



# ISB NEWS REPORT

COVERING AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY DEVELOPMENTS

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## NEWS AND NOTES

### **WILL FOOD PRODUCTS FROM CLONED ANIMALS BE COMMERCIALIZED SOON?**

*Eric M. Hallerman*

The development of somatic cell nuclear transfer (SCNT) cloning offers significant benefits for genetic improvement of production herds, but poses concerns regarding food safety and animal welfare.<sup>1</sup> The US Food and Drug Administration (FDA) contacted companies developing SCNT clones for agricultural use and requested that food products from cloned animals or their progeny not be entered into the human or animal food supply until evaluation of these issues is completed.<sup>2</sup> Against this background, the FDA and the Pew Initiative on Food and Biotechnology hosted a public workshop, "Animal Cloning and the Production of Food Products: Perspectives from the Food Chain,"<sup>3</sup> in Dallas, Texas on September 26, 2002. The workshop was a forum for 150 participants from industry, academia, non-governmental organizations, and the public to hear presentations on key issues and to provide input to FDA as it develops its policy on foods from cloned animals.

SCNT cloning. Somatic cell nuclear cloning involves transferring the nucleus from a differentiated cell of a donor animal, frequently an adult, into an enucleated egg. The introduced nucleus is reprogrammed by the cytoplasm of the egg and directs the development of a new embryo, which is transferred to a recipient female to develop to term. The offspring formed will be identical to the donor in terms of nuclear DNA, but will differ in initial patterns of methylation of nuclear genes and in mitochondrial genes. Spatial and temporal patterns of gene expression in cloned embryos differ from those in non-cloned embryos.<sup>4</sup> After cloning of sheep was reported in 1997, SCNT was applied to many species, including cattle, goats, pigs, mice, and, more recently, rabbits and cats. At present, production of SCNT clones is inefficient, with less than one percent of the nuclear transfers resulting in a live, cloned offspring. Even with low efficiency, there are many potential applications for reproducing highly desired genotypes, including rare or endangered species, pets, elite sires or dams, breeds with desirable production traits but low fertility, or transgenic animals that have high value, for which rapid propagation is desirable. The success rate is increasing with refinement of SCNT protocols.

Highlights of the workshop. One key technical issue is whether the composition of food products from cloned animals is the same as that from non-cloned

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animals. Erik Forsberg of Infigen (Deforest, WI) presented results of a study comparing the composition of milk from 10 cloned and six control cows with values published in the scientific literature. Milk composition was quantified at laboratories at the University of Wisconsin and Utah State University where researchers did not know which samples pertained to clones. Concentrations of solids, fat, protein, lactose, caseins, lactalbumin, and most minerals, and somatic cell counts did not differ significantly between cloned and non-cloned groups. Significant differences in certain fatty acids and minerals were attributed to differences in feeds consumed by the two groups. The results are reported in detail in a manuscript submitted to a peer-reviewed journal.

Ron Gillespie (Cyagra, Worcester, MA) and Don Coover (SEK Genetics, Galesburg, KS) placed cloning of animals within an agricultural perspective. At a price of \$19,000, commercial producers are not the ones who are buying cloned calves—rather the consumers of this technology are selective breeding programs that are marketing genetically improved germplasm. Producers of selectively bred cattle are interested in better utilizing the genetics of high performance females; cloning of selected females will allow more matings with high performance sires, providing more opportunities to produce fine candidate sires to improve future production. Should the price decline dramatically, dairy producers may become interested in cloning in order to produce only female cattle. For beef cattle, it takes so much time to identify an outstanding sire that his productive life is limited; cloning offers the possibility of extending the availability of his genes. Producers of selectively bred pigs are interested in cloning because porcine semen does not cryopreserve well, and supplies of semen from boars in high demand are limited. Gillespie concluded that cloning will benefit producers and consumers when the price is right. Coover mentioned that small producers are concerned that they will prove less able to benefit from cloning than large producers.

A number of workshop speakers presented data showing high rates of perinatal mortality or clinical signs of distress in neonatal cloned animals. It is difficult to determine whether such problems are due to cloning by nuclear transfer, to embryo culture or transfer, or to some combination of cloning, culture, and transfer methods. Workshop attendees representing the Humane Society of America, Animal Welfare Institute, and United Poultry Concerns expressed concern that impacts of cloning would exacerbate animal welfare problems imposed by conventional production of animals in confinement facilities.

Carol Tucker Foreman (Food Policy Institute, Consumer Federation of America, Washington, DC) said that a hasty decision to approve clones as food carries "incendiary potential for public debate."<sup>5</sup> Poor public acceptance of cloned animals may limit commercial application of the technology.



