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Animal Productivity and Genetic Diversity: Cloned and Transgenic Animals

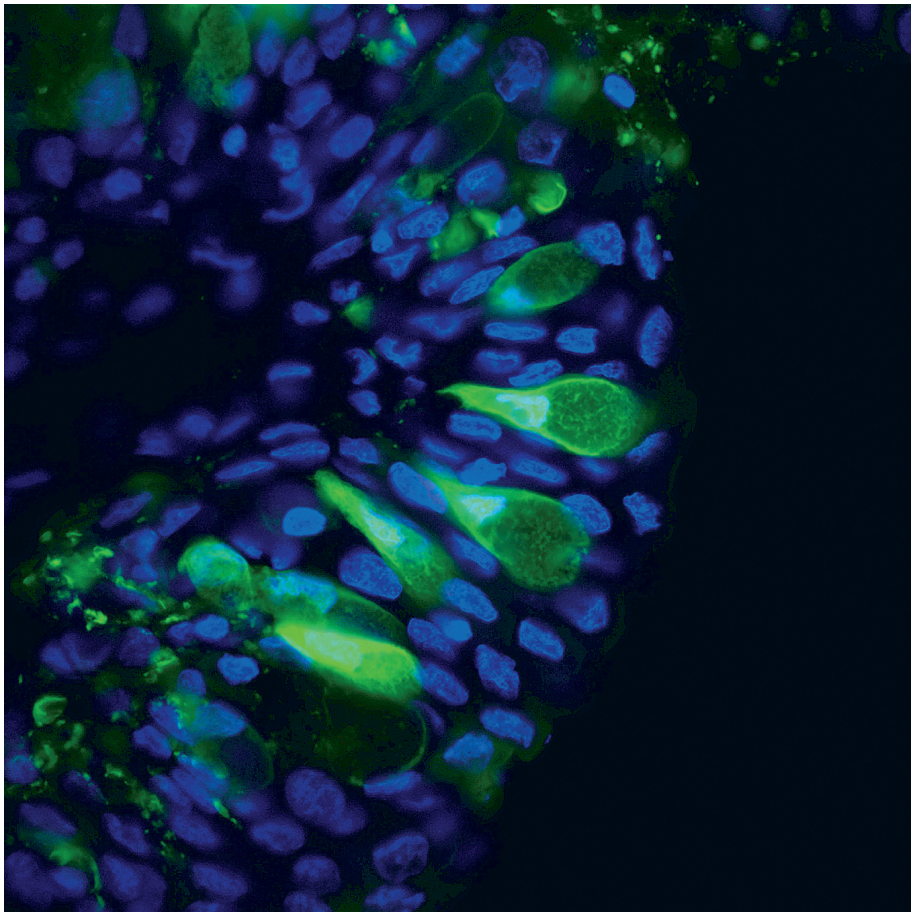
Animal Agriculture's Future through Biotechnology, Part 8

Abstract

Improvements in agronomic traits in all livestock species have been achieved during the past several decades using reproductive technologies. Cloning and transgenesis are the most recent of these technologies, providing geneticists with additional tools to influence population genetics. This Issue Paper describes both of these technologies, addresses their strengths and limitations, and provides a framework for discussion about their future use.

Cloning is a reproductive tool that can be used to narrow or broaden genetic diversity. Somatic cell nuclear transfer is the most common method of animal cloning and is more efficient than other procedures for some applications, resulting in the use of fewer experimental animals to achieve success. Other cloning methods include embryonic cell nuclear transfer, using nuclei from cryopreserved, genetically superior cell lines, and bisecting and trisecting preimplantation embryos. The value of cloning genetically superior animals will vary depending on the situation. Cloning could increase the frequency of a desirable trait in the cattle population, but because of the diverse nature of animal agriculture, one phenotype of cattle will not fit all needs.

Whereas a cloned animal is genetically identical to the one from which it came, a transgenic animal is one into which a new gene has been introduced or in which an existing gene has been modified by human intervention. This technology offers potential solutions to some limitations of selective breeding while simultaneously providing opportunities for increasing the genetic diversity of populations. Applications of transgenic technology can create animals that are better able to combat or resist infection, improve food safety and quality, increase production efficiency, decrease the environmental footprint of livestock production, and introduce new characteristics into the gene pool. The technology may eventually be used to manipulate complex traits controlled by multiple genes.



A photomicrograph of cells from a transgenic pig; the transgenic cells show up in green. (Photo courtesy of Drs. Douglas Vasey and Bruce Whitelaw, The Roslin Institute.)

One main limitation to the development of cloning and transgenic technologies has been the lack of public acceptance. The public has been tentative to accept cloning as an animal breeding method, even though there is scientific consensus that no difference exists between food products of cloned animals and the same products of noncloned animals; this perspective recently has been supported by both a National Research Council study and

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by U.S. Food and Drug Administration evaluations. The authors of this paper suggest that proponents of biotechnological approaches consider consumers' concerns, and that the government develop a regulatory process that addresses consumers' apprehension while offering realistic expectations of biotechnology.

Introduction

From a population genetics standpoint, *cloned*[1] animals and *transgenic* animals might be thought of as polar opposites. The most obvious goal of cloning is to make genetic copies of an existing individual, thus potentially decreasing genetic diversity of a population. Transgenic animals by definition are endowed with new genetic information that had not existed previously in the genetic makeup of their parents. Thus, by their very existence, transgenic animals increase the genetic diversity of a population. The reality of these two technologies, however, is a little more complicated.

Cloning is a recent addition to the tools available for geneticists to influence population genetics. This relatively new procedure complements other reproductive technologies used in animal breeding for decades, such as artificial insemination, in vitro fertilization, and embryo transfer. Training in animal science is not required to appreciate the efficiencies that cloning might bring about in livestock production, by replacing herd mates of average productivity with animals that possess superior production traits. Cloning provides a mechanism by which those superior animals can be produced, knowing at the outset that the genotype being copied contains the genetic information capable of producing the desired traits. Furthermore, the cloning process can achieve a surge in genetic gain in significantly less time than would be required by other breeding approaches. Cloning, however, is not likely to be used to produce animals for food, at least not in the near future, because it is too expensive and too inefficient. Rather, cloning most likely will be used to produce breeding stock to generate animals for food.

The most common livestock cloning method, *somatic cell nuclear transfer* (SCNT), not only has potential to increase animal production efficiency by generating groups of animals with desirable traits, but also can achieve feats that other breeding tools cannot. Nuclear transfer technology can assist in maintaining genetic diversity by providing a means to rescue endangered breeds. And the technology can increase genetic diversity by generating reproductively competent copies of animals that were made reproductively incompetent early in life, such as steers.

The mixing of parental genetics resulting from conventional reproduction increases genetic variability, providing diversity in *phenotypic* traits; geneticists can use the genetic variability in their breeding strategies. Selective breeding can generate individuals of superior genetic potential. The dramatic improvement in agronomic traits in all livestock species achieved during the past half century can be attributed, in large part, directly to the algorithms developed by quantitative geneticists to predict the potential genetic merit of offspring.

This approach to improving production efficiency, however, has limitations as well. For one, selective breeding relies almost exclusively on the existing genetic variation in the trait of interest in the current population. For example, if milk fat in Holstein dairy cows normally ranges from 3 to 5%, it is unlikely a cow could be bred that produces 12% milk fat. Likewise, if a trait such as disease resistance does not exist in the population, it is not possible to select for it. No amount of selective breeding, for example, can produce pigs that are able to synthesize lysine de novo (from scratch), because the multistep biochemical pathway to "fabricate" lysine does not exist in the swine population. It is also possible, while selecting for a desirable trait, to inadvertently select for an undesirable trait at the same time.

