Chapter 1
Suitability of Medaka Fish as a Test Organism and New Medaka Strains

Yuko WAKAMATSU and Kenjiro OZATO
Bioscience Center, Nagoya University
Furo-cho, Chikusa-ku, Nagoya, Japan

1- Suitability of medaka fish as a test organism

Medaka has been used as a model in various fields of environmental research for the past several decades, namely, radiation biology in the 1970s, carcinogenesis in the 1980s, and toxicology in the 1990s. More recently, about half the number of publications dealing with medaka is on toxicology (1, 2). Thus, it is apparent that medaka has a high potential as a model fish for use in the testing of environmental chemicals. In particular, medaka is useful for studying the effects of endocrine-disrupting substances on reproduction, because of its characteristic reproductive system in which the sex is determined by XY chromosomes. Several medaka stocks developed for reproduction research are powerful tools for investigating the effects of those substances. Here, we will review the potential of medaka as a model fish based on its biological characteristics, resources and strains for use in endocrine-disrupting substance testing. This review is based on our two papers published in ENVIRONMENTAL SCIENCES (3, 4).

1-1. Medaka

Medaka (*Oryzias latipes*) is a small, egg-laying freshwater teleost that is widely used as a laboratory fish (5). Its oogenesis, fertilization, and embryonic development were extensively studied (6, 7). Medaka, typically 3 cm long, is among the smallest vertebrates known. The generation time is short, 2 to 3 months, comparable to that of zebrafish and mice. Spawning is daily and year-round under artificial conditions, and the timing of spawning can be controlled by regulating light conditions during a 24-hr period. In addition, the transparency of the eggs is a distinct advantage for embryonic observation and manipulation. Its chromosome number is 48. It is the only fish species with more than ten inbred strains established (8), and about 100 natural mutant strains collected and maintained (9). Physical maps (10) and BAC libraries (11) are available for use in gene analysis. A large-scale mutagenesis using medaka has been started and is progressing rapidly (12). Its small genome size, about 800Mb, which is one-half that of zebrafish and one-fourth that of mammals, enables sequencing of the whole genome. The whole-genome shotgun sequencing has been started very recently (13). The databases are available at the Medakafish Homepage of medaka biology (1) and at the Medaka ToxiNet of toxicology in medaka (2). The biological characteristics and resources of medaka are summarized in Tables 1 and 2, respectively.

Table 1 Biological characteristics of medaka and zebrafish.

	Medaka	Zebrafish
Scientific name	*Oryzias latipes*	*Brachydanio rerio*
Growth		
Body length	3 cm	4 cm
Generation time	2-3 months	2-3 months
Life span	2 years	2 years
Development		
Spawning cycle	Daily	Twice a week
Brood size	20-30	200-300
Egg size	1 mm	1 mm
Transparency of eggs	High	High
Hardness of chorion	Hard	Soft
Days to hatching	7-10 days	2-3 days
Sex determination		
Sex chromosome	XY	Unknown
Y chromosome	Identified	Unknown
Male-determining gene	*Dmy*	Unknown
Breeding temperature	26°C	26°C
Inhabitable temperature	0-40°C	20-30°C
Chromosomes		
Chromosome number	48	50
Genome size	800Mb	1,600Mb

Table 2. Biological resources of medaka and zebrafish.

	Medaka	Zebrafish
Scientific name	*Oryzias latipes*	*Brachydanio rerio*
Strains		
Inbred	13	
Natural mutants	120	
Sex reversal mutants	Several	
Strains with sex markers	Several	
See-through	Some	
Wild populations	100	
Genome-wide mutagenesis	Primary step	Extensive
Transgenic	Available	Available
ES cells	Ongoing	Ongoing
Nuclear transplantation	Ongoing	Ongoing
Genome		
BAC library	Available	Available
Physical map	1,760	15,353
EST cloned	44,531	214,214
Genes cloned	4	50
Genome project ranking	41st	12th

1-2. Sex Determination and Differentiation in Medaka

Sex in medaka is genetically determined by XY chromosomes, i.e., the female and male sexes are determined by XX and XY chromosomes, respectively (14). The first experimental sex reversal in vertebrates following exposure to hormones was demonstrated in medaka (15). Since then, many studies on sex determination and differentiation have been conducted using medaka as the model fish (16). Genes for xanthophores (14) and leucophores (17) are located on the sex chromosomes. Some PCR markers have been developed to identify sex chromosomes (18). Using these markers, the Y chromosome is identified microscopically by the FISH technique (19). Very recently, the male-determining gene (*Dmy*) has been identified on the Y chromosome and cloned (20, 21). Thus, medaka is an ideal model for determining the effects of endocrine-disrupting substances on fish reproduction.

