

Transgenic Fish as Models in Environmental Toxicology

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Abstract

Historically, fish have played significant roles in assessing potential risks associated with exposure to chemical contamination in aquatic environments. Considering the contributions of transgenic rodent models to biomedicine, it is reasoned that the development of transgenic fish could enhance the role of fish in environmental toxicology. Application of transgenic fish in environmental studies remains at an early stage, but recent introduction of new models and methods demonstrates progress. Rapid advances are most evident in the area of in vivo mutagenesis using fish carrying transgenes that serve as recoverable mutational targets. These models highlight many advantages afforded by fish as models and illustrate important issues that apply broadly to transgenic fish in environmental toxicology. Development of fish models carrying identical transgenes to those found in rodents is beneficial and has revealed that numerous aspects of in vivo mutagenesis are similar between the two classes of vertebrates. Researchers have revealed that fish exhibit frequencies of spontaneous mutations similar to rodents and respond to mutagen exposure consistent with known mutagenic mechanisms. Results have demonstrated the feasibility of in vivo mutation analyses using transgenic fish and have illustrated their potential value as a comparative animal model. Challenges to development and application of transgenic fish relate to the needs for improved efficiencies in transgenic technology and in aspects of fish husbandry and use. By taking advantage of the valuable and unique attributes of fish as test organisms, it is anticipated that transgenic fish will make significant contributions to studies of environmentally induced diseases.

Key Words: *Fundulus*; lambda; medaka; plasmid; transgenic

Introduction

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 dis-

ease. 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 contamination 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 environment.

The development of transgenic animal models represents a revolutionary advance in the study of a variety of disease processes (Pinkert 1997). Using introduced genes, rodent genomes are routinely modified to express novel gene products or to overexpress endogenous gene products. Endogenous genes, such as oncogenes or tumor 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 (reviewed by Mahler 2000). Since then, the emphasis has continued to be on developing transgenic models to improve and expedite carcinogenicity testing (Battalora and Tennant 1999; Friedberg et al. 1998). Development of new transgenic rodent models for measuring other endpoints, such as xenobiotic metabolism and xenobiotic ligand/receptor interactions, is expanding (Mahler 2000).

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 nonmammalian animal models in comparative biology. Fish have played historically significant roles in monitoring and assessing risks of exposure to chemicals in aquatic environments. In some applications, such as 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 (Powers 1989). Fish are also embraced as cost-effective and important animal models in genetics, developmental biology, and toxicology (Hawkins et al. 1995; Mullins et al. 1994; Solnica-Kretzel et al. 1994). Development of transgenic fish has been

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envisioned as a means to enhance the utility of fish models in reducing, refining, or replacing selected mammals used in toxicity testing.

In the following review, progress in the development of transgenic fish for studies of environmental toxicology is evaluated. Many advantages of fish as animal models are highlighted, and through an example of a transgenic fish model developed for *in vivo* mutagenesis, important issues are illustrated that apply widely to the use of transgenic fish in environmental toxicology.

Transgenic Fish Models

Scientists have generated numerous transgenic fish using a variety of species and transgenes since the first transgenic fish were introduced in 1985 (reviewed by Maclean 1998). 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.

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 (Carvan et al. 2000; Gibbs et al. 1994; Maclean 1998).

Numerous groups have reported difficulties with sustaining gene expression in transgenic lineages, which has slowed the introduction of new transgenic fish. Expression of foreign DNA is commonly observed in the founder fish, but expression is rare or unpredictable in subsequent generations (Chen and Powers 1990; Fletcher and Davies 1991; Hackett 1993; Iyengar et al. 1996). Suboptimal expression may result from various factors including the use of heterologous transcription units derived from nonfish species (Foecking and Hofstetter 1986; Gorman et al. 1982a,b), from methylation of CpG dinucleotides (Boyes and Bird 1991; Ehrlich et al.

1990), from incorporation of multiple transgenes into a single site (Amsterdam et al. 1995), or from integration of transgenes in loci susceptible to gene silencing (Eissenberg and Elgin 1991). As more groups gain experience using a variety of methods, such as insulating border elements to stabilize the expression of transgenes (Stief et al. 1989; Udvardy et al. 1985), 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. Legler and colleagues (2000) introduced an estrogen binding sequence linked to a TATA box and a luciferase reporter gene in zebrafish (*Danio rerio*). Binding of the chemical substance to endogenous estrogen receptors and subsequent transactivation of the estrogen 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 estradiol. In adult males, the testis was the target tissue most sensitive and responsive to estrogens.

