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Review Article

Medaka as model animal and current status of medaka biological resources

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Abstract - The medaka species is used as a model animal throughout the world. As an experimental animal, it is similar to zebrafish but has important distinguishing features. As it lives in the temperate zone, medaka spawning is influenced by photoperiods and circadian rhythms. Medaka can adapt to lower temperatures in winter and higher temperatures in summer. Adaptation to high salinity has also been observed. Medaka research has been supported by important biological resources hosted by the National Bio-Resource Project (NBRP) since 2002. NBRP Medaka is a central repository of medaka resources that provides the research community with fish, cDNA/BAC/fosmid materials, hatching enzyme, useful databases and, more recently, a TILLING library for mutant screening and a genome editing platform based on CRISPR-Cas9. NBRP Medaka continues to support the medaka research community by enhancing productivity, continuity and reproducibility.

Keywords: Medaka, photoperiod, salinity, lower temperature, resources

1. Common and unique futures of medaka as model animal

Medaka serves as an alternative model that is both comparable and complementary to zebrafish (Table 1) (Wittbrodt *et al*., 2002). The two fish species have many similar features, such as a short generation time, established tools for embryonic manipulations, genome resources (high-density genetic map and draft genome sequence), transgenic technology and genome editing methods such as TALENs and CRISPR-Cas9 (Kawahara *et al*., 2015). Recent progress in medaka genetics and genomics tools has been reviewed by Kirchmaier *et al*. (2015). The generation time of medaka is 6 to 10 weeks, depending on feeding conditions such as the frequency of feeding and space for rearing. Medaka is a daily spawner and lays 10 to 30 eggs/day, resulting in 70-210 eggs/week, which is similar to zebrafish fecundity (Naruse *et al*., 1994). The breeding strategy of medaka is quite different from other model fishes, such as zebrafish and stickleback. As medaka is native to the temperate zone, the maturation of oocytes is strongly influenced by day and night length (Koger *et al*., 1999). Medaka is a long-day animal, and a daytime period >13.5 hours is essential for LH/FSH release through GnRH secretion (Urasaki, 1975) (Awaji and Hanyu, 1988). Even at higher temperatures (greater than 20 °C), a shorter period of daylight (≤13 hours) inhibits oocyte maturation and breeding is arrested (Awaji and Hanyu, 1988). Regional variations in the critical period of day length have also been reported (Sawara and Egami, 1977). Oocyte maturation is also strictly controlled by the circadian rhythm during the breeding season (Iwamatsu, 1978) (Ueda and Oishi, 1982). Thus, the day/night cycle must be fixed to obtain eggs at a fixed time. Alternatively, the timing of oocyte maturation can be controlled by changing the start and end times of the daylight period. Medaka can adapt to a low temperature (4 °C) in winter and a high temperature (37 °C or higher) in summer. Adaptation to a lower temperature occurs even in embryos, and embryonic development can thus be reversibly controlled by a temperature shift from 25 °C to 4 °C (Sampetrean *et al*., 2009; Valdez *et al*., 2005). The relationship between temperature and the rate of development at each stage is illustrated in Figure 1. Medaka also displays tolerance to high-salinity conditions (Inoue and Takei, 2002). Medaka can survive direct transfer from freshwater to 1/2 sea water. After acclimatization in half seawater for five days, medaka can survive in seawater (Ogoshi *et al*., 2012) (Kang *et al*., 2008). In addition to *Oryzias latipes, O. dancena* (previously named *O. melastigma*) and *O. javanicus* are actively used to study seawater adaptation (Kang *et al*., 2008; 2013) and marine ecotoxicology (Imai *et al*., 2007). *O. javanicus* lives in mangrove swamps from Thailand to Indonesia and thus it is a marine fish; its life cycle can only be completed in seawater. *O. dancena* lives in both

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freshwater and seawater from the east coast of India to Malaysia (Yusof *et al*., 2012), and its life cycle can be completed in freshwater and seawater (Inoue and Takei, 2003). Medaka and its relatives provide a unique opportunity to analyze the mechanisms of adaptation to various environments.

Table 1. Comparison of charactors between zebrafish and medaka.

Character	Zebrafish	Medaka
Generation time	12 weeks	6-10 weeks
Sex determination	ZZ-ZW (not identified)	XX-XY (DMY/DMRT1Yb gene)
Eegg envelope	Soft	Hard
Fecundity	100-200 eggs / week	10-30 eggs /day
Chromosome numbers	25 pairs	24 pairs
Genome size	1700Mbp	800Mbp
Number of inbred lines	3	13
SNP rate among strains	1%	4%
High density genetic map	Aavailable	Available
Genome analysis	Finished	Finished
Transgenic technology	Well established	Well established
Upper and lower survival temp.	35-20°C	37-4 °C
ES like cells	Not available	Available
Upper and lower salinity range	No data	Survive in the half sea-water
Sperm cryopreservation	Well established	Well established
Gene and/or enhancer trap method	Well established	Available
Reverse genetic approach	Genome editing /TILLING	Genome editing/TILLING