Transgenic technology offers potential solutions to some of the limitations of selective breeding while simultaneously increasing genetic diversity. With use of well-established recombinant deoxyribonucleic acid (DNA) protocols, new genetic information can be intro-

[1] Italicized terms (except genus and species names) are defined in the Glossary.

duced precisely in the desired location in a *genome*. Furthermore, the new genetic information can be introduced into a genome of a well-characterized animal with highly desirable traits. But perhaps the most important feature of transgenic technology is its unique ability to use genetic code information from almost any source. Because the code by which DNA spells out genetic information is universal, instructions (genes) from bacteria can be introduced into and processed correctly by a pig or a cow. This ability to cross species barriers with genetic information potentially allows the geneticist to use any desirable genetic solution that nature has devised.

This Issue Paper describes the potential agricultural applications of these two new breeding tools. The potential benefits of animal cloning are notable, though they remain substantially untested. *Transgenesis*, the more mature technology of the two, has been shown, at least in principle, to have a positive impact on a variety of livestock production parameters, from feed utilization to animal well-being.

Cloning Farm Animals

Definition of Clone

There are many definitions of clone and quite a few methods of cloning. For mammalian reproduction, cloning implies genetic identity, either in the context of making genetic copies or having two or more genetically identical animals. The latter concept is not limited to cloning; for example, individuals of highly inbred lines of laboratory mice and rats are genetically identical (except for gender), as are their noninbred first generation offspring when lines are crossed. Naturally occurring identical twins and triplets are genetically identical and represent the "gold standard"; all methods of cloning mammals yield less-identical animals than identical twins, and there can be considerable phenotypic differences between identical twins for some traits (Seidel 2002).

Thousands of identical twin and triplet mammals have been manufactured using a variety of techniques, the simplest being bisecting and trisecting preimplantation embryos (Williams, Elsden, and Seidel 1984). This cloning method seems not to have evoked much public concern; neither did nuclear transfer, which involved embryonic cell cloning. Meat and milk from more than 1,000 cattle and sheep cloned by nuclear transplantation entered the food chain in the 1990s, and no one paid attention from a food safety perspective. But with the attention that accompanied the cloned sheep named Dolly, and the use of nuclei from somatic cells rather than embryonic cells, concerns arose.

Safety of Food from Clones

For most nonscientists, and some scientists, cloning elicits an uneasy reaction, often with negative connotations. There are legitimate ethical concerns, mostly of the "slippery slope" nature, that cloning procedures may be inappropriately applied to people. Concerns regarding food safety, however, have been addressed by the U.S. Food and Drug Administration (FDA) in its recent report, which indicated that milk, meat, and other products from cloned animals are as safe as those from noncloned animals (USFDA 2008).

The FDA's evaluation, however, highlights the struggle to achieve acceptance of cloned products in the food supply. The FDA statement contained a few caveats, such as "Cloned sheep have not been studied rigorously with respect to carcass composition, so it is best not to extrapolate from other species without more data." From a scientific perspective, food from cloned animals such as cattle and pigs is as safe to eat as that from noncloned animals, as documented by both a National Research Council study (NRC 2002) and the extensive FDA evaluations (USFDA 2008). Although no study can evaluate all cloned animals exhaustively, there is no reasonable scientific evidence to suspect that food products from any cloned animal would be less safe than those same products from noncloned animals, regardless of species.

Are Clones Normal?

Essentially, all the trillions of somatic cells in a given animal, other than red blood cells, have a nucleus with chromosomes that contain exactly the same DNA sequence. But there are hundreds of different kinds of cells in the body, and they are different because each cell type selectively uses different parts of the genome. Thus, skin cells are programmed to use specific parts of the DNA that differ from parts that leukocytes use, which in turn differ from cells in the embryo that will form the placenta. Scientists are just beginning to understand how such programming is accomplished.

Somatic cell nuclear transfer is now the most commonly used method for animal cloning. The DNA in the nucleus transferred into an *oocyte* requires reprogramming, for example, from functioning as a skin *fibroblast* to functioning as a one-cell embryo. Little is known about how this reprogramming occurs, except that it often does not get done correctly. This is not surprising, because the one-cell embryo normally programs sperm and oocyte DNA, not DNA from somatic cells.

Most malprogrammed embryos result in embryonic or fetal death. With current SCNT procedures, this result occurs in nearly 90% of embryos; it is one of nature's ways of weeding out problems. (For perspective, embryonic fetal death rates are normally 20 to 30% with farm animals and seem to exceed 50% in women.) The additional embryonic and fetal death with SCNT is a major reason for low success rates and high costs of the procedure.

Despite the mostly successful weeding out of problems during pregnancy, some abnormal animal pregnancies go to term, both with conventional reproduction and with SCNT. The types of abnormalities are similar with both kinds of reproduction, but the incidence is much higher with cloning and intermediate with procedures such as in vitro fertilization. Most abnormalities with cloning seem to be because of abnormal placentas; fetuses and newborns are mostly normal but have problems because of development and birth from abnormal placentas. This problem, called abnormal offspring syndrome (AOS) (Farin, Piedrahita, and Farin 2006), includes *hypoxia* and hypoglycemia, conditions that normalize if newborns are given special care for a few days.

The most striking of these abnormalities is oversized offspring. This condition likely is because of inappropriate placenta function, which also occurs in human *macrosomic* babies born to diabetic mothers who do

not appropriately monitor their blood glucose concentrations. The larger-than-normal animal offspring occur at an incidence of 20 to 30% with some procedures in some species, and the degree of oversize can be 30% or greater. These young may require delivery by Caesarian surgery. With time, such offspring normalize in size and do not pass this trait on to their own offspring.

There are several important points to emphasize with AOS.

- Incidence varies widely depending on specific procedures used and the species.
- The same kinds of abnormalities occur with conventional reproduction, but at a lower incidence, and with certain assisted reproductive procedures, such as in vitro fertilization, at an intermediate incidence.
- Clones that survive result in meat and milk that is indistinguishable from that of nonclones.
- Cloning procedures continue to improve, resulting in a decreased incidence of AOS.

Clones for Breeding Purposes

Current costs for cloning cattle exceed $10,000 per animal produced, and although efficiency may increase considerably with more research, mass producing clones for meat or milk is unprofitable and likely will remain so for many years. Numerous applications do exist, however, that already would be profitable for breeding purposes. For example, there are hundreds of bulls in the United States whose current value exceeds $100,000 because of the value of their semen. None of them is worth even $2,000 for their carcass, even though most would produce a huge amount of meat. Their value as lactating animals is zero, although their genetic value for lactation of their daughters often is huge, even for bulls of beef breeds.

Most of these same principles apply to the value of females because of the value of their offspring, either born naturally or by procedures such as superovulation, embryo transfer, or in vitro fertilization. The most effective use of genetically valuable females usually is by artificial insemination using semen of their sons.

From both genetic and commercial perspectives, cloning genetically valuable animals like those described previously will be of great value in some situations and of no value or only marginal value in others. Consider the issue of premature death, for example. With dairy bulls, it takes nearly half a decade to determine whether a bull is truly genetically superior and a few years more to determine precisely how superior he is. Bulls often die, become seriously injured, or have declining semen quality by the time they are proven, a process costing more than $30,000 per proven bull produced. Reproducing the truly superior bull that died early makes good sense. A variation of this situation would be a bull insured for $100,000; it would be more profitable to the insurance company to make a cloned copy of that bull than to pay $100,000 when it dies.

