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).

including the enzyme at 94 °C for 3 min, and then 30 cycles of denaturation (0.5 or 15 min at 98 °C)—annealing/extension (4 min at 70 °C or 15 min at 68 °C), and that with HotStar Taq Master Mix was activation of the enzyme at 95 °C for 10 min then 35 cycles of denaturation (1 min at 95 °C)—annealing (1 min at 70 °C)—extension (3 min at 72 °C), followed by a final extension at 72 °C for 5 min.

2.3. Nucleic acid sequence determination

PCR-amplified DNA, purified using a QIAEX II Gel Extraction kit (Qiagen) after gel electrophoresis on Nusieve GTG agarose (Cambrex Bio Science Rockland, Rockland, ME), was cloned using pT7 Blue T vector (Novagen, Madison, WI) or a TOPO XL PCR Cloning kit (Invitrogen, Carlsbad, CA). Recombinant plasmid DNA was prepared by the alkaline-SDS method (Mierendorf and Pfeffer, 1987) and sequenced on both strands using a Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). DNASIS-Pro (Hitachi Software Engineering, Yokohama, Japan) and Genetyx-Mac (Software Development, Tokyo, Japan) were used for processing the sequence and searching for repetitive sequences. Homology searches were done using the FASTA analysis program (http://www.ddbj.nig.ac.jp/search/fasta-e.html). In both *POC* and *POM* genes, 5′-ends were assigned by determination of capping sites of corresponding mRNAs according to the method of Ohtake et al. (2004).

3. Results

Clones POC-1 to 5 were amplified from genomic DNA by PCR using primers based on the nucleotide sequence of POC cDNA and the cassette primer (Fig. 1). The length of the clones excluding primers are 426 bp for POC-1, 1669 bp for POC-2, 348 bp for POC-3, 475 bp for POC-4, and 1604 bp for POC-5. The merging of these five DNA fragments provided a *POC* gene consisting of 2882 bp and its 5′-flanking region of 1153 bp (Fig. 1).

Clones POM-1 to 4 were amplified from genomic DNA by PCR using primers based on the nucleotide sequence of POC cDNA (Fig. 1). The clone POM-5 was amplified from self-ligated genomic DNA by inverse PCR after digestion with the restriction enzyme *Nde*I. The length of the clones excluding primers are 2454 bp for POM-1, 2088 bp for POM-2, 1158 bp for POM-3, 582 bp for POM-4, and 2843 bp for POM-5. The merging

