in vivo *24*: 457-462 (2010)

Review

In Vivo Research Using Early Life Stage Models

RITA SEABRA and NIRMALA BHOGAL

FRAME, 96-98 North Sherwood Street, Nottingham, NG1 4EE, U.K.

Abstract. *Scientists, for a variety of reasons, need to carry out in vivo research. Since experiments that require the use of adult animals pose various logistical, economical and ethical issues, the use of embryonic and larval forms of some organisms are potentially attractive alternatives. Early life stages are appealing because, in general, large numbers of individuals can be maintained in relatively simple housing, minimising costs, and their use involves fewer legal formalities. With this succinct review, we aim to provide an overview of different biological issues that have been successfully explored with the help of eggs, embryos and larvae from the frog, zebrafish and chicken.*

Eggs, embryos and larvae offer several advantages compared to adult animals as research models. The early life stages of three animals, the African clawed frog, the chicken and the zebrafish, are widely used as models during their early developmental stages and represent valuable assets to both fundamental and applied research. Their small size simplifies housing and the experimental apparatus requirements. Furthermore, the adults of these species tend to produce many offspring, which develop externally and relatively fast, simplifying the replenishment of egg, embryo and larval stocks. These traits, plus the fact that animal regulations are less strict (or non-applicable) for such specific developmental stages, make these *in vivo* models appealing to scientists. Collectively, eggs, embryos and larvae of these species have been successfully used in various research areas, including developmental biology, physiology, and toxicology, and have the potential to yield information of relevance to human health. Here, we give a brief account of how these species have contributed to advances in science.

Correspondence to: Rita Seabra, FRAME, 96-98 North Sherwood Street, Nottingham, NG1 4EE, U.K. e-mail: rita@frame.org.uk

Key Words: Animal experiments, embryos, larvae, replacement, reduction, *Xenopus*, zebrafish, CAM.

Xenopus

The African clawed frog, *Xenopus laevis*, became a popular laboratory animal in the 1950s. It was first used to test for pregnancy hormones but, nowadays, it is a promising model in a range of fields. Upon stimulation, the animals produce eggs all year round, which can be fertilized *in vitro*, and development takes place in medium. Manipulations can be carried out with relative ease because of the large size of the egg and the embryos. This is the case, for example, of tissue grafting and oligonucleotide injections. *X. tropicalis*, a relative of *X. laevis,* with a faster development and a diploid genome, is also frequently used. The availability of molecular biology techniques, which allow alterations in gene expression, has contributed to make the frog a useful vertebrate animal model. With such tools, studying frog organogenesis has contributed to improving the knowledge of the development of certain human organs. The frog larval kidney is a pronephros, which human embryos never have in a functional form but instead progresses to form the metanephric human kidney. *Xenopus*, therefore, is a model in which to investigate genes that play a role in kidney development and which may contribute to kidney disease (1).

Cardiac function can also be studied in frog embryos. In order to gain a better understanding of some heart conditions, it may be advantageous to investigate the embryonic heart, as developmental abnormalities often lead to congenital cardiac disease. For example, the heart conduction system, which is essential for normal function, has been pharmacologically manipulated in *Xenopus* embryos and found to share many characteristics with the human system (2). Furthermore, certain mutations of the gene nkx2-5, which are linked to human congenital heart defects, have been shown to interfere with *Xenopus* heart development (3). For instance, *pdx1*, an essential gene for pancreatic development, was first identified in the frog (4). Tadpoles are also candidates for the study of vertebrate lymph vessel development and for the identification of potential compounds affecting lymphangiogenesis (5). Vessel formation and remodelling occurs in a range of biological events such as cancer dissemination,

inflammation and tissue repair. Being able to modulate angiogenesis according to the clinical situation would be advantageous in medical care. *X. tropicalis* has recently been suggested as a model to study spinal muscular atrophy (SMA), a human degenerative neuromuscular disease. Antisense morpholinos oligonucleotides were used to disrupt the production of survival motor neuron (SMN), a protein that when present at low levels is associated with the disease. Since in the frog embryo reduced amounts of SMN gave rise to muscle defects, this system may prove useful to investigate the human condition (6). Developmental research in these models can, therefore, be of value to understand the mechanistic basis of some human diseases.

