

## **Transgenic Medaka, Small Fresh Water Teleost (*Oryzias latipes*), is Now Available for Environmental Science**

Masato KINOSHITA

*Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University,  
Kyoto 606-8502, Japan*

(Received 29 September 2011; accepted 14 November 2011)

**Abstract**—Recently, small model fish, medaka and zebrafish, have been casted a light on environmental science. Transgenic technique is a method that introduce exogenous gene into host genome and enables to generate unique model creature. Adapting transgenic technique on these fish, they become more powerful tool for evaluating aquatic contamination. Chemical analysis and *in vitro* bioassay, such as ELISA, and analysis with wildlife are popular methods to assess contamination in aquatic environment. In addition to these conventional methods, transgenic fishes are casted a spotlight to facilitate to evaluate aquatic contamination with living creature. In this paper, recent works of transgenic medaka on this issue are introduced.

Keywords: medaka, taransgenic, GFP, pollution, estorogen

### INTRODUCTION

Water pollution of anthropogenic origin may have harmful effects on aquatic ecosystem and is considered a serious problem for aquatic and also terrestrial vertebrates including man. Therefore, accurate and prompt detection of anthropogenic chemicals and natural chemicals is a primary task to identify water with contamination and to produce waste cleanup methods that will reduce contamination of surface waters.

Chemical analysis is a widely used and sensitive method for the detection of contaminants in water samples. On the other hand, whole organism analysis enables us to assess the bioavailability, the effect of complex mixtures, toxicokinetics, or metabolic conversion of the original compounds to products with increased or reduced toxic properties. Biotests using whole organisms have also employed to detect contaminants: (1) Morphological and histological analysis of related organs and tissues from wildlife and laboratory animals is used. (2) Detection of biomarkers, related mRNA and protein, is quantified. In addition to these methods, more simple method that can evaluate water contamination with living creature has been required.

Medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*) have been accepted as

## E2 conc.

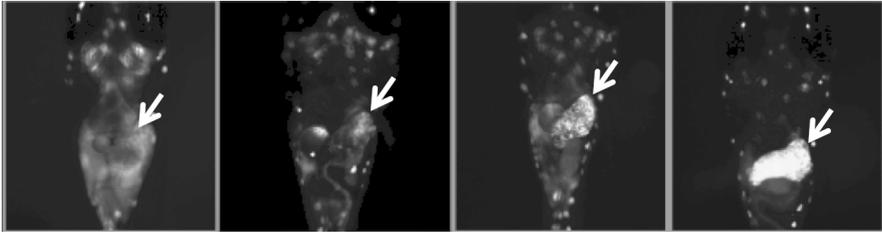


Fig. 1. Increasing of intensity of green fluorescence in liver of ChgH-GFP medaka as increasing estrogen concentration in exposure water. Fluorescent images of ventral view of larva exposed to various concentration of 17beta-estradiol (E2). Arrows indicate liver.

splendid model organisms in various scientific fields because of their advantages: small size (*ca.* 3 cm), short generation time, transparency of their embryo, availability of their genome information. In addition to these, transgenic technique is available to these fish, which enable to generate new model organism with suitable characteristics for evaluation water contaminants. Indeed, some transgenic medaka and zebrafish for environmental science have been generated and the simplicity to detect contaminants with these transgenic fish has been proofed.

In this paper, I introduce some of transgenic medaka for environmental science field and discuss for future prospects of transgenic medaka in this field.

### TRANSGENIC MEDAKA FOR DETECTION OF ESTROGEN-LIKE SUBSTANCES

We have established three types of transgenic medaka lines for detection of estrogen-like substances (ELSs), ChgH-GFP medaka, olvas-GFP medaka, and 42Sp50-GFP/RFP medaka.