For the examples mentioned, only one or two cloned copies would capture most of the genetic value, but there are situations in which producing a dozen copies also might make sense, particularly if the cost per copy were less than $5,000. For example, clones of truly superior bulls—originally proved by having hundreds of thousands of offspring through artificial insemination—could be produced for natural breeding. In this circumstance, usually fewer than 100 offspring per year per bull would be produced. As with any commodity, the less abundant, the higher value per unit; this principle is true genetically as well as commercially.

Creating a breeding animal from a castrate is a special circumstance. The ideal end product for beef production is the carcass of a steer (steers grow more efficiently than heifers), and much of the beef produced in the United States is from steers. One cannot judge the beef quality from an animal until the meat is available for testing flavor, juiciness, tenderness, intramuscular fat content, and waste fat. Additionally, health, feed efficiency, and numerous other production characteristics can be evaluated for individual animals. With cloning, one can make a fertile copy, even with cells of the dead carcass (if collected within a day of slaughter) (Heyman 2005), and use that animal to sire superior beef animals. In practice, one would make many such bulls from steers and determine their genetic superiority by progeny testing, perhaps only using semen extensively from the best 10 to 20% of the bulls.

What about narrowing the gene pool, inbreeding, and the overall statistical value of cloning in a breeding population? Fundamentally, cloning is a genetic tool, and like any tool used in selective breeding, it can be used to narrow or broaden genetic diversity (Seidel 2001). Making a castrate into a breeding animal is an example of broadening genetic diversity, as is reproducing an animal lost to early death. Selective breeding, however, is by definition narrowing the gene pool statistically, because the process increases the frequency of desirable *alleles* and decreases the frequency of undesirable ones.

The frequently used practice of crossbreeding to produce *heterosis* increases the genetic diversity within an individual animal but can increase or decrease the genetic diversity of the population, depending on how it is used. An especially valuable use of cloning would be to copy high performing F_1 *crossbred* animals; when F_1 animals themselves reproduce, the next generation often is undesirable. But the current costs of cloning are prohibitive for this application.

If cloning were a routine and relatively inexpensive tool like artificial insemination, one potentially could increase the genetic value of a population for traits typically of value to cattle producers and consumers by approximately 30% in one generation. Such an increase would take five or six generations by conventional breeding (Smith 1989). Because the average generation interval in cattle is approximately 6 years, 30 years of genetic progress would be accomplished in only 6 years. Current costs and considerations such as AOS prevent this kind of implementation.

Some of the genetic applications mentioned previously, however, are already possible, genetically valuable, and commercially viable. One other important characteristic of the increase in genetic value of a cloning program is that it is mostly a "one-time boost." That is, no matter how many times one clones a particular animal, the clones—at least theoretically—all will exhibit the same incremental genetic gain. Sexual repro-

duction is necessary to obtain additional genetic variation to make further progress. In other words, the 30% boost in genetic value cannot be built upon with another 30% in the next generation; with or without cloning, one returns to the noncloning rate of genetic progress (Smith 1989).

Cloning of cattle has been emphasized primarily because a large amount of information is available, and because this is the food animal species for which cloning likely will have the most immediate application. The same considerations apply to other food animals such as sheep, goats, swine, rabbits, and camels. But cloning is unlikely to be as useful for most of these species as it is for cattle because of shorter generation intervals, more offspring per female per pregnancy, and higher heritabilities for some traits. Nevertheless, there are situations for each species for which cloning will be a valuable genetic tool.

Cloning for Direct Food Consumption

In a decade or two, it may be possible to set up mass cloning systems whereby hundreds of millions of oocytes from slaughtered females will be used to clone nuclei from cryopreserved, genetically superior cell lines. The embryos generated would be frozen, and instead of inseminating females artificially, technicians would thaw and transfer these embryos nonsurgically.

Although cloning of cryopreserved cells can be done already, cost and inefficiency make this procedure impractical. Additionally, a large-scale program would require thousands of farmers as customers or cooperative participants. A similar plan likely would be easier for pig producers than for cattle producers, but as indicated previously, cloning is a less valuable tool for pork production than for beef and milk production.

Producing more-uniform animal products would be desirable from the agricultural producer perspective, as well as from the points of view of the food processor and the food consumer. The objective of cloning, however, is more than simply uniformity. Cloning could, for example, speed the introduction of a desirable trait, such as decreased incidence of *Escherichia coli* O157:H7, into the cattle population. But it will not be possible to produce one *phenotype* of cattle that fits subtropical, Western range land, and Corn Belt environments and simultaneously is optimal for producing steak, hamburger, and milk for cheese production. The ideal carcass for steak is different from that for hamburger, so there are myriad ideal animals, and dozens of clonal lines, that would be required for optimal commercialization of cloning for food production.

Animal Clones versus Transgenic Animals

What is the difference between clones and transgenic animals? Sometimes the only difference is a single gene. Sometimes a transgenic animal is made using cloning technology. An animal can be a clone, however, and not transgenic or can be transgenic without being a clone.

A transgenic animal is (1) one into which a new gene has been introduced by human intervention, or (2) an "organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or recombination" (European 2001). The new gene is intended to alter some physiological characteristic of the animal, such as increased resistance to disease, enhanced carcass quality, improved feed conversion efficiency, increased food safety, or decreased *environmental footprint*. The new gene can be transferred into the animal by several technologies. Almost all the approaches introduce the new genetic information when embryos have only a few cells, with the objective that the new genetic information will be in every cell of the developing individual.

The first successful method for introducing new genetic information into mammals, *pronuclear microinjection*, involved injecting a solution containing the new gene into a recently fertilized egg (Hammer et al. 1985a). The most recently developed method involves using *lentiviruses*, a type of virus genetically engineered to carry the new genetic information, to infect early stage embryos (Hofmann et al. 2003, 2004).

Both of these methods, and numerous others, have weaknesses (Niemann and Kues 2007). First, genes introduced by these methods are inserted randomly into the genome, which can have negative consequences, depending on where the gene lands. Second, especially in the instance of the viral approach, the new gene can end up being inserted into a number of locations in the genome. Such a result can turn into a logistical challenge when trying to keep track of the gene in subsequent generations of offspring.

Cloning can help resolve those issues. One application of SCNT cloning is as a gene transfer technology. While the cells to be used as nuclear donors are being cultured in the laboratory, special techniques can be used to introduce new genetic information into the cells. These gene transfer techniques provide a means for genetically engineered precision. Thus, it is possible to direct the new genetic information to the exact desired location in the genome of these cells. And if such a genetically engineered cell is used for cloning, the resulting cloned animal will carry the accurately positioned transgene (Kuroiwa et al. 2004).

Therefore, using cloning technology as a means of producing a transgenic animal provides a method for genetic engineering to be done with more precision than could be achieved by the original transgenesis techniques. Furthermore, cloning technology is more efficient than many other means of producing transgenic farm animals, resulting in the use of fewer experimental animals to achieve success.

Disease-Resistant Transgenic Animals

Perhaps the most appealing use of transgenic technologies in farm animals will address the welfare of the animals themselves. These technologies also will have a positive impact on commercial activities. Since the first demonstration of transgenic technologies in livestock in the mid-1980s (Hammer et al. 1985a), the goal of enhancing an animal's ability to resist or combat a disease has been debated (Muller and Brem 1991; Whitelaw and Sang 2005). This goal is now reality in cattle, with studies in sheep, pigs, and chickens at an advanced stage (Whitelaw and Sang 2005). This work will promote fundamental understanding of both the dis-

ease process and the causative agents, directly benefiting the animal.

