Lecture 20 - Animal Pharming and Nuclear Transfer (AMG text pp. 223-229)

Development of transgenic livestock
Transgenic animals can be used as bioreactors for the production of recombinant proteins that have pharmaceutical applications. Such animals are sometimes called "Pharm Animals". Transgenic sheep, goats, pigs, and cattle have been generated over the last 15 years using standard DNA microinjection of fertilized eggs based on methods initially developed for laboratory model systems such as mice.

While this has been shown to work for various livestock animals, the isolation of single founder animals is a difficult task, and even then, entire herds of transgenic offspring from the founder animal takes many generations.

However, more recent developments using whole nuclear transfer methods, have revolutionized the field of agribiotechnology through animal "cloning". Animal cloning is based on the ability to inject (or fuse) enucleated unfertilized egg cells with diploid nuclei from somatic cells grown in culture. The somatic cells can be stably transfected and thus provide a means to theoretically produce hundreds of identical animals in a single generation.

Animal pharming using transgenic livestock
More successful strategies exploit the properties of milk as a renewable resource to produce functional enzymes, antibodies and structural proteins using existing dairy manufacturing processes. The approach is to use the transcriptional promoters of mammary-specific genes to direct the expression of soluble transgenic proteins.

Cloning animals by nuclear transfer
Developmental biologists in agrobiotechnology have been trying for years to find conditions under which nuclear material from somatic diploid cells could be used as pluripotent source of genetic information. If the donor cell could be genetically manipulated in cell culture using standard stable DNA transfection protocols, then transgenic farm animals would be a common as transgenic mice.

As a first step towards this goal, Ian Wilmut and Keith Campbell, along with their colleagues at the Roslin Institute and the Edinburgh biotechnology company, PPL therapeutics, used a modified nuclear transfer method to produce a viable sheep using genetic material from a somatic cell that had been isolated from the mammary gland of a pregnant 6 year old ewe. These reconstituted egg cells subsequently divided and gave rise to 29 multicellular embryos that were implanted into 13 surrogate female sheep. A single lamb was born 148 days later that the Roslin Institute researchers named "Dolly", in honor of their favorite country singer, Dolly Parton.

Flow scheme depicting how Dolly the sheep was cloned from a reconstituted egg cell of a Scottish Blackface ewe, using genetic material derived from cells that had been isolated from the mammary gland of an adult Finn Dorset ewe.
A number of Pharm animals have now been produced by whole nuclear transfer proving that Dolly was not a fluke. The various techniques and range of efficiencies suggest that there is much more flexibility in the genome of a differentiated cell than previously thought. Some scientists have reported success with "knock-in" strategies in which therapeutic genes have been targeted to specific loci by homologous recombination, although this has not yet become routine.

Flow scheme illustrating how the Roslin Institute researchers used lipofection-mediated gene transfer to stably transfected diploid fetal donor cells, isolated from a Poll Dorset sheep embryo, with a mammary gland-specific expression vector containing human Factor IX cDNA (pMIX1). The ovine b-lactoglobulin (BLG) promoter upstream of the Factor IX coding sequence on pMIX1 had previously been shown to direct high level expression of a heterologous gene in sheep mammary glands. Molly and Polly represent the first two cloned transgenic pharm animals shown to contain a human gene of pharmaceutical importance.

The Honolulu nuclear transfer technique utilizes microinjection to generate diploid egg cells for animal cloning. In vitro oocyte activation is done by placing the injected eggs into calcium-free media containing strontium. Cytochalasin B is included in the oocyte activation media to prevent polar body formation which results in chromosome loss.

Despite early successes, albeit few and far between, it has been difficult to develop routine animal cloning methods. For a recent summary of the long road ahead in animal cloning, see this recent Science article by Pennisi and Vogel.

What is a "Pharm animal" and what advantages will they provide over conventional bacterial or yeast over-expression systems currently in use to produce recombinant therapeutic proteins such as insulin?

What is the primary difference between the method used to create Dolly and that used by the group in Hawaii to generate Cumilina the mouse?

What does the agrobiotech industry project as the primary uses of Pharm animals in the future?

Cite two reasons why the development of Pharm animals for commercial use may be years away despite the proof of principle experiments that have shown it is possible?

Recent updates on nuclear transfer applications:
- What is the latest on Missy the dog?
- Formation of Genetic Savings & Clone Incorporated.
- What is Genzyme Transgenics Corporation up to?
- There is always Infigen corporation to consider.
- Overview of animal cloning and future of the industry from Canada with commentary.
- Don’t forget Clonaid, the first human cloning company.
- What is The Human Cloning Foundation?