NOTE: Read Text Chapter 6 on Animal Biotechnology (pg. 165-177) For Background Reading

1

Transgenic Livestock as Drug Factories

By introducing key human genes into mammals, biologists can induce dairy animals to produce therapeutic proteins in their milk

by William H. Velander, Henryk Lubon and William N. Drohan

Exactly one year after her own birth, Genie, our experimental sow, was serenely nursing seven healthy piglets, her milk providing the many nutrients these offspring needed to survive and grow. But unlike other pigs, Genie's milk also contained a substance that some seriously ill people desperately need: human protein C. Traditional methods of obtaining such blood proteins for patients involve processing large quantities of donated human blood or culturing vast numbers of cells in giant stainless-steel reactor vessels. Yet Genie was producing copious amounts of protein C without visible assistance. She was the world's first pig to produce a human protein in her milk.

Genie's ability to manufacture a therapeutic drug in this way was the outcome of a research project conceived almost a decade ago. In collaboration with scientists from the American Red Cross who specialized in providing such blood proteins, we began to consider the possibility of changing the composition of an animal's milk to include some of these critically needed substances. In theory, this approach could generate any required quantity of the various therapeutic blood proteins that are regularly in short supply.

Demand for such drugs comes from many quarters. For instance, hemophiliacs may lack any of several different clotting agents, particularly blood proteins called Factor VIII and Factor IX. Certain people with an inborn deficiency require extra protein C (which acts to control clotting) to supplement their body's meager stores, and patients undergoing joint replacement surgery can benefit from this protein as well. Another important example of the need for therapeutic blood proteins involves people suffering strokes or heart attacks: these cases often demand quick treatment with a protein called tissue plasminogen activator, a substance that can dissolve blood clots. And some people suffering from a debilitating form of emphysema can breathe more easily with infusions of a protein called alpha-1-antitrypsin.

All these proteins are present in donated blood only in tiny amounts, and hence they are currently so difficult to produce that their expense precludes or severely limits their use as drugs. For example, treatment with purified Factor VIII (restricted to those times when someone with hemophilia is actually bleeding) typically costs the patient tens of thousands of dollars every year. The cost of continuous replacement of this blood protein for the same period—a desirable but rarely available option—would exceed $100,000.

Such enormous sums reflect the many problems involved in extracting these proteins from donated blood or establishing specialized production facilities using cultured cells—an enterprise that can require an investment of $25 million or more to supply even modest amounts of a single type of protein. Developing "transgenic" animals such as Genie (that is, creatures that carry genes from other species) demands only a small fraction of such costs. Yet the new breeds simplify procedures enormously and can produce vast quantities of human blood protein. Replacing conventional bioreactors with transgenic livestock thus offers immense economic benefits.

Creating blood proteins in this fashion also stands to better the other cur-

BARRY L. WILLIAMS (*pig*); JOHN HORNER ARCHITECTURAL PHOTOGRAPHY (*bioreactor*)

BIOREACTORS are typically large stainless-steel tanks with complicated controls for maintaining the broth in which countless individual cells are grown. But a new strategy for producing protein-based medicines circumvents the need for such elaborate, and often costly, machinery by using transgenic livestock, such as the pig (*inset*) engineered by the authors to produce one such protein in its milk.

rent practice—purifying them from donated blood—because it would circumvent the risk of contamination with infectious agents. Although blood proteins derived from pooled blood plasma are considered relatively safe now that donors are carefully screened and virus inactivation treatments are routinely applied, the threat from some pathogens always looms. For example, the fear of inadvertently spreading HIV (the AIDS-causing agent) and the hepatitis C virus is spurring researchers to seek substitutes for drugs now derived from human blood. Similarly, recent concerns about Creutzfeldt-Jakob disease (a degenerative disease of the nervous system) has caused some blood products to be withdrawn from the U.S. and Europe. Creating human blood proteins with transgenic livestock that are known to be free of such diseases would deftly sidestep these difficulties.

The many gains that would result from the use of transgenic animals as bioreactors gave us ample reason to pursue our vision of tidy stalls occupied by healthy livestock carrying a few key human genes. But at the outset of our work, we had many worries about the technical hurdles facing us in breeding such transgenic animals and garnering usable quantities of protein from their milk. Fortunately, we were able to progress rapidly, benefiting from a body of trailblazing research that had already been done.

Prior Mousing Around

As early as 1980, Jon W. Gordon and his colleagues at Yale University had determined that a fertilized mouse embryo could incorporate foreign genetic material (DNA) into its chromosomes—the cellular storehouses of genetic material. Shortly afterward, Thomas E. Wagner and his associates at the University of Ohio demonstrated that a gene (a segment of DNA that codes for a particular protein) taken from a rabbit could function in a mouse. Using a finely drawn glass tube of microscopic dimensions, these researchers devised a way to inject a specific fragment of rabbit DNA into a single-cell mouse embryo. Amazingly, that DNA would often become integrated into the mouse's chromosomes, perhaps because it was recognized by the cell as a broken bit of DNA that needed to be repaired.

These researchers then implanted the injected embryos in a surrogate mother mouse and found that some of the mice born to her contained the rabbit gene in all their tissues. These transgenic mice in turn passed the foreign gene on to their offspring in the normal manner, following Mendel's laws of inheritance. The added gene functioned normally in its new host, and these mice made rabbit hemoglobin in their blood.

Another milestone on the road to transgenic animal bioreactors was passed in 1987. Along with their respective colleagues, both Lothar Hennighausen of the National Institute for Kidney and Digestive Diseases and A. John Clark of the Institute of Animal Physiology and Genetics at the Edinburgh Research Station in Scotland established means for activating foreign genes in the mammary glands of mice. Foreign protein molecules created in this way were then secreted directly into a transgenic mouse's milk, where they could be easily collected. These researchers accomplished this feat by combining the foreign gene of interest with a short segment of DNA that normally serves to activate a gene for a mouse milk protein.

Whereas Hennighausen's mice produced the desired human protein (in that case, tissue plasminogen activator) at disappointingly low concentrations, Clark's mice produced 23 grams of a sheep milk protein (known as beta-lactoglobulin) in each liter of milk—approximately matching a mouse's own major milk proteins in abundance. But beta-lactoglobulin was not a human protein in short supply, nor were these tiny mice the proper vehicle to provide useful quantities of milk. So Clark and his colleagues went to work injecting sheep embryos with DNA that contained a medically important human gene.

They used the gene that codes for a blood-clotting factor (Factor IX), along with a segment of sheep DNA that normally switches on the production of beta-lactoglobulin in the mammary gland. Two years later Clark's transgenic sheep secreted Factor IX in their milk—but at barely detectable levels. It was at that juncture that we began our attempts to realize the potential of such pioneering work. But we decided to take a gamble and try a novel strategy.

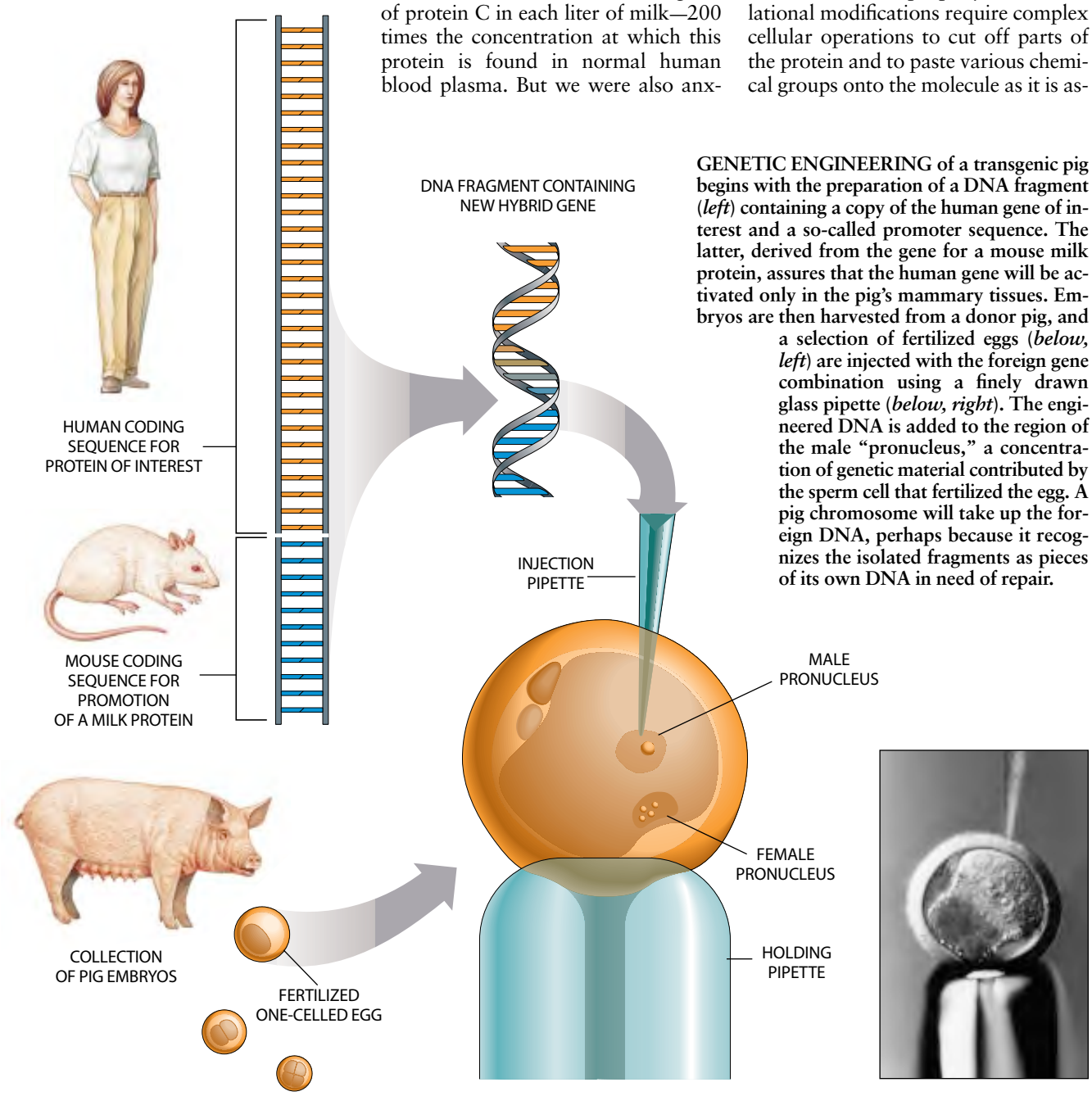
A Pig in a Poke

Whereas other research groups had picked sheep, goats or cows as suitable dairy animals for producing human proteins, we chose to work with pigs instead. Swine offer the advantages of short gestation periods (four months), short generational times (12 months) and large litter sizes (typically 10 to 12 piglets). Thus, producing transgenic pigs is relatively quick compared with transforming other types of livestock. And despite their lack of recognition as dairy animals, pigs do produce quite a lot of milk: a lactating sow generates about 300 liters in a year. The real question for us was whether this unconventional choice of transgenic animal could in fact be made to produce appreciable levels of human protein in its milk.

