THE ROLE OF GENE TRANSFER IN ANIMAL AGRICULTURE AND BIOTECHNOLOGY

THOMAS E. WAGNER

Edison Animal Biotechnology Center, Ohio University, Athens, Ohio 45701. Received 1 Mar. 1985, accepted 24 May 1985.


Biotechnology in general, and mammalian recombinant genetics in particular, assuredly will have a major impact on all aspects of animal science and commercial livestock production. A review of the basic molecular biology and technology involved in gene transfer in mammalian species is presented in the content of potential commercial application of these new procedures for enhancing productivity in livestock. Special emphasis is given to near-term possibilities for the application of mammalian recombinant genetics to animal growth and lactation.

Key words: Biotechnology, gene transfer, livestock production

[Le transfert de gènes en production animale et biotechnologie.] Titre abrégé: Transfert de gènes chez les animaux.

Le biotechnologie et, en particulier, la génétique des recombinants chez les mammifères auront assurément une incidence majeure sur tous les aspects de la zootechnie et de la production commerciale du bétail. Nous passons en revue les principes fondamentaux de la biologie et de la technologie moléculaire mis à profit dans le transfert de gènes chez les mammifères, dans le contexte général de leur utilisation commerciale possible pour l’amélioration de la productivité du bétail. Nous mettons particulièrement l’accent sur les possibilités à court terme de l’application des principes de la génétique des recombinants chez les mammifères, pour l’amélioration de la croissance et de la lactation chez les animaux.

Mots clés: Biotechnologie, transfert de gènes, production du bétail

The advent of recombinant genetic technology has fundamentally and permanently altered biological research in general and agricultural technology in particular. The term biotechnology has been applied to the application of the "new biology" to both industrial and agricultural bioproduction. An exciting area of biotechnology relating to research aimed at improving agricultural livestock production, known as animal biotechnology, has emerged during the past several years. Animal biotechnology is an outgrowth of experiments carried out during the early 1980s which demonstrated that specific cloned genes could be permanently transferred into the genetic makeup of mammals. These experiments in turn followed more fundamental results of genetic recombination in microbial and animal cell culture systems. The purpose of this paper is to review these foundation studies and to suggest how modern recombinant genetic technology might be integrated into the fabric of animal science, as animal biotechnology, to improve the productivity of agricultural livestock.

Although biotechnology has already influenced and is predicted to impact further in a major way on the pharmaceutical, spe
cially and heavy chemical, food processing, and resource recovery industries, the greatest impact of biotechnology will surely be in agriculture, where new genetically engineered plants and animals might well improve farming methods more in a few decades than previously achieved over thousands of years of traditional plant and animal breeding methods. The Office of Technology Assessment of the United States Congress estimates that over $100 billion dollars in increased production and product sales could result within a decade from the inclusion of biotechnology into U.S. farming practices alone. Also, agriculture, which was traditionally used living organisms for product production, is positioned better than other industries to utilize biotechnology.

Utilizing bacterial plasmids, gene recombination was first achieved in bacteria (Cohen et al. 1972) and later in simple eukaryotic organisms such as yeasts (Beggs 1978). Microorganisms transformed with recombinant plasmids containing cloned prokaryotic or eukaryotic genes are presently being used to produce various product protein molecules. Although gene recombination in animal and plant cells was known by the later 1970s as a result of random recombinational events in cell culture, the selection methods used to rescue recombinant cell lines held no promise for transferring genes into the permanent genetic makeup of whole plants and animals. The goal of transferring specific functional cloned genes into complete mammals was not achieved until a series of experiments was carried out in several laboratories using molecular biological and mammalian embryological procedures, in conjunction with genetically transformed embryonic tissue from which intact genetically transformed animals resulted.