## TECHNOLOGY NEWS

Regulatory prospects. John Matheson (FDA) discussed FDA actions anticipated over the next year. A detailed assessment of food safety data on SCNT clones and offspring will be prepared. An assessment will consider animal safety issues for clones and progeny, as well as genetic diversity impacts of cloning on populations of cattle and hogs. Release of both white papers is targeted for January 1, 2003. A proposed policy and guidance document considering risk management, legal, and enforcement issues will be published during spring 2003. At that time, comments on the proposed policies will be invited. Depending on the level of interest and the nature of comments, a public meeting may be held in spring or summer 2003, possibly before the Veterinary Medicine Advisory Committee. A "final" guidance policy will be published, accompanied by monitoring for compliance. The guidance policy will be revised as needed.

Combining numbers presented by the various speakers, on the order of 100-200 SCNT cloned animals have been produced and transferred to farms in the United States. Any role they might play in agricultural production will be determined within the next year.

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### EMERGING BIOTECHNOLOGIES: UPGRADING THE TERMINATOR

Zac Hanley and Kieran Elborough

Terminator technology has been a lightning rod for debate and confrontation on the issue of genetic modification. Most of the discussion, and all of the hypothesising over potential consequences, has taken place while Terminator and similar technologies were largely theoretical concepts. While it is proper to discuss the ethical ramifications at the early stages, in this case it has often led to the technical advances being relegated to the introduction or the appendix. We have previously described the benefits, risks, impacts, and opportunities of Terminator (more properly known as Genetic Use Restriction Technologies, or GURTs<sup>1</sup>). Now, here is the appendix.

#### Traitors and Terminators

Two types of GURTs have been described.<sup>2</sup> The first GURT patent (USP# 5,723,765 - see footnote 3) outlined the concept later described as Variety-restriction GURT, or V-GURT. This type is the emotively named 'Terminator', as it causes the production of sterile seeds. The reproductive viability of the plant is under the proprietary control of the owning company, ensuring that viable seed is not available for the farmer to harvest. This is achieved via triggering a disrupter gene prior to the sale of seed, which has a delayed effect, rendering the next generation non-viable. In 'Terminator', this triggering is by commission, that is, treatment prior to seed sale with an activating stimulus. In the technology dubbed 'Traitor' by critics, this is by omission; a suppressing stimulus is withdrawn and the disrupter is then able to act.

The concept of a V-GURT is not tied to any particular disrupter gene; there are many possibilities. Examples are genes encoding proteins which break down essential cellular components such as RNA or the cell wall; or which activate the apoptosis-like programmed cell death pathway in plants; or which perturb fundamental aspects of metabolism such as osmolyte balances or proton gradients. The aim is to disrupt the creation of the next generation or to render that generation incompetent to grow and survive. This is where real ingenuity is required. It is vital to arrange the genetic elements so that the disrupter has its effect in a timely manner.

The essential component is the promoter, which must respond to an exogenous compound or some triggering stimulus. One interesting example is the oestrogen receptor transactivation system described by Zuo and co-workers.<sup>4</sup> Their paper discusses the application of this system to marker excision; marker excision systems are but one application of GURTs.<sup>5</sup> In the oestrogen receptor transactivation system, application of  $\beta$ -oestradiol causes the promoter to express strongly; this promoter could be used to drive a suitable disruptor gene. Without  $\beta$ -oestradiol, expression is extremely low and disruption does not occur. However there are several obstacles to the successful use of exogenous chemical regulators in GURTs. Firstly, the regulatable promoter must remain physically linked in the genome to the disrupter gene, else they may on rare occasions separate during the development of the next generation. This would lead to viable and fertile trait- and modification-bearing offspring. Secondly, it is not clear how a chemical treatment can be 100% effective, as some cells or even whole seeds may not be penetrated.

### Trait Transfer Termination

The second GURT concept is the Trait-restriction GURT, or T-GURT, where it is the inheritance of the elite trait differentiating the plant from other germplasm that is under control, rather than the plant's reproductive ability. T-GURTs can be described as less crude than V-GURTs since their effects are localized to one area of the genome, and such finesse is increasingly desirable given public concerns over the process of germplasm enhancement. Control over the inheritance of a trait may occur via regulating the expression of genes conferring the trait, or ensuring the disruption or loss of these genes in the next generation (as deployed in marker excision systems such as described in footnote 4).

To achieve such relative finesse, a different range of disrupter genes is required. Genes encoding essential components of flowering or embryogenesis are suitable leverage points, and the inducible promoter here must drive the expression of an antisense construct. Alternatively, T-GURTs can employ marker excision-type mechanisms to remove the elite trait and dispense with disrupter genes altogether. This may be an important consideration if the plant is part of the human food chain. The site of action for T-GURTs is the reproductive tissues of the first generation, so tissue- or developmental stage-specific promoters are used. The triggering may be by omission or commission, as for V-GURTs (above).