Toward that end, we decided to use a DNA segment made up of a human gene and the so-called promoter for a major mouse milk protein (called whey acidic protein) that had been characterized by Hennighausen and his colleagues. By injecting this DNA combination into mouse embryos, those researchers were able to augment a mouse's chromosomes so that the creature would produce the desired human protein in its milk. To take advantage of this approach, we, too, fashioned a fragment of DNA that contained the human gene for the target protein (in our case, protein C) and the mouse promoter for whey acidic protein. But we injected this DNA into a set of pig embryos.

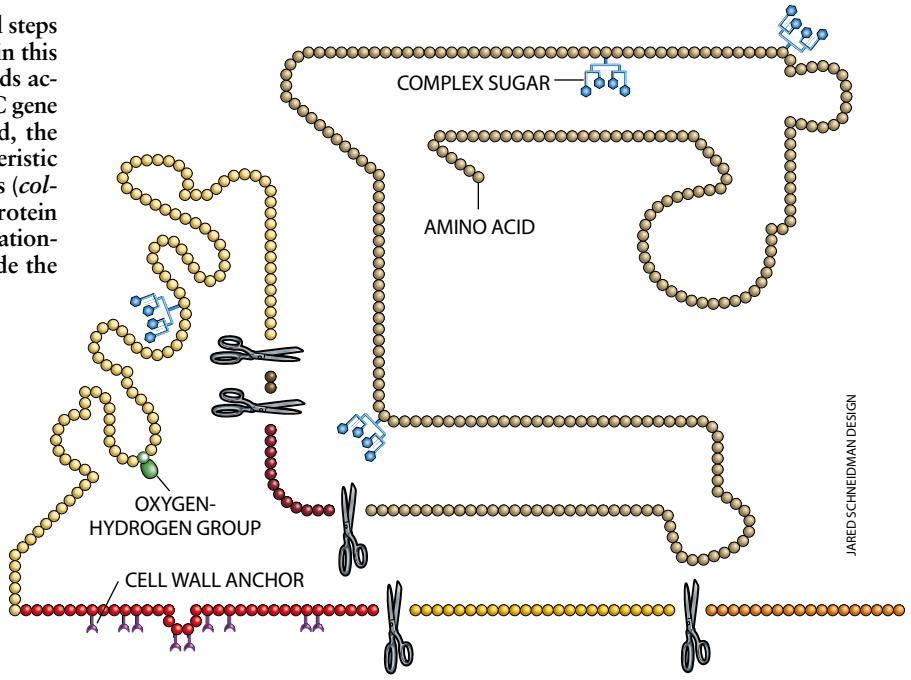
By implanting these fertilized cells in a surrogate mother pig, we could identify—after four months of nervous waiting—a newborn female piglet that carried the foreign DNA in all its cells. But even with this accomplishment, we had to remain patient for another year as our transgenic piglet, Genie, matured. Only then could we find out whether she would indeed produce the human protein in her milk. To our delight, Genie's milk contained protein C. Although the human protein was not as abundant as some of the pig's own milk proteins, it was nonetheless present in substantial amounts, with about one gram of protein C in each liter of milk—200 times the concentration at which this protein is found in normal human blood plasma. But we were also anxious to find out if this pig-made human protein would be biologically active.

We were concerned because the details of protein synthesis inside cells remain somewhat mysterious. The workings of the cellular machinery for reading the genetic code and translating that information into a sequence of amino acids—the building blocks for protein molecules—is, for the most part, well understood by biologists. But there are some subtle manipulations that need to be done by cells after the amino acids are joined together. These so-called post-translational modifications give a newly constructed protein molecule the final shape and chemical composition it needs to function properly. Post-translational modifications require complex cellular operations to cut off parts of the protein and to paste various chemical groups onto the molecule as it is as-



GENETIC ENGINEERING of a transgenic pig begins with the preparation of a DNA fragment (*left*) containing a copy of the human gene of interest and a so-called promoter sequence. The latter, derived from the gene for a mouse milk protein, assures that the human gene will be activated only in the pig's mammary tissues. Embryos are then harvested from a donor pig, and a selection of fertilized eggs (*below, left*) are injected with the foreign gene combination using a finely drawn glass pipette (*below, right*). The engineered DNA is added to the region of the male "pronucleus," a concentration of genetic material contributed by the sperm cell that fertilized the egg. A pig chromosome will take up the foreign DNA, perhaps because it recognizes the isolated fragments as pieces of its own DNA in need of repair.

ROBERTO OSTI (*animals*); JARED SCHNEIDMAN DESIGN (*diagram*); STEPHEN P. BUTLER (*photomicrograph*)



HUMAN PROTEIN C is synthesized in several steps within a cell. The cellular machinery involved in this task starts by stringing together 461 amino acids according to a prescription coded by the protein C gene (a step known as translation). As it is created, the nascent protein molecule folds into a characteristic configuration, forming several distinct domains (*colored regions*). But to function properly, the protein must also undergo several so-called post-translational modifications. These additional steps include the cleaving and removal of certain sections of the protein, as well as the addition of particular chemical groups to specific sites on the amino acid chain.

sembled. Would the cells of Genie's mammary tissue be able to carry out those modifications well enough to make a working version of the human blood protein?

To determine the answer, we had to tackle the new problem of isolating a human blood protein from pig milk. First we removed the milk fat by centrifugation. Then we purified the remaining whey using a procedure that would extract only the biologically active part of the human protein. To our amazement, this component amounted to about one third of the total complement of protein C present. Never before had functional protein C been produced and harvested at such high levels from a transgenic animal—or from a conventional bioreactor. Genie had passed a major test, providing the first practical demonstration that a complex human protein could be produced in the milk of livestock.

Next Year's Model?

We devoted several years to studying Genie and many of her extant offspring and then began to focus our efforts on increasing the concentration of active human protein in the milk. Our intent was to overcome the limitations of mammary tissue in making the needed post-translational modifications. In principle, breaking through those final barriers could triple the output of useful protein molecules produced.

With some painstaking research into the problem, we discovered that most of the protein C remained in an immature, inactive form because there were insufficient amounts of a key processing enzyme named furin—itself a complex protein—within these cells. Hence, we immediately asked ourselves whether we could improve the situation by introducing another foreign gene, one that would allow more of the needed processing enzyme to be made.

To test this possibility quickly, we switched our efforts temporarily from pig to mouse, the fast-breeding mainstay of most transgenic mammal experiments. In 1995 we succeeded in engineering a line of transgenic mice that contained two human genes—one for protein C and one for furin. We arranged for both of these transgenes to switch on in the mammary gland by attaching them to the DNA promoter we had previously incorporated in Genie.

After months of tedious effort in the lab, we were ecstatic to find that these mice were able to secrete the mature form of protein C in their milk. We have thus started development of a new and improved transgenic pig that contains human genes for both protein C and furin. We expect soon to see a pig that produces three times more active protein C than Genie did, and we anticipate that other researchers working with transgenic livestock will also be able to fashion genetic modifications that cause the manufacture of processing enzymes along with the target protein.

Chimerical Visions

The notion of obtaining essentially unlimited quantities of scarce human blood proteins at reasonable cost would have seemed pure fantasy just a short time ago. For more than two decades, molecular biologists and biochemical engineers have labored to overcome the problems of producing even modest amounts of human proteins from large-scale cell culture facilities. Yet making biological pharmaceuticals in huge stainless-steel vats of genetically engineered cells seemed destined to remain an awkward and expensive undertaking.

Such bioreactors are enormously costly to construct, and they prove in operation to be extremely sensitive to small changes in the temperature and composition of the broth in which the cells are grown. In contrast, transgenic livestock bioreactors can be created merely by breeding more animals. Transgenic livestock need only routine attention to control their living conditions and nutrient supply, and yet they can easily produce the desired proteins at much higher concentrations than their metallic counterparts.

Although some risk exists that pathogens could be transmitted from livestock to humans, formal procedures are available to establish pedigreed animals that are free of known diseases. Indeed, such specific-pathogen-free herds are a well-established part of the agriculture industry. In addition, decades of the clinical use of pigs to produce insulin for diabetics give us confidence that swine can readily serve as bioreactors for therapeutic human proteins without presenting undue hazard.

Still, like all new medicines, the human proteins produced in this way need to be carefully tested for safety and effectiveness before the government approves them for widespread use. The

What's Good for Genie…

The advent of transgenic techniques for manipulating livestock also raised legitimate concerns about the health and welfare of the animals altered in this rather unorthodox way. After all, engineered "transgenes" of the kind we implanted in pig embryos can ultimately become part of each and every cell of the mature animals. What if an introduced gene turns on inappropriately and produces the foreign protein in a way that damages the surrounding tissue?

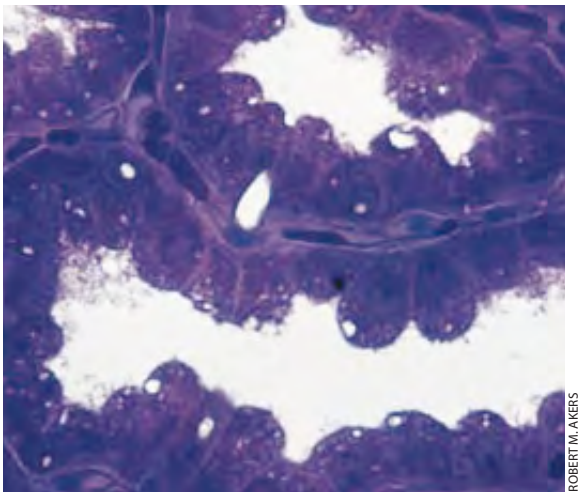
Such worries made it critically important that we design our genetic manipulations so that the foreign gene would be driven into action only in the mammary gland—that is, within tissues that have a natural ability to produce and export protein without harming themselves or their host. We could expect to achieve such targeted control of protein production in our transgenic pigs because we used a promoter from a milk gene—a genetic switch of a type that is present in all mammals.

