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Review:

TOWARD THE ESTABLISHMENT OF THE TOOLS FOR MONITORING COASTAL ENVIRONMENTS UTILIZING GENE RESPONSE IN *ORYZIAS* FISHES

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ABSTRACT

Southeast Asia is known as a center of biodiversity of the earth. As economic growth of this region is remarkable, conservation of biodiversity is a top priority issue. For this purpose, it is important to monitor the environmental condition in effective ways. We are trying to detect pollutants in coastal and estuary waters through the expression level of pollutant-responsive genes of rice fishes of the genus *Oryzias*, widely distributed in Asia. Japanese medaka *O. latipes* is a useful model because whole genome sequence is available. Javanese medaka *O. javanicus* and Indian medaka *O. dancena*, both of which are widely distributed in Southeast Asia and adaptable to seawater, are also potential models. One possible method for pollution monitoring is the use of transgenic fish bearing artificial gene construct containing the pollutant-responsive promoter and a reporter gene. For example, transgenic strains that can detect estrogen-like substances have already been established. It is also probable to detect pollutants by quantifying mRNA or proteins expressed from the pollutant-responsive gene. In any case, the most important point is to identify the gene that responds to specific pollutants. Comprehensive transcriptomic analyses are powerful tool for this purpose. Organotin-responsive genes are being screened at present.

Keywords: Enzyme-Linked Immunosorbent Assay (ELISA), medaka, pollution, transgenic.

INTRODUCTION

Southeast Asia, containing Indochina and Malay Peninsula and many large and small islands with different geological background, is known to be "the center of biodiversity". For terrestrial ecosystems, specific fauna and flora reflecting the geological history of each area have been reported. Southeast Asia also has a variety of coastal and marine environments, where unique ecosystems with high biodiversity, such as coral reefs, mangroves, and seagrass beds, are observed. On the other hand, Southeast Asia is a region whose economy is growing rapidly. Thus, special care must be taken for conservation of the biodiversity, the precious treasure of the earth.

To minimize the negative impact on the ecosystems caused by economic growth, it is important to monitor the status of the environments effectively and continuously. In the case of the monitoring of the marine environment, one way is to measure the level of pollutants in water by chemical analyses. However, chemical analyses of water require expensive equipment and reagents as well as skilled techniques. In addition, it is difficult to evaluate the environmental status correctly

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from the analysis on limited amount and number of samples.

A possible solution of such problems is the use of bioindicators. Blue and green mussels, for example, have been utilized as the sample for chemical analyses of pollutants (Goldberg et al., 1978; Monirith et al., 2003). As the mussels attach themselves to a substrate and do not move frequently, the result of analyses indicates the pollution status of the point where the mussels were collected. In addition, the mussels bio-concentrate the pollutants and thus the data represent the long-term status of the sampling points. Their characteristics to bio-concentrate pollutants also increase the sensitivity of the analysis. Thus, the use of the mussels has become popular for pollution analyses. However, even using mussels, problems accompanying chemical analyses still remain.

We are trying to detect the pollution through the expression of genes that respond to pollutants. For this purpose, possible models are small fish species, of which genomic information is abundant. Unlike direct chemical analyses of pollutants, the small size is not an obstacle for mRNA analyses; it is rather advantageous as it is easy to catch and handle such fish. Here we propose that small medaka fishes (rice fishes) are ideal models for environmental monitoring through gene expression.

MEDAKA FISHES

Medaka fishes of the genus *Oryzias* inhabit fresh and salt water in Asia (Parenti 2008; Roberts, 1998). About 20 species have been recorded in Asia; all species are small, even adult fish are usually less than 4 cm. They prefer shallow still water or slow stream near shore or bank and often found in rice paddy, ponds, irrigation channels and small creeks. Most species are surface swimmer, and relatively large eyes at sides of the slightly flattened head are easily recognizable when looking down the swimming fish from above.

Among *Oryzias* species, Japanese medaka *O. latipes* has already been established as an experimental model (Wittbrodt et al., 2002; Kinoshita et al., 2009). For molecular-biological studies, its whole genome sequence information (Kasahara et al., 2007), and established technique for transgenesis (see below) (Ozato et al., 1986; Inoue et al., 1989; Inoue et al., 1990; Inoue et al., 1992; Kinoshita et al., 1994) is especially useful. An enormous number of studies have been conducted using this species.