#### *ChgH-GFP medaka*

This transgenic medaka contains green fluorescence protein (GFP) gene regulated by regulatory sequence of choriogenin H (ChgH) gene (Fig. 1). ChgH is a female specific protein, which expresses in liver with stimulus of estrogen and its expression after estrogen stimulus is faster than that of vitellogenin, which is one of famous proteins specific for female (Lee *et al.*, 2002). Therefore, male and immature fish don't express ChgH while mature female which produces estrogen in its ovary expresses ChgH in liver. In case of ChgH-GFP medaka, male and immature fish express GFP only when they exposed to estrogen or ELSs in breeding or environmental water. The advantage of this transgenic medaka is that ELS contamination can be notified easily: if contamination occurs, GFP is expressed in liver and is detectable by observing its fluorescence with fluorescent

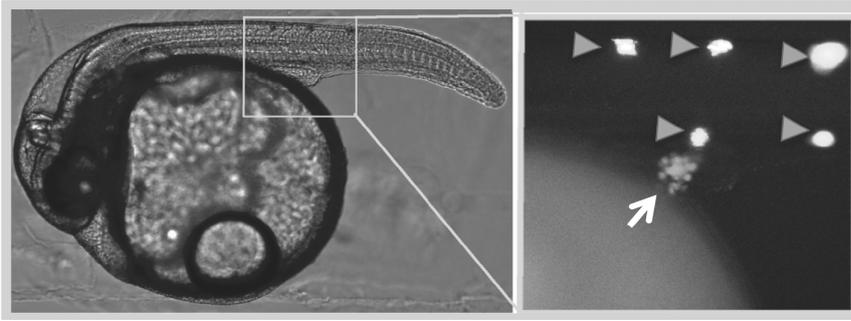


Fig. 2. *olvas-GFP* medaka. Left hand image: bright light image of embryo whose egg envelope was removed. Right hand image: magnified and fluorescent image of boxed portion of left hand image. Arrow indicates primordial germ cells. Arrowheads indicate auto fluorescence of leucophores.

microscope without any treatment and sacrificing fish (Kurauchi *et al.*, 2005). Kurauchi *et al.* (2005) and Kinoshita *et al.* (2010) successfully detected ELS contamination in sewage treatment effluent in Japan and in river water in urban area in Thailand and Malaysia, respectively. These results indicated that this transgenic medaka line is useful for simple detection of ELS contamination in surface water.

#### *olvas-GFP* medaka

This transgenic medaka contains GFP gene regulated by regulatory sequence of medaka *vasa (olvas)* gene (Tanaka *et al.*, 2001) (Fig. 2). In this transgenic medaka, primordial germ cell shows green fluorescence. In medaka, as in many vertebrates, male and female phenotypes can be distinguished by the proliferative activity of germ cells soon after the day of hatching. Germ cells in female larva start meiotic cell division within 24 h after hatch, whereas in males, germ cells proliferate much later (15 day post hatch). Namely, gender of larva is identified the number of germ cells and the area of gonad. In case of this transgenic medaka, female larva expresses green fluorescence brighter and in wider area of gonad than male larva, enabling us easy to identify its gender. With this transgenic medaka line, Hano *et al.* (2005) clearly shows the transgenerational effect of ethynilestradiol (EE2) using nanoinjection method. They used *olvas-GFP/ST-II* YI strain, which carries a genotypic marker, leucophore. The leucophore marker appears in the skin of 2-day post fertilized male embryos but not in female embryos (Wakamatsu *et al.*, 2003). Therefore, in *olvas-GFP/ST-II* YI, leucophore indicates genetic gender and the area with green fluorescence in gonad tells phenotypic gender. This means that *olvas-GFP/ST-II* YI medaka is good model for detection of reverse-sex phenomenon. In their report, the EE2 injected into an oil-drop in fertilized egg of *olvas-GFP/ST-II* YI medaka increased the area with green fluorescence in gonad and generated oocytes and mature ovary in genetically

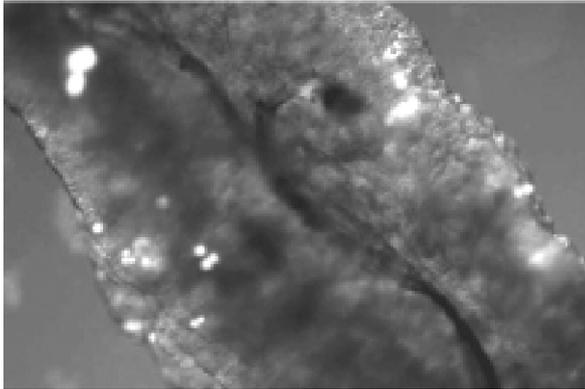


Fig. 3. Fluorescent image of testis-ova induced in testis of 42Sp50-GFP medaka. White spots are oocytes that were induced in male testis by the exposure to estrogen.

male gonad, indicating that maternally introduced ELSs, in this case injected EE2 in fertilized egg, may cause reverse-sex phenomenon in aquatic creature.

