The complete nucleotide sequence of the growth-hormone gene from the common carp (*Cyprinus carpio*)

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We have isolated and sequenced a phage clone from a common carp (*Cyprinus carpio*) genomic library that carries a gene encoding growth hormone (GH). This gene consists of five exons and four introns spanning a region of about 3 kilobasepairs. Its exons correspond with one of two reported cDNAs of carp GH except for nine differences in the nucleotide sequence, while the encoded amino-acid sequences are identical. The sequence upstream from the transcription start point contains two tandem repeats of AACTCTCATG (from –85 to –62) and the typical TATA box. All the introns start with a consensus GT dinucleotide and end with AG. The arrangement of exons and introns is very similar to that seen in mammalian GH, but quite different from the GH genes of rainbow trout and Atlantic salmon.

Fig. 1. The restriction map of the carp GH gene in EMBL 3 (A) and the sequencing strategy for the subcloned fragment in pGEM4 (B). Arrows indicate sequencing by the chain-termination method. P TATA box; MCS multiple cloning site; open box untranslated region; solid box coding region; the lines between the boxes indicate introns.

Growth hormones (GH) are secreted by the pituitary glands of vertebrate species and are essential for normal growth and development of pre-adult. The genes encoding GH have been studied extensively in many species.

Abbreviations: GH, growth hormone; kb, kilobasepairs. The sequence data in this paper have been submitted to the EMBL/Genbank Data Libraries under the accession number X51969.

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Nucleotide sequences have been determined for GH genes from human [1,2], rat [3,4], porcine [5], bovine [6], ovine [7], rainbow trout [8] and Atlantic salmon [9]. Recently two cDNAs for carp GH have been reported [10,11] which show almost identical sequences in the coding region and remarkable differences in the 3'-untranslated region. Apparently, at least two genes encoding GH must exist in the carp. This report describes the cloning and characterization of a carp GH gene corresponding to one of the two reported cDNAs.

Genomic DNA was prepared from carp eggs and partially digested with *Bam*HI. Fragments of 10–20

kilobasepairs (kb) were recovered from 0.5% agarose gel and cloned into lambda phage EMBL 3. Procedures from the commercial supplier (Promega Inc.) were followed for in vitro packaging and transfection.

The genomic library was screened using nick-translated ³²P-labeled carp GH cDNA [10] as a probe. The positive clones were further analyzed by repeated hybridization with the probe and the positive fragments

were subcloned into plasmid pGEM4. The recombinant phage and plasmid DNA were purified according to standard procedures [12,13]. The DNA sequence was determined using a chain-termination procedure [14] with denatured plasmid templates [15].

The transcription start site was determined by primer extension using carp pituitary mRNA as template. About 2 µg of mRNA prepared as previously described

