Expressed sequence tag of Japanese flounder Paralichthys olivaceus skin cells

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Japanese flounder is one of the most important food fish of both the fishing and aquaculture industries in Japan. In addition, a cloned population of Japanese flounder has been produced and established. Such a cloned population is useful for a molecular biological analysis of fish. For characterization of molecular biological aspects of fish, molecular tools, such as genes and cDNA, are necessary. In addition, to characterize the function of a cloned gene, transgenic technology is useful. To conduct transgenic technology of fish, availability of several different gene promoters is necessary. However, information on the molecular biological aspects of fish is rather limited compared with that available for mammals. Recently, it has been confirmed that expressed sequence tag (EST) analysis of several different tissues and cells is highly effective for obtaining genes from Japanese flounder Paralichthys olivaceus. EST are short sequences a few hundred base pairs in length, which are derived by partial, single-pass sequencing of inserts of randomly selected cDNA clones. Partial sequences of DNA clones randomly selected from a cDNA library have been shown to be an excellent way of identifying novel genes, and characterizing the expressed genes in cells, tissues and species. We found several differences of gene expression pattern in different tissues and cells including liver, spleen, and peripheral blood leukocytes. In the present study, we conducted an EST analysis of skin cells and tried to find a gene that is expressed in several tissues and organs or that is specifically expressed in skin in order to isolate its promoter.

mRNA of skin was isolated using a micro mRNA purification kit (Amersham-Biotech, Piscataway, NJ, USA). cDNA was synthesized using a cDNA synthesis kit (Amersham-Biotech) with an oligo dT primer. The cDNA library was constructed in λZAP II vectors (Stratagene, La Jolla, CA, USA) according to the instructions of the manufacturer. We conducted an EST analysis of the cDNA library. Conversion of the recombinant λZAP II into the pBluescript plasmid was carried out by in vivo excision according to the protocol of the manufacturer (Stratagene). After conversion of phage clones into plasmids, we randomly selected clones from the library and sequenced them. cDNA clones were sequenced using ThermoSequenase (Amersham-Biotech) with M13 forward and/or M13 reverse primers and an automated DNA sequencer LC4200 (Li-Cor, Lincoln, NE, USA). Each determined sequence was compared with all sequences available in DDBJ/EMBL/GenBank using the BLAST (blastn and blastx) program ver.2.0. We sequenced 112 clones from this cDNA library. The average sequence length was 650 bp. Thirty-nine (35%) of these clones revealed homologies with nucleic acid and/or amino acid sequences of known genes, whereas the remaining 73 clones (65%) did not show any significant homology. In 39 homolog genes, there were 29 different genes (Table 1).

We found a cytokeratin cDNA (JFSK-4-30) that was a candidate gene for a specifically expressed gene in epidermis and its appendages. Keratins expanded from a single gene to a multigene family. Of the approximately 30 keratin genes in the human genome, at least 18 of these are expressed in skin. Some keratin genes, like housekeeping genes, are expressed in several tissues and organs. Based on previous studies of human keratin genes, we expect that studying the expression patterns of...
Fish keratin genes will be valuable for developing transgenic technology for fish as well as for understanding the biology of fish. Recently, Ju et al.\textsuperscript{15} reported that the zebrafish type II cytokeratin gene was specifically expressed in skin epithelia in early embryos and prominently expressed in adult skin tissue. In addition, they introduced cytokeratin gene promoter-GFP constructs to zebrafish embryos. The cytokeratin gene promoter was sufficient to direct GFP expression in skin epithelia of zebrafish. We chose a cytokeratin gene homolog (JFSK-4-30) for further study in order to find a gene promoter for making transgenic flounder and to characterize the functions of unknown genes from our EST studies.

