# Genetic Engineering and Its Impact on the Food Industry

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I would like to thank the organizers of the Reciprocal Meat Conference and especially Dr. Michael E. Dikeman for inviting me to speak on Genetic Engineering and its impact on the food industry. Since 1972 when the first gene was cloned by recombinant DNA techniques, the field has had an impact on every area of molecular biology and holds great potential for the commercial sector. I hope, in this short presentation today, to convey some of the excitement shared by scientists in this field, review the basic technology, discuss some of the "problem" areas that remain to be solved, and indicate how this technology may be applied in the food industry.

At first, it may appear that the field of molecular biology has little application to the meat industry, however, the potential application of this technology was recognized earlier by the members of the Reciprocal Meat Conference when they invited Dr. Don Carlson to discuss recombinant DNA as a tool in animal research at the 33rd annual meeting in 1980. In that review, (Carlson, 1980) Dr. Carlson defined the field and indicated some of the applications. Since then, the field has advanced so far that it would now be appropriate to consider what has been achieved with recombinant DNA techniques and to briefly review the history of the technology to put these advances in perspective to this new field.

The field of recombinant DNA can trace its history back to the fundamental studies of Avery, MacLeod, and McCarty (1944) when they documented that DNA was indeed the "transforming principle". However, it was not until 9 years later that Watson and Crick (1953) elucidated the structure of DNA. Another 17 years passed until the seminal discovery, in 1970 by Dr. Hamilton Smith, and his co-workers, (Smith and Wilcox, 1970; Kelly and Smith, 1970) of the existence of site-specific restriction endonucleases. It was the coming together of the fields of DNA chemistry, molecular biology of extra-chromosomal elements and enzymology of nucleic acids that allowed Dr. Paul Berg and co-workers to construct the first recombinant DNA molecule (Jackson et al, 1972). Shortly thereafter in 1973, Cohen et al cloned the first gene by recombinant DNA techniques and expressed it in a foreign cell. The field developed at an explosive rate and by 1979 a human gene composed of naturally occurring nucleotide sequences joined to a chemically synthesized oligonucleotide was cloned and expressed in *E. coli* (Goeddel, et al 1979) This was truly a remarkable feat to be accomplished in such a short time.

The basic cloning technology is illustrated in Figure 1. DNA isolated from chromosomes can be cut or cleaved by restriction endonucleases and joined to vector molecules or plasmids by the use of DNA ligase. The site specificity of the restriction endonucleases allows the short single stranded sequences to hybridize specifically with each other to form the appropriate substrate for DNA ligase. Following ligation, these molecules are then introduced into *E. coli* or some other appropriate host microorganism to replicate and express the newly introduced foreign DNA or cloned genes.

Figure 1

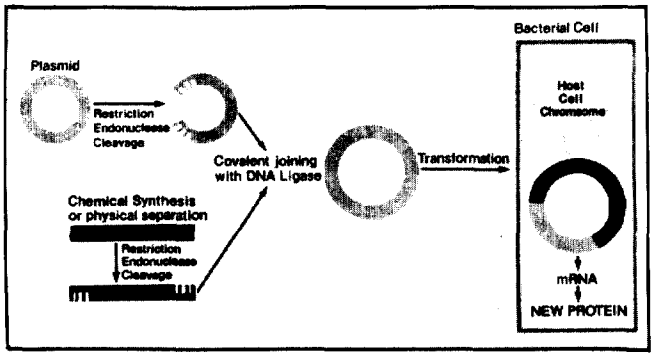


Figure 1. General scheme to clone Foreign DNA sequence.

If the process is examined more closely, the importance of the discovery of restriction endonucleases cannot be over emphasized. With the use of these enzymes, it became possible, for the first time, to prepare large quantities of uniformly fragmented DNA. These enzymes recognize a specific nucleotide sequence in the DNA and hydrolyze the phosphodiester bond to yield uniform molecules. There are now over 100 such enzymes that recognize specific sequences. Two of these enzymes that Frank Young, I, and our associates were fortunate enough to discover illustrate the specificity and usefulness of these enzymes (Wilson and Young, 1975; Wilson and Young, 1976). *Bam* H1, an enzyme isolated from *Bacillus amyloliquefaciens* is one that recognizes the hexa-nucleotide double-stranded sequence GGATCC/CCTAGG and hydrolyzes the phosphodiester bond between the two guanine nucleotides, leaving a single stranded tetra-nucleotide. As illustrated in Table 1, when DNA is treated with another enzyme, *Bg1* II, from *Bacillus globigii*, DNA is hydrolyzed leaving the same single-stranded tetra-nucleotide. The common tetra-nucleotide that remains after treatment with either of these two