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gttaacttagtgccaacatcatcccagagtcagtgaaatagccatacacataaatgaccggaaccgctacactgggttcgtatggtgtctaaatc -122
acgcagcaatgcataatcaaacatagttctcatgga aactctcatgtttcaactctcatg cattaagatgcataaaacatggttcagccaca -29
                                     +1                                     M A
tataaa tatcagtacctgagcctgaaaa A CAAACATTACAAAGCTCTTAACCTAAGCCTGCAAGAGTTGTCTACCTGAGCGAA ATG GCT 62
R
AGA G gtatgtctgtgatgctttctgttgcattgtttttagcacttaggacatggatttcatcttatttttgacatatccctatttagttat 155
ttatgtaatacatgtatttatatatttttgttacctgtaaaagtaattttatagtttaaagaggataaaaaaaaaaattggataaaaaaaaaat 250
atccaaaatcttgatttcattggttatttggtaacttttcaggagccttgaacaacaatgtttcaaattaaaaataaccctctctttctcttc 345
                                     *
V L V L L S V V L V S L L V N Q G R A S D N Q
ctag TA TTA GTG CTA TTG TCG GTG GTG CTG GTT AGT TTG TTG GTG AAC CAG GGG AGA GCA TCA GAC AAC CAG 417
                                     A
R L F N N A V I R V Q H L H Q L A A K M I N D F
CGG CTC TTC AAT AAT GCA GTC ATT CGT GTA CAA CAC CTG CAC CAG CTG GCT GCA AAA ATG ATT AAC GAC TTT 489
gtaagattctcattaaaatctattcagaactagtagattctaaactgtaatttagagcaaacattatttagtcacacctattaagggttaaaagt 584
aaatgtgcttaggactcacctgtaaacctctatcagatgcaattatttattagttatctgagtgagttaaatgtattataaataagcaatttta 679
                                     E D S L L P E E R R Q L S K
aatacaataactaaatacaatttacccttttgtttatcaag GAG GAC AGC CTG TTG CCT GAG GAA CGC AGA CAG CTG AGT AAA 759
I F P L S F C N S D Y I E A P A G K D E T Q K S
ATC TTC CCT CTG TCT TTC TGC AAT TCT GAC TAC ATT GAG GCG CCT GCT GGA AAA GAT GAA ACA CAG AAG AGC 831
S
TCT gtgagtaaaactatattataaaaaattcaatcccaacatgttttgcaggggttcgaactggggtctgaggaccggggggccagaaaag 925
ggtattatggtgtctgtggaaggtttggagaaaaacagtgtaaaaaataaatatgatttgactacataaatatgattaaaataagctgcctttta 1020
aatttttaggatgaggtccctcttatgaggatgatcatatttgggtgtacgtgcaatctgaaatattgaaaagccctgtttttattcaacaacata 1115
caactataaaacaaacogtatcaacaaattaaataatacaaaaagatgcagtgagtgaaatatttgacaatttgatgacaattttactcat 1210
aaattgcttataaatcagagaaatctttaagaacataatttaccacaccagtagatgctttccatcattaagcatctttgcatattatagtttg 1305
gaagcacttggaattgatcaaatatgggcagcataaaacataaaaaatggccaatggtctgaagcctccaattttatcatttatggtttcttttag 1400
                                     *
M L K L L R I S F H L I E S W E F P S Q S L S G
ATG CTG AAG CTT CTT CGC ATC TCT TTT CAC CTC ATT GAG TCC TGG GAG TTC CCT AGC CAG TCC CTG AGC GGA 1472
                                     A
T V S N S L T V G N P N Q L T E K L A D L K M G
ACC GTC TCA AAC AGC CTG ACC GTA GGG AAC CCC AAC CAG CTC ACT GAG AAG CTG GCC GAC TTG AAA ATG GGC 1544
I S V L I Q
ATC AGT GTG CTC ATC CAG gtgagaaagacaagcaagtgtctagtggctgaccagaaggtgtgacagcatcctcaaatcttatttggac 1633
                                     A C L D G Q P N M D D N D S L
cgctctaagttcattttacatttgtttctgcacag GCA TGT CTC GAT GGT CAA CCA AAC ATG GAT GAT AAC GAC TCC TTG 1713
P L P F E D F Y L T M G E N N L R E S F R L L A
CGG CTG CCT TTT GAG GAC TTC TAC TTG ACC ATG GGG GAG AAC AAC CTC AGA GAG AGC TTT CGT CTG CTG GCT 1785
C F K K D M H K V E T Y L R V A N C R R S L D S
TGC TTC AAG AAG GAC ATG CAC AAA GTC GAG ACC TAC TTG AGG GTT GCA AAT TGC AGG AGA TCC CTG GAT TCC 1857
N C T L
AAC TGC ACC CTG TAG ATGGCACGGTGTATTGTAGTCAATGCCTGTAAACACATTGTGCTTTGCTGCAAACTCTAAGACCAGTTTACAAT 1947
                                     A
                                     *
CTGGTCTTATATGTGCAGGAAATGTCAACGAGCATGCCTAGGTCTGTGTTTTCTTTTCCCTCCCATATTTAAACCTTACCTATTGTATTAT 2042
                                     A
TCTTCTCATTGTTGGAGTG-CTCATAAATT-AAAACCGTTCCTTTAAACGTAAGGGATGGATCTGGAACATTTACAGTGGTG-CTAAGCAATTT 2134
T T T
ATGGCAATATTTTAAATGTGGCCAAATTGACCTTAGTCAAAGTCTGACAATATGTTAAAAAAGGGCTAAAGATCAGTGTACGTGGAAATTTG 2229
TAATTTAAATCGGATGTGTCACTCTTGGTGTATGCATGTTAAACATTGTCTCATATATTATGCTCTTATTATACTCATCGTATCCTCTTCA 2324
AGCGCTGTGCTTTCTCTATTAAAGTTTAAATTGCATCCAAatattcttgcgtgtggttcaattttcctgctatctcaacatgcaaaaatg 2419
ttgaactggattttcagatcagaatctgttgttgcctatcatgagctgtaatgcgtcccaaaaacaatgagcaggtctaaaattggccgaaaat 2514
gttactgttgcatttaagctacaaacattggcacattgaacaagacagccaaaaaaaacataaatgtaaatattatgaaaaataaaa 2609
gcaataggtogac 2623