#### *42Sp50-GFP/RFP medaka*

This transgenic medaka contains GFP or red fluorescence protein (RFP) gene regulated by regulatory sequence of medaka 42Sp50 gene (Kinoshita *et al.*, 2009) (Fig. 3). 42Sp50 gene expresses exclusively in oocytes which have started meiosis, so in 42Sp50-GFP/RFP medaka, oocytes are easily noticed with fluorescence without sacrificing fish. By exposing this transgenic medaka to estrogen, testis-ova, which are ectopically induced oocytes in testis, were observed in testis. This finding suggested that 42Sp50-GFP/RFP medaka is useful to detect the efficiency of chemicals and water pollutants to derange gonad formation on adult medaka.

#### *Transgenic medaka for detection of mutagens*

Winn and colleagues produced a transgenic medaka line, which carries multiple copies of a bacteriophage  $\lambda$  vector that harbors the *cII* gene as a mutational target in medaka genome (Winn *et al.*, 2000; Hobbie *et al.*, 2009). The bacteriophage  $\lambda$  DNA can be recovered from medaka genome into host bacteria and the mutation in the *cII* gene can be evaluated by drug resistance of host bacteria based on the *cII* gene. They have successfully evaluated the contamination in mutagens in drinking-water and sediments (Geter *et al.*, 2004; Cachot *et al.*, 2007)

#### *Transgenic medaka as a candidate for detection general stress and xenobiotics*

Recently, Ng and Gong (2011) produced two transgenic medaka lines which may notice aquatic pollution with green fluorescence: One is Tg (*hsp70:gfp*)

medaka, which contains GFP gene driven by the regulatory region of heat shock protein 70 gene, and the other is Tg (*cyp1a1:gfp*) medaka, which contains GFP gene driven by the regulatory region of *cyp1a1* gene. HSP 70 is one of stress response proteins and is induced by general stress stimuli such as physical and chemical stress, for example heat-shock and heavy metals. Therefore, they suggest that Tg (*hsp70:gfp*) medaka may be useful for monitoring these stressor. On the other hand, *cyp1a1*, one of cytochrome P450 enzymes, which catalyze the oxidation of organic substances including xenotoxins to mediate their toxicity, is induced by exposure to some pollutants. They demonstrated the induced green fluorescence by exposing Tg (*cyp1a1:gfp*) medaka to TCDD suggesting the usefulness of this transgenic medaka for screening active xenobiotic compounds in surface water.

### FUTURE PROSPECTS

Recently, transgenic technique, genome modification technique, and the number of annotated gene have been developing day by day. These advance of technique and knowledge are surely beneficial on environmental science. In transgenic medaka for detection of water contaminants, expression regulatory sequence of certain gene is used as a sensor of contaminants. Expanding the knowledge of gene expression spectra means expanding the number of sensor for contaminants, leading expansion of spectra of detectable contaminants with transgenic fish.

As for the reporters that notice the existence or effects of water contaminants, fluorescent protein genes, such as GFP gene, are mainly subjected. The detection system of contaminants with GFP is much simpler compared with conventional detection methods such as chemical analysis. However, to detect and evaluate the fluorescence of GFP special instruments, fluorescence microscope and attached computer system, are essential. Simpler and less expensive detection system is favorable for researchers in developed and developing countries. To address this issue, melanin-concentrating hormone (MCH) gene may be a candidate, which promotes lightening of fish body color by shrinking melanophore on body surface (Kinoshita *et al.*, 2001).

At present, transgenic technique is available to limited aquatic animals. When this technique is adapted to many kinds of aquatic animals for example benthos, it helps simple and convenient monitor of aquatic environment including surface water and sediments.