MAMMALIAN GENE TRANSFER
Germline gene recombination in complete animals must be approached by the genetic transformation of early embryonic cells from which a recombinant animal may develop. Since these cells may not be collected in large numbers and may not be effectively cultured over many cell generations, the techniques used to genetically transform mammalian cells in culture cannot be used. In 1981, in the Laboratory of Mammalian Recombinant Genetics at Ohio University, a team of scientists successfully accomplished the first functioning germine genetic transformation of laboratory mice by microinjection of cloned DNA into the male pronucleus of fertilized mouse eggs at the one-cell stage (Wagner et al. 1981a). The importance of using a single cell was to reduce chances of producing mosaic mice with genes in only some tissues and to insure foreign gene integration into all cells of the developing animal, including the germ cells. The relatively large male pronuclear region of the fertilized egg also provided an easy target for microsurgical injection. In addition to these reasons for pronuclear microinjection, earlier studies in our laboratory at Ohio University had suggested that the early male pronucleus may provide a highly specialized nuclear environment for the incorporation of DNA sequences into a functional chromosomal region (Wagner et al. 1977; Wagner and Yun 1979, 1980, 1981). The period of time during which sperm chromosomes lose their protamine-regulated structure and acquire a maternally determined histone structure may be a fortuitous time for incorporation of foreign DNA into a structure that is transcriptionally active. Although very little, except for these studies, is known about the molecular events within the developing male pronucleus of mammalian species, the molecular biology of these events has been investigated in the sea urchin, and these studies support a role for male pronuclear function in facilitating DNA chromosomal integration. It has been suggested that sperm chromatin dispersion and male chromosomal gene expression may be manifestations of changes in the nucleoprotein content of the paternally derived chromatin
within the developing male pronucleus. Kunkle et al. (1978) have shown that soon after fertilization of the sea urchin egg, the male pronuclear chromatin acquires proteins, probably maternally inherited, of molecular weights greater than 80,000 daltons and a nuclear protein composition similar to that of the female pronucleus. These workers postulate that such changes in composition of the male pronucleus may allow the paternal genome to participate in RNA synthesis (Longs and Kunkle 1977). Also, Laskey et al. (1977, 1978) have isolated a group of enzymes from the oocyte which organizes cellular histones into nucleosomal chromatin units and which may function in structuring the sperm chromatin into transcriptionally functional chromosomal units during early male pronuclear development. Both of these observations suggest that extensive "rebuilding" of functional chromosomes occurs within the early male pronucleus following removal of protamines from the sperm and DNA decondensation. Therefore, similar early molecular events within the mouse male pronucleus might assist in assuring an appropriate nucleoprotein structure for the microinjected DNA and integration of exogenous DNA sequences placed into the early male pronucleus.

In order to take advantage of the unique chromosomal processing systems present in the early male pronucleus, we microinjected the cloned rabbit b-globin gene into the early male pronuclei of several hundred mouse eggs (Wagner et al. 1981a). Of the 46 mice born following transfer of the microinjected eggs to pseudopregnant foster mother mice, five animals contained intact rabbit b-globin DNA sequences in their chromosomal DNA. Each of these animals also showed traces of rabbit globin in hemolysates prepared from blood samples. In addition, offspring from one of these animals were studied and showed the presence of the rabbit gene and the protein product of the gene. Subsequent studies in our laboratories have definitively demonstrated the presence of the rabbit gene, rabbit globin m-RNA and rabbit b-globin in successive generations of these transgenic mice (Wagner et al. 1982). More significantly, four other independent laboratories have since confirmed our findings with a variety of other cloned genes (Brinster et al. 1981; Palmiter et al. 1982; Constantini et al. 1983; McKnight et al. 1983). From these experiments, there are now mouse lines that contain stably integrated Herpes simplex virus thymidine kinase genes, rabbit b-globin genes, human leukocyte interferon genes, metallothionein/rat growth hormone genes, metallothionein/Herpes virus thymidine kinase genes, and chicken transferrin genes in their chromosomal complement.

