Genetically Modified Salmon Vying for a Spot at the Dinner Table

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The Food and Drug Administration is reviewing a first: an advanced hybrid salmon intended for the human food table. The fish is actually a genetically modified (GM) Atlantic salmon made to reach market growth in half the time of Atlantic salmon now grown routinely in today's aquaculture ocean-pen farms.

The underlying technology for producing GM Atlantic salmon was developed in 1989 when Garth Fletcher, Peter Davies, and Choy Hew, all at Memorial University of Newfoundland at the time, created a gene construct for creating transgenic Atlantic salmon. Research papers from the team began appearing in journals in the early 1990s describing the evolution of genetically modified salmon with rapid growth curves.

Today's commercial application is championed by AquaBounty Technologies, based in Waltham, MA. The company hopes to sell its AquaAdvantage genetically modified fish eggs to approved land-based growth facilities should the FDA provide the regulatory go-ahead, an unknown at the time this article went to press.

"To make our fish eggs, we have inserted a specific heritable gene and promoter construct into the fish eggs that allows the salmon to grow more rapidly than nontransgenic salmon," explains Ronald Stotish, Ph.D., AquaBounty's CEO. In effect, with a single gene addition, the fish grow more quickly. It does not make the fish grow to larger sizes, just more quickly.

Expediting the Natural Life Cycle

In the first year of an Atlantic salmon's life, it does not grow much at all. Its growth, particularly its growth hormone, is regulated by sunlight, food availability, and water temperature because it is a cold water fish. In its first year, the salmon grows from an egg sac to small fry that is a few grams. Trout, by comparison, grow immediately and rapidly in the first year. "We've allowed AquaAdvantage salmon to grow more like a trout in its first year of life," says Stotish. The AquaAdvantage fish reaches the desirable nearly 9 lb weight limit considered prime by market standards in about 18 months compared with 30 months or more for conventionally farmed Atlantic salmon.

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Growth Hormone Gene and Promoter Construct

The technology for making the GM salmon is straightforward compared with today's complicated genomics research. Nearly 20 years ago, Fletcher and Hew were researching antifreeze genes that allow some fish, such as winter flounder and cod, to survive in cold water. They honed in on a gene promoter, a short DNA sequence, from the antifreeze gene in the ocean pout fish. A promoter is a DNA sequence that allows a gene to be transcribed. It is a recognition site that binds to the cells' RNA polymerase, allowing transcription of the growth hormone gene to produce functional growth hormone proteins.

The second half of the recipe includes the growth hormone gene from the Chinook salmon, a variety of naturally harvested West Coast salmon. This GH is nearly identical to that of the Atlantic salmon—nearly 98% homologous. This gene was chosen for practical purposes: to see if the endogenous resident growth hormone was being expressed in cultured fish or the transgene. Both produce the same functional protein, but this choice allowed the early researchers to know which gene was being expressed.

In the case of AquaAdvantage salmon, the ocean pout promoter acts as a switch that allows the growth hormone gene to be continually transcribed consistently. In native Atlantic salmon, the growth hormone gene is normally only expressed in the pituitary gland of the fish. They knew they had to find a way to modify it so that it would be expressed in other tissues to avoid downregulation by the pituitary. Because antifreeze protein genes are expressed in liver and other tissues, Fletcher and Hew selected the promoter region from an ocean pout antifreeze gene and spliced it to the coding region of the Chinook salmon growth hormone gene.

The gene/promoter construct is then microinjected into developing embryos of Atlantic salmon. Over a series of generations, they systematically scored the fish that had incorporated the gene into their genomic DNA and looked to see which fish were capable of transferring it in a heritable fashion. This proved the construct had been incorporated into the germ line of the fish.

Then they identified fish that were expressing the gene in a heritable fashion and sorted through those fish to find one that had the phenotype of choice: rapid growth. That fish was used to establish the line and became the founder fish of what is now known as the AquaAdvantage salmon.