The next generation of inducible promoters useful for GURTs are likely to respond to more complex, multi-

factorial signals involving environmental conditions, such as those which initiate flowering in overwintering plants. Or they may be switched on and off during certain developmental stages with great precision. Control via such triggers would circumvent some of the problems inherent in activation via exogenous chemicals. Early studies on such promoters can be found in the literature.<sup>6</sup>

### The Third Type?

Tobacco transgene containment has been demonstrated by Kuvshinov and coworkers,<sup>7</sup> devisors of the 'Recoverable Block of Function' (RBF) GURT concept. They attempt to differentiate it from previous GURTs, although it is conceptually identical to mechanisms described by the Food and Agriculture Organization working party.<sup>2</sup> RBF can be used in T-GURTs or V-GURTs, depending on the DNA constructs and target genes chosen, and is applicable to vegetatively propagated plants as well as seed. Kuvshinov and co-workers give a V-GURT example in their publication.

The RBF is a gene construct that includes a disrupter gene (the 'blocker' of reproductive 'function') and a rescue gene, which disrupts the disrupter and is under the control of an inducible promoter. In Kuvshinov *et al.* (2001), the BARNASE protein is expressed during embryogenesis and destroys most cellular RNA at this critical developmental stage, leading to the production of sterile seed. The *barstar* gene is also present in the same plant and can be triggered by a particular treatment. The authors used a heat-shock-specific promoter. On production of the BARSTAR protein, BARNASE is inhibited and viable seed are formed. However, *barnase* is reset to be expressed during the next embryogenesis, so the block is 'recovered' and the heat treatment would be required in the next generation, if it is not to be the last. A different 'blocker' and rescuer combination could control a T-GURT; for example, an antisense construct could 'block' the trait-conferring gene and be rescued by a gene, which specifically targets the promoter of the 'blocker'.

### Trends for Tomorrow

Today's GURTs have been criticized on a number of fronts, and most commentators have been concerned over their economic, social, and environmental impacts. These were addressed in our previous article.<sup>1</sup> But it is also important to say that ongoing research and development of new and improved GURTs continues, is exciting, and suggests remedies for many of the technical objections. However, GURTs do not have quite the necessary specifications and mostly remain interesting concepts or elegant creatures residing in the protective environment of the laboratory or glasshouse. This is the best reason for continuing research,



not inhibiting it. Widespread application in different species and extensive field testing are required. Government funding should be placed into programs developing GURTs. Publication in peer-reviewed journals of new developments, as with the RBF concept, is to be encouraged and should be recognized by proponents and critics as laudable. As an aside, the current public mistrust over patent protection often extends to the misunderstanding that patents are secrets; the current revolution in public access to patent databases (as in footnote 3) should help the public image of companies who wish to protect their investment by publishing their discoveries at the patent offices. At the toolkit level, more and different promoters are needed for T- and V-GURT systems, and there are many research groups in academic institutions that could apply their discoveries here. Lastly, a demonstration of the RBF system in a T-GURT application would be most helpful.

Taking insurance and mitigating risk through 'belt and braces' approaches are the best practises where public perceptions of safety, however distorted for political ends, are a factor. For this reason, further investment and research into GURTs are needed but emphatically do not imply that genetic modification is dangerous. GURTs developed as described herein are one component of several in a portfolio of technologies that can and should be employed (others include apomixis, transplastomics, male sterility, and enforced cleistogamy). Such technologies could be shared between companies. Some customers seek assurances; GURTs and the biotechnology industry are well on the way to providing them.

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## PLANT RESEARCH

### CROP IMPROVEMENT THROUGH ALTERATION IN THE PHOTOSYNTHETIC MEMBRANE

Peter Horton

Photosynthesis is a pivotal process in plant growth—it is not only the primary process of energy conversion in the plant, but the machinery involved requires a large investment from external resources such as nitrogen, its function impacts upon water-use *via* stomatal conductance, and it is a main site for plant/environmental interactions. Therefore, for many different reasons, manipulation of photosynthesis is considered of central importance in efforts to increase crop yield.<sup>1</sup> Firstly, the efficiency of conversion of intercepted radiation into biomass is a major yield parameter; many facets of the photosynthetic process that can give rise to inefficiency have been identified. Secondly, the photosynthetic apparatus is of prime importance in considerations of yield reduction by abiotic stress, common when crops are grown under sub-optimal conditions and detectable even under optimal conditions at light saturation. The latter topic is the subject of this article.

