

Developments in transgenic fish in the People's Republic of China

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Summary

In the People's Republic of China, genetically modified (GM) fish are being developed primarily to produce desirable alterations to growth rates or feed-conversion efficiency. Up to the present, no transgenic fish have been commercially approved for human consumption. This review introduces advances in the People's Republic of China in transgenic fish studies, biosafety studies of fast-growth GM fish, and the regulation of GM fish.

Keywords

Common carp – Growth hormone – People's Republic of China – Transgenic fish.

Introduction

With the expansion of the global population and overfishing, advanced aquaculture is needed to meet the increasing demand for high-quality fish protein. Genetically modified (GM) fish (transgenic fish) offer the opportunity of improving both the production and characteristics of conventional fish strains currently exploited in aquaculture. Since Zhu *et al.* (41) produced the first batch of fast-growth transgenic fish, many laboratories throughout the world have been successful in generating transgenic fish in a variety of species using different foreign gene constructs. At present, biotechnology has made considerable advances in producing transgenic animals, and fish may be considered the best candidate for the first marketable transgenic animal for human consumption (36). Before such a product can enter the marketplace, many aspects of the biosafety of transgenic fish have to be carefully evaluated (16, 18, 36). The Food and Drug Administration in the United States of America is reviewing the application of a fast-growth transgenic salmon (19). Because growth-enhanced transgenic fish are near the point of application to aquaculture, many countries are discussing or developing safety assessment strategies for fast-growth transgenic fish.

Recently, many reports have focused on the biosafety considerations of transgenic fish (5, 21, 22, 27, 28), which are also a major concern for the government, the public

and scientists in the People's Republic of China. To overcome these concerns, it is important to develop a reliable and widely accepted method of assessing the potential for harm that might be caused by GM fish escaping into the wild. Recently, the National Natural Science Foundation of China and the Ministry of Science and Technology of the People's Republic of China have provided funding to Chinese scientists for the assessment of the biosafety of a fast-growth transgenic common carp that contain 'all-fish' growth hormone (GH) gene constructs. In this review, the authors will introduce advances in transgenic fish studies in the People's Republic of China, biosafety studies of fast-growth GM fish, and Chinese regulations regarding GM fish.

Transgenic fish studies in the People's Republic of China

Integration, expression and inheritance of transgenes

In the late 1980s, the integration and expression of foreign genes were thoroughly studied in a model system in which the common carp (*Cyprinus carpio*) was the host fish and a recombinant human growth hormone (hGH) gene was the transgene (43). Using *in situ* Dot blotting, Southern blotting, Northern blotting and radio-immunoprecipitation analysis experiments, the study found that

the foreign gene underwent a dynamic process during embryogenesis. The *in situ* Dot blotting experiment showed that the replication of the foreign gene started as soon as it was introduced into the fertilised eggs, and the strongest signal of replication occurred from late blastula to early neurula. The Southern blotting experiment showed that the integration of the foreign gene was most likely to occur at the early blastula stage and last for a long time, resulting in transgenic mosaicism; in other words, the integrated foreign genes were distributed in different tissues and organs of the transgenic fish. Only those foreign genes integrated into the genome of germlines could be transmitted to the offspring via sexual reproduction. The transcription of the foreign gene could be observed at the late-gastrula stage by Northern hybridisation, and radio-immuno-precipitation analysis revealed that different individuals had different levels of foreign gene expression. Depending on the positional effects related to the expression and function of the transgene, foreign gene integration can be categorised in three different ways: functional integration, silent integration and toxic integration. Silent integration shows no visible effect and toxic integration blocks the normal development of the fish; only functional integration results in hGH expression that leads to growth enhancement (43).