Yet we recognized that even such well-behaved genes can show some promiscuous activity. The genes we introduced into pigs, for example, also produce small amounts of their foreign proteins in the animals' salivary glands. These tissues are, in fact, quite similar in composition to mammary tissue. So we fully expected this incidental production, and we are quite sure that this minor side effect does not harm the pigs in any way.

The lack of detrimental side effects is crucial—for the animals involved and also for the success of this pioneering method. One of the primary reasons for developing transgenic livestock to supply human proteins is to limit the possibility of transmitting diseases to the recipients of these drugs. Using anything but the healthiest livestock to produce these substances could increase the animals' susceptibility to disease as well as the possibility that they might accidentally pass on some unknown pathogen. Genetically engineering weakened livestock would thus, in the end, only prove self-defeating in the quest to produce safe and plentiful medicines. *—W.H.V.*

first example to be so examined (an anticlotting protein called antithrombin III, manufactured by Genzyme Transgenics Corporation using transgenic goats) began clinical trials just a few months ago.

It is possible that the subtle differences between human and animal cells in the way post-translational modifications are carried out may affect how such proteins function in people. For example, certain modifications cause proteins to be cleared from the blood quickly by the liver, and so we suspect that some of the differences between the animal and human forms of these proteins could actually constitute improvements in the way these substances function as long-lived therapeutic drugs.



ROBERT M. AKERS

MAMMARY TISSUE from a genetically engineered pig contains a dense array of cells (*purple*) that produce a therapeutic human protein. The structure of the mammary gland allows the human protein produced in this way to flow through the secretory channels (*white*), along with other components in the animal's milk.

It is tempting to view the development of transgenic livestock bioreactors purely as a triumph of technology. But the history of this science also highlights the limits of what people can do with sophisticated machines. The mammary gland is optimized to maintain a high density of cells, to deliver to them an ample supply of nutrients and to channel the valuable proteins produced into an easily harvested form. Mammary tissue proves far superior to any cell-culture apparatus ever engineered for these tasks. Despite all their efforts to improve industrial cell-culture facilities, it turns out that a generation of biochemical engineers were unable to match the abilities of a tool for making proteins that nature had already honed. SA

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Further Reading

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The Land of MILK & MONEY

By Gary Stix

The first drug from a transgenic animal may be nearing approval

Proteins are biotechnology's raw crude. For much of its 30-year history, the industry has struggled to come up with a steady source of supply, squeezing the maximum out of these large-molecule commodities from cell lines isolated from hamster ovaries and the like. In the late 1990s—with the advent of a new class of protein-based drugs, monoclonal antibodies—demand sometimes outstripped supply. For decades, the scientists who created recombinant erythropoietin to rejuvenate red blood cells and monoclonal antibodies to combat cancer have sought out alternative forms of manufacture.

A new bioreactor—an animal genetically engineered to produce a therapeutic protein in its milk—may finally be ready to fulfill its long-awaited promise. The European Medicines Evaluation Agency (EMEA) may decide early next year on approval of an anticoagulant protein, human antithrombin, that is produced in goat's milk to treat a hereditary disorder. If the drug, ATryn, finally gets a nod from regulators, its approval will mark the culmination of a meandering 15-year journey for GTC Biotherapeutics, a Framingham, Mass., spin-off of the biotech giant Genzyme.

The idea of making transgenic drugs occurred to a number of scientists during the mid-1980s, when the new industry began to wrestle with the challenge of making complex proteins: ensuring that these big molecules were folded into the proper shape and that they had all their sugars in the right places on the surface of the proteins' amino acids. Chinese hamster ovary cells do the job, but getting enough product has been a constant frustration and one reason why biotech drugs today cost so much. In addition, mammalian cell cultures are not always an ideal medium: at times, it is simply too hard to produce proteins in this manner.

In their quest for greater efficiencies, researchers noticed that the mammary glands of cows, rabbits and goats, among others, are capable of becoming ideal protein manufacturing plants because of their ability to make high volumes of complex proteins. Milk glands, moreover, do not need the constant coddling required for cell cultures.

Genzyme got involved after its purchase in 1989 of Integrated Genetics, which had a portfolio of drugs and diagnostics products. To head up its program, Genzyme recruited one of the pioneers in this technology from another company, Biogen. Harry Meade, along with Nils Lonberg, had patented a method of extracting therapeutic proteins from mice.

In the early 1990s Genzyme's program was targeted at producing drugs in goat's milk. Genzyme, though, was not focusing on transgenics and decided to spin off its operation into a separate entity, Genzyme Transgenics (later re-

BRAD DECECCO

TRANSGENIC GOAT gets milked at a farm owned by GTC Biotherapeutics, headquartered in Framingham, Mass. The animal secretes a valuable pharmaceutical protein in its milk.

named GTC Biotherapeutics), in which the parent still holds an equity interest. The new company could thus produce its drugs for other firms without the inevitable conflicts of interest that would have arisen had it remained within the bosom of a large drugmaker.

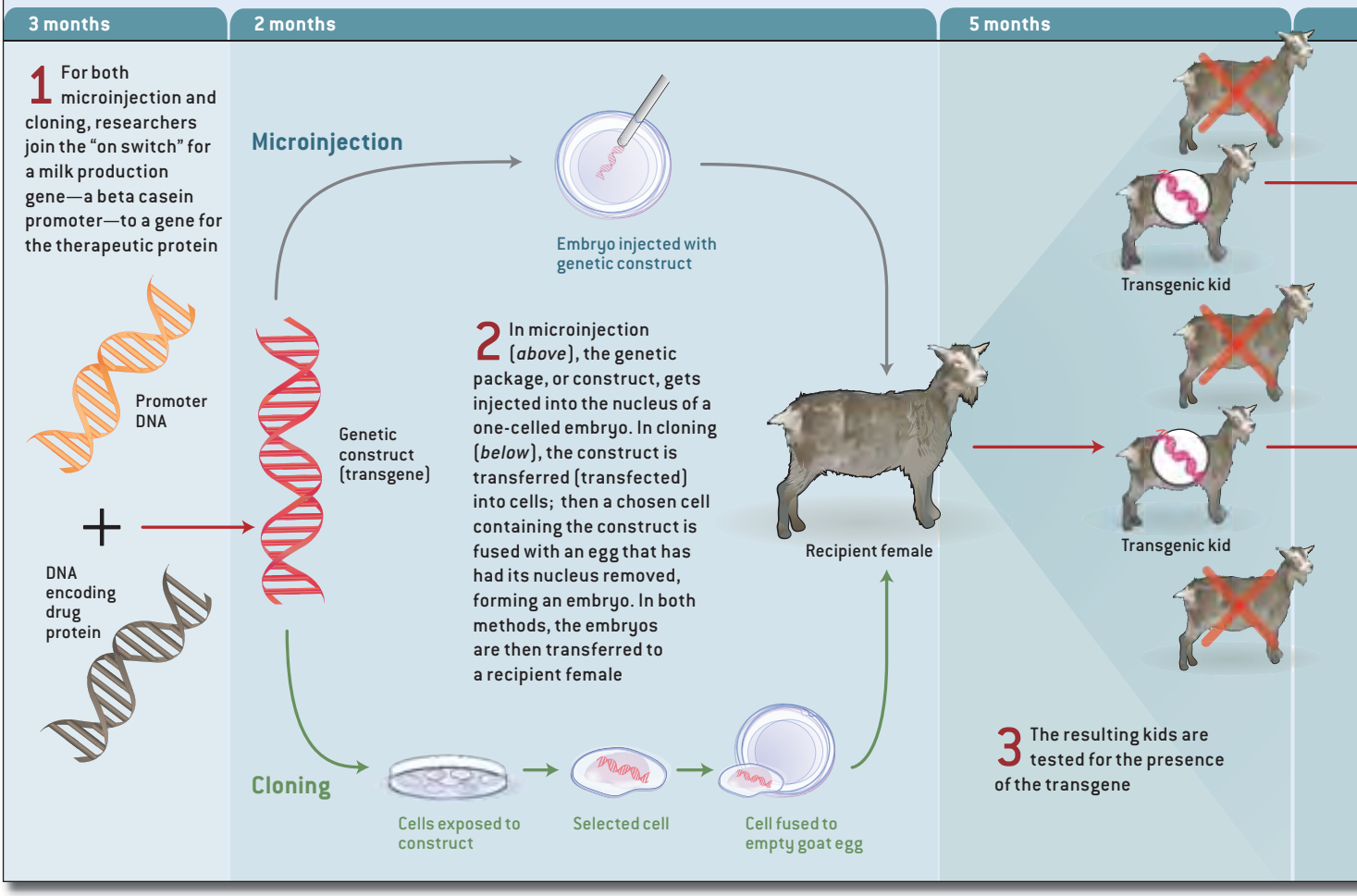
Goats as Drug Factories

INITIALLY, GTC generated transgenic goats by microinjecting into the developing nucleus of a one-cell embryo a gene encoding the desired human protein (along with DNA that promotes activation of that gene in milk). Such embryos were transferred into female goats, which produced offspring that were then tested for the presence of the newly integrated gene. The milk of these "founder" animals contains the therapeutic protein, which must then undergo a purification process. The mature transgenic animals were bred usually with nontransgenic goats as a first step toward producing a herd [*see box on next two pages*]. Microinjection, however, is an inefficient process. Only 1 to 5 percent of the embryos result in transgenic animals. For newer drugs in its portfolio, GTC has adopted somatic cell nuclear transfer, a.k.a. cloning, which ensures that an animal will carry the desired transgene. Dolly the sheep was cloned, in fact, with the intention of eventually using this procedure to create transgenic animals having useful properties, not as a means to make carbon copies of baseball legend Ted Williams or a favorite dead pet.