Infectious disease adversely affects livestock production and animal welfare, thereby affecting a community's sustainability and competitiveness. The costs of existing *endemic* diseases are estimated at 17% of turnover of livestock industries in the developed world and 35 to 50% in the developing world. Individual diseases, such as mastitis in cattle, can have multibillion dollar impacts. Epidemics, particularly in developed countries, can incur further costs and have profound impacts on the rural economy and on public confidence in livestock production. This problem occurred during the foot-and-mouth disease virus outbreak in the United Kingdom in the spring and summer of 2001. In addition, *zoonotic* diseases such as bovine spongiform encephalopathy (BSE), known as mad cow disease, also can impact human health.

Notwithstanding the success of traditional strategies such as vaccination or culling in combating specific diseases, there are many continuing challenges relating to animal health and disease. Many previously used control strategies, particularly antibiotic use, are now less available because of legislation, or because the pathogen has evolved to avoid the control strategy. Furthermore, new issues arise continually, and some disease control problems simply remain unsolved. Diseases such as BSE cannot be controlled with antibodies but are amenable to transgenic approaches. Powerful genetic tools now exist to assist in combating disease.

Transgenic technology is delivering animals that challenge both commercial attitudes and public opinion. These technologies will lead to new opportunities for diagnosis, intervention, and selective breeding of animals for disease resistance. The combination of these technologies with traditional disease control measures should allow for more effective and sustainable animal disease control.

Attacking the Pathogen

Transgenesis offers the opportunity to incorporate novel disease prevention strategies. Currently, perhaps the most attractive strategy is ribonucleic acid interference (RNAi), which involves the use of short ribonucleic acid (RNA) molecules to interfere with the activity of a gene. This technology weakens the activity of a given target gene (*knockdown*). Several forms of RNAi molecules are able to interfere with gene activity (Rana 2007). Additionally, RNAi molecules designed specifically to target a pathogen's gene can interfere either with that pathogen's ability to infect or with some other aspect of the disease process, significantly decreasing the disease burden (Pfeifer et al. 2006).

For some viruses, farm animals provide a reservoir for *viral reassortment*. The consequence of this situation can be globally devastating when a human tropic virulent strain is produced; for example, more people died of Spanish influenza in 1918 than died in the preceding World War. Given the world's current mobility, the global spread of new influenza viruses can occur extremely rapidly.

Central to this cycle of influenza virus evolution are domestic species such as pigs and chickens where the viruses from these animals and from humans exchange their genetic information, evolving as a new and potentially pathogenic strain. Therefore, strategies that target these species could have a dramatic effect on decreasing the risk of influenza epidemics (Chen et al. 2008). Transgenic chickens currently are being studied for the ability of RNAi strategies to decrease significantly the ability of influenza to progress through a population of birds (Hunter, Tiley, and Sang 2005). Several current studies are evaluating RNAi approaches to control other virus-caused disease in pigs, sheep, cattle, and horses.

Ribonucleic acid interference is a robust method to decrease the activity of a gene, by destroying its *messenger RNA* (mRNA), but it is unlikely to prevent the activity completely. That is because the mechanism by which RNAi interferes with gene expression relies on there being a nearly perfect concentration match between the RNAi molecules and its target mRNA—not something easily achieved. Therefore, RNAi probably will not halt a disease entirely, and the technique would be applied in conjunction with more standard disease mitigation strategies, if time permits.

A more dramatic strategy would aim to eliminate the possibility for the disease to develop. If infection or disease requires a specific host genetic factor, transgenic technology could be used to remove that factor. For example, the development of BSE requires the host animal to have the prion protein "prp" in its cells. But transgenic cattle have been generated that lack this protein completely (Kuroiwa et al. 2004; Richt et al. 2007), so these animals are resistant to transmissible spongiform encephalopathy development and do not show any disease symptom. The initial analysis of a number of parameters—including immune function, behavior, growth, and reproduction—indicates that these animals are otherwise normal. If similar nonessential genes can be identified that are essential for infection by other pathogens, this powerful strategy could be applied to other diseases.

Transgenic Animals Better Able to Combat Disease

The prevention of disease can be tackled in two ways: (1) by attacking the pathogen, as described previously; and (2) by generating transgenic animals that are better able to combat or resist the infection. (For other benefits of using genetic modification [GM] to combat disease, see Textbox 1.) The second approach includes enhancing the immune response of an animal, conferring additional innate protection, or blocking the route of pathogen entry into the animal.

Transgenic cows have been generated that have elevated concentrations of the antimicrobial protein lysostaphin in their milk (Wall et al. 2005). Lysostaphin is an enzyme that can degrade bacterial cell walls, thereby destroying the bacterium *Staphylococcus aureus*. Nearly complete protection against intramammary challenge with *S. aureus* was observed in these transgenic animals. Thus, these transgenic cattle are substantially less susceptible to mastitis, an inflammation of the udder that is usually the result of bacterial infection and the most consequential disease of dairy cattle. Decreasing the incidence of mastitis in the U.S. dairy herd alone will provide multibillion dollar savings and will improve the well-being of these animals. Although *S. aureus*

Textbox 1. Benefits of using genetic modification to combat disease

Animals more able to resist infection or display decreased disease symptoms will have better well-being, which is an important welfare advantage. More healthy animals will have a significant impact on the socioeconomics of the region where they are bred, whether in developed or underdeveloped countries. Before transgenic animals are used in the field, studies involving these animals will provide new information on the biology of pathogens and the mechanisms of disease progression. This information will be invaluable as the underpinning knowledge for the development of better disease intervention strategies, usually not even involving transgenic methodology in the application phase.

is one of the main pathogens leading to mastitis, it is not the only one. The next step in this technology will be to generate animals expressing a range of antimicrobials that are able to resist various microbial pathogens.

Additionally, the entry of a pathogen sometimes can be prevented. This strategy has successfully allowed transgenic animals to resist infection by the pseudorabies virus (Ono et al. 2004). Pseudorabies virus disease in commercial swine has been eradicated from the United States by vaccination but remains endemic in many parts of the world.

To become the preferred disease prevention policy, any GM strategy would need to compete on a cost–benefit basis against established vaccination and other disease prevention strategies. In challenge studies comparing disease resistance of transgenic animals with animals protected by vaccination, the GM animals were the more protected population (Wall et al. 2005). In this example, animals were generated that expressed a soluble form of the cell surface factor used by the virus to enter the host. The virus binds to this form that, because it is not attached to any cell, acts to block entry of the virus into the host. This strategy could be applicable to a variety of pathogens.

Perspectives

Traditionally, control of animal disease focused on destruction of the pathogen or vector outside the animal by spraying pest breeding grounds with pesticides to kill disease-carrying mosquitoes, for example, or after infection but before development of disease symptoms with vaccination and/or drugs. To complement these more traditional strategies, transgenic animals that are better able to combat disease have now been generated, and it is likely that GM animals can be incorporated into successful disease management schemes.

Transgenic strategies can provide novel intervention approaches not possible through established prevention schemes. In some instances, GM animals could provide the ultimate disease prevention strategy, such as cattle that have no prp protein and are therefore unable to develop BSE. For other diseases, transgenic animals such as transgenic chickens with decreased susceptibility to influenza could be incorporated into combined strategies that are currently only partly effective at disease prevention. Both approaches use the specific advantage that GM provides: permanent genetic advantages to the animal.