Given that organ development can be inspected with relative ease, this organism also plays an important role in toxicology. Indeed, *Xenopus* embryos have been used to investigate the teratogenic effects of ethanol (7). While it is recognised that exposing human embryos to alcohol may lead to individuals with physical and psychological abnormalities, many of the molecular processes affected remain unclear. Thus far, research has shown that ethanol causes comparable phenotypic abnormalities in frog and human embryos, with both species most vulnerable during early embryogenesis (7). In the field of environmental toxicity, *Xenopus* embryos are a promising tool, for instance, they have been included as part of studies evaluating hazardous wastes (8).

One of the main scientific contributions of the *Xenopus* model has been to the area of signal transduction. The suitability of frog oocytes for cellular investigations has made these cells popular for the study of signal transduction mechanisms, particularly of receptors, ion channels and transporters. Mutated ion transporters are implicated in a range of diseases such as cystic fibrosis and congenital hearing impairment. Pendrin is an ion transporter whose mutations are associated with the latter condition (9). By expressing human pendrin on frog oocytes, it has been possible to gain a better understanding of its function and potential role in inner ear physiology (10). Experiments that shed light into the functioning of such proteins and why mutations lead to disease might contribute to the development of potential therapies. *Xenopus* eggs have also been used to study the roles of localized RNAs as structural elements within cells. A suitable model to study the biology of RNA may be useful given that small interfering RNAs (siRNAs) are becoming increasingly important molecular biology tools and are the underlying technology being developed to treat a range of diseases (11).

During a limited period in their larval stage, *Xenopus* are able to regenerate their tails (12). This transition from regenerative to non-regenerative capabilities makes *Xenopus* a suitable model for understanding the mechanisms that underlie tissue repair (13). Retinal degeneration, a common cause of vision loss in humans, has also been studied in these models, which might contribute to the development of new treatments for retinopathies (14).

Chicken

Chicken (*Gallus* species) embryos have been used for decades to study vertebrate embryonic development. The availability of the chicken genome sequence, which has fewer genes than those of other model organisms, has given rise to a number of new opportunities, making these species an attractive choice for research. For example, a large amount of what is known about congenital malformations in humans came from studies using chicken embryos. Chick limb formation has been a heavily researched topic and genes involved in chick embryonic limb development are of relevance to human clinical genetics (15).

Experiments often involve the embryonic avian chorioallantoic membrane (CAM), an extra-embryonal membrane that functions as a respiratory organ for the developing embryo. The CAM has a number of useful qualities, particularly with respect to toxicity testing. Dermatological research and skin toxicity testing can be carried out on chicken eggs. This is possible because human skin grafted onto the CAM maintains its integrity and phenotype (16).

A vascular system, which resembles the one in the human eye, is essential for the CAM's function and the thickness of the human retina and the chick CAM are comparable. These similarities have encouraged the use of this membrane as an alternative to animals in a number of areas of ophthalmic research. It is now used for detecting potential eye irritants (17). An investigation concerning the ideal timing of therapy for wet age-related macular degeneration has recently been carried out using the CAM (18). This *in vivo* system can also be used to develop and test tools and techniques for retinal surgery. The highly vascularized membrane is particularly suitable for assessing the cutting capacity of surgical tools, and to test coagulation, cannulation and injection procedures (19).

For similar reasons, this model is also a promising aid in other surgical areas, for example in research and training for specific endolaryngeal surgery. The treatment of certain vocal cord lesions relies on laser surgery and, using the CAM, it is possible to determine the ideal laser settings to maximize clinical efficacy and minimize tissue damage (20). Unfertilized chicken eggs have been recently used to mimic the human amniotic cavity and to test compounds for their ability to preseal the CAM before a surgical procedure. This simple model might be useful in the development of foetoscopic surgery, as premature membrane rupture is still a threat when such operations are carried out (21). In a recent experiment, chicken embryos were used to mimic the embryonic damage resulting from diabetic pregnancy, as an

alternative to invasive procedures in mammals. The embryos were cultured in a shell-less environment (in glass bowls), which allows visualization of growth and malformations at various stages of embryo development, in the presence of different osmolarities of glucose. Although the reported use of this model was for the detection of glucose-induced malformations, it may be suitable for studying the effects of a range of chemicals from the early stages of embryo development (22).