3. Strains

2-1. Orange-red strain

The medaka with the wild-type body color has four main types of pigment cells in the skin, namely, melanophores, xanthophores, leucophores and iridophores (22, 23). Xanthophores and leucophores show sex-linked pigmentation. The strain with an orange-red body is commercially available and widely used in research and testing of environmental chemicals, because it is the easiest strain to maintain under laboratory conditions. The body color is due to the wild-type phenotype of orange-red pigmentation in xanthophores, determined by the sex-linked *r* locus (X^RX^R for females and X^RY^R for males) (14), and the recessive phenotype of black pigmentation in melanophores, determined by the *b* locus (24). Most studies of endocrine-disrupting substances in medaka were conducted using this strain (25). However, this orange-red strain lacks markers for identifying the genotypic sex at the embryonic or early stages of its life cycle. Thus, the occurrence of sex reversal, which is used as an endpoint of exposure experiments, is evaluated from the deviation in the phenotypic sex ratio of experimental fish. If genotypic sex identification is possible, the sex reversal evaluation becomes more reliable. Furthermore, we can use the female or male populations distinguished from each other at the embryonic or larval stage before experiments and thus reduce the number of fish required.

2-2. Strains with body-color and PCR sex markers

2-2-1. d-rR strain with *r* marker

This strain was developed by Yamamoto (15). The d-rR stock has a genotypic sex marker of the orange-red body color, in which females (X^rX^r) exhibit a recessive phenotype for *r* expressing the white body color and males (X^rY^R) exhibit a wild-type phenotype expressing the orange-red body color (Fig. 1). The marker can be observed at the later stages of embryonic development under a microscope and two to three weeks after hatching by the naked eye. The frequency of recombination between the *r* locus and the sex-determinant (*SD*) locus by crossing over of the X and Y chromosomes is 0.2 %, that is, an error of 0.2% is unavoidable in sex identification based on this color marker. d-rR has been used in studies of fish reproduction (16) and recently, in the testing of endocrine disrupters (26, 27, 28). A similar strain, s-rR, is also used in substance exposure experiments (29, 30).

Figure 1.

d-rR strain. The female (upper) has a white body (X^rX^r) and the male (bottom) has an orange-red body (X^rY^R) (4).

2-2-2. FLF stock with *lf* and *SL1* markers

The *lf* (leucophore free) locus is sex-linked and determines leucophore expression in the skin (17). In the wild-type strain, leucophores first appear in two-day-old embryos in both females ($X^{LF}X^{LF}$) and males ($X^{LF}Y^{LF}$) (31). The lf

strain is homozygous recessive at the *lf* locus and has no visible leucophores in the skin throughout life (9).

In addition to color markers, some PCR markers that are linked to sex chromosomes were reported (18, 19). One of these markers, sex-linked 1 (*SL1*), is closely linked to the *SD* locus. In some medaka stocks, the molecular sizes of PCR products of *SL1* are different between the X and Y chromosomes, resulting in detection of only one band in samples from females whereas two bands in those from males in PCR analyses.

The lf stock and a wild-type stock (Nagoya) were crossed to generate the FLF stock. In this stock, females ($X^{lf}X^{lf}$) exhibit the recessive phenotype for *lf*, that is, having no visible leucophores in the skin, and their samples show one band in PCR analysis. On the other hand, males ($X^{lf}Y^{LF}$) exhibit the wild-type phenotype, that is, having leucophores and their samples show two bands. The skin of the FLF stock is dark due to the wild-type nature of the autosomal *b* locus (24).

In males of the FLF stock, leucophores appear first on the ventral side of the midbrain of stage-26 embryos two days after fertilization and then along the dorsal and ventral midlines at later stages five days after fertilization. No visible leucophores are observed throughout the embryonic stages in females. The pattern of leucophore expression in males is the same as that in the wild-type strain (17). Leucophores appear reddish brown under transmitted light and rather difficult to distinguish from differentiating melanophores which also appear dark in color. Under reflective light, leucophores appear yellow to white. Under a fluorescence stereoscopic microscope, leucophores are easily identified by their intense yellow autofluorescence.