Currently, there are no treatments for more than half of all diseases that affect animals. Even for those diseases for which a treatment is available, contradicting issues limit effectiveness of treatments. For example, the use of some vaccines is compromised by virus variants against which the vaccine does not confer protection. With some vaccines, the expense and logistics of repeated administration must be considered, and with other vaccines, international trade is compromised when the presence of viral antibodies in animals is used as a tool to monitor disease status.

There also are global concerns about the current extensive use of antibiotics in animal agriculture and whether this use will speed the development of antibiotic-resistant organisms. Because GM animals are better able to combat disease, and thereby display better well-being with fewer intervention strategies, they will contribute to more efficient food production with less environmental impact.

Transgenics for Improved Food Safety and Quality

Food safety and quality are important issues for both the consumer and the producer. Current production systems provide safe animal food products with good nutritional qualities, but there is room for improvement. Precautions often are taken postharvest (e.g., the pasteurization of milk and vacuum packaging of meat) to ensure the safety of many food products and to preserve their quality. The transgenic approach, however, can be used to improve food safety and quality preharvest as well. Animal production traits can be modified by the addition of transgenes to act on the food product itself or to alter existing pathways in the animal to improve the safety and/or quality and healthfulness of the animal food product.

Modified Milk Protein

One of the first suggestions for the use of genetic engineering was to modify the milk protein system to alter the functional and physical properties of milk (Jimenez-Flores and Richardson 1988). For instance, altering the ratios of the milk proteins present or making single amino acid changes to improve the functional characteristics of individual milk proteins could result in milk with increased heat stability, improved milk properties for production of cheese, or the development of novel milk products. Similar methods have been used to apply transgenic technology to improve food safety and quality, such as the production of transgenic goats that produce milk with increased shelf life, transgenic pigs with more human-healthy fats in their muscle, and, potentially, dairy animals that produce milk that lacks key allergenic proteins.

The shelf life and stability of milk and milk products is important for food safety and quality. Milk proteins and fat globules break down with time, thereby decreasing the quality of the product. Bacteria naturally present in the milk and those contaminating the milk also can affect its shelf life. Fortunately, pasteurization effectively kills bacte-

rial pathogens and inactivates many of the protein- and fat-degrading enzymes. Even with heat treatment, the shelf life of milk is limited because of the growth of nonpathogenic cold-spoilage organisms—those that can live at refrigerator temperatures.

One approach to prolonging milk's shelf life is by enhancing expression of antimicrobials. Lysozyme is a naturally occurring antimicrobial found in the milk, saliva, and tears of all mammals as part of the bacterial innate defense system. Concentrations of lysozyme in the milk of dairy animals are 1,600 to 3,000 times less than those in human milk. Transgenic goats have been generated that express human lysozyme in the mammary gland, elevating lysozyme in the milk to approximately two-thirds that of concentrations found in human milk (Maga et al. 2006a). Milk from these animals was found to be *bacteriostatic* against bacteria responsible for causing mastitis and the cold-spoilage of milk and had an increased shelf life (Maga et al. 2006b). Milk could be left at room temperature for at least two days before bacterial growth was detected. This property could benefit milk consumers in industrialized countries by slowing the growth of bacterial contaminants and could be especially important in developing countries where refrigeration is limited and transport time is long.

Improved Nutritional Quality

The nutritional quality of food also can be improved by using the transgenic approach. Animal food products such as meat and milk contain high concentrations of saturated fatty acids (SFA). Consumption of SFA has been associated with an increase in blood cholesterol concentrations and subsequent increased risk of atherosclerosis and cardiovascular disease in humans. In contrast, fish contain high concentrations of omega-3 long-chain polyunsaturated fatty acids (n-3 PUFA). These types of fatty acids are important for human development and the prevention of cardiovascular disease. Mammals cannot synthesize n-3 PUFA, specifically linoleic acid (18:2n-6) and linolenic acid (18:3n-3), and thus rely on dietary sources of these essential fatty acids. Livestock have high concentrations of n-6 PUFA from which n-3 PUFA can be derived in plants, but like all mammals, livestock lack the enzyme necessary for this conversion.

The nematode *Caenorhabditis elegans*, however, possesses the required enzyme (n-3 fatty acid desaturase) for this conversion. The enzyme is encoded by the fat-1 gene, and transgenic pigs have been generated that express *C. elegans* fat-1 systemically (Lai et al. 2006). Because of the expression of the fat-1 gene, these pigs were able to convert some of their skeletal muscle n-6 PUFA to n-3 PUFA and raise the n-3 PUFA content of muscle from 1 or 2% to 8%. Therefore, these pigs can produce meat with improved nutritional quality and can act as a source of beneficial n-3 PUFA.

Transgenic technology also can be used to produce milk for people who are lactose intolerant. Lactose is the main sugar in milk and is required for proper milk production and secretion in the animal. Lactose is hydrolyzed into its component sugars (glucose and galactose) in the intestine by the action of the enzyme lactase. Lactose intolerance results when there is a decrease of lactose hydrolysis in the intestine due to a normal decrease in lactase activity because of age, damage to the intestinal lining, or a congenital lactase deficiency. Lactose nondigestion in the intestine has an osmotic effect, resulting in bloating, pain, and diarrhea.

Currently, low-lactose milk can be generated by the postharvest treatments of *ultracentrifugation* or enzyme addition, but these methods can be expensive and time consuming. With this technology, lactase could be expressed in the mammary gland and could catalyze hydrolysis of the lactose after it is secreted in the milk, without disrupting milk production. This concept has been tested in mice, and the expression of active lactase in the mammary gland resulted in a 50 to 85% decrease in the amount of lactose in the milk (Jost et al. 1999). If these findings could be extrapolated to dairy cattle, people with lactose intolerance could consume this modified milk more easily, taking advantage of this natural nutritional source of protein and calcium.

Additional Applications

Other applications of transgenic technology to improve food safety and quality include

- generating transgenic dairy animals that lack the milk protein β-lactoglobulin (BLG),
- producing nutraceuticals in milk, and
- combining applications to produce tailor-made food products that deliver good nutritional value.

Present in the milk of dairy animals but not in that of humans, BLG is thought to be the main allergen in bovine milk. With current gene engineering techniques, it is possible to *knock out* the gene responsible for producing BLG in bovine cells and then use those cells in nuclear transfer cloning techniques to produce transgenic cows lacking BLG. Because bovine milk often is the source for infant formula, much postharvest processing currently must occur to remove or degrade the BLG component. With BLG knockout transgenic cows, milk could be used directly because the main allergen already would be removed.

Nutraceuticals—compounds that can impart a medical benefit to humans—also could be produced in the milk or meat of livestock. For instance, lysozyme and lactoferrin could be produced in milk and, when consumed, impart their antimicrobial benefits at the intestinal level to result in a healthier gut microbiota. In contrast to breeding and selection, genetic engineering allows for the manipulation of specific traits. Therefore, animals could be generated with several gene additions and/or modifications to result in the desired food product.

Transgenics for Decreased Environmental Impact

The impact of livestock production on the environment is one of the most important issues facing producers today.

Animals impact the environment by

- requiring land for cultivation of their feed;
- generating greenhouse gases;
- excreting phosphorus, nitrogen, and metals;
- being hosts for zoonotic diseases; and
- contributing to the diminished efficacy of broad-spectrum antibiotics.

Transgenic technology may offer solutions to all these issues. For example, improved feed efficiency (the amount of lean gained per unit of feed) through growth-enhancing genes (Hammer et al. 1985a; McPherron, Lawler, and Lee 1997) would require fewer resources—land, fertilizer, and energy for cultivation—to support production of animal feed. Likewise, better feed utilization by improving the digestibility of glucans in barley, oats, and rye (Zhang et al. 1999) or of cellulose in poor-quality feed (Hall et al. 1993) would use fewer resources.