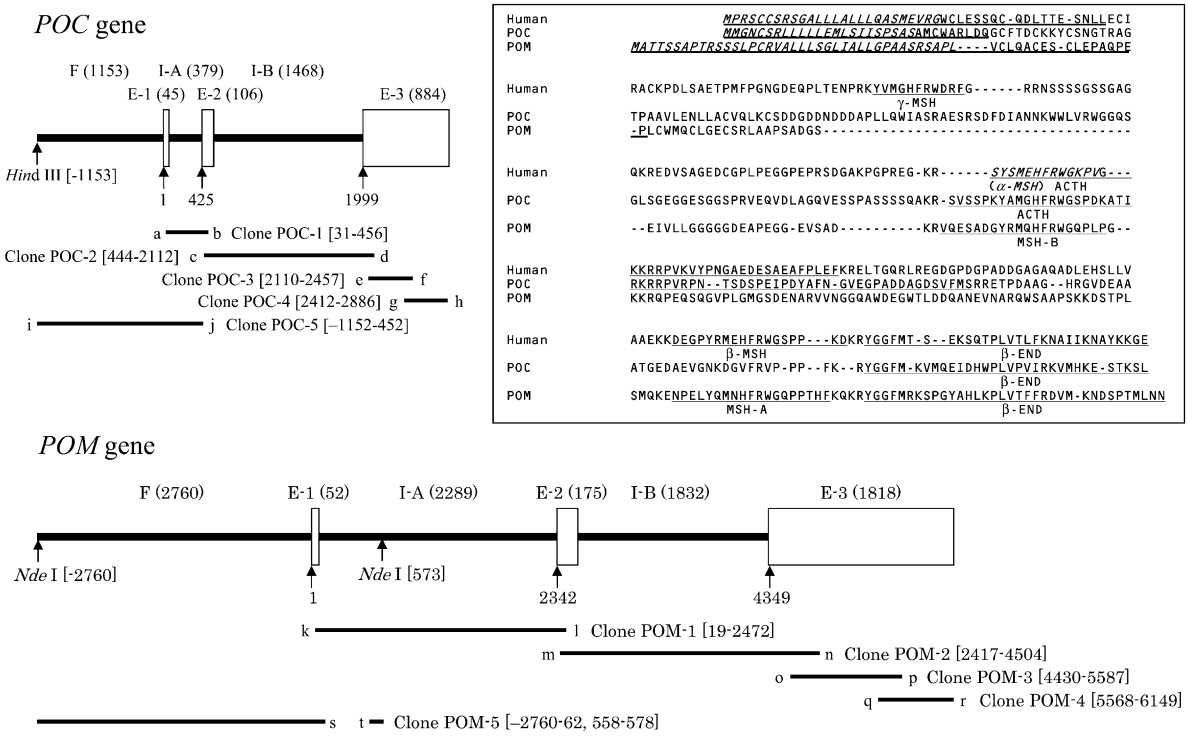

Fig. 1. Schematic diagrams of sea lamprey *POC* and *POM* genes. Introns are marked with I, 5′-flanking regions with F, and exons with E. Numbers in parentheses show segment size in base pairs. Arrows indicate first positions of exons or restriction sites of *Hin*dIII and *Nde*I. Thin bars show relative positions of DNA clones amplified by PCR. Lower cases on both sides of thin bars show primers (see Table 1 for sequences). The numbers in square brackets show 5′ and 3′ ends excluding primers. For details of nucleotide sequences, see Accession No. AB206464, for the *POC* gene, and AB206465, for the *POM* gene, in the DDBJ/EMBL/GenBank nucleotide sequence database. (Inset) Amino acid sequences of prePOC and prePOM compared with the sequence of human prePOMC. Bold bars show the part of prePOC or prePOM encoded on exon 2. Other parts without bold bars are encoded on exon 3. Italics show signal peptides. Thin bars show ACTH, MSH, and β-END segments.

of these five DNA fragments provided a *POM* gene consisting of 6166 bp and its 5′-flanking region of 2760 bp (Fig. 1). The 5′-flanking region and exon one of both *POC* and *POM* are shown in Fig. 2.

Nucleotide sequence comparison of *POC* and *POM* with corresponding cDNAs (Takahashi et al., 1995b) reveals that these genes are composed of three exons and two introns (Fig. 1). In the *POC* gene, exons 1, 2, and 3 are composed of 45, 106, and 884 bp, respectively. Exon 2 encodes a signal peptide consisting of 23 aa and POC (1–9), and exon 3 encodes POC (10–246) including ACTH and β-END (Fig. 1 inset). In the *POM* gene, exons 1, 2, and 3 are composed of 52, 175, and 1818 bp, respectively. Exon 2 encodes a signal peptide consisting of 39 aa and POM (1–17), and exon 3 encodes POM (18–189) including MSH-A, MSH-B, and β-END (Fig. 1 inset).

The *POC* intron-A and -B are composed of 379 and 1468 bp, respectively, and the *POM* intron-A and -B are composed of 2289 and 1832 bp, respectively (Fig. 1). There are three transposon-like sequences (TnLs) in the *POM* intron-A, each of which is characterized by the presence of an inverted repeat accompanying the direct repeat (Fig. 3). Among the three direct repeats, two are palindromes identical to the restriction site of *Bal*I or *Sna*BI. The number of nucleotides composing TnL-1 and -2 containing a nested TnL-3 is 1055 and 726 bp, respectively.

