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—Review—
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Zebrafish Research in Japan and the National BioResource Project

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Abstract: The zebrafish is the simplest model vertebrate amenable to genetics, and genome information and methods of embryo manipulation have been accumulated worldwide. The numbers of mutant and transgenic zebrafish strains are rapidly increasing, and these strains will play important roles in the basic biology research and as model systems of the human diseases in the future. Although researchers who had established zebrafish strains, were distributing the fish on a discretionary basis, a well-established system for distributing the strains did not exit in Japan prior to 2003. Due to these circumstances, a system to collect, preserve, and provide zebrafish strains was established as part of the National BioResource Project useful model vertebrates in Japan and to the world.
Key words: Japanese contribution, national bioresource project, zebrafish research

A Brief History of Zebrafish as a Vertebrate Model Animal

Since George Streisinger first reported in his monumental paper on the production of clones of homozygous diploid zebrafish by hydrostatic pressure treatment, zebrafish have been widely used as a vertebrate model animal (Fig. 1A) [28]. They are suitable for genetic analysis because of their relatively short generation (about three months) and the easiness of their breeding. They are also suitable for developmental analysis because they are transparent throughout the entire embryonic period (Fig. 1B). This characteristic makes possible the precise time-lapse imaging of a single embryonic cell for lineage tracing, or a single neuron for analyses of axonal pathfinding in live embryos [17, 33]. Zebrafish were the first vertebrate for which a large scale mutagenesis study and screening were performed to the saturation level with a chemical mutangen, ethyl nitrosourea (ENU) [20, 34]. A large-scale insertional mutagenesis study using a retrovirus followed this [2]. For the genetic analysis of the mutants, the zebrafish research community has made concerted efforts to establish several key technologies such as radiation hybrid panels [6, 10], BAC libraries [1], and whole genome sequencing [25]. The development of antisense morpholino oligonucleotides has made it easy to repress translation or splicing of a target gene, making it easy and quick to confirm the candidate genes identified by positional cloning as the genes responsible for the mutant phenotypes [21]. The results obtained from analyses of zebrafish mutants, including the positional cloning of responsible genes, have not just brought about novel knowledge in the study of early embryonic development, but have also opened

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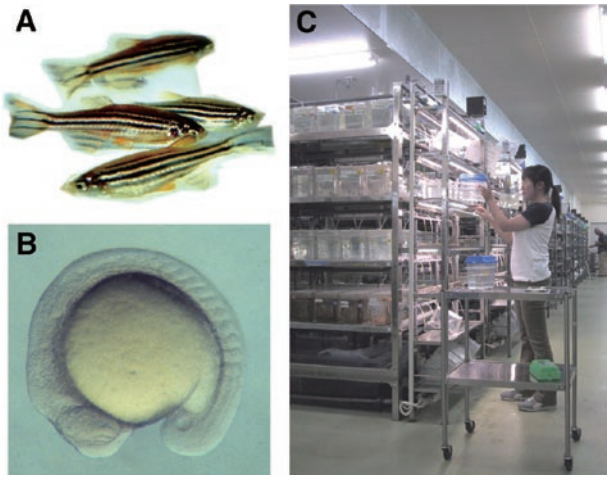

Fig. 1. Zebrafish and the facility for National BioResource Project Zebrafish. A) Adult zebrafish. B) A zebrafish embryo at the 16-somite stage. C) The central facility for National BioResource Project Zebrafish at RIKEN.

totally new research fields such as the studies of molecular mechanisms of organogenesis, including those of the intestinal organs, heart, blood vessels, bones, and brain [16, 22, 24, 27, 32].

Contribution of the Japanese Community for Advancement of Zebrafish Research

Since the first research paper on zebrafish development by researchers in Japan was published in 1992 [19], researchers in Japan have made several significant and unique contributions to the advancement of zebrafish research. Theses include the establishment of a transgenic fish line [*Tg*(*isl1:GFP*)] expressing green fluorescent protein (GFP) in a specific subset of neurons under the regulation of the motor neuron-specific enhancer of the *isl1* gene (Fig. 2A) [8], a large-scale mutant screening using *Tg*(*isl1:GFP*) which led to identification of