Due to the mosaic integration of transgenes in the founder fish, the frequencies of transgene transmission to F_1 progeny are usually less than the frequencies in Mendelian ratios. When 'all-fish' GH-transgenic carp founders were crossed with non-transgenic controls, the transgenic ratios in the F_1 generation were from 72% to 88%, and genetic analysis showed that two or three chromosomes of each founder were integrated with transgenes (33). No matter how the integration of the transgene occurs or how many integration sites the transgenic founder owns, a transgenic line with stable germline transmission has to be established. Many studies have shown that F_1 transgenics produced from crosses between a wild type female and a transgenic male are in a heterozygous state, since they give birth to the next generation with a transgene positive ratio of about 50% (3, 9, 13, 25). In the authors' laboratory, it was also recently discovered that frequencies of transgene transmission to F_2 progeny from a fast-growth 'all-fish' GH F_1 transgenic germline were about 50% (unpublished findings). To mate a pair of F_2 transgenic siblings from the same parents has been shown to be an efficient way to establish a homozygous line of transgenic fish (3, 10). In another study of metallothionein-1 (MThGH)-transgenic common carp, transgenics were mated to each other and reproduced generation by generation, producing F_4 offspring. Most transgenes were reported to be steadily inherited over four generations of transmission, although a small proportion of the transgenes were rearranged, deleted and/or inserted with host sequences, and appeared to be highly polymorphic (38). It was also observed that a MThGH-transgene in the F_4 generation could still initiate transcription properly, and

produced faithful transcript products (26). Therefore, after several generations of transmission, the transgene tends to be stably inherited and to function as an endogenous gene. This phenomenon could be called the 'endogenous domestication' of a novel gene.

Growth of enhanced transgenic fish

In the middle 1980s, a research group led by Dr Zuoyan Zhu successfully transferred a recombinant human growth gene under the control of mouse MThGH into the fertilised eggs of goldfish (41) and loach (42), which led to the birth of fast-growth transgenic fish.

For application purposes, a new 'all-fish' genomic construct (pCAGcGH) was made, which included the common carp β -actin gene, CA (15), and grass carp growth hormone gene (gcGH) (44). This 'all-fish' GH construct was microinjected into the fertilised eggs of yellow river carp, a local strain of common carp, and the growth performance was examined at different growth stages (33). The study found that the frequency distribution of body weight (BW) of non-transgenic fish ($n = 359$) was normal, while that of transgenics ($n = 324$) was not normal at the 120-day stage. The BW of the largest non-transgenics was 1,414 g, and that of the smallest was 264 g. The BW of the largest transgenics was 2,750 g, although that of the smallest was only 84 g. Among the transgenic individuals, 8.7% had a higher BW than the largest non-transgenic individual, and 6.4% of the transgenics weighed more than 2 kg, which is more than double the mean BW of non-transgenics. Fast-growth transgenic founders were crossed with non-transgenics. Results showed that the BW frequency distribution of F_1 individuals was normal at the 80-day stage, with a mean BW of 417.89 ± 79.72 g. Among all F_1 individuals, 60% were above the average BW of the controls (260.4 ± 22.47 g). The study also found that fast-growth individuals of the P_0 transgenic fish had much thicker muscles on the back and an obvious hump behind the head (33). The study indicated that 'all-fish' GH-transgenic common carp could attain higher growth rates than the controls.

In addition, our laboratory successfully produced P_0 GH autotransgenic blunt-snout bream (*Misgurnus mizolepis*) with a construct of blunt-snout bream β -actin gene and its GH complementary deoxyribonucleic acid (cDNA) in 2003. Preliminary studies showed that some individual P_0 GH autotransgenic blunt-snout bream displayed fast-growth (12).

