

Cloning and Characterization of Insulin-like Growth Factor I cDNA from Black Seabream (*Acanthopagrus schlegeli*)

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Mark Hung-Chih Chen, Gen-Hwa Lin, Hong-Yi Gong, Chiou-Yueh Lee, Chi-Yao Chang, Thomas T. Chen and Jen-Leih Wu (1998) Cloning and characterization of insulin-like growth factor I cDNA from black seabream (*Acanthopagrus schlegeli*). *Zoological Studies* 37(3): 213-221. A cDNA library was constructed in Uni-ZAP XR using mRNA from the liver of black seabream, *Acanthopagrus schlegeli*. In this study, we designed a pair of primers from the C and E domains of trout IGF-I cDNA, and synthesized an internal probe of IGF-I from the liver of black seabream by reverse transcription/polymerase chain reaction (RT/PCR). Using the internal probe to screen the cDNA library, we obtained 16 positive clones. Subsequent restriction enzyme map analysis suggested that about 6 forms ranging in size from 1.8 kilo-base pair (kb) to 2.3 kb of IGF-I were present. We took 2 longer cDNA clones to read the full sequence. Sequences of 2 clones of IGF-I cDNAs were found to be 2238 base pairs (bp) and 2299 bp in length. Except for a sequence of 61 nucleotides missing in the 5'-untranslated region (5'-UTR) and 6 nucleotides being different in the 3'-untranslated region (3'-UTR), the other nucleotides of these 2 clones are identical. This showed that the black seabream IGF-I gene contains polymorphism or gene duplication. Both cDNAs containing an open reading frame (ORF) encode 185 amino acids, including a 44-amino acids leader peptide, the 67-amino acids mature peptide in the B, C, A, and D domains, and a 74-amino acids extended carboxyl-terminal peptide in the E domain. The predicted propeptide of IGF-I can be divided into B, C, A, D, and E domains. Owing to the conserved mature peptide of IGF-I, we compared IGF-I of other teleosts with that of black seabream. The comparisons showed 100%, 91%, 91%, 87%, 87%, 58%, and 48% amino acid identity, respectively, with the IGF-I of *Sparus*, salmon, trout, carp, catfish, hagfish, and amphioxus. Moreover, we compared the E domain of IGF-I of Salmonidae with that of black seabream. The comparisons revealed that the E domain of black seabream belongs to the largest Ea form. These data imply that black seabream cDNAs encode a particular subtype of IGF-I from liver, IGF-I Ea-4. Furthermore, this IGF-I is phylogenetically most closely related to that of Sparidae.

Key words: IGF-I, Sparidae, cDNA.

Insulin-like growth factor I (IGF-I) is a member of the insulin gene superfamily (Nagamatsu et al. 1991). IGF-I production is stimulated by growth hormone that acts on hepatic and extrahepatic tissues. The resultant stimulation of growth by IGF-I has been widely demonstrated in mammals (Walker et al. 1991, Kiess et al. 1993), chickens (Kajimoto and Rotwein 1989), *Xenopus* (Kajimoto and Rotwein 1990), and teleosts (Bern et al. 1991, Chen et al. 1994, Shambloott et al. 1995). The pro-

tein molecule of IGF-I can be divided into B, C, A, D, and E domains. The mature form of IGF-I is a basic protein consisting of 70 amino acid residues (Rinderknecht and Humbel 1978). The amino acid sequences are highly conserved. IGF-I cDNAs have been cloned from a number of fish species and from a primitive chordate species, amphioxus (*Branchiostoma californiensis*) (Chan et al. 1990). IGF-I amino acid sequences are now known for the agnathan Atlantic hagfish (Nagamatsu et al. 1991)

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and for the following bony fish species: salmon and rainbow trout (Cao et al. 1989, Duguay et al. 1992, Shambloott and Chen 1992, Wallis and Devlin 1993), catfish (McRory and Sherwood 1994), common carp (Liang et al. 1996), and gilthead seabream (Duguay et al. 1996). The homology of B and A domains of IGF-I between these teleosts and mammals is above 85%. So IGF-I is highly conserved among a variety of vertebrate species (Rotwein 1991, Chan et al. 1992).

Regulation of growth is very important to aquaculture production (Powers 1989, Chen and Powers 1990, Du et al. 1992, Chen et al. 1995). More and more research on IGF-I related to nutrition, osmoregulation, reproduction, embryonic development, and growth in teleosts has been conducted (Gac et al. 1993, Sakamoto and Hirano 1993, Shambloott and Chen 1993, Duguay et al. 1994, 1996, Kagawa et al. 1994, Perez-Sanchez et al. 1994). IGF-I appears to be a key link to nutritional condition, environmental adaptation, embryonic development, and growth regulation of teleosts. The study of IGF-I is therefore basically important for aquaculture. Toward better understanding of the molecular biology, function, and phylogeny of IGF-I in other teleosts, we have started to characterize it in the commercially important Sparid fish, the protandrous black seabream (Chang and Yueh 1990, Jean et al. 1995), found in estuarine and coastal waters of Taiwan. Herein we report the isolation and characterization of cDNA clones encoding a prepro-IGF-I from black seabream (*Acanthopagrus schlegelii*).

