Identification of an Olfactory Imprinting-Related Gene in Sockeye Salmon

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Salmon accurately home to their natal river and spawning areas. Many behavioral and electrophysiological studies have addressed the important functions of olfactory system (olfactory epithelium, olfactory nerve and olfactory bulb) in salmon (Dittman and Quinn 1996; Shoji et al. 2000). Hasler and his co-workers proposed the olfactory hypothesis on salmon homing in 1950s.

In mammals, the olfactory memory is thought to be formed by the long-term potentiation (LTP) in synapse (Martin et al. 2000). LTP was also detected in the olfactory bulb of lacustrine sockeye salmon (*Oncorhynchus nerka*) at the smolt stage (Satou et al. 1996). Recently, odorant receptors have been isolated from Atlantic salmon (Wickens et al. 2001; Dukes et al. 2006). Dukes et al. (2004) also reported that odorant receptor gene expression changes during the parr-smolt transformation in salmon. However, the molecular basis of olfactory imprinting of salmon is poorly understood.

The cDNA representational difference analysis (cDNA-RDA) is a PCR based subtractive enrichment procedure. It has been adapted to enable the isolation of genes with an altered expression between various tissues or cells (Hubank and Schatz 1994). This technique offers several advantages over other approaches for assessing gene expression, including a low number of false positives. Thus, unwanted difference products can be competitively eliminated and genes producing rare transcripts, which may not be represented in the currently available database, are also detectable. In this study, we attempted to identify imprinting specific genes in the olfactory system of lacustrine sockeye salmon by using the cDNA-RDA method.

We used one and three-year-old lacustrine sockeye salmon reared at the Toya Lake Station, Field Science Center for Northern Biosphere, Hokkaido University. We sampled one-year-old (1^+) smolts fish in the May 2002 and three-year-old (3^+) fish in June 2002. The body color of smolts became silver, with fins that had intense black pigment. All the sub-adults sampled did not have smolt characteristics. Twenty fish were used from each age group. Fish were anesthetized with eugenol (4-ally-2-methoxyphenol) and the olfactory epithelium, gill, liver, heart, head, kidney, spleen, muscle and brains were surgically isolated. The brain was cut into small regions consisting of olfactory bulb, telencephalon, hypothalamus, optic tectum, cerebellum and medulla oblongata.

In the forward subtraction experiment, the double strand cDNA of the olfactory bulb from 1^+ fish was used as a tester, and in reverse subtraction, the tester consisted of 3^+ fish. The cDNA-RDA technique was performed according to the protocol of Niwa et al. (1997). A forward subtractive (1^+) cDNA library was constructed after three cycles of subtractive enrichment. Approximately 1000 white colonies from the forward subtraction library were randomly picked up and differential screening was performed using forward and reverse subtraction product probes. As a result, we obtained a clone that showed a positive reaction in the forward subtraction probe from differential screening. Semi-quantitative RT-PCR was used to find a difference in the expression levels of mRNA between the olfactory bulb of 1^+ and 3^+ fish. The clone was expressed only in the olfactory bulb of 1^+ fish (Fig. 1). We named this partial clone "Sockeye salmon Olfactory system Imprinting related Gene" (SOIG). Expression of SOIG mRNA in the brain and body tissues from 1^+ lacustrine sockeye salmon was analyzed by northern blotting. A strongly hybridized signal corresponding to about 1.7 kb was only detected in the olfactory epithelium (Fig. 2). No signal was detected by northern blot analysis in six

Fig. 1. Results of 3% agarose gel electrophoresis of semi-quantitative RT-PCR product. Specific product for clone and ß-actin mRNAs were amplified from aliquots of the same mRNAs of the olfactory bulb of one-year-old (1^+) and three-year-old (3^+) fish.