A FASTA homology search revealed similarity in nucleotide sequence between a part of TnL-3 and some genomic sequences in sea lamprey. The sequence identity of *POM* (1886–2102) versus a segment of the intron of an ABCB9-like TAP-family protein gene was 77% in a 220-bp overlap, that of *POM* (1918–2094) versus a segment of the intron of the P-opsin gene was 78% in a 179-bp overlap, and that of *POM* (1917–2095) versus an upstream segment of the lamprin L-1.8 gene is 73% in a 184-bp overlap.

Some putative transcriptional elements were observed in the 5′-flanking regions of the *POC* and *POM* genes (Fig. 2). The 5′-flanking region of the *POC* gene contains two TATA boxes (TATAWAW), one CCAAT box (CCAAT), one binding site for Ptx1 (CTAAGCC), one binding site for Tpit (TCACAC), one binding site for pituitary transcription factor-1 (Pit-1, TATCCAT), eight E boxes (CANNTG), one signal transducer and activator of transcription response element (STAT, TTCNN NGAA), and one retinoic acid-inducible element (RAIE, AGGTCA). The 5′-flanking region of the *POM* gene contains four TATA boxes, one binding site for Pit1, eight E boxes, three STATs, two RAIEs, and two cyclic AMP response element-like elements (CRE-like, CTGCA).

4. Discussion

The sea lamprey *POC* and *POM* genes are each composed of three exons and two introns. The gene organization is the same as that of the gnathostome *POMC* genes (Cochet et al., 1982; Deen et al., 1992; Drouin et al., 1985; Gonzalez-Nunez et al., 2003; Hansen et al., 2003; Nakanishi et al., 1981; Notake et al., 1983; Takeuchi et al., 1999). The genetic information encoded in each exon of *POC* and *POM* is also similar to that of the *POMC* gene; exon 1 encodes an untranslated nucleotide sequence, exon 2 encodes a signal peptide and N-terminal short region, and exon 3 encodes the remaining segment including ACTH, MSH or END (Fig. 1). The similarity between *POC* and *POM* supports our interpretation that the two genes may have originated from a common ancestral *POMC* gene during the course of lamprey evolution (Takahashi and Kawauchi, 2005; Takahashi et al., 1995b, 2001). Moreover, the similarity in the genomic structure of POMC between lampreys and gnathostomes suggests that the gene organization may have been established early in the evolution of vertebrates, probably before the divergence of the gnathostome lineage from lampreys.

In addition to sea lamprey, POMC subtypes have been identified in barfin flounder, sockeye salmon, rainbow trout, carp, zebrafish, sturgeon, and paddlefish. (Alrubaian et al., 1999; Arends et al., 1998; Danielson et al., 1999; Gonzalez-Nunez et al., 2003; Okuta et al., 1996; Salbert et al., 1992; Takahashi et al., 2000, 2005). However, *POC* and *POM* in sea lamprey differ from gnathostome *POMC* subtype as to the tissue distribution and function. Namely, *POC* and *POM* are expressed specifically in the PD and PI, respectively, in sea lamprey, while *POMC* subtypes are expressed in both the PD and PI in the gnathostomes (Salbert et al., 1992). Moreover, *POC* encoding ACTH and one form of β-END is functionally different from *POM* encoding MSH and the other form of β-END, while gnathostomes *POMC* subtypes commonly encodes ACTH, MSH, and β-END. Despite these differences, the roles of the PD and PI are conserved between sea lamprey and gnathostomes in a sense that ACTH and MSH are major products in the PD and PI, respectively (Castro and Morrison, 1997; Nozaki et al., 1995; Smith and Funder, 1988).

The presence of the TnLs in intron-A of *POM* suggests that the intron has expanded through three successive insertions of DNA after the duplication of a common ancestral gene. One of the three events may have resulted in a nested structure, TnL-3 within TnL-2. Although the intron-A of *POM* (2289 bp) is six times longer than that of *POC* (379 bp), its length excluding TnL-1 (1055 bp) and TnL-2 (726 bp) is 508 bp. This value is comparable to that of the *POC* intron-A. Therefore, the intron-A of the ancestral *POM* gene would have been much shorter than the present one, and hence similar to that of *POC*. The similarity in nucleotide sequence between of TnL-3 and other segments within the sea lamprey genome suggests that intron-A of *POM* evolved through the insertion of DNA originating from other parts of the genome.