MATERIALS AND METHODS

Black seabream liver cDNA library construction

The liver was dissected from a rapidly growing 1-yr-old male black seabream specimen. Total RNA was isolated by the modified phenol/chloroform/NaDodSO₄ method (Agellon et al. 1986). Affinity chromatography on oligo(dT)-cellulose was carried out to enrich for poly(A)⁺ RNA (Aviv and Leder 1972). Double-stranded cDNA(ds cDNA) was generated from about 5 µg of poly(A)⁺-enriched RNA by a commercially available synthesis method (Stratagene manual). Of the about 1.5 µg of ds cDNA generated, 100 ng was ligated into the Uni-ZAP XR vector. After in vitro packaging, about 1 million recombinant phages were generated and amplified by the plate lysate method (Stratagene manual).

Reverse transcriptase/PCR for internal probe

To isolate cDNA coding for seabream insulin superfamily members, including IGFs, a strategy was used that was based on regions of strong nucleotide conservation in trout. Two oligonucleotides were designed as 18-mers: [IGF-IC, 5' TCACG GCGGT CACAT AAC 3'; IGF-IE, 5' CGCTG TGCGC GCACA GAG 3']. Approximately 100 ng of seabream liver single-stranded cDNA (ss cDNA) was used as a PCR template. PCR was carried out under the following standard conditions: 95 °C denaturation, 42 °C primer annealing, and 72 °C polymerization for 30 cycles. Single-stranded cDNA was generated from seabream liver RNA by using avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) and oligo(dT) primer. A small fraction of the ss cDNA was used as the 1st PCR template. PCR primers were de-

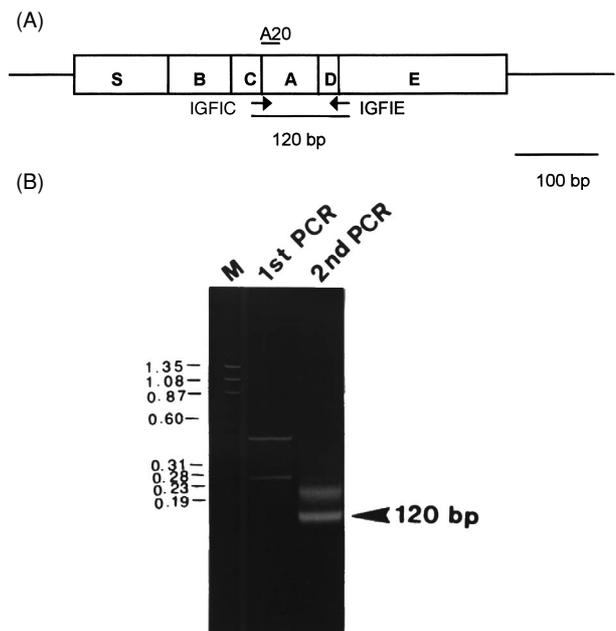


Fig. 1. (A) Diagrammatic presentation of RT/PCR primers for IGF-I internal probe. One 120 bp internal probe was developed from primers IGF-IC and IGF-IE by PCR. A20 degenerated oligomers were used to screen the internal probe by colony hybridization. (B) PCR amplification of black seabream IGF-I cDNA fragment with IGF-IC and IGF-IE primers. First strand cDNA was reverse transcribed from 10 µg of total RNA. Two microliters of the resulting product was directly used in the 1st PCR amplification. The desired band of 120 bp (lane 1) was recovered from the gel. For 2nd PCR amplifications (lane 2), about 10 ng of diluted DNA from the 1st PCR was used as a template. The gels were analyzed on a 2% agarose gel, stained with ethidium bromide, and photographed under UV light. Molecular size standards correspond to ϕ x 174 digested with *Hae*III (lane M).

signed to amplify a region (internal probe) between the C and E domains of rainbow trout IGF-I (see Fig. 1A. for positions of these primers). Oligonucleotides A20[5' CA(G or A)CA CT(C or G)(C or A)T C(C or A)AC(G or A) ATGCC 3'] used in screening of internal probe clones were end-labeled with [γ - 32 P]ATP by a standard polynucleotide kinase method (Sambrook et al. 1989).

Radioactive probe labeling and screening of IGF-I cDNA

The internal probe was end-labeled with the [γ - 32 P]ATP polynucleotide kinase method. Approximately 1 million recombinant bacteriophages from the seabream liver cDNA library were plated, lifted in duplicate onto nylon membranes, and incubated overnight at 42 °C in a hybridization solution (0.25 M phosphate buffer, pH 7.2; 10% PEG8000; 50% Formamide; 0.25 M NaCl; 0.5 mM EDTA, pH 8.0; 7% NaDodSO₄) containing 1 x 10⁶ cpm of internal probe/ml. In vivo excision of Lambda ZAP XR phage to a plasmid was used to facilitate recombinant DNA preparation and characterization.