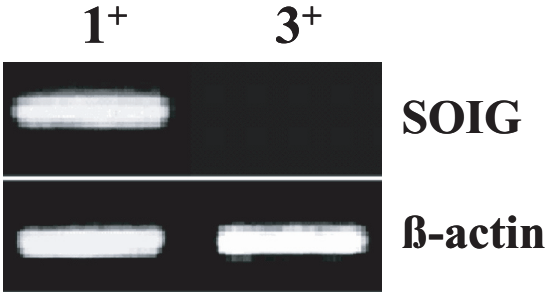

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Fig. 2. Northern blot analysis of 1⁺ lacustrine sockeye salmon SOIG mRNA in the body tissues. Total RNA (10 µg) isolated from body tissues was blotted to nylon membrane. The position of SOIG transcripts is indicated by arrow. The relative positions of 28S and 18S ribosomal RNA are indicated.

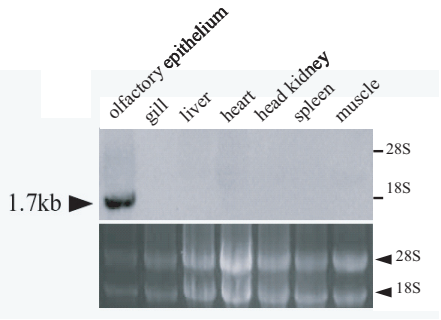

Fig. 3. Expression of SOIG mRNA demonstrated by *in situ* hybridization in the olfactory epithelium of lacustrine sockeye salmon. (A) Adjacent sections were stained with Carazzi's hematoxylin and eosin counter-stain. (B) Section hybridized with antisense probes. (C) Section hybridized with sense probes as a negative control. Olfactory receptor cell and basal cell are indicated a and b. Scale bar = 50 µm.

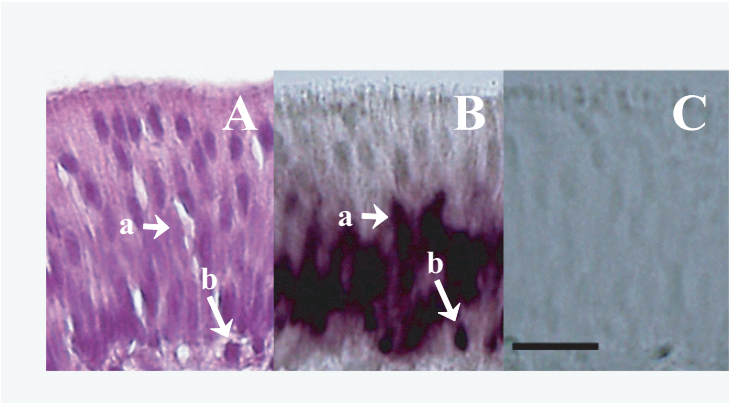

Fig. 4. Comparison of SOIG amino acid sequence with monkey and bovine uPAR. Red characters indicate cysteine residues. Black characters indicate residuse that are conserved throughout all sequences. Grey characters indicate residues that are the same for 2/3 sequences. Dotted underlines denote CCXXXXCN motif. Sequence alignments and analysis were done with ClustalW.

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Monkey MGHPLLLPLLLLLLLHTGVPASWGLRCMQCNGHGNCRVEECALGQNLCRTTSVRHWEEGEEVEMVEKSCTHSEKTNRTMSFRTGVRITTLT  90
bovine MGQPLLLL---LLVYTYIPGSWGLRCLQCENTTSCSVEECTPGQDLCRTTVLSVWEGGNEMNVVRKGCTHPDKTNRSMSYRAADQIITLS  87
SOIG   MKHTIILL---------------LACMLL-----CIAVSCSAALQ-------------------------------------------  25

