Biotechnology in 1990s

John J. Kopchick
Elizabeth Parks
Edison Biotechnology Institute
Ohio University
Athens, USA

1. Introduction

Biotechnology has been practiced in one form or another since the beginnings of the domestication of animals. Selective breeding techniques have long been used with both livestock and plants in order to propagate certain desired traits. The advent of molecular biology and recombinant DNA technology brought new developments not only to agriculture, but also to medicine and the basic sciences. Generally, the terms recombinant DNA technology, genetic engineering, and biotechnology can be used interchangeably. In this report, we will review the basic aspects of genetic engineering and discuss its applications to several scientific disciplines.

In the past twenty years, the field of genetic engineering as applied to biomedicine, diagnosis of disease, and improvement of food related products has proved to be valuable, as well as controversial. Food related products under development encompass a broad spectrum of commodities from genetically modified food producing animals and plants to food additives such as bacteriocins. A vide variety of therapeutic products, including growth hormone (GH), insulin, hepatitis B vaccine, and interferon have been synthesized via recombinant DNA techniques and have subsequently been approved by the United States Food and Drug Administration (FDA). Recombinant protein products such as these are being marketed for the treatment of a number of human disorders. To date fifteen therapeutic recombinant products have been approved for use in the United States, while hundreds of others are in various stages of clinical development. Additionally, human immunodeficiency virus, *Mycobacterium tuberculosis*, and *Salmonella spp.* are examples of pathogens for which recombinantly derived diagnostic procedures have been developed (11). Numerous other types of genetically engineered products are in various stages of research development and await FDA approval. Together, agricultural, therapeutic, and diagnostic products resulting from recombinant DNA technology will continue to benefit and revolutionize the fields of science and medicine and will remain economically important.

2. Recombinant DNA technology

Recombinant DNA technology refers to laboratory techniques that enable two distinct molecules or fragments of DNA to be spliced or "recombined". Usually, these two DNA molecules are 1) the DNA of interest and 2) the vector used for amplification in bacteria. For example, the DNA of interest, which encodes a particular protein, is spliced into a vector. This recombinant DNA is then amplified in bacteria and usually expressed in an appropriate cell system (such as mammalian, yeast, or bacterial cells) which facilitates the production of the recombinant protein.

Modern molecular biology also allows for the modifications, or site-directed mutagenesis, of DNA that encode a given protein, thus leading to alterations in the protein depending on the changes in the DNA. Protein analogs generated in this manner may be used to improve protein stability, modulate the biological activities of the protein, improve product pharmacokinetic properties, and simplify purification procedures.

3. Biotechnology and livestock enhancement

Selective breeding has long been employed in the food producing animal industry. The techniques of molecular biology in conjunction with selective breeding will lead to refinements in efficiency and composition of animal products for human consumption. Specifically, scientists are in the process of generating gene maps, identifying gene markers responsible for certain desirable traits and quantifying those traits. Animals with designated traits can then be selected and propagated. Identification and amplification of the disease resistance capabilities of livestock can be accomplished with the aid of biotechnology. These types of breakthroughs will become increasingly important to the global food supply due to our current population explosion and longer lifespans.

Strategies for animal growth regulation have the goals of increasing the total meat and milk production, improving the nutrient composition of animals products, and augmenting feed use efficiency. An important aspect of this program is to reduce carcass fat, increase lean meat, and enhance growth of animals used for meat production. An endogenous animal protein, GH or somatotropin, when administered to animals will achieve these ends. Currently, the most widely used method of increasing GH concentration in livestock is by way of exogenous chronic administration (12). Biotechnology has facilitated the production of relatively large quantities of pure GH at reasonable prices, as compared to pituitary derived GH. Animals receiving GH injection have improved production characteristics. For example, pigs have increased weight gain, increased feed efficiency, decreased lipid accumulation, and increased protein content (5,7). The most significant effect of GH administration in cattle is increased milk production (16). By combining traditional selective breeding with the recombinant derived products and gene mapping techniques, the enhancement of desirable animal production traits will continue.

