BIOPROCESSING

Transgenic Animal R&D Steadily Progresses

Benefits of Transgenics Being Realized

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In science, ideas come and ideas go. At one time, the idea of making human pharmaceuticals from bacteria or transformed cell lines seemed far-fetched, unfeasible, and maybe even repulsive. Naysayers warned of insurmountable technical obstacles and long approval times. Similarly, just a few years ago the idea of harvesting therapeutic human proteins from the milk of transgenic animals seemed like a 25-year project.

Today, with more than two dozen biotech and pharmaceutical companies exploring joint ventures with about half a dozen transgenic specialty companies, transgenic animals as protein expression systems have arrived.

Transgenics holds the potential for low-cost protein production through increased capacity, expression of complex proteins with mammalian or humanlike, post-translational modifications, simplified downstream processing, and lower capital investment for expression system equipment. Because the field of animal transgenics is so new and so few companies are involved, it will take time to realize many of these benefits.

As protein expression systems, transgenic animals could alleviate the cell-culture capacity shortage through a type of scalability that cell-culture specialists can only dream about: "If you have the need, breed." Since transgenic traits are hereditable, scaling from one animal to a herd is no more difficult than breeding animals for any other purpose.

Depending on the drug and transgenic platform, one animal may easily produce enough material for preclinical studies, a few animals may support Phase III trials, and a small herd may express tons of material per year. Output depends only on the animal's milk production and the protein expression level.

Using Large Farm Animals

Because of milk's high protein expression levels and ease of collection, most transgenics firms, like **GTC Biotherapeutics** (Framingham, MA), specialize in large farm animals. GTC's preferred platform is cows, which produce tons of protein-laden milk per year per animal.

GTC is working on recombinant human anti-thrombin III (rhATIII), an anti-coagulation protein found in blood. Current markets for plasma-derived product are just $250 million worldwide but, since the protein's only U.S. indication is for hereditary deficiency, only $10 million worth of rhATIII is sold annually in the U.S. Tom Newberry, vp of corporate communications at GTC believes the U.S. market alone could reach today's world figures.

Demand for another GTC product, recombinant human serum albumin (rHSA), approaches 400 metric tons per year. Virtually all this material is isolated from plasma, which raises issues of supply and safety. Newberry says rHSA is difficult to make through fermentation. "Besides," he asks, "how do you make 400 metric tons of a protein through fermentation or cell culture without covering the face of the Earth with bioreactors?"

At the other end of the volume spectrum is GTC's third in-house project, malaria vaccine MSP-1, from goat milk. According to Newberry, eight goats can produce enough of this vaccine to inoculate 20 million individuals.

In addition to its in-house R&D, GTC has twelve industrial collaborations with such companies as **Abbott, Centocor, Progenics Pharmaceuticals, Alexion, Immunogen,** and **Merrimack Pharmaceuticals**. "We're taking a lot of shots on goal but have managed to keep our cash burn rate down," says Newberry.

Pharming Group (Leiden, The Netherlands) also relies mostly on transgenic cows although its lead product, a C1 inhibitor esterase for hereditary angioedema, comes from rabbits. Pharming's principal cattle-derived products are fibrinogen and collagen—two high-volume proteins used in wound healing and cosmetic applications —and lactoferrin, an anti-infective. It takes fewer than 20 cows to produce the entire world's fibrinogen supply, about 150 kg, notes vp Samir Singh.

Pharming Group has established cattle production lines expressing high levels of recombinant human fibrinogen, human collagen, and other human proteins, in milk.

"Cattle are ideally suited for large-scale production," Singh says, "and the animals' post-translational modifications resemble humans' much more closely than do CHO cells."

With advances in nuclear transfer (cloning) technology, Pharming has been able to reduce development timelines to the point of competitiveness with other transgenic systems, "but clearly there's no way yet to reduce gestation time for cattle," Singh notes. "Any advances in that area would be most welcome."

Singh believes that transgenics could alleviate periodic fermentation capacity shortfalls, which are inevitable in bioprocessing, without entirely replacing older production methods. Rather, he views transgenics as a complementary manufacturing method, tailor-made for situations where its quality or quantity considerations demand it.