These mice, derived from one-cell embryos microinjected with specific cloned genes, have been scrutinized on the molecular level. Experiments to determine copy number, integrity, stability of integration and germline transmission of the introduced genes have been performed to determine the fate of the foreign DNA. The functional expression of each of the introduced genes has also been determined. Wagner et al. (1981a,b) demonstrated chromosomal integration of rabbit b-globin and Herpes virus thymidine kinase genes in transgenic mice by Southern hybridization of undigested DNA. Hybridization was only associated with high-molecular-weight DNA. Using similar procedures and digestion with restriction endonuclease enzymes showing flanking chromosomal sequences, Brinster et al. (1981), Palmiter et al. (1982), Gordon and Ruddle (1981), and McKnight et al. (1983) demonstrated chromosomal integration for metallothionein/thymidine kinase genes, metallothionein/rat growth hormone genes, human leukocyte interferon genes, and chicken transferrin genes. The strongest evidence for chromosomal integration of introduced genes in transgenic mice is provided by the in situ hybridization studies of Constantini et al. (1983) showing integration of tandem multiple copies of the rabbit b-globin genes in several different
mouse chromosomes. The copy number of integrated genes in all these transgenic mice varies from one to two copies per genome to as many as 25 or more copies. Following the initial experiments to introduce genes into whole mammals, the main thrust of research involving transgenic mice has been to establish lines of animals that not only contain stable integrated foreign DNA, but express the integrated genes. The Ohio University Laboratory of Mammalian Recombinant Genetics (Wagner et al. 1981a) as well as several other laboratories (Brinster et al. 1981; Wagner et al. 1981a; Palmiter et al. 1982; Constantini et al. 1983; McKnight et al. 1983) have demonstrated expression of injected sequences in transgenic mice. The stable integration of intact foreign genes into the mouse genome allows analysis of foreign gene expression in these mouse lines. We found the protein gene product present in some of our mice containing the integrated rabbit b-globin genes. (Wagner et al. 1981a). Immunodiffusion analyses showed the presence of a protein in the blood that reacted with mouse antirabbit globin antiserum. This finding was further corroborated by two-dimensional protein gel electrophoresis. Gel patterns showed an additional protein in the blood of transgenic mice at the molecular weight and isoelectric point for rabbit b-globin (Wagner et al. 1982). Northern hybridization of the m-RNA from bone marrow cells from these mice also showed in excess of 100 copies per cell of rabbit b-globin m-RNA. Brinster et al. (1981) have demonstrated thymidine kinase activity in the livers of transgenic mice containing the *Herpes* viral thymidine kinase gene and the metallothionein/*Herpes* viral thymidine kinase gene. Recently, Constantini et al. (1983) have demonstrated the presence of rabbit b-globin m-RNA in the tissues of mice containing the rabbit gene, supporting the earlier work of our laboratory. Also, the chicken transferrin gene has been shown to produce high levels of chicken transferrin in the livers of transgenic mice containing this cloned gene (McKnight et al. 1983). Perhaps the most convincing demonstration of the expression of a foreign added gene integrated in a mouse germ line is the strong metallothionein promoter fused to the structural gene for rat growth hormone and introduced into mice by Palmiter et al. (1982). A high percentage of these mice produce between 5 and 100 times the concentration of growth hormone normally present in mice and have grown substantially larger than control mice. In 1983, scientists in the Ohio University Laboratory of Mammalian Recombinant Genetics introduced similar gene constructs containing the metallothionein promoter sequence fused to the human growth hormone gene, and a viral promoter sequence fused to the bovine growth hormone gene, into the germlines of several mice. These mice are over 100% larger than control animals. The gene constructs in these mice are of particular interest for agricultural applications since the products of expression of these genes, human and bovine growth hormone, will stimulate growth and efficient feed utilization in all major breeds of mammalian livestock.

Interestingly, the same genetic constructs presently in the genetic makeup of these transgenic mice, if introduced into certain species of livestock, could dramatically improve actual field performance of these farm animals. Since these genetic constructs produce continuous high levels of growth hormone throughout the lifespan of animals which contain these sequences in their genetic makeup, increased growth rates, efficiency of feed utilization, and size will result (Evans and Simpson 1931; Lee and Shaffer 1934; Machlin 1972).

**IMMEDIATE POSSIBILITIES AND FUTURE PROSPECTS FOR GENE TRANSFER IN LIVESTOCK**

**Swine**

Although it is possible to genetically engineer several species of livestock, one species is the clear choice for immediate ap-
Swine are marketed at an immature stage of development, thus making it possible to improve to any degree the rate and efficiency of weight gain without producing an altered sale product. Finally, swine producers are acutely aware of the need to improve the efficiency of production and are keenly interested in even small increments of improvement.