We have focused upon the photosynthetic membrane since it is not only the primary site of oxidative damage but also the main source of the reactive oxygen species (ROS) that cause widespread cell destruction under stress conditions (drought, extremes of temperature, nutrient deficiency). Our approach contrasts with that of many laboratories and consortia who focus on the plant cell processes that might confer resistance to abiotic stress by altering cell osmotic potential, expression of heat shock proteins, the levels of

enzymes that scavenge ROS, and so on. We are interested in the processes in the photosynthetic membrane that are the origins of oxidative stress.

Our approach in turn leads to a consideration of the pigments in the membrane. The absorption of light by chlorophyll is the main origin of oxidative damage associated with stress. Photosynthesis has evolved into a process tuned to absorb and store light energy very efficiently. However, in many environmental conditions the level of sunlight is in excess of that which can be used in photosynthesis, and this excess light energy is potentially highly damaging to plants. Photosystem II (PSII), the starting point for photosynthetic electron transfer since it oxidizes water, is particularly susceptible to damage. In fact, throughout the lives of all leaves, proteins damaged by oxidative stress in the PSII reaction center (the site of photochemical reactions) are continuously being replaced by new ones. Under stress, the repair process can be overloaded and the plant suffers photoinhibition. However, the problem is more widespread than just the PSII reaction center proteins—excess light energy increases the probability of forming ROS species in the pigment – protein complexes of the light harvesting system (where most of the chlorophyll is found). Furthermore, any damage to a constituent of the photosynthetic system increases the probability of further ROS production, a potentially lethal downward spiral of damage to protein, lipid, and nucleic acids, terminating in plant death.

It is therefore not surprising to learn that the photosynthetic membrane contains specific constituents and processes that are designed for “photoprotection.”<sup>1,2</sup> In recent years, enormous strides have been made in understanding the complex function of this membrane. High-resolution structural information is available for most of the main protein complexes, and new methods of image analysis are showing how these are assembled into giant macrostructures containing hundreds of proteins. One essential feature of the light harvesting system is its structural flexibility, which allows it to switch between two functional states—one for light harvesting, another to dissipate excess light energy harmlessly as heat. The latter, known as the state of feedback de-excitation, is readily measured non-invasively by chlorophyll fluorescence (nonphotochemical quenching or NPQ) and has been the subject of intensive study.

To dissipate excess light energy, two molecules in the membrane are needed. The first is a very hydrophobic membrane protein known as PsbS. When PsbS is absent from *Arabidopsis* plants, photoprotection is inhibited, and

the plant becomes more susceptible to oxidative stress and suffers a large yield penalty in the field.<sup>3</sup> Secondly, a particular type of carotenoid plays a crucial regulatory role, since it determines the rate of the switch between these two states.

Carotenoids are molecules widely known to protect against oxidative damage in all cells. In plants, the xanthophyll cycle (the reversible interconversion of two particular carotenoids, violaxanthin and zeaxanthin) has evolved to play this essential role in photoprotection. In fact, the xanthophyll cycle has two roles. Zeaxanthin accumulates under conditions of excess light because of the activation of violaxanthin de-epoxidase by the high transthylakoid pH gradient (DpH) generated under these conditions. In order for zeaxanthin to carry out its role in feedback de-excitation, it is bound to one or more of the proteins that constitute the macromolecular complexes of the chloroplast membrane. This binding, which has the characteristics of an allosteric regulator, is also dependent upon, or stimulated by, the increase in thylakoid DpH. Zeaxanthin is thought to bind to PsbS. In addition, zeaxanthin has a second protective role. Since zeaxanthin is hydrophobic, it is found mostly at the periphery of the light-harvesting complexes, and there it functions to prevent damage to the membrane lipids from ROS, lipid peroxidation.

These elements of photoprotection, therefore, are promising targets for genetic engineering to enhance stress tolerance in crop plants.<sup>4</sup> From studies in comparative ecology, it is known that the capacity for photoprotection is subject to genetic variation—plants inhabiting harsh environmental conditions tend to have a higher capacity for feedback de-excitation. We have focused on the manipulation of the xanthophyll cycle, since the level of zeaxanthin is always higher in stressed plants, and took a metabolic engineering approach to increase the xanthophyll cycle pool size in *Arabidopsis thaliana*. Given the nutritional importance of carotenoids, several groups have manipulated carotenoid biosynthesis in plants. Although *Arabidopsis* mutants with an increased xanthophyll cycle pool size are available, they have large alterations in the content of other carotenoids and lesions in the biosynthesis of the plant growth regulator, abscisic acid.

