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Review Article

TRANSGENIC FISH MODEL IN ENVIRONMENTAL TOXICOLOGY

Madhuri Sharma[1], A.K. Mandloi[2], Govind Pandey[3]* and A.B. Shrivastav[4]
[1]Guest Lecturer, Department of Zoology & Biotechnology, Govt. MH College of Home Science & Science for Women, Jabalpur, MP, India
[2]Professor & Head, Department of Fisheries, College of Veterinary Science & A.H., Madhya Pradesh Pashu Chikitsa Vigyan Vishwavidyalaya (MPPCVV), Jabalpur, India
[3]Deputy Director/Associate Professor/Senior Scientist, Directorate of Research Services, MPPCVV, Jabalpur, India
[4]Director, Center for Wildlife Forensic & Health, MPPCVV, Jabalpur, MP, India

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*E-mail: drgovindpandey@rediffmail.com, drgovindpandey@yahoo.co.in

ABSTRACT
A number of experiments and the use of drugs have been performed in fish. The fish may be used as model organism in various biological experiments, including environmental toxicology. Aquatic animals are being engineered to increase aquaculture production, for medical and industrial research, and for ornamental reasons. Fish have been found to play an important role in assessing potential risks associated with exposure to toxic substances in aquatic environment. Hence, it has been thought that the development of transgenic fish can enhance the use of fish in environmental toxicology. India has developed experimental transgenics of rohu fish, zebra fish, cat fish and singhi fish. Genes, promoters and vectors of indigenous origin are now available for only two species namely rohu and singhi for engineering growth. Development of fish model carrying identical transgenes to those found in rodents is beneficial and has shown that several aspects of in vivo mutagenesis are similar between the two classes of vertebrates. Fish shows the frequencies of spontaneous mutations similar to rodents and respond to mutagen exposure consistent with known mutagenic mechanisms. The feasibility of in vivo mutation analysis using transgenic fish has been demonstrated and the potential value of transgenic fish as a comparative animal model has been illustrated. Therefore, the transgenic fish can give the significant contribution to study the environmental toxicity in animals as a whole.

KEY WORDS: Transgenic fish models, mutations, chemical contaminants, aquatic environments, toxicology.

INTRODUCTION

As the field of genetic engineering advances, we are beginning to see increased commercial application of this technology. Aquatic animals are being engineered to increase aquaculture production, for medical and industrial research, and for ornamental reasons. While some of these alterations may provide some benefits, the potential effects on human health and the environmental risks that transgenic fish pose to native ecosystems remain unstudied and unknown[1]. Transgenic organisms can be defined as those which have received sequences of DNA by artificial means, followed by integration of one or more transgenic organisms of the novel sequences into their chromosomal DNA.

India has developed experimental transgenics of rohu fish, zebrafish, cat fish and singhi fish. Genes, promoters and vectors of indigenous origin are now available for only two species namely rohu and singhi for engineering growth. Transgenic rohu recently produced from indigenous construct at Madurai Kamaraj University (MKU) has proved to be eight times larger than the control siblings. This transgenic rohu attains 46 to 49 g body weight within 36 weeks of its birth. In India, research in transgenic fish was initiated in MKU, Centre for Cellular and Molecular Biology, Hyderabad and National Matha College, Kollam with borrowed constructs from foreign scientists. The first Indian transgenic fish was generated in MKU in 1991 using borrowed constructs. Taking the research further to promote the transgenic fish programme, the Indian Council of Agricultural Research plans to develop autotransgenesis in commercially important fish species with growth hormone gene[2]. Fishes not only play an important role in the demand of food for humans but they have also emerged as major model organisms for different biomedical researches[3]. The zebrafish (*Danio rerio*) is very popular freshwater tropical fish, which has emerged as a major model organism for biomedical research, especially in developmental genetics, neurophysiology, oncology and biomedicine[3-4]. A number of experiments and the use of drugs have been performed in fish. Therefore, fish may be used as model organism in the experimental pharmacology and toxicology[5].

