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Brief Report

Genetic mapping of the medaka *pectoral-finless* (*pl*) mutant locus

Masato Ohtsuka*, Natsuko Kikuchi*, Minoru Kimura*, Kenjiro Ozato and Kinya Yoda****

**Division of Basic Molecular Science and Molecular Medicine, School of Medicine, Tokai University, Kanagawa 259-1193, Japan and **Laboratory of Freshwater Fish Stocks, Bioscience and Biotechnology Center, Nagoya University, Nagoya, 464-8601, Japan*

The spontaneously isolated *pectoral-finless* (*pl*) mutant of medaka lack pectoral fins (Tomita, 1993). Inheritance of the *pl* phenotype is autosomal and recessive. In the *pl* mutant, the pectoral fin bud disappears in the early stages of development following the appearance of the apical ectodermal ridge (AER) (Okamoto and Kuwada, 1991). From the fact that pectoral fins of fish correspond to the forelimbs of higher vertebrates (Coates, 1995), *pl* mutant is regarded as a good model for the study of limb development. The causative gene of the *pl* mutant, however, has not yet been isolated.

Mapping of the mutant locus is the first prerequisite to isolating the causative gene of a mutant using positional cloning or positional candidate gene approach. Although linkage of the *pl* locus to eight phenotypic markers were examined by Tomita (1993), none of them were linked to the *pl* locus. Therefore, mapping of the *pl* locus remains to be determined. Recent development of several kinds of polymorphic DNA markers enable us to rapidly accumulate genome-wide markers. Utilizing these markers, genetic linkage maps of the medaka have been constructed (Ohtsuka *et al.*, 1999, Naruse *et al.*, 2000). Because each marker is randomly distributed in the medaka genome, any gene or mutation is expected to be mapped.

To map the *pl* mutant locus, we generated 58 backcross progeny from the cross between

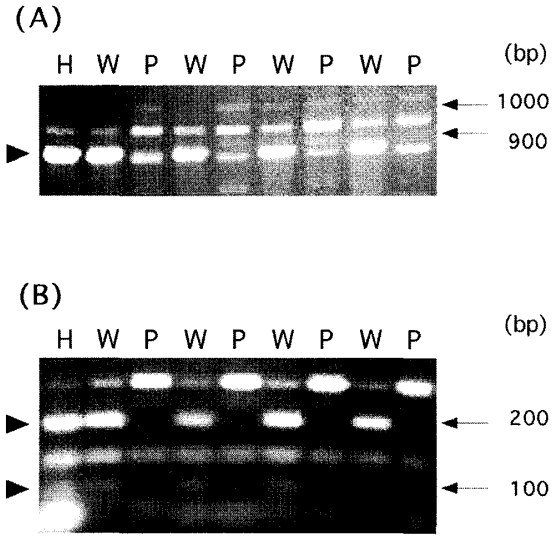

Fig. 1 Polymorphic marker linked to *pl* locus. (A) RAPD marker "M76-2" (arrowhead). (B) STS marker "stsM76-2" derived from the original RAPD marker "M76-2" was digested with *Msp*I. HNI strain-specific polymorphisms (arrowheads) were cosegregated with backcross progeny showing the wildtype phenotype. H: HNI strain, W: backcross progeny with wildtype phenotype, P: backcross progeny with *pl* phenotype.

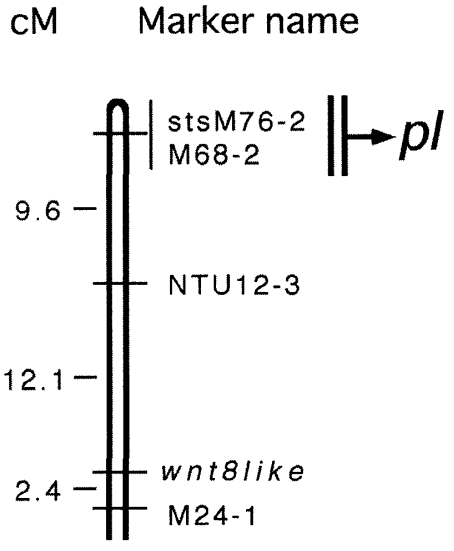

Fig. 2 *pl* locus is on LG XIII.

* Corresponding to: Dr. Masato Ohtsuka, Division of Basic Molecular Science and Molecular Medicine, School of Medicine, Tokai University, Kanagawa 259-1193, JAPAN. Tel.: 81-463-93-1121 (ext. 2730), Fax: 81-463-91-9358, E-mail: masato@is.icc.u-tokai.ac.jp

Table 1. STS primer set

Marker Name	Primer pair (5' - 3')	Size (bp)	Polymorphism
stsM76-2	TGGCGTTCCTCATCATC CATCAAGAGTTCAGTAGGGGA	299	RFLP after cutting with *Msp*I

the HNI strain (wildtype; northern population) and the *pl* mutant (southern population). In this study, recombination based on female meioses was examined. After phenotyping at the hatching stage, genomic DNA was isolated and used for mapping. To find markers that linked to the *pl* locus, we screened the random amplified polymorphic DNA (RAPD) primers used for our map construction (Ohtsuka *et al.*, 1999, MEDAKAFISH HOMEPAGE; http://biol1.bio.nagoya-u.ac.jp:8000/RAPD.html). Most of the RAPD markers of our map also showed polymorphisms between the HNI strain and *pl* mutants. Among these markers, M76-2 completely cosegregated with the *pl* locus (Fig. 1A). This indicates that the *pl* locus is located 0 cM (–6.4 cM; 95% confidence level) from the M76-2 on LG XIII of our map (Fig. 2). Regarding this marker, the original RAPD marker was converted to the sequence tagged site (STS) marker (stsM76-2). The STS primer sequences are listed in Table. 1. StsM76-2 was amplified from both strains (HNI and *pl*), and polymorphism was detected by cleaving with *Msp*I (Fig. 1B).

In summary, we mapped the *pl* mutant locus on LG XIII and the closest marker stsM76-2 was obtained. Although a more high resolution mapping around the *pl* locus will be required, stsM76-2 will be a good marker for typing, and may provide a clue in initiating chromosome walking.

Acknowledgements

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