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WILL TRANSGENIC FISH BE THE FIRST AG-BIOTECH FOOD-PRODUCING ANIMALS?

by John Matheson

The FDA Center for Veterinary Medicine (CVM) regulates, in whole or in part, diverse animal biotechnology products. Two general areas that involve genetic modification are germ line transgenic modifications and non-heritable modifications (a.k.a.: somatic cell therapy and gene therapy).

Non-heritable modifications are still in early stages of development for animals, although this is a very active area in human medicine. These products are anticipated to be individual animal injections that would modify only some of the cells of the body to express a protein, protein hormone or enzyme. For example, individual steers could be modified to produce more muscle mass without having to modify the breeding herd, where additional muscle mass could cause calving difficulties.

Germ line transgenic modifications of animals, including fish and shellfish, have already begun to receive public attention in the U.S. and abroad. Most of the modifications currently relate to improving animal productivity. The biology of fish and shellfish facilitates more early work in the area of agronomic traits, compared to other farm animals.

One example is the inclusion of a gene cassette for increased expression of growth hormone in fish for increased growth rate and improved feed efficiency. This is a gene-based version for fish of CVM's first recombinant DNA product - recombinant bovine somatotropin (BST) for dairy cows.

Most, but probably not all, gene-based modifications of animals for production or therapeutic claims fall under CVM regulation as new animal drugs. As strange as it may seem at first, many of the modifications being investigated involve the addition of new animal drug substances. For example, adding growth hormone to a cow can be accomplished through use of BST injections, through gene therapies to create BST-producing regions in the body of the cow, or through germ-line modification, making a transgenic variety that contains extra BST-coding genes in every cell of the body, including reproductive cells. It all amounts to adding an animal drug, but the conditions are different - dose, areas of the body where the drug is released, opportunity for a withdrawal time, etc. The substances being added are for the purpose of improving animal health or productivity.

Absent a new, special law for regulating transgenic animals, the Federal government is directed to apply the existing laws. The animal drug provisions of the Federal Food, Drug, and Cosmetic Act best fit transgenic animals that have agronomic traits now being investigated and developed. Other transgenics will no doubt come along that could be viewed as containing food additives, color additives, vaccines, and nutritional supplements. Development of site-specific gene insertion techniques and animal genome projects could change the scope of potential genetic modifications to yield a wider variety of products than are currently being investigated.

As there is active investigation of transgenic fish abroad, as well as in the U.S., the public and the research community are occasionally exposed to predictions of the imminent commercial release of transgenic fish into the food supply. This should not occur without the pre-market approval from CVM, for those fish that have an added gene-based animal drug. No transgenic fish have been approved for producing food in the U.S., although a variety of transgenic fish species can be found in laboratories around the world.

It might be useful to describe how transgenic fish varieties are currently being produced. In addition to the genes being added, the techniques used are the source of some of the safety questions being raised at FDA.

Steps for Making a Transgenic Fish

Step 1

Decide on which gene to add, e.g., tilapia growth hormone gene. **Step 2.** Decode the growth hormone protein and translate it into the corresponding DNA code, i.e., make an artificial gene to express growth hormone. **Step 3.** Add the DNA code for a promoter gene and/or other regulatory genes and prepare a gene construct. **Step 4.** Insert the gene construct into a bacterial plasmid (plasmids are small, self-replicating, circular pieces of DNA that carry a number of genes, often including drug resistance genes). **Step 5.** Insert the plasmid into a bacterial cell line, such as an *E. coli* K-12, and grow up billions of copies of the plasmid. **Step 6.** Isolate the plasmid from the bacteria and cleave it into linear cassettes. **Step 7.** Inject a million or so cassettes into each newly fertilized, undivided egg of the fish to be modified; repeat hundreds of times. **Step 8.** Incubate eggs and grow up surviving fry. **Step 9.** Survivors will include many transgenic individuals, each one unique in the site of integration of the cassette, the number of cassettes integrated in the genome, and the degree to which the added genes are expressed, i.e., the amount of extra growth hormone produced. Find the transgenics and pick out the individuals that have the growth characteristics that are potentially marketable. **Step 10.** Embark on breeding program to stabilize the genetic construct in the new fish variety and to obtain enough individual fish to assess for commercial value, food safety, etc. **Step 11.** Now that there is a reasonable example of the final product, seek regulatory approval for it. Develop safety and other data and convince a number of governments and the citizens of those countries that your transgenic fish strain is commercially desirable, safe to eat and safe when lost into the environment. Design biological or physical containment for the new variety of transgenic fish to protect wild populations from gene introgression and competition.