The frequency of recombination between the *lf* and *SD* loci by crossing over of the X and Y chromosomes is 4.2% and an error at this rate is unavoidable in the sex identification based on the leucophore marker. This error can be reduced to 0 %, if necessary, by utilizing the *SL1* marker, because there is no recombination between the *SL1* and *SD* loci (18, 19). Thus, the genotypic sex can be identified first by the leucophore marker at early embryonic stages and then the error of identification can be reduced by utilizing the *SL1* marker. The FLF stock has been used for testing of endocrine-disrupting substances (32).

The Qurt stock carrying the genotypic sex marker of leucophore is similar to the FLF stock, although it does not carry a PCR marker (17).

2-2-3. FLFII stock with *lf*, *r* and *SL1* markers

The Hd-rR.Y^{HNI} strain (8) has *r* and *SL1* markers for identifying the genotypic sex. The FLF stock was crossed with this strain to generate the FLFII stock (3). In the case of the FLFII stock, the females show the recessive phenotype of $X^{lf,r}/X^{lf,r}$ and the males show the wild-type phenotype of $X^{lf,r}/Y^{LF,R}$ for both the *lf* and *r* characteristics. Leucophores appear in males in the same spatiotemporal manner as that in the wild-type strain, whereas there are no leucophores in females. In embryos, leucophores are more easily observed in this stock than in FLF because of the absence of melanophores in the skin. Xanthophore pigmentation is microscopically observed in the skin of male embryos at late embryonic stages, but not in that of female embryos. The orange-red pigmentation in the skin is easily observed by the naked eye in the male fry around two to three weeks after hatching (Figs. 2 and 3).

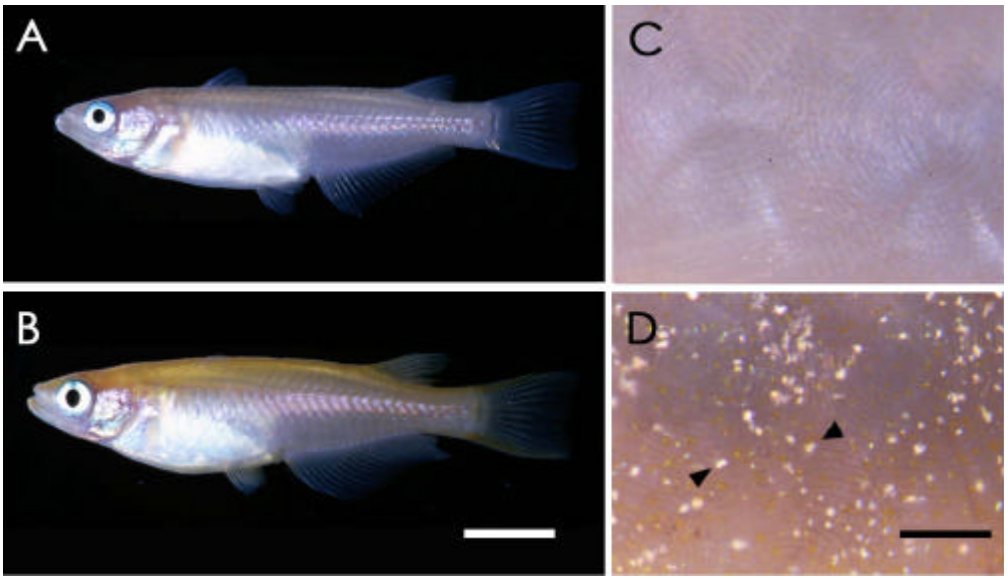

Figure 2.

Body colors in adult fish of the FLFII stock. A: a female showing a white body. B: a male showing an orange-red body. C: the skin of a female exhibiting no visible leucophores. D: the skin of a male exhibiting many leucophores (arrowheads). The magnification is the same for pairs A and B, and C and D. The bars in B and D represent 5 mm and 300im, respectively (3).

The frequency of recombination between the *lf* locus and the SD locus by crossing over of the X and Y chromosomes is 1.4 % in this stock, which is apparently lower than that in the FLF stock. No recombinant for the *r* locus and

the *SD* locus is obtained. For the *SL1* marker, one band is detected in all samples from females examined whereas two bands are detected in all samples from males examined (Fig. 4).

In the FLFII stock, the genotypic sex can be screened at early embryonic stages based on the presence of leucophores, confirmed at larval stages by the presence of xanthophores, and finally reconfirmed by presence of the SL1 marker, eliminating errors due to the use of color markers (3). Furthermore, the FLFII stock may be superior to the FLF stock because of its lower frequency of recombination between the *lf* and *SD* loci.