With protein expression levels topping off at 10 g/L, quantities of product obtainable from cattle are significantly higher than from almost any other source, says Singh. For example, a single animal producing 10,00 L of milk per year generates 100 kg of protein. "Obviously, you don't need a large herd to match the 200–300 kilogram-per-year output of a typical bioprocessing plant," Singh states. "It's possible to obtain tons of protein from a modest herd of cows."

A much-touted, but still unproven, advantage of transgenics is much lower capital costs, at least for the expression system equipment. "Not many small biotech firms have the $40 million to $200 million needed to build a production facility in the hope that a product will be approved," Singh notes. Of course, companies must still purify proteins, but transgenics firms believe a combination of high protein expression and a relatively homogeneous medium (e.g., milk) will offer distinct downstream benefits as well.

Breeding Like Rabbits

Departing from the trend line of using large animals, transgenics-based contract manufacturer **BioProtein Technologies** (Paris) specializes in proteins from rabbit milk. The main advantage of using rabbits is shorter development time and time-to-market, according to Alexandre Fouassier, business development manager at BioProtein.

Rabbits' gestation is only one month, animals mature sexually in four (females) or five (males) months, and rabbits are prolific breeders. "It's possible to generate a line of transgenic rabbits within six months and have the first grams of protein from those rabbits in less than a year," Fouassier states, "which is a time frame similar to that for CHO cells."

Apparently, rabbits' legendary reproductive skills and potential for high protein expression levels more than compensates for their small size and relatively low (compared with cows) milk production. Lactating rabbits produce up to 250 mL of milk per day— "nowhere near the 20 L that cows produce," notes Fouassier, "but volume is not a big problem because you can produce so many animals and milking is automated. Hundreds of rabbits can produce kilograms of therapeutic proteins per year."

Bioprotein's potential products include antibodies, plasma proteins, and hormones. The company has had some results with human growth hormone and extracellular-superoxide dismutase, but the focus for now is improving transgenic vectors and demonstrating their manufacturing capability to potential customers.

Safety Concerns Overblown?

Biotech firms are experienced with standard sterilization and safety measures used with cell or microorganism fermentations. CHO cells and *E. coli* are known quantities. To many of these companies, transgenics opens up new, unfamiliar safety concerns related to difficult-to-detect pathogens (e.g., prions) and the unknown impact of endogenous animal viruses on humans.

Transgenics firms counter that milk-producing animals do not typically harbor viral pathogens that plague humans. Besides, animal lineage can be traced as rigorously as can that of any cell line. Once a pedigree is deemed clear of prions, the controlled environment in which resulting animals are reared guarantees continued safety.

If anyone is especially concerned about pathogens from transgenic animals, it is not regulatory agencies. Neither European nor U.S. regulatory guidelines on using transgenic animals raise any special red flags vis-à-vis viruses or prions.

In its 1995 guidance, "Points to Consider in the Manufacture and Testing of Therapeutic Products for Human Use Derived from Transgenic Animals," the FDA raises the issue of "potential safety concerns about adventitious agents," noting that these "will be considered on a case-by-case basis." The FDA notes that an animal's apparent health, while a good barometer of infection, is not foolproof, so that "health monitoring is necessary, but not sufficient to guarantee absence of these contaminants."

With regard to infectious-agent clearance, the guidance states that validation should be conducted similarly to the way it is done for cell culture and fermentation. In fact, the document specifically refers readers to viral clearance guidelines in its guidance on cell line safety.

Cost Benefits Could Decide

For a given level of protein production, raising animals is anywhere from 33–80% less expensive than cell culture, depending on who's doing the estimating. Viewed another way, it costs about $300,000 to produce a functional transgenic cow and approximately $30,000 to create a transgenic pig.

Cloning, one technique used to duplicate transgenic animals, costs between $100,000 and $200,000 per animal, with related facilities costing between $5 million and $7 million. Once created, however, a transgenic animal could theoretically produce a hundred million dollars worth of pharmaceuticals or more in its lifetime and serve as breeding stock for a herd.

A facility with 100,000-L capacity costs somewhere between $200 million and $400 million to build, with validation times approaching five years. This generally

includes the cost for housing downstream operations as well, so a direct comparison with transgenics manufacturing is not entirely appropriate.