GTC stuck with goats because they reproduce more rapidly than cows and can yield more protein than mice or rabbits. Other efforts, including a more nascent GTC endeavor, have opted for cows. Pharming, a Netherlands-based company, aims to milk both cows and rabbits for drugs. Yet others have pursued distinctive forms of bioreactors: making drugs in chicken eggs, for instance. After undertaking basic development of the technology during the 1990s,

MILKING GOATS FOR DRUGS

GTC Biotherapeutics, which is counting on European approval of an anticlotting drug produced in goats, has used two major approaches to create a transgenic animal. The older technique, microinjection, employed for the drug undergoing regulatory review called ATryn,



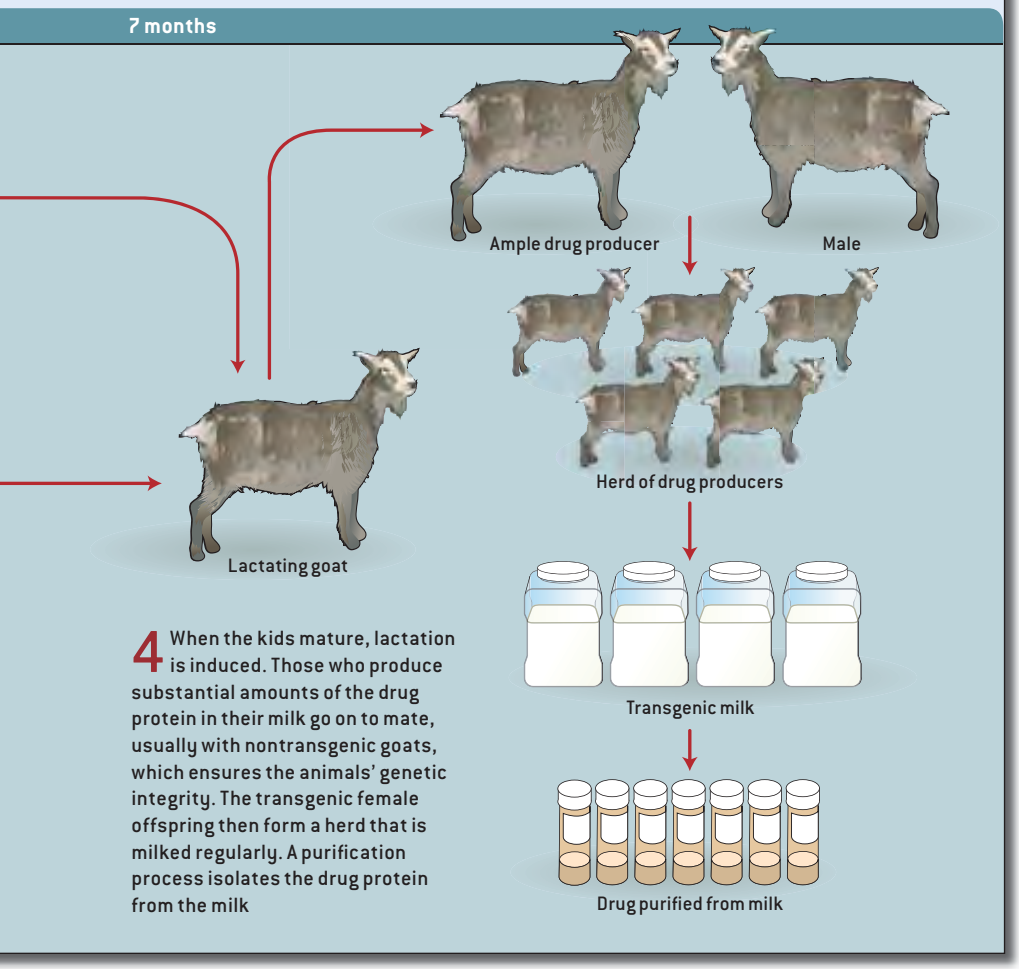
GTC hung out a shingle, marketing itself as a technology platform for companies that either wanted to produce difficult-to-make pharmaceutical proteins or needed large quantities at low cost. The one catch was that regulators had never approved a transgenically produced drug, and the more than a dozen partners that GTC took on tended to view the technology as a backup in case other protein-drug development strategies did not work out. They were unwilling to accept the expense and risk of an arduous regulatory process for a pioneering form of drug manufacture.

GTC recognized the need to demonstrate on its own the potential for the technology and, in the late 1990s, began a clinical trial of human antithrombin for patients undergoing bypass surgery who develop resistance to the anticoagulant drug heparin. Transgenic antithrombin was intended to improve supply and address concerns about pathogens in the form of the drug isolated from human blood. The company completed the required clinical trials. But when the Food and Drug Administration asked for more data in late 2000—which would have necessitated additional testing—then chief executive Sandra Nusinoff Lehrman scrapped the effort. In mid-2001 Nusinoff Lehrman left, and her replacement, Geoffrey Cox, decided to proceed with development of transgenic antithrombin—this time in European clinical trials for patients with inherited antithrombin deficiency. Regulators there had recently issued guidelines that set out the requirements for getting approval for antithrombin.

The company still has a few partnerships. It also has a preliminary program to make other blood proteins, such as alpha-1 antitrypsin, and a clinical trial in the U.S. for ATryn. But its future hinges on the European approval. The company, which went public in 1993, has flirted with penny-stock status (less than $1 a share), and its cash levels are much depleted from what they were at the start of the decade. It has also experienced "restructurings," layoffs that occurred in 2003 and 2004. "This is an important moment," says Cox of the upcoming EMEA decision. "This isn't a business for the faint of heart."

LUCY READING-IKKANDA

involves introducing a gene directly into an embryo. The company has also been a pioneer in producing transgenic drugs through cloning.



4 When the kids mature, lactation is induced. Those who produce substantial amounts of the drug protein in their milk go on to mate, usually with nontransgenic goats, which ensures the animals' genetic integrity. The transgenic female offspring then form a herd that is milked regularly. A purification process isolates the drug protein from the milk

Bioreactor Blues

OTHER TRANSGENIC companies have also had a rough haul. The Scottish company PPL Therapeutics, which helped to clone Dolly, encountered difficulties and sold its remaining intellectual property to Pharming in 2004. The latter has staged a comeback since filing for protection from creditors in 2001. It hopes to get approval soon for a treatment for hereditary angioedema, a genetic disease that causes swelling from the absence of the C1 inhibitor protein.

If GTC survives, it could become the leader in transgenics. The impetus for starting the company still appears justified. The capital costs for a drug production facility using hamster cells can amount to $400 million to $500 million, Cox says, whereas a herd of goats can produce the comparable amount of drug for $50 million. "There's still a need for alternative production methodologies," says Philip Nadeau, who tracks GTC as an analyst with S. G. Cowen. "There are still proteins that are difficult to produce using traditional methods, and therefore a company like GTC should certainly have a niche." ATryn's uses could be broadened to encompass an array of treatments—for coronary bypass, burn or sepsis patients—that might, in total, bring in as much as $700 million annually, Cox estimates.

The drug appears to have surmounted an important technical hurdle: so far it has not created any adverse immune response in patients. But such events will always remain a worry. Researchers administering inhaled transgenic alpha-1 antitrypsin from sheep bred by PPL discovered that some patients suffered pulmonary symptoms that caused them to leave the trial—a possible immune reaction to residual proteins from the animal that remained after purification of the drug. The PPL drug, given on a longer-term basis than ATryn is, needed to be better purified, notes Meade, GTC's chief scientific officer.

Producing drugs in goats has so far elicited less criticism than the debate over genetically modified plants. Goats cannot drift with the wind like corn pollen, spreading their transgenes to unexpected places. "If it's able to make drugs available that are not otherwise available by other methods and if it would make drugs cheaper, it would be certainly advantageous to consumers," notes Jane Rissler of the Union of Concerned Scientists. "Frankly, consumers have not benefited very much [so far] from biotechnology in the agricultural sector."

At GTC, the scrapie-free goats brought in from New Zealand are penned within a 190-acre enclosure on a 300-acre plot in Charlton, Mass. The animals are fed—and not permitted to graze—to diminish the possibility of contracting disease from contaminants in other animals. Thirty goats are devoted to making ATryn among a transgenic herd of more than 300, and an additional 1,200 nontransgenic animals are kept for breeding. "We have more veterinarians than M.D.s," Cox says. If ATryn finally receives approval, traditional dairy farmers flirting with insolvency may gaze in astonishment at a product made in milk that commands thousands of dollars per gallon. SA

MORE TO EXPLORE

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ATryn, an anticoagulant, is the first ever biologic product produced by a genetically engineered (GE) animal – a goat. It is for patients who have a rare disease known as antithrombin (AT) deficiency. ATryn (Antithrombin [Recombinant]) is an important and welcome drug to prevent blood clots in patients who lack sufficient antithrombin (AT). About 1 in 5,000 Americans has AT deficiency, and these patients are at high risk for clotting during surgery and childbirth. Therefore, ATryn will be given in the hospital. Up to now a product derived from human blood donors was the only AT drug available in the U.S.

"ATryn is a welcome therapeutic option", says NBCA's Medical Director, Dr. Stephan Moll, "as it is recombinantly produced and, thus, the risk of transmittable diseases from human blood donors, however low it may be with plasma-derived products, is eliminated". People with hereditary antithrombin deficiency are at increased risk for blood clots in legs and lung, referred to as deep vein thrombosis (DVT) and pulmonary embolism (PE), which can be life-threatening. Antithrombin occurs naturally in healthy humans - it helps keep blood from clotting in blood vessels. Antithrombin normally acts to inhibit coagulation, so a deficiency in anthithrombin makes the blood more prone to clot. This is the first time federal officials approved the sale of a drug made in animals genetically modified to secrete a compound in their milk. The goats have been genetically engineered by introducing a segment of DNA into their genes with instructions for the goat to produce human antithrombin in its milk. The human antithrombin is then extracted from the goats' milk to manufacture ATryn. Using animals to produce medications needed by humans has been a long-standing goal of the FDA, and federal officials emphasized that this genetic technique not only has vast potential for patients, but can be carried out without harm to the animals.

GTC Biotherapeutics, Inc., the manufacturer of ATryn, received approvals from two FDA centers. The Center for Biologics Evaluation and Research (CBER) approved the human biologic based on its safety and efficacy, and the Center for Veterinary Medicine (CVM) approved the rDNA construct in the goats that produce ATryn.

ATryn is manufactured by GTC Biotherapeutics, Inc., Framingham, Mass. GTC has granted Ovation Pharmaceuticals, headquartered in Deerfield, IL, the right to market ATryn in the U.S. and pursue further clinical development. The companies expect ATryn to be available in the second quarter of 2009. ATryn previously received approval from the European Medicines Agency in 2006 for use in preventing clotting conditions during surgical procedures in patients with hereditary AT deficiency.