DNA sequencing and analysis

Nucleotide sequences of the cDNA inserts were determined in both directions by the commercially available dideoxynucleotide chain-termination method (United States Biochemical Corp., Cleveland, OH, USA) and confirmed by an ABI autosequencer. The various seabream cDNAs were analyzed using the alignment program GCG (Genetics Computer Group, Version 7.0; Devereux et al. 1991) and some related programs. Comparisons of nucleic acid and predicted amino acid sequences between the 2 clones of IGF-I were made using the GAP alignment program. Predicted amino acid multiple sequence alignments were analyzed using the PILEUP, DISTANCES, and GROWTREE programs. The DISTANCES software was used for calculating the pairwise sequence distances using the Kimura protein model. The FASTA alignment program was used in a nucleotide search of GenBank (December 1996). A word size value of 6 was used. The program provided the top 40 homologies ranked by their initial score (initn).

RESULTS

Generation of homologous probe with RT/PCR

Upon resolution of the RT/PCR product, a band of predicted size (120 bp) was evident (Fig. 1B). This fragment was cloned into the pBluescript II SK⁻ (Stratagene) plasmid vector according to the Stratagene manual. We picked up colonies containing inserts for further analysis. Colony hybridization (Sambrook et al. 1989) was utilized to screen clones by the degenerated A20 oligo probes with the 5'-end radioactive. We obtained a positive clone (IGF-IP), and its nucleotide sequence was determined and analyzed using FASTA. The resultant nucleotide sequence had about 98% identity to the corresponding region of the cDNA sequence of trout IGF-I.

Identification and characterization of seabream IGF-I cDNAs

Screening the seabream liver cDNA library with IGF-IP resulted in identification of 20 positive clones, 16 of which were chosen for further plaque purification. Subsequent restriction enzyme map analysis of the 16 cDNA clones suggested that about 6 forms ranging in size from 1.8 kilo-base pair (kb) to 2.3 kb of IGF-I were present (data not shown). Partial nucleotide sequence determination of their 5' and 3' ends showed that 6 forms represent different insert sizes in the 5' ends, whereas the 3' ends were almost identical. In the shorter 5' ends, cDNA clones even have truncated N-terminal residues of predicted IGF-I. So we took the 2 largest cDNA clones to read the full sequence by the commercially available dideoxynucleotide chain-termination method and ABI autosequencer.

The different restriction enzyme map of both cDNAs was verified from sequence data. The sequences of the longer clone (2299 bp) are represented in Fig. 2A. The shorter clone showed the transversion G to C at 2228 bp of Fig. 2A. So it appeared to be the cutting site of the PstI recognition site (CTGCAG). The structures of both cDNAs have different 5'-UTR (184 bp or 123 bp), but the same ORF (558 bp) and 3'-UTR (1557 bp). At the nucleotide level, both the seabream cDNA clones were 100% identical to each other except in the length of the 5'-UTR (missing 61 nucleotides) and 3'-UTR (6 different nucleotides). In the 5'-UTR of the seabream IGF-I cDNA, there is a TCTCCT (19~24) motif (Koval et al. 1994) that is the consensus motif of the transcriptional start site. A putative polyadenylation signal sequence AATAAA was found in the 3'-UTR (1746~1751).

At the amino acid level, the clone was identified as black seabream preproIGF-I (bbIGF-I)

(A)

