploitation of the ability to engineer proteins. Objectives are to create superior enzymes for use as catalysts in the production of high-value specialty chemicals, to produce biologicals that are superior to natural ones, and to produce enzymes for large-scale use in the chemical industry.

The necessary genes are synthesized by joining nucleotides together. This takes time and effort, but at AMGen a 500-member oligonucleotide for a γ-interferon was made in 2 months by a two-person team. Later it became feasible to modify portions of the interior of this gene, and a variety of artificial γ-interferons were expressed in *E. coli*.

The number of potential proteins approaches infinity. In moving out into the unknown, a good starting point is furnished by natural proteins with known functions. In the future, as new proteins are isolated by the current powerful separative methods, their amino acid sequences will be of fundamental interest. In this issue, Hunkapiller and Hood describe the performance of a new modification of the Edman procedure. Automated microsequencing of proteins can be carried out on samples as small as 5 to 10 picomoles. For a given sample, successful runs are limited to 30 to 70 cycles. The sequence of the remainder of a protein can be determined after it is fragmented by chemical or enzymatic methods to generate a set of overlapping peptides. Another way to obtain the amino acid sequence of a protein is to analyze the nucleotide sequence of the gene that codes for the protein. This is very useful when the gene is available.

Sutcliffe, Lerner, and colleagues describe work which extends Lerner's observation that relatively small peptide chains from a protein antigen can elicit an antiserum against the native protein. Using a virus genome sequence as a blueprint, they synthesized peptides which corresponded to different regions of the genome. From immunological tests they established some primitive rules. Peptides that were extremely hydrophobic and those with six or fewer residues were ineffective. Longer, soluble peptides, particularly those containing proline, were effective. This study was extended to other viruses and the results conformed to the rules. Further studies showed that in some, but not all, instances, peptide fragments were capable of serving as effective vaccines.

Ulmer's article in this issue is an interesting analysis of the opportunities and techniques available for protein engineering. In nature, specific catalytic functions are served by enzymes of differing properties. For example, enzymes of thermophilic organisms can withstand temperatures approaching 100°C, while those of mesophilic organisms may be destroyed at much lower temperatures. Great differences among organisms exist with respect to turnover rates, *p*H optima, and other characteristics. The testimony from evolution is that it is possible to select compositions and structures that are superior for particular applications. Through the use of synthetic genes and recombinant DNA techniques, it is now possible to begin to improve on the evolutionary process. In this effort x-ray crystallography and computer capabilities, including graphics, will be important. Such studies will provide solid knowledge about the aspects of protein structure that are crucial to protein functions. Many stable and useful industrial catalysts will be produced.

Agricultural Research

Plant breeders such as Borlaug have worked diligently for about four decades and have been able to obtain varieties in which such characteristics as yield, pest resistance, and nutritive qualities are greatly enhanced. Their techniques will continue to be important, but will be supplemented by new ones now under development.

Tissue culture techniques for growing plant cells have been very successfully developed. Already plants produced in tissue culture are being sold commercially. In this issue, Chaleff reviews work on tissue culture and notes that when single cells are allowed to proliferate, the progeny often have a chromosome content that differs from the original. Polyploidy, aneuploidy, and chromosomal rearrangements have been identified. Additional evidence is furnished which makes it clear that tissue culture will facilitate development of many strains of plants, some of which will probably prove superior. Masses of cells respond to herbicides or plant toxins as mature plants would respond.

Another method of creating new plants is to bring together chromosomes from two different plant species. This was done in the 1960's with wheat and rye. The first plants were inferior with respect to yield and other characteristics, but breeding programs have now achieved varieties that in important respects, such as tolerance to poor soils, are superior to wheat. At the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) crosses of wheat and wild grasses are under study.

Shepard and associates describe work with protoplasts which also yields new forms. The protoplasts are prepared by removing cell walls that are largely cellulosic. When brought together, two protoplasts fuse and the new entity contains nuclear material from both. In this way sexually incompatible pairings can be made. Combinations of unrelated genomes are regularly followed by elimination of chromosomes from either or both of the parental cell lines. However, total elimination of chromosomes from either species does not necessarily occur. Thus, there is a potential for the creation of novel plants. A more immediate possibility is the use of protoplast fusion to create hybrids between related but sexually incompatible species. In this way, a broad spectrum of resistance to disease in a primitive species might be incorporated into a commercial cultivar.