\begin{table}
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\begin{tabular}{|l|l|l|l|l|l|}
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Clones & Accession No. & Homologous genes & Closest species & Identities\textsuperscript{1} & Redundancy\textsuperscript{2} \\
\hline
\multicolumn{5}{|c|}{Cell signaling/cell communication} \\
JFSK-1-104 & AB079728 & Ankyrin 1 & Bovine (AF222766) & 24/29 (82\%) & 1 \\
\hline
\multicolumn{5}{|c|}{Cell structure/motility} \\
JFSK-4-30 & AB079729 & Keratin & Flounder (AB049616) & 84/98 (86\%) & 1 \\
JFSK-2-2 & AB079730 & Very low-density lipoprotein receptor VLDL-R2 & Bovine (AF016537) & 24/52 (46\%) & 1 \\
\hline
\multicolumn{5}{|c|}{Cell/organism defense} \\
JFSK-4-1 & AB079731 & Heat shock protein 70 & Flounder (AF053059) & 46/50 (92\%) & 2 \\
\hline
\multicolumn{5}{|c|}{Gene/protein expression} \\
JFSK-5-6 & AB079732 & 40S ribosomal protein S17 & Catfish (AF402826) & 128/133 (96\%) & 1 \\
JFSK-4-37 & AB079733 & 40S ribosomal protein S27-1 & Catfish (AF048363) & 64/85 (75\%) & 1 \\
JFSK-5-23 & AB079734 & 40S ribosomal protein S30 & Catfish (AF028441) & 81/129 (62\%) & 1 \\
JFSK-5-21 & AB079735 & PAI-1 mRNA-binding protein & Human (BC017449) & 49/111 (44\%) & 1 \\
JFSK-1-114 & AB079736 & Reverse transcriptase protein & Fugu (AF086712) & 71/111 (63\%) & 1 \\
JFSK-1-134 & AB079737 & Ribosomal protein L13a & Catfish (AF401568) & 156/202 (77\%) & 2 \\
JFSK-4-2 & AB079738 & Ribosomal protein L34 & Catfish (AF401588) & 60/94 (63\%) & 2 \\
JFSK-5-29 & AB079739 & Ribosomal protein L36a & Catfish (AF401592) & 94/106 (88\%) & 1 \\
JFSK-5-13 & AB079740 & Ribosomal protein L7 & Catfish (AF401559) & 46/49 (93\%) & 2 \\
JFSK-4-17 & AB079741 & Ribosomal protein large P2 & Flounder (AF220554) & 48/51 (94\%) & 4 \\
JFSK-4-36 & AB079742 & Ribosomal protein P0 & Catfish (AF401551) & 122/172 (70\%) & 2 \\
JFSK-5-8 & AB079743 & Ribosomal protein P1 & Catfish (AF401552) & 73/113 (64\%) & 2 \\
JFSK-1-144 & AB079744 & T-box transcription factor txb2 & Zebrafish (AF719405) & 33/33 (100\%) & 1 \\
\hline
\multicolumn{5}{|c|}{Metabolism} \\
JFSK-4-49 & AB079745 & ATPase subunit-6 & Flounder (AB028664) & 117/149 (78\%) & 1 \\
JFSK-1-153 & AB079746 & Ferritin middle subunit & Atlantic salmon (S77386) & 81/95 (85\%) & 1 \\
JFSK-4-21 & AB079747 & Organic cation transporters OKB1 & Human (NM_033125) & 30/59 (50\%) & 1 \\
JFSK-4-18 & AB079748 & Succinate dehydrogenase Ip subunit & Mouse (NM_023374) & 21/25 (84\%) & 1 \\
\hline
\multicolumn{5}{|c|}{Unclassified} \\
JFSK-1-109 & AB079749 & Caenorhabitis elegans ZK678.4 & C. elegans (Z79605) & 16/16 (100\%) & 1 \\
JFSK-5-3 & AB079750 & Calumenin & Human (AF346537) & 125/251 (49\%) & 1 \\
JFSK-1-137 & AB079751 & Coronin-like protein & Human (D44497) & 138/214 (64\%) & 2 \\
JFSK-4-29 & AB079752 & dnaK-type molecular chaperone & Mouse (A26283) & 32/87 (36\%) & 1 \\
JFSK-1-113 & AB079753 & KIAA1644 protein & Human (AB051431) & 52/106 (49\%) & 1 \\
JFSK-5-38 & AB079754 & NO27 & X. laevis (AB018189) & 31/34 (91\%) & 1 \\
JFSK-5-39 & AB079755 & Similar to hypothetical protein & Human (XM_058539) & 24/30 (80\%) & 1 \\
JFSK-4-41 & AB079756 & Similar to KIAA0144 & Mouse (BC007179) & 48/63 (76\%) & 1 \\
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\end{tabular}
\caption{Putative identified EST of Japanese flounder skin cDNA library}
\end{table}

\textsuperscript{1}Identities, no. identical amino acid residues/no. compared amino acid residues (% identity).
\textsuperscript{2}Redundancy, no. detection time/112 sequences.
Fig. 1 Agarose gel electrophoresis of reverse transcriptase–polymerase chain reaction (RT-PCR) products of cytokeratin and β-actin which were expressed in the Japanese flounder. Lane 1, spleen; 2, fin; 3, liver; 4, brain; 5, head kidney; 6, trunk kidney; 7, skin; 8, erythrocyte; 9, intestine; 10, heart; 11, muscle; 12, ovary; 13, gill; and 14, peripheral blood leukocytes.

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