Another way to lessen the impact of animal agriculture on the environment is to develop a means of liberating nutrients already present but not available through the digestive process. Most phosphate in feedstuffs is in the form of phytate (Forsberg et al. 2003), a nondigestible form of phosphorus in *monogastrics*. As a result, approximately 60% of the phosphorus in the feed ends up in the manure. When the phosphate concentration in manure-based fertilizer is too high, phosphate runoff from fields results in *eutrophication* of streams and other marine environments, depleting oxygen in the water because of the accelerated plant growth.

Scientists at the University of Guelph and the Ontario Ministry of Agriculture and Food have used transgenic technology to deal effectively with this phytate issue in pigs. Transgenic pigs expressing phytase in their saliva (Golovan et al. 2001) are able to catalyze hydrolysis of phytate and use the otherwise unavailable phosphate. An important result of the improved phosphorus utilization is a decrease of up to 75% in fecal phosphate. The consequences of this decrease in phosphorus waste are widespread. Because the phytase transgenic pigs are able to use the *endogenous* phytate phosphate in the feed, supplemental phosphate is not needed in the diet.

Furthermore, because the phosphate concentration is lower in the feces, smaller plots of land are needed on which to spread manure to achieve optimal phosphate levels for crops. This important strategy for mediating the environmental impact of livestock production is in the early stages of being extended to fish (Hostetler et al. 2005) and chickens (Cho et al. 2006).

Transgenics for Increased Production Efficiency

The majority of important livestock production traits are complex and often controlled by multiple genes. Despite the continual increase in understanding of the functional relationship between livestock genes and production traits, this complexity makes it challenging to modify the appropriate gene(s) accurately enough to generate desired phenotypes, novel animal products, or specific animal adaptations. Applications of transgenic technology have aided efforts to improve major livestock production traits, including the quality of meat, milk, and fiber components (Table 1), and to expand farming areas into new habitats (Textbox 2).

Improved Meat Production

Researchers have been encouraged by the dramatic growth characteristics of transgenic mice engineered with transgenes for the expression of growth factors, including growth hormone (GH) (Palmiter et al. 1982, 1983), GH-releasing factor (GRF) (Hammer et al. 1985b), and insulin-like growth factor I (IGF-I) (Mathews et al. 1988). Therefore, many of the initial transgenic livestock studies focused on modifying body composition for increased meat production by stimulating growth rates with the introduction of genes for these growth factors.

Pigs seemed to be well-suited for this approach, as demonstrated by the remarkable growth effects achievable with the administration of exogenous GH (Chung, Etherton, and Wiggins 1985). But the pioneering studies with transgenic GH pigs, rather disappointingly, resulted in only slightly increased growth rates (Pursel et al. 1989).

Textbox 2. Expanding farming areas into new habitats

Some farmed fish of high economic value, such as salmonids, are unable to survive in environments of frigid waters. This situation poses a significant limitation to the farming of these fish in open waters. A solution that could expand the usable farming areas into the colder waters of the northern hemisphere might be provided by introduction of genes for antifreeze proteins (AFPs) from polar fish species. These proteins provide protection against freezing by binding to emerging ice surfaces, thereby inhibiting the growth of ice crystals and lowering the freezing temperature. Antifreeze proteins derived from winter flounder (Hew et al. 1999; Shears et al. 1991) and ocean pout (Wang et al. 1995) have been used to attempt production of freeze-tolerant fish. This approach has been hampered, however, by low expression levels for the AFPs, essentially resulting in lower AFP concentrations than those found in polar fish and, therefore, insufficient to confer freeze resistance (Zbikowska 2003).

Another, more dramatic effect observed in the few transgenic pigs that were responsive to the GH transgene was a decrease of carcass fat by as much as 80% at market weight (Pursel et al. 1990). Poor control of the transcriptional regulation of the GH transgene severely hampered the transgenic approach, resulting in high systemic GH concentrations. As a consequence, the transgenic pigs were suffering from a range of deleterious side effects including lameness, susceptibility to stress, and decreased fertility. Similar contemporaneous studies in sheep (Murray et al. 1989; Rexroad et al. 1989) essentially mirrored these findings (Pursel and Rexroad 1993).

More desirable effects on growth rate and body composition in the absence of adverse health effects have been achieved with approaches offering better control, such as targeting growth factor expression to skeletal muscle (Pursel et al. 1999) or applying inducible expression strategies with the ability to switch transgene expression on or off (Nottle et al. 1999).

Table 1. Transgenic technology applications aimed at the improvement of agricultural production characteristics

Introduced Modification	Application	Species	Reference
Meat production			
Insulin-like growth factor 1	Increased meat production	Pig	Pursel et al. 1999
Human and porcine growth hormone releasing factor	Increased meat production	Pig	Draghia-Akli et al. 1999; Pursel et al. 1990
Human growth hormone releasing factor	Increased meat production	Sheep	Rexroad et al. 1989
Bovine, human, and porcine growth hormone	Increased meat production	Pig	Nottle et al. 1999; Pursel et al. 1989; Pursel et al. 1990
Ovine growth hormone	Increased meat production	Sheep	Adams, Briegel, and Ward 2002; Ward and Brown 1998
Inducible myostatin knock out	Increased postnatal muscle growth	Mouse	Grobet et al. 2003
Myostatin disruption	Increased muscle growth	Mouse	Yang et al. 2001
Sex-specific disruption of myostatin	Efficient cattle production system for dairy cows and superior beef bulls	Mouse	Pirottin et al. 2005
Growth rate			
Piscine growth hormone	Shorter time to market	Fish	Devlin et al. 1994; Du et al. 1992; Nam et al. 2001; Rahman and Maclean 1999
Milk production			
Bovine α -lactalbumin	Increased milk yield and piglet survival	Pig	Wheeler, Bleck, and Donovan 2001
Bovine β- and κ-casein	Improved milk composition	Cattle	Brophy et al. 2003
Fiber production			
Ovine insulin-like growth factor 1	Improved wool production	Sheep	Damak et al. 1996
Ovine growth hormone	Improved wool production	Sheep	Adams, Briegel, and Ward 2002
Ovine keratin intermediate filament	Improved wool processing and wearing properties	Sheep	Bawden et al. 1998
Bacterial serine transacetylase and O-acetylserine sulfhydrylase	Improved wool production	Sheep	Ward 2000
Feed conversion			
Bacterial isocitrate lyase and malate synthase	Increased glucose supply	Sheep	Ward 2000
Human glucose transporter 1 and rat hexokinase II	Improved glucose utilization	Fish	Krasnov et al. 1999
New habitat			
Piscine antifreeze protein	Fish farming in colder waters	Fish	Hew et al. 1999; Wang et al. 1995
Disease resistance / food safety			
S. simulans lysostaphin	Mastitis resistance	Cattle	Wall et al. 2005
Human lysozyme	Food spoilage	Goat	Maga et al. 2006b

The modest success of improving growth characteristics in sheep and pigs with GH-based transgenes is in stark contrast to the massive change in phenotype that has been achieved with GH-enhanced transgenic fish using all piscine DNA constructs (Devlin et al. 1994; Du et al. 1992; Nam et al. 2001; Rahman and Maclean 1999). Overexpression of GH increased growth rates up to 35 times normal rates in transgenic loach and salmonids and resulted in large size and weight differences (five to eleven times normal) of transgenic and control fish. In the majority of fish species, the GH transgene-dependent growth acceleration results in fish that reach double the normal body size in half the normal time (Zbikowska 2003). Thus, these fast-growing transgenic fish could provide an enormous commercial benefit through greatly shortened production cycles and significantly heightened food production. *Pleiotropic effects*, however, including skin color change, modified skull shape, decreased fertility, and decreased viability have been detected in some of these growth-enhanced fish.