In native Atlantic salmon, the endogenous growth hormone gene is downregulated, a unique evolutionary adaptation of the fish. It is shut off for periods of 4–5 months of the year in young salmon at times when there is likely to be low food, shorter days, and colder water. In spring, when food is more abundant and the water warms a bit, growth begins again. Once the salmon reach adulthood, they do not demonstrate this sort of downregulation, continuing to grow and feed.

"The idea is to have the fish produce a sustained level of growth hormone transcripts, not high levels," explains Stotish. He points out differences of the AquaBounty salmon compared with other transgenic salmon species. Research from Dr. Robert Devlin of the Center for Aquaculture and Environmental Research at Fisheries, part of Fisheries and Oceans Canada (DFO), an internationally known researcher in the field of salmon aquaculture and genetics, found that transgenic salmon produced much higher levels of growth hormone compared with non-transgenic fish. "In contrast, in our fish, the promoter is allowing the GH hormone to be produced at low but sustained levels," says Stotish. "The levels of GH are basically the same as found in a traditionally raised Atlantic salmon, but it is enough to allow the fish to grow year-round."

Single-Sex, Sterile Fish

The AquaAdvantage salmon is purposely made to be all female, all of which are sterile. ""We did it for containment and environmental reasons," explains John Buchanan, AquaBounty's Director of Research and Development. "By breeding all female fish—which are also sterile—they cannot breed with other local populations in the case of escape and create self-sustaining populations."

Triploidy technology is a decades-old technology used to make the fish sterile. It is a process that allows the fish to retain the polar body during cell division that allows an extra copy of the chromosomes to be incorporated into the offspring. The cells of triploid individuals have three sets of chromosomes. These daughter fish, also known as 3N fish, are therefore unable to reproduce.

"We use female fish since the sterilization process we use is more effective on the female sex," adds Buchanan. Many commercial fish species, including farm-raised trout and tilapia, are bred with triploidy technology.

The Data

The FDA is currently reviewing a variety of safety and study data pending its decision on allowing the AquaAdvantage salmon to be introduced into the human food supply. The fish are presented to the FDA under the following description: triploid hemizygous, all-female Atlantic salmon (*Salmo salar*) bearing a single copy of the α-form of the *opAFP-GHc2* rDNA construct at the α-locus in the EO-1α lineage.

In mid-September 2010, the FDA Veterinary Medicine Advisory Committee (VMAC) held a two-day meeting to discuss the AquaAdvantage salmon. Detailed information can be found at the VMAC website (http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/ucm201810.htm), including the FDA briefing packet describing the scientific design and test data of the GM salmon.

Aquabounty Technologies

When Fletcher and Hew created what would become the construct for the AquaAdvantage salmon, Aquabounty Technologies did not exist. It evolved from a company called A/F Protein, which was interested in a variety of antifreeze proteins for controlling cold-induced damage to food medical products. When they learned of Drs. Fletcher and Hew's discovery, the company shifted focus to developing it as a tool for the salmon industry.

Aquabounty claims that the fish's rapid growth is not its only benefit. Contrary to farm-raised Atlantic salmon, AquaAdvantage salmon are grown in land-based systems. This would theoretically allow the fish to be cultivated locally near food consumption sites, potentially reducing the environmental impact compared with coastal-based farms. "By growing them efficiently in land-based system, you regulate their environment extremely closely," says Stotish. "This limits exposure to diseases common in communal ocean pens and provides another measure of security against into wild populations." It should be noted, however, that AquaBounty's FDA submission only includes an application for growing the salmon out in a facility in Panama. To be grown in other sites, additional approvals will need to be sought and granted.

Today, nearly all commercial Atlantic salmon is farm-raised in ocean pens in various sites worldwide, notably Norway, Chile, Scotland, and the Canadian Maritimes. The wild Atlantic salmon fishery has never been commercially viable.

Aquabounty has second and third generation technologies in development for creating sterile fish. "We have a small zebrafish group mainly for that purpose," explains Buchanan. Should the company become viable in the long-term, they hope to use them not only for creating sterile salmon but perhaps other fish and arthropod species.

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