Recently, a candidate male-determining gene, *Dmy*, has been isolated from the *SD* locus on the Y chromosome in medaka (20, 21). *Dmy* could be a tool for identifying the genotypic sex of wild-type populations or laboratory strains of medaka with or without the known genotypic sex markers such as genes for color and *SL1*. Further studies of *Dmy* are required to determine its applicability as a genotypic sex marker for general use in medaka.

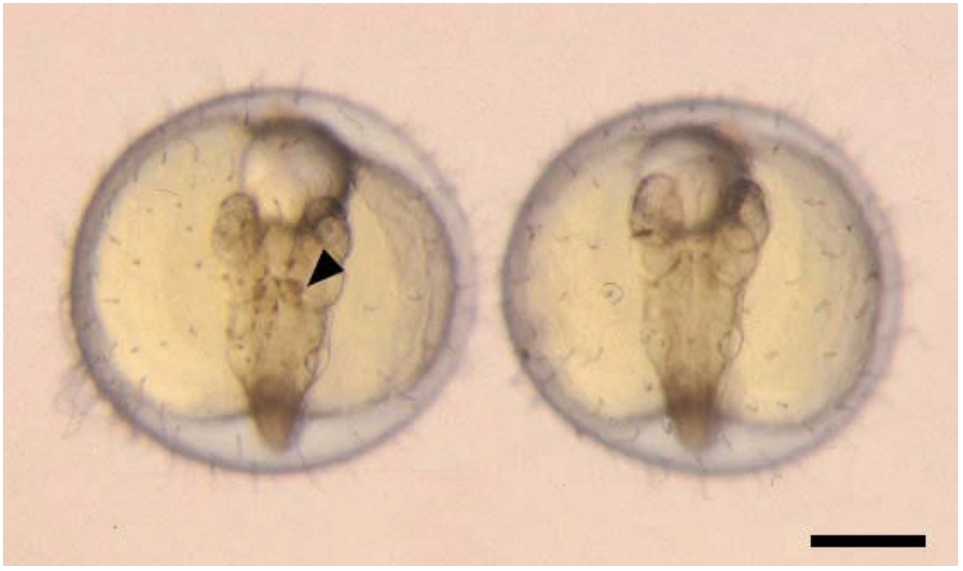

Figure 3.

First appearance of leucophores in embryos of the FLFII stock at stage 26 (two days after fertilization). Leucophores are observed as reddish-brown cells (arrowhead) on the ventral side of the midbrain in a male embryo (left) under transmitted light. A female embryo (right) at the same developmental stage shows no leucophores. The bar represents 300ìm (3).

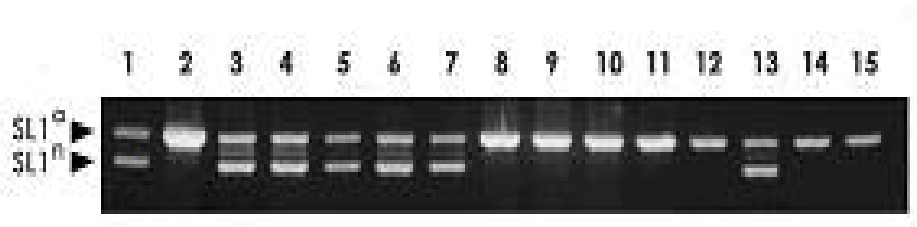

Figure 4.

PCR analysis of *SL1* from the FLFII stock. Each lane contains the sample from each fish. Lane 1; sample from a control male of the Hd-rR.Y^{HNI} stock exhibiting two bands corresponding to $SL1^a$ and $SL1^n$. Lane 2; sample from a control female of the Hd-rR.Y^{HNI} stock exhibiting one band corresponding to $SL1^a$. Lanes 3-7; samples from males of the FLFII stock exhibiting two bands corresponding to $SL1^a$ and $SL1^n$. Lanes 8-12; samples from females of the FLFII stock exhibiting one band corresponding to $SL1^a$. Lane 13; sample from a male recombinant for *lf* and *SD* loci exhibiting two bands corresponding to $SL1^a$ and $SL1^n$. Lanes 14 and 15; samples from female recombinants for *lf* and *SD* loci exhibiting one band corresponding to $SL1^a$ (3).