The CAM model also has applications in the field of cancer research. It has been exploited to determine the role of laminins in extravascular migratory metastasis of melanoma cells. The expression of particular genes was shown to be dependent on the localization of such malignant cells, with overexpressed genes associated with tumour progression and metastasis (23). Given that graft vascularization usually occurs quickly and no immune-mediated rejection occurs, a functional immune system is only present from day 17 (24), various tissues can be successfully grafted onto the CAM. This has allowed the development of several vascularized solid human-derived tumours (25) and the identification of proteases that may thwart metastic progression. In fact, enzymatic activity differed depending on whether the cells were grown *in vitro* (on plastic) or in the CAM as solid tumours, with the latter representing a more realistic model (26). Cancer research using chicken embryos can use a range of tissues. For example, glioma cell lines injected in embryonic chicken brain have been shown to be able to grow into vascularized tumours and migrate. This promising strategy might provide an alternative to some of the currently used xenograft rodent models (27).

Various drugs and formulations can also be screened on the CAM or on CAM-grafted tissues using a few different administration routes (28). The existence of actively proliferating capillaries makes the chicken embryo a suitable *in vivo* model to study angiogenesis. It is possible, for example, to test substances for their anti- or pro-angiogenic effects by intravascular inoculation or topical application onto the CAM (29). This model also shows potential for the study of short-term ovarian tissue transplantation, a necessary step to preserve the fertility of women undergoing some types of cancer treatment. Using the CAM as support for human ovarian tissue grafts, it has been possible to investigate the early steps of graft transplantation (24). Neovascularization is essential for a successful transplant and an understanding of ischaemic damage and of the processes leading to blood vessel formation in grafted tissue is crucial.

Endometriosis, a common gynaecological condition, occurs when endometrial-like tissue grows outside of the uterus. To overcome the limitation of endometriosis only being found in some primate species, scientists have relied on artificially inducing endometriosis by surgically transplanting uterine or endometriotic tissue fragments onto the peritoneal cavity of study animals. The CAM of fertilized eggs has surfaced as an alternative model to study the early phases of this disease. Human endometrial fragments can be grafted onto the CAM and lesions develop within three days (30, 31). Even though this model is not useful for long-term investigations or those assessing immunological or inflammatory events, it can replace animal models for certain experiments.

Animal studies can be avoided by using an optimized *in vivo* CAM model to test biomaterials and implants. The way in which the chicken embryonic membrane responds to materials is similar to the reactions seen in mammalian tissues, making it an option for preliminary screening of biomaterials and devices. In fact, the CAM model is cheaper, quicker and more convenient than animal tests and has potential to accelerate and optimize research in this area (32). Cell-seeded scaffolds, used in tissue engineering research, consist of viable cells within a biodegradable material. When they are implanted, if blood supply is not quickly restored, the cell-scaffold loses viability. The CAM allows a preliminary *in vivo* assessment of implants, particularly regarding neovessel formation, and could therefore enhance progress in this important research area (33, 34).