With increasing numbers of synthetic chemicals introduced into the environment each year, concerns remain regarding our understanding of the linkages between exposure to toxic agents and potential disease. Although health effects associated with exposure to most chemicals are thought to be benign, actual risks posed by a majority of substances, particularly those entering ground water, streams, rivers, estuaries and other aquatic systems, are largely unknown. Chemical contaminants of aquatic environments is of significant concern because although it is understood that aquatic systems serve as major conduits for distribution and deposition of many toxic agents, relatively few methods are available that provide sufficient sensitivity, accuracy and practicality necessary for routine assessment of chemical toxicity. As a consequence, new approaches are needed to improve the assessment of health risks associated with exposure to chemical contaminants in the aquatic environments[6].

The development of transgenic animal models represents a revolutionary advance in the study of a variety of disease processes[7]. Using introduced genes, rodent genomes are routinely modified to express novel gene products or to over express endogenous gene products. Endogenous genes, such as oncogenes or tumour suppressor genes, can be selectively inactivated or knocked out to produce animals that do not express normal amounts of a specific gene product. Beginning in the late 1980s, researchers began to use genetically modified mice in toxicological studies of chemical carcinogenesis and in vivo mutagenesis. Since then, the emphasis has continued to be on developing transgenic

models to improve and expedite carcinogenicity testing[8]. Development of new transgenic rodent models for measuring other endpoints, such as xenobiotic metabolism and xenobiotic ligand/receptor interactions, is expanding[9].

Considering the achievements of transgenic rodent models, it is reasoned that the development of transgenic fish could enhance the utilization of fish as indicators of chemical exposure and as non-mammalian animal models in comparative biology. Fish have played significant roles in monitoring and assessing risks of exposure to chemicals in aquatic environments[6]. In some applications, e.g., assessment of toxicity associated with exposure to complex chemical mixtures or in low-dose chronic exposure regimens, fish are recognized as test organisms with distinct and superior benefits in providing insights to disease processes[10]. Fish are also embraced as cost-effective and important animal models in genetics, developmental biology, and toxicology[11]. Development of transgenic fish has been envisioned as a means to enhance the utility of fish models in reducing, refining or replacing selected mammals used in toxicity testing[6].

In the present review, the importance and use of transgenic fish for studies of environmental toxicology has been discussed. Many advantages of fish as animal models have been highlighted, and through a transgenic fish model developed for in vivo mutagenesis, important issues have been illustrated that apply widely to the use of transgenic fish in environmental toxicology.

MODELS OF TRANSGENIC FISH AND THEIR DEVELOPMENT

Scientists have generated numerous transgenic fish using a variety of species and transgenes since the first transgenic fish were introduced in 1985. Despite wide recognition of fish as sensitive subjects for chemical toxicity testing and as valuable comparative animal models, the field of environmental toxicology has only recently begun to benefit from transgenic technology. The emphasis for transgenic fish research has been on refining transgenic methodology or developing novel strains of fish with commercially beneficial traits. However, recent developments indicate that transgenic fish are promising as new research animal models[6]. To expand the utility of fish as environmental sentinel organisms (i.e., as indicators of the presence or biological impact of a hazardous substance), transgenic fish have been envisioned carrying reporter genes driven by promoters that are responsive to chemical exposure. In a generalized conceptual approach, the transgenic fish would be placed in the water containing the chemical to be tested. Following uptake, distribution, and accumulation of the substance in fish tissues, the genomically integrated response elements would be activated and the reporter genes would be upregulated. The fish would be removed from the water and assayed for reporter gene activity that would be proportional to the concentration of the chemical to which the fish had been exposed. Using this approach, various transgenic fish have been conceived or are at early stages of development. Examples include fish carrying metal-responsive or heat-shock promoters spliced into green fluorescent or LUC reporter genes to provide an in vivo indicator of exposure to heavy metals or other pollutants[12]. As more groups gain experience using a variety of methods, such as insulating border elements to stabilize the expression of transgenes, it is anticipated that new fish models with improved transgene expression will be introduced. A promising example is demonstrated by a transgenic fish assay recently introduced to determine the effects of estrogenic chemicals at critical life stages on sensitive target organs in fish[6]. An oestrogen binding sequence linked to a TATA box and a luciferase reporter gene in zebrafish (*D. rerio*) was introduced. Binding of the chemical substance to endogenous oestrogen receptors and subsequent transactivation of the oestrogen receptors induced the luciferase gene as measured in tissue lysate. The study revealed that the period of gonad differentiation was highly sensitive in juvenile fish exposed to oestradiol. In adult males, the testis was the target tissue most sensitive and responsive to oestrogens[13].