These extraordinary growth pheno-

types seem to be restricted to wild fish and are not achievable in domesticated fish (Devlin et al. 2001), possibly because domesticated fish already produce their own GH at high levels due to genetic selection. This difference might indicate that intrinsic limitations for further growth enhancement by GH exist in domesticated animals, consistent with the only modestly increased growth rates observed in transgenic GH pigs and sheep.

A promising alternative strategy to improve growth performance of large farm animals that could offer greater control is direct interference with regulators of skeletal muscle development. The functional loss of myostatin, a negative regulator of muscle growth, resulted in muscle mass increase in knockout mice of two to three times normal rates (McPherron, Lawler, and Lee 1997). This loss also was shown to be the underlying cause of the distinctive double-muscling phenotype characterized by an approximately 20% increase in muscle mass in some beef cattle breeds (Grobet et al. 1997; Kambadur et al. 1997; McPherron and Lee 1997). Because these known double-muscling breeds are associated with major calving difficulties and resulting welfare concerns, transgenic technology could provide the opportunity to limit the myostatin-related effects to only postnatal muscle growth. This limitation could decrease or eliminate the adverse health effects of an otherwise attractive production phenotype by using a conditional myostatin knockout strategy. The feasibility of this concept has been validated in a mouse model (Grobet et al. 2003).

But additive strategies to interfere with the myostatin pathway, including expression of myostatin inhibitors (Lee et al. 2005; Yang et al. 2001) or myostatin-specific RNAi molecules (Magee et al. 2006), might offer even more flexibility. Using precise, site-specific molecular tools, transgenic mice were engineered for the Y chromosome-linked muscle-specific expression of a competitive myostatin inhibitor (Pirottin et al. 2005). The males of these lines showed a 5 to 20% increase in skeletal muscle mass, whereas females, which lack the male-specific Y chromosome, are neither transgenic nor affected in their growth characteristics. This strategy, combined with a postnatal or inducible expression strategy, could provide a more efficient cattle production system, enabling the concurrent production of elite dairy cows and bulls with superior meat production ability. This approach can be enhanced further by using sex-sorted semen to increase the number of males, because only the males express the trait.

Improved Milk Production

In addition to providing nutrition and promoting health and growth for the suckling young, milk from dairy animals is an important food source for human nutrition, not only in its natural form but also in a variety of processed products. For millennia, dairy animals have been selected for milk production characteristics, but milk composition, in particular, proved to be relatively resistant to change by conventional means. Transgenic technology allows for the potential to introduce desired characteristics into livestock at unprecedented speed and magnitude. Although this situation prompted intense discussions on the application of transgenic strategies more than a decade ago (Jimenez-Flores and Richardson 1988; Wilmut et al. 1990), most of the concepts only have been tested or are being evaluated in mice. Only a few studies have been extended into target species, including pigs and cattle.

In pigs, milk production capacity is a limiting factor for piglet growth and survival and thus has a detrimental impact on the efficiency of pig production. To boost lactational performance of sows, overexpression of α-lactalbumin, which plays a key role in lactose synthesis and regulation of milk volume, has been proposed. Indeed, transgenic gilts overexpressing bovine α-lactalbumin had higher milk lactose and thus carbohydrate content in early lactation associated with a 20 to 50% increase in milk yield. As a result, growth and survival of piglets suckling transgenic gilts was improved greatly compared with growth and survival of control piglets (Wheeler, Bleck, and Donovan 2001).

Milk protein is important for the quality and yield of several dairy products. Therefore, it is not surprising that protein content is a major target to improve productivity, aid human nutrition, and alter various processing properties designed to suit the manufacture of specific protein-containing food products (Karatzas 2003; Wall, Kerr, and Bondioli 1997). In an attempt to increase the *casein* content of bovine milk and improve its composition for greater processing efficiency, additional gene copies encoding bovine β- and κ-casein were introduced into the bovine genome. Milk derived from these transgenic cows had an altered composition—most strikingly, a κ-casein content two to three times higher than in control milk (Brophy et al. 2003). Interestingly, this increase in κ-casein was at the expense of the production of some other milk proteins, emphasizing a biological ceiling in terms of protein output in high-producing dairy cows.

The combined changes also affected the physical appearance of the milk with a distinctive color change from ordinary white to yellow for the modified milk (Laible et al. 2007), an indication of an altered composition and novel milk with unique processing properties. The higher κ-casein content causes a size reduction of the casein *micelles*, which is probably the main reason for the observed change in color.

More importantly, however, this size reduction is an attribute that has been associated with increased heat stability and improved cheese manufacture (Jimenez-Flores and Richardson 1988). Initial analyses confirmed the presence of smaller casein micelles and, in cheese manufactured with the high κ-casein milk, increased concentrations of essential amino acids and thus greater nutritional value (Laible et al. 2007). But the functional properties of this novel milk still remain to be evaluated fully. Nonetheless, this study demonstrates the potential of the technology to alter milk composition dramatically in modern dairy cows within a single generation.

Improved Fiber Production

Some Australasian research groups have explored using transgenic strategies for the improvement of wool production in sheep. Sheep were engineered for the targeted expression of IGF-1 in wool follicles to increase wool growth. Shearing the transgenic sheep at one year of age resulted in an average 6% increase in clean fleece weight compared with that of nontransgenic sheep (Damak et al. 1996). The improved

wool growth, however, was associated with a decrease in wool quality and a tendency for coarser wool of lower staple strength. Surprisingly, the slight production advantage was only detectable in the first year and also failed to be transmitted into the next generation (Su et al. 1998). Efforts to boost wool production by overexpression of ovine GH were hampered initially by high plasma GH concentrations, causing adverse effects in the animals (Ward and Brown 1998). An improved construct, associated with decreased plasma GH concentrations, resulted in increased (12%) fleece weights in transgenic Merino sheep.

In a different breed, Poll Dorset cross sheep, the opposite outcome was observed: lower fleece weights from transgenic sheep indicating a significant interaction with breed type (Adams, Briegel, and Ward 2002). Moreover, the approach had a negative impact on wool quality with transgenic sheep producing wool with greater, thus less desirable, fiber diameter. To improve wool fiber quality, an ovine intermediate filament keratin transgene was expressed in wool follicles. Both microstructure and macrostructure of wool fibers, which had higher luster and decreased crimp, were substantially altered, demonstrating the potential of this approach to generate novel fiber types with improved processing and wearing qualities (Bawden et al. 1998).

A progression from these single transgene approaches to enhance wool production in sheep has resulted in the much more ambitious strategy to introduce a bacterial-derived biosynthetic pathway for the amino acid cysteine, which is a rate-limiting factor for wool growth. Although this novel biosynthetic pathway approach comprising two bacterial genes was validated successfully in mice (Ward et al. 1994), transfer of the strategy to sheep was unsuccessful because only unsuitably low expression levels of the biosynthetic enzymes could be achieved (Bawden et al. 1995; Ward 2000).

Improved Feed Conversion

On the basis of an analogous concept, it has been proposed that increased feed utilization efficiency in ruminants might be achieved by introducing a new biochemical pathway to increase the supply of glucose, which is at least partly responsible for the low feed utilization of ruminants. But efforts to complement sheep with two bacterial genes to establish a glyoxylate cycle in ruminants, enabling the synthesis of glucose directly from acetate produced in the rumen, failed because of the inability to insert these genes into the sheep genome (Ward 2000).