Current Technology for Producing Transgenic Fish Has Limitations

The current technology has limitations that affect what types of transgenics can be developed.

The "transgenes" are limited to short gene constructs and are inserted randomly and in variable numbers of copies in each individual. This creates difficulty in stabilizing genetic modifications in a breeding population. There may be uncontrolled expression of the transgene. It may be expressed all the time; it cannot be turned off. Insertion sites for the transgenes may inadvertently affect the expression of other genes by disabling them or turning them on at an inappropriate time. The incidental insertion of drug resistance genes from bacterial plasmids introduces further uncertainties as to food safety.

Breeding programs are needed to stabilize the transgenes in a patentable variety and to produce numbers necessary for regulatory approvals and for marketing. Biocontainment strategies, both from an engineering and biological point of view, are necessary to prevent escape of the transgene into wild fish populations and to provide a means of control over the unlicensed breeding of the patented variety. These features add to the costs of development and affect competitiveness of the approach versus other, more traditional, breeding approaches.

Biocontainment needs are specific for each species and the location where it would be reared. The primary environmental concerns about releases of transgenic fish, for example, include competition with wild populations, movement of the transgene into the wild gene pool, and ecological disruptions due to changes in prey and other niche requirements in the transgenic variety versus the wild populations. For example, transgenic tilapia (with cold tolerance similar to the unmodified species) might require little containment in the northern tier of the U.S., but might be excluded from the Gulf States altogether, where tilapia may be a serious exotic invader of freshwater streams and ponds. These site-specific concerns may make it necessary to control the sites where transgenic fish are reared and the level of biocontainment required might differ from site to site. Any biocontainment other than absolute containment will have to be assessed for specific proposed sites.

Public acceptance of foods derived from transgenic animals will be important to the success of any transgenic variety introduction. Approval by FDA or a food regulatory group in another country does not guarantee public acceptance. Labeling of food from transgenic animals will likely be even more important to consumers desiring a choice than has been observed for milk derived from BST-treated dairy cows or for transgenic plant varieties. Ethical concerns among the public over the appropriate use of animals are issues, not evident with transgenic plants, that may affect public acceptance of transgenic animals as food sources. There is also expected to be variation among the citizens of different countries as to their acceptance of transgenic animals.

Competition from Alternative Approaches

Finally, alternative approaches to breed improvement are in competition with transgenic approaches. This is especially true in fish and shellfish species, where there has been only a short history of attempts at breed improvement through selective breeding. The use of new genetic screening techniques in combination with selective breeding could produce even faster results. The strains of animals produced through these means face less regulatory or public scrutiny than transgenic varieties.

Improved nutrition and management techniques may also yield increased productivity, disease control and profitability of aquacultured species. Orally active growth promoters that can be withdrawn pre-harvest are currently not available for aquaculture. Such classical animal drug approaches, while regulated by the same process as transgenic fish, could be applied to any individual of the species, a potentially larger market than a transgenic variety could penetrate.

Does this mean that transgenic fish are not likely to be commercialized in the U.S.? Not at all! It means only that the improvements offered by transgenic fish, and any other transgenic animals, must be dramatic when compared to what is possible by other, better-accepted, approaches. Benefits to producers and consumers must be tangible and outweigh the costs in royalties, license fees, biocontainment, and regulatory and public acceptance. The technology for creating transgenic animals is constantly improving and

will soon begin to reduce the limitations of the current approaches and improve the competitive balance with other approaches to breed improvement.

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