We decided to overexpress the thylakoid membrane enzyme  $\beta$ -carotene hydroxylase, which catalyses the conversion of  $\beta$ -carotene to zeaxanthin, in order to specifically manipulate the xanthophyll cycle pool size. We transformed *Arabidopsis* with the *chyB* gene under the control of a constitutive promoter and established several stable lines derived from independent transformation



events. These lines displayed up to 4-fold overexpression of  $\beta$ -carotene hydroxylase. Overexpression of the *chyB* gene specifically increased the xanthophyll cycle pool size two-fold, from 14% to nearly 30% of total carotenoid in low light grown plants, and from 22% to over 40% for moderate light plants. The latter value is significantly larger than any reported value for wild type *Arabidopsis*. The levels of other carotenoids were not perturbed, and so photosynthetic function was maintained at wild type levels and plant growth proceeded normally.

The extra xanthophyll was mostly associated with the photosystem II light-harvesting complexes (LHCII), which show a 3-fold increase in the amount of bound violaxanthin. The extra violaxanthin was also available for de-epoxidation when the plants were exposed to high light, so that the levels of zeaxanthin were at least twice as high in the sense *chyB* lines as in the wild type. We conclude that at least some of the extra xanthophyll is biologically active, allowing us to test whether an increased xanthophyll cycle pool will confer any improvement in stress tolerance.

Plants were grown for five weeks under low light conditions (100 mmol photons  $m^{-2} sec^{-1}$  / 20°C) and then were switched to stress conditions, 1000 mmol photons  $m^{-2} sec^{-1}$  and air temperature 40°C, for two weeks. One indicator of stress is the presence of the purple flavonoid anthocyanin, and sense *chyB* plants had significantly less anthocyanin than the wild type control (mean value  $1.0 \pm 0.47$  and  $1.6 \pm 0.49$ ). This difference in anthocyanin levels was obvious in the whole plants where the sense *chyB* lines were clearly greener and healthier, showing less leaf necrosis. An estimate was made of general lipid peroxidation as determined by the amount of malondialdehyde (MDA), a secondary end product of the oxidation of polyunsaturated fatty acids, in wild type and sense *chyB* plants exposed to stress. The amount of lipid peroxidation differed between the wild type and sense *chyB* lines—the mean values were  $9.2 \pm 4.3$  and  $6.6 \pm 3.9$  respectively; the almost 30% drop in the amount of lipid peroxidation in the sense *chyB* lines was statistically significant.

The results show that increasing expression of the  $\beta$ -carotene hydroxylase enzyme brings about an increase in the content of the xanthophyll cycle and zeaxanthin in the chloroplast membrane, and, most importantly, that this manipulation leads to an improved tolerance of high light and high temperature conditions. It is important to point out that these effects on anthocyanin production and lipid peroxidation were observed in plants exposed to stress conditions for two weeks; that is, it was a sustained property of the transformed plants. We conclude that

genetic manipulation of a single enzyme in carotenoid metabolism can bring about a pronounced increase in the stress tolerance of plants and represents a potentially powerful way forward in the production of stress-tolerant crops.

The exact mechanism by which the xanthophyll cycle provides increased stress tolerance remains undetermined. No increase in feedback de-excitation was found in these plants. Most likely, therefore, the increase in zeaxanthin provides direct protection against lipid peroxidation. Further work is needed to confirm this. The next phase in the research and development is to introduce this genetic manipulation into crop plants and to test its effect on yield in the field.

This research, showing a positive effect from the alteration of  $\beta$ -carotene hydroxylase level, represents the first time that a change in any feature of the chloroplast thylakoid membrane has resulted in an enhancement of plant performance. Whilst there is widespread acknowledgement that the basic features of the photosynthetic process *per se* do not offer opportunities for genetic improvement, there is an increasing likelihood that manipulation of the secondary “regulatory” processes in the thylakoid membrane, associated with the large number of low abundance proteins and other molecules, such as carotenoids, minor lipids and tocopherols, has considerable untapped potential.

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## ANIMAL RESEARCH

### ABNORMAL GENE EXPRESSION IN CLONED MICE

*Eric Wong*

Most cloned mammals derived by nuclear transfer die in gestation or show physical abnormalities at birth, including enlarged placenta and large offspring syndrome. The genetic basis for these problems is unknown but may be due to abnormal gene expression. During cloning by nuclear transfer, the donor nucleus must be reprogrammed to accurately regulate gene expression in a developing embryo, otherwise developmental defects will occur.

In the October 1, 2002, issue of the *Proceedings of the National Academy of Sciences USA*, a team of researchers examined gene expression in cloned mice. Using microarray analysis, the expression patterns of over 10,000 genes expressed in the placenta or liver were compared between neonatal control and cloned mice. The effect of the nuclear transfer process *per se* or the source of the donor nuclei on the gene expression profile was examined.