Initial work to modify metabolic pathways to improve feed conversion in fish has been equally unsuccessful, albeit for different reasons. To enhance glucose utilization, genes encoding mammalian enzymes implicated in rate-limiting roles for glucose transport and phosphorylation were introduced into rainbow trout. Although their enzymatic activities were confirmed in transfected embryos (Krasnov et al. 1999), expression could not be detected in transgenic fish, possibly because of a random, uncharacterized failure of transgene expression across all cells and tissues (Krasnov, Pitkänen, and Mölsä 1999).

It is noteworthy that despite the limitations discussed here, the transgenic GH approach did demonstrate that expression of GH can improve feed utilization significantly. Compared with nontransgenic littermates, transgenic GH pigs showed up to 18% higher feed conversion efficiencies (Pursel et al. 1990). Similarly, GH-enhanced fish, when compared with nontransgenic fish, seemed to be more efficient metabolically (Zbikowska 2003) and achieved increased growth rates through better feed conversion efficiency, at least with high protein diets (Fu et al. 2005). No changes in feed utilization, however, were observed in GH sheep (Ward and Brown 1998).

Conclusions

Like any tools, cloning and transgenesis have their strengths and limitations. Cloning can be used to make rapid genetic gains, but eventually breeders will have to rely on conventional breeding to create new genetic variation from which the next generation of elite animals will be selected. Transgenic technology can be used to address a variety of problems and introduce new characteristics into the gene pool. But, so far, the technology has not been applied to manipulate complex traits that are controlled by multiple genes. Furthermore, until researchers have a better understanding of how genes control phenotypic traits, designing transgenes to alter specific functions will remain part art and part science.

The technological limitations of these tools, however, are not the most significant hurdles restricting their use in food production systems. Although these technologies can enhance animal production in a number of important ways, they are not commonly used because of the lack of public understanding and acceptance. Proponents of biotechnology have not convinced consumers that including these technologies in food production systems is in the consumer's best interest, and consumers want to weigh the risks of new technologies in relation to the benefits they provide. Currently, only a few transgenic livestock projects are sufficiently mature for in-depth evaluation. So far, no hazards have been identified that would constitute a risk to consumers. Therefore, the risk currently perceived by consumers likely is associated with the unknown rather than with a genuine hazard.

Regarding food products, the government plays a role in managing risk by alerting consumers to potential hazards. When regulators are silent, as they have been for the past decade regarding their criteria for evaluating transgenic animals, both consumers and entrepreneurs are reluctant to pursue new technology.

Recently, regulators have begun to fashion a framework designed to assure consumers that a strategy for dealing with cloning and transgenesis is being developed. In 2008, the FDA concluded that "edible products from healthy clones that meet existing requirements for meat and milk in commerce pose no increased food consumption risk(s) relative to similar products from sexually derived animals" (USFDA 2008).

The regulatory apparatus is not as advanced for transgenic animals. The FDA recently issued a guidance document to outline the process by which transgenic animals will be regulated (USFDA 2009). If the same science-based approach used to evaluate the potential risks associated with eating meat and milk from cloned animals is applied to transgenic animals, consum-

ers and the livestock industry will be well served. Some people, however, believe that a different and somewhat unprecedented evaluation standard is being applied (Miller 2008). The FDA has taken a nontraditional approach to the regulation of transgenic animals, defining the transgene as a drug. This approach establishes an unusually high hurdle for the approval of a food product. Once the first few applications are processed by the FDA, it will become clear whether the regulations for transgenic animals will be able to achieve the delicate balance between assuring safe and healthful food products and not inappropriately delaying the application of these new technologies.

A regulatory process in which consumers have confidence and with which biotechnology companies can afford to comply must be in place for transgenic technology to be applied to livestock. In addition to generating the solid research necessary to demonstrate safety of products from these technologies, livestock producers must assure consumers that there is a compelling benefit to, or certainly no negative impact from, consuming transgenic products. The desirability of the product needs to be great enough to overcome natural concerns associated with trying something new.

Some of the most compelling projects enhance animal well-being and decrease the environmental impact of animal production. Will consumers perceive these approaches—those that only indirectly benefit the consumer—as sufficiently enticing to overcome their apprehension of the unknown? Or will approaches that consumers perceive to offer more direct benefits—such as enhanced food safety or human health—be required before consumers demand these products? The answers to these questions likely will be revealed within the next decade.

Glossary

Allele. Any of the alternative forms of a gene that may occur at a given location.

Bacteriostatic. The slowing of bacterial growth, usually by an antimicrobial, antibiotic agent.

Casein. One of the two main protein fractions of milk.

Clone. Individuals produced by asexual reproduction whose genomes are identical because they came from the same source.

Endemic. Characteristic of or prevalent in a particular location or population.

Endogenous. A product or process synthesized by an organism itself.

Environmental footprint. Impact of an individual, population, or system on natural resources and/or the ecosystem as a whole.

Eutrophication. Excessive accumulation of nutrients into an ecosystem resulting in accelerated growth and decay.

F_1 crossbred. A term used by geneticists to describe the first generation offspring of parents with distinctive genetic backgrounds.

Fibroblast. One of the most common cell types in the animal body; this type of cell secretes a number of proteins that aid wound healing and maintain the structural integrity of connective tissue.

Genome. The set of heritable information passed from one generation to the next, encoded by DNA molecules and usually thought of as the complete set of genetic information of an individual.

Heterosis. Increased or enhanced expression of a trait or traits in a hybrid individual, relative to its parents. Also known as hybrid vigor.

Hypoxia. A deficiency of oxygen in the tissues of the body.

Knockdown/Knock out. Attenuation of gene function usually resulting in a diminished amount of synthesis of the protein that the gene encodes.

Lentivirus. A member of the family of retroviruses; a type of virus whose genome is encoded by RNA molecules.

Macrosomic. Abnormally large body.

Messenger RNA (mRNA). A molecule of RNA encoding a chemical "blueprint" for a protein product.

Micelle. A unit of structure built up from polymeric molecules or ions.

Monogastric. An animal with a single-chamber stomach.

Oocyte. A female gamete or germ cell, otherwise known as an egg.

Phenotype/Phenotypic. A measurable characteristic of an animal such as hair color, growth rate, or degree of carcass marbling. These traits are the product of genetics and the environment.

Pleiotropic effect. The phenomenon of a single gene having influence(s) on multiple traits.

Pronuclear microinjection. A pronucleus is an organelle of a recently fertilized egg containing the genome of either the fertilizing sperm or the egg itself. Microinjection is the act of injecting a solution, usually containing transgenes, into one of the pronuclei.

Somatic cell nuclear transfer (SCNT). A method of animal cloning, which uses a body cell rather than an embryonic cell as a nuclear donor.

Transgenesis. The process by which a transgenic organism is made.

Transgenic. An animal, plant, or microbe that carries a recombinant DNA molecule (a gene spliced together in a laboratory) introduced by human intervention.

Ultracentrifugation. An analytical procedure for separating molecules or cellular components based on their mass at very high centrifugal forces.

Viral reassortment. A naturally occurring process in which a virus acquires a genetic segment or segments from a closely related virus. This acquisition may occur when two related viruses infect the same individual. The acquisition of the new genetic information may allow the newly constituted virus to have a broadened host range, such as when avian influenza virus acquires the ability to infect pigs.

Zoonotic. An infectious disease that can be transmitted between animal species and between animals and humans.

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