Fig. 2. Structures of 5′-flanking regions of sea lamprey *POC* and *POM* genes. Exon 1 of each gene is shown in lower case. ▼, 5′ end of exon 1, which is designated as position 1.

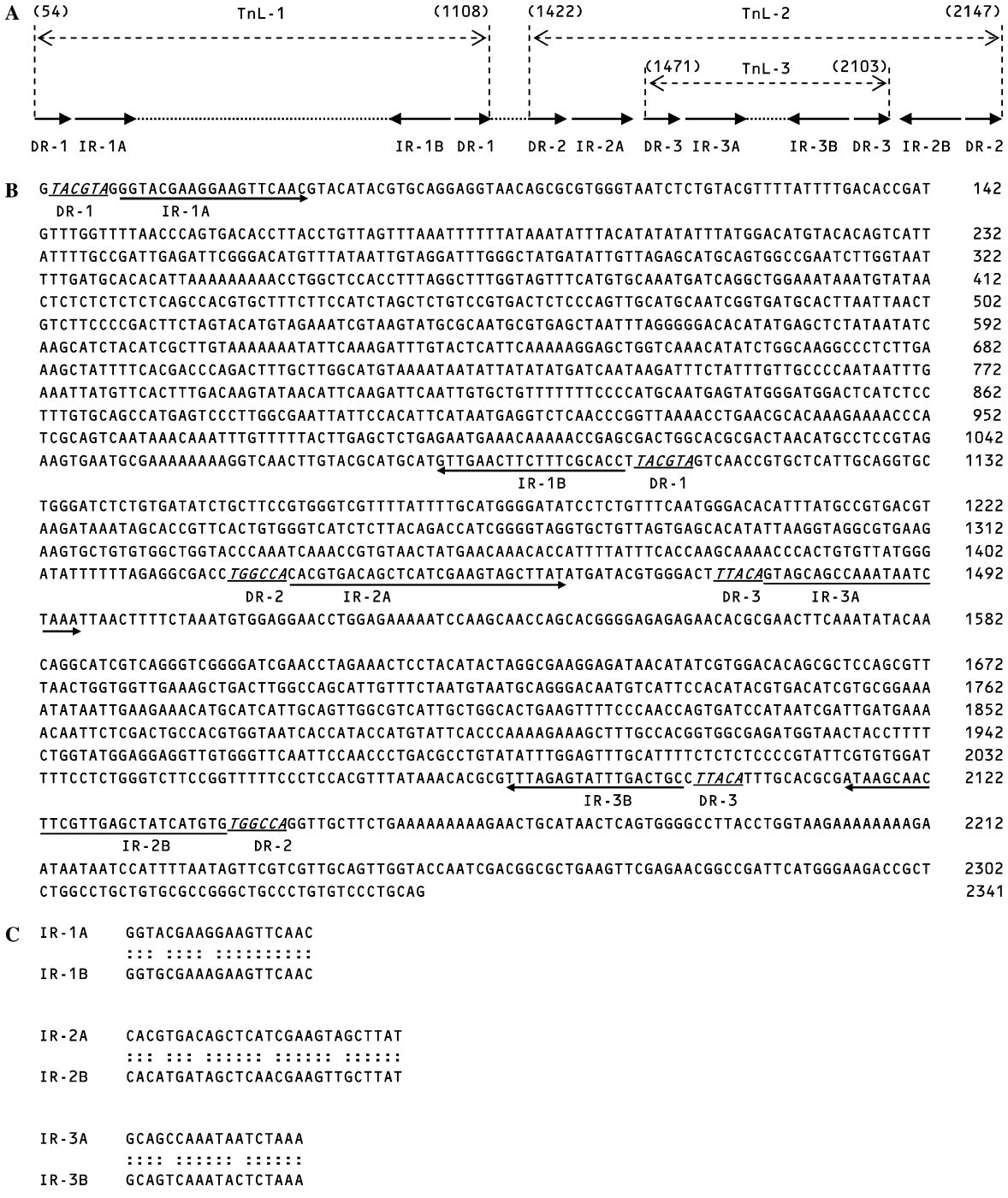

Fig. 3. Structure of intron-A of the sea lamprey *POM* gene. (A) Schematic diagram showing relative positions of the TnLs. The numbers in parentheses show positions of 5′ or 3′ ends of TnLs on the *POM* gene. DR, direct repeat. IR, inverted repeat. (B). Nucleotide sequence of intron-A showing the structures of direct repeats with italics and underlines, and inverted repeats with arrows. DR-1 and DR-2 are identical to *Sna*BI and *Bal*I sites, respectively. (C) Sequence comparison of inverted repeats. The sequence of "B" is reversed and complementary.

There are several kinds of transcriptional elements in the 5′-flanking region of *POC* and *POM*. A Pit-1-binding site, an E box, STAT, and RAIE are observed in the 5′-flanking regions of both genes. However, virtually no similarity was observed between the two genes in the distribution of these elements. Moreover, while the *POM* gene contains a TATA box in the vicinity of the transcription initiation site, which is conserved in the zebrafish, *Xenopus laevis*, human, rat, and bovine *POMC* genes, the *POC* gene does not. Based on the tissue-specific expression of the *POC* and *POM* genes in sea lamprey pituitary gland, we proposed that after duplication of the ancestral *POMC* gene, each copy evolved in concert with the specialization of tissue

function (Takahashi and Kawauchi, 2005; Takahashi et al., 2001). It is conceivable that changes in regulatory elements after gene duplication resulted in the expression of *POC* and *POM* gene in the PD and PI, respectively, which synchronized with the mutations in the coding regions so that the two distinct precursors diverged to encode ACTH and the two MSHs separately.