While other commodity animals share or exceed some of the characteristics outlined above for swine, no other livestock species combines all the attributes. For example, beef cattle, while having a higher inventory value, are not produced as intensively as swine and, because of low reproductive rate, a very large proportion of the inventory is used for reproduction. Furthermore, beef cattle are marketed on the basis of maturity, because of consumer preferences in degree of intramuscular fat (marbling) and tenderness, rather than size or weight. This creates problems in that growth hormone recombinant cattle would grow more rapidly and therefore would be likely to reach larger sizes than is true of normal cattle; and since cattle must reach a physiological stage of maturity in which fat is relatively heavily deposited intramuscularly, it would be more difficult to produce marketable beef cattle in the normal weight range. This is already somewhat of a problem with “exotic” breeds, such as Simmental, that are much larger than traditional breeds such as Angus or Hereford. Thus, production of beef cattle with recombinant growth hormone genes is feasible, but it would result in several problems unless regulation of expression of the transgenes were possible. While regulation is likely in the future, it is not currently available. This restriction is not an obstacle in swine because, as described above, swine are marketed at a specific weight range and the current market hog is immature. Furthermore, breeding swine are used at wide variety of ages and sizes. For example, in operations in which sows are maintained in the breeding herd as long as they are productive, it is not uncommon for sows to weigh 200 kg and similarly 300 or 350 kg boars are used. If growth hormone gene recombinant swine are produced and grow to larger than normal size, it would not be out of the ordinary to have such large animals in the breeding herd. The producer could decide at what point such breeding females were too large for use. Large boars can be used for breeding even small gilts through artificial insemination, so there is no foreseeable limit to the size of breeding boars. Production of growth hormone gene recombinant swine can be accomplished in the short-term while efforts are underway to design genes that can be regulated for use in the other species. Furthermore, as described below, production of growth hormone gene recombinant swine will meet some of the current needs of the swine industry which exist in the U.S.A. and in the world. The cost of feeding animals is the single most expensive item in the cost of production. Even with the highly efficient swine that are produced currently, the feed cost is approximately 50% of the sale value of the pig at slaughter weight. Modern swine can achieve an overall feed to gain ratio of 2.5–3 (Isler 1983). Assuming the lower ratio (2.5), which is currently achieved by few entire herds, and assuming an average feed cost of $16.5/100 kg, the cost of feeding a pig from 10 to 90 kg would be $33.0, or approximately 35% of the sale price of slaughter pigs. Clearly, a significant improvement in the efficiency of growth (less feed per unit of weight gain) would have a dramatic impact on swine production costs.

Generally, animals that grow more effi-
ciently also grow more rapidly (Isler 1983). This is of economic significance, particularly in modern swine production operations where facilities are expensive, because the length of time required for pigs to reach market weight directly affects the effective holding capacity of the housing. Each day a pig remains in production facilities affects cost because it is using space that otherwise could be occupied by another pig. A significant decrease in the time required to reach market weight would reduce the cost of production because facilities of a given size could support more growing pigs per unit time (or a smaller facility could be designed for a given number of pigs).

A third factor of economic significance in swine production is the proportion of lean meat to fat in the carcass. The most striking effect of the efforts of swine geneticists is the dramatic shift in carcass composition of swine from fat to lean. The emphasis is still very much on producing lean pigs because consumers demand large lean cuts of pork and they are acutely aware of the highly publicized disadvantages of animal fat in human diets. Currently, there is concern that the trend toward ever decreasing proportions of fat in pork carcasses is reversing. Therefore, it is important to maintain and further improve the lean body composition of pigs because many packers purchase swine on a quality and yield basis and because of human health considerations.

Improvements in any or all of the above factors in swine production would be highly sought advantages, since producers, like other livestock producers, are acutely aware of the impact of feed and facility cost and end product acceptability on their ability to produce a profit.

The concept that improved feed efficiency, growth rate, and body composition is well worth achieving is not new. The vast majority of research emphasis over the past 50 yr in animal science has been directed toward breeding more efficient swine and in designing diets that promote the highest possible efficiency at low cost (Smith et al. 1980). While progress viewed over the past half century has been impressive, it has been relatively slow and represents the cumulative effect of selective breeding without knowledge of the genetic loci involved. It is now possible to engineer swine and other livestock genetically to be more efficient by specific alteration of the genome of the animals. Obviously, to do this, one must have knowledge of a gene or genes that can bring about the desired alteration, and one must have the technical capability of placing the gene or genes into the genome of the animal.

One gene product, about which a great deal is known and for which the gene is already cloned for use in recombinant genetics is growth hormone (Woychik et al. 1982). The effects of exogenous growth hormone on animal growth have been studied for more than 50 yr. Studies dating to those of Evans and Simpson (1931) have demonstrated that long-term treatment of animals with growth hormone or adenohypophysis extracts can substantially improve both growth rate and feed efficiency in animals. Evans and Simpson (1931) found that female rats receiving chronic daily injections of an extract from the anterior pituitary gland rich in growth hormone reached approximately 475 g at 200 days of age while controls were approximately 270 g in weight. At the same age, male rats weighed 530 and 410 g, respectively. At 400 days of age, growth-hormone-treated females weighed about 570 g while controls weighed 240 g. At the same age, males weighed 850 and 520 g, respectively. These are dramatic increases in growth. Evans and Simpson (1931) also noted that the effect of growth hormone on body weight in rats was not particularly evident until after the animals had reached 100 days of age. Thus, this early study provides evidence that the physiological stage on the growth curve of animals is important in determining the degree of response to exogenous growth hormone. Therefore, use of growth hormone in young animals might be expected to yield
poor responses.