Bioenergetic analysis of growth-enhanced transgenic fish

Why and how can the growth rate of GH transgenic fish be dramatically improved? Is there any difference in body

composition between transgenics and controls? Several studies have been carried out to analyse the bioenergetics of transgenic fish to try to answer these questions. There has been a thorough bioenergetic analysis of F₂ MThGH transgenic common carp as compared with controls (2). When fed with fresh tubificid species, the energy budget of transgenic and control common carp can be expressed by the following equations:

a) F₂ MThGH-transgenic common carp: $100C = 8.9F + 0.63U + 49.03R + 41.44G$

b) control carp: $100C = 7.37F + 1.14U + 53.36R + 38.13G$

where C is the total energy from food, F is the energy lost in faeces, U is the energy lost in nitrogen excretion, R is the energy channelled to metabolism, and G is the energy channelled to growth.

Compared with the controls, transgenic fish had a significantly higher proportion of food energy channelled to G and a significantly lower proportion channelled to R and U. The transgenic fish saved 6.62% of the total energy from food for growth improvement. This phenomenon was named the 'fast-growing and less-eating' effect.

A study on the growth and feed utilisation by F₄ MThGH-transgenic common carp fed with diets containing 20%, 30% and 40% protein has also been carried out (6). The study showed that at each protein level the transgenics had higher specific growth rates than the controls. Feed intake was significantly higher in the transgenics than in the controls fed a low protein diet (20%), but feed intake did not significantly differ between transgenics and the controls when the diets contained levels of 30% or 40% protein. It was thus demonstrated that at a lower dietary protein level, transgenics achieved higher growth rates mainly by increasing feed intake; but at higher dietary protein levels, transgenics achieved higher growth rates mainly through higher energy conversion efficiency. The study also showed that the transgenics contained significantly higher amounts of dry matter and protein, but lower amounts of lipids than the controls at all dietary protein levels. The apparent digestibility of amino acids tended to be higher in the transgenics than in the controls, especially in fish that were fed diets with lower protein levels. When studying the whole-body amino acid pattern in transgenics and controls, Fu *et al.* (7) found no differences in 17 amino acids between the transgenics and controls.

Field trials of transgenic fish

Although great advances have been made in fast-growth GH transgenic fish in the People's Republic of China, no GH transgenic strains have yet been applied for commercial purposes. To determine whether transgenic fish have the potential for commercial exploitation, trials

must be performed in conditions that are similar to those of commercial aquaculture. Recently, the authors' laboratory has completed a medium-scale trial of 'all-fish' (CAGcGH) transgenic common carp in Wuhan, a major city in the centre of the country, authorised by the Ministry of Agriculture of the People's Republic of China (40). New rearing ponds with an area of 2 hectares were built by professional aquaculturists, with banks high enough to withstand floods and enclosed with wire netting. All of the water channels into and out of the ponds were carefully designed to prevent the escape of transgenic fish, and strict measures were adopted to ensure no escapes occurred. Each pond was equipped with oxygen supplies and auto-feeding machines, which greatly facilitated aquatic rearing. In the spring of 2000, two-year-old mature founders of 'all-fish' GH transgenic common carp were examined with polymerase chain reaction (PCR) to detect those transgene carriers whose sexual gonads were transgene positive. Two hundred transgenic individuals that had shown significant growth enhancement were artificially spawned to produce F₁ transgenics. The F₁ transgenics were reared in the ponds with a total area of 1.67 hectares, and non-transgenics were reared as controls under the same conditions. From 16 June to 7 September 2000, 50 transgenics and 50 controls were randomly sampled for analysis at 20-day intervals. The results showed that on average the transgenics had growth rates of 80%, 55%, 77%, 60%, and 42% greater than the controls in the serial samplings. On 7 September, when the fish were 142 days old, most of the transgenics had reached market size, while the controls needed another year to reach the same size. Furthermore, the feeding coefficient (total food weight per unit of gained BW) of the transgenics was 1.10 and that of the controls was 1.35. The study indicated that GH transgenic common carp not only had faster growth rates but also were more efficient at feed utilisation than the controls in the pond culture. Relatively detailed descriptions of studies on fast-growth GH transgenic common carp have also been recorded by Wu *et al.* (35).