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**Table 1**
**Restriction Enzymes from Bacilli**

| *Endonuclease* | *Recognition Sequence* | *Product of Ligation* |
|---|---|---|
| Bam HI | G GATCC<br>CCTAG G | GGATCC<br>CCTAGG |
| Bgl II | A GATCT<br>TCTAG A | AGATCT<br>TCTAGA |
| Bam/Bgl Fusion | | AGATCC<br>TCTAGG |

enzymes allowed Duncan et al (1978) to select directly for recombinant hybrid molecules. Basically, he demonstrated that it was possible to treat the vector DNA with *Bam* H1 and the chromosomal DNA with *BG1* II. Following hybridization and ligation, three products were formed: 1) circularized vector DNA; 2) circularized chromosomal DNA, and 3) the desired hybrid molecule consisting of the vector DNA and the chromosomal insert. The first two products could be eliminated when the hybridization and ligation reactions were allowed to proceed in the presence of both *Bam* H1 and *Bg1* II. Since hybrid site was AGATCC/TCTAGG, neither *Bam* H1 nor *Bg1* II could recognize the hybrid junction. This example illustrates that it is possible, even without selective techniques, to be able to insert foreign pieces of DNA specifically into restriction sites of plasmid vectors.

DNA fragments that were generated after treatment with the endonucleases could be readily separated on the basis of size by agarose-ethidium bromide electrophoresis, a procedure developed by Sharp, Sugden and Sambrook (1973). The combination of the use of restriction endonucleases and separation procedures in agarose gels made it possible to isolate specific DNA fragments that harbored unique genes. Preparations of DNA obtained after these procedures are quite homogeneous as indicated in Figure 2. This figure illustrates a 1.8 kb fragment from *B. subtilis* that was cloned into a plasmid by E.M. Rubin in our laboratory at the University of Rochester. Fragments such as these are typical of purified DNA sequences that can be obtained by these techniques. All that remains in the cloning procedure is to insert these fragments into an autonomously replicating plasmid to allow expression of the foreign gene.

Figure 2

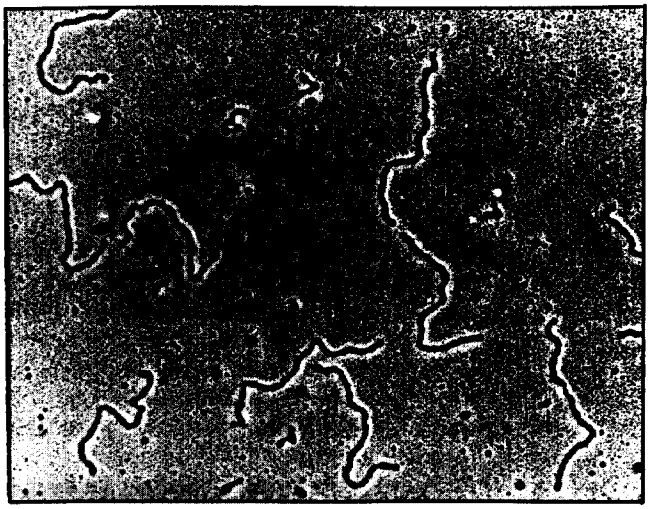

Figure 2. Electron micrograph of DNA fragments isolated after treatment with a restriction endonuclease.

The plasmid vectors themselves have been constructed to facilitate cloning in a number of diverse species. A typical shuttle plasmid that was constructed by Ehrlich (Primrose and Ehrlich, 1981) is illustrated in Figure 3. This plasmid contains origins of replication that allow the plasmid to be expressed in both *Bacillus subtilis* and *E. coli.* In addition, it contains three antibiotic resistance markers, chloramphenicol resistance, tetracycline resistance, and ampicillin resistance. These antibiotic resistances are particularly useful for cloning fragments of foreign DNA since they allow an easy screening procedure. The general method is referred to as insertional inactivation and is illustrated in Figure 4. The plasmid pHV33 is treated with *Bam* H1, thus cleaving the tetracycline resistance gene. This treatment leaves a tetra-nucleotide extension that can