FDA officials said that although their primary responsibility was to make sure the antithrombin produced in goats is safe, the agency had also taken care to assure itself that animals involved in this genetic engineering are not harmed.

For more information on antithrombin deficiency (click here): **http://www.stoptheclot.org/natt_publications/antithrombin_def.pdf**

For more information on the FDA's decision go to: **http://www.fda.gov/bbs/topics/NEWS/2009/NEW01952.html**

Click here to return to our news page.

Cloning

Now that genetically modified and copied mammals are a reality, biomedical researchers are starting to develop imaginative ways to use this technology

JOHN CHADWICK PHOTOGRAPHIC AND PPL THERAPEUTICS

RODDY FIELD *Roslin Institute*

In the summer of 1995 the birth of two lambs at my institution, the Roslin Institute near Edinburgh in Midlothian, Scotland, heralded what many scientists believe will be a period of revolutionary opportunities in biology and medicine. Megan and Morag, both carried to term by a surrogate mother, were not produced from the union of a sperm and an egg. Rather their genetic material came from cultured cells originally derived from a nine-day-old embryo. That made Megan and Morag genetic copies, or clones, of the embryo.

Before the arrival of the lambs, researchers had already learned how to produce sheep, cattle and other animals by genetically copying cells painstakingly isolated from early-stage embryos. Our work promised to make cloning vastly more practical, because cultured cells are relatively easy to work with. Megan and Morag proved that even though such cells are partially specialized, or differentiated, they can be genetically reprogrammed to function like those in an early embryo. Most biologists had believed that this would be impossible.

We went on to clone animals from cultured cells taken from a 26-day-old fetus and from a mature ewe. The ewe's cells gave rise to Dolly, the first mammal to be cloned from an adult. Our announcement of Dolly's birth in February 1997 attracted enormous press interest, perhaps because Dolly drew attention to the theoretical possibility of cloning humans. This is an outcome I hope never comes to pass. But the ability to make clones from cultured cells derived from easily obtained tissue should bring numerous practical benefits in animal husbandry and medical science, as well as answer critical biological questions.

How to Clone

Cloning is based on nuclear transfer, the same technique scientists have used for some years to copy animals from embryonic cells. Nuclear transfer involves the use of two cells. The recipient cell is normally an unfertilized egg taken from an animal soon after ovulation. Such eggs are poised to begin developing once they are appropriately stimulated. The donor cell is the one to be copied. A researcher working under a high-power microscope holds the recipient egg cell by suction on the end of a fine pipette and uses an extremely fine micropipette to suck out the chromosomes, sausage-shaped bodies that incorporate the cell's DNA. (At this stage, chromosomes are not enclosed in a distinct nucleus.) Then, typically, the donor cell, complete with its nucleus, is fused with the recipient egg. Some fused cells start to develop like a normal embryo and produce offspring if implanted into the uterus of a surrogate mother.

In our experiments with cultured cells, we took special measures to make the donor and recipient cells compatible. In particular, we tried to coordinate the cycles of duplication of DNA and those of the production of messenger RNA, a molecule that is copied from DNA and guides the manufacture of proteins. We chose to use donor cells whose DNA was not being duplicated at the time of the transfer [*see box on page 60*]. To arrange this, we worked with cells that we forced to become quiescent by reducing the concentration of nutrients in their culture medium. In addition, we delivered pulses of electric current to the egg after the transfer, to encourage the cells to fuse and to mimic the stimulation normally provided by a sperm.

After the birth of Megan and Morag demonstrated that we could produce viable offspring from embryo-derived cultures, we filed for patents and started experiments to see whether offspring could be produced from more completely differentiated cultured cells. Working in collaboration with

for Medicine

by Ian Wilmut

MEGAN AND MORAG (*above*) were the first mammals cloned from cultured cells. That basic technique has allowed the creation of cloned sheep carrying human genes. Such animals produce milk that can be collected and processed (*left*) to yield therapeutic human proteins.

PPL Therapeutics, also near Edinburgh, we tested fetal fibroblasts (common cells found in connective tissue) and cells taken from the udder of a ewe that was three and a half months pregnant. We selected a pregnant adult because mammary cells grow vigorously at this stage of pregnancy, indicating that they might do well in culture. Moreover, they have stable chromosomes, suggesting that they retain all their genetic information. The successful cloning of Dolly from the mammary-derived culture and of other lambs from the cultured fibroblasts showed that the Roslin protocol was robust and repeatable.

All the cloned offspring in our experiments looked, as expected, like the breed of sheep that donated the originating nucleus, rather than like their surrogate mothers or the egg donors. Genetic tests prove beyond doubt that Dolly is indeed a clone of an adult. It is most likely that she was derived from a fully differentiated mammary cell, although it is impossible to be certain because the culture also contained some less differentiated cells found in small numbers in the mammary gland. Other laboratories have since used an essentially similar technique to create healthy clones of cattle and mice from cultured cells, including ones from nonpregnant animals.

Although cloning by nuclear transfer is repeatable, it has

Is Quiescence the Key to Cloning?

All the cells that we used as donors for our nuclear-transfer experiments were quiescent—that is, they were not making messenger RNA. Most cells spend much of their life cycle copying DNA sequences into messenger RNA, which guides the production of proteins. We chose to experiment with quiescent cells because they are easy to maintain for days in a uniform state. But Keith H. S. Campbell of our team recognized that they might be particularly suitable for cloning.

He conjectured that for a nuclear transfer to be successful, the natural production of RNA in the donor nucleus must first be inhibited. The reason is that cells in a very early stage embryo are controlled by proteins and RNA made in the precursor of the parent egg cell. Only about three days after fertilization does the embryo start making its own RNA. Because an egg cell's own chromosomes would normally not be making RNA, nuclei from quiescent cells may have a better chance of developing after transfer.

A related possibility is that the chromosomes in quiescent nuclei may be in an especially favorable physical state. We think regulatory molecules in the recipient egg act on the transferred nucleus to reprogram it. Although we do not know what these molecules are, the chromosomes of a quiescent cell may be more accessible to them. *—I.W.*

limitations. Some cloned cattle and sheep are unusually large, but this effect has also been seen when embryos are simply cultured before gestation. Perhaps more important, nuclear transfer is not yet efficient. John B. Gurdon, now at the University of Cambridge, found in nuclear-transfer experiments with frogs almost 30 years ago that the number of embryos surviving to become tadpoles was smaller when donor cells were taken from animals at a more advanced developmental stage. Our first results with mammals showed a similar pattern. All the cloning studies described so far show a consistent pattern of deaths during embryonic and fetal development, with laboratories reporting only 1 to 2 percent of embryos surviving to become live offspring. Sadly, even some clones that survive through birth die shortly afterward.

Clones with a Difference

The cause of these losses remains unknown, but it may reflect the complexity of the genetic reprogramming needed if a healthy offspring is to be born. If even one gene inappropriately expresses or fails to express a crucial protein at a sensitive point, the result might be fatal. Yet reprogramming might involve regulating thousands of genes in a process that could involve some randomness. Technical improvements, such as the use of different donor cells, might reduce the toll.

The ability to produce offspring from cultured cells opens up relatively easy ways to make genetically modified, or transgenic, animals. Such animals are important for research and can produce medically valuable human proteins.

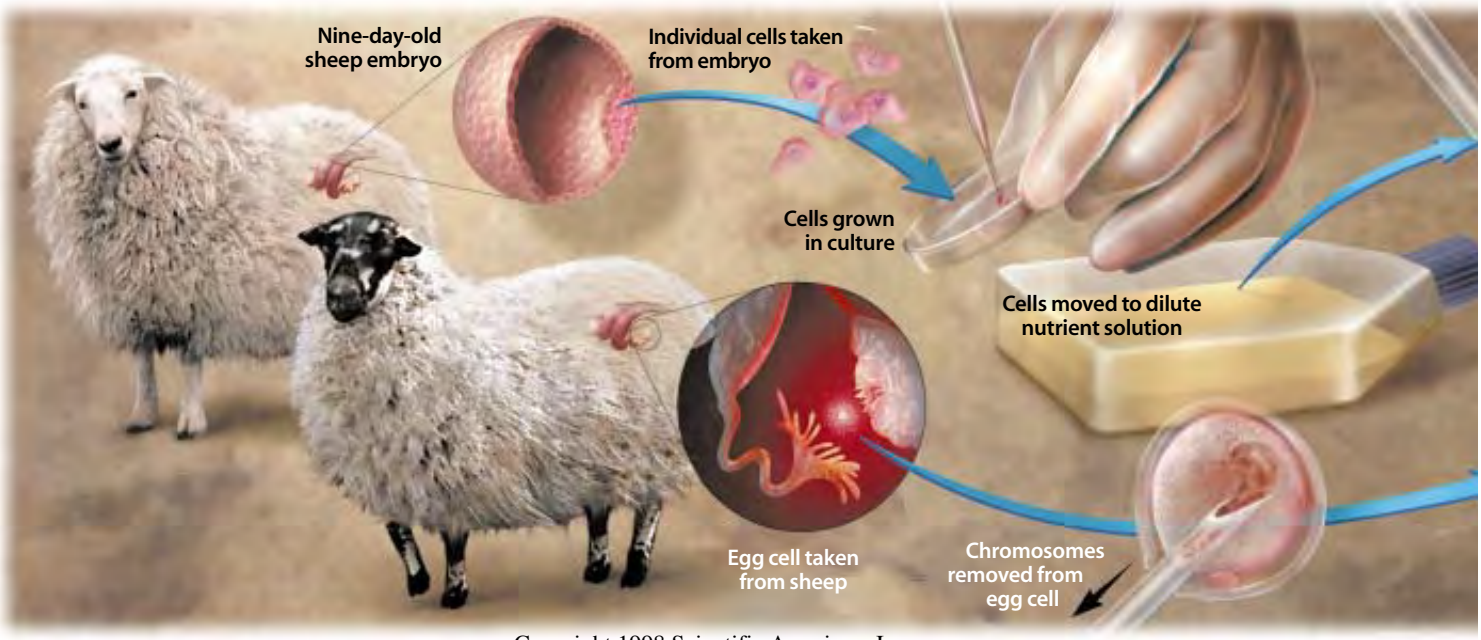
The standard technique for making transgenic animals is painfully slow and inefficient. It entails microinjecting a genetic construct—a DNA sequence incorporating a desired gene—into a large number of fertilized eggs. A few of them take up the introduced DNA so that the resulting offspring express it. These animals are then bred to pass on the construct [see "Transgenic Livestock as Drug Factories," by William H. Velander, Henryk Lubon and William N. Drohan; Scientific American, January 1997].