Biosafety studies of genetically modified fish

Producing sterile transgenic fish

Sterile transgenic fish pose no risks of any genetic impacts on the local gene pools. In the People's Republic of China, work on the production of sterile transgenic fish has focused on polyploidy manipulation and use of transgenesis.

Polyploidy manipulation techniques are easily applied to fish, thus offering fish biologists an approach for producing various useful reproductive characteristics for commercial

aquaculture. In many fish species, triploid males and females generally fail to produce mature gonads and turn out to be sterile. In the People's Republic of China, sterile transgenic fish are produced by hybridising tetraploids and diploids. A study of interspecific hybridisation between red crucian carp (*Carassius auratus* red var.) and common carp found that the F₃-F₈ generations of the hybrids were allotetraploids, and capable of producing tetraploid offspring (14). 'All-fish' GH gene construct had been transferred into the fertilised eggs of the allotetraploids, and the transgenic tetraploids showed significantly better growth performance than non-transgenic tetraploids. Transgenic tetraploids could produce spermatozoa at the age of 240 days (37). Subsequently, transgenic triploids were successfully produced by crossing transgenic diploid common carp with tetraploids (40). Transgenic triploids were found to be sterile, and had higher growth rates than non-transgenics. Since transgenic tetraploids are able to produce successive generations of offspring and still maintain the tetraploidy, the technology is available to produce sterile transgenic triploids for aquaculture.

It is obvious that the triploid strategy is not suitable for all species of transgenic fish, and more feasible techniques need to be developed to ensure the environmental safety of transgenic fish. Fish gonadotropin-releasing hormones (GnRHs) are well known to be decapeptides that play critical roles in fish gonadal development and in regulation of the reproductive cycle (1, 31). Repressing the expression of GnRHs is likely to cause sterility in the fish. To this end, the most promising method at present appears to be through antisense technology, which has achieved success in transgenic plants (23, 32). The introduction of short DNA or ribonucleic acid (RNA) sequences corresponding to part of the coding sequence can block the expression of a particular gene, either by binding the double-stranded DNA to form a triplex to block transcription, or by binding the mRNA to block the processing of transportation. After introducing constructs containing salmon GnRH promoter fused to GnRH antisense cDNA into rainbow trout, Uzbekova *et al.* (29) found the expression of antisense GnRH RNA in the brain and a decrease in the production of endogenous GnRH mRNA in the brain and pituitaries. Unexpectedly, the levels of the gonadotropins, follicle-stimulating hormone and luteinising hormone, were not affected in the antisense GnRH transgenics, and the fish reached maturity at the same time as non-transgenic individuals. On the other hand, when histone H3 promoter was used to drive antisense GnRH cDNA, some transgenic rainbow trout were sterile, although their fertility could be restored by hormonal treatment (30). In the People's Republic of China, sterile transgenic fish have been produced by antisense technology to repress the expression of GnRHs. GnRH cDNA derived from the common carp was isolated, and a construct with common carp β -actin promoter and antisense GnRH cDNA was

subsequently generated. Introducing this antisense GnRH construct into fast-growing transgenic common carp may cause sterility. Furthermore, the fertility of antisense GnRH-transgenics with desirable performance could be restored by exogenous hormone administration, resulting in a physiologically reversible fertility strain of transgenic fish that could serve as brood stock for aquaculturists (11).

Food safety of transgenic fish

At present, the generally accepted principle for evaluating the safety of foods produced by modern biotechnology is the 'substantial equivalence principle', which was proposed by the Organization for Economic Cooperation and Development in 1993 (20). In 1995, a World Health Organization (WHO) consulting group that convened to provide practical guidance for the safety evaluation of plants derived from modern biotechnology used the substantial equivalence principle in this guidance (34). In 1996, the WHO and the Food and Agriculture Organization came together to provide practical and concrete recommendations for international guidelines for the safety assessment of foods derived from biotechnology, and suggested that the substantial equivalence principle be used as a general guidance for the safety evaluation of all genetically modified organisms (GMOs) (4).