Researchers have developed mutation assays that are not reliant on the expression of a transgene in the animal to assess potential DNA damage after environmental chemical exposure. Using procedures similar to that developed for rodents, scientists also produced several transgenic fish models which carry prokaryotic vectors harboring specific genes that serve as targets for quantifying spontaneous and induced in vivo mutations. The common approach to analyzing mutations in transgenic rodent and fish models entails treating the animals with a chemical and allowing sufficient time for manifestation of the mutations. Genomic DNA is then isolated from various tissues, and the vectors are separated and recovered upon transfer into specialized indicator bacteria, where the mutant and non-mutant target genes are readily distinguished and quantified[6]. Transgenic mutation assays provide numerous benefits for analyzing mutations in vivo not available using other approaches. A significant challenge to detecting mutations in whole animals is the practical problem of recovering and identifying mutant genes. The need to detect mutations at very low frequencies demands highly efficient means of recovering and distinguishing mutant genes among a very large number of non-mutant genes. Transgenic mutation assays afford efficient recovery and screening of large numbers of copies of a transgene target, providing statistically meaningful results as well as reducing the numbers of animals required in a chemical treatment[14].

Transgenic mutation assays facilitate comparisons of mutational responses among identical loci recovered from different cells, tissues, organs and species. In contrast to assays based on endogenous genes in which mutations are detected only in specific developmental stages or specific tissues, mutations in transgene targets can be examined in virtually any tissue from which DNA may be isolated. Mutation analyses can also be combined with measures of other endpoints, such as sequencing of specific mutations to aid in disclosing possible mechanisms of mutagen action. The utilization of transgenic mutation models in a broad range of comparative analyses is improving our understanding of the influences of cell proliferation, metabolism, toxicity, and DNA repair in mutagenesis[15]. Before the advent of in vivo assays with the capability of detecting mutations directly in transgenic targets, researchers relied on genetic toxicity assays that focused on endpoints other than mutation. Among these endpoints, researchers have used induction of DNA adducts, DNA repair, DNA strand breakage, and chromosomal damage as indices of genotoxicity in vivo in fish. These analyses have provided valuable information regarding exposure of organisms to genotoxic agents and indirect information on alterations of DNA or chromosome structure induced by a chemical. However, these methods have limited sensitivity in detecting mutations at the level of the DNA, the ultimate endpoint of DNA damage and/or attempted repair[6]. Mutations in

transgenic assays are detected in genetically neutral targets thereby avoiding potential selective pressures on the mutant frequency in vivo and allowing the accumulation and persistence of mutations[16].

As a consequence of the accumulation of mutations in these loci over time, repeated or chronic chemical treatments will increase the sensitivity of the mutation assay. The amenability of fish to a wide range of chemical treatment regimens indicates that fish will be well suited to mutation studies using treatments that closely approximate environmental exposure conditions. Although development of new transgenic fish models has increased steadily in recent years, transgenic fish are not currently being produced as routinely as rodents. Production and perpetuation of lineages of transgenic fish remain a time-consuming process requiring combinations of specialized personnel, equipment and facilities, which are not universally available in research laboratories. Procedures and facilities for developing and maintaining the transgenic fish are nevertheless undergoing continual improvement to increase the efficiency of this process. Selection of a fish species for development of a transgenic model is dictated by whether the species has certain characteristics, which will enable efficient production of the transgenic fish that can be used to address fundamental research questions. Small laboratory aquaria fish species such as medaka, mummichog and zebrafish share numerous desirable traits for transgenic development and environmental toxicology. Small size, short generation time and cost-effective husbandry contribute to efficient transgenic development and aquarium-based laboratory culture. Well-described embryology, controlled year-round spawning, transparent chorion, and short embryogenesis and generation time are advantageous for transgenic production. Medaka fish has several characteristics, which are especially well suited for environmental toxicology, including well-characterized histopathology[6]. They have been used extensively in chemical hazard testing, carcinogenesis bioassays[17] and germ cell mutagenesis studies[18]. As a common inhabitant of coastal environments, the mummichog is one of the most extensively characterized and utilized organisms in studies of marine environmental contaminant risk[6].