Transgenic Fish Mutation Models

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 the ones Mirsalis and colleagues (1995) developed for rodents, Amanuma and colleagues (2000) and scientists in my laboratory (Winn et al. 1995, 2000, 2001) produced several transgenic fish models that carry prokaryotic vectors harboring specific genes, which 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 nonmutant target genes are readily distinguished and quantified (Gossen et al. 1989; Summer et al. 1989).

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 (e.g., ~ 1 spontaneous mutation/ 10^5 – 10^7 loci) demands highly efficient means of recovering and distinguishing mutant genes among a very large number of nonmutant 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. Typically, hundreds of thousands of mutation target loci are recovered from as little as 1 to 5 μg of DNA, and as few as six to 10 animals/treatment are required to

detect significant induction of mutations above a background mutation frequency (Boerrigter et al. 1995; Winn et al. 2000).

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 (Bielas and Heddle 2000).

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 (reviewed by Shugart 1995). 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.

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 (Cosentino and Heddle 1996; Swiger et al. 1999; Tao et al. 1993). 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 (Heddle et al. 1995). 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.

Mutation Assays Using a Bacteriophage λ LIZ Vector

Colleagues and I (Winn et al. 2000) produced transgenic medaka (*Oryzias latipes*) that carry multiple copies of the bacteriophage λ LIZ vector, which harbors the *lacI* and *cII* bacterial genes as mutational targets (Winn et al. 2000). The most widely used transgenic rodent mutation assay is based on this bacteriophage vector (Kohler et al. 1991b). By adapting a mutagenesis system originally developed for rodents to fish, the process of transgenic fish model development was made more efficient and increased the potential utility of the fish model for comparative mutagenesis studies. The two

mutational target transgenes, *lacI* and *cII*, contained in the λ LIZ transgenic animals are analyzed using different assay procedures.

lacI Mutation Assay

Colleagues and I (Winn et al. 2000) produced a transgenic medaka lineage that carries ~75 copies/haploid genome of the bacteriophage λ LIZ vector (~45 Kb). Shih and colleagues (2001) recovered mutations in the *lacI* gene (1089 bp) from transgenic medaka using procedures adapted for the transgenic rodent *in vivo* mutation assay (Kohler et al. 1991a). In this approach, genomic DNA is mixed with *in vitro* packaging extracts, which excise the intact λ LIZ vector sequence from the animal's genomic DNA and simultaneously package the vector into viable bacteriophage. Individually packaged phage are then allowed to infect and lyse an *Escherichia coli* host. Mutations that inactivate the *lacI* target allow expression of the α *lacZ* reporter producing the α portion of β -galactosidase. This protein combines with the remaining portion of the protein produced in the *E. coli* to form a functional β -galactosidase and to form blue plaques on indicator agar containing X-gal (5-bromo-4-chloro-3-indolyl β -D-galactoside). Plaques with nonmutated targets remain colorless. The ratio of blue plaques to the total number of plaques is used as a measure of the mutant frequency. In rodent models, the *lacI* assay has been well characterized, and the mutants collected have been sequenced to produce a mutation spectrum (De Boer et al. 1998). Piergorsch and colleagues (1995) have developed quantitative and statistical analyses for rodent models. Cariello and colleagues (1997) and De Boer (1995) have established databases that contain thousands of *lacI* mutations. The feasibility of analyzing spontaneous and chemically induced mutations in the *lacI* locus recovered from these fish was demonstrated recently (Shih et al. 2001). Frequencies of spontaneous *lacI* mutants in whole fish were comparable with ranges observed in rodent tissues (Dycaico et al. 1994; Kohler et al. 1991b). Shih and colleagues (2001) have recently shown that the *lacI* gene in the fish is highly responsive to chemical mutagen treatment. Fifteen days after the fish were exposed to the model mutagen ethylnitrosourea (ENU¹), the mutant frequency in those fish was significantly greater than in untreated fish. Sequencing of spontaneous and ENU-induced *lacI* mutants revealed mutational spectra from these fish consistent with ENU mutagenesis in rodent studies. This finding confirmed that the *lacI* assay is capable of detecting most classes of mutations, including base substitutions, single-base frame-shifts, insertions, duplications, and deletions (Mirsalis 1993; Provost et al. 1993).