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1 attttctctctaaatccgctctcctggtcgctaaatctcacttctccaaaacgagcctgcgcaatggaaacaaagtcggaat
81 attgagatgtgacattgccccgatctcatctcttctccccgttttaatagacttcaaaacaagttcattttcgccgggc
*
161 tttgtcttgcggagaccctggggatgtctagcgtcttctcctttcagtgccatttatgtgatgtcttcaagagtgccgat
1 M S S A L S F Q W H L C D V F K S A M
B→
241 gtgctgtatctcctgtagccacacctctcactactgctgtgcgtcctcaccctgactccgacggcaacagggggcgagcc
20 C C I S C S H T L S L L L C V L T L T P T A T G A S P
321 cagagaccctgtgccccgggagctggtcgacacgctgcagtttgtgtggagagagaggtttttatttcagtaaacct
47 E T L C G A E L V D T L Q F V C G E R G F Y F S K P
C→
401 ggctatggccccaatgcaacggcggtcacgtggcattgtggacgagtgctgcttccaaagctgtgagctgccccgtctgga
73 G Y G P N A R R S R G I V D E C C F Q S C E L R R L E
D→
481 gatgtactgtgcaacctgccaagactagcaaggctgctcgtctgtgctgcaacgcccacacagacatgccaaagcac
100 M Y C A P A K T S K A A R S V R A Q R H T D M P R A P
561 ccaaggttagtaccgagggcacaagtggaacagggcagagcgttaggacagcacagcagccagacaagacaaaaac
127 K V S T A G H K V D K G T E R R T A Q Q P D K T K N
641 aagaagagaccttacctggacatagtcattcatccttcaaggaagtgcacccgaaaaactcaagtcgaggcaacgcaggg
153 K K R P L P G H S H S S F K E V H P K N S S R G N A G
#
721 gggcagaaactacagaatgtagggacggagcaaatggacaaatgccaccgacttgggaagagagaaggaggatggcctta
180 G R N Y R M
801 cctggtaccctgtggaatggttcaactgtaaaacaaaacagagaggaggctaaacaatgggtccgaaacgctcttcagaatg
881 attgaacgctgagagctaaagtgggtttaaaggtttgatgagggatcttggattattttatacactgcaccattccata
961 tcgggaggaattcttggtaatgcaatgtaacagactagtttagctgctgagacacgaaacaagagcttattatcctcc
1041 atgtgtgagctgcagcaaccctggctccaggaagggtgggaacggatctgggcttcagccaatcagagagctgcagact
1121 gcgttttttttttttggtaacaacggctccatccccctcccttgaactctgagggagtaaatcctttttctctgaga
1201 gagtgcagctcttcccccgtcacatattacagttaaaaatcagatcaacttaccgtcaagcgttgttcaggcaccgtgc
1281 gttactgagatattttaataagttaaaaaaaacagtttgcctaaagcacaagaccaggtggttcatctcagactt
1361 ttaacattgtagttatttccataaaatccctctcgtctcattgactgaaggaaagcgttcttcttcaagtggagtcagt
1441 cgaaggcttgagtcacaatcccaatccctcctaagctgcaggaagtgttgagaacctgaggtctgagctcacaatccaaac
1521 acctctataacaacagatcaagcaccat tagaggggattttaaggacagtagtactccaaaaccaaagccgtgctt
1601 ttgctgttttccaaaacagagaatcaaattaaccagaagctaaattgaaataaagtcttcttggccgctcgttgcgtgg
1681 cgcttgtttggttgcagaaatgggtcattcagtgattcagaagtgatttggcataagccagtggaataaatcaaatag
1761 cgacaatcaggcaaatgttgcgtctgcatcctttcacatgtcaccacccgcccgaatctgattttctgtcaacaa
1841 tccagtcggcagtgatcaacatttgagtcagatggaggcccggttcaagggttaaacacaagctcatgaaagtttagtgg
1921 cagaaagagccaagagctcgaagagaagtatacaaatatggtaaaatcttactcgtgctctcttgaagaactagcagatg
2001 gtccccaaagcttccataaagtgcgagagggctcgaacagtagccgctccacagacgtagaagctaatgatcgatcgatc
2081 ggtgtgtaataatttgcagtttaacttcgcagcttcagtcagtcgttgccttgggcccacatcgttacgagagtgcaacga
2161 aacccaaagtccaacaaagaaccggttgcagatgatttggttaaatagaaacaaagtgactcgtcctcgtgcagatgtgag
2241 atacggcaacgcaatttaacagtgcaaaaacacacatgcaaaagaaaaaaaaaaaaaaaaa
    
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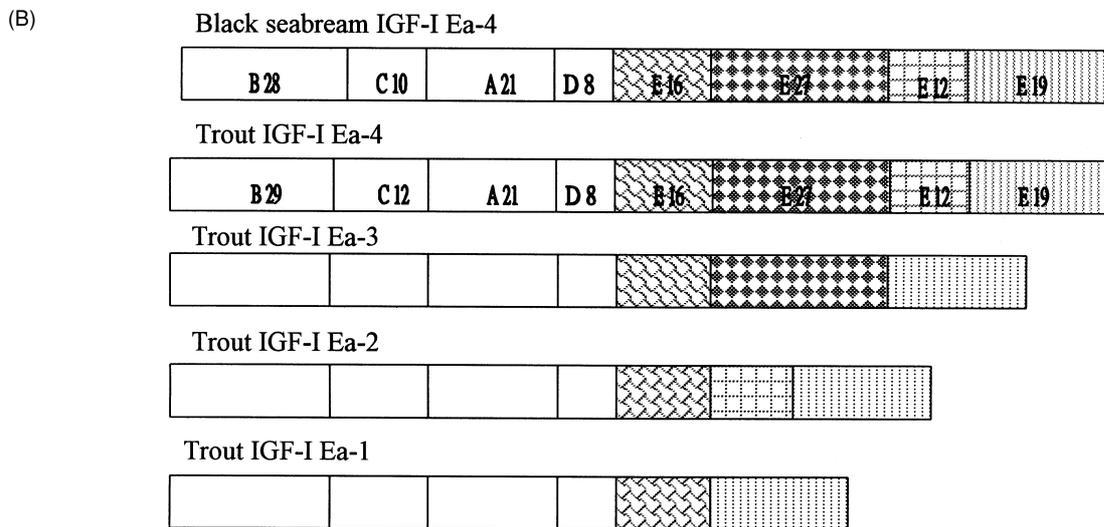