2-2-4. Stability of newly established stocks

Both of the FLF and FLFII stocks are healthy and easy to breed. They mature approximately two months after hatching and show no abnormalities in morphology and behavior. Their reproductive activity is normal, that is, adult females spawn 20 to 30 eggs per day everyday under controlled temperature and lighting conditions. These stocks are stably maintained in our laboratory at Nagoya University. Sex markers for seven medaka strains or stocks are summarized in Table 3.

Table 3 Medaka stocks and genetic sex markers

Strains	*lf*	*r*	*SL1*
Orange-red	-	-	-
d-rR	-	H14 days 0.2%	-
Hd-rR	-	H14 days ?	-
Hd-rR.Y^{HNI}	-	H14 days ?	0%
Qurt	E2 days 2.2%	-	?
FLF	E2 days 4.2%	-	0%
FLFII	E2 days 1.4%	H14 days 0%	0%

Hd-rR: inbred strain of d-rR (8).
E2 days: embryos two days after fertilization.
H14 days: fry 14 days after hatching.
%: percentage of recombination frequency.

3. Medaka model for testing based on bioimaging

3-1. See-through medaka

The bodies of most vertebrates are opaque, and thus internal organs are not visible from the outside. This makes noninvasive studies of internal organs difficult in vertebrate models. The opacity of the fish body is mainly due to the existence of pigment cells in the skin, peritoneum, and some other tissues. In the see-through medaka , most of the pigments are genetically removed by crossing selected color mutants (33). In this stock, the main internal organs, namely, the heart, spleen, blood vessels, liver, gut, gonads, kidney, brain, spinal cord, lens, air bladder, and gills, in a living adult fish are visible to the naked eye or can be visualized under a simple stereoscopic microscope (Fig. 5).

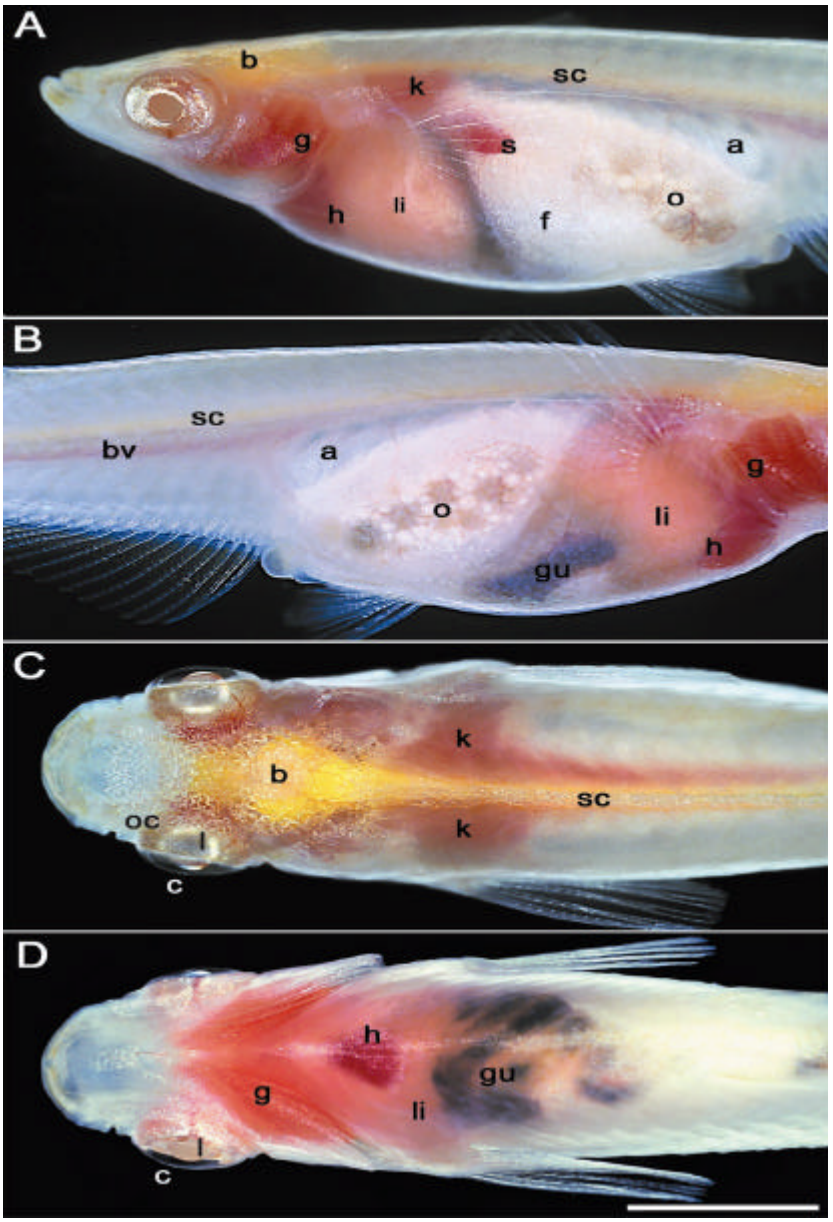

Figure 5.