¹Abbreviations used in this article: DMN, dimethylnitrosoamine; ENU, ethylnitrosourea; Kan, kanamycin; Sm, streptomycin.

cII Mutation Assay

The λ LIZ transgenic medaka also carry the *cII* gene, which can be analyzed for mutations. The *cII* mutation assay is a positive-selection assay originally developed for transgenic rodents, which uses the *cII* gene target as a logistically simple and cost-effective alternative to the *lacI* mutation assay (Jakubczak et al. 1996). The assay is based on the role the *cII* protein plays in the commitment of bacteriophage λ to the lysogenic cycle in *E. coli*. Selection of mutant *cII* λ is facilitated using a specialized *E. coli* strain (*hfl*-) that extends the longevity of the *cII* product. After isolation of fish genomic DNA, in vitro packaging procedures similar to those used in the *lacI* assay simultaneously excise and package the vector into viable phage particles. To determine the total number of packaged phage, a subsample of the packaged phage solution is mixed with the *E. coli* cells, mixed with top agar, and incubated on (titer) plates at 37°C overnight. To select *cII* mutants, the packaged phage are mixed with *E. coli* host cells, plated, and incubated at 24°C for 40 hr. The phage with wild-type *cII* produce lysogens that are indistinguishable in the *E. coli* lawn, whereas phage that carry a mutation in *cII* form plaques on the bacterial lawn when incubated at 24°C. The mutant frequencies are calculated by dividing the total number of *cII* mutant plaque-forming units on the selective mutant screening plates by the estimated total λ + and *cII* phage on the titer plates.

Results from fish (Winn et al. 2000) and rodent studies (Gollapudi et al. 1998; Harbach et al. 1998; Watson et al. 1998; Zimmer et al. 1999) support the continued use of *cII* locus as a target for mutations as an acceptable alternative to *lacI*. Spontaneous mutant frequencies in the identical *cII* locus from fish and rodents were in a similar range of 2 to 8×10^{-5} . Colleagues and I (Winn et al. 2000, 2001) have recovered *cII* mutants with high efficiency from a variety of fish tissues, including whole fish, liver, testes, eyes, whole blood, and skin. This ability to compare mutations among tissues provides an important tool for understanding differential responses to mutagens. Treatment with ENU resulted in concentration-dependent, tissue-specific, and time-dependent mutation inductions consistent with the known action of this mutagen. The mutagen exposure studies illustrated the importance of mutation manifestation time, or the interval between mutagen treatment and analyses. The time required for mutations to manifest is affected by several variables, including tissue/cell type, mutagen, and mutagen treatment regimen, which must be considered in designing and interpreting mutation studies (Hara et al. 1999; Sun et al. 1999; Walker et al. 1999). Colleagues and I (Winn et al. 2000) showed in the fish that frequencies of mutants in liver do not increase significantly 5 days after ENU exposure but instead, increase progressively to more than six-fold above background by 30 days. In contrast, we showed that mutants in the testes are induced significantly 5 days after ENU exposure and attain a 10-fold induction peak within 15 days. In other studies in my laboratory (Winn et al. 2001), we exposed fish to dimethylnitrosoamine (DMN¹), a potent liver carcinogen that requires

cell proliferation for DMN-produced methyl DNA adducts to become fixed as mutations (Mirsalis et al. 1993). The mutant induction observed in the DMN-exposed fish (Winn et al. 2001) supports results from recent studies that demonstrated cell proliferation is a requisite for DNA repair and mutation (Bielas and Heddle 2000).

An added benefit of the *cII* mutation analyses is the small size of the *cII* target (296 basepairs vs. 1080 basepairs for *lacI*), which facilitates efficient characterization of the mutations by directly sequencing the entire gene. Sequencing of the *cII* gene revealed similar spontaneous and chemically induced mutational spectra in fish and rodents, with different single base substitutions comprising the majority of mutations and reflecting different modes of action of ENU and DMN.

Bacteriophage ϕ X174 Assay

Colleagues and I (Winn et al. 1995) developed a transgenic fish mutation assay based on a bacteriophage vector using medaka (*Oryzias latipes*) and mummichog (*Fundulus heteroclitus*) carrying the bacteriophage ϕ X174*am3cs70* vector (Winn et al. 1995). Using the system that Burkhart and colleagues (1993) had originally developed in rodents, we detected mutations by reversion of *am3* to wild-type phage by one transition and two transversions of a single A:T base pair. We were able to show that the spontaneous mutant frequencies in the fish are comparable with those of transgenic mice carrying the identical vector. This mutation assay has not had wide use; however, results of the study demonstrated the feasibility of generating transgenic mummichog, which should encourage expanded development of transgenic marine species as models for environmental studies.