Another two species, Javanese medaka *O. javanicus* (Fig. 1) and the Indian medaka *O. dancena* are becoming popular especially in the

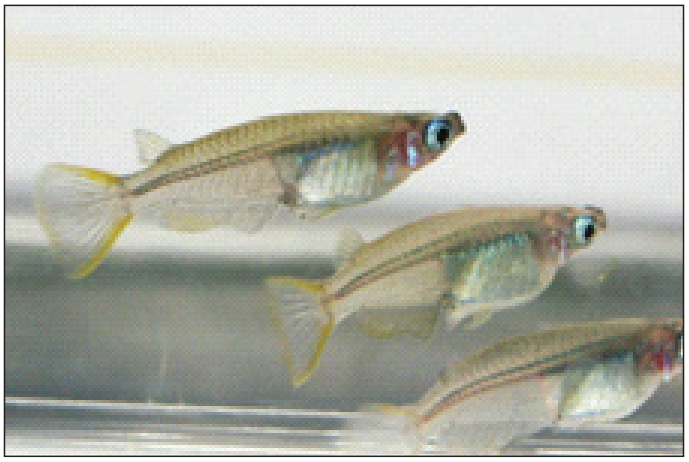

Figure 1. Javanese medaka *Oryzias javanicus*. It was obtained from National Bioresource Project Medaka (NBRP) in National Institute for Basic Biology, Okazaki, Japan. Most *Oryzias* species have relatively flat back, perhaps reflecting their surface swimming habitat, and a small dorsal fin locates near the tail fin.

field of environmental sciences. For scientists in Southeast Asia, these two species are easily obtainable because of their wide distribution in the region (Parenti, 2008; Roberts 1998; Yusof et al., 2012). They can adapt to both fresh and salt water (Inoue and Takei, 2002, 2003) although the former prefers hyperosmotic environment and the latter hypoosmotic (Yusof et al., 2012). Therefore, these species can be used for experiments in seawater.

POLLUTANT DETECTION USING TRANSGENIC FISH

"Transgenic fish" is the fish to which foreign gene(s) are introduced. It is commonly used to examine regulation and function of genes. It also becomes a powerful tool to detect pollutants in water by introducing a construct of pollutant-responsive promoter with appropriate reporter gene. The possibility of the use of transgenic fish has been proposed in 1990s (Inoue et al., 1992; Kinoshita et al., 1994). Recently, one of the authors of this article (MK) established a transgenic Japanese medaka strain bearing the construct of the choriogenin H (Chg-H) promoter, which is responsive to estrogens, with green fluorescence protein (GFP) gene; and demonstrated that it can detect estrogens in the water in a dose-dependent manner (Kurauchi et al., 2005; 2008). Using the strain, we surveyed the pollution status of some areas in Thailand and Malaysia (Kinoshita et al., 2010). As Japanese medaka can adapt to seawater after adequate acclimation process, it is possible to use it for the assay of estrogenic substances in seawater.

As the endogenous Chg-H promoter was used in this transgenic strain, it is expected that the promoter of the transgene is regulated by the same manner as the original Chg-H gene. It implies that the transgenic strain detect all the substances that activate the Chg-H gene even if the pollutants were unknown substances. In addition, it is also visualize the process of bio-concentration of pollutants by the host fish. So, it may be possible to detect low concentration of pollutants in the case of long-term exposure.

For the use of the transgenic strains, the simplest way is the direct exposure to the water samples. The pollution status becomes visible just putting the fish into the water and monitor the fluorescence by a fluorescent microscope. However, as transgenic fish are genetically modified organisms (GMOs), it may cause serious problems on natural ecosystem if they escaped to natural environment, and thus the use of transgenic animals in non-enclosed environment is prohibited in most countries under The Convention on Biological Diversity. Therefore, we do not intend in situ use of the transgenic strains in the field. Instead, the sample water should be brought to the laboratory where transgenic fish are maintained. Transportation of water sample is not a problem when the sampling point is close to the laboratory for transgenic assay. However, when it is distant, transportation of water is costly, and the chemical composition may change during transportation. To solve these, mini-columns such as a Sep-Pak C-18 cartridge are used to trap the pollutants (Kurauchi et al., 2005; Kinoshita et al., 2010). The use of the columns reduces the volume of samples and the possibility of the degradation of the components. The use of the column is also advantageous as the pollutants can be concentrated. It increases sensitivity of the assay when the concentration of the pollutants is low.