Adult STIII fish. The left (A) and right (B) sides of a female body. The dorsal (C) and ventral (D) views of a male body. a, air bladder; b, brain; bv, blood vessels; c, conjunctiva; f, fat tissue; g, gill; gu, gut; h, heart; k, kidney; l, lens; li, liver; o, ovary; oc, optic cup; s, spleen; sc, spinal cord. The dark color of the gut is due to ingested feed. The bar represents 4 mm (33).

3-2. *GFP* transgenic see-through medaka

A transgenic see-through medaka carries the *GFP* gene fused to the promoter region of the medaka *vasa* gene that exhibits a germline-specific expression. In the *GFP* transgenic see-through medaka, the expression of *GFP* is evident in the gonad not only at embryonic stages, but also at postembryonic stages through the transparent body wall and peritoneum (33). The testis and ovary can be clearly observed under excitation light (Fig. 6). The fluorescent tag is effective in visualizing the internal organs and affords a unique opportunity to monitor in vivo expressions of tissue-specific genes during all stages of the life cycle of the see-through medaka. Using these transgenic see-through medaka, we can evaluate the effects of endocrine-disrupting substances as changes in the fluorescence image. Research on the applicability of *GFP*-transgenic see-through medaka in in vivo testing is in progress (34, 35).

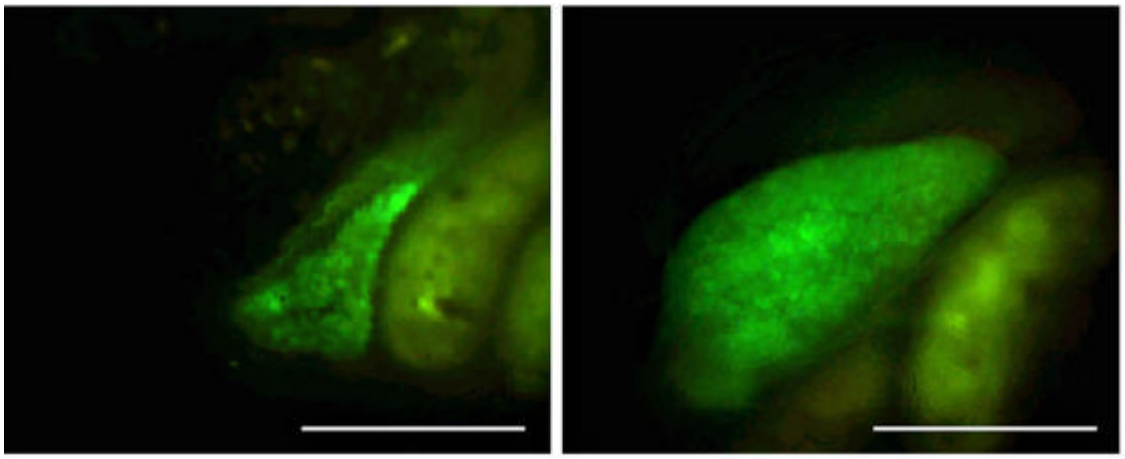

Figure 6.

The testis (left) and ovary (right) of a young adult of the transgenic see-through stock expressing the *olvas-GFP* gene (STII-Y1). This stock carries the leucophore marker (Arrowhead). The fish are oriented with its head on the right side. The bar represents 1 mm.(Unpublished data)

Acknowledgements

The Figure 6 is a photograph of the *olvas-GFP* transgenic see-through medaka generated by Wakamatsu, Y., Pristyazhnyuk, S., Kinoshita, M., Tanaka, M., and Ozato, K.

4. Conclusions

Medaka is a useful model for testing endocrine-disrupting substances by virtue of its biological characteristics. In particular, medaka is advantageous for determining the effects of these substances on reproduction, because the molecular and cellular mechanisms of sex determination and differentiation have been extensively studied in this species. The male-determining gene, first discovered in nonmammalian species, is a key tool for future toxicogenomics. Medaka stocks whose sex markers had been identified make testing reliable. The see-through medaka enables visualization of the effects of chemicals in living fish from the embryonic to the adult stage.