Figure 3

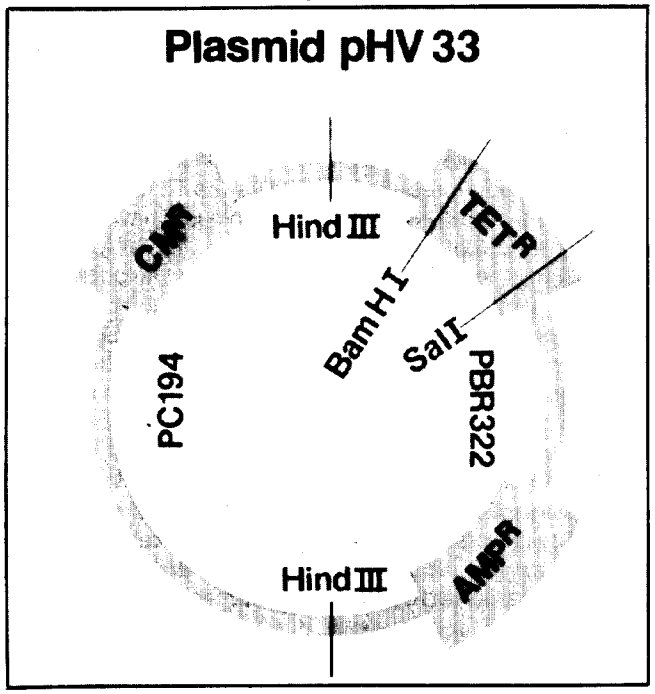


Figure 3. Structure of shuttle vector pHV33.

Figure 4

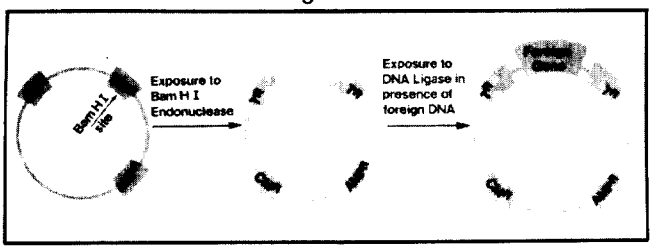


Figure 4. Insertional inactivation of tetracycline resistance gene.

hybridize to fragments of foreign DNA. Due to the insertion of foreign DNA into the tetracycline resistance gene, the gene is inactivated and the bacterial cell that receives this new chimeric plasmid can be recognized by the fact that it is sensitive to tetracycline and resistant to chloramphenicol and ampicillin. Without the insertion of the foreign DNA, upon ligation, the tetracycline resistance gene could reform exactly and restore the tetracycline resistance to the transformed cell.

Vector plasmids have also been designed to lack a replication function in particular cells. Therefore, in order to be maintained by the cell and express the foreign DNA, the plasmid must integrate into the host chromosome since it lacks the ability to replicate autonomously. The exact mechanism by which the plasmid integrates into the chromosome is unknown. However, from the work of Duncan et al (1978) plasmids that are not capable of replication in *Bacillus subtilis* integrate into the host chromosome through areas of homology. Not only is the foreign gene integrated into the chromosome, but the entire plasmid now resides in the chromosome and the cell can express the foreign DNA as if it had been part of the original chromosome. By the techniques I have outlined, it should be possible to select any sequence of DNA, insert it into a plasmid, and have it expressed either on autonomously replicated plasmids or as part of the host chromosome. In some cases, however, the bacterial cell is not capable of reading the sequences on the foreign DNA that control the expression of the cloned gene. These control signals regulate the activity of the gene and the ability to recognize these regulatory sequences may be a unique property of the host cell. Therefore it has become necessary to develop a wide variety of recipient hosts for the foreign DNA. No one particular host will be universally acceptable for the production of foreign products from cloned DNA sequences. For example, expression of foreign genes has been most successfully achieved in the bacterium *E. coli.* However, due to the nature of some of the cell wall constituents, and the inherent problems in disposing of the *E. coli* cells following fermentation, *E. coli* will probably be the organism of choice only for products that have a high profit margin. In other cases, a Gram-positive microorganism, such as *Bacillus subtilis* that is non-pathogenic, free of toxins, and excretes proteins into the medium may be the host of choice for commodity products since the foreign products can be excreted into the fermentation beer. Thus industrial enzymes such as amylase may be produced more economically in *Bacillus subtilis* rather than *E. coli*, since industrial enzymes are generally not highly purified. In other cases, such as in the production of human growth hormone, expression may be maximized in *E. coli* and due to the necessary purification and separation techniques that will be required, foreign substances that would be contributed by the host organism will be removed and therefore are of no significance in the final product. For these reasons, a number of hosts have been developed for recombinant DNA techniques. Among the most promising candidates are *E. Coli*, *Bacillus subtilis*, the common yeast, *S. cerevisiae*, animal cells in tissue culture, and Streptomyces. Consideration has been given to plant cells to produce certain products in plant tissue culture or to modify agricultural crops. However, the vectors appropriate to plant cells are not as widely developed as those developed for bacterial cells.