Cloned mice were generated following the transfer of nuclei from cultured embryonic stem cells or newly isolated cumulus cells. As expected, many cloned mice exhibited large offspring syndrome and/or an enlarged placenta. Analysis of placental gene expression revealed that 286 genes showed at least a 2-fold increase or decrease in expression level in cumulus cell-derived clones compared to normally fertilized controls. Similarly, 221 genes were 2-fold over- or underexpressed in embryonic stem cell-derived clones compared to controls.

The majority of the abnormally regulated placental genes were common to both cumulus-derived and embryonic stem cell-derived clones. A smaller subset of genes was abnormally regulated in only one clone type or the other. Overall about 76% of the genes that showed abnormal regulation were common among the cumulus-derived and embryonic stem cell-derived clones. The same genes, however, that were overexpressed in cumulus cell-derived clones were not necessarily overexpressed in embryonic stem cell-derived clones and vice versa. A wide array of genes is represented in this list of abnormally expressed genes, but no definitive unifying theme is readily apparent.

In addition to an analysis of placental gene expression, expression of neonatal liver genes was also compared between the clones and controls. As expected, the liver

results were similar to that observed with placenta, except to a lesser extent. Some genes in the clones were overexpressed while others were underexpressed, with parallel over- or underexpression between clone types seen for some genes but not others. A subset of genes was only abnormally expressed in one clone type or the other. In general, the genes abnormally expressed in the fetal liver were different from those abnormally expressed in the placenta.

Of the over 10,000 genes examined in this study, approximately 4% show abnormal gene expression in cloned mice compared to normal control mice. Most of the abnormal gene expression is related to the nuclear transfer process *per se*, since mice generated from both cumulus cells and cultured embryonic stem cells showed many common abnormally regulated genes. However, the source of the nuclei for nuclear transfer also affected the pattern of abnormal gene expression. Presumably a similar pattern of abnormal gene expression occurs in livestock cloned by nuclear transfer. Understanding why these genes are abnormally regulated and how this can be avoided remains a major challenge to improving the efficacy of the cloning process.

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### WHERE'S THE BEEF? TRANSGENIC CATTLE PRODUCE HUMAN IMMUNOGLOBULINS

*Eric Wong*

Human polyclonal antibodies (hPAB) are valuable therapeutics for treating many human diseases because they are less likely to elicit an immune response compared with antibodies from other species. However, the supply of hPAB is limited because they are derived from human donors and people cannot be hyperimmunized to boost production of an important therapeutic antibody. Furthermore, any product purified from human blood always runs the risk of viral contamination. Therefore, an alternative method for producing large quantities of hPAB in a non-human system would be highly desirable.

In the September 2002 issue of *Nature Biotechnology*, a team of researchers from Japan and the US report the generation of cloned calves that express human immuno-



globulins from a human artificial chromosome. These calves were generated using a combination of human artificial chromosome and nuclear transfer technologies. The resultant transgenic calves are called transchromosomal because they contain a human artificial chromosome in addition to their normal complement of bovine chromosomes. An artificial chromosome is a reconstructed, truncated version of a natural chromosome that retains the properties of a natural chromosome.

Immunoglobulins or antibodies consist of a large and a small protein subunit, known as the heavy and light immunoglobulin chains, respectively. The genes encoding these heavy and light immunoglobulin chains are located on different chromosomes. During the course of antibody production, the heavy and light immunoglobulin chain genes are rearranged by genetic recombination and altered by somatic mutation to generate a large variety of heavy and light protein chains with different amino acid sequences. The pairwise combination of one heavy chain with one light chain results in an almost unlimited number of immunoglobulin molecules that are capable of recognizing a vast array of foreign molecules.

A human artificial chromosome of approximately 10 megabases in size (one megabase equals one million base pairs of DNA) was constructed to contain the unrearranged human heavy and light immunoglobulin chain genes. The human immunoglobulin artificial chromosome was transferred into primary bovine fibroblasts. Because standard methods for introducing foreign DNA into cells are limited by the size of the transferred DNA fragment, a microcell-mediated chromosome transfer procedure was utilized to introduce the megabase-sized artificial chromosome.

Fibroblasts containing the human DNA fragment were isolated and used in a subsequent nuclear transfer step to produce cloned fetal calves. Analysis of the fetuses revealed that 1) the human artificial chromosome was stable in cells; 2) the heavy and light immunoglobulin chains had undergone functional rearrangement; and 3) the human immunoglobulin chain genes were transcribed in cells. Of particular note was the correct functional rearrangement of the immunoglobulin genes. Because the site for maturation for antibody-producing B cells differs between humans (bone marrow) and cattle (spleen), it was unknown if human genes could be rearranged in cattle. These data show that rearrangement appears to be more a property of the DNA sequence than the cellular environment in which maturation occurs.