DETECTION OF EXPRESSION WITHOUT USING TRANSGENICS

Transgenic strains are very convenient tools for detection of pollutants. However, as mentioned above, their use is limited to the laboratory where the biological containment is possible. Thus, we are also considering the way of pollutant detection without using transgenics. In pollution-detecting transgenic fish, a foreign reporter gene (e.g., GFP) is used to make the detection of gene expression easier but the promoter of the transgene is derived from the host genome sequence. It means that, when GFP is expressed in the transgenic fish, the transcription of the original gene also occurs. For example, the Chg-H-GFP transgenic fish express fluorescence, the transcripts of the original Chg-H gene also increases. By detecting Chg-H mRNA, the estrogenic pollutants can be detected. In fact, Yu et al. (2006) and Chen et al. (2008) reported that Chg-H mRNA increased after estrogen exposure in Javanese and Indian medaka, respectively. Thus, the detection of mRNA is a possible solution

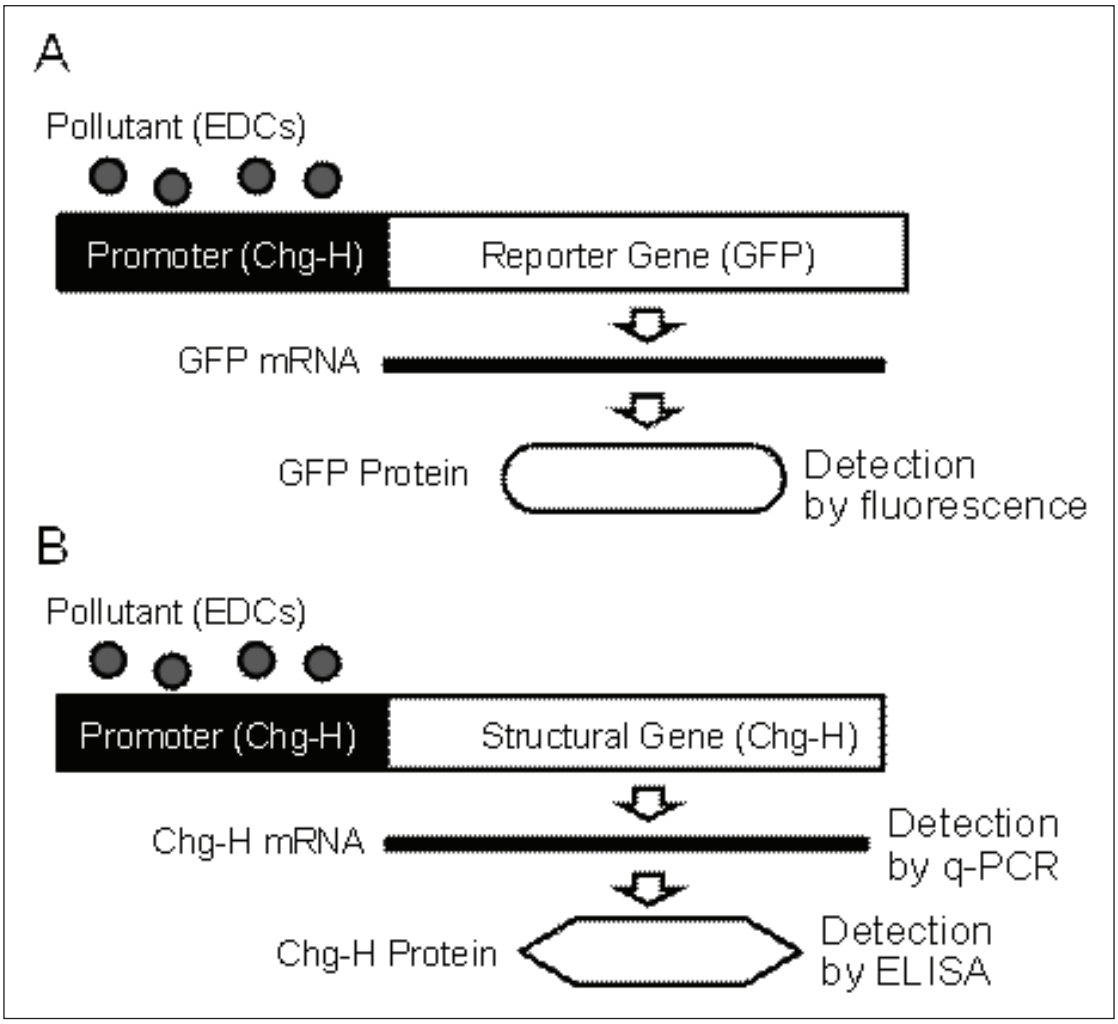

Figure 2. Principle of the detection of the expression of a pollutant-responsive gene in transgenic and wild fish. Choriogenin H (Chg-H) gene that responds to estrogen-like endocrine disrupting chemicals (EDCs) was used as an example. A, transgene consisting of Chg-H promoter and green fluorescence protein (GFP), which was introduced into transgenic fish. The translated protein is detected by fluorescence. B, normal (wild type) gene. The transcribed mRNA and translated protein are detected by quantitative reverse-transcription PCR (q-PCR) and enzyme-linked immunosolvent assay (ELISA), respectively.

although the equipment for quantitative real-time PCR is necessary.