Lee and Shaffer (1934) performed experiments similar to those reported by Evans and Simpson (1931), but they also evaluated treatment effects on body composition of experimental rats. They found, in pair-feeding experiments, that while consuming the same quantity of feed as controls, rats treated with pituitary gland extracts gained more weight than the controls. At the same time, body composition revealed an increase in water, nitrogen and ash content, but a decrease in fat (ether extract) content. Even with small numbers of animals, it is evident from the data presented that growth hormone exerted little effect on the younger rats (rats started on treatment at 52 days of age and slaughtered at 129 days of age gained a mean net of 2.5 g, while rats started at 171 days of age and slaughtered at 227 days of age gained a mean net of 45 g in response to growth hormone). This is potentially an important point in that it suggests that response to increased growth hormone may depend on age at treatment. In consideration of the results of both Evans and Simpson (1931) and Lee and Shaffer (1934), it has been clear for half a century that daily treatment with exogenous growth hormone can greatly increase nonfat body growth in experimental animals.

Presumably due to lack of available hormone in sufficient quantity, studies of the effect of exogenous growth hormone on farm species were done only recently. Machlin (1972) reported that exogenous porcine growth hormone positively affected growth. Exogenous porcine growth hormone increased muscle growth and decreased fat deposition. Five experiments with various numbers of pigs and various doses of porcine growth hormone were reported, and in one experiment, with 18 growing pigs in each treatment group, effects of porcine growth hormone on rate of gain and feed efficiency were significantly improved by 16.2 and 13.2%, respectively. Daily gain and feed efficiency were improved to a similar degree in all experiments, although statistical significance was not always observed because of low numbers of animals per treatment group in some experiments. The data clearly demonstrate that elevated levels of growth hormone in the pig are advantageous in terms of rate of growth, feed efficiency, and body composition. As described above, all these attributes are extremely important in swine production.

**Poultry**

In addition to swine, poultry production could benefit from birds with transgenes producing constantly high levels of growth hormone. As is true for other farm species, the cost of feed is the largest single expense in poultry production. For example, 4 000 000 broilers are produced annually in the United States. Broilers have an average feed efficiency of about 1.9 kg feed per kg gain. Even a small improvement in feed efficiency would result in a large reduction in the cost of production. A 10% improvement in feed efficiency would result in a savings of 1 374 000 metric tons of feed at a cost of $180 per metric ton. This would produce a savings to be shared by producers, suppliers, retailers, and consumers of $22 000 000 annually. Similar computations could be made for other types of poultry production. Thus, while the degree of improvement in feed efficiency that will result from development of growth hormone gene recombinant poultry is not known, it is certain that improvements of even small increments are worth producing. However, there are other worthwhile reasons for genetic engineering of poultry.

As discussed above for swine, animals that are more efficient in growth tend to grow more rapidly. The efficiency in using facilities would be improved in a linear manner by more rapid growth rate. Thus, more weight of poultry could be produced within facilities of a given size.

Another important problem in broiler production is the over-fat broiler. In the in-
Industry's pursuit of improved growth rate in broiler chickens by selective breeding, increased appetite, among other things, has been selected. Along with this is an increased ability to convert energy to fat. As reviewed above for swine, it is known that growth hormone tends to promote protein synthesis and decrease fat deposition. Therefore, it is likely that growth hormone gene recombinant broilers would have less fat in their carcasses and, thus, become a superior product. This might well be the most important accomplishment resulting from producing broilers with recombinant growth hormone genes, especially if significant improvement in feed efficiency occurs concurrently, as would be expected.

Increases in growth rate resulting from mass selection are negatively associated with egg production in chickens (Kinney 1969), turkeys (Nestor 1971), and Japanese quail (Nestor and Bacon 1982). These improvements in body weight are the result of selection for increased frequency of many genes, not just those associated with growth hormone. Growth-selected chickens (Jaap and Clancy 1968), and turkeys (Nestor et al. 1970) produce an excess of ovarian follicles which are lost during egg formation. This results in reduced total production of settable eggs. It is possible that through the production of growth hormone gene recombinant poultry, the advantages of increased body weight can be obtained without sacrificing egg production. This would be a significant benefit to the poultry industry. The potential benefits to be derived from genetically engineered poultry are large, and the technology, once developed, can be expanded to exploit additional genes when time and availability allow. Certainly the opportunity for genetic progress at a rate heretofore impossible will be available.

