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Genomic structure of the sea lamprey growth hormone-encoding gene

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Abstract

Growth hormone (GH) belongs to a family of pituitary hormones together with prolactin and somatolactin. In our previous study, GH and its cDNA were identified in the pituitary gland of the sea lamprey, *Petromyzon marinus*, an extant representative of the most ancient class of vertebrates, and isolated GH stimulated expression of insulin-like growth factor in the liver. The evidence suggests that GH is the ancestral hormone in the molecular evolution of the GH/PRL/SL family and that the endocrine mechanism for growth stimulation was established at an early stage in the evolution of vertebrates. To further understand the molecular evolution of the GH/PRL/SL gene family, we report the genomic structure of sea lamprey GH including its 5′-flanking region, being cloned by PCR using specific primers prepared from its cDNA. The sea lamprey GH gene consists of 13,604 bp, making it the largest of all the GH genes. The 5′-flanking region within 697 bp contains consensus sequences for a TATA box, two Pit-1/GHF-1, three TRE, and a CRE. The sea lamprey GH gene consists of five exons and four introns, the same as in mammals, birds, and teleosts such as cypriniforms and siluriforms with the exception of some teleosts such as salmoniforms, percififorms, and tetradontiforms, in which there is an additional intron in the 5th exon. The 5-exon-type gene organization might reflect the structure of the ancestral gene for the GH/PRL/SL gene family.

Keywords: Growth hormone; Sea lamprey; Genomic structure; Exon–intron organization; 5′-Flanking region

1. Introduction

Growth hormone (GH), prolactin (PRL), and somatolactin (SL) form a family of pituitary hormones, which share similarity in structure and function, and thus are believed to have evolved from a common ancestral gene through duplication and subsequent divergence. In terms of the molecular evolution of the GH/PRL/SL gene family, it is not known which member of this family is the closest relative to the ancestral hormones. Recently, we identified GH and its cDNA in the sea lamprey, *Petromyzon marinus*, an extant representative of the most ancient class of vertebrates, the Agnatha (Kawauchi et al., 2002). This study provides conclusive evidence that GH is present in all classes of vertebrates. The sea lamprey GH is far removed from the gnathostome GHs, suggesting that GH diversified after the separation of agnathans and gnathostomes. On the other hand, there has been no evidence to support that PRL and SL are present in agnathans. The identification of GH in the most ancient lineage of vertebrates indicates that GH is the ancestral hormone in the molecular evolution of the GH/PRL/SL gene family (Kawauchi et al., 2002).

Although GH and/or its cDNA have been identified in over 110 species from all taxonomic groups of vertebrates, GH-encoding genes have been characterized in only 10 mammals; [human (DeNoto et al., 1981), rat (Barta et al., 1981), mouse (Das et al., 1996), bovine (Woychik et al., 1982), pig (Vize and Wells, 1987), sheep (Byrne et al., 1987), rabbit (Wallis and Wallis, 2001), red deer (Lioupis and Wallis, 1997), chevrotain (Wallis and Wallis, 2001), and dolphin

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(Maniou et al., 2002)]; one birds species [chicken (Tanaka et al., 1992; Wallis and Wallis, 1995)]; and 15 teleost species [cypriniforms such as common carp (Chiou et al., 1990), grass carp (Zhu et al., 1992), silver carp (Hong and Schartl, 1993), and mud loach (Noh et al., 1999); siluriforms such as channel catfish (Tang et al., 1993); salmoniforms such as rainbow trout (Agellon et al., 1988a,b), Atlantic salmon (Johansen et al., 1989; Male et al., 1992), chinook salmon (Du et al., 1993), and sockeye salmon (Devlin, 1993); percififorms such as tilapia (Ber and Daniel, 1992, 1993), flounder (Tanaka et al., 1992), barramundi (Yowe and Epping, 1995), yellowtail (Ohkubo et al., 1996), and gilthead seabream (Almuly et al., 2000); tetradontiforms such as pufferfish (Venkatesh and Brenner, 1997)].