The presence of several kinds of transcriptional elements suggests that the expression of *POC* and *POM* is regulated by synergistic interactions of several regulatory factors as in mammals (Therrien and Drouin, 1991). Among several possible factors, Ptx1 and Tpit, are known to be *POMC*-specific transcription factors and to activate cooperatively *POMC* transcription in mammals (Lamolet et al., 2001). Ptx1 and Tpit may also cooperate in the case of the *POC* gene because their binding sites adjoin in the 5′-flanking region. Although Pit-1 is primarily responsible for the expression of growth hormone family genes in gnathostome pituitaries (Bodner et al., 1988; Ingraham et al., 1988; Ono et al., 1994), both *POC* and *POM* have a Pit-1-binding site in the 5′-flanking region in sea lamprey, suggesting the importance of Pit-1 in the transcription of the two genes. In addition to their roles as transcription factors, Ptx1, Tpit, and Pit-1 have been shown to be involved in the differentiation of pituitary cells during organogenesis (Castrillo et al., 1991; Lamonerie et al., 1996; Pulichino et al., 2003). The presence of binding sites for Ptx1, Tpit, and Pit-1 in the *POC* gene and Pit-1 in the *POM* gene suggests that the corresponding factors also have important functions during the development of pituitary cells into corticotrophs in the PD and melanotrophs in the PI in lamprey.

In conclusion, we determined the structure of the genes of *POC* and *POM* in sea lamprey. These genes are composed of three exons and two introns as in mammals, suggesting the early establishment of the *POMC* gene during the evolution of vertebrates. Intron-A of *POC* is thought to have expanded with the insertion of TnLs. The area upstream of the *POC* and *POM* genes showed no similarity in terms of the distribution of putative transcriptional elements. The transcriptional regulation of *POC* and *POM* may have diverged with the functional differentiation of the genes.

Acknowledgments

We thank Ms. Kayo Akiyama and Mr. Hiroho Komatsu, Kitasato University, and Mr Eiji Ohara, Nippon Gene, Co., for technical assistance. This study was supported in part by a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, to H.K.

References

Alrubaian, J., Danielson, P., Fitzpatrick, M., Schreck, C., Dores, R.M., 1999. Cloning of a second proopiomelanocortin cDNA from the pituitary of the sturgeon, *Acipenser transmontanus*. Peptides 20, 431–436.