In contrast, a simple chemical treatment can persuade cultured cells to take up a DNA construct. If these cells are then used as donors for nuclear transfer, the resulting cloned offspring will all carry the construct. The Roslin Institute and PPL Therapeutics have already used this approach to produce transgenic animals more efficiently than is possible with microinjection.

We have incorporated into sheep the gene for human fac-

How Megan and Morag Were Made

Cultured cells were combined with egg cells to yield embryos that developed into cloned offspring.



KEITH KASNOT

tor IX, a blood-clotting protein used to treat hemophilia B. In this experiment we transferred an antibiotic-resistance gene to the donor cells along with the factor IX gene, so that by adding a toxic dose of the antibiotic neomycin to the culture, we could kill cells that had failed to take up the added DNA. Yet despite this genetic disruption, the proportion of embryos that developed to term after nuclear transfer was in line with our previous results.

The first transgenic sheep produced this way, Polly, was born in the summer of 1997. Polly and other transgenic clones secrete the human protein in their milk. These observations suggest that once techniques for the retrieval of egg cells in different species have been perfected, cloning will make it possible to introduce precise genetic changes into any mammal and to create multiple individuals bearing the alteration.

RODDY FIELD *Roslin Institute*

DOLLY
(*right*) shot to worldwide fame in 1997 as the first mammal cloned from an adult's cells. Now mature, Dolly has given birth to a healthy lamb, Bonnie (*left*), the product of a normal mating and gestation.

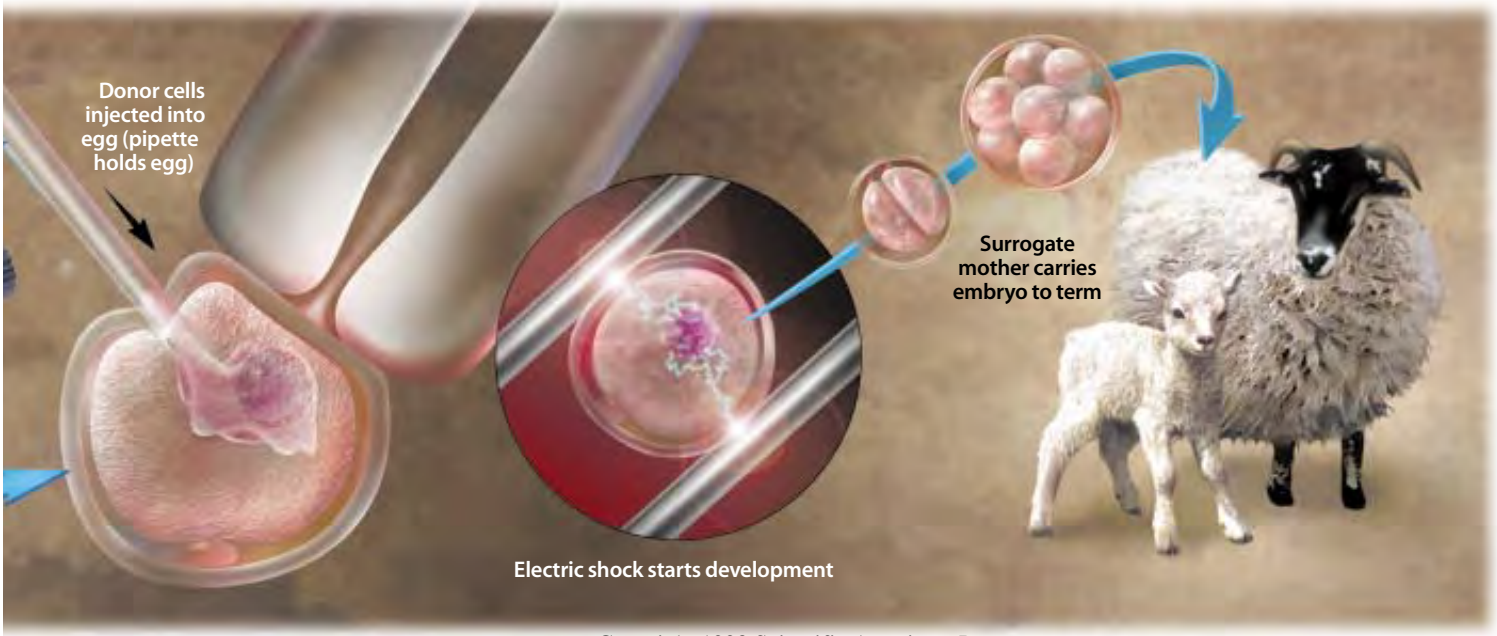
Cultures of mammary gland cells might have a particular advantage as donor material. Until recently, the only practical way to assess whether a DNA construct would cause a protein to be secreted in milk was to transfer it into female mice, then test their milk. It should be possible, however, to test mammary cells in culture directly. That will speed up the process of finding good constructs and cells that have incorporated them so as to give efficient secretion of the protein.

Cloning offers many other possibilities. One is the generation of genetically modified animal organs that are suitable for transplantation into humans. At present, thousands of patients die every year before a replacement heart, liver or kidney becomes available. A normal pig organ would be rapidly destroyed by a "hyperacute" immune reaction if transplanted into a human. This reaction is triggered by proteins on the pig cells that have been modified by an enzyme called alpha-galactosyl transferase. It stands to reason, then, that an organ from a pig that has been genetically altered so that it lacks this enzyme might be well tolerated if doctors gave the recipient drugs to suppress other, less extreme immune reactions.

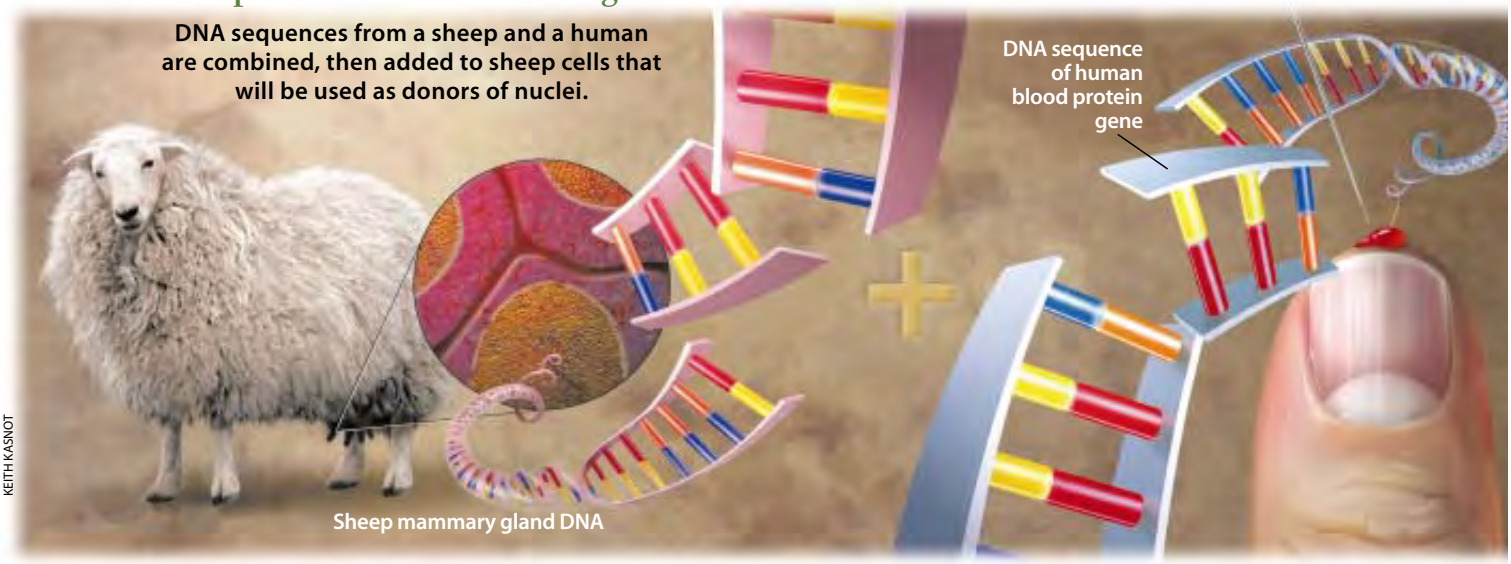
Another promising area is the rapid production of large animals carrying genetic defects that mimic human illnesses, such as cystic fibrosis. Although mice have provided some information, mice and humans have very different genes for cystic fibrosis. Sheep are expected to be more valuable for research into this condition, because their lungs resemble those of humans. Moreover, because sheep live for years, scientists can evaluate their long-term responses to treatments.

Creating animals with genetic defects raises challenging ethical questions. But it seems clear that society does in the main support research on animals, provided that the illnesses being studied are serious ones and that efforts are made to avoid unnecessary suffering.

The power to make animals with a precisely engineered genetic constitution could also be employed more directly in cell-based therapies for important illnesses, including Parkinson's disease, diabetes and muscular dystrophy. None of these conditions currently has any fully effective treatment. In each, some pathological process damages specific cell populations, which are unable to repair or replace themselves. Several novel approaches are now being explored that would provide



How to Prepare Cells to Make Transgenic Clones



KEITH KASNOT

new cells—ones taken from the patient and cultured, donated by other humans or taken from animals.

To be useful, transferred cells must be incapable of transmitting new disease and must match the patient's physiological need closely. Any immune response they produce must be manageable. Cloned animals with precise genetic modifications that minimize the human immune response might constitute a plentiful supply of suitable cells. Animals might even produce cells with special properties, although any modifications would risk a stronger immune reaction.

Cloning could also be a way to produce herds of cattle that lack the prion protein gene. This gene makes cattle susceptible to infection with prions, agents that cause bovine spongiform encephalitis (BSE), or mad cow disease. Because many medicines contain gelatin or other products derived from cattle, health officials are concerned that prions from infected animals could infect patients. Cloning could create herds that, lacking the prion protein gene, would be a source of ingredients for certifiable prion-free medicines.

The technique might in addition curtail the transmission of genetic disease. Many scientists are now working on therapies that would supplement or replace defective genes in cells, but even successfully treated patients will still pass on defective genes to their offspring. If a couple was willing to produce an embryo that could be treated by advanced forms of gene therapy, nuclei from modified embryonic cells could be transferred to eggs to create children who would be entirely free of a given disease.