However, it is probably fair to say that hundred-million dollar capital investments demand exquisite timing with regard to approvals and commercialization timelines, whereas transgenic animal breeding is a lot more forgiving.

What's Good for the Cow is Good for the Cell Line

Gala Design (Middleton, WI) was founded as a transgenic animal company but has switched its focus to gene insertion, using retroviruses, to create highly expressing mammalian cells. Gala's retroviral vectors are the same ones it used to transform cattle embryos and similar to those used in human gene therapy.

The principal benefit of Gala's technology, whether used for transgenic animals or transformed mammalian cells, is a transfection rate that routinely reaches 50% and sometimes approaches 100%. "Researchers using conventional transgenic systems using microinjection are lucky to get two or three percent transformation rate," says Robert Bremel, Ph.D., chief scientist at Gala.

"When creating a transgenic herd of cattle, that's a big difference because a big cost associated with making transgenic livestock is maintaining a recipient herd, from which typically only a fraction will express the desired gene. A success rate of 50–100% means companies can get by with much smaller herds."

Gala's technology has been used to transform cattle, pigs, and, recently, monkeys. Genes are inserted into eggs before fertilization, through a technique known as transgametic—as opposed to transgenic—transformation. "Retroviruses are an efficient way to target desired regions of a genome."

Which Came First?

Although animal milk is probably the best near-term bet for large-batch transgenic protein manufacturing, other tissues and fluids, such as organs, urine, and eggs, are potential protein sources. **Avigenics** (Athens, GA) makes a good case for chicken eggs.

Thus far, Avigenics can express proteins in chicken eggs at concentrations of about 1 mg in an egg containing about 4.5 g of protein. Although one milligram seems paltry, Anthony P. Cruz, vp of corporate development at Avigenics, points out that, at the equivalent of 20 mg/kg, chickens are in the same ballpark as cows, which express between 1 g/L and 10 g/L.

The advantages of using chickens include their early sexual maturity, high egg output, and prolific breeding. A typical hen starts laying at five months and produces between 600 and 700 eggs in her lifetime.

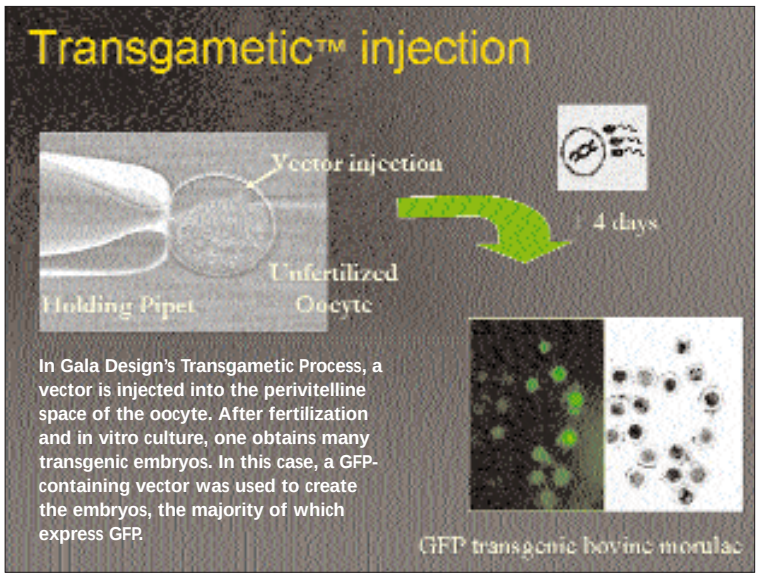
Borrowing breeding and processing techniques from the huge U.S. egg and poultry industries, which produce 30 billion eggs and 8 billion food chickens per year, it is possible to raise a flock of 100 million chickens from a single rooster in two years. Each egg contains 4.5 g of egg white protein "in a nice, sterile package," notes Cruz. "Eggs can be stored in their natural form without stabilizing them."

Downstream processing also makes egg proteins attractive. At 88% protein, egg white is a remarkably homogeneous substance containing no fat. Just 11 proteins make up 95% of the egg white, which Cruz called "a very consistent matrix." Modern egg processing machinery, when adapted to pharmaceutical-grade operations, are able to crack and separate 200,000 eggs per hour.

