MEETING REPORT

ALTERING THE GENETIC MAKEUP OF ANIMALS

DAVIS, Calif.—The latest advances in gene transfer technology, vaccine development, gene mapping and karyotyping, and embryology all have potential applications to bioengineering in animals. Present and future possibilities were discussed at a meeting on Genetic Engineering of Animals: An Agricultural Perspective, held here in September.

A potential genetic resource for improving fertility in animals, the Booroola *F* gene, was described by B. M. Bindon of the Commonwealth Scientific & Industrial Research Organization (CSIRO, Armidale, Australia). This genotype confers on Booroola Merino sheep increased ovulation rates and large litter sizes (as many as seven per litter) without detriment to body size or quality or quantity of wool. The *F* gene is expressed primarily in the ovary: an abnormal number of follicles mature and ovulate during each estrous cycle. This is due to prolonged production of elevated levels of follicle stimulating hormone (FSH). Booroola females have high levels of FSH because they are deficient in inhibin—which normally depresses FSH levels—and in the granulosa cells that produce inhibin.

Introduction of genes into animals has so far met with limited success. Enhanced growth of mice after transfer of the human growth hormone (hGH) gene is an effect that has not been duplicated in other animals. Robert Hammer (University of Pennsylvania, Philadelphia) reported that there is no evidence of enhanced growth in transgenic pigs *despite* high expression of the hGH gene. Instead, arthritis-like symptoms and other pathologies were noted in high expressors. It is apparent that the hGH gene cannot be assumed to work in general as it did in mice. Thomas Wagner (Ohio University, Athens) and others at the conference agreed that a deeper understanding of genetic regulation is essential to the successful development of transgenic animals.

Dean Hamer and coworkers (National Cancer Institute, Bethesda, MD) are manipulating metallothionein (*mt*) genes *in vitro*—by gene replacement to study function, by mutagenesis to locate DNA sequences important for regulation, and by using fusion genes to facilitate studies of gene regulation. Fusion genes can also be used to overproduce useful gene products in cell types equipped to process them properly. Hamer reported that by fusing the mouse *mt*-1

promoter to the hGH gene, 200 to 300 milligrams of authentically processed hGH can be produced in five weeks by a liter of cells in culture. John Kopchick and associates (Merck Institute, Rahway, NJ) have exploited retroviral long terminal repeats (LTRs) to produce bovine growth hormone (bGH). They have established a stable mouse fibroblast cell line that constitutively synthesizes and secretes biologically active bGH at a rate of 75 micrograms per 5×10^6 cells per day.

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Frank Ruddle (Yale University, New Haven, CT) reported that recent progress in mapping human genes has yielded a genetic map that is at least comparable in complexity to that of the mouse. He suggested that a major contribution to understanding the genetics of domestic animals would be to prepare detailed genetic maps using newer technologies together with classical Mendelian genetics. Restriction fragment mapping of chromosomes retained by cross-species hybrids of somatic cells could be performed using probes already existing for human and mouse genes.

The transfer of DNA into fertilized mammalian eggs by microinjection into the male pronucleus is one method for introducing novel DNA into the germ line. (The other is to use retroviral vectors.) Although single copies of DNA are desirable, microinjection of DNA results in multiple copies that become integrated as concatemers at single, random sites (Richard Hammer). Several speakers mentioned that the presence of prokaryotic sequences in introduced DNA reduces the expression of linked mammalian genes. In addition, understanding the negative effects of some introduced genes on fertility is essential before practical implementation of gene introduction

can be feasible. Clearly, the development of methods for targeting the introduced gene so that it integrates in controlled number of copies at the site of its endogenous homologue—perhaps replacing it—is an important goal.

David Whittingham (Medical Research Council, Surrey, England) delineated some species differences in the manipulability of mammalian eggs that affect the success of obtaining viable embryos for reimplantation following microinjection or fertilization *in vitro*. Pig embryos cease dividing in culture at the four-cell stage, but sheep and cow embryos can divide to become blastocysts. These cell division blocks may be partially relieved by discovering the different nutritional requirements of embryos from different species at various stages of development.

In contrast to the futuristic possibilities for gene transfer, immediate benefits to domestic animal agriculture are accruing from the production of reagents needed for the diagnosis, treatment, and prevention of disease. Charles Muscoplat (Molecular Genetics, Minnetonka, MN) documented that simply reducing the incidence of neonatal death from infectious diseases in farm animals would have a substantial impact on the economics of animal production.

Stable, regulatable and heritable alterations of genes in food animals will be far more difficult to effect, so it is essential to make farsighted choices about the goals of genetic manipulations. George Seidel (Colorado State University, Fort Collins) predicted that both increased productivity and increased concerns about animal welfare will be important factors in animal agriculture forty years hence. Food animals will have to be smaller, because of constraints of handling and shipping, yet will be selected for rapid growth and maturation for economy of production. Here, uncontrolled expression of growth hormone would be disastrous. Seidel pointed out that the need for small cows to give birth to large calves illustrates but one of the conflicts between efficient production and successful reproduction. He predicted that, at least for the next forty years, production of farm animals will continue to use existing methods of gene transfer such as artificial insemination and embryo transfer, with help from bioengineering to improve fertility and reduce disease.

—Kathryn Radke and Donna Lagarias