Microinjection into the pronucleus or even into the whole avian zygote poses special problems. This is because, like mammalian ova, avian ova undergo second maturation division and fertilization in the oviduct (Olsen 1942; Olsen and Fraps 1944; Romanoff 1960). Unlike mammalian ova, however, avian ova contain a massive vitellus, supported only by the vitelline membrane. This makes the egg very fragile if removed from the oviduct and the extremely large amount of vitellus precludes transmitted light microscopy of the nuclear region. Furthermore, the avian egg rapidly develops and is rapidly moved through the oviduct. Sperm entry into the egg occurs very soon after ovulation (within 30 min) and formation of male and female pronuclei and fusion of the two occur within 3 h after sperm penetration. First, cleavage occurs at approximately 5 h after fertilization, and when ovoposition occurs at about 25–26 h after ovulation, the developing embryo is composed of about 512 cells. Therefore, the time interval in which to perform microsurgery is very short and the fragility of newly ovulated eggs magnifies the problem. An additional problem to be overcome is that avian species ovulate one egg at a time in response to photoperiod, and it is not possible to superovulate birds unless hypophysectomy is performed (Blair and Nalbandov 1977). Therefore, it is not readily possible to obtain large numbers of ova at the same stage of development from the same hen. Fortunately, because poultry can be kept in large numbers and because they ovulate in response to photoperiod, it is possible to obtain a sufficient number of ova from a group of hens.

Current technology, as developed in the mouse (Wagner et al. 1981a), based upon microinjection of the mice pronucleus will be extremely difficult to implement in birds. An alternative approach to gene insertion may be provided by an altered retrovirus to transect the avian zygote, inserting the desired recombinant gene into the genome of the zygote. This strategy would involve development of a retroviral growth hormone fusion gene construct for use in avian species and development of methods of reliably injecting this gene construct into the cytoplasm (near the blastodisc) of the zygote, and development of
procedures for incubation and normal embryo development to hatching.

**Dairy**

The introduction of genetic sequences alone is not sufficient, in some cases, to produce useful recombinant livestock. Control of the genetic expression of added genes is often a requirement necessary for the fine control of economically important livestock traits. Although this is true in a number of livestock production situations, it is especially relevant in dairy cattle applications. Although the dairy animal will be the major recombinant product resulting from the development of regulable genes in transgenic livestock, other applications in swine, beef cattle, and other species will result.

The dairy industry of today is facing a very difficult battle between high costs of production, relatively low product price, excess product in the market place, and failure to develop significant new markets for the existing product resulting in market saturation for the foreseeable future. The best estimates indicate a contracting market as efforts to reduce the surplus of milk are implemented. The only segment of the dairy industry which is gaining strength with increasing sales is the cheese industry.

For the fluid milk dairy industry, product price pressure and increasing production costs will reduce margins to hazardous levels. Every emphasis is given to cutting both production and production costs in order to maintain cash flow for the industry and the individual farmer. Any measure which could significantly reduce production costs would be welcomed. The largest costs in the production of milk are feed costs (currently about $3/(cow·day) for a typical farm) and capital expenses for land, buildings, and equipment. While increasing milk production alone is undesirable because of the current oversupply, the means to produce the same amount of milk with fewer cows, and therefore less barn and equipment, and with less feed would do much to improve the economic position of fluid milk producers.

For the cheese industry, access to higher protein milk with an increased cheese yield would be an immense asset. The immediate benefits would be reduced transportation costs, higher manufacturing plant efficiency, and reduction of waste disposal requirements. All are costly. Further, if milk proteins could be altered to produce alternate products better suited to specialized needs of the food processing industry, this would increase the market for dairy products and open new opportunities for food processing or cheese manufacturing industries.

Recombinant genetics offers much promise for the problem areas outlined above in the dairy industry. As early as 1973, Machlin (1973) showed that injected growth hormone could increase milk production in dairy cattle. Several subsequent studies have shown that exogenous growth hormone administered over several day periods to lactating dairy cattle can increase milk production by 15% to more than 30%, depending on the stage of lactation, even in high-producing dairy cows (Peel et al. 1981, 1982, 1983). These studies demonstrate the dramatic results possible by administering exogenous hormones such as growth hormone to lactating dairy cattle. Milk production from these studies was generally increased a minimum of 15% for early lactation and as much as 30% or more by late lactation in lactating cows given growth hormone injections compared to sham-injected control herdmates. Other striking features of these experiments are that feed efficiency increased dramatically; +18% for early and +5% for late lactation (Peel et al. 1983). However, milk protein concentration decreased substantially in two studies (Peel et al. 1983; Fronk et al. 1983) but not in two other similar studies (Peel et al. 1981, 1982).