References

1. Medakafish Homepage. (1995) http://biol1.bio.nagoya- u.ac.jp:8000/
2. Medaka Toxinet (2001) http://medaka.fish.agr.kyushu-u.ac.jp/~ishidu/bbs/flashlst.cgi
1. Wakamatsu, Y., Inoue, C., Hayashi, H., Mishima, N., Sakaizumi, M. and Ozato, K. Establishment of new medaka (*Oryzias latipes*) stocks carrying genotypic sex markers. Env. Sci. in press.
2. Wakamatsu, Y. and Ozato, K. (2002) Medaka (*Oryzias latipes*) as a fish model for endocrine-disrupting substance testing. Env. Sci. 9:419-426.
3. Yamamoto, T. (1975) Medaka (*Killifish*) Biology and Strains. Keigaku Publishing. Tokyo.
4. Ozato, K., Wakamatsu, Y. (1994) Developmental genetics of medaka. Develop. Growth Differ., 36: 437-443.
5. Wittbrodt, J., Shima, A., and Schartl, M.(2002) Medaka-a model organism from the Far East. Nat. Rev. Genet., 3:53-64
6. Hyodo-Taguchi Y. and Egami N. (1985) Establishment of inbred strains of the medaka *Oryzias latipes* and the usefulness of the strains for biomedical research. Zool. Sci., 2: 305-316.
7. Tomita, H. (1992) The lists of the mutants and strains of the medaka, common gambusia, silver crucian carp, goldfish, and golden venus fish maintained in the Laboratory of Freshwater Fish Stocks, Nagoya University. Fish Biol. J. Medaka 4: 45-48 (1992).
8. Naruse, K., Fukamachi, S., Mitani, H., Kondo, M., Matsuoka, T., Kondo, S., Hanamura, N., Morita, Y., Hasegawa, K., Nishigaki, R., Shimada, A., Wada, H., Kusakabe, T., Suzuki, N., Kinoshita, M., Kanamori, A., Terado, T., Kimura, H., Nonaka, M., Shima, A. (2000) A detailed linkage map of medaka, *Oryzias latipes*: comparative genomics and genome evolution. Genetics 154:1773-1784.
9. Matsuda, M., Kawato, N., Asakawa, S., Shimizu, N., Nagahama, Y., Hamaguchi, S., Sakaizumi, M., Hori, H. (2001) Construction of a BAC library derived from the inbred Hd-rR strain of the teleost fish, *Oryzias latipes*. Genes Genet. Syst., 76:61-63.
10. Ishikawa, Y. (2000) Medakafish as a model system for vertebrate developmental genetics. Bioessays, 22(5):487-495.
11. International Symposium on Medaka Genome (Organizers, Akira Kudo and Hiroyuki Takeda), Jan. 15, 2003, Hitotsubashi Memorial Hall, Tokyo.

12. Aida, T.(1921) On the inheritance of color in a freshwater fish, *Aplocheilus latipes* Temminck and Schlegel, with special reference to sex-linked inheritance. Genetics, 6:554-573.
13. Yamamoto, T.(1953) Artificially induced sex reversal in genotypic males of the medaka (*Oryzias latipes*). J. Exp. Zool., 123:571-594.
14. Nakamura, M., Chang, X-T., Kobayashi, T., Kajiura, H., and Nagahama, Y. (1998) Gonadal sex differentiation in fish. J Exp Zool. 281: 1-13.
15. Wada, H., Shimada, A., Fukamachi, S., Naruse, K., Shima, A. (1998) Sex-linked inheritance of the lf locus in the medaka fish (*Oryzias latipes*). Zool. Sci., 15: 123-126.
16. Matsuda M., Kusama T., Oshiro T., Kurihara, Y., Hamaguchi, S., Sakaizumi, M. (1997) Isolation of a sex chromosome-specific DNA sequence in the medaka, *Oryzias latipes*. Genes Genet. Syst., 72: 263-268.
17. Matsuda, M., Matsuda, C., Hamaguchi, S., Sakaizumi, M. (1998) Identification of the sex chromosomes of the medaka, *Oryzias latipes*, by fluorescence in situ hybridization. Cytogenet. Cell Genet., 82:257-262.
18. Matsuda, M., Nagahama, Y., Shinomiya, A., Sato, T., Matsuda, C., Kobayashi, T., Morrey, C.E., Shibata, N., Asakawa, S., Shimizu, N., Hori, H., Hamaguchi, S., Sakaizumi, M. (2002) *DMY* is a Y-specific DM-domain gene required for male development in the medaka fish. Nature, 417: 559-563
19. Nanda, I., Kondo, M., Hornung, U., Asakawa, S., Winkler, C., Shimizu, A., Shan, Z., Haaf, T., Shimizu, N., Shima, A., Schmid, M. and Schartl, M. (2002): A duplicated copy of DMRT1 in the sex-determining region of the Y chromosome of the medaka, *Oryzias latipes*. *Proc. Natl. Acad. Sci. USA* 99: 11778-11783.
20. Bagnara, J. T. (1998): Comparative anatomy and physiology of pigment cells in nonmammalian tissues. In: J, J. Nordlund et al. Eds. The Pigmentary System: Physiology and Pathophysiology. Oxford Univ. Press, Oxford, pp. 9-40.
21. Fujii, R. (1993): Cytology of fish chromatophores. *Int. Rev. Cytol.* 143: 191-255.
22. Fukamachi, S., Shimada, A. and Shima, A. (2001): Mutations in the gene encoding *B*, a novel transporter protein, reduce melanin content in medaka. *Nature Genet.* 28: 381-385.
23. Gray, M. A. and Metcalfe, C. D.(1997) Induction of testis-ova
(i) in Japanese medaka (*Oryzias latipes*) exposed to *p*-nonylphenol.

