Genetic Engineering and Competitiveness of Livestock Production

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SUMMARY

Our ability to modify whole animal genetics has grown considerably in the last two decades. We have seen concerns regarding food safety and protection of breeding rights of genetically modified animals compel redirection of genetic engineering experimentation toward biomedical applications. Indeed, it has been nearly twenty years since the first transgenic livestock appeared in the literature, yet at this time, there are no commercially viable agricultural species. In contrast to commercialization concerns, in a variety of existing transgenic animal models, basic research into the regulation and function of specific genes (including both gain-of-function and ablation of potentially deleterious gene products) has persevered. Pioneering efforts in transgenic animal technology have markedly influenced our appreciation of the factors that govern gene regulation and expression, and have contributed significantly to our understanding of the biology of mammalian development.

KEY WORDS

Transgenic animals, genetic engineering, nuclear genome, mitochondrial genome

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INTRODUCTION

Animal modeling and technologies for genetic modification of animals have experienced a tremendous growth rate and central presence in the sciences over the last 25 years. From the first transgenic animal models reported in the early 1980s, to cloning technologies in the 1990s, to the current utilization of functional genomics and bioinformatics technologies – genetic engineering technologies have and will continue to evolve at an ever increasing pace.

Transgenic animals have provided us with powerful tools to explore cellular and physiological processes in vivo. We have been able to modify cell-, tissue-, or organ-specific gene expression as well as ubiquitous (whole-body) expression in literally thousands of different animal models that were produced to date. There are a multitude of reviews across the life sciences that touch on a broad cross-section of the sciences, including a number of animal science targeted reviews and a number of new references since my summary at the 2002 KRIMVA Conference (Pinkert et al., 2002; see also, Pinkert, 1994, 2002; Houdebine, 1997; Pinkert et al., 1997b, 2002; Pinkert and Murray, 1998; Nagy et al., 2003).

A number of methods exist for gene transfer in mammalian species (Table 1). Genetic engineering technologies were reported in a variety of farm animal species including rabbits, swine, ruminants (including sheep, goats and cattle), poultry and fish. With advances in the characterization of factors that control gene expression, gene transfer technology has become an important means of dissecting gene regulation and developmental pathways in vivo. Normally, endogenous gene function is regulated by a variety of molecular factors. Analysis of transgenic animals has illustrated molecular underpinnings and events associated with developmental timing, tissue distribution, and consequences of modifying gene expression. Additionally, transgenic animals have also proven quite useful in validating a array of in vitro and theoretical model systems.

Development of Transgenic Animals. The ability to introduce functional genes or to modify endogenous genes in animals provides very powerful tools in engineering production animals as well as dissecting complex biological processes and systems. Transgenic animals represent unique models that are custom tailored to address specific biological questions. Genetic engineering experimentation in farm animals can ultimately surpass classical breeding practices where long generational intervals will slow the rate of genetic improvement. Additionally, with evolving technologies, experiments that are proven in pilot laboratory animal studies can be validated and introduced into livestock species in a very timely manner.

Sequentially, early cell biology and both in vitro and in vivo studies laid the groundwork for the development of the first genetically engineered TRANSGENIC ANIMALS. From late 1980 through 1981, six different groups reported success at gene transfer and the development of transgenic mouse models using microinjection or viral transfection protocols. In gene transfer, animals harboring new genes (foreign DNA sequences integrated into their genome) are referred to as transgenic – a term coined by Gordon and Ruddle in 1981. The term "transgenic" has since been adapted to include chimeric and knock-out animals in which gene(s) have been selectively ablated from the host genome (Fig 1; see also Beardmore, 1997).

A few key terms are important in developing an appreciation for many of the current state-of-the-art technologies in genetic engineering studies (outlined in greater detail in Pinkert, 2002; Nagy et al., 2003). As noted in previous reviews, GENE TRANSFER is defined as one of a set of techniques directed toward manipulating biological function via the introduction of foreign DNA sequences (genes) into living cells. DNA MICROINJECTION is a gene transfer technique where DNA constructs (transgenes) are microinjected directly into pronuclei or nuclei of fertilized ova (zygotes) (Fig. 1 & 2). In contrast, EMBRYONIC STEM (ES) CELL TRANSFER involves the transfer and incorporation of pluripotent embryonic stem cells into developing embryos (Fig. 1). ES cell transfer also provides for gene targeting capability (allowing for creation of KNOCK-OUT or KNOCK-IN modeling, where endogenous genes are selectively ablated or replaced

• Blastomere/embryo aggregation	Whole genome transfer only
• Teratocarcinoma cell transfer	
• Retroviral infection	Primary advantage allowing for addition or modification of discrete gene sequences
• Electrofusion	
• Microinjection	
• Embryonic stem (ES) cell transfer	
• Nuclear transplantation	
• Spermatozoa - and spermatogonial cell - mediated transfer	
• Particle bombardment and jet injection	

Table 1.
Gene transfer methods.