Arends, R.J., Vermeer, H., Martens, G.J.M., Leumissen, J.A.M., Wendellar Bonga, S.E., Flik, G., 1998. Cloning and expression of two proopiomelanocortin mRNAs in the common carp (*Cyprinus carpio* L.). Mol. Cell. Endocrinol. 143, 23–31.

Bodner, M., Castrillo, J.L., Theill, L.E., Deerinck, T., Ellisman, M., Karin, M., 1988. The pituitary-specific transcription factor GHF-1 is a homeobox-containing protein. Cell 55, 505–518.

Castrillo, J.L., Theill, L.E., Karin, M., 1991. Function of the homeodomainprotein GHF1 in pituitary cell proliferation. Science 253, 197–199.

Castro, M.G., Morrison, E., 1997. Post-translational processing of proopiomelanocortin in the pituitary and in the brain. Crit. Rev. Neurobiol. 11, 35–57.

Cochet, M., Chang, A.C., Cohen, S.N., 1982. Characterization of the structural gene and putative 5′-regulatory sequences for human proopiomelanocortin. Nature 297, 335–339.

Danielson, P.B., Alrubaian, J., Muller, M., Redding, J.M., Dores, R.M., 1999. Duplication of the POMC gene in the paddlefish (*Polyodon spathula*): analysis of γ-MSH, ACTH, and β-endorphin regions of ray-finned fish POMC. Gen. Comp. Endocrinol. 116, 164–177.

Deen, P.M., Bussemakers, M.J., Terwel, D., Roubos, E.W., Martens, G.J., 1992. Comparative structural analysis of the transcriptionally active proopiomelanocortin genes A and B of *Xenopus laevis*. Mol. Biol. Evol. 9, 483–494.

Drouin, J., Chamberland, M., Charron, J., Jeannotte, L., Nemer, M., 1985. Structure of the rat pro-opiomelanocortin (POMC) gene. FEBS 193, 54–58.

Drouin, J., Charron, J., Gagner, J.P., Jeannotte, L., Nemer, M., Plante, R.K., Wrange, O., 1987. Pro-opiomelanocortin gene: a model for negative regulation of transcription by glucocorticoids. J. Cell Biochem. 35, 293–304.

Forey, P., Janvier, P., 1993. Agnathans and the origin of jawed vertebrates. Nature 361, 129–134.

Gagner, J.P., Drouin, J., 1987. Tissue-specific regulation of pituitary proopiomelanocortin gene transcription by corticotropin-releasing hormone, 3′, 5′-cyclic adenosine monophosphate, and glucocorticoids. Mol. Endocrinol. 1, 677–682.

Gonzalez-Nunez, V., Gonzalez-Sarmiento, R., Rodriguez, R.E., 2003. Identification of two proopiomelanocortin genes in zebrafish (*Danio rerio*). Brain Res. Mol. 120, 1–8.

Gorbman, A., Dickhoff, W.W., Vigna, S.R., Clark, N.B., Ralph, C.L., 1983. Comparative Endocrinology. John Wiley and Sons, New York.

Hansen, I.A., To, T.T., Wortmann, S., Burmester, T., Winkler, C., Meyer, S.R., Neuner, C., Fassnacht, M., Allolio, B., 2003. The pro-opiomelanocortin gene of the zebrafish (*Danio rerio*). Biochem. Biophys. Res. Commun. 303, 1121–1128.

Ingraham, H.A., Chen, R.P., Mangalam, H.J., Elsholtz, H.P., Flynn, S.E., Lin, C.R., Simmons, D.M., Swanson, L., Rosenfeld, M.G., 1988. A tissue-specific transcription factor containing a homeodomain specifies a pituitary phenotype. Cell 55, 519–529.

Lamolet, B., Pulichino, A.M., Lamonerie, T., Gauthier, Y., Brue, T., Enjalbert, A., Drouin, J., 2001. A pituitary cell-restricted T box factor, Tpit, activates POMC transcription in cooperation with Pitx homeoproteins. Cell 104, 849–859.

Lamonerie, T., Tremblay, J.J., Lanctot, C., Therrien, M., Gauthier, Y., Drouin, J