Now that we have considered all of the components necessary for gene cloning: the isolation of foreign DNA which contains a desired gene, the purification and modification of the bacterial plasmid to accept the foreign DNA, the recipient host to express the foreign DNA and finally the expression and isolation of the product itself, it is time to examine the applications of recombinant DNA technology.

Although no product has been commercialized by recombinant DNA techniques, a variety of proteins, enzymes and small molecular weight compounds have been either produced by recombinant DNA techniques or the yield of production has been increased due to recombinant DNA means. One of the early targets of recombinant DNA technology was the production of enzymes in bacterial cells. This choice is logical since a single gene often encodes a polypeptide chain or protein or enzyme. The greatest impact that Genetic Engineering will have in this area will be the introduction of novel enzymes to be produced by bacterial cells. Human growth hormone has already been demonstrated to be produced by bacterial cells. It is now possible to isolate more human growth hormone from a liter of fermentation culture than from a single human pituitary gland. The impact of this achievement is obvious. The cost savings and the high yield should result in a less expensive, more highly purified, human growth hormone.

Examples of some of the proteins that have been produced in bacterial cells by recombinant DNA techniques are listed in Table 2. Human insulin, human interferon, human growth hormone, and human urokinase are all enzymes that may be the pharmaceuticals of the 1980's. The human derived proteins are thought to be superior because they will be less antigenic in the recipient population. In addition, human growth hormone is species specific and therefore porcine or bovine growth hormone cannot be used to treat people that are deficient in the production of this hormone.

**Table 2**
**Examples of Poly-Peptides Made by Recombinant DNA/Fermentation Processes**

| |
|---|
| HUMAN INSULIN |
| HUMAN INTERFERON (SEVERAL TYPES) |
| HUMAN GROWTH HORMONE |
| HUMAN UROKINASE |
| BOVINE GROWTH HORMONE |
| ANTIGEN FOR FOOT AND MOUTH DISEASE VIRUS |
| ANTIGEN FOR HEPATITUS VIRUS |

One of the interesting applications of the use of recombinant DNA technology has been in the production of antigens from highly infectious or difficult-to-propagate viruses. In the case of Foot and Mouth Disease, the virus is not allowed into the United States for production or experimental purposes. Prior to the advent of the recombinant DNA technology, the infectious virus had to be propagated to be used as the antigen for vaccination. It is possible, instead of using the entire virus, to clone the genes that express the antigens specific for the Foot and Mouth Disease virus. These antigens can then be expressed in bacterial cells and either the antigens or the

bacterial cells can be used as a vaccine and circumvent the use of the infectious virus.

A different reason for the use of recombinant DNA techniques is illustrated in the case of the hepatitis virus. It has not been possible to propagate the hepatitis virus under laboratory conditions. Cloning of the antigens for this particular virus for use in a vaccine has been shown to be possible by recombinant DNA techniques. In the future, we will see many more vaccines produced by recombinant DNA techniques due to the infectious nature of whole virus, or our inability to produce large amounts of the virus under laboratory conditions.

In the food processing industry, a number of commercially important microbial enzymes have been considered to be candidates for recombinant DNA research. These industrially important enzymes are listed in Table 3. The estimated sales of these enzymes, that are used in the food processing industry, is over $200 million dollars, annually. Although these enzymes represent those that are currently most widely used in the industry, recombinant DNA offers the possibility that additional enzymes that are not produced commercially, to be

**Table 3**
**Industrially Important**
**Microbial Enzymes**

| | *Tons of Enzyme Produced* | *Estimated Sales ($ MM)* |
|---|---|---|
| *Bacillus* Protease | 500 | 88 |
| Glucoamylase | 300 | 31 |
| *Bacillus* Amylase | 300 | 26 |
| Glucose Isomerase | 50 | 26 |
| Microbial Rennin | 10 | 15 |
| Calf Rennin | 29 | 40 |

available to the food industry in the future. For instance, collagenases and elastinases that are used in the flaked and formed meat processing industry could perhaps be produced by fermentation rather than by isolation from tissue organs. In addition, these enzymes and those of the future could be modified to more specifically meet the needs of the industry. They could be made more heat labile or heat resistant, or be combined with other enzyme activities to more thoroughly complete the processing step for which they are intended.