To generate the cloned transchromosomal calves, the

transgenic fetal cell lines were used in a second round of nuclear transfer. Six calves were produced following nuclear transfer, of which four survived and were healthy and phenotypically normal. Again in the transchromosomal calves, the human artificial chromosome was stable and the human immunoglobulin chain genes were rearranged and expressed. Human immunoglobulin proteins were secreted at levels ranging from 13-258 ng/ml in blood samples.

These results demonstrate that human immunoglobulin heavy and light chain genes can be stably transferred and functionally rearranged and expressed in cattle cells. This is an exciting first step towards the large-scale production of human polyclonal antibodies for therapeutic purposes. However, a number of technological hurdles remain. For example, in these transchromosomal cattle, the endogenous bovine heavy and light chain genes are still functional. Thus chimeric antibodies containing combinations of human and bovine heavy and light chains likely exist, which would complicate efforts to selectively purify the product to obtain just the human immunoglobulin molecules. The deletion of the endogenous bovine immunoglobulin genes by gene targeting technology prior to human artificial chromosome transfer would alleviate this problem.

Nevertheless the development of a "humanized" bovine immunoglobulin system would be tremendously useful because cattle produce large quantities of antibodies and can be hyperimmunized with any antigen. With the generation of cattle containing human genes, the late Clara Peller, of the endearing Wendy's hamburger ads, might be asking "Where's the beef?" for an entirely different reason.

#### Reference

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## PRODUCTION NEWS

### LET THE FACTS SPEAK FOR THEMSELVES

Kimball Nill

Farmers in the American Midwest have suffered their worst drought in decades this year. Agricultural productivity will be substantially reduced and water reservoirs

severely depleted. Yet the situation could have been much more serious had it not been for the adoption of herbicide-resistant biotech crops, which have allowed the increased practice of no-till farming. No-till allows a farmer to plant a new crop directly into the soil through the residue of the previously harvested crop; the plant residue breaks down, helping to increase soil organic matter.

Traditionally, growers would have cleared their previous crop and deep plowed, in part to hinder the re-growth of weeds that would otherwise smother the young crop plants. Deep plowing leaves fields exposed to wind and erosion. No-till not only minimizes erosion, but also maintains the natural moisture in the soil so crops get a good start with less need for watering. Additionally, less carbon escapes from the soil to contribute to greenhouse gas accumulation in the atmosphere. There is also a considerable reduction in the amount of tractor fuel used in field operations.

Farmers in the US and in an increasing number of countries around the world are seeing the benefits of a technology that provides cleaner, less laborious planting with ecological advantages. But in Europe, environmental activists and Green party groups have attacked the technology on the grounds that it is unnatural or unsafe. US farmers are aware of the debate in the European Union over the adoption of agricultural biotechnology. They are also aware of the many issues and controversies surrounding the debate, as well as the myths and misinformation that have been put forward by opponents of agricultural biotechnology, fueling much of the discussion and often leading to a misunderstanding of the use of the technology and the advantages it can bring.

Recent reports from activists state that widespread growing of biotech herbicide-tolerant crops has harmed the environment, ruined US agricultural exports, and cost US agriculture billions of dollars a year. Such reports, especially that many farmers are opposed to biotechnology, prompted American farmers to defend their use of genetically modified crops. In September, nine major farmer organizations of the United States, representing nearly 60 percent of total US agricultural production, published a report: 'Let the Facts Speak for Themselves.' It attempts to correct current misinformation and allows farmers to present their perspective on the contribution of biotechnology and genetically modified crops to American farming.

The report lists a series of 'factoids'—a term commonly referring to unverified or inaccurate information which is presented, mostly by activists and media, as factual and

accepted as true because of frequent repetition. Each factoid is then redressed in the report with factual and verifiable information from technical and scientific points of view as well as knowledge garnered from the farmers' personal experience. The nine groups supporting the report are: American Agri-Women, American Soybean Association, National Chicken Council, National Corn Growers Association, National Cotton Council, National Milk Producers Federation, National Potato Council, National Turkey Federation, and the United Soybean Board.

According to the report's author, Kimball Nill, Technical Director of the American Soybean Association, various pressure groups and some media are deceiving the public by making unsubstantiated assertions about US farmers' adoption of biotechnology. "Their random statements were ludicrous, untrue, and deliberately misleading," he said at the launch of the report in London, UK, on September 16, 2002. The report points out that biotech crops benefit every link in the food chain from the farmer to the consumer.