Mutation Assays Using Plasmid Vectors

rpsL Mutation Assay

Amanuma and colleagues (2000) recently adapted to zebrafish a mouse mutation assay based on the pML4 plasmid vector. The pML4 vector contains the *rpsL* gene as a mutational target that converts kanamycin (Kan^r)-sensitive and streptomycin (Sm^r)-resistant host *E. coli* cells to Kan-resistant and Sm-sensitive, respectively. When a mutation occurs in the *rpsL* transgene, *E. coli* host cells remain streptomycin resistant, thereby resulting in Kan- and Sm-resistant colonies. Amanuma and colleagues (2000) showed that the spontaneous mutant frequency in the zebrafish is similar to transgenic mice containing the same target, and they observed a dose-dependent induction of mutants after treatment of fish embryos with ENU. Sequencing of the recovered mutants revealed a mutation spectrum that was in agreement with known mechanisms of ENU mutagenesis. Treatment of embryos with two mutagenic compounds, benzo[a]pyrene or 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, which are

often present in contaminated water, also induced mutations in the *rspL* target gene.

***lacZ* Mutation Assay**

Another plasmid-based mutation system adapted from a rodent mutation assay (Boerrigter et al. 1995) is in early stages of development. The mutation assay is based on transgenic medaka and mummichog, which carry the plasmid pUR288 vector harboring the *lacZ* gene (3096 bp) as a mutation target (Winn et al. 2001). After exposure of the animal to a mutagen, genomic DNA is isolated and digested with a restriction enzyme (*HindIII*) to release monomeric plasmid sequences. The plasmid is then separated from the genomic DNA by affinity capture using magnetic beads. After circularization by ligation, plasmids are transferred into *lacZ*, *galE*-*E. coli* by means of electroporation in the presence of phenyl- β -D-galactoside (Gossen et al. 1992). Results from transgenic rodent studies demonstrate that this assay affords a significant advantage over other approaches in the ability to detect point mutations, small deletions, and insertions, as well as large-scale deletions and rearrangements induced by clastogenic agents such as radiation (Boerrigter 1998). Analyses reveal that the plasmid vector can be recovered from fish tissues with exceptional efficiency and that the spontaneous mutant frequency in the fish is similar to that of the transgenic rodent carrying the identical transgene. Further optimization of assay procedures for this transgenic fish model is under way.

Transgenic Fish Development and Care

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 that are not universally available in research laboratories. Procedures and facilities for developing and maintaining transgenic fish are nevertheless undergoing continual improvements to increase the efficiency of the process.

Choice of Species

Selection of a fish species for development of a transgenic model is dictated by whether the species has certain characteristics that 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 has several characteristics that are especially well suited for environmental toxicology, including well-characterized histopathology. They have been used extensively in chemical hazard testing, carcinogenesis bioassays (Bunton 1999), and germ cell mutagenesis studies (Shima and Shimada 1994). 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 (Atz 1986; Eisler 1986) and ecotoxicology (Lotrich 1975; Vogelbein et al. 1990).

Gene Transfer

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 (Chourrout et al. 1986; Maclean et al. 1987; Winn et al. 1995; Zhu et al. 1985). A variety of methods have also been used, including microinjection of the germinal vesicle (Ozato et al. 1986), electroporation of embryos (Inoue et al. 1990; Powers et al. 1992) or sperm (Muller et al. 1992), retroviral infection (Gaiano et al. 1996; Lin et al. 1994), and particle gun bombardment (Kolenikov et al. 1990). 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. Unfortunately, fish culture facilities are generally not integrated into larger institution-wide animal resource programs. As a result, fish researchers are often solely responsible for supporting their program and meeting the demands associated with long-term perpetuation of wild-type and transgenic lineages. These burdens can ultimately hamper production of new fish models and limit the availability of established lineages of animals for use by other researchers. 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, further impeding introduction of new fish models. 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 programs. 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 programs will aid in expanding and developing new models and ensure continuity in long-term care.

Before embarking on the development of transgenic fish, researchers are advised to address issues regarding assessment of the potential risks associated with environmental release of transgenic fish. 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 (USDA 1995) to ensure that precautions are taken to safeguard against releases of transgenic fish into the environment.

Application 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 (Walker et al. 1996) to static-renewal and flow-through chronic exposures (Kane et al. 1996), 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.

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 (Gulezian et al. 2000; Heddle et al. 2000; Mahler 2000).

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. The speed with which these new models can be developed and the extent to which they will be adopted will be influenced largely by advances in transgenic technology and improvements in fish care and use.

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