The prospect of simultaneously improving milk production and feed efficiency is very exciting for the dairy industry. How-
ever, the administration of growth hormone requires daily injections since no sustained release implant is available for delivering proteinaceous pharmaceuticals (Fronk et al. 1983). This means that in a practical setting, the procedure is unlikely to be adopted by dairy farmers in spite of its production merits because of insufficient labor to inject each lactating cow every day. If endogenous growth hormone production could be elevated in dairy cattle by insertion of activated growth hormone genes with recombinant DNA technology, we would expect similar results in increased milk production and feed efficiency.

Calculations on the economic effects of the successful application of recombinant growth hormone in dairy cattle, as estimated from conservative changes reported by Peel et al. (1983), suggest a sizable impact. The injection of exogenous growth hormone into lactating dairy cows caused, among other benefits, an increase in milk production of 15% in early lactation and over 30% in late lactation. Using the conservative increase of 15% in milk production and applying it throughout the lactation, one calculates that this amounts to an increase in milk production from the current DHIA average for Ohio (about 7000 kg milk per 305-day lactation) to about 8000 kg per lactation for a net gain in milk production of 1000 kg per lactation. For milk with a market value conservatively estimated at $22/100 kg, the value of 1000 kg milk would be $220 per cow per lactation.

For the total number of dairy cows in Ohio (290,000) this is equivalent to an increased income value of 65.25 million dollars a year. For the United States as a whole with 11.1 million dairy cows, this is an increased value of 2.5 billion dollars a year. Since milk is currently in excess supply, it would be better to think of achieving this added value by reducing by 15% the herd size necessary for a given income level. Further, the increased production potential is attained with increased feed efficiency, thereby reducing the cost of milk production and increasing the profit margin. Capital expenditures for buildings and equipment would be reduced accordingly. Collectively, this would amount to a dramatic restructuring of the cost of milk production, allowing investments in other areas.

Although increased growth hormone levels in lactating dairy cattle would be of decided economic advantage to the dairy producer, continuous, uncontrolled production of growth hormone in the dairy animal would present problems negating the positive advantages. Unlike swine, the product of the dairy animal is derived from a mature animal. Also, dairy animals are kept in production throughout most of their mature life. Although a swine management system utilizing young females as breeding stock and marketing immature animals as a product lends itself to an animal which continuously produces growth hormone, dairy management systems using mature cows are incompatible with such a recombinant animal. Excessively large dairy cows are unlikely to be advantageous, economical, or desirable and the result of continuous growth hormone expression throughout the life of an animal includes continuous stimulation of somatomedin release in the recombinant animal resulting in increased skeletal growth and gigantism.

Therefore, in order for the dairy industry to take full advantage of the enhanced feed efficiency for milk production provided by excess growth hormone during lactation, it will be necessary to either control the time of expression of recombinant growth hormone in transgenic dairy cattle or to remove the somatomedin releasing effect of the recombinant growth hormone.

Mechanisms of Genetic Regulation

Although cloned genes have been introduced into transgenic animals and have been shown to function to produce their protein products, demonstration of external control of the genetic expression of these genes has not, as yet, been achieved. The
design, construction, and testing of genetic control sequences in transgenic animals is the largest and most significant challenge facing recombinant animal biotechnology. Although several economically important animal agricultural products could result from the addition of continuously expressing genes (i.e., growth hormone) to transgenic livestock (i.e., poultry and hogs), the most far-reaching animal agricultural applications or gene transfer technology require specific control of the time and site of expression of added genes.

Advances in molecular biology and recombinant genetic technology over the past year or less suggest that accurate control of the expression of added genes in transgenic animals may be a real possibility in the near future. Important aspects of this work are being carried out by the Ohio University Laboratory of Mammalian Recombinant Genetics in collaboration with researchers at Case Western Reserve University School of Medicine in the Departments of Molecular Biology and Biochemistry. Since the basic mechanisms of regulation of gene expression in mammalian cells are not yet understood, studies of the control of genes in transgenic mammals are dependent on basic research findings resulting from studies of cultured transformed cells. But transgenic experimental animals are also contributing in large measure to our understanding of fundamental mechanisms controlling gene expression in intact animals.

Although genetic control mechanisms in lower prokaryots are beginning to be understood, less of this knowledge than expected is directly relevant to an understanding of mammalian and other higher eukaryotic mechanisms of gene control. In large measure, control of prokaryotic gene expression is “negative”. The ability of RNA polymerase enzymes to read the genetic message is blocked by the presence of specific repressors in order to “shut down” gene expression. Activation of gene expression is accomplished in these organisms by removal of repression. A similar general mechanism does not seem to explain any large proportion of the genetic control modes in higher species. Rather, the promoters, or sites for the binding of RNA polymerase enzymes and initiation of transcription in higher eukaryotes, appear to, alone, only allow very inefficient and slow transcription or expression of the structural gene. Without additional influences the genes continuously express very low levels of their protein products. These constitutive levels of protein production are probably the same for all cell types throughout the life span of the organism. Some genes have significantly higher constitutive levels of expression if the products of these genes are continuously required for maintenance of all cells (i.e., “housekeeping genes”).

