Available online at www.sciencedirect.com
SCIENCE DIRECT®
General and Comparative Endocrinology 144 (2005) 174–181
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

Communication in Genomics and Proteomics

Structures for the proopiomelanocortin family genes *proopiocortin* and *proopiomelanotropin* in the sea lamprey *Petromyzon marinus*

Akiyoshi Takahashi [a,*], Osamu Nakata [a], Makoto Kasahara [a], Stacia A. Sower [b], Hiroshi Kawauchi [a]

[a] *Laboratory of Molecular Endocrinology, School of Fisheries Sciences, Kitasato University Sanriku, Ofunato, Iwate 022-0101, Japan*
[b] *Department of Biochemistry and Molecular Biology, University of New Hampshire, NH 03824, USA*

Received 19 March 2005; revised 2 May 2005; accepted 5 May 2005
Available online 24 June 2005

Abstract

Gnathostomes express a common *proopiomelanocortin* (*POMC*) gene in the pars distalis (PD) and the pars intermedia (PI) of the pituitary gland. In contrast, the sea lamprey *Petromyzon marinus* expresses one distinct gene in each lobe; *proopiocortin* (*POC*) encoding adrenocorticotropic hormone (ACTH) and β-endorphin (END) is expressed in the PD and *proopiomelanotropin* (*POM*) encoding melanophore-stimulating hormone (MSH), and a different β-END is expressed in the PI. We characterized the genomic structure of the sea lamprey *POC* and *POM* genes including their 5′-flanking regions. Both genes have two introns at positions similar to those of gnathostomes. Each exon encodes genetic information seen in the gnathostome *POMC* gene: exon 1 encodes an untranslated nucleotide sequence, exon 2 encodes a signal peptide and the N-terminal short part of POC or POM, and exon 3 encodes all other parts including ACTH, MSHs or β-END. Intron-A of *POM* (2289 bp) is six times longer than that of *POC* (379 bp). The *POM* intron-A has three transposon-like sequences (TnL-1, -2, -3), the total length of which is 1781 bp, suggesting that it has expanded via the insertion of TnLs. The 5′-flanking region of the *POC* gene contains two TATA boxes, a CCAAT box, eight E boxes, STAT, RAIE, and one binding site each for Ptx1, Pit-1, and Tpit. The *POM* gene contains four TATA boxes, eight E boxes, three STATs, two RAIEs, two CRE-like elements, and one binding site for Pit1. However, there is virtually no similarity between the two genes in the distribution of the elements. The transcriptional regulation of *POC* and *POM* may have diverged with the functional differentiation of the two genes.

Keywords: Lamprey; Proopiocortin; Proopiomelanotropin; Gene structure; Nucleotide sequence; Intron; Exon; 5′-flanking region; Transposon-like sequence; Transcriptional element; Adrenocorticotropic hormone; β-Endorphin; Melanophore-stimulating hormone

1. Introduction

Adrenocorticotropic hormone (ACTH), melanophore-stimulating hormone (MSH), β-endorphin (β-END), and other peptides are encoded on a common *proopiomelanocortin* (*POMC*) gene expressed in both the pars distalis (PD) and pars intermedia (PI) of the pituitary gland in gnathostomes (Takahashi and Kawauchi, 2005). Tissue-specific posttranslational processing of POMC results in the production of ACTH and β-END in the PD, and MSHs and *N*-acetyl-β-END in the PI (Castro and Morrison, 1997; Smith and Funder, 1988).

Lampreys, which are descendants of agnathans, appeared over 500 million years ago (Forey and Janvier, 1993). The pituitary gland of the lamprey is composed of the PD and PI, the former subdivided into the rostral and proximal PD as seen in teleost fish (Gorbman et al., 1983). We have previously isolated ACTH and two

* Corresponding author. Fax: +81 192 44 3934.
E-mail address: akiyoshi@kitasato-u.ac.jp (A. Takahashi).

0016-6480/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2005.05.002

different forms of MSH from sea lamprey pituitary glands (Takahashi et al., 1995a), and identified ACTH-producing cells and MSH-producing cells immunocytochemically in the PD and PI, respectively (Nozaki et al., 1995). The occurrence and topological distributions of these POMC-derived peptides appear comparable to those in gnathostomes. In the sea lamprey, however, ACTH and one form of β-END are encoded by one gene called *proopiocortin* (*POC*), whereas the two forms of MSH and the other form of β-END are encoded by the other gene *proopiomelanotropin* (*POM*). The *POC* is expressed in the PD and *POM* in the PI (Takahashi et al., 1995b). On the basis of sequence comparison, we suggested that an ancestral *POMC* gene may have duplicated and differentiated into the PD-specific *POC* gene and PI-specific *POM* gene in concert with the specialization of pituitary function during the course of lamprey evolution (Takahashi and Kawauchi, 2005; Takahashi et al., 2001).