Monkey EAVCGLDLCNQDSSGPAVTFPRSRFLECISCG---------------SSDMSCERGRHQSLQCTSPKEQ---------CLDMVTHRTSEA 156
bovine ETVCRSDLCNKPNPGRDATVSRNRYLECASCS---------------STDLSCERGWDQTMQCLKSRDQ---------CVDVITHRSLKE 153
SOIG   ---------------------------CFTCKDLADTHCLEQTLETCSDGQVCSTNSSSSLRSSVVVNDRGRIHFSIWDVDIDIRWGKDR  88

Monkey EEG-------RPKDDHHIRGCGHLPGCPGIAGFHSEDTFHFLK--CCNTTKCNGGPILSLANLPKNGHRCYSCQGNSTHGCSSENTVLTD 237
bovine N----------PGDERHIRGCGILPGCPGPTGFHNNHTFHFLR--CCNTTKCNAGSVLELQNLPPNGLQCYSCEGNGAHRCSSEETFLID 231
SOIG   TATNPNVVSNSSINERIVKGCMDDHFCQSSPKMSLNIGFHQFTSSCCNSSGCNSD---TFSDDPHNGLECFSCTDPEDEVCN----QAVT 171

Monkey CRGPMNQCLEATGIYEPLSESYMVRGCATSSMCQHDHVSDAFSMSHIDVACCTENDCNNPAEDIQHRSEAAPQPGPAHLSLTITGLMTAR 327
bovine CRGPMNQCLEATGTKGLRNPSYTIRGCAAPSWCQSLHVAEAFDLTHVNVSCCTGSGCNHPARDDQPGKGGAPKTSPAHLSFFVSLLLTTL 321
SOIG   CQGVQDHCLNDT-VTASNGRSVTLRGCVSRSMCGSGSG---------SVSCCEGSLCNR-----NRAMNSAQPVALTVLTLLVGITTTTL 246

Monkey LWGGTLLWT 336
bovine LWGATLLCT 330
SOIG   LQSVDQ--- 252
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regions of brain (data not shown). To determine the distribution of SOIG mRNA expression in the olfactory system (olfactory epithelium and olfactory bulb), we performed *in situ* hybridization using DIG-labelled RNA probes. The signals for SOIG were observed mainly in the olfactory receptor cells and basal cells in the olfactory epithelium of 1⁺ lacustrine sockeye salmon (Fig. 3). We did not obtain the signal for SOIG mRNA by *in situ* hybridization in the olfactory bulb (data not shown). To isolate full length SOIG cDNA, we constructed a cDNA library from 1⁺ lacustrine sockeye salmon olfactory epithelium. SOIG cDNA was 1700 bp in length, having an open reading frame of 759 bp encoding 252 amino acids. Database searches showed that the SOIG amino acid from 102 to 220 share amino acid sequence similarity with urokinase plasminogen activator receptor (Fig. 4). Overall, SOIG shares 28.2 and 30.3% similarity, respectively, with the amino acid sequence of urokinase plasminogen activator receptor from bovine (Kraetzschmar et al. 1993) and monkey (Engelholm and Behrendt 2001).

The urokinase plasminogen activator receptor (uPAR) is one of the members of uPAR/LY-6/CD59/snake toxin-receptor (Ly-6) superfamily. The detailed biological function of the superfamily members is not known, except for uPAR, which has an important role in proteolysis of extracellular matrix proteins (Tarui et al. 2001). Ly-6 superfamily has a unique structure showing conserved 8–10 cysteine residues with a characteristic spacing pattern and shares the consensus sequence motif CCXXXXCN at the carboxy-terminal end (Palfree 1996). SOIG contains the CCXXXXCN motif (Fig. 4; positions 134–141 and 212–219), indicating that SOIG has may belong to Ly-6 superfamily.

The present study demonstrated that a strongly hybridized SOIG mRNA signal in the olfactory epithelium

was detected by northern blot analysis. SOIG mRNA was also expressed in the olfactory receptor cells. No SOIG mRNA signal was detected in other tissues or organs. These results suggest that SOIG may play important role on the imprinting function in the lacustrine sockeye salmon.

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