Mouse modeling techniques evolved from procedures for non-specific (whole genome) transfer to the transfer of discrete genes and the modification of endogenous genes. From mice to domestic animal studies, significant inroads to existing technologies continue to advance (adapted from Pinkert, 1997).
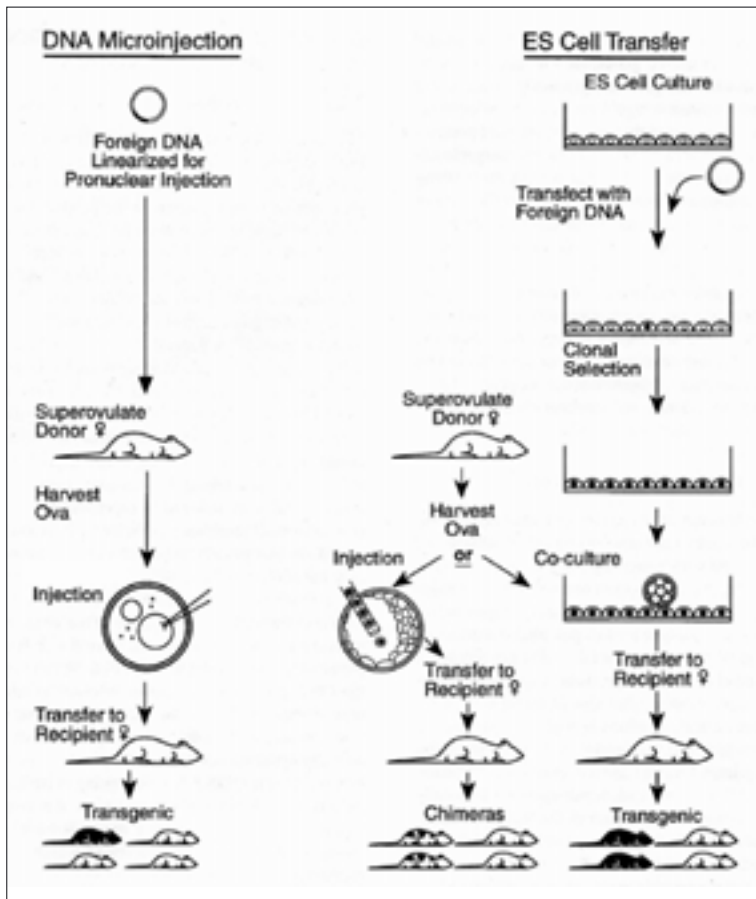
Figure 1.
DNA microinjection and ES cell transfer in mice.

For DNA microinjection, an in vitro culture step is not required (left). DNA is injected directly into a pronucleus of a zygote. Generally, when transgenic mice (represented in black) are created by DNA microinjection, all of their cells contain the new transgene(s). On the right, after clonal selection of transfected ES cells, either embryo injection or co-culture is employed. The ES cells are either injected directly into a host blastocyst or co-cultured with 8-cell to morula-stage ova. With blastocyst injection, transgenic offspring are termed "chimeric", as some of the cells are derived from the host blastocyst and some from the transfected ES cells (denoted by mice with black patches). Yet, using co-culture and tetraploid ova, founders may be obtained and derived completely from the transfected ES cells (solid black mice). [Reprinted with permission, Pinkert et al., 1997b]
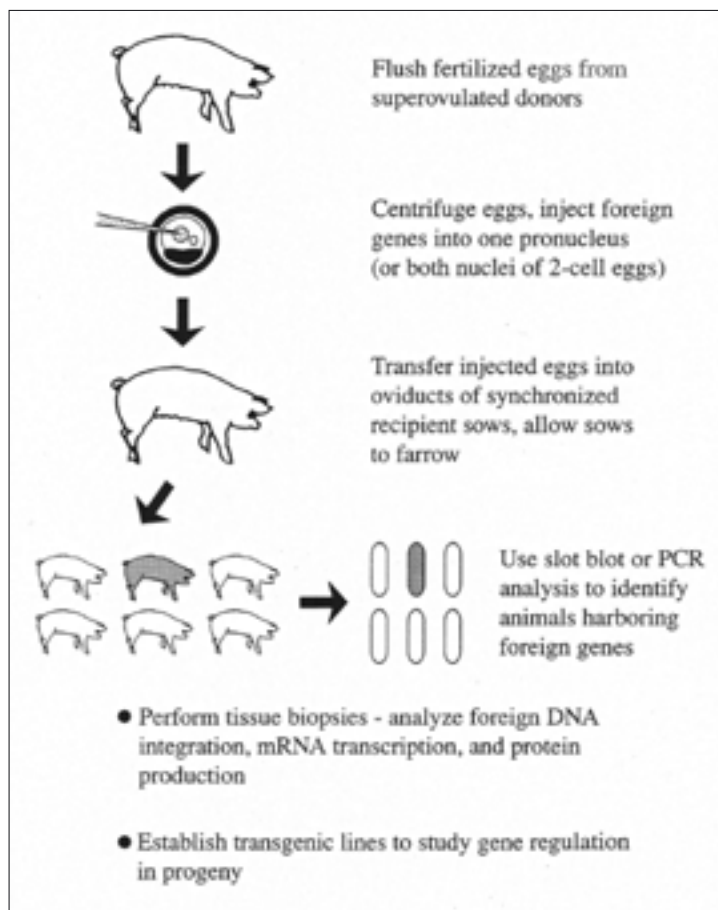

Figure 2.
Transgenic pig production by DNA microinjection.

In contrast to mouse modeling, for microinjection into zygotes (or later-stage ova) for most domestic animal species, visualization of the pronuclei (or nuclei) is necessary. This is accomplished by centrifugation of the ova to stratify the opaque lipids (Wall et al., 1985), making pronuclei or nuclei readily visible. Here, one of six founder pigs is represented as transgenic following initial analysis (reprinted with permission, Pinkert et al., 1990).

by modified sequences, respectively). CLONING is generally associated with NUCLEAR TRANSFER whereby a nucleus provided by a donor cell is introduced into an enucleated oocyte (unfertilized ovum) or zygote allowing for reprogramming of the developing embryo (Fig. 3). Today, a TRANSGENIC ANIMAL can be represented as an animal harboring foreign DNA sequences integrated into its genome following gene transfer, or b) resulting from the molecular manipulation of endogenous genomic DNA (hence, not only gain-of-function models are represented in the definition, but ablation or loss-of-function models are included as well). A TRANSGENIC LINE includes the founder and any subsequent offspring inheriting the specific GERM-LINE manipulation. Lastly, the majority of genetic engineering studies have focused all attention on nuclear-encoded genes. The NUCLEUS of the cell is generally found within a defined nuclear envelope within the cell, containing the genetic directives of the animal and its development; "the genome". As we have learned from recent efforts at mapping the human and other animal-specific genomes – mammalian genomes contain on the order of 30,000 genes. However, beyond the nucleus, MITOCHONDRIA are found in the cytoplasm of cells, are critical in energy production and metabolism, and actually code for 37 additional genes. Over the course of the last 7 years, mitochondrial populations and mitochondrial DNA (mtDNA) genes have become targets of genetic engineering research as well.