Fig. 2. (A) Black seabream IGF-I cDNA sequence with an open reading frame (ORF). Small letters indicate nucleotide sequences. Small letters in bold indicate different nucleotides between 2 cDNA clones. Capital letters indicate amino acid residues. Capital letters in bold following the arrow indicate different domain start sites of IGF-I prepropeptide. The putative transcription initiation motif (TCTCCT) is underlined. Double underlining indicates the potential polyadenylation signal (AATAAA). An asterisk (*) indicates the translation start site: methionine. An octothorpe (#) indicates a stop codon. Untranslated regions are represented as UTR. 5'UTR: 184 nucleotides, ORF: 558 nucleotides, 3'UTR: 1557 nucleotides. The nucleotide sequence has been assigned GenBank accession no. AF030573. (B) Schematic comparison of the cloned black seabream IGF-I cDNA and the 4 subtypes of rainbow trout IGF-I cDNA (Chen et al. 1994). B, C, A, D, and E indicate different domains of the IGF prohormone. The numbers indicate amino acid residues. Different regions of the E domains are indicated in different shades.

feature of mRNAs in higher vertebrates is the presence of the sequence AATAAA in the region from 11 to 30 nucleotides upstream of the site of poly(A) addition. A putative polyadenylation signal sequence AATAAA was found in the 3'-UTR (1746~1751) of black seabream IGF-I. But the poly(A) is located at 2282~2299. These 2 sites are separated from the above by 500 nucleotides. This indicates that black seabream may use a different polyadenylation mechanism (Fig. 2A).

The IGF-I peptide is an important cellular regulator, with structural and biological actions similar to those of insulin. Residues 45-72 of bblGF-I are homologous to the B-chain of insulin and are thus known as the B-domain of IGF-I. Then follows the C-domain (73-82), analogue in location to the C-domain of proinsulin, but shorter and with no sequence homology with proinsulin. These lead to the A-domain, residues 83-103 of bblGF-I, which is homologous to the A-chain of insulin. The short sequences at the carboxyl termini have no insulin analogue and are known as the D-domain (104-111) (Fig. 2A). bblGF-I has 3 amino acid deletions included in the carboxyl-terminal B domain (threonine) and C domain (asparagine, histidine). Among teleosts, IGF-I comparisons (Fig. 3) show that these regions (carboxyl-terminal B domain, C domain, and D domain) are very distinctive. It is not clear whether these regions function in receptor binding or not. An IGF-I derivative exhibited a single cutting following C7. This derivative possessed decreased binding to the IGF-I receptor, whereas it has a little changeable binding to the insulin receptor (Jansen et al. 1990). So these

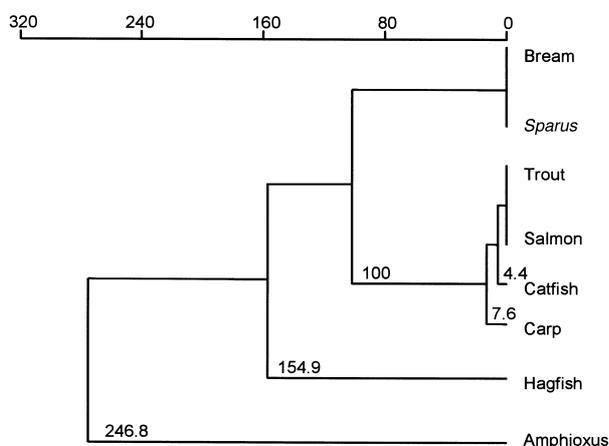


Fig. 4. Phylogenetic tree of 8 species of teleosts based on Kimura protein distance using the UPGMA clustering method. The PILEUP, DISTANCES, and GROWTREE programs (GCG VER. 7.0) aligned IGF-I.

variable regions should be considered and investigated separately and specifically.