Teleost GH genes can be grouped into two types. Those of cypriniforms and siluriforms consist of five exons and four introns (5-exon-type) as in mammals and birds, while those of salmoniforms, percififorms, and tetradontiforms consist of six exons and five introns (6-exon-type). The 6-exon-type has an intron inserted in the 5th exon of the 5-exon-type. On the other hand, all known PRL genes of mammals [e.g., human (Truong et al., 1984), bovine (Camper et al., 1984), and rat (Gubbins et al., 1980)], birds [turkey (Kurima et al., 1995) and chicken (Au and Leung, 2002)], and teleosts [carp (Chen et al., 1991), chinook salmon (Xiong et al., 1992), tilapia (Swennen et al., 1992), and gilthead seabream (Astola et al., 2003)] are of the 5-exon-type. Moreover, chum salmon and gilthead seabream SL genes are also of the 5-exon-type (Astola et al., 2004; Takayama et al., 1991). These results provide evidence that the 5-exon-type gene organization reflects the structure of the ancestral gene for the GH/PRL/SL gene family. However, the GH gene has yet to be characterized in early evolved fish such as chondrichthyes and agnathans. To further understand the molecular evolution of the GH/PRL/SL gene family, we report here the structure of the sea lamprey GH-encoding gene including its 5′-flanking region, which was cloned by PCR using specific primers prepared from its cDNA, and discuss the evolutionary implications for the GH/PRL/SL gene family.

2. Materials and methods

2.1. Animals

Sampling and tissue collection were done in accordance with the UNH IACUC animal care guidelines. Adult, sea-run sea lampreys, *P. marinus*, were collected in a trap on the Cocheco River in Dover, New Hampshire in May and June, 2000 during their migration from the ocean upstream to coastal rivers where they undergo their final spawning. The sea lamprey were transported to the freshwater fish hatchery at the University of New Hampshire. They were killed by decapitation, and the liver was collected, immediately frozen in liquid nitrogen, and stored at −80 °C prior to use.

2.2. Isolation of genomic DNA

Genomic DNA was prepared from the liver (50 mg) of adult, sea-run, sea lamprey using ISOTISSUE (Nippon Gene, Tokyo, Japan) according to the manufacturer's directions. The concentration of genomic DNA was estimated by measuring the absorbance at 260 nm, and the purity was determined from the ratio of absorbance at 260/280 nm.

2.3. Polymerase chain reaction

*Xba*I cassette DNA and cassette-specific primers were purchased from Takara (Tokyo, Japan). Templates for inverse PCR were prepared after the digestion of genomic DNA with *Hind*III (Nippon Gene, Tokyo, Japan) according to the method of Ochman et al. (1990). To clone introns of the sea lamprey GH gene, insertion positions of introns on the precursor mRNA were assumed by sequence comparison of sea lamprey GH cDNA (Kawauchi et al., 2002) with GH genes of teleosts such as catfish (Tang et al., 1993), carps (Chiou et al., 1990; Ho et al., 1991), salmonids (Agellon et al., 1988a,b; Devlin, 1993; Du et al., 1993; Johansen et al., 1989; Male et al., 1992), and flounder (Tanaka et al., 1992). A set of primers specific for each intron was prepared from the sea lamprey GH cDNA sequence (Kawauchi et al., 2002). Primers were ordered from Nihon Gene Research Laboratories (Sendai, Japan). DNA was amplified using AmpliTaq Gold PCR Master Mix (Applied Biosystems, Forster City, CA) and GeneAmp XL PCR Kit (Applied Biosystems). PCR was done using a thermal cycler (PC-808, Astec, Fukuoka, Japan) with a combination of the gene-specific primers listed in Table 1. The PCR profile using the AmpliTaq Gold PCR Master Mix was enzyme activation at 95 °C for 15 min, 30 cycles of denaturation (1 min at 94 °C), annealing (1 min at 60 °C), extension (2 min 30 s at 72 °C), and a final extension at 72 °C for 7 min. The PCR profile using the GeneAmp XL PCR Kit was enzyme

Table 1
Custom-made oligonucleotide primers used for PCR to amplify DNA fragments of the sea lamprey GH gene

Primer	Nucleotide sequence
a	5′-GTGTTTGGACTTGGGATATTGTTTTTGC-3′
b	5′-CCAGGTTGAGGTCACGGAGAGGATCA-3′
c	5′-GCGCTTGCCCCAAAAGTCTATTCAA-3′
d	5′-GGACCTCGTCCTTCTTGCTGG-3′
e	5′-TGTCGTGTCACCCAGCCAGCT-3′
f	5′-CTGCCGCTCATTGCGTAGGAGGCTCT-3′
g	5′-TGGAGTCGTGGAGAGGGGTGTT-3′
h	5′-CTGCTCAGCTGCTTCAAGAA-3′
i	5′-CCAGTGCATTGCGCGTCCAA-3′
j	5′-CGTTAGAACGCGTAATACGACTCACTATAGGGAGA-3′
k	5′-CAACAGAGTGTCTGAGCTTCT-3′
l	5′-CGACGTTGGCCCTCTTGGCTTTAGAAGG-3′
m	5′-GGGCAATGCCTTTATTCAAGCAAACGTGT-3′

Primers were synthesized by Nihon Gene Research Lab's. (Sendai, Japan) Excluding j, which was purchased from Takara (Tokyo, Japan).