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Fig. 2. The complete nucleotide sequence of the carp GH gene. Exons are shown by capitals and introns by lower-case letters. The numbering is given in the right margin and begins at the cap site (+1). Encoded amino-acid residues are represented by one-letter codes above the codons. Asterisks indicate the positions at which the bases in mRNA [11] are different as shown below the genomic sequence; hyphens indicate deletions.

Two tandem repeats, TATA box and polyadenylation signal are underlined.

[10] was annealed to 1 pmol of a synthetic primer: CGCTCAGGGTAGACAACTC which is complementary to the genomic sequence from nucleotides 54–35 (Fig. 2). The primer extension reaction was carried out with the mouse Moloney leukemia virus reverse transcriptase. The 32 -labeled reaction products were analyzed by electrophoresis in 10% polyacrylamide sequencing gel.

Fig. 3. Determination of the transcription start site. Lanes G, A, T and C: size markers from dideoxy sequencing reaction products of vector mp18 as template with a suitable primer. Lane GH: primer-extension products generated using synthetic oligonucleotide primer and mRNA. The adenine residue corresponding to the 5' end of carp GH mRNA is indicated by an asterisk.

Fig. 4. Comparison of the *GH* structure of human [1,2], porcine [5], ovine [7], rat [3,4], trout [8] salmon [9] and carp (this study). Open box, untranslated region; solid box, coding region; lines between exons, introns.

Of the $1.5 \cdot 10^6$ recombinant plaques screened, two clones hybridized strongly with the 32 P-labeled cDNA probe. Restriction analysis of the DNA showed that they carried the same *GH* gene in opposite orientation (data not shown). Both clones contained an inserted 15 kb DNA fragment. The exon-containing region was narrowed down further to a 4.3 kb fragment produced by *SalI* digestion. This fragment was subcloned into pGEM 4 and sequenced (Figs. 1 and 2).

The transcription start site was determined by the primer extension method (Fig. 3) and was found to be an adenine residue (Fig. 2). As is often the case, the primer extension also produced some shorter oligonucleotides, probably due to the dissociation of reverse transcriptase caused by RNA secondary structures. The primary transcript is 2364 nucleotide long, with the transcription start site located at almost the same position as the *GH* of rainbow trout [8] and Atlantic salmon [9]. The sequence upstream from the transcription start point contains two tandem repeats of a decanucleotide AACTCTCATG (from -85 to -62) and a typical TATA box at -28 (Fig. 2). All introns start with a GT dinucleotide and end with an AG, analogous to other reported splice sequences [16]. The carp *GH* gene consists of five exons and four introns spanning a region of about 3 kb. It is interesting that the arrangement of exons and introns in this gene is more similar to that of mammalian *GH*s than that of the other two fish genes (Fig. 4), which are much longer in sequence and have one more intron and exon. Comparison between the nucleotide sequences of the exon regions and the cDNAs [10,11] reveals that this genomic clone corresponds almost perfectly to the cDNA reported by Koren et al. [11] with only nine differences in the nucleotide se-

quence, while the encoded amino-acid sequences are identical (Fig. 2).

This genomic clone is certainly not that which would give rise to the mRNA described in our previous report [10], as the genomic sequence is quite different from the mRNA immediately after the translation stop codon down to the end of the last exon. The gene corresponding to our reported cDNA [10] still awaits further screening and characterization.

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