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Knocking Down Prion Genes in Livestock

Researchers have demonstrated that they can nearly eliminate production of infectious prion proteins in livestock by using an innovative approach based on RNA interference (RNAi).

The technique could enable scientists to genetically engineer livestock that are resistant to prion-caused diseases such as mad cow disease or bovine spongiform encephalopathy (BSE). A similar strategy might be used to protect animals against influenza or foot-and-mouth disease.

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— **Gregory J. Hannon**

Although careful monitoring of animal health and appropriate safety precautions are a current approach to containing such diseases, there is theoretical potential for creating genetically engineered strains of animals with a natural resistance to numerous diseases. However, genetic methods for altering livestock have thus far been lacking, write the authors in an article published on March 20, 2006, in the early online edition of the *Proceedings of the National Academy of Sciences (PNAS)*.

Unlike bacteria and viruses, prions consist only of aberrant proteins that misfold themselves into forms that can induce misfolding of normal molecules. In mammalian prion infections, these abnormal, insoluble proteins trigger protein clumping that can kill brain cells. In humans, clumping causes fatal brain-destroying human diseases such as Creutzfeldt-Jakob disease and kuru, and in animals it causes BSE and scrapie.

The new study extends the use of RNAi beyond mice to larger animals for the first time. The researchers say that the success of the technique suggests that manipulating genes with RNAi in plants and livestock may be an important alternative to traditional breeding or genetic engineering techniques to

enhance production of meat, dairy, or fiber products. Current techniques can be costly, inefficient, and time-consuming.

While this is very much a research effort, given the ability to manipulate these organisms in this way, I think it will be possible to do more quickly what selective breeders have been doing for a long time — creating animals with disease resistance and more advantageous properties for agriculture, said Howard Hughes Medical Institute investigator Gregory J. Hannon, one of the senior authors of the study.

Hannon and first author Michael C. Golding, who are at Cold Spring Harbor Laboratory (CSHL), collaborated on the studies with senior author Mark E. Westhusin of Texas A & M.

RNA interference uses tiny microRNAs to shut down targeted genes by interfering with the messenger RNA they produce. While researchers have long used RNAi as a method for manipulating gene activity for research purposes, they have discovered recently that cells use RNAi as an important mechanism for regulating their own genetic activity. Due to recent advances in RNAi techniques from Hannon's lab, the study's authors suspected it might also be possible to apply the approach to agricultural challenges.

Several factors have, until now, limited the use of RNAi to smaller animals. But in the new collaborative study, which was initiated by Westhusin, the researchers were able to use techniques developed in Hannon's laboratory to design genes that would produce interfering RNAs that could then be introduced into goat cells. The researchers conducted their experiments with goats because they are susceptible to the prion disease scrapie, which requires less stringent biosafety precautions than for those for studying BSE in cattle.

The team used a computer algorithm developed in Hannon's laboratory to identify genetic sequences encoding short RNAs that would effectively shut off the prion gene. These were screened to identify those that would be effectively processed by the cell — transforming the newly made microRNA into its functional, interfering form and loading it into the cell's silencing machinery.

The researchers next used a viral transfer technique developed in Hannon's laboratory to introduce the gene for the interfering RNA into goat fibroblast cells. Those cells - which they then used to produce goat embryos - used the gene to generate the interfering RNA that would target the prion gene. When the scientists looked for the prion protein in the brain of the cloned fetal goat, they found that it had been reduced by greater than 90 percent. Earlier studies have shown that this is enough to prevent prion infection. In subsequent experiments, they also confirmed that the viral transfer technique also was effective in the fertilized eggs of cattle.

In many ways, this is a simple proof of principle, said Hannon. Our objective was to ask whether we could generate a large animal through a cloning procedure in which our RNAi technology could be used to knock down a gene. While this is very much a basic experiment, since cows have been

cloned before, it would seem that the leap to practical application could be rather short, he said.

Hannon said that his laboratory, however, will continue to concentrate on the basic development and use of RNAi techniques in mice to understand tumor progression. For example, he and his colleagues are continuing to explore findings they published in a 2005 paper, in which they reported that a specific cluster of microRNAs can cause lymphomas in mice.