Zebrafish

Zebrafish (*Danio rerio*) are extensively used in genetic research and to study the role of genes in embryogenesis. Their embryos are small, transparent, develop in freshwater and the egg becomes a free swimming larva within three days of fertilization. These features make the model logistically attractive since, during this time, all the stages of organ development can be observed without the need for invasive *in utero* imaging of embryos and the larvae can be accommodated within the wells of tissue culture multi-well plates, making them amenable for high-throughput phenotype screens. Widely used by developmental biologists, these fish have also been exploited for both fundamental and applied research. Progress in molecular biology techniques has given scientists options regarding forward and reverse mutagenesis of zebrafish. Alternatives include the creation of genetically modified (GM) fish by random or insertional mutagenesis and by altering gene expression with morpholinos or siRNAs, resulting in radical to slight changes in gene expression (35). By coupling expression of a reporter gene (for example, of green fluorescent protein) to the expression of specific developmental genes, such as the GATA-1 promoter (36), the islet-1 neuron-specific promoter (37) or the muscle-specific alpha-actin promoter (38), it is possible to monitor spatial and temporal changes in gene expression relating to haematopoiesis, axonal development and muscle development, respectively. Genetic manipulations may also contribute to elucidation of the molecular mechanisms behind various human disorders. An example is

the use of zebrafish in research aiming to clarify the role of presenilins in the pathogenesis of Alzheimer's disease (39). The *presenilin* genes identified in zebrafish are evolutionary highly conserved genes and share a high degree of sequence and structural similarity to their human counterparts, making them attractive research targets in this fish model (40).

Zebrafish possess a functional pronephric kidney, mainly used for osmoregulation, that is composed of the same cell types as those seen in higher vertebrates. This similarity in cellular and molecular elements gives this simplistic model enormous potential in improving our understanding of the mechanisms underlying the development of kidney disease (41). Polycystic kidney is a common human genetic disease which can lead to renal failure. Insertional mutagenesis screening in zebrafish has identified several genes that contribute to the formation of cystic kidney and shed light onto human genes potentially involved in polycystic kidney (42). Steatohepatitis, a fatty liver disease for which there is no effective cure, has also been modelled in zebrafish. The livers of embryos treated with thioacetamide exhibited histological, biochemical and molecular characteristics similar to those of human non-alcoholic steatohepatitis (43).

The study of blood vessel development is an area of intense research and angiogenesis-affecting drugs are already on the market. Rodents have been the traditional models but the zebrafish has the advantage of being a simpler system. As genetic manipulations are relatively straightforward, the signalling pathways involved in blood vessel development can be investigated and specific genes manipulated. Angiogenesis is an essential element of actively growing tumours and scientists are investigating anti-angiogenic compounds as cancer therapies. Like the embryonic CAM, zebrafish embryos can be recipients of human or mouse cancer cells, with xenografts inducing neo-vascularization (44). The knowledge obtained using fish embryos may lead to the identification of novel therapeutic targets and the model offers the possibility to screen potential angiogenesis-modifying compounds in an *in vivo* vertebrate system.

Anoxia, a condition that occurs when tissues do not receive oxygen, can have drastic consequences on human tissues and also contributes to making cancer cells more resistant to treatment. Zebrafish embryos are capable of tolerating anoxic conditions and, as they develop, the embryos become increasingly sensitive to low oxygen levels (45). This vertebrate animal model may thus be useful to investigate the biochemical pathways involved in protecting cells from anoxia and also the factors that affect the transition between anoxia-tolerant and -sensitive stages. This information may contribute to improve the treatment of diseases where oxygen deprivation plays a role (46).

For toxicological studies, assays with zebrafish embryos may be very useful in predicting potential *in vivo* effects. For example, some human drugs show serious cardio vascular toxicity, which can lead to market withdrawal. Zebrafish may help identifying compounds likely to cause human cardio-vascular toxicity early during drug development (47). At the neurological level, severe defects can be visually detected but certain compounds exert poorly detectable toxic effects. To overcome this, assays exist to monitor defective functions, such as vision (48) and audition (49). Increasingly, nanomaterials are included in products destined for humans, animals and the environment. Assessing the dangers of such miniscule components is a challenge for toxicologists – the small size of nanoparticles often causes their physicochemical properties to differ from those of their constituent chemicals. The need for additional animal studies could be reduced by using zebrafish embryos, an already established toxicology model (50). This fish model may also be useful to examine the mechanisms by which microbial toxins and virulence factors, many of which still have unidentified *in vivo* targets, exert their biological activity (51).