PRODUCTION OF TRANSGENIC ANIMALS

Mouse Models. The relative importance of using particular strains or breeds of animals in gene transfer experimentation will vary dramatically according to the species under consideration. Probably the most complex system is encountered in the production of transgenic mice, simply because so much work has been done with this species. Well-documented differences in reproductive productivity, behavior, related husbandry requirements, and responses to various experimental procedures influence the efficiency and degree of effort associated with production of transgenic founder animals.

Beyond the mouse model, other laboratory animal species may be necessary to study a particular biological phenomenon. However, independent of the specific techniques employed, the significance and critical importance of optimizing the experimental protocols cannot be underestimated. In any given strain or species, selection and management of donor females that respond well to hormonal synchronization and superovulation, embryo transfer recipients that are able to carry fetuses to term and then care for neonates appropriately, and the effective use of males in a breeding regimen will all add to the relative experimental efficiencies. In turn, transgenic animal protocols developed in mice
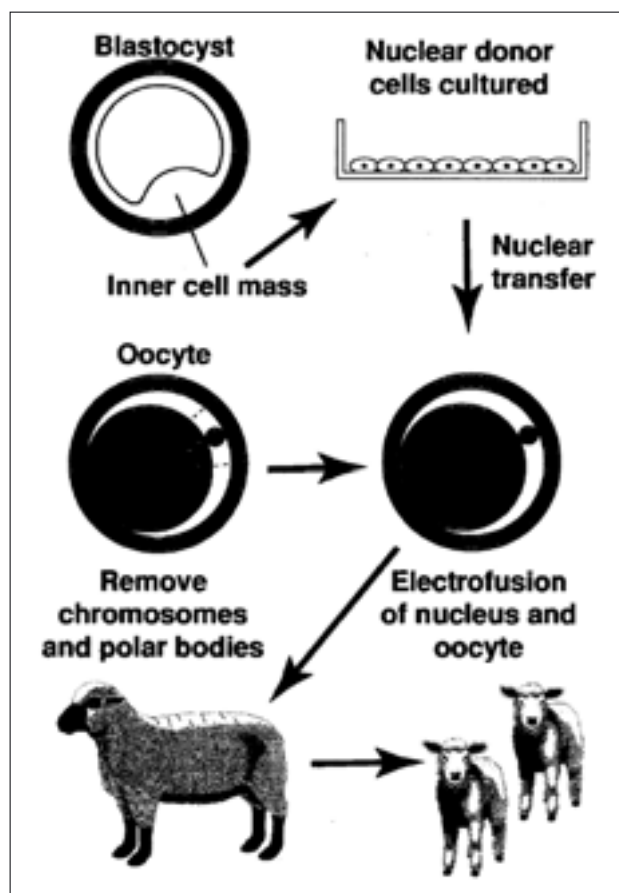

Figure 3.
Nuclear transfer and cloning of sheep.
Cells from blastocysts (e.g., inner cell mass cells) or other somatic tissues are obtained and propagated in culture. These cells are used as nucleus donors for transfer into enucleated oocytes. In contrast to DNA microinjection, a fusion step is generally employed Here, electrofusion is used to fuse couplets (transferred nucleus plus enucleated oocyte) that are transferred to recipients for the remainder of gestation. (reprinted with permission, Pinkert, 2000).

have been modified to accommodate production of other transgenic species.

DNA Microinjection. DNA microinjection, prior to nuclear transfer in domestic animals, was the most direct and reproducible method for producing transgenic animals (Fig. 1). DNA microinjection generally involves physical injection of a DNA construct solution into embryos (Polites and Pinkert, 2002) (Fig. 1 & 2). It was this technology and the dramatic growth characteristics of transgenic mice produced by Palmiter and Brinster (Palmiter et al., 1982; see also Brinster, 1993) that ignited the animal science field (Pursel et al., 1989; Wall et al., 1992; Pursel and Rexroad, 1993).

Virtually any cloned DNA construct can be used. With few exceptions, microinjected gene constructs integrate randomly throughout the host's genome. Yet, multiple integration sites per founder are rarely observed, with a single chromosomal "integration

site" the general rule. Host DNA near the site of integration frequently undergoes various forms of sequence duplication, deletion or rearrangement as a result of transgene incorporation. Such alterations, if sufficiently drastic, may disrupt the function of host genes at the integration site and constitute insertional mutagenesis, and result in an aberrant phenotype. Such events are generally not purposefully designed, but have led to the serendipitous discovery of previously unanticipated genes and gene functions. Because DNA microinjection is usually accomplished in pronuclear zygotes, transgene incorporation occurs in essentially every cell in the developing embryo. Incorporation of the transgene into cells that will eventually contribute to development of germ cells (sperm or ova) is a common occurrence with this method, and makes heritability of the transgene by offspring of (germline-competent) founder animals likely within one generation. However, integration of the microinjected DNA construct into the host's genome occasionally may be inexplicably delayed. In such a case, if cells of the early embryo (blastomeres) undergo mitosis before the transgene-integration event occurs, some but not all of the cells will contain the transgene, and the founder animal, although still considered to be transgenic, will be classified as a mosaic or chimera.

