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Cloning and Gene Targeting in Domestic Species

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While transgenic manipulation in mice have been very successful the same is not true for cattle and pigs. The inability to isolate ES cells from the bovine and porcine has precluded the utilization of the gene targeting technology in these species. Fortunately new advances in cloning by nuclear transfer have opened up a unique opportunity to undertake precise genetic modification in cattle and pigs. The ability of a number of different laboratory groups to successfully clone cattle is due to numerous research programs focused on nuclear transfer in cattle, and the enormous base of knowledge developed over the last 20 years involving the application of assisted reproductive techniques in cattle. Successful and repeatable procedures for in vitro oocyte maturation, in vitro fertilization, and in vitro embryo culture are now well established for cattle. In our laboratory we have utilized nuclear transfer to reproduce the genotypes of several animals, selected for cloning based on their inherent genetic value. Results that we have obtained to date are similar to those reported by other laboratories.

More recently, we have modified existing technology to increase the efficiency of cloning in pigs and have been able to obtain multiple live births and litter sizes as large as nine. The availability of a highly efficient cloning process in pigs, coupled with the ability to genetically transformed cultured cells will greatly facilitate the development of transgenic pigs that can be used in human medicine and agriculture.

In addition, we have successfully developed the gene targeting technology in cultured fetal fibroblasts. Thus, the combination of our ability to undertake precise genetic modification in somatic cells and utilize those cells in a nuclear transfer procedure ensures that the proposed experiments will be completed in the time frame indicated.

At present we are applying these technologies to the control of infectious disease in cattle and the development of universal organ donors in swine. In addition, we are developing animal models of human disease.

One of our projects in cattle entails the development of an animal resistant to mad cow diseases (BSE). BSE, a form of TSE, represents a critical and emerging issue to world agriculture. As demonstrated by the drastic effect of this disease on the cattle industry in Europe,

entry of BSE into a country's cattle population can be devastating. More importantly, it negatively influences the public perception of the safety of our animal food supply, and has long-term consequences for animal agriculture. It is imperative, therefore, that the tools of agricultural biotechnology and genomics are utilized to increase the level of safety of our cattle and pig population both from a direct economic need, and a public perception need. In addition, with the emerging threat of bioterrorism, new technologies and approaches need to be developed to create safety mechanisms that can diminish or abolish such a threat. The approach we propose can serve as a blueprint for future developments in related areas, and the information generated will benefit any future efforts to utilize the tools of biotechnology to improve the safety of our food supply.

Prions are highly infectious pathogens recognized as causing transmissible spongiform encephalopathies (TSEs) in humans and animals. Among the invariably fatal neurodegenerative diseases caused by these pathogens are bovine spongiform encephalopathy (BSE), scrapie in sheep and goats, chronic wasting disease in mule deer and elk, and Creutzfeldt-Jakob disease in humans. The pathogenic agent is an abnormal form of an endogenous protein (PrP^{C}), distinct from viruses and viroids in that prions are not associated with nucleic acids and appear to be composed entirely of an abnormal protein (PrP^{Sc}). Bovine PrP encodes a protein of either 256 or 264 amino acids with 5 or 6 Gly/Pro-rich octapeptide repeats, respectively. High levels of expression of PrP are detected by Northern analysis in the brain, intermediate levels in heart and lung and low levels in the liver and spleen. At the gene levels there is a high degree of conservation among mammalian species. Inactivation of both endogenous PrP alleles in mice by homologous recombination results in animals that are completely resistant to spongiform encephalopathy. Subsequent results suggest that although knockout mice are physically normal, they exhibit altered sleep-wake cycles and circadian rhythms . Altered sleep regulation may have behavioral consequences for cattle. Naturally occurring sheep PrP genotypes have been discovered that confer resistance to scrapie. In each case the resistant animals displayed either a Gln/Arg171 or Arg/Arg171 genotype. In the bovine, PrP is not known to be polymorphic at the Gln 171(179) position, its genotype corresponding to the susceptible genotype. Not only does the resistance genotype confer resistance to natural transmission of scrapie, but also there is complete resistance to experimental transmission of both BSE and natural scrapie to sheep having the Arg/Arg 171 genotype. It is clear that there are several forms of the PrP capable of conferring resistant to BSE in cattle. Unfortunately, no naturally occurring resistant forms have been identified in cattle. Thus, the alternative approach of artificially creating such a polymorphism by transgenic means is warranted.

In short, application of transgenic and cloning technology to problems of medical and agricultural importance can have a drastic and positive impact on both the economic and health status of society. As such it is imperative that we continue to develop this important technology and in the process inform the public of the benefits, as well as potential risks, of animal biotechnology.