A working hypothesis among scientists studying mammalian gene control mechanisms is that, unlike prokaryotes, higher species utilize a positive mode of gene control. According to this hypothesis, activation of gene expression in higher species is the result of an “opening” of the gene promoter allowing much easier access of the RNA polymerase to the initiation site for transcription. Although the actual molecular mechanisms for the “opening” of the gene promoter in positive gene regulation are not fully understood they appear to affect the local helical structure of the promoter, making helical unwinding by RNA polymerase a more thermodynamically favorable process.

Because of the existence of several levels of gene control (i.e., tissue specificity, temporal control, hormonal regulation, etc.) several different types of control elements may be associated with a gene, functioning to activate the gene by promoter “opening” or chromosomal conformational mechanisms. Recently, several systems of gene regulation associated with the growth hormone gene and other genes have begun to be elucidated. Studies of the immunoglobulin genes have shown that tissue specificity of gene expression in this system
may be explained by tissue specific enhancer sequences. DNA sequences within the intervening sequences (introns) of immunoglobulin genes specifically interact with specific immune cell proteins to "open" the promoters of immunoglobulin genes only in immunoglobulin-producing cells.

Similar enhancer and regulator sequences may be used in conjunction with the growth hormone genes to impart regulated, tissue-specific growth hormone expression from introduced transgenes. Some examples of possible sequences which may be used include prolactin promoter/regulator sequences to regulate growth hormone expression in dairy animals for significant expression only during lactation; somatomedin promoter/regulator sequences to restrict the expression of transgenic growth hormone only to the period of normal growth in beef and other animals; and the use of hormonally regulated gene promoter/regulator sequences to control the expression of growth hormone transgenes by addition of simpler synthetic hormones or by control of feed composition which indirectly could regulate hormonal levels and therefore growth hormone levels. The major future challenge in livestock gene transfer lies in this area of regulation of transgene expression. Already, major strides have been taken in this direction and details of these experiments will soon appear in the molecular biological literature. Therefore, the future looks most encouraging not only for the introduction of transgenes into livestock, but for the controlled regulation of these genes to give the livestock producer control over the use of this powerful new aspect of biotechnology to increase the efficiency and profitability of livestock production.

**TOWARD AN INTEGRATED BIOTECHNOLOGY EFFORT**

The greatest challenge facing the new science of animal biotechnology is the integration of existing animal science expertise and emerging animal recombinant genetic technology into a unified discipline. Animal scientists who intimately understand the physiology of the farm animal systems and the relation of these physiological processes to agricultural productivity do not usually have the training or experience in molecular biology to use the new gene transfer technologies, while molecular biologists working on gene transfer have very little knowledge of the specific physiology of farm species. Both of these groups are hindered by lack of interaction with the other. In some unique cases outstanding animal scientists are working side by side with molecular biologists in recently established centers founded to generate this interaction, but these situations remain the exception. True progress in animal biotechnology will occur only when animal scientists begin to orient their studies towards the new possibilities offered by recombinant genetics. This does not mean that animal scientists will have to become directly involved in molecular biology, but that they must use their knowledge of animal physiology to point out specific pathways and regulation points in the physiological mechanisms of farm species which may be amenable to enhancement by a recombinant genetic approach. These studies must focus on specific protein molecules and their role in physiological processes involved in agricultural production. Just as animal scientists must direct their work towards specific molecular processes, so also must the molecular geneticist become more aware of the animal science and animal physiology literature. Only when a specific protein gene product is known to have a significant influence on an important aspect of the physiology of an animal should the molecular biologist begin the laborious task of cloning the gene for this protein and attempting to introduce an appropriately regulated expressing genetic construct of this gene into the animal with the goal of improving animal performance. If animal biotechnology is to attain a mature status as a significant part of the agricultural sciences, it will be
the result of a special partnership between the animal physiologist, identifying sites for useful recombinant genetic input, the molecular biologist, isolating and introducing these useful genes, and the agricultural animal geneticist evaluating the long-term value of the resulting product animals. It is hoped that this review and others like it will stimulate the beginnings of such an exciting partnership.


Olsen, M. W. and Fraps, R. M. 1944. Maturation, fertilization, and early cleavage of the egg