Transgenic fish are produced through the introduction of foreign DNA sequences into zygotes or embryos resulting in the genomic integration of the DNA in a stable and heritable manner. Manual injection of DNA using drawn capillary needles via the cytoplasm of newly fertilized eggs remains the most commonly used method of introducing DNA into the fish genome[19]. A variety of methods have also been used, including micro-injection of the germinal vesicle[20], electroporation of embryos[21], or sperm[22], retroviral infection[23] and particle gun bombardment[24]. Additional methods will likely be introduced in the near future to exploit the genetic diversity offered by fish as a group more fully.

ESTABLISHING AND MAINTAINING TRANSGENIC FISH LINEAGES

Transmission of a transgene through the germ line is a requisite for most transgenic animal studies. Whereas the process of gene transfer can be technically challenging, the identification of transgenic founders, performance of appropriate crosses and perpetuation of desired lineages are not technically difficult. However, these tasks can burden resources and personnel. Mosaic integration of a transgene in founder animals (i.e., integration in only a portion of the animal's cells) is the rule in transgenic fish. Although the presence of the transgene may be demonstrated in excised fin tissue, the transgene is often not transmitted, or is transmitted only at a low frequency to the next generation. In addition, the need to identify transgenic fish that meet specific experimental requirements, places additional demands on the process of establishing lineages. Problems may arise relating to integration or function of a transgene, or the animal may have poor reproduction or an undesirable phenotype thereby reducing the utility of the lineage. Consequently, it is advisable to establish multiple lineages to allow for these differences. Fish researchers, being most familiar with the requirements for care, handling and maintenance of laboratory fish populations, are typically the primary animal care providers. Similarly, other researchers may recognize the value of fish as models and may want to pursue the production of transgenic fish but may not have the necessary experience with fish husbandry or transgenic fish technology. To assist in promoting optimal use of fish models and to bring standards, practices and facilities for care and utilization of fish up to that of mammalian models, various improvements are needed in aquatic animal resource programmes. In particular, improvements in training of personnel in fish care and transgenic techniques, increased research on diet, disease diagnosis and prevention, and increased institutional support of aquatic research programmes will aid in expanding and developing new models and ensure continuity in long-term care. It may be argued that most small aquaria fish species are especially dependent on the laboratory environment for survival. For most transgenic models developed for applications in environmental toxicology, fish would conceivably carry new genes that are either neutral or deleterious to the animal's health. Nonetheless, it is advisable that procedures and facilities used to maintain transgenic fish be reviewed in accordance with guidelines proposed for the development, care and use of genetically modified fish and shellfish to ensure that precautions are taken to safeguard against releases of transgenic fish into the environment[6].

USE OF TRANSGENIC FISH IN ENVIRONMENTAL TOXICOLOGY

Transgenic animal models hold significant promise for enhancing studies on the genetic and molecular basis of disease and for improving the scientific basis for environmental health risk assessment. Studies from transgenic fish can yield reliable and reproducible data, using precisely defined toxicological endpoints while reducing costs and numbers of animals. By taking full advantage of the amenability of fish to a variety of manipulations, ranging from microinjection of embryos[25] to static-renewal and flow-through chronic exposures[26], transgenic fish can improve the assessment of realistic risks related to exposure to waterborne and sediment-associated contaminants. As the utilization of transgenic fish models intensifies, it is anticipated that transgenic fish will contribute significantly to emerging issues related to environmentally induced, reproductive, developmental and heritable diseases for which such studies in mammalian models are exceedingly difficult[6].

The attraction of transgenic fish models is moderated by the uncertainty of how these new models will be applied most effectively in environmental toxicology. Limited availability of lineages of fish beyond laboratories from which they were generated and limited databases on responses under a variety of test conditions will potentially slow wide adoption by researchers. For each new model and application, it will be necessary to resolve complex issues related to establishing optimal study designs, such as appropriate exposure

regimens, chemical concentrations, routes and duration of exposures, and number of animals required for a test. In addition, requirements for reproducibility of responses in individual test animals will demand rigid husbandry practices, many of which are not standardized among different laboratories, to reduce variability in culture conditions. Guidance in addressing some of these issues may be obtained from following examples provided by application of transgenic rodent models in toxicology[6,9].

CONCLUSION

In conclusion, the increasing number of substances introduced into the environment each year, combined with the prevalent need for improved methods to assess potential adverse health effects, will be an impetus to continue to develop new transgenic fish models. India has developed experimental transgenics of rohu fish, zebra fish, cat fish and singhi fish, etc. The transgenic fish can give the significant contribution to study the environmental toxicity in animals.

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