

Molecular Biological Study on Olfactory Imprinting Related Genes in Salmon

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Anadromous salmon are well known for accurate homing migration to their natal river. Since Hasler and his co-workers proposed the olfactory hypothesis of salmon homing in 1950's, many behavioral and electrophysiological studies have reported the important functions of the olfactory system (olfactory epithelium, olfactory nerve, and olfactory bulb) in salmon.

Research on olfactory memory has been mainly investigated in mammals. It is generally thought to be formed by the long-term potentiation (LTP) in synapse. In teleost fish, LTP was detected in the olfactory bulb of lacustrine sockeye salmon (*Oncorhynchus nerka*) during the smolt stage (Satou et al. 1996).

It has been suggested that the down stream migration of salmonids is controlled by various hormones including growth hormone, thyroid hormone and adrenal cortex hormone (Iwata and Hirano 1991). Moreover, the thyroid hormone is thought to be necessary for imprinting to the natal river, because it is driven by facilitation of smolting and down stream migration (Dittman and Quinn 1996).

It is thought that the olfactory bulb of salmonids in term of imprinting may express specific genes in relation with nerves and endocrine systems, since more active nervous activities may occur in the olfactory bulb. By using subtractive method, we have tried to identify olfactory imprinting and homing specific genes in the olfactory bulb of lacustrine sockeye salmon.

Lacustrine sockeye salmon of 1-year-old (1⁺) and 3-year-old (3⁺) reared at the Toya Lake Station, Faculty of Fisheries, Hokkaido University were used. We sampled 1⁺ fish from April to June 2002 (parr-smolt transformation period) and 3⁺ fish in June 2002 (feeding migration period). Fish were anesthetized with 4-allyl-2-methoxyphenol (eugenol), and then olfactory epithelium, gill, liver, heart, head kidney, testes and brain were surgically isolated. Brain tissue was cut into small regions; olfactory bulb, telencephalon, hypothalamus, optic tectum, cerebellum and medulla oblongata. Total RNA isolated from each tissue was reverse-transcribed into cDNA.

To identify a specific gene in the olfactory bulb of 1⁺ fish, the subtractive hybridization technique of representational difference analysis (RDA) was carried out using the olfactory bulbs cDNA of 1⁺ fish in May and 3⁺ fish in June. The basic protocol of RDA method was followed from Niwa et al. (1997). After three cycles of subtractive enrichment, subtractive (1⁺) cDNA library was constructed. Approximately 1,000 clones from this library were picked up and differential screening was performed using subtractive (1⁺, 3⁺) cDNA as a probe. As a result, we obtained 10 clones which showed only the probe (1⁺) positive reaction. Sequence of these clones analysis was carried out using Genetix software (Software Development CO., LTD). The similarity search of the obtained cDNAs was done using the Internet server of the DNA Data Bank of Japan (DDBJ).

Four kinds of partial clones, which were selected from the result of similarity search, were analyzed by semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). A partial sequencing clone which showed the strongest identity to unknown genes of mouse, were expressed only in the olfactory bulb of 1⁺ fish and designated as DNA 138 since its a 138 bp amplified fragment by RT-PCR (Fig. 1). Moreover, the expression of DNA 138 was analyzed in tissues of 1⁺ fish in May, and in the olfactory bulb during the parr-smolt transformation (April–June). Semi-quantitative RT-PCR analysis showed that DNA 138 was expressed in the olfactory epithelium, hypothalamus, and medulla oblongata, but not in other body organs and brain regions (Fig. 2, 3). In the olfactory bulb, the gene expression was down-regulated as the fish transformed from a parr to a smolt (Fig. 4).

Further studies are being carried out to isolate the full-length of DNA 138 clone from cDNA libraries of the olfactory epithelium and whole brain, as well as to observe localization of DNA 138 mRNA using an *in situ* hybridization technique.

Fig. 1. Results of agarose gel electrophoresis of semi-quantitative RT-PCR products. DNA 138 (A) and beta-actin (B). Specific products for DNA 138 and beta-actin mRNAs were amplified from aliquots of the same mRNAs of the olfactory bulb of 1+ in May (lane 1) and 3+ fish (lane 2).

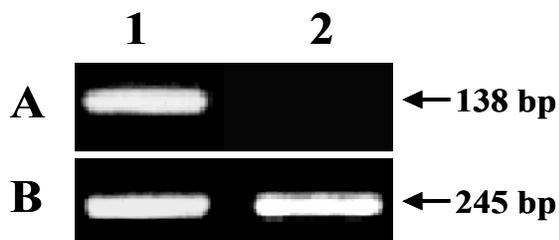


Fig. 2. Results of agarose gel electrophoresis of semi-quantitative RT-PCR products. DNA 138 (A) and beta-actin (B). Specific products for DNA 138 and beta-actin mRNAs were amplified from aliquots of the same mRNAs of the olfactory epithelium (lane 1), gill (lane 2), liver (lane 3), heart (lane 4), head kidney (lane 5) and testis (lane 6) of 1+ fish in May.

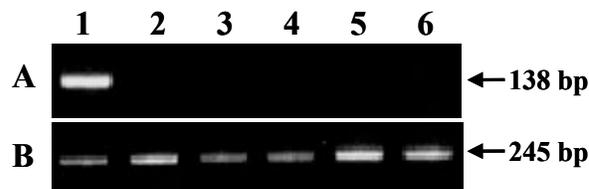


Fig. 3. Results of agarose gel electrophoresis of semi-quantitative RT-PCR products. DNA 138 (A) and beta-actin (B). Specific products for DNA 138 and beta-actin mRNAs were amplified from aliquots of the same mRNAs of the telencephalon (lane 1), hypothalamus (lane 2), optic tectum (lane 3), cerebellum (lane 4) and medulla oblongata (lane 5) of 1+ fish in May.

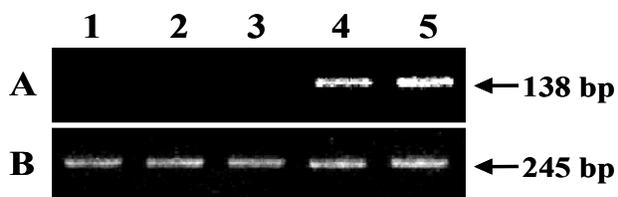
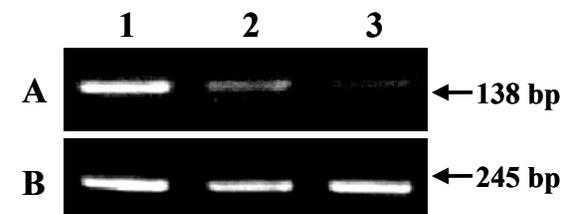


Fig. 4. Results of agarose gel electrophoresis of semi-quantitative RT-PCR products. DNA 138 (A) and beta-actin (B). Specific products for DNA 138 and beta-actin mRNAs were amplified from aliquots of the same mRNAs of the olfactory epithelium (lane 1), gill (lane 2), liver (lane 3), heart (lane 4), head kidney (lane 5) and testis (lane 6) of 1+ fish in May.



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