In the People's Republic of China, the food safety of 'all-fish' GH-transgenic common carp was evaluated through studies of three groups of mice that were fed with 'all-fish' GH-transgenic common carp, non-transgenic common carp, and physiological saline respectively for six weeks. The experiments strictly followed the pathological rules for testing new medicines issued by the Ministry of Health of the People's Republic of China. The results indicated that the test mice showed no significant differences when compared with the two groups of control mice, including in terms of growth performance, biochemical analysis of blood, histochemical assay of twelve organs, or reproductive ability (39). Information for assessing the food safety of transgenic fish is still limited, and more studies may need to be undertaken.

Environmental risk assessments of fast-growth genetically modified fish

At present, Chinese scientists are developing environmental safety assessment strategies for fast-growth GM fish. Since the beginning of 2005 many studies have been taking place to compare the morphology, life history and behaviour of fast-growth GM fish with those of wild fish. Scientists hope to obtain complete information on these aspects of fast-growth transgenic fish in order to develop a generally accepted model for assessing the environmental risk of such fish.

In the authors' laboratory, some preliminary information has been obtained on the morphology and life history of fast-growth transgenic carp. In a study conducted with fast-growth 'all-fish' GH-transgenic common carp, the gonadosomatic index (ovary weight/BW), fertilisation rate (number of fertilised eggs/number of eggs) and hatchability (number of hatched fry/number of fertilised eggs) were compared with wild-type non-transgenics. The results showed that the quality of eggs of the transgenics was similar to that of the non-transgenics, while the gonadosomatic index of the transgenics was significantly lower than that of the non-transgenics. For both transgenics and non-transgenics, the fertilisation rate was higher than 80% and hatchability was over 60%, and there were no significant differences in these parameters between the transgenics and non-transgenics (33). Fast-growth 'all-fish' GH-transgenic common carp also promoted thymus development and thymocyte proliferation, and retarded thymus degeneration (8). Recently, the authors found that sexual maturity in non-transgenic carp male individuals occurred at the age of six months (body mass = 1,079 ± 46 g), while sexual maturity in the fast-growth 'all-fish' GH-transgenic common carp male individuals had still not occurred at seven months (body mass = 3,143 ± 405 g) (unpublished findings). The results indicated that fast-growth 'all-fish' GH-transgenic carp did not have a lower age of sexual maturity.

Regulation of genetically modified fish

There is a comprehensive framework of committees and legislation to regulate and provide advice on GMOs in the People's Republic of China. Under existing legislation, all GMOs developed in the People's Republic of China, whatever their intended use, must be assessed by the committees. The regulations in place are outlined briefly here.

On 24 December 1993, the first 'Safety Administration Regulation on Genetic Engineering' was issued by the State Science and Technology Commission of the People's Republic of China. The aims of this regulation were as follows:

- to promote research and development of biotechnology in the People's Republic of China
- to tighten safety controls on genetic engineering work
- to protect the health of both the public as a whole and genetic engineering workers
- to prevent environmental pollution
- to maintain ecological balance.

The regulation made specific stipulations about the management of technologies that utilise carrier systems to reorganise DNA and that import the DNA from other sources into organisms in physical and chemical ways.

On 10 July 1996, the second measure, the 'Safety Administration Implementation Regulation on Agricultural Biological Genetic Engineering', was issued by the Ministry of Agriculture of the People's Republic of China. This regulation provides a classification of safety considerations relating to different genetic engineering carriers, and prescribes relevant management measures. In particular, it designates the procedures and rules for the registration and safety assessment of agricultural biological genetic engineering. The regulation is effectively enforced in the management of agricultural biological genetic engineering across the country. The above-mentioned regulations are available at www.biosafety.gov.cn (English-language versions are also available: 17, 24).