Enzymes are not the only products that are used in the food processing industry. A few of the commercially significant bioscience products, that either could be altered by recombinant DNA technology or produced more economically, are indicated in Table 4. Collectively, these products constitute a significant market in the food processing or animal feed areas. While many of these products are already produced by biotechnology, new products that could fulfill the same function in the process may be produced by recombinant DNA techniques in the future. In many cases, the yields of these bioscience products have been increased by recombinant DNA techniques.

In addition to the obvious impact to the pharmaceutical industry, two other industries will find wide application of bioscience products. These two industries are the food manufacturing industry and the agricultural industry. In Tables 5 and 6, I have indicated some of the applications of bioscience products to these two major industries. Amino acids are already being used as flavoring agents and as feed additives. If recombinant DNA techniques can be applied to increase the yield of amino acids in the fermentation, we will see increased use of amino acids as feed supplements. One group of products that has received considerable attention is that of peptides, such as hormones for the agricultural industry. It is anticipated that these natural products will fulfill the same

**Table 4**
**Market Values of Selected**
**Bioscience Products**

| | *$ MM* |
|---|---|
| Enzymes | 218 |
| Citric Acid | 575 |
| Amino Acids | 1703 |
| Vitamins | 668 |
| Antibiotics | 4240 |
| Nucleotides | 72 |

**Table 5**
**Applications of Recombinant DNA**
**Technology to Food Manufacturing**

| *Product* | *Application* |
|---|---|
| Amino acids | Food enrichment and flavoring agents |
| Vitamins | Food enrichment |
| Petrochemicals | Food preservatives (e.g. benzoic and propionic acids) |
| Short peptides | Artificial sweeteners (e.g. aspartame) |
| Enzymes | Manufacturing processes (e.g. amylases and glucose isomerase) |
| Nucleotides | Flavoring agents |

**Table 6**
**Applications of Recombinant DNA**
**Technology to the Agricultural Industry**

| *Product* | *Application* |
|---|---|
| Amino acids | Feed additives |
| Vitamins | Feed additives |
| Petrochemicals | Feed preservatives (e.g. sorbic acid) |
| Antibiotics | Feed additives and prophylactics |
| Peptide hormones | Growth promoters (e.g. bovine growth hormone) |
| Viral antigens | Vaccines |
| Enzymes | Feed additives (e.g. papain) and feed preservatives (e.g. glucose oxidase) |
| Pesticides | Insecticides (e.g. Bacilli spores) |
| Gases | Fertilizer (e.g. due to ammonia) |

function that antibiotics did in feed efficiency and weight gain. In addition, a number of micro-organisms are being altered by recombinant DNA means to overproduce insecticides and pesticides, and these may replace some of the petrochemical products that are in current use today.

The examples I have selected for the application of recombinant DNA products to the food and agricultural areas are not complete. In the next decade, we will see many new products replacing the traditional ones that are used in the industries. The field of recombinant DNA technology to the commercial sector has great promise. We will see new products produced economically and widely applied in fields that are dominated now by petrochemical products and products derived from expensive substrates. I truly believe that the 1990's will be regarded as the biotechnology years, due largely to the impact of recombinant DNA on our abilities to produce biological compounds by fermentation processes. This period is an exciting one and one that gives us the opportunity to reshape our traditional fields of food and agricultural industries to take full advantage of the full advances in biotechnology.

## Discussion

*Andy Milkowski, Oscar Mayer Foods Corp.:* In what type of time scale do you see a lot of genetic engineering products coming on the market with respect to replacement of animal by-products as sources for pharmaceuticals? When human insulin is produced by gene splicing technology, pancreas glands will no longer become a salable product to the meat producer and they will take a loss in dollars in terms of what their animal is used for.