Another possible solution is the detection of translation products, i.e., proteins. For quantitative detection of proteins, enzyme-linked immunosolbent assay (ELISA) may be useful as it does not special equipment except for a microplate reader. For example, ELISA for Chg-H detection has been established in several fish species to detect estrogenic activities in environment (Fujita et al. 2004; Prakash et al. 2007; Brander et al. 2012)

SCREENING OF POLLUTANT RESPONSIVE GENES

For establishing the systems to detect pollutants mentioned above, the most important point is to identify the gene that respond to the pollutant. Although some pollution-responsive genes have been discovered, for example, metallothioneins for metals (Inoue et al., 1992; Kinoshita et al., 1994; Woo et al., 2006), cytochrome P450 (CYP) proteins for many organic pollutants (see Uno et al., 2012 for a review). For discovery of new genes respond to pollutants, comprehensive transcriptomic analyses may be the most efficient way. For such analyses, several techniques have been

used such as differential display, (Liang and Pardy, 1992), microarray (Schena et al., 1995), high coverage expression profiling (HiCEP) (Fukumura et al., 2003), but the "next generation" sequencing (Mardis, 2008), the new technologies rapidly developed recently, will accelerate the research further. Screening of the genes that respond to organotin compounds, which have been included in antifouling paints (Harino et al., 2013), is in progress. We expect that a variety of systems that can detect various pollutants will be established by using appropriate model species such as *Oryzias* fishes, which are easily obtainable and culturable, and gene expression profiling techniques.

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REFERENCES

Brander, S.M, B.J. Cole and G.N. Cherr. 2012. An approach to detecting estrogenic endocrine disruption via choriogenin expression in an estuarine model fish species. Ecotoxicology 21: 1272–1280.

Chen, X.P., V.W.T. Li, R.M.K. Yu and S.H. Cheng. 2008. Choriogenin mRNA as a sensitive molecular biomarker for estrogenic chemicals in developing brackish medaka (Oryzias melastigma). Ecotoxicol. Environmental Safety 71: 200–208.

Fujita, T., H. Fukada, M. Shimizu, N. Hiramatsu and A. Hara. 2004. Quantification of serum levels of precursors to vitelline envelope proteins (choriogenins) and vitellogenin in estrogen treated masu salmon, Oncorhynchus masou. Gen. Comp. Endocrinol. 136: 49–57.

Fukumura, R., H. Takahashi, T. Saito, Y. Tsutsumi, A. Fujimori, S. Sato, K. Tatsumi, R. Araki and M. Abe. 2003. A sensitive transcriptome analysis method that can detect unknown transcripts. Nucleic Acids Res. 31: e94.

Goldberg, E.D., V.T. Bowen, J.W. Farrington, G. Harvey, J.H. Martin, P.L. Parker, R.W. Risebrough, W. Robertson, E. Schneider and E. Gamble. 1978. The mussel watch. Environ. Conserv. 5: 101–125.

Harino, H., E. Yatsuzuka, Z. Arifin, I.F.M. Rumengan, A. Ismail, G. Wattayakorn and K. Inoue. 2013. A review of antifouling biocides contaminations in Indonesia, Malaysia, Thailand and Vietnam. Mar. Res. Indonesia, this issue.

Inoue, K. and Y. Takei. 2002. Diverse adaptability in Oryzias species to high environmental salinity. Zool Sci 19: 727–734.

Inoue, K. and Y. Takei. 2003. Asian medaka fishes offer new models for studying seawater adaptation. Comp. Biochem. Physiol. B 136: 635–645.

Inoue, K., N. Akita, T. Shiba, M. Satake and S. Yamashita. 1992. Metal-inducibleactivities of metallothionein promoters in fish cells and fry. Biochem. Biophys. Res. Commun. 185: 1108–1114.

Inoue K., K. Ozato, H. Kondoh, T. Iwamatsu, Y. Wakamatsu, T. Fujita and Okada T.S. 1989. Stage-dependent expression of the chicken δ-crystallin gene in transgenic fish embryos. Cell Differ. Dev. 27, 57–68.

Inoue, K., S. Yamashita, J. Hata, S. Kabeno, S. Asada, E. Nagahisa and T. Fujita. 1990. Electroporation as a new technique for producing transgenic fish. Cell. Differ. Dev. 29: 123–128.