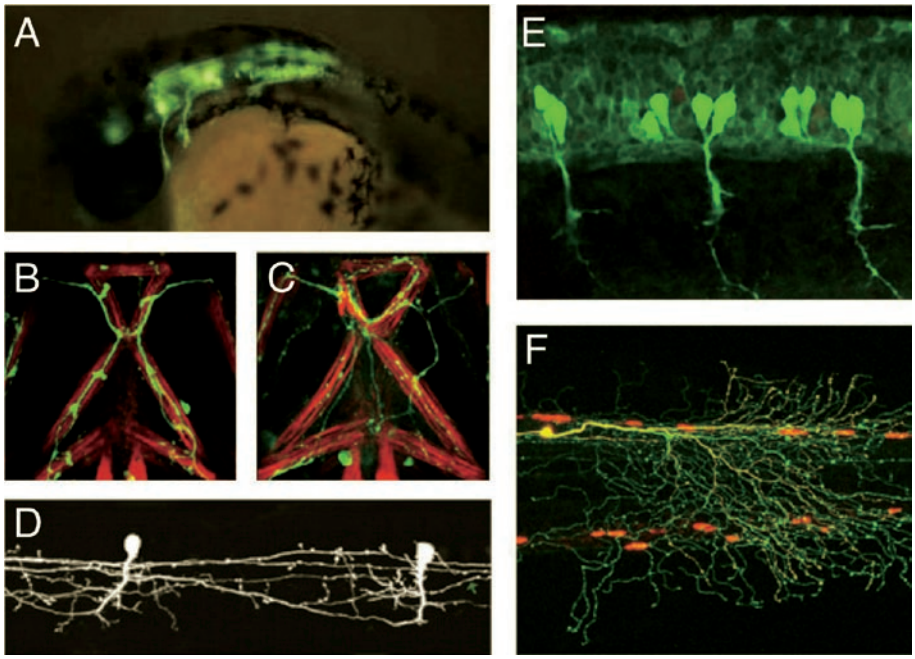

Fig. 2. Representative zebrafish strains stored by National BioResource Project Zebrafish. A) A *Tg*(*isl1:GFP*) transgenic embryo at 2-day post fertilization [8]. B, C) The axons of the trigeminal and facial motor nerves of the wild type (B) and mutant (C) *Tg*(*isl1:GFP*) transgenic embryo innervating the jaw muscles [29]. D) A transgenic line expressing GFP in the spinal interneurons [9], courtesy of Dr. Shinichi Higashijima. E) An enhancer trap line expressing GFP in the spinal primary motor neurons [15], courtesy of Dr. Koichi Kawakami. F) A transgenic line expressing Kaede in all spinal primary neurons. Exposure of a single Rohon-Beard sensory neuron to ultraviolet light turns the color of its axon from green to red, enabling precise tracing of the peripheral axons [26].

many mutants with defects in differentiation and axogenesis of motor neurons (Figs. 2B and 2C) [23, 29–31], development of a method to regulate the activity of exogenously applied genes arbitrarily in embryos by photo-activation of caged mRNA [3, 4], and the establishment of a method to drastically improve the efficiency of transgenesis using a transposon, Tol2 [11].

Establishment of the Tol2-mediated transgenesis method is particularly worthy of a special remark as it was the culmination of close collaboration between two research communities in Japan, one, a newly established research community using zebrafish and the other, a long-established research community, using medaka, fresh water fish with a long history of study in Japan. The Tol2 transposon was first identified in the Medaka genome by Koga and Hori [18]. The insertion of this transposon was responsible for the phenotype of the *albino* mutant, which was maintained in the mutant collection made and maintained by Yamamoto and Tomita at Nagoya University. In collaboration with Koga and Hori, Kawakami showed the activity of Tol2 transposase in zebrafish [12], and Kawamai finally succeeded in establishing a system to use Tol2 for the transgenesis of zebrafish [13, 14].

National BioResource Project (NBRP) of Japan for Zebrafish

As the number of researchers using zebrafish increased in Japan, a problem emerged. Reflecting the short history of the zebrafish as a model animal, the researchers who were using zebrafish as a research animal were generally young and working in relatively small groups in Japan. They were finding difficulty in maintaining the transgenic and mutant strains once reports on these strains had been published and if the researchers who created the strains left their positions. Especially after establishment of the enhancer and gene trap methods by Kawakami [15], the number of the strains worth maintaining dramatically increased. This situation increased demands for the establishment of the National BioResource Project of Japan for Zebrafsh (NBRP Zebrafish). This project included the establishment of a central stock center in 2003 at the campus of RIKEN in Wako, Saitama which is run by Hitoshi Okamoto and Yoshihiro Yoshihara. Its mission is to collect and store zebrafish strains created in Japan and to supply them to meet not only domestic but also worldwide demands (Fig. 1C). The stock center at RIKEN stores more than 400 strains, and supplies about 100 strains a year to users both inside and outside Japan (Figs. 2A–C and 2F). In addition to RIKEN, two other groups have joined this project: a group led by Koichi Kawakami at the National Institute for Genetics, Mishima, Shizuoka, takes responsibility mainly for the enhancer trap and gene trap strains generated by Tol2-mediated methods (Fig. 2E); and another group led by Shinichi Higashijima at the Institute for Integrative Bioscience, Okazaki, Aichi, takes responsibility for maintaining and supplying transgenic strains for analyzing spinal neurons (Fig. 2D) [7].

The web site for NBRP Zebrafish can be found at http://www.shigen.nig.ac.jp/zebra/index_en.html by way of the NBRP web site at http://www.nbrp.jp/report/reportProject.jsp?project=zebrafish.

For Further Readings

Readers who are interested in various experimental techniques for studies using zebrafish should refer to the two volumes of books edited by Detrich, Westerfield, and Zon [5].

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