Retrovirus-Mediated Gene Transfer. Transfer of foreign genes into animal genomes has also been accomplished using retroviruses (Chan et al., 1998). Although embryos can be infected with retroviruses up to midgestation, oocytes to 16-cell stage ova are generally used for infection with one or more recombinant retroviruses containing a foreign gene of interest. After infection, the retrovirus produces a DNA copy of its RNA genome using the viral enzyme, reverse transcriptase. Completion of this process requires that the host cell undergoes the S phase of the cell cycle. Therefore, retroviruses effectively transduce only mitotically active cells. Modifications to the retrovirus frequently consist of removal of structural genes, such as gag, pol, and env, which support viral particle formation. Additionally, most retroviruses and complementary lines are ecotropic in that they infect species-specific cell lines, limiting risk to humans in animal experimentation (Kim, 2002).

Embryonic Stem (ES) Cell Technology. ES cell transfer technologies have been used to produce either random or targeted insertions as well as ablation of discrete DNA fragments within the whole animal (Fig. 1). In contrast to microinjection, the use of ES cells was quite effective in targeting genes to specific chromosomal locations (Brinster et al., 1989a; Capecchi, 1989). Procedures for ES cell transfer take advantage of an in vitro selection of a specific chromosomal integration event via homologous recombination in cell cultures prior to introduction into preimplantation embryos (Capecchi, 1989). The ability to screen ES cell clones in vitro has led to the production of animals that a) incorporate novel foreign genes into their genome, b) carry modified endogenous genes ("knock-in" models), and c) lack specific endogenous genes ("knock-out" models) (Doetschman, 2002; Rucker et al., 2002).

Pluripotential ES cells are derived from early pre-implantation embryos and characterized as to their state of differentiation (i.e., undifferentiated cells based on morphology, various molecular markers, and ability to contribute to the germline of animals) before in vitro manipulations. After characterization, ES cells are transfected with various vector systems to effect a given genetic modification of interest, and then injected directly into blastocyst stage or earlier stage embryos, or incubated in association with preimplantation stage ova (Doetschman, 2002; Rucker et al., 2002). The host embryos are then transferred into intermediate hosts or surrogate females for further development. The use of ES cells to produce transgenic mice faced a number of procedural obstacles before becoming competitive with DNA microinjection as a standard technique in animal modeling. Additionally, the use of co-culture techniques involving tetraploid host embryos (8-cell stage to morula stage) has resulted in animals completely derived with ES cell genetics in one generation (Wood et al., 1993). Yet, while various ES cell lines were identified for species other than the mouse, the production of germline-competent ES cell-derived/chimeric farm animals was not reported. With the advent of nuclear transfer-related and somatic-cell technologies, the need to identify ES or primordial germ cells (PGCs) to induce targeted genetic modification has lessened considerably (see also Matsui et al., 1992; Piedrahita et al., 1998).

Nuclear Transfer. Nuclear transfer involves a technique where nuclei of various cell origins are introduced into enucleated oocytes (unfertilized eggs), thereby reprogramming future development (Fig. 3). The successful "cloning" of a sheep was reported in the mid-1990s following technological difficulties in mouse studies in the early 1980s, and rekindling the imagination of researchers struggling with microinjection and related technologies (Wilmut et al., 1997). This landmark paper, followed by a number of technological feats, rapidly led to the production of transgenic domestic animals using a variety of cloning technologies (see also Godke et al., 2002; Paterson et al., 2002; Tsunoda and Kato, 2002). It should be noted that nuclear transfer, with nuclei obtained from either mammalian stem cells or differentiated adult cells, is an especially important development in species beyond the mouse model. In particular, a technological barrier was surpassed in allowing the characterization of in vitro cell cultures, and simultaneously obviating the difficulties in identifying

pluripotential farm animal stem cells. Unfortunately, at this time, the relative efficiencies for nuclear transfer experimentation still pale in comparison to other techniques (Pinkert et al., 2001; Honaramooz et al., 2002; Lavitrano et al., 2002). While nuclear transfer might be considered inefficient in its current form, major strides in enhancing experimental protocols have been envisioned (Godke et al., 2002; Paterson et al., 2002).

Sperm and Spermatogonia. In contrast to progress in embryo manipulation, a completely different avenue was taken with the advent of sperm-related transfer procedures. In 1989, sperm-mediated gene transfer was reported but hotly disputed when many laboratories around the world were unable to duplicate the outlined procedures (Brinster et al., 1989b). Yet, by 1994, the sperm-mediated story generated interest that resulted in the development of spermatogonial cell transplantation procedures as feasible alternatives for in vivo gene transfer (Brinster and Avarbock, 1994; Nagano et al., 2001). Additionally, studies over the past year have now illustrated procedures that were successful in transgenic pig production using either sperm-mediated gene transfer (Lavitrano et al., 2002) or spermatogonial transplantation (Honaramooz et al., 2002).

Production of Transgenic Domestic Animals. The success of transgenic mouse experiments led a number of research groups to study the transfer of similar gene constructs into the germ-line of domestic animal species. These efforts have been directed primarily toward three general endpoints: improving the productivity traits of domestic food animal species, development of transgenic animals for use as bioreactors (i.e., producers of recoverable quantities of medically or biologically important proteins), and in transplantation-related modeling efforts. Toward these goals, a number of strategies have been employed, including systems designed to study: dominant gene expression, homologous recombination/gene targeting and the use of ES cells, efficiency of transformation of eggs or cells, disruption of gene expression by antisense or RNAi transgene constructs, gene ablation or knockout models, reporter genes, and marking genes for identification of developmental lineages. Since 1985, numerous studies have focused on transgenic farm animals created using growth-related gene constructs. Unfortunately, for the most part, desirable growth phenotypes devoid of deleterious consequences were not achieved because of an inability to coordinately regulate gene expression – in deference to the ensuing cascade of endocrine events that unfolded. In contrast, efforts at using farm animals as bioreactors or in transplantation-related efforts have continued to progress, although viable products have yet to pass regulatory approval and gain market acceptance at this time (see also Godke et al., 2002; Martin and Pinkert, 2002; Niemann et al., 2002)