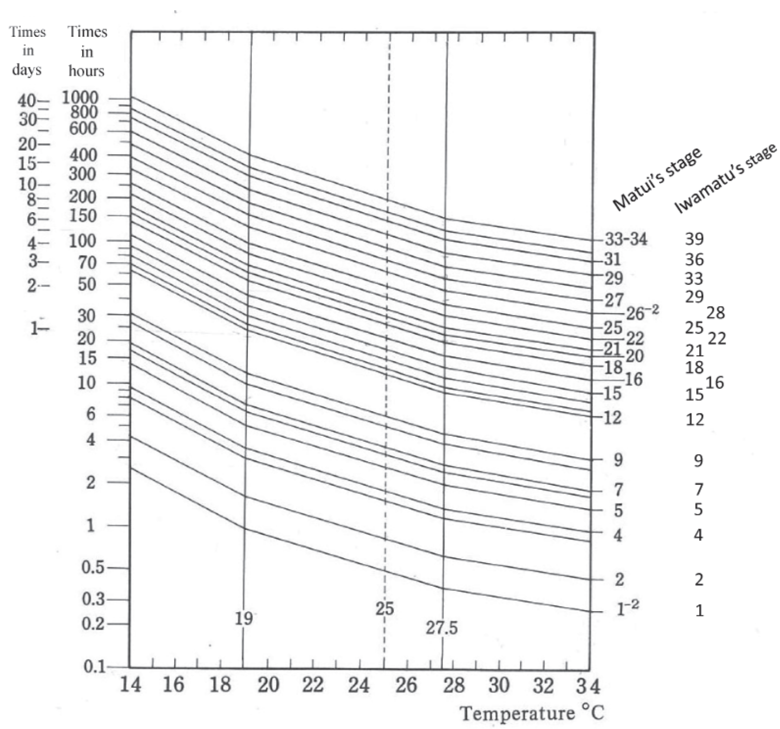

Figure 1. Time required to reach different developmental stages at different temperatures. The left ordinate axis indicates the time needed to reach the developmental stage in hours and days. The right ordinate axis shows the developmental stages according to Matui's developmental staging series (Yamamoto, 1975) and Iwamatsu's developmental staging (Iwamatsu, 1994; 2004). The abscissa indicates temperature in degrees Celsius. Modified from MEDAKA (KILLIFISF) Biology and Strains (Yamamoto, 1975).

2. National BioResource Project Medaka (NBRP Medaka)

NBRP was launched by MEXT in 2002 to support the research community in Japan as well as other countries, and the project is now in its third term. NBRP Medaka (https://www.shigen.nig.ac.jp/medaka/) is a central repository of medaka resources such as transgenic lines, mutants, inbred lines, wild stocks and related species and cDNA/BAC/fosmid clones. NBRP provides three requestable resources (medaka strains, cDNA/BAC/fosmid clones and hatching enzyme). Each resource can be ordered online using a shopping cart system. The provision of inbred lines, congenic/consomic lines and related medaka species are unique to NBRP Medaka. There are 300,000 full-length cDNA and EST clones available, as well as 55,000 BACs and 230,000 fosmid clones providing 20x coverage of the genome. BAC/fosmid resources are useful for establishing transgenic fish by in vivo homologous recombination. The hatching enzyme, which can digest the hard medaka chorion (egg envelope), enables embryonic manipulations such as chimera formation and somite transplantation. In addition to these resources, NBRP Medaka provides several databases. There are atlases of the brain and vascular system at embryonic stages 24 to 30 and in the adult (https://www.shigen.nig.ac.jp/medaka/medaka_atlas/). A phylogenetic tree (http://www.actioforma.net/nibb/medaka/index.html) that describes the relationships of wild medaka populations and closely related species. A physical map of the genome is provided in a browser (http://viewer.shigen.info/medakavw/mapview/) that can display the genomic positions of the cDNA/BAC/fosmid clones. This database is useful for identifying clones that contain a gene of interest as identified by a BLAST search (http://viewer.shigen.info/cgi-bin/blast/blast.cgi). It is also possible to search for CRISPR target sites with micro-homology, with links to CCTOP-CRISPR/Cas9 target online prediction (http://crispr.cos.uni-heidelberg.de/index.html) (Stemmer *et al*., 2015); these features are helpful for creating gene knock-outs by genome editing. Exp VD (http://www.shigen.nig.ac.jp/medaka_vd_cgi-bin/main.pl?name=medaka_091023_ex10&type=c) shows the expression profile of each gene across 11 cDNA libraries and is used in a full-length cDNA sequencing project. This application can be used to identify genes with similar expression patterns. The Medaka Book and Laboratory Manual provides several experimental protocols, such as sperm cryopreservation and artificial insemination, and describes the husbandry of see-through medaka, which can be raised on paramecia (https://www.youtube.com/watch?v=hNvHPSnbxHM) (https://www.shigen.nig.ac.jp/medaka/download/strain/150205_Chisada_ver2_Breeding_for_See-through_medaka.pdf). Screening for mutants using a TILLING library is also described (https://www.shigen.nig.ac.jp/medaka/strain/aboutTilling.jsp), and a TALEN assembly protocol is provided (https://www.shigen.nig.ac.jp/medaka/download/TALEN_protocol.pdf).

3. Support for visiting researchers

NBRP Medaka provides laboratory facilities for screening the TILLING library, constructing sgRNA plasmids and microinjecting sgRNA and Cas9 into embryos (Fig. 2). Users are able to work in the NBRP Medaka facility as visiting researchers. The National Institute for Basic Biology has individual collaborative research projects (http://www.nibb.ac.jp/en/collabo/collabo.html) that are collaborations between researchers from other institutions and the professors, associate professors and assistant professors of NIBB. These projects are established and conducted on a case-by-case basis. NIBB can cover travel and accommodation costs for researchers within Japan who submit successful applications for support.

Figure 2. Fish facility (left) and microinjection apparatus (right) for visiting researchers in NBRP Medaka. Users have access to their own fish racks during their stay at NIBB. Fish caretakers and lab technicians assist with screening of the TILLING library, constructing sgRNA plasmids for CRISPR-Cas9 genome editing and microinjecting eggs.

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