Variants of Pro-IGF-I in teleosts

Salmon and trout have been shown to express alternatively spliced IGF-I mRNA transcripts coding for at least 4 different IGF-I prohormones (Duguay et al. 1992 1994, Wallis and Devlin 1993). These transcripts now are designated as Ea-1, Ea-2, Ea-3, and Ea-4 in trout (Shamblott and Chen 1993, Chen et al. 1994) (Fig. 2B). In salmon, Ea-1, Ea-2, Ea-3 and Ea-4 mRNA transcripts were detectable in the liver, and Ea-1 and Ea-3 levels increased dramatically in response to GH treatment, whereas amounts of Ea-2 and Ea-4 mRNA were unchanged. Heart, fat, brain, kidney, spleen, and ovary expressed only the Ea-4 transcript, and expression was not influenced by GH stimulation (Duguay et al. 1994). Ea-2 is the form predominantly expressed in common carp (Liang et al. 1996). In Sparid fish, the E domain of *Sparus* (Duguay et al. 1996) should predominantly belong to the IGF-I Ea-4 group in the liver, as we reported for *Acanthopagrus*. These studies show that more complicated degrees of E domain transcripts exist in teleosts. It is still not clear whether all teleosts can express all 4 subtypes of IGF-I Ea form in the liver. This question may need more extensive studies to define the gene structures and alternative splicing patterns. Although the presence in black seabream of analogues to trout IGF-I Ea-1, Ea-2, and Ea-3 were not detected after 2 screenings (2×10^6 pfu total) of a black seabream liver cDNA library, we still cannot rule out their presence because the more sensitive RT-PCR assay was not used for analysis of black seabream liver. The E domain is cleaved before mature IGF-I is secreted. The E domain has a different N-link glycosylation site in mammals, so its physiological function remains unclear. proIGF-I can be synthesized and secreted in human fibroblast cells (Conover et al. 1989). As in mammals, black seabream contained an N-link glycosylation site at 172-174 (Fig. 2A). However, additional studies will be necessary to reveal whether the E domain of proIGF-I has its own specific physiological function in teleosts.

Evolution of piscine IGF-I peptide

Among vertebrates, fish have the largest number of species. At least 28 000 fish species have been described, based on morphological, bio-

chemical, and molecular characters. However, when differences occur in classification are based on 1 kind of taxonomic character, other sets of taxonomic characters must be used. Our study comparing ubiquitous IGF-I amino acid sequences obtained from different teleosts (Fig. 3) has indicated that this peptide is evolving very slowly compared to an outgroup including amphioxus and hagfish. The amphioxus is a primitive cephalochordate that occupies a key position in chordate phylogeny as a possible extant relative of the invertebrate progenitor from which vertebrates emerged (Chan et al. 1990). The estimated evolutionary divergence time of the hagfish group (myxinooids) is from 550 million years ago (Nagamatsu et al. 1991). Both amphioxus and hagfish appear as outgroups in the cladogram (Fig. 4). The reconstructed phylogenetic tree is based on the predicted IGF-I mature peptide sequences analyzed by Kimura protein distance using the UPGMA clustering method (Fig. 4). It shows similar clustering in the present hierarchy of higher categories of fish (Nelson 1984). These sequence similarities allow for comparison of substitutions and deletions in IGF-I which have occurred during teleost evolution. ORF sequence comparisons among teleosts show that the B and A domains of IGF-I have been highly conserved (more than 80% identity), but the signal peptide, C, D, and E domains of IGF-I have been variable among the teleosts (38%~100%). Even black seabream and *Sparus* are differences at the E domain (96%). This study suggests that IGF-I may have an important role in the evolutionary rate of teleosts. It is still not known whether the ubiquitous IGF-I serves as 1 molecular character in taxonomy responding to different evolutionary pressures or not. The uniform IGF-I sequences of other teleosts may need more extensive studies to determine their usefulness in classification.