Some of the most ambitious medical projects now being considered envision the production of universal human donor cells. Scientists know how to isolate from very early mouse embryos undifferentiated stem cells, which can contribute to all the different tissues of the adult. Equivalent cells can be obtained for some other species, and humans are probably no exception. Scientists are learning how to differentiate stem cells in culture, so it may be possible to manufacture cells to repair or replace tissue damaged by illness.

Making Human Stem Cells

Stem cells matched to an individual patient could be made by creating an embryo by nuclear transfer just for that purpose, using one of the patient's cells as the donor and a human egg as the recipient. The embryo would be allowed to develop only to the stage needed to separate and culture stem cells from it. At that point, an embryo has only a few hundred cells, and they have not started to differentiate. In particular, the nervous system has not begun to develop, so the embryo has no means of feeling pain or sensing the environment. Embryo-derived cells might be used to treat a variety of serious diseases caused by damage to cells, perhaps including AIDS as well as Parkinson's, muscular dystrophy and diabetes.

Scenarios that involve growing human embryos for their cells are deeply disturbing to some people, because embryos have the potential to become people. The views of those who consider life sacred from conception should be respected, but I suggest a contrasting view. The embryo is a cluster of cells that does not become a sentient being until much later in development, so it is not yet a person. In the U.K., the Human Genetics Advisory Commission has initiated a major public consultation to assess attitudes toward this use of cloning.

Creating an embryo to treat a specific patient is likely to be an expensive proposition, so it might be more practical to establish permanent, stable human embryonic stem-cell lines from cloned embryos. Cells could then be differentiated as needed. Implanted cells derived this way would not be genetically perfect matches, but the immune reaction would prob-

Now, Cloned Mice

Recently Ryuzo Yanagimachi of the University of Hawaii at Honolulu and his colleagues successfully cloned mice by transferring donor nuclei—not whole cells—into eggs. The group took nuclei from cells called cumulus cells, which surround the ovary. These cells are naturally quiescent. So far we believe that no one has shown that offspring can be produced from differentiated cells that are not quiescent. —*I.W.*

PROBIO AMERICA

Surrogate mother (*center*) is flanked by cloned offspring of nucleus donor.

ably be controllable. In the longer term, scientists might be able to develop methods for manufacturing genetically matched stem cells for a patient by "dedifferentiating" them directly, without having to utilize an embryo to do it.

Several commentators and scientists have suggested that it might in some cases be ethically acceptable to clone existing people. One scenario envisages generating a replacement for a dying relative. All such possibilities, however, raise the concern that the clone would be treated as less than a complete individual, because he or she would likely be subjected to limitations and expectations based on the family's knowledge of the genetic "twin." Those expectations might be false, because human personality is only partly determined by genes. The clone of an extrovert could have a quite different demeanor. Clones of athletes, movie stars, entrepreneurs or scientists might well choose different careers because of chance events in early life.

Some pontificators have also put forward the notion that couples in which one member is infertile might choose to make a copy of one or the other partner. But society ought to be concerned that a couple might not treat naturally a child who is a copy of just one of them. Because other methods are available for the treatment of all known types of infertility, conventional therapeutic avenues seem more appropriate. None of the suggested uses of cloning for making copies of existing people is ethically acceptable to my way of thinking, because they are not in the interests of the resulting child. It should go without saying that I strongly oppose allowing cloned human embryos to develop so that they can be tissue donors.

It nonetheless seems clear that cloning from cultured cells will offer important medical opportunities. Predictions about new technologies are often wrong: societal attitudes change; unexpected developments occur. Time will tell. But biomedical researchers probing the potential of cloning now have a full agenda. SA

JOHN CHADWICK PHOTOGRAPHIC AND PPL THERAPEUTICS

POLLY
(*left*) is a transgenic clone of a poll Dorset sheep. A gene for a human protein, factor IX, was added to the cell that provided the lamb's genetic heritage, so Polly has the human gene. The ewe that carried Polly (*right*) is a Scottish blackface.

The Author

IAN WILMUT pursues research on the genetic engineering of livestock at the Roslin Institute near Edinburgh in Midlothian, Scotland. After obtaining a Ph.D. from the University of Cambridge for research on methods of freezing boar semen, he did postdoctoral work at Cambridge on techniques for freezing animal embryos. Later Wilmut identified developmental and physiological causes of prenatal death in sheep and pigs, before turning to studies of ways to improve economically important animals.

Further Reading

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GENOMIC POTENTIAL OF DIFFERENTIATED CELLS. Marie A. Di Berardino. Columbia University Press, 1997.

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HUMAN FACTOR IX TRANSGENIC SHEEP PRODUCED BY TRANSFER OF NUCLEI FROM TRANSFECTED FETAL FIBROBLASTS. E. Schnieke et al. in *Science,* Vol. 278, pages 2130–2133; December 19, 1997.

GM salmon: FDA's assessment of environmental risks

BEATRICE DE GEA / LOS ANGELES TIMES

Some salmon steaks. Genetically modified salmon drew closer to FDA approval with publication last week of a long-awaited assessment of environmental effects of the fish.

BY ROSIE MESTEL
December 24, 2012, 3:42 p.m.

Genetically modified salmon moved closer to the market last week with release of draft documents from the Food and Drug Administration that assessed the environmental risks posed by AquAdvantage salmon, which grow faster than regular Atlantic salmon.

The agency found, on a preliminary basis, that the GM fish, produced by AquaBounty Technologies of Massachusetts, posed no significant threat.

Both documents -- an environmental assessment and preliminary "finding of no significant impact," known by the policy wonks as a FONSI -- will be published Dec. 26 in the Federal Register and be available for public comment for 60 days.

The assessment focused on the environmental questions. Food safety came earlier. Back in 2010, the FDA concluded that the salmon "is as safe as food from conventional salmon, and there is a reasonable certainty of no harm from consumption." For example, the flesh of the fish contain no more growth hormone than regular Atlantic salmon, the FDA said -- a concern of opponents to the fish because of the manner in which they were genetically engineered.

The new documents aren't exactly light Yuletide reading, filled as they are with regulatory-agency speak and charts of containment facilities and weather reports from sundry Canadian islands. They go over in some detail the way the fish were created, how and where they will be reared and whether the proposed strategy poses risks to wild salmon or otherwise on the environment.

Here are a few of the points, but anyone who's really interested or concerned should probably wade through the entire document, fortified perhaps with some brandy-spiked eggnog.

How is the salmon genetically modified?

AquAdvantage salmon is Atlantic salmon engineered with a gene from chinook salmon. The gene carries instruction for making growth hormone; that gene is attached to -- and activated by -- a piece of DNA from an ocean pout. The hybrid DNA was microinjected into fertilized salmon eggs back in 1989, to create the first "founder" GM fish. Because of the growth hormone supplied by the added gene, the salmon reaches smolt stage in its lifestyle faster than other Atlantic salmon. (Smolt is the stage when the salmon becomes silvery and would be ready to migrate to the ocean.) Faster growth time to smolt cuts down on feed costs and time to market and thus would make land-based salmon farming more economical, says AquaBounty Technologies, makers of the GM salmon. The FDA notes that 99% of the Atlantic salmon we eat in this country comes from farmed salmon operations in Canada Chile, Norway and Scotland.

How would it be farmed?

Unlike conventionally farmed salmon, the proposal the company put before the FDA doesn't involve farming the fish in net pens in the ocean. Instead, fertilized eggs would be created in inland tanks in Canada (on Prince Edward Island) and the eggs would be transported to an

inland facility in Panama to reach maturity in tanks. The farmed fish would be 100% female and almost all triploid — meaning they carry three copies of every chromosome in each cell instead of the normal two. That makes them sterile. They would be processed in Panama, and salmon fillets and steaks would then be transported to the U.S.

What did the environmental assessment look at?

It reviewed the scientific evidence to draw conclusions on a number of essential questions: the likelihood that the salmon would escape from the facilities, and, if they did escape, how likely they'd be to survive, disperse, reproduce and establish themselves in the wild; and the likely environmental effects within the U.S. if all those things happened. Environmental effects in Canada and Panama are not within the FDA's purview, but the FDA noted that potential physical effects on the U.S. would have to depend on security and containment of the facilities in Panama and Canada, so it looked at those. Those countries also have their own rules and regulations for assessing genetically modified animals.

The FDA wrote that the plans for these salmon confine them physically, geographically and biologically: The fish are sterile, grown in facilities on land with redundant containment measures, and the report goes into detail about all of these. For example, you can read about the confinements starting on Page 13 and later on in more detail, including a table (on Page 46) and figure (Page 47) that describes the various physical containment measures planned at Prince Edward Island; followed by a table and figure for the Panama facility on pages 52 and 53.

The agency concluded that the probability of escape, reproduction and establishment in the wild or harm to the Atlantic salmon or the human environment in the U.S. were very unlikely.

Some opponents of the AquAdvantage salmon expressed concerns, given the distressed state of wild Atlantic salmon fisheries, that the U.S. Fish and Wildlife Service and National Marine Fisheries Service were not involved in the environmental assessment. FDA spokeswoman Shelly Burgess said that both agencies (as well as the U.S. Department of Agriculture) provided comments on the draft. You can read their comments on pages 135 and 136 of the report. And starting on Page 100, you can read about all the various agencies who were consulted during the process.

The Fish and Wildlife Service noted that approval would be only for the planned two facilities on Prince Edward Island and in Panama. And it wrote: "Concern for effects on listed Atlantic salmon would arise if there were a detectable probability that the transgenic salmon could interbreed or compete with or consume the listed fish. Given the nature of the facilities described, any of these outcomes appears to be extremely unlikely, and your 'no effect' determination seems well supported for approval."

But the Fish and Wildlife Service also noted that this was based on the farming scheme as currently laid out. If more facilities were planned, or facilities different in kind were planned, or facilities in the United States planned, AquaBounty would have to apply to the FDA each time and the FDA would review any major or moderate changes in plans. The FDA said in the draft environmental assessment that ocean-based pens were a nonstarter because farmed salmon escape from them.

Would the genetically modified fish carry labels?

The hard plastic coolers transporting fish eggs in transit from Prince Edward Island to Panama would be labeled. But there no decision has been made on labeling of the final product. "Should FDA approve the application related to AquAdvantage Salmon, the agency will make a determination on whether food derived from AquAdvantage Salmon requires additional labeling," Burgess said.