4. Transgenic animal technology

Transgenic animal generation is an another means of achieving the genetic modification of food producing animals. Transgenic animals are those which have a new, or foreign gene, introduced into the germ line of the animal. The foreign gene ("transgene"), which may have been altered by site-directed mutagenesis, is then passed on to subsequent generations of animals. A variety of genes have been introduced into the germ lines of rabbits, sheep, mice, chicken, fish, and pigs (8,9). Several methods may be used to incorporate the foreign gene into the animal. These include direct microinjection of the DNA of interest into the pronucleus of fertilized mammalian eggs, retroviral mediated gene transfer, and embryonic stem cell (ESC) technology.

Direct pronuclear microinjection of a fertilized embryo is the principal means by which genes are transferred into mammalian embryos. Myriad lines of transgenic mice have been generated by this method for a wide variety of research purposes since the early 1980's (27). Employing this technology, we have used altered GH genes to determine the activity of GH *in vivo* (13).

Retroviral-mediated gene transfer involves the use of mammalian or avian retroviruses as delivery systems for foreign DNA into the genome of the animal cell. Typically, the retroviral vector is rendered replication incompetent or defective. Vectors such as these have been used to successfully transfer genes to genes to both chickens and mice (2). One slight risk inherent in this method is the opportunity for the replication defective virus to recombine with endogenous viral sequences and form new replication competent viruses.

Embryonic stem cell technology pertains to the use of *in vitro* cultured cells derived from an embryo, which when reintroduced into a developing embryo, can give rise to a population of cells in numerous tissues. Recombinant DNA can be introduced into the ESCs which are then used to create the transgenic animals. Mosaic animals are generated by this approach since only a portion of the tissues arise from the cultured ESCs. This technology has been successfully used in mice, and similar avenues of research for larger animals are being explored (15).

5. Transgenic livestock

Early research into transgenic farm animals involved the introduction of genes encoding GH. GH releasing factor, and insulin-like growth factor one (18,25). Several laboratories have produced GH transgenic pigs (17,26). The overall efficiency rate of transgenic pig production is much lower than that of mice. GH transgenic pigs did not have the enhanced growth phenotype typical for GH mice. However, the transgenic pigs did have a 30% decrease in backfat and were 15% more efficient at converting food into body mass. Interestingly, pigs that were given exogenous porcine GH (pGH) exhibit a similar outcome. Problems have been reported in transgenic livestock that express high concentrations of GH; including loss of appetite, arthritis, sterility, and diabetes (17). It has been suggested that these abnormalities may be due to the use of a heterologous transgene. In this regard, pigs expressing pGH (a homologous transgene) seem to be devoid of some of these problems (23). Future research in this area will focus on the establishment of healthy transgenic animals.

6. Transgenic animals in biomedical research

Livestock production is not the only important application of transgenic animals. Currently, a great deal of research employing transgenic animals is in the broad field of biomedical research. Again, the method most commonly used to generate transgenic is direct microinjection (27). Endocrinology research has benefitted in the past several years from the variety of emerging molecular biology technologies, including transgenic animals production. Mice which expressed foreign GH genes were among the earliest transgenic animals developed. Insulin-like growth factor one, insulin, and other genes which influence the endocrine status of the animal have also been successfully introduced using transgenic technology (13,24).

We have successfully used a structure/function approach in our laboratory to examine the mechanisms of GH action (13). Growth promotion, lactation, carbohydrate and lipid metabolism are among the wide array of biological activities associated with the hormone. The mechanisms by which GH coordinates the complex metabolic processes has not been fully elucidated. Using a site-directed mutagenesis approach, numerous GH analogs were developed. In transgenic mice, expression of wild type bovine (b) GH or human (h) GH genes caused an enhanced growth phenotype independent of the serum levels of GH. However, mice expressed certain GH analogs did not show an enhanced growth phenotype. Furthermore, expression of specific GH analogs (bGH-G119R or hGH-G120R) resulted in mice with a dwarf phenotype (6,13). This finding led to the discovery of a GH antagonist which is currently a potential therapeutic product to be used in patients with elevated levels of GH.