Although involved and at times quite tedious, the steps in the development of transgenic models are relatively straightforward. In contrast to gene transfer in mice, the efficiency associated with the production of transgenic livestock, including swine, remains low (Martin and Pinkert, 2002). However, two advantages offered by swine over other domestic species include a favorable response to hormonal superovulation protocols (20-30 ova can be collected on average) and as a polytocous species, they have a uterine capacity to nurture more offspring to term. For studies where the pig may be the desired model, the use of outbred domestic pigs is the most practical way to produce transgenic founders. However, miniature or laboratory swine are now used with increasing frequency in biomedical research, where their well-characterized background genetics make them more suitable for human modeling studies (e.g., xenotransplantation research). Reproductive efficiency in miniature swine is low compared to commercial swine and is characterized by a low ovulation rate, low birth weight and small litter size. Estrous cycles and gestation length are similar to standard commercial swine, though sexual maturity in males and females actually occurs between 4 and 6 months of age in some breeds. This is significantly earlier than observed in commercial swine; thereby hastening the generational interval.

In comparison to swine modeling, the relative experimental efficiencies associated with the production of transgenic ruminants (including goats, sheep and cattle) are even lower (Rexroad and Hawk, 1994; Niemann et al., 2002). While the different techniques from DNA microinjection to nuclear transfer require a large number of embryos to ensure varying levels of success, there are a number of pertinent considerations that will influence experimental yields. Such factors include the rate of embryo survival following manipulation, uterine capacity (generally these species are monotocous), generational interval, and animal maintenance, all impacting on experimental costs and efficiencies. In many laboratories, in vitro maturation (IVM), in vitro fertilization (IVF) and culture of ova (in surrogate hosts or incubators, although culture conditions are not optimal for embryo survival at this time) prior to final transfer aid in maximizing resources for production of genetically engineered ruminants.

EVOLVING TECHNOLOGIES

Nuclear Genes. Today, DNA microinjection, retroviral transfection, nuclear transfer and passive transfer procedures (e.g., sperm-mediated transfer) have been used to successfully produce transgenic livestock

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(Fig. 2 & 3). Our genetic engineering capabilities will continue to mature, in concert with advances in whole animal and somatic-cell techniques (including liposome-mediated gene transfer, jet injection, and particle bombardment), together with novel vector systems and innovative use of bioinformatics data. Envisioned progress and expansion of our existing knowledge base will allow us to better maximize and engineer production traits in farm animals in a most effective fashion.

From the Nucleus to the Mitochondrion. Gain-of-function and loss-of-function modeling have, for the most part concentrated on introducing specific mutations into the nuclear genome. From a gene ablation standpoint, creation of loss-of-function models will be facilitated by the emerging technology of RNA interference (RNAi). Short, interfering RNA (siRNA) exists in a double-stranded state and inhibits endogenous genes (and/or exogenous sequences as in viral genes) due to a complementary sequence homology (Fire et al., 1998; Hamilton and Baulcombe, 1999). RNAi technology has potential agricultural applications including the inhibition of viral gene transcription and inhibition of endogenous genes coding for deleterious gene products (Novina et al., 2002). Transgenic mouse and rat models using RNA interference were germline competent and did recapitulate null phenotypes (Hasuwa et al., 2002).

Using the mouse as a basis for comparison, RNAi has several advantages over homologous recombination/ ES cell mediated gene knock-out methodologies. Construction of an RNAi DNA fragment can be synthesized directly (including a small RNA Polymerase III promoter) avoiding time consuming and laborious cloning steps. Similarly, confirmation of homologous recombination in ES cell clones by PCR or Southern analysis would be obviated. Beyond nuclear transfer, this methodology provides the most significant advance or alternative for efficient loss-of-function experimentation for farm animal species at this time (either knock-out models or decreasing gene expression in quantitative terms).

In contrast to various methods to target modification of the nuclear genome, until recently, little attention was focused on the importance of mitochondrial genetics and the mitochondrial genome in animal production. This omission, in part, related to the difficulty associated with in vivo mitochondrial transfer. Without an approach in hand, a significant technological hurdle remained in the identification of mitochondrial gene targets appropriate for engineering or modification. In domestic animals, various production traits were associated with specific mitochondrial populations. Long-established cytoplasmic-based traits (inferring mitochondrially-controlled or regulated traits) in domestic animals have included growth, reproduction and lactation.

In addition, mitochondrial restriction fragment length polymorphisms (RFLPs) were identified and associated with specific lactational characteristics in a number of dairy cattle lineages (Brown et al., 1989; Koehler et al., 1991). Most recently, productivity and adaptability-related characteristics were explored in cloning studies that resulted in complete mitochondrial transfer in cattle (Bos Taurus:Bos Indicus; Meirelles et al., 2001). Therefore, for a host of applications, the ability to manipulate the mitochondrial genome and to regulate mitochondrial gene function would provide an additional target in modifying mammalian development.

We and others have initiated studies revolving around mitochondrial transfer and techniques to produce animals harboring foreign mitochondrial genomes (Pinkert et al., 1997a; Irwin et al., 1999, 2001; Levy et al., 1999; Marchington et al., 1999; Inoue et al., 1999; Sligh et al., 2000; Meirelles et al., 2001; Pinkert and Trounce, 2002; Takeda et al., 2002). The creation of transmitochondrial animals represents a new model system that will provide a greater understanding of mitochondrial dynamics, leading to the development of genetically engineered production animals as well as therapeutic strategies for human metabolic diseases affected by mitochondrial mutation or function.