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REFERENCES

- Agellon LB, TT Chen, RJ van Beneden, RA Sonstegard, GF Wagner, BA Mckeown. 1986. Rainbow trout (*Salmo gairdneri*) growth hormone: in vitro translation of pituitary RNA and product analysis. *Can. J. Fish. Aquatic Sci.* **43**: 1327-1331.
- Aviv H, P Leder. 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc. Natl. Acad. Sci. USA* **69**: 1408-1412.
- Bern HA, SD McCormick, KM Kelley, ES Gray, RS Nishioka, SS Madsen, PI Tsai. 1991. Insulin-like growth factors under water: role in growth and function of fish and other poikilothermic vertebrates. In EM Spencer, ed. *Modern concepts of insulin-like growth factors*. New York: Elsevier, pp. 85-96.
- Cao QP, SP Duguay, EM Plisetskaya, DF Steiner, SJ Chan. 1989. Nucleotide sequence and growth hormone-regulated expression of salmon insulin-like growth factor I mRNA. *Mol. Endocrinol.* **3**: 2005-2010.
- Chan SJ, QP Cao, DF Steiner. 1990. Evolution of the insulin superfamily: cloning of a hybrid insulin/insulin-like growth factor cDNA from amphioxus. *Proc. Natl. Acad. Sci. USA* **87**: 9319-9323.
- Chan SJ, S Nagamatsu, QP Cao, DF Steiner. 1992. Structure and evolution of insulin and insulin-like growth factors in chordates. In J Joosse, RM Buijs, FJH Tilders, eds. *Progress in brain research*. New York: Elsevier, pp. 15-24.
- Chang CF, WS Yueh. 1990. Annual cycle of gonadal histology and steroidal profiles in the juvenile males and adult females of the protandrous black porgy, *Acanthopagrus schlegelii*. *Aquaculture* **91**: 179-196.
- Chen TT, JK Lu, MJ Shambloott, CM Cheng, CM Lin, JC Burns, R Reimschuessel, N Chatakondi, RA Dunham. 1995. Transgenic fish: ideal models for basic research and biotechnological applications. *Zool. Stud.* **34**: 215-234.
- Chen TT, A Marsh, MJ Shambloott, KM Chan, YL Tang, CM Cheng, BY Yang. 1994. Structure and evolution of fish growth hormone and insulin-like growth factor genes. In CL Hew, N Sherwood, eds. *Fish physiology*. New York: Academic Press, pp. 179-209.
- Chen TT, DA Powers. 1990. Transgenic fish. *TIBTECH* **8**: 209-215.
- Conover CA, BK Baker, RL Hintz. 1989. Cultured human fibroblasts secrete insulin-like growth factor IA prohormone. *J. Clin. Endocrinol. Metab.* **69**: 25-30.
- Devereux J, P Haeberli, P Marquess. 1991. Genetic computer group manual. version 7.0. Madison, WI: Univ. Wisconsin.
- Du SJ, Z Gong, GL Fletcher, MA Shears, MJ King, DR Idler, CL Hew. 1992. Growth enhancement in transgenic Atlantic salmon by the use of an "All Fish" chimeric growth hormone gene construct. *Bio/Technology* **10**: 177-181.
- Duan C, SJ Duguay, EM Plisetskaya. 1993. Insulin-like growth factor I (IGF-I) mRNA expression in coho salmon, *Oncorhynchus kisutch*: tissue distribution and effects of growth hormone/prolactin family peptides. *Fish. Physiol. Biochem.* **11**: 371-379.
- Duguay SJ, J Lai-Zhang, DF Steiner, B Funkenstein, SJ Chan. 1996. Developmental and tissue-regulated expression of IGF-I and IGF-II mRNAs in *Sparus aurata*. *J. Mol. Endocrinol.* **16**: 123-132.
- Duguay SJ, LK Park, M Samadpour, WW Dickhoff. 1992. Nucleotide sequence and tissue distribution of three insulin-like growth factor I prohormones in salmon. *Mol. Endocrinol.* **6**: 1202-1210.
- Duguay SJ, P Swanson, WW Dickhoff. 1994. Differential expression and hormonal regulation of alternatively spliced IGF-I mRNA transcripts in salmon. *J. Mol. Endocrinol.* **12**: 25-37.
- Furlanetto RW, JN DiCarlo, C Wisheart. 1987. The type II insulin-like growth factor receptor does not mediate deoxyribonucleic acid synthesis in human fibroblasts. *J. Clin. Endocrinol. Metab.* **64**: 1142-1149.
- Gac FL, Q Blaise, A Fostier, PYL Bail, M Loir, B Mourot, C Weil. 1993. Growth hormone (GH) and reproduction: a review.