The use of transgenic animals is flexible and has expanded the possibilities for research in many fields (3,24). For example, research into the pathophysiology of diabetes mellitus has used transgenic mice models with altered states of glucose homeostasis. Diverse diabetes models include the over expression of an insulin gene which causes a disease state that mimics non-insulin dependent diabetes (19). Likewise, in nutrition research, transgenic animals may be employed to examine the effects of nutrition or nutrients on gene expression, or to study the effects of gene expression on various aspects of metabolism, including nutrient utilization (10). Transgenic animals are also used in studies of cytokine function and cell physiology research (14,20). Creation of new animal models that might overexpress a given gene, express the gene in an inappropriate tissue, express a mutated gene, or specifically "knockout" an endogenous gene are all achievable and valuable to biomedical research.

7. Ethical concerns and safety issues

Molecular biology and genetic engineering technologies have not only accelerated progress in science, but have also heightened the potential safety risks and ethical concerns associated with these products. Unlike traditional breeding strategies, transgenic animal technology allows for interspecies gene transfer. Environmental consequences and food product safety issues should be examined in the case of genetically modified livestock or other genetically engineered products. A vast majority of issues associated with meat producing animals and their surroundings are independent of biotechnology. Food safety concerns for transgenic animals greatly overlap with the general concerns for all animals used for nourishment. Healthy, transgenic livestock are likely to be as safe as their non-transgenic counterparts (1). Currently, transgenic fish have the greatest potential for unwanted environmental impact due to the fact that fish are not domesticated and cannot be easily contained (4). Overall, the safety of food products should be enhanced by innovations in biotechnology (11).

A number of genetically engineered pharmaceutical products have already proven to be safe trough clinical trials and continued use throughout the world. Despite encouraging safety records for a wide variety of therapeutic, diagnostic, and food related products, safety and ethical concerns abound. The two major controversies surrounding genetic engineering are 1) concerns that the technology might somehow cause an unintended or unforseen outcome 2) moral and religious concerns about interspecies gene transfer (22). Objection such as these can only be dealt with by conscientious integration of scientific information with the ethical discussions.

8. Conclusions

The field of biotechnology has increased at a rapid rate. New techniques involving recombinant DNA have had extraordinary impact on medicine, basic science, and agriculture. Therapeutic proteins important for treating humans and animals have been developed and are used world wide. Furthermore, the use of recombinant DNA technology has and will continue to be used for the diagnosis of disease, gene therapy type strategies, as well as in forensic arenas. Twenty years ago, recombinant DNA technology was "born" and has now developed into its early "teenage years". In the future, biotechnology will continue to mature into a vibrant, healthy "adult".

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Summary

Biotechnology has been practiced in one form or another since the beginnings of the domestication of animals. Selective breeding techniques have long been used with both livestock and plants in order to propagate certain desierd traits. The advent of molecular biology and recombinant DNA technology brought new developments not only to agriculture, but also to medicine and the basic sciences. Molecular biology and genetic engineering technologies have not only accelerated progress in science, but have also heightened the potencial safety risks and ethical concerns associated with these products. Objectives such as these can only be dealt with by conscientious integration of scientific information with the ethical discussions.

The use of recombinant DNA technology has and will continue to be used for the diagnosis of disease, gene therapy type strategies, as well as in forensic arenas. Despite many problems associated with, biotechnology will continue into a vibrant, healthy 'adult'.

Biotechnologia w latach 1990

Streszczenie

Za początek rozwoju biotechnologii można uznać moment, w którym udomowiono pierwsze zwierzęta. Od dawna bowiem w celu uzyskania pożądanych cech stosowano metody selektywnego krzyżowania wśród zwierząt i roślin. Rozwój biotechnologii molekularnej oraz techniki rekombinacji DNA wniósł nowe wartości zarówno do rolnictwa czy medycyny, jak i do nauk podstawowych. Biologia molekularna i inżynieria genetyczna oprócz przyśpieszania postępu w nauce stwarzają jednocześnie szereg niebezpieczeństw i problemów natury etycznej. Mogą być one rozwiązywane poprzez zacieśnienie wymiany informacji naukowej połączonej z dyskusją na tematy etyczne.

Technika rekombinacji DNA, ze względu na możliwości jakie stwarza, jest i będzie stosowana w diagnostyce chorób, terapii genowej czy kryminalistyce. Pomimo wielu problemów, biotechnologia będzie się nadal rozwijać w silny, zdrowy „organizm".

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Adres dla korespondencji:
John J. Kopchick, Elizabeth Parks, Edison Biotechnology Istitute, Ohio Univerity, Athens, Ohio 45701, USA.