Cruz points out that industry's familiarity with eggs, both from eating them and using them to make flu and measles, mumps, and rubella (MMR) vaccine, works in the egg's favor as a transgenic medium.

Avigenics is working on several egg-derived products. Thus far, the company has expressed interferons, antibodies, and several

See Transgenic Animals on page 43



In Gala Design's Transgametic Process, a vector is injected into the perivitelline space of the oocyte. After fertilization and in vitro culture, one obtains many transgenic embryos. In this case, a GFP-containing vector was used to create the embryos, the majority of which express GFP.

tein, even if the protein has not been purified," explains Jack Foster, the company's antibody manager. "To date we have successfully generated antibodies to several viral targets and the new facility greatly expands our capacity."

Akubio (Cambridge, U.K.) reported the full commissioning of its premises on the Cambridge Science Park, following completion of a Class 10,000 pilot production facility. Established in 2001, Akubio is exploiting proprietary technology, originally developed at the University of Cambridge, that, in essence, can detect the sounds made when molecular bonds break.

A scientist at Akubio's Cambridge Science Park facility.

Genetastix (San Jose) and **Monsanto Protein Technologies** (MPT; St. Louis), a unit of **Monsanto Co.,** will collaborate to evaluate production in plants of two fully human monoclonal antibodies that were generated by Genetastix using its yeast-based HuMYTech™ technology platform.

Genetastix will transfer two antibody leads to MPT that have been isolated and tested against two publicly recognized infection and inflammatory disease targets, the firms report. MPT will express the antibodies in its plant-based production system and purify the antibodies for drug development studies, to be performed by Genetastix and its China joint venture, **Shanghai IgCon Therapeutics Co.** (Shanghai, China).

MPT and Genetastix/IgCon say they will then explore development and commercialization options for these plant-made therapeutic antibodies in major pharmaceutical markets including the U.S. and China.

Chromos Molecular Systems (Burnaby, Canada) entered a nonexclusive license agreement with **Cambridge Antibody Technology** (CAT; Cambridge, U.K.) to develop protein-expressing cell lines for commercial manufacture of antibodies and biologics using Chromos' ACE (artificial chromosome expression) System.

Under the agreement, CAT will use the ACE System platform to generate cell lines expressing Mabs in the context of its therapeutic programs, and will have the right to manufacture and market any emerging therapeutic products.

Chromos will receive an upfront payment and annual maintenance fees, as well as milestone and royalty payments on resulting products.

Transgenic Animals

Continued from page 41

commercial cytokines in eggs, and is speaking with potential commercial partners.

What's Next?

Despite their attractiveness, transgenic animals have not exactly caught on like wildfire. The technology appears to work and regulators do not seem overly concerned about safety, so where's the beef?

One problem is perception, says Fouassier of Bioprotein. Because products have not yet reached the marketplace, the biotech industry, regulators, and customers all need to be convinced that safe, effective, reasonably-priced drugs can come from animal milk. "For now, everyone is watching GTC closely, since its products are the closest to market. When GTC breaks through with a biological license agreement, that will probably be the end of those perception problems and safety questions."

We may also see some resistance to transgenic-derived pharmaceuticals from anti-GM activists, and perhaps managed care as well—especially for new products. "Insurers and Medicare are already trying to replace biotech medications with less expensive small molecule drugs," notes Denise Kettelberger, Ph.D., an intellectual property attorney with Merchant & Gould (Minneapolis, MN). Ultimately, transgenics will overcome, says Dr. Kettelberger, because "these are products that the consumer wants."

In an industry where success always seems to trump perception, there may be a simpler explanation for going slowly: economics. It would be foolhardy not to acknowledge the risks of any new technology, and with venture money drying up, companies are becoming risk-averse. "There is more risk with transgenics than in standard mammalian cell programs," says Gregory Bleck, Ph.D., of Gala Design.

"Plus, the financial situation in biotech has deteriorated over the past three years. When we started in the late 1990s a half-dozen companies had plans for transgenic animals, and many more were interested in transgenic plants. Most of them are still in business, but these days it's just too difficult to raise capital to expect the number of interested companies to grow." GEN