Barton and Brill examine the subject of plant genetic engineering with the hope of using recombinant DNA techniques to improve genomes. Progress will be slow at first because the state of knowledge of plants lags behind that of animals and some microorganisms. Cloned DNA is routinely transferred between microorganisms, but lack of comparable vector systems has inhibited similar experiments in plants. However, progress is now being made in this area. Also lacking has been a transformation marker—a gene present on a vector that permits easy identification of transformed cells. This problem seems solvable, but obstacles remain. Success in plant genetic engineering will rely on a thorough knowledge of the genetics and regulation of the traits to be transferred. At this time, the mechanisms of regulation are not understood. Barton and Brill provide examples which illustrate the problems involved in making a substantial change, such as incorporating nitrogen fixation in plants. As an alternative approach, they emphasize the possibility of improving microorganisms that fix nitrogen and are associated with roots of plants. Other major objectives that seem possible are improvement of photosynthesis and pest and pathogen resistance.

Much of the earth's surface is not suitable for agriculture, but can support growth of trees. Ultimately, forest yields will be substantially increased. In addition to the present product slate, wood will be available for energy and feedstocks to produce food or chemicals. In this issue, Farnum, Timmis, and Kulp describe how forest yields are being greatly improved and estimate what can be attained eventually through good forestry practice. They also discuss tissue culture proliferation of superior trees, which shortens the time required to achieve superior forests.

Microbiological Engineering

For several decades, industrial microbiology has been producing antibiotics. Excellent antibiotics have been discovered and, through a laborious process involving mutagens and selection, their yields have been substantially increased. The major pharmaceutical firms have equipment and expertise for effective large-scale production with microorganisms, once the appropriate strains are available. For the production of small-volume, high-value specialty chemicals, small companies will be competitive. One can anticipate that many new products will emerge fairly soon. Many vectors exist for the introduction of genetic material into host microorganisms, and generation times are short. Vournakis and Elander describe some of the methods being employed to increase production of antibiotics and to discover new ones. Current screening methods are more efficient than those employed earlier, and knowledge of detailed synthetic pathways is applied. Protoplast fusion of related and correlated organisms has led to new products. Use of recombinant DNA techniques has begun.

Over the past 30 years, the pharmaceutical industry focused largely on the production of antibiotics through fermentation. Many substances isolated from the fermentation of beer were inactive as antibiotics. However, many were found to have other applications, which Demain surveys in this issue. For example, nearly a score have physiological effects in humans that give them medicinal value. Another set of these substances is effective against nematodes. One fermentation product, monensin, is a potent coccidiostat in chickens. In addition, when monensin is incorporated in the feed of ruminants, it suppresses the formation of methane, thereby improving dietary efficiency. A scanning of Demain's compilation of microbiological products must leave the reader with admiration for the microbes' capabilities.

Bacteria, viruses, and fungi grow in a wide variety of circumstances. Some favor insects as a medium, often secreting toxins within them. Miller, Lingg, and Bulla discuss the increasing use of microbiological insecticides. In general, they are ecologically much more benign than the chemicals that have hitherto been employed.

Earlier in this overview, I mentioned the prospects for protein engineering and cited possible applications in the production of superior enzymes to catalyze specific reactions. In practice, it is usually desirable to immobilize either the separated enzymes or the cells containing them. Klibanov describes techniques for doing this and cites the advantages that accrue. For example, the enzymes are not lost and their stability is often improved when they are chemically bound to a support. Immobilized enzymes are being used commercially. Glucose isomerase is being employed on a large scale to convert corn sugar (glucose) into a mixture of glucose and fructose.

It should be evident that, in future, fermentation will have an increasing role. For many products it will become competitive with conventional chemical processing. The decisive consideration will be comparative costs. In biological processing, bioreactors will have a key role, and Cooney discusses design factors for these reactors.

Current annual production of organic chemicals is close to 100 million metric tons, about 99 percent of which is oxychemicals. Many of these could be produced by microbial fermentation with or without chemical processing. These chemicals and their derivatives represent nearly half of the total organic chemical production and their current value is over $15 billion. America's largest chemical company, DuPont, owns abundant reserves of hydrocarbons and coal, but it is conducting substantial research on the conversion of biomass into products. In this issue, Ng, Hardy, and colleagues provide an assessment of feedstocks, costs, potential products, and separative processes. They point out that ethyl alcohol produced by fermentation is today cost-competitive with that obtained from ethylene. They mention fermentations resulting in acetic acid, acetone, *n*-butanol, and isopropyl alcohol. One of the handicaps of fermentation is that it is generally conducted in dilute solution. Accordingly, the energy and dollar cost of isolating products is a burden. With time, improvements in bioengineering are likely to make the picture more favorable.

Many microorganisms have fastidious requirements for carbon and energy sources. But one or more organisms can prosper by using a large number of organic chemicals, CO, CH_3OH, CH_4, $CO_2 + H_2$, and others. This capability can be exploited to produce single-cell proteins that are useful for animal and human food. The subject is reviewed by Litchfield in this issue.