

Screening for Induced Point Mutations in Medaka with TILLING

Takeshi Todo

(Osaka University, Department of Radiation Biology and Medical Genetics, Graduate School of Medicine, Professor)

【Outline of survey】

One of the most important tools in biological research is mutational analysis. Our understanding of the basic mechanism of disease has been transformed by the systematic application of mutational analysis. One of the most widely used approaches is forward genetics, which is driven by the identification of mutant phenotypes. Another approach is reverse genetics. The most widely used reverse genetics method in zebrafish and Medaka is undoubtedly the use of morpholinos, but this method is not a substitute for mutations because it is a transient method and only suited for early developmental stages. Recently a general reverse genetics method was reported, which can identify mutations in genes that are known only by their sequence. The method, called TILLING (Targeting Induced Local Lesions IN Genome), includes random mutagenesis, followed by screening for induced mutations in target genes at the genomic DNA level. Adult Cab male Medaka were mutagenized with ENU and then outcrossed with Cab female to generate F1 progeny for the library. We established 5771 ENU-mutagenized F1 male fishes. To construct a library, genomic DNA and testis samples were isolated and cryopreserved from each F1 fish. We have also established a system for screening of mutations. A pilot screening revealed that the average per-base mutation frequency was 1 in 300 kbp. This reverse genetic approach will be combined with transgenic technique to establish in vivo system for analyzing gene function in Medaka. In this proposal we will focus on damage response.

【Expected results】

Establishment of reverse genetics will make Medaka a useful model animal not only for classical Genetics but also for Molecular Genetics. We will apply this method to establish the Medaka model for human diseases.

【References by the principal investigator】

- Taniguchi Y, Kamei Y, Todo T, et al. Generation of medaka gene knockout models by target-selected mutagenesis. *Genome Biol.* 2006, 7(12):R116
- H. Mitani, Y. Kamei, T. Todo, N. Shimizu et al. Medaka: a model fish in comparative and functional genomics. In: "Genome dynamics vol.2 Vertebrate Genomes" (ed. By Jean-Nicolas Volf) KARGER, Basel, Switzerland, pp165-182 (2006)
- M. Furutani, T. Todo, H. Mitani, H. Kondoh et al. A systematic genome-wide screen for mutations affecting organogenesis in Medaka, *Oryzias latipes*. *Mech Dev.* 121:647-58.2004

【Term of project】 FY2007 – 2011

【Budget allocation】 21,000,000 yen
(2007 direct cost)

【Homepage address】

<http://www.med.osaka-u.ac.jp/pub/radbio/www/index-jp.html>