(ii) Environmental Toxicology & Chemistry, 16: 1082-1086.

24. Edmunds, J. S., McCarthy, R. A., Ramsdell, J. S(2000) Permanent and functional male-to-female sex reversal in d-rR strain medaka (*Oryzias latipes*) following egg microinjection of o,p'-DDT. Environmental Health Perspectives, 108: 219-24.

25. Scholz, S. and Gutzeit, H. O. (2000) 17-alpha-ethinylestradiol affects reproduction, sexual differentiation and aromatase gene expression of the medaka (*Oryzias latipes*). Aquatic Toxicology, 50: 363-373.

26. Villalobos, S. A., Papoulias, D. M., Meadows, J., Blankenship, L., Pastva, S. D., Kannan, K., Hinton, D. E., Tillitt, D. E., Giesy, J. P. (2000) Toxic responses of medaka, d-rR strain, to polychlorinated naphthalene mixtures after embryonic exposure by in ovo nanoinjection: A partial life-cycle assessment. Environmental Toxicology & Chemistry, 19: 432-440.

27. Hagino, S., Kagoshima, M., Ashida, S. (2001a) Effects of ethinylestradiol, diethylstilbestrol, 4-t-pentylphenol, 17-estradiol, methyltestosterone and flutamide on sex reversal in S-rR strain medaka (*Oryzias latipes*). Environmental Sciences, 8: 75-87.

28. Hagino, S., Kagoshima, M., Ashida, S. (2001b) Evaluation of anti-androgenic effect of flutamide on sex reversal of S-rR medaka strain. Environmental Sciences, 8: 174-175.

29. Iwamatsu, T. (1994): Stages of normal development in the medaka *Oryzias latipes*. *Zool. Sci.* **11**: 825-839.

30. Hatakeyama, S., Sugaya, Y., Takagi, H., Ishikawa, E., Ozato, K., Wakamatsu, Y. (2001) Effects of 17â-estradiol(E_2) on sex reversal of male medaka (*Oryzias latipes*), FLF strain, and reproductive potential of the sex-reversed or not reversed individuals. Jpn. J. Environ. Toxicol., 4, 99-111.

31. Wakamatsu, Y., Pristyazhnyuk, S., Kinoshita, M., Tanaka, M., Ozato, K. (2001) The see-through medaka: A fish model that is transparent throughout life. Proc. Natl. Acad. Sci. USA, 98:10046-10050.

32. Hano, T., Ohshima, Y., Oe, T., Kinoshita, M., Tanaka, M., Wakamatsu, Y., Ozato, K., and Honjo, T. (2002). Rapid assay for detecting the alteration of sexual differentiation caused by ethinylestradiol using transgenic medaka. Abstract Book. SEATAC 23rd Annual Meeting in North America. pp.65.

35. Wakamatsu, Y., Pristyazhnyuk, S., Kinoshita, M., Tanaka, M., Ozato, K. (2002). The see-through medaka: A fish model for bioimaging throughout life. Abstract Book. SEATAC 23rd Annual Meeting in North America. pp.76.