Summary

Due to conserved developmental and physiological processes among species, lower animal models can provide insights into the development and progression of certain human diseases. The definition of an ideal animal model is a controversial one and depends on a plethora of factors. In many cases, early life stage models can mimic human biological events and yield a great deal of relevant data without the need to resort to experiments on adult animals or mammals.

References

1 Jones EA: *Xenopus*: a prince among models for pronephric kidney development. J Am Soc Nephrol *16*: 313-321, 2005.

2 Bartlett HL, Scholz TD, Lamb FS and Weeks DL: Characterization of embryonic cardiac pacemaker and atrioventricular conduction physiology in *Xenopus laevis* using noninvasive imaging. Am J Physiol Heart Circ Physiol *286*: 2035-2041, 2004.

3 Bartlett HL, Sutherland L, Kolker SJ, Welp C, Tajchman U, Desmarais V and Weeks DL: Transient early embryonic expression of Nkx2-5 mutations linked to congenital heart defects in human causes heart defects in *Xenopus laevis*. Dev Dyn *236*: 2475-2484, 2007.

4 Wright CV, Schnegelsberg P and De Robertis EM: XlHbox 8: a novel *Xenopus* homeo protein restricted to a narrow band of endoderm. Development *105*: 787-794, 1989.

5 Ny A, Koch M, Schneider M, Neven E, Tong RT, Maity S, Fischer C, Plaisance S, Lambrechts D, Héligon C, Terclavers S, Ciesiolka M, Kälin R, Man WY, Senn I, Wyns S, Lupu F, Brändli A, Vleminckx K, Collen D, Dewerchin M, Conway EM, Moons L, Jain RK and Carmeliet P: A genetic *Xenopus laevis* tadpole model to study lymphangiogenesis. Nat Med *11*: 998-1004, 2005.

6 Ymlahi-Ouazzani Q, Bronchain OJ, Paillard E, Ballagny C, Chesneau A, Jadaud A, Mazabraud A and Pollet N: Reduced levels of survival motor neuron protein leads to aberrant motoneuron growth in a *Xenopus* model of muscular atrophy. Neurogenetics *11*: 27-40, 2010.

7 Yelin R, Kot H, Yelin D and Fainsod A: Early molecular effects of ethanol during vertebrate embryogenesis. Differentiation *75*: 393-403, 2007.

8 Pablos MV, Fernández C, del Mar Babín M, María Navas J, Carbonell G, Martini F, García-Hortigüela P and Vicente Tarazona J: Use of a novel battery of bioassays for the biological characterisation of hazardous wastes. Ecotoxicol Environ Saf *72*: 1594-1600, 2009.

9 Glaser B: Pendred syndrome. Pediatr Endocrinol Rev *1*: 199-204, 2003.

10 Scott DA and Karniski LP: Human pendrin expressed in *Xenopus laevis* oocytes mediates chloride/formate exchange. Am J Physiol Cell Physiol *278*: 207-211, 2000.

11 Kloc M: Teachings from the egg: New and unexpected functions of RNAs. Mol Reprod Dev *76*: 922-932, 2009.

12 Beck CW, Christen B and Slack JM: Molecular pathways needed for regeneration of spinal cord and muscle in a vertebrate. Dev Cell *5*: 429-439, 2003.

13 Yokoyama H: Initiation of limb regeneration: the critical steps for regenerative capacity. Dev Growth Differ *50*: 109-120, 2008.

14 Vergara MN and Del Rio-Tsonis K: Retinal regeneration in the *Xenopus laevis* tadpole: a new model system. Mol Vis *15*: 1000-1013, 2009.

15 Davey MG and Tickle C: The chicken as a model for embryonic development; Cytogenet Genome Res *117*: 231-239, 2007.

16 Kunzi-Rapp K, Rück A and Kaufmann R: Characterization of the chick chorioallantoic membrane model as a short-term *in vivo* system for human skin. Arch Dermatol Res *291*: 290-295, 1999.

17 Luepke NP: Hen's egg chorioallantoic membrane test for irritation potential. Food Chem Toxicol *23*: 287-291, 1985.

18 Debefve E, Pegaz B, Ballini JP and van den Bergh H: Combination therapy using verteporfin and ranibizumab; optimizing the timing in the CAM model. Photochem Photobiol *85*: 1400-1408, 2009.