### REFERENCES

- Cachot, J., M. Law, D. Pottier, L. Peluhet, M. Norris, H. Budzinski and R. Winn (2007): Characterization of toxic effects of sediment-associated organic pollutants using the  $\lambda$  transgenic medaka. *Environ. Sci. Technol.*, **41**, 7830–7836.
- Getz, D. R., R. Winn, J. W. Fournie, M. B. Norris, A. B. DeAngelo and W. E. Hawkins (2004): MX [3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone], a drinking-water carcinogen, does not induce mutations in the liver of cII transgenic medaka (*Oryzias latipes*). *J. Toxicol. Environ. Health A*, **67**, 373–383.
- Hano, T., Y. Oshima, T. Oe, M. Kinoshita, M. Tanaka, Y. Wakamatsu, K. Ozato and T. Honjo (2005):

- Quantitative bio-imaging analysis for evaluation of sexual differentiation in germ cells of olvas-GFP/ST-II YI medaka (*Oryzias latipes*) nanoinjected in ovo with ethinylestradiol. *Environ. Toxicol. Chem.*, **24**, 70–77.
- Hobbie, K. R., A. B. DeAngelo, L. C. King, R. N. Winn and J. M. Law (2009): Toward a molecular equivalent dose: use of the medaka model in comparative risk assessment. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, **149**, 141–151.
- Kinoshita, M., T. Morita, H. Toyohara, T. Hitata, M. Sakaguchi, M. Ono, K. Inoue, Y. Wakamatsu and K. Ozato (2001): Transgenic medaka overexpressing a melanin-concentrating Hormone exhibit lightened body color but no remarkable abnormality. *Mar. Biotechnol.*, **3**, 536–543.
- Kinoshita, M., G. Okamoto, T. Hirata, A. Shinomiya, T. Kobayashi, Y. Kubo, H. Hori and A. Kanamori (2009): Transgenic medaka enables easy oocytes detection in live fish. *Mol. Reprod. Dev.*, **76**, 202–207.
- Kinoshita, M., M. P. Zakaria, A. Ismail, S. Yusof, C. Boonphakdee, T. Boonphakdee and K. Inoue (2010): An attempt to detect contamination with estrogenic compounds in river water of urban area in Thailand and Malaysia using transgenic medaka. *Coast. Mar. Sci.*, **34**(1), 216–222.
- Kurauchi, K., Y. Nakaguchi, M. Tsutsumi, H. Hori, R. Kurihara, S. Hashimoto, R. Ohnuma, Y. Yamamoto, S. Matsuoka, S. Kawai, T. Hirata and M. Kinoshita (2005): In vivo visual reporter system for detection of estrogen-like substances by transgenic medaka. *Environ. Sci. Technol.*, **39**, 2762–2768.
- Lee, C., J. G. Na, K. C. Lee and K. Park (2002): Choriogenin mRNA induction in male medaka, *Oryzias latipes*, as a biomarker of endocrine disruption. *Aquat. Toxicol.*, **61**, 233–241.
- Ng, G. H. B. and Z. Gong (2011): Maize Ac/Ds transposon system leads to highly efficient germline transmission of transgenes in medaka (*Oryzias latipes*). *Biochimie*, **93**, 1858–1864.
- Tanaka, M., M. Kinoshita, D. Kobayashi and Y. Nagahama (2001): Establishment of medaka (*Oryzias latipes*) transgenic lines with the expression of green fluorescent protein fluorescence exclusively in germ cells: A useful model to monitor germ cells in a live vertebrate. *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 2544–2549.
- Wakamatsu, Y., C. Inoue, H. Hayashi, N. Mishima, M. Sakaizumi and K. Ozato (2003): Establishment of new medaka (*Oryzias latipes*) stocks carrying genotypic sex markers. *Environ. Sci.*, **10**, 291–302.
- Winn, R., M. Norris, K. Brayer, C. Torres and S. Muller (2000): Detection of mutations in transgenic fish carrying a bacteriophage  $\lambda$  cII transgene target. *Proc. Natl. Acad. Sci. U.S.A.*, **97**, 12655–12660.

---

M. Kinoshita (e-mail: kinoshit@kais.kyoto-u.ac.jp)