Kasahara, M., K. Naruse, S. Sasaki, Y. Nakatani, W. Qu, B. Ahsan, T. Yamada, Y. Nagayasu, K. Doi, Y. Kasai, T. Jindo, D. Kobayashi, A. Shimada, A. Toyoda, Y. Kuroki, A. Fujiyama, T. Sasaki, A. Shimizu, S. Asakawa, N. Shimizu, S. Hashimoto, J. Yang, Y. Lee, K. Matsushima, S. Sugano, M. Sakaizumi, T. Narita, K. Ohishi, S. Haga, F. Ohta, H. Nomoto, K. Nogata, T. Morishita, T. Endo, T. Shin-I., H. Takeda, S. Morishita and Y. Kohara. 2007. The medaka draft genome and insights into vertebrate genome evolution. Nature 447: 714–719.

Kinoshita, M., K. Murata, K. Naruse, M. Tanaka. 2009. A laboratory manual for medaka biology. Wiley-Blackwell, Ames, Iowa, USA.

Kinoshita, M., H. Toyohara, M. Sakaguchi, N. Kioka, T. Komano, K. Inoue, S. Yamashita, M. Satake, Y. Yakamatsu and K. Ozato. 1994. Zinc-induced activation of rainbow trout metallothionein-A promoter in transgenic medaka. Fish. Sci. 60: 307–309.

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. Coastal Mar. Sci. 34: 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. Env. Sci. tech nol. 39: 2762–2768.

Kurauchi, K., T. Hirata and M. Kinoshita. 2008. Characteristics of ChgH-GFP transgenic medaka lines, an in vivo estrogenic compound detection system. Mar. Pollut. Bull. 57: 441–444.

Mardis, E.R. 2008. Next-generation DNA sequencing methods. Annual Review of Genomics and Human Genetics 9: 387–402.

Liang, P. and A.B. Pardee. 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257: 967–971.

Monirith, I., D. Ueno, S. Takahashi, H. Nakata, A. Sudaryanto, A. Subramanium, S. Karuppiah, A. Ismail, M. Muchtar, J. Zheng, B.J. Richardson, M. Prudente, N.Y.D. Hue, T.S. Tana, A.V. Thalin and S. Tanabe. 2003. Asia-Pacific Mussel watch: Monitoring contaminant of Persistant Organochlorine Compounds in Coastal Waters of Asian Countries. Marine Pollution Bulletin; 46: 281–300.

Ozato, K., H. Kondoh, H. Inohara, T. Iwamatsu, Y. Wakamatsu and T.S. Okada. 1986. Production of transgenic fish: introduction and expression of chicken delta-crystallin gene in medaka embryos. Cell Differ. 19: 237–244.

Parenti, L.R. 2008. A phylogenetic analysis and taxonomic revision of rice fishes, Oryzias and relatives (Beloniformes, Adrianichthyidae). Zool J. Linnaean Soc. 154: 494–610.

Prakash, O., S.V. Goswami and N. Sehgal. 2007. Establishment of ELISA for murrel vitellogenin and choriogenin, as biomarkers of potential endocrine disruption. Comp. Biochem. Physiol. C-Toxicology 146: 540–551.

Roberts, T.R. 1998. Systematic observations on tropical Asian medakas or rice fishes of the genus Oryzias with descriptions of four new species. Ichthyol Res. 45: 213–224.

Uno, T., M. Ishizuka and T. Itakura. 2012. Cytochrome P450 (CYP) in fish. Environmental Toxicology and Pharmacology 34: 1–13.

Schena, M., D. Sharon, R.W. Davis and P.O. Brown. 1995. Quantitative monitoring of gene-expression patterns with a complementary-DNA microarray. Science 270: 467–470.

Wittbrodt, J., A. Shima and M. Schartl. 2002. Medaka—A model organism from the Far East. Nat. Rev. Genet. 3: 53–64.

Woo, S., S. Yum, L.H. Jung, W.J. Shim, C.H. Lee and T.K. Lee. 2006. Heavy metal-induced differential gene expression of metallothionein in Javanese medaka, Oryzias javanicus. Marine Biotech. 8: 654–662.

Yu, R.M.K., M.M.L. Wong, R.Y.C. Kong, R.S.S. Wu and Cheng S.H. 2006. Induction of hepatic choriogenin mRNA expression in male marine medaka: A highly sensitive biomarker for environmental estrogens. Aquatic Toxicology 77: 348–358.

Yusof, S., A. Ismail, T. Koito, M. Kinoshita and K. Inoue. 2012. Occurrence of two closely related ricefishes, Javanese medaka (Oryzias javanicus) and Indian medaka (O. dancena) at sites with different salinity in Peninsular Malaysia. Env. Biol. Fish. 93: 43–49.