The genomic structure of the *POMC* gene has been reported in human (Cochet et al., 1982), bovine (Nakanishi et al., 1981), rat (Drouin et al., 1985), mouse (Notake et al., 1983), chicken (Takeuchi et al., 1999), *Xenopus laevis* (Deen et al., 1992), and zebrafish (Gonzalez-Nunez et al., 2003; Hansen et al., 2003) and shown to be well conserved. Two introns are present at homologous positions and all functional segments, ACTH, MSHs, and β-END, are encoded on exon 3.

In mammals, transcription of *POMC* in the PD and PI is controlled by hypothalamic hormones and glucocorticoids (Drouin et al., 1987; Gagner and Drouin, 1987). Several transcription factors synergistically participate in the initiation of transcription of the POMC gene (Therrien and Drouin, 1991). Among them, pituitary homeobox 1 (Ptx1) and pituitary cell-restricted T box factor (Tpit) are essential for cell-specific transcription of the POMC gene (Lamonerie et al., 1996; Lamolet et al., 2001). These factors also participate in the development of POMC cells (Lamonerie et al., 1996; Pulichino et al., 2003).

The present study was undertaken to determine the nucleotide sequences of introns and 5′-flanking regions of *POC* and *POM* to investigate the diversity and evolutional differentiation of these genes.

2. Materials and methods

2.1. Lampreys and preparation of nucleic acid

Sampling and tissues collection were done in accordance with the UNH IACUC animal care guidelines. Up-migrating adult sea lampreys, *Petromyzon marinus*, were collected in a trap of the fish ladder at the Cocheco River, New Hampshire. The lampreys were transported to the freshwater fish hatchery at the University of New Hampshire and maintained in an artificial stream. They were killed by decapitation, and the liver was removed and frozen on dry ice until transferred to a −80 °C freezer. Genomic DNA was prepared from adult liver using Isotissue (Nippon Gene, Tokyo, Japan).

2.2. Polymerase chain reaction

*Hin*dIII cassette DNA and cassette-specific primers were purchased from Takara (Tokyo, Japan). Templates for inverse PCR were prepared after digestion of genomic DNA with *Nde*I (Nippon gene, Tokyo) according to the method of Ochman et al. (1990). DNA was amplified using AmpliTaq Gold Master Mix (Applied Biosystems, Foster City, CA), Takara LA Taq with GC Buffer (Takara, Tokyo, Japan), or HotStar Taq Master Mix (Qiagen, Hilden, Germany). PCR was done using a thermal cycler (PC-808, Astec, Fukuoka, Japan) with a combination of gene-specific primers listed in Table 1. Profile of PCR with the AmpliTaq Gold Master Mix was activation of the enzyme at 94–95 °C for 10–15 min then 30 cycles of denaturation (1 min at 94–95 °C)—annealing (0.5–1 min at 50–55 °C)—extension (1–2 min at 72 °C), followed by a final extension at 72 °C for 10 min; that with Takara LA Taq with GC buffer was preheating of the reaction mixture excluding an enzyme at 94 °C for 2 min, subsequently heating of the reaction mixture

Table 1
Custom oligonucleotide primers used for PCR to amplify DNA fragments of sea lamprey *POC* and *POM* genes

Primer	Target	Nucleotide sequence
a	POC-1	5′-CTGCAACGCAAAGCAACACT-3′
b	POC-1	5′-GACAGCATCTCCAGCAGAA GCAGCA-3′
c	POC-2	5′-GTGCTGCTGGAATGATGGGA-3′
d	POC-2	5′-GTCGTCGTCGTTGTCATC-3′;
e	POC-3	5′-AGCTCAAATGCAGCGACGAC-3′
f	POC-3	5′-ACCCCATTGAAGGCGTAGTC-3′
g	POC-4	5′-GATAAGGCCACCATCCGCAA-3′
h	POC-4	5′-TTGAAGCGATTAATAGAT-3′;
i	POC-5	5′-CGTTAGAACGCGTAATACG ACTCACTATAGGGAGA-3′
j	POC-5	5′-TGACAGCATCTCCAGCAGAA GCAGCAGTCG-3′
k	POM-1	5′-ACCCGCCTTTGCTCACAA-3′
l	POM-1	5′-AGCTCTCGCACGCCTGTA-3′
m	POM-2	5′-TGTCGCTCTCCTACTGTC-3′
n	POM-2	5′-GAAGTGTTGCATCCGGTA-3′
o	POM-3	5′-GAGATTGTGCTCCTTGGA-3′
p	POM-3	5′-CTGTCCACTCTTTGGTTG-3′;
q	POM-4	5′-ACACCTACAGTGTGGTTGGT-3′
r	POM-4	5′-TAAGTGGGTTACATGTGT-3′
s	POM-5	5′-TGCACGTATGTACGTTGAACT TCCTTCGTA-3′
t	POM-5	5′-AGAAATCGTAAGTATGCGCA ATGCGTGAGC-3′

Synthesis of primers was performed by Nihon Gene Research Lab's (Sendai, Japan) excluding i, which was purchased from Takara (Tokyo Japan).