CONCLUSIONS AND FUTURE DIRECTIONS

Gene Transfer and Genetic Engineering Today. The expertise and effort associated with genetic modification in farm animals have proven significant and most challenging. Innovative solutions to enhance experimental efficiencies in domestic species are needed to bring potential products into the marketplace and every day usage. The outlined techniques for genetic engineering technologies represents what is currently envisioned and on the horizon for animal science. These techniques will continue to evolve, particularly with animal studies complementing targeted human bioinformatics and genomics inroads. In this regard, microarray technologies (Fig. 4), coupled with developments on genomic, proteomic, and metabonomic platforms will provide noteworthy direction in our outlook at modifying animal productivity and utility.

Future Directions. Much has been learned about various physiological processes in transgenic farm animals created to date. Unfortunately, the "better" production animal has not reached the marketplace as yet. It may very well be that creation of value-added genetics (e.g., animals used as bioreactors or in biomedical modeling) may provide a stronger avenue for propelling transgenic technologies forward in the near-term. However, the pioneering studies performed since the mid-1980s have provided far reaching insights in redefining many in vivo regulatory and developmental processes that were
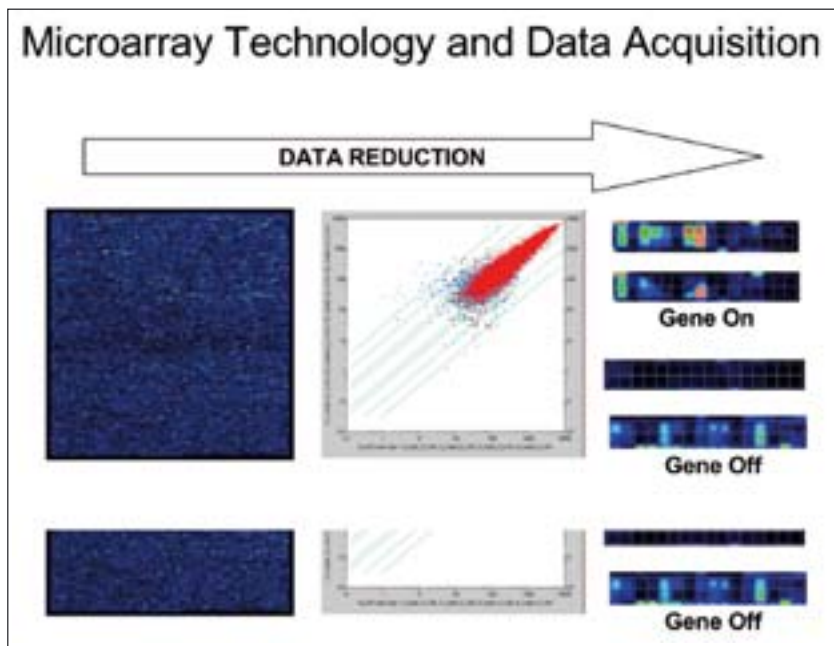
Figure 4.
Microarray technology.

Until recently, gene discovery was performed studying individual genes, one gene at a time. With microarray technology, molecular principles are employed on an industrial scale, allowing generation of quantitative and qualitative gene expression profiles for many genes simultaneously. Gene expression profiles are then be used to decipher molecular mechanisms that underlie comparative differences in various biological systems (reprinted with permission, Pinkert et al., 2002).

poorly understood in farm animal species. The utility of genetically-modified animal models still holds considerable promise – and the value to production agriculture cannot be underestimated. The Croatian indigenous Turopolje pig provides an ideal example of what might be targeted and feasible for genetic engineering studies in respect to both production agriculture and conservation biology (Robic et al., 1996; Dikic et al., 1999). In collaboration with Professors Marija Dikic and Ivan Juric, the Turopolje breed, found only in Europe and principally in Croatia, is a pig breed with important and useful genetic traits that might otherwise be lost due to its endangered status (with limited numbers of breeding sows in existence) and most worthy of further characterization and preservation. In the end, the development of rationale and safe food products, together with opportunities for establishing biomedical and value-added trait modifications, will provide demanding but exciting challenges for animal scientists in the 21st century.

REFERENCES

Beardmore J. A. (1997). Transgenics: autotransgenics and allotransgenics. Transgenic Res 6: 107-108

Brinster R. L. (1993). Stem cells and transgenic mice in the study of development. Intl J Devel Biol 3: 89-99

Brinster R. L., Avarbock, M. R. (1994). Germline transmission of donor haplotype following spermatogonial transplantation. Proc Natl Acad Sci USA 91: 11303-11307

Brinster, R. L., Chen, H. Y., Trumbauer, M. E., Avarbock, M. R. (1980). Translation of globin messenger RNA by the mouse ovum. Nature 283: 499-501

Brinster R. L., Braun R. E., Lo D., Avarbock M. R., Oram F., Palmiter R. D. (1989a). Targeted correction of a major histocompatibility class II Eα gene by DNA microinjected into mouse eggs. Proc Natl Acad Sci USA 86: 7087-7091

Brinster, R. L., Sandgren, E. P., Behringer, R. R., Palmiter R. D. (1989b). No simple solution for making transgenic mice. Cell 59: 239-241

Brown D. R., Koehler C. M., Lindberg G. L., Freeman A. E., Mayfield J. E., Myers A. M., Schutz M. M., Beitz D.C. (1989). Molecular analysis of cytoplasmic genetic variation in Holstein cows. J Anim Sci 67: 1926-1932

Cappechi M. R. (1989). Altering the genome by homologous recombination. Science 244: 1288-1292