19 Leng T, Miller JM, Bilbao KV, Palanker DV, Huie P and Blumenkranz MS: The chick chorioallantoic membrane as a model tissue for surgical retinal research and simulation. Retina *24*: 427-434, 2004.

20 Broadhurst MS, Kobler JB, Burns JA, Anderson RR and Zeitels SM: Chick chorioallantoic membrane as a model for simulating human true vocal folds. Ann Otol Rhinol Laryngol *116*: 917-921, 2007.

21 Carnaghan HK and Harrison MR: Presealing of the chorioamniotic membranes prior to fetoscopic surgery: preliminary study with unfertilized chicken egg models. Eur J Obstet Gynecol Reprod Biol *144S*: S142-S145, 2009.

22 Datar S and Bhonde RR: Shell-less chick embryo culture as an alternative *in vitro* model to investigate glucose-induced malformations in mammalian embryos. Rev Diabet Stud *2*: 221-227, 2005.

23 Lugassy C, Torres-Muñoz JE, Kleinman HK, Ghanem G, Vernon S and Barnhill RL: Overexpression of malignancy-associated laminins and laminin receptors by angiotropic human melanoma cells in a chick chorioallantoic membrane model. J Cutan Pathol *36*: 1237-1247, 2009.

24 Martinez-Madrid B, Donnez J, Van Eyck AS, Veiga-Lopez A, Dolmans MM and Van Langendonckt A: Chick embryo chorioallantoic membrane (CAM) model: a useful tool to study short-term transplantation of cryopreserved human ovarian tissue. Fertil Steril *91*: 285-292, 2009.

25 Kunzi-Rapp K, Genze F, Küfer R, Reich E, Hautmann RE and Gschwend JE: Chorioallantoic membrane assay: vascularized 3-dimensional cell culture system for human prostate cancer cells as an animal substitute model. J Urol *166*: 1502-1507, 2001.

26 Bérubé M, Deschambeault A, Boucher M, Germain L, Petitclerc E and Guérin SL: MMP-2 expression in uveal melanoma: differential activation status dictated by the cellular environment. Mol Vis *11*: 1101-1111, 2005.

27 Cretu A, Fotos JS, Little BW and Galileo DS: Human and rat glioma growth, invasion, and vascularization in a novel chick embryo brain tumor model. Clin Exp Metastasis *22*: 225-236, 2005.

28 Vargas A, Zeisser-Labouèbe M, Lange N, Gurny R and Delie F: The chick embryo and its chorioallantoic membrane (CAM) for the *in vivo* evaluation of drug delivery systems. Adv Drug Deliv Rev *59*: 1162-1176, 2007.

29 Ribatti D, Gualandris A, Bastaki M, Vacca A, Iurlaro M, Roncali L and Presta M: New model for the study of angiogenesis and antiangiogenesis in the chick embryo chorioallantoic membrane: the gelatin sponge/chorioallantoic membrane assay. J Vasc Res *34*: 455-463, 1997.

30 Malik E, Meyhöfer-Malik A, Berg C, Böhm W, Kunzi-Rapp K, Diedrich K and Rück A: Fluorescence diagnosis of endometriosis on the chorioallantoic membrane using 5-aminolaevulinic acid. Hum Reprod *15*: 584-588, 2000.

31 Maas JW, Groothuis PG, Dunselman GA, de Goeij AF, Struijker-Boudier HA and Evers JL: Development of endometriosis-like lesions after transplantation of human endometrial fragments onto the chick embryo chorioallantoic membrane. Hum Reprod *16*: 627-631, 2001.

32 Klueh U, Dorsky DI, Moussy F and Kreutzer DL: Ex ova chick chorioallantoic membrane as a novel model for evaluation of tissue responses to biomaterials and implants. J Biomed Mater Res A *67*: 838-843, 2003.