In the future, even more exciting agbiotech innovations will continue to improve food quality, make farming more sustainable, increase productivity, reduce pressure on land use, and reduce the environmental footprint of agriculture. A number of examples of likely innovations are:

#### **Low-phytate soybeans and corn**

Poultry and swine producers in most countries currently add mined and processed phosphate to their feed rations to encourage optimal animal growth. The added phosphate is required to supplement the natural phosphate already present in traditional soybean and corn varieties, because the phosphate extant in traditional soybeans and corn exists in the form of an insoluble phytate. Monogastric animals such as chickens and pigs lack the phytase enzyme needed for digestion of phytate. Virtually all of the extant corn/soy phytate and part of the added (mined) phosphate is excreted by the animals, which can sometimes contribute to pollution problems. Innovations in biotechnology can likely reduce phytate in most feed crops. When low-phytate soybean meal is mixed with low-phytate corn to comprise animal feed rations, phosphate emissions in swine and poultry manure can be reduced by half. The iron, calcium, and protein in the ration are also absorbed more completely by the animal, which reduces anemia and nitrogen excretion.

#### **High-phytase soybeans and corn**

In those countries and regions where pollution from manure phosphate causes severe problems (e.g., The Netherlands), it has become common practice to reduce phosphate



excretions by including a microbial-source phytase enzyme supplement in animal feed. Research shows that adding the phytase supplement to feed rations can cut phosphate levels in swine and other monogastric animals' manure by as much as half, since the animal is able to digest the extant plant-source phosphorous; thereby allowing less (mined) phosphate to be added as a feed ingredient. Phytase has also been produced in transgenic crops grown as feed. Use of the crops has been shown to be as beneficial as adding purified enzyme to animal feed.

### High-oleic soybeans

High-oleic soybeans contain more than 80% oleic acid content in their oil. This contrasts with the 23% oleic acid content of traditional soybean oil. Because oleic acid has greater heat and oxidation resistance than the other fatty acids in soybean oil, high-oleic soybean oil is naturally more resistant to degradation by heat and oxidation over time so it requires less or no hydrogenation, depending on the intended oil application. Other research has shown that feeding of high-oleic soybean meal full-fat (i.e., with the oil in it) to cows and chickens results in a lowering of the saturated fat levels in the milk and poultry meat thereby produced. Such fat changes are also produced through feeding of traditional canola oil. High oil grain developed through biotechnology may be commercially available within five years.

### High lysine, high-methionine, high-threonine, etc. corn and soybeans

In the future, American farmers will be able to grow biotech corn and soybean varieties that contain higher levels of the amino acids lysine, methionine, threonine, and cystine. Poultry and swine can only utilize amino acids from their feed protein in highly specific ratios. Those animals metabolize and excrete, in the form of nitrogen pollution, the amino acids that are caused to be "in excess" by a shortfall in the primary amino acids required in those ratios. The primary requirements for corn/soymeal-based feed rations are usually lysine and methionine. High-lysine/high-methionine corn and soybean meals could allow feed ration formulations that reduce animal nitrogen excretion by providing an improved balance of essential amino acids. That can be accomplished now, but only by adding synthetic lysine and methionine to the feed ration, which increases feed costs.

'Let the Facts Speak for Themselves' makes the case for how biotech crops used worldwide are the product of genetic modification and selection that has taken place for centuries, and that today's use of biotechnology is simply extending plant breeding techniques used for the past 100

years to ensure a safe and wholesome food supply.

Widespread adoption by farmers is evident from the numbers. This year's US soybean harvest is expected to be more than 75% biotech, with herbicide-resistant varieties planted on 90% of soybean farms—a clear indication that US farmers have adopted a technology that works well in their individual operations. Argentina reports that 98 percent of their soybean crop is planted to biotech herbicide resistant varieties, and farmers in India, China, South Africa and even several European countries are now seeing first-hand the advantages afforded by agricultural biotechnology.

'Let the Facts Speak for Themselves,' which refutes the attack on a technology embraced by the farming community, has been well-received in Europe and the UK. It is available at <http://www.tomorrowsbounty.org>. By answering questions and challenging unsubstantiated assumptions, it represents an important step in the process of educating consumers and media alike.

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## INTERNET NEWS

### FAO E-MAIL CONFERENCE - BIOTECHNOLOGY AND RESEARCH

The FAO Electronic Forum on Biotechnology in Food and Agriculture is hosting its next e-mail conference on "What should be the role and focus of biotechnology in the agricultural research agendas of developing countries?" The conference begins in early November, lasting for four weeks, and will be moderated. The conference is organized by the FAO Working Group on Biotechnology and is the second to be held this year. All messages posted during the conference will be placed on the Forum website (<http://www.fao.org/biotech/forum.asp>). To join the Forum (and also register for the conference), send an e-mail to [mailserv@mailserv.fao.org](mailto:mailserv@mailserv.fao.org) leaving the subject blank and entering only the following two-line text message:

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