On 9 May 2001, the Chinese State Council promulgated the 'Regulations on Safety of Agricultural Genetically Modified Organisms'. The purpose of these regulations was to strengthen the safety administration of agricultural GMOs, safeguard human health and the safety of animals, plants and microorganisms, protect the environment, and promote research on agricultural GMOs. The activities of research, testing, production, processing, marketing, import or export with respect to agricultural GMOs within the territories of the People's Republic of China must conform with these regulations (an English-language version is available: www.biosafety.gov.cn/image20010518/3107.doc).

On 5 January 2002, the 'Implementation Regulations on the Labelling of Agricultural Genetically Modified Organisms' were issued by the Ministry of Agriculture. The purpose of these regulations was to strengthen the labelling administration of agricultural GMOs, standardize the marketing activities of agricultural GMOs, guide the production and consumption of agricultural GMOs, and protect consumers' rights to full access to information about the product. All listed agricultural GMOs for marketing should be labelled. Any agricultural GMO that bears no label or whose label is not in conformity with the requirements of the regulation shall be banned from import or marketing (an English-language version is available: www.biosafety.gov.cn/image20010518/3110.doc).

On 8 April 2002, the 'Health Administration Regulation on Transgenic Food' was issued by the Ministry of Health. The aims of this regulation were to strengthen supervision and management, and to protect consumers' rights to access information about GM food. This regulation licenses and monitors all GM food from the human safety point of view. It also designates the procedures for safety assessment of GM food (a Chinese-language version is available: www.moh.gov.cn/public/open.aspx?n_id=7504&seq=).

On 8 July 2002, the 'National Transgenic Biosafety Committee of the People's Republic of China' was founded. The committee is responsible for the biosafety assessment of GMOs and for biotechnology consultation and supervision.

All these regulations are used to regulate, license and monitor the use of GM fish in the People's Republic of China. At some point in the future, when sterility-reversible transgenic fish have been developed and environmental release testing has been successfully performed, a scientist or company wishing to market a GM fish for food will have to apply to the National Transgenic Biosafety Committee of the People's Republic of China.

Conclusion

In the People's Republic of China, GM fish are being developed primarily to produce desirable alterations to growth rates or feed-conversion efficiency. Up to the present, no transgenic animals, and certainly no transgenic fish, have been commercially produced or approved for

human consumption. The Chinese government and scientists are very cautious in assessing the biosafety of GM fish. Further studies will be conducted to comprehensively assess the environmental impacts and food safety of fast-growth GM carp. Those involved in the technology, whether in the development of legislation or in the application of scientific developments, need to engage in an open and frank debate with the public, and to recognise and address public concerns about these issues.

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Progrès réalisés dans le domaine des poissons transgéniques en République populaire de Chine

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Résumé

En République populaire de Chine, les poissons génétiquement modifiés (GM) sont créés essentiellement pour produire une modification souhaitable des taux de croissance ou du coefficient de transformation des aliments. À ce jour, la commercialisation de poissons transgéniques destinés à la consommation humaine n'a pas été autorisée. Le présent article examine les avancées réalisées en République populaire de Chine en matière d'études sur les poissons transgéniques, d'études de biosécurité des poissons GM à croissance rapide et de réglementations relatives aux poissons GM.

Mots-clés

Carpe commune – Hormone de croissance – Poisson transgénique – République populaire de Chine.

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Avances en el área de los peces transgénicos en la República Popular China

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Resumen

En la República Popular China se están generando peces modificados genéticamente con el objetivo básico de lograr cambios deseables en la tasa de crecimiento o el coeficiente de conversión de alimento de los animales. Hasta la fecha, no se ha aprobado ningún pez transgénico para consumo humano. Los autores pasan revista a los avances que han experimentado en la República Popular China los estudios sobre los peces transgénicos y sobre la bioseguridad de peces transgénicos de crecimiento rápido, así como la reglamentación sobre el tema.

Palabras clave

Carpa común – Hormona del crecimiento – Pez transgénico – República Popular China.



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