Chan A. W. S., Homan E. J., Ballou L. B., Burns J. C., Bremel R. D. (1998). Transgenic cattle produced by reverse-transcribed gene transfer in oocytes. Proc Natl Acad Sci USA 95: 14028-14033

Dikic M., Juric I., Robic Z., Henc Z., Gugic G. (1999). Litter size and weight of piglets of the Turopolje pig breed in the suckling period. Agric. Conspectus Scientificus 64: 97-102

Doetschman T. (2002). Gene targeting in embryonic stem cells: I. History and methodology. In: Transgenic Animal Technology: A Laboratory Handbook, 2nd ed (CA Pinkert, ed), Academic Press, San Diego, 113-141

Fire A., Xu S., Montgomery M. K., Kostas S. A., Driver S. E., Mello C. C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391: 806-811

Godke R. A., Sansinena M., Youngs C. (2002). Assisted reproductive technologies and embryo culture methods in farm animals. In: Transgenic Animal Technology: A Laboratory Handbook, 2nd ed (CA Pinkert, ed), Academic Press, San Diego, 513-568

Gordon J. W., Ruddle F. H. (1981). Integration and stable germ line transmission of genes injected into mouse pronuclei. Science 214: 1244-1246

Hamilton A. J., Baulcombe D. C. (1999). A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 286: 950-952

Hasuwa H., Kaseda K., Einarsdottir T., Okabe M. (2002). Small interfering RNA and gene silencing in transgenic mice and rats. FEBS Letters 532: 227-230

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Honaramooz A., Megee S. O., Dobrinski, I. (2002). Germ cell transplantation in pigs. Biol Reprod 66: 21-28

Houdebine L.M. (1997). Transgenic Animals: Generation and Use. Harwood Academic Publishers, Amsterdam

Inoue K., Nakada K., Ogura A., Isobe K., Goto Y.- I., Nonaka I., Hayashi J.- I. (1999). Generation of mice with mitochondrial dysfunction by introducing mouse mtDNA carrying a deletion into zygotes. Nature Genet 26: 176-181

Irwin M. H., Johnson L.W., Pinkert C.A. (1999). Isolation and microinjection of somatic cell-derived mitochondria and germline heteroplasmy in transmitochondrial mice. Transgenic Res 8: 119-123

Irwin M. H., Parrino V., Pinkert C. A. (2001). Construction of a mutated mtDNA genome and transfection into isolated mitochondria by electroporation. Adv Reprod 5: 59-66

Kim T. (2002). Retrovirus-mediated gene transfer. In: Transgenic Animal Technology: A Laboratory Handbook, 2nd ed (CA Pinkert, ed), Academic Press, San Diego, 173-193

Koehler, C. M., Lindberg, G. L., Brown, D. R., Beitz, D. C., Freeman, A. E., Mayfield, J. E., Myers A. M. (1991). Replacement of bovine mitochondrial DNA by a sequence variant within one generation. Genetics 129: 247-255

Larsson, N.- G., Clayton, D. A. (1995). Molecular genetic aspects of human mitochondrial disorders. Annu Rev Genet 29: 151-178

Lavitrano M., Bacci M. L., Forni M., Lazzeresch, D., Di Stefano C., Fioretti D., Giancotti P., Marfe G., Pucci L., Renzi L., Wang H., Stoppacciaro A., Stassi G., Sargiacomo M., Sinibaldi P., Turchi V., Giovannoni R., Della Casa G., Seren E., Rossi G. (2002). Efficient production by sperm-mediated gene transfer of human decay accelerating factor (hDAF) transgenic pigs for xenotransplantation. Proc Natl Acad Sci USA 98: 13090-13095

Levy S. E., Waymire K. G., Kim Y. L., MacGregor G. R., Wallace D. C. (1999). Transfer of chloramphenicol-resistant mitochondrial DNA into the chimeric mouse. Transgenic Res 8: 137-145

Marchington D. R., Barlow D., Poulton J. (1999). Transmitochondrial mice carrying resistance to chloramphenicol on mitochondrial DNA: Developing the first mouse model of mitochondrial DNA disease. Nature Med 5: 957-960

Martin M. J., Pinkert C. A. (2002). Production of transgenic swine by DNA microinjection. In: Transgenic Animal Technology: A Laboratory Handbook, 2nd ed (CA Pinkert, ed), Academic Press, San Diego, 307-336

Matsui Y., Zsebo K., Hogan B. L. (1992). Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. Cell 70: 841-847

Meirelles F. V., Bordignon V., Watanabe Y., Watanabe M., Dayan A., Lobo R. B., Garcia J. M., Smith L. C. (2001). Complete replacement of the mitochondrial genotype in a Bos indicus calf reconstructed by nuclear transfer to a Bos taurus oocyte. Genetics 158: 351-356

Nagano M., Brinster C. J., Orwig K. E., Rye B.- Y., Avarbock M. R., Brinster R. L. (2001). Transgenic mice produced by retroviral transduction of male germ-line stem cells. Proc Natl Acad Sci USA 98: 13090-13095

Nagy A., Gertsenstein M., Vintersten K., Behringer R. (2003). Manipulating the Mouse Embryo: A Laboratory Manual. 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Niemann H., Dopke H. H., Hadeler K. G. (2002). Production of transgenic ruminants by DNA microinjection. In: Transgenic Animal Technology: A Laboratory Handbook, 2nd ed (CA Pinkert, ed), Academic Press, San Diego, 337-357

Novina C. D., Murray M. F., Dykxhoorn D. M., Beresford P. J., Riess J., Lee S.- K., Collman R.G., Lieberman J., Shankar P., Sharp, P. A. (2002). siRNA-directed inhibition of HIV-1 infection. Nature Med 8: 681-686