- Fish Physiol. Biochem. **11**: 219-232.
- Jansen J, SC Van Buul-Offers, CM Hoogerbrugge, JL Van Den Brande. 1990. Effects of a single cleavage in insulin-like growth factors I and II on binding to receptors, carrier proteins and antibodies. *Biochem. J.* **255**: 513-518.
- Jean CT, CF Hui, SC Lee, CT Chen. 1995. Variation in mitochondrial DNA and phylogenetic relationships of fishes of the subfamily Sparinae (Perciformes: Sparidae) in the coastal waters of Taiwan. *Zool. Stud.* **34**: 270-280.
- Kagawa H, M Kobayashi, Y Hasegawa, K Aida. 1994. Insulin and insulin-like growth factors I and II induce final maturation of oocytes of red seabream, *Pagrus major*, in vitro. *Gen. Comp. Endocrinol.* **95**: 293-300.
- Kajimoto Y, P Rotwein. 1989. Structure and expression of a chicken insulin-like growth factor I precursor. *Mol. Endocrinol.* **3**: 1907-1913.
- Kajimoto Y, P Rotwein. 1990. Evolution of insulin-like growth factor I (IGF-I): structure and expression of and IGF-I precursor from *Xenopus laevis*. *Mol. Endocrinol.* **4**: 217-225.
- Koval A, V Kulik, S Duguay, E Plisetskaya, ML Adamo, CT Roberts Jr., D Leroith, V Kavsan. 1994. Characterization of a salmon insulin-like growth factor I promoter. *DNA Cell Biol.* **13**: 1057-1062.
- Kiess W, U Kessler, S Schmitt, B Funk. 1993. Growth hormone and insulin-like growth factor I: basic aspects. *In A Flyvbjerg, H Ørskov, G Alberti, eds. Growth hormone and insulin-like growth factor I in human and experimental diabetes.* Chichester: J. Wiley, pp. 1-21.
- Liang YH, CHK Cheng, KM Chan. 1996. Insulin-like growth factor IEa2 is the predominantly expressed form of IGF in common carp (*Cyprinus carpio*). *Mol. Mar. Biol. Biotechnol.* **5**: 145-152.
- McRory JE, NM Sherwood. 1994. Catfish express two forms of insulin-like growth factor-I (IGF-I) in the brain. *J. Biol. Chem.* **269**: 18588-19592.
- Nagamatsu S, SJ Chan, S Falkmer, DF Steiner. 1991. Evolution of the insulin gene superfamily: sequence of a preproinsulin-like growth factor cDNA from the Atlantic hagfish. *J. Biol. Chem.* **266**: 2397-2402.
- Nelson JS. 1984. *Fishes of the world.* New York: J. Wiley.
- Perez-Sanchez J, H Marti-Palanca, PY Le-Bail. 1994. Seasonal changes in circulating growth hormone (GH), hepatic GH-binding and plasma insulin-like growth factor-I immunoreactivity in a marine fish, gilthead sea bream, *Sparus aurata*. *Fish Physiol. Biochem.* **13**: 199-208.
- Powers DA. 1989. Fish as model systems. *Science* **246**: 352-358.
- Rinderknecht E, RE Humbel. 1978. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *J. Biol. Chem.* **253**: 2769-2776.
- Rotwein P. 1991. Structure, evolution, expression and regulation of insulin-like growth factor I and II. *Growth Factors* **5**: 3-18.
- Sakamoto T, T Hirano. 1993. Expression of insulin-like growth factor I gene in osmoregulatory organs during seawater adaptation of the salmonid fish: possible mode of osmoregulatory action of growth hormone. *Proc. Natl. Acad. Sci. USA* **90**: 1912-1916.
- Sambrook J, EF Fritsch, T Maniatis. 1989. *Molecular cloning: a laboratory manual.* 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press.
- Shamblott MJ, TT Chen. 1992. Identification of a second insulin-like growth factor in a fish species. *Proc. Natl. Acad. Sci. USA* **89**: 8913-8917.
- Shamblott MJ, TT Chen. 1993. Age-related and tissue-specific levels of five forms of insulin-like growth factor mRNA in a teleost. *Mol. Mar. Biol. Biotechnol.* **2**: 351-361.
- Shamblott MJ, CM Cheng, D Bolt, TT Chen. 1995. Appearance of insulin-like growth factor mRNA in the liver and pyloric ceca of a teleost in response to exogenous growth hormone. *Proc. Natl. Acad. Sci. USA* **92**: 6943-6946.
- Sussenbach JS, PH Steenbergh, P Holthuizen. 1992. Structure and expression of the human insulin-like growth factor genes. *Growth Regulation* **2**: 1-9.
- Walker JL, M Ginalska-Malinowska, TE Romer, JB Pucilowska, LE Underwood. 1991. Effects of the infusion of IGF-I in a child with GH insensitivity syndrome (Laron dwarfism). *New Engl. J. Med.* **324**: 1483-1488.
- Wallis AE, RH Devlin. 1993. Duplicated insulin-like growth factor I genes in salmon display alternative splicing pathways. *Mol. Endocrinol.* **7**: 409-422.

黑鯛類胰島素生長因子基因之選殖與定序

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由於肝臟是 IGF 基因表現最多的部位，本研究擬由黑鯛肝臟組織，建立 Uni-ZAP XR cDNA 基因庫，並進行 IGF 基因家族之選殖。本實驗利用鱒魚 IGF-I cDNA 的 C 和 E 區，設計引子，以反轉錄聚合酶連鎖反應來合成黑鯛 IGF-I cDNA 探針，以此內生性探針自黑鯛肝臟 cDNA 基因庫中篩選出 IGF-I cDNA 基因。

以限制酶圖譜分析，篩選到的十六個選殖株，整理為大小自 1.8 至 2.3 kb 的六種 cDNA 片斷之選殖株，其中兩個較長的 IGF-I cDNA 基因，經定序分析後，大小分別為 2238 bp 及 2299 bp，發現這兩個基因除了一個在 5' 端少了 61 個核苷酸序列和在 3' 端非轉譯區發現六個核苷酸不同，其餘序列皆相同，推測是一種多型態 (polymorphism) 現象或基因雙倍化 (duplication) 所形成的。

黑鯛 IGF-I cDNA 可轉譯出 185 個胺基酸，其中包含 44 個胺基酸的訊息勝肽，67 個胺基酸含 B、C、A、D 區的成熟勝肽，以及 74 個胺基酸的 E 區。而原勝肽 IGF-I 由 B、C、A、D、和 E 區所組成。其中值得注意的是已知物種的 IGF-I 成熟勝肽保留性相當高，比較黑鯛與其它魚類 IGF-I 胺基酸序列分別為鯛，鮭，鱒，鯉，鱈，盲鰻和文昌魚有 100%，91%，91%，87%，87%，58%，和 48% 的同源性。而黑鯛 IGF-I 的 E 區相較於鮭科魚類是屬於最長的，故此選殖出之黑鯛 IGF-I cDNA 基因應屬 IGF-I Ea-4 家族而且序列比對符合現行之魚類種源關係圖，是為鯛科。

關鍵詞：類胰島素生長因子，鯛科，互補去氧核糖核酸。

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