What happens next?

The FDA could decide to move ahead and finalize that FONSI, paving the way toward ultimate approval of the salmon, or it could decide to do a more detailed environmental analysis. (Page 9 provides a figure describing the steps involved approval of a genetically engineered animal.)

In an email, FDA Burgess said: "FDA will review the comments it receives from the public regarding this draft [Environmental Assessment] and preliminary FONSI before making a decision on whether to prepare a final [Environmental Assessment] and FONSI, or to prepare an [Environmental Impact Statement]. In addition, FDA will complete the review of the AquAdvantage Salmon application and will reach a decision on approval. At this point it is not possible to predict a timeline for when these decisions will be made."

Though "environmental assessment" and "environmental impact statement" may sound very much alike, they are not. An environmental assessment is a more concise document that is prepared, in part, to determine whether agencies need to take a more detailed look. Not that these are exactly slim documents: The one prepared for the GM salmon was 145 pages long.

As explained by the EPA, environmental impact statements "are generally prepared for projects that the proposing agency views as having significant prospective environmental impacts." The FDA doesn't see that being the case for the salmon.

Trust, a non-profit organization based in East Grinstead, UK, that opposes the killing of badgers. He adds that controlling cattle movements and increasing TB screening on farms would have a greater impact. Adam Quinney, a beef farmer and vice-president of the National Farmers Union in Stoneleigh, which is lobbying for the cull, disagrees. "If I said to you, 'I'm going to give you an increase in income of 16%,' would you say that was significant?"

In July 2011, the Department for Environment, Food and Rural Affairs (DEFRA) laid out a plan for bovine TB eradication in England. The plan included increased surveillance and security measures on farms, as well as what the government described as a "science-led policy" of killing badgers in areas of high bovine TB. The plan reflects the reality that "this little micro-organism is really getting the better of us", says Ian Boyd, DEFRA's chief scientific adviser, who supports the cull. Politicians do not expect that the cull alone will eradicate bovine TB, but they hope that it will at least help to stabilize infection rates. Boyd insists that the new policy is rooted in the science of the RBCT trial.

Test culls will begin in Somerset and Gloucestershire, two of the most heavily infected regions in the country. The cull areas will be larger than those in the original trial, and will use physical boundaries, such as rivers and roads, to prevent infected badgers from roaming in or out of the cull zone. For many scientists, however, the new cull seems too distant from the RBCT to deserve the title of 'science-led' policy. The 70% reduction is a particular sticking point, as it is virtually impossible to determine badger populations in advance of actually killing them. On 14 October, 31 academics warned in a letter to *The Observer* newspaper that if the targets are missed, then levels of bovine TB could actually increase, because infected badgers will begin to roam more widely. "They say that their policy will be science-based but that's simply not true," says Krebs, who signed the letter. "They feel they have to do something, and the easiest something to do is to shoot badgers."

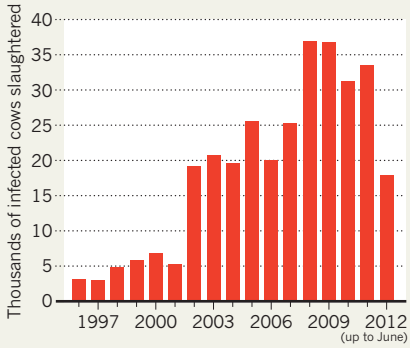
Other parts of the British Isles have already taken action. The Irish have used targeted snare-trapping to all but eliminate badgers from selected areas. That system would be more affordable but it is considered unethical in England. In Wales, officials have begun an expensive campaign to immunize badgers against TB. Both techniques depend on the peculiarities of local geography and badger populations, but they reflect the range of approaches that can be supported by the scientific evidence.

Policy-makers, meanwhile, are frustrated. "Politicians feel that the scientists have let them down," says Phil Willis, a Liberal Democrat and member of the House of Lords Science and Technology Committee. "They've not come with clarity, not just in terms of the science but in terms of the solution." Willis says that based on his understanding of the data, the government policy is unlikely to work.

As both farmers and protesters gird themselves, Donnelly acknowledges that science has given few straight answers. But, she says, it has helped to shift the debate: farmers now admit that tougher biosecurity standards will be instrumental in controlling bovine TB, and conservationists concede that badgers are a major reservoir for the disease. "They may not be singing from the same hymn sheet," she says, "but at least they're looking at the same data table." ■ SEE EDITORIAL P.310

BOVINE BURDEN

English farmers have struggled to control bovine tuberculosis over the past decade.



SOURCE: DEFRA

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FOOD SCIENCE

Politics holds back animal engineers

Funds and approvals lag for transgenic livestock in US.

BY AMY MAXMEN

When she saw the trailer for the documentary *Genetic Roulette*, Alison Van Eenennaam wanted to laugh, then cry. The film touts the risks of genetically engineered (GE) organisms, calling them "the most dangerous thing facing human beings in our generation". For Van Eenennaam, a geneticist at the University of California, Davis, the scientifically unfounded assertions — that transgenic foods are responsible for increased incidence of autism, Alzheimer's disease and type 2 diabetes in the United States — cannot be taken seriously. But the film reflects attitudes that have thwarted Van Eenennaam's research into the genetic modification of animals to reduce food costs and improve quality.

"Twenty years ago, the technology was our hurdle," says Mark Westhusin, who works on GE animals at Texas A&M University in College Station. "Now the technology is great and the sky is the limit," he says, "but good luck getting money for GE animals."

Inquiries by *Nature* reveal that fewer than 0.1% of research grants from the US Department of Agriculture (USDA) have gone to work on GE food animals since 1999, in part because of a poor public image. In one case, James Murray, another geneticist at the University of California, Davis, was told in 2003 that the USDA had rejected his proposal to develop a goat that produces milk rich in human lysozymes — enzymes that fight diarrhoeal disease — because the agency felt that "the general public would not accept such animals".

Van Eenennaam once hoped to engineer a cow that produced milk rich in omega-3 fats, but the USDA rejected her proposals, and she ended the project because of a lack of funding. The agency now funds her work on conventional breeding techniques to create dairy cows without horns, sparing farmers the danger and expense of removing them. Van Eenennaam says that she might do better by disrupting the genes that lead to horns, but there is no money for that. "I've got plenty of funding now, but the project is completely inefficient compared to genetic engineering," she says.

The USDA supports research to improve livestock and agriculture, but a spokesperson says that it has not considered work on GE animals to be the best use of its funding. The US National Institutes of Health (NIH) occasionally supports research on transgenic pigs that model human diseases, but rarely funds proposals to produce drugs or vaccines

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OFF THE TABLE
A brief history of some of the genetically engineered food animals submitted to the US Food and Drug Administration (FDA) for review. No such animal has yet been approved.

Animal	Purpose	Created	History
Salmon	Grows to market size faster than conventional salmon	1989 (Massachusetts)	1995 FDA receives application 2008 Fish farm moved to Panama 2010 Cleared by FDA scientific advisory panel
Pig	Produces more milk to nurse healthier young	1993 (Illinois)	1999 FDA receives application
Goat	Milk has human lysozymes to treat diarrhoeal disease	1999 (California)	2003 Funding denied by USDA 2008 FDA receives application 2011 Research moved to Brazil
Pig	Efficiently digests plant phosphorus, reducing pollution	1999 (Ontario, Canada)	2007 FDA receives application 2012 Pigs killed owing to lack of commercial interest
Cow, sheep, goat, pig	Increased muscle mass without reduced fertility	2010 (Texas)	2009 FDA receives application

in the milk of transgenic livestock. An NIH spokesperson says that decisions are based on many factors, including the needs of the research community.

For GE animals that have been developed despite these hurdles, market approval has stalled. On 27 September, Van Eenennaam was a panellist at a meeting in Washington DC, where advocates of GE animal research aired their frustrations with the US Food and Drug Administration (FDA), which has yet to issue a decision on any GE food animal submitted for approval (see 'Off the table'). A fast-growing salmon developed by AquaBounty in Maynard, Massachusetts, has been under review since 1995; in 2010, an FDA scientific advisory panel evaluated 21 years of data on the fish and deemed it safe for the environment and human consumption (see *Nature* **467**, 259; 2010), yet the agency has still not announced a final decision. The FDA will not comment on its process.

"AquaBounty has done everything they are legally required to do, and, yes or no, now we just want an official word from the FDA," says Van Eenennaam, who was on the advisory panel. "We will never have investment in this field if there is no way to move it forward." She was one of 56 biotechnology advocates who wrote to US President Barack Obama on 15 September, asking why there has been no update.

The White House has not responded, and AquaBounty's salmon is swimming against the tide of politics. Legislation introduced last year in the US House of Representatives and the Senate would ban the FDA from approving it. The protest in Congress comes mainly from salmon-exporting states such as Alaska, Washington and Oregon, amid fears that an inexpensive new source of salmon would undermine the industry. Politicians also reference unforeseen dangers from GE foodstuffs.

The FDA evaluates animals as strictly as it does drugs. In the 17 years that the salmon has been under review, AquaBounty has spent more than US$60 million on, for example, showing that its allergenic potential is no greater than that of Atlantic fish. To ensure that the mainly sterile GE salmon can't mate with native species, the company keeps them in multiwalled tanks on a mountain in Panama. If the fish were to be sold commercially, they would be reared similarly isolated from the ocean.

The prospects for research are better outside the United States. Last year, Murray moved his goat project to Brazil, where the government funds his research; the childhood diarrhoea that the goats' milk is intended to treat is a serious problem in the north of the country. And China invested nearly $800 million in transgenic pigs, cattle, sheep and crops between 2008 and 2012, says Ning Li, director of the State Key Laboratories for AgroBiotechnology in Beijing. More than 20 GE food animals are in development in China, he says, including a fast-growing carp and cows that produce milk with reduced allergenic potential. However, a Chinese researcher who asked to remain anonymous because he did not have permission to speak to the press predicts that approval for the animals will lag because the government has not determined how to ensure that the products are safe.

"The technology is great and the sky is the limit, but good luck getting money."

Even in the United Kingdom, where public opposition to GE plants and animals has been fierce, researchers seem to be better off than their US counterparts. The Biotechnology and Biological Sciences Research Council (BBSRC) supports work on GE food animals, including chickens engineered to be resistant to the bird-flu virus. A BBSRC spokesperson told *Nature*: "We consider it important to fund research that provides a range of technological options that can be applied to the challenges that we face as a society." ■