*Wilson:* The point you raised is significant. It affects not only the meat producer, but also has an impact on the sales of any by-product that is used as a source for pharmaceuticals. For instance, a number of factors including serum albumin are extracted from plasma. If one of these factors becomes available through gene cloning technology, then the profits that can be made on other factors that are extracted from blood may not justify purchasing the raw material to extract the limited other products. Another aspect is the amount of money spent on research. For instance, if a company is currently producing and selling a particular product from a raw material they may find it prudent to invest considerable research resources in producing the product by recombinant DNA means to maintain a competitive position. I would speculate that a number of companies have entered the recombinant DNA field from a strictly defensive position to maintain their current product line.

The time scale that will be required to place a product made by genetic engineering on the market consists of two factors. The first is the amount of time it takes for research and development to have a product ready for production. And the second contributing factor is the amount of time it takes to have the pharmaceutical approved by the regulatory agencies so that it can be sold. In many instances, the time required for research and development has been three to five years for a pharmaceutical derived by genetic engineering. The amount of time that will be required to test the product before it is approved by the regulatory agencies is at this time uncertain. In the case of insulin, the FDA has not determined whether insulin produced by recombinant DNA means is a new drug and therefore must go through the full regimen of testing or if less extensive testing will be required due to the nature of the product itself. I would speculate, however, that to bring a pharmaceutical that has been derived by recombinant DNA means to market will not take less than five years nor more than ten years in most cases.

*R. G. Kauffman, Wisconsin:* I would like to ask you two questions. You didn't say anything about the major limitations such as the cost of energy to do this. Second, what would be the ultimate potential of this sort of thing? You have talked about a number of potentials but, in your imagination and from where you see it, where will we be 50 years from now and will we have a meat industry, for instance?

*Wilson:* In the near future, I do not see recombinant DNA technology endangering the existence of the meat industry. I would imagine that in three to five years there will be a rather inexpensive source of single cell protein but I do not know what impact this would have on the whole meat industry. As far as the technology is concerned, the major limitations are on the yield of the product by fermentation. The product may be produced in considerable amounts in a test tube but one may find it difficult to scale up to a 40,000 liter fermenter. In addition, some of the products produced by recombinant DNA techniques have not proved to be as stable as the product isolated from its natural source. Therefore, stability of the product and of the cloned gene in the host bacterium are presently limitations in the system. I do not believe that energy consumption is a limitation in the system. Considerable energy is expanded in the isolation of a product such as insulin; fermentation and isolation of the product from the fermentation beer will probably be less costly in terms of energy and raw material.

In my wildest imagination, I can imagine that recombinant DNA technology will affect many different areas. For instance, we could be witnessing the production of protein as a food source that was produced strictly by fermentation. One could imagine that we would be entering an entirely new field of medicine where recombinant DNA technology would be used in the treatment of disease. One could imagine that the gene for insulin production could be inserted into a diabetic individual somehow so it could be regulated to produce insulin as needed. This would be far better than providing insulin by injection. One could imagine that the treatment of diabetes would evolve from a single injection of insulin to small amounts of insulin injected by a computer that senses the amount of blood glucose and compensates for this by small injections of insulin. These two methodologies are currently being tested. The next step might be to construct a biological reactor that actually provides insulin to the organism on the basis of the blood glucose. This may take the form of a small fermenter located within the body. Finally, we may witness the incorporation of a cloned gene that encodes insulin into the genetic structure of the cell itself to correct the metabolic defect.

The field of recombinant DNA technology is only ten years old and when it began, I did not have great expectations for the applications of recombinant DNA technology although I felt it would be a powerful investigative tool. If someone had said they could actually clone human genes, put them into bacteria and have them function, I would have suspected that this

advance would take twenty to forty years. I am really amazed at how fast the technology has advanced.

*G. Trout, Colorado:* A reasonable amount of genetic engineering effort has tried to incorporate oxygen fixation type genes into plants. Can you foresee any type of applications similar to this in the animal area? I don't mean putting nitrogen fixation genes into cattle, but it is separating that concept.

*Wilson:* Although the genes involved in nitrogen fixation have been cloned, they have not been incorporated into plant cells. It is conceivable that the energy requirements for the amount of nitrogen that can be fixed could not be supported by having the genes cloned into a plant cell. Unfortunately, no vectors are available for corn which is one of the major crops that could be aided by nitrogen fixation. I have not really considered how applications of this nature could be applied in animal cells unless it were to provide essential vitamins, such as ascorbic acid, that higher organisms are not capable of producing.

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