Palmiter R. D., Brinster R. L., Hammer R. E., Trumbauer M. E., Rosenfeld M. G., Birnberg N. C., Evans R. M. (1982). Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. Nature 300: 611-615

Paterson L., Ritchie W., Wilmut I. (2002). Nuclear transfer technology in cattle, sheep and swine. In: Transgenic Animal Technology: A Laboratory Handbook, 2nd ed (CA Pinkert, ed), Academic Press, San Diego, 395-416

Piedrahita J. A., Moore K., Oetama B., Lee C. K., Scales N., Ramsoondar J., Bazer F. W., Ott T. 1998. Generation of transgenic porcine chimeras using primordial germ cell-derived colonies. Biol Reprod 58: 1321-1329

Pinkert C.A. (1994). Transgenic swine models for xenotransplantation. Xeno 2: 10-15

Pinkert C.A. (1997). The history and theory of transgenic animals. Lab Anim 26: 29-34

Pinkert C.A. (2000). Genetic engineering of farm mammals. In: Reproduction in Farm Animals, 7th ed (ESE Hafez, B Hafez, eds), Williams & Wilkins, Baltimore, Chapter 21, 318-330

Pinkert C. A. (2002). Transgenic Animal Technology: A Laboratory Handbook. Academic Press, San Diego

Pinkert C. A., Murray J. D. (1998). Transgenic farm animals. In: Transgenic Animals in Agriculture (JD Murray, GB Anderson, AM Oberbauer, MM McGloughlin, eds), CAB Intl, Wallingford UK, 1-18

Pinkert C. A., Trounce I.A. (2002). Production of transmitochondrial mice. Methods 26: 348-357

Pinkert C. A., Dyer T. J., Kooyman D. L., Kiehm D. J. (1990). Characterization of transgenic livestock production. Dom Anim Endocrinol 7: 1-18

Pinkert C. A., Irwin M. H., Johnson L. W., Moffatt R. J. (1997a). Mitochondria transfer into mouse ova by microinjection. Transgenic Res 6: 379-383

Pinkert C. A., Irwin M. H., Moffatt R. J. (1997b). Transgenic animal modeling. In: Encyclopedia of Molecular Biology and Molecular Medicine, vol 6 (RA Meyers, ed), VCH, New York, 63-74

Pinkert C. A., Johnson L. W., Irwin M. H., Wong S., Baetge E. E., Wolfe D. F., Simpkins S. A., Owsley W. F., Bartol F. F. (2001). Optimization of superovulation and fertilization protocols in the production of transgenic swine. Adv Reprod 5: 45-53

Pinkert C. A., Kooyman D. L., Martin M. J. (2002). Genetic engineering of farm animals: From transgenesis to gene mapping, what does the future hold? Proc. KRMIVA 2002, vol 9. Opatija, Croatia, pp 18-34

Polites H. G., Pinkert C. A. (2002). DNA microinjection and transgenic animal production. In: Transgenic Animal Technology: A Laboratory Handbook, 2nd ed (CA Pinkert, ed), Academic Press, San Diego, 15-70

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Pursel V. G., Rexroad C. E. Jr. (1993). Status of research with transgenic farm animals. J Anim Sci 71(Suppl. 3): 10-19

Pursel V. G., Pinkert C. A., Miller K. F., Bolt D. J., Campbell R. G., Palmiter R. D., Brinster R. L., Hammer R. E. (1989). Genetic engineering of livestock. Science 244: 1281-1288

Rexroad C. E. Jr., Hawk H. W. (1994). Production of transgenic ruminants. In: Transgenic Animal Technology: A Laboratory Handbook (CA Pinkert, ed), Academic Press, San Diego, 339-355

Robic, Z., Dikic, M., Juric, I., Stipic, N., Rupic, V., Muzic, S., Bozac, R., Liker, B. (1996). The Turopolje pig, one of the oldest European races: Its saving and renewal. In: Animal Production, Healthy Nutrition, Environment. 4th Intl Symp Anim Sci Days, Pannon Univ, pp 1-5

Rucker E. B. III, Thomson J. G., Piedrahita J. A. (2002). Gene targeting in embryonic stem cells: II. Conditional technologies. In: Transgenic Animal Technology: A Laboratory Handbook, 2nd ed (CA Pinkert, ed), Academic Press, San Diego, 143-171

Sligh J. E., Levy S. E., Waymire K. G., Allard P., Dillehay D. L., Heckenlively J. R., MacGregor G. R., Wallace D. C. (2000). Maternal germ-line transmission of mutant mtDNAs from embryonic stem cell-derived chimeric mice. Proc Natl Acad Sci USA 97: 14461-14466

Takeda K., Akagi S., Takahashi S., Onishi A., Hanada H., Pinkert C.A. (2002). Mitochondrial activity in response to serum starvation in bovine (Bos taurus). cell culture. Cloning Stem Cells 4: 223-230.

Tsunoda Y., Kato Y. (2002). Nuclear transfer technologies. In: Transgenic Animal Technology: A Laboratory Handbook, 2nd ed (CA Pinkert, ed), Academic Press, San Diego, 195-231

Wall R. J., Pursel V. G., Hammer R. E., Brinster R. L. (1985). Development of porcine ova that were centrifuged to permit visualization of pronuclei and nuclei. Biol Reprod 32: 645-651

Wall R. J., Hawk H. W., Nel N. (1992). Making transgenic livestock: genetic engineering on a large scale. J Cell Biochem 49: 113-120

Wilmut I., Schnieke A. E., McWhir J., Kind A. J., Campbell K. H. (1997). Viable offspring derived from fetal and adult mammalian cells. Nature 385: 810-813

Wood S. A, Allen N. D., Rossant J., Auerbach A., Nagy A. (1993). Non-injection methods for the production of embryonic stem cell-embryo chimaeras. Nature 365: 87-89

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