33 Falkner E, Eder C, Kapeller B, Fröschl W, Schmatz C, Macfelda K and Losert UM: The mandatory CAM testing of cells and scaffolds for tissue engineering: benefits for the three Rs of cooperation with the vaccine industry. Altern Lab Anim *32*: 573-580, 2004.

34 Steffens L, Wenger A, Stark GB and Finkenzeller G: *In vivo* engineering of a human vasculature for bone tissue engineering applications. J Cell Mol Med *13*: 3380-3386, 2009.

35 Nasevicius A and Kker SC: Effective targeted gene 'knockdown' in zebrafish. Nat Genet *6*: 216-220, 2000.

36 Long Q, Meng A, Wang H, Jessen JR, Farrell MJ and Lin S: GATA-1 expression pattern can be recapitulated in living transgenic zebrafish using GFP reporter gene. Development *124*: 4105-4111, 1997.

37 Higashijma S, Hotta Y and Okamoto H: Visualisation of cranial motor neurons in live transgenic zebrafish expressing green fluorescent protein under the control of islet-1 promoter/enhancer. J Neurosci *20*: 206-281, 2000.

38 Higashijma S, Okamoto H, Ueno N, Hotta Y and Eguchi G: High frequency generation of transgenic zebrafish which reliably express GFP in whole muscles or the whole body by using promoters of zebrafish origin. Dev Bio *192*: 289-299, 1997.

39 Newman M, Musgrave IF and Lardelli M: Alzheimer disease: amyloidogenesis, the presenilins and animal models. Biochim Biophys Acta *1772*: 285-297, 2007.
40 Groth C, Nornes S, McCarty R, Tamme R and Lardelli M: Identification of a second presenilin gene in zebrafish with similarity to the human Alzheimer's disease gene presenilin2. Dev Genes Evol *212*: 486-490, 2002.
41 Drummond IA: Kidney development and disease in the zebrafish. J Am Soc Nephrology *16*: 299-304, 2005.
42 Sun Z, Amsterdam A, Pazour GJ, Cole DG, Miller MS and Hopkins N: A genetic screen in zebrafish identifies cilia genes as a principal cause of cystic kidney. Development *131*: 4085-4093, 2004.
43 Amali AA, Rekha RD, Lin CJ, Wang WL, Gong HY, Her GM and Wu JL: Thioacetamide-induced liver damage in zebrafish embryo as a disease model for steatohepatitis. J Biomed Sci *13*: 225-232, 2006.
44 Nicoli S, Ribatti D, Cotelli F and Presta M: Mammalian tumor xenografts induce neovascularization in zebrafish embryos. Cancer Res *67*: 2927-2931, 2007.
45 Padilla PA and Roth MB: Oxygen deprivation causes suspended animation in the zebrafish embryo. Proc Natl Acad Sci USA *98*: 7331-7335, 2001.
46 Mendelsohn BA, Kassebaum BL and Gitlin JD: The zebrafish embryo as a dynamic model of anoxia tolerance. Dev Dyn *237*: 1780-1788, 2008.
47 Langheinrich U, Vacun G and Wagner T: Zebrafish embryos express an orthologue of HERG and are sensitive toward a range of QT-prolonging drugs inducing severe arrhythmia. Toxicol Appl Pharmacol *193*: 370-382, 2003.
48 Brockerhoff SE: Measuring the optokinetic response of zebrafish larvae. Nat Protoc *1*: 2448-2451, 2006.
49 Bang PI, Yelick PC, Malicki JJ and Sewell WF: High-throughput behavioral screening method for detecting auditory response defects in zebrafish. J Neurosci Methods *118*: 177-187, 2002.
50 Usenko CY, Harper SL and Tanguay RL: *In vivo* evaluation of carbon fullerene toxicity using embryonic zebrafish. Carbon NY *45*: 1891-1898, 2007.
51 Hamm EE, Voth DE and Ballard JD: Identification of *Clostridium difficile* toxin B cardiotoxicity using a zebrafish embryo model of intoxication. Proc Natl Acad Sci USA *103*: 14176-14181, 2006.

Received October 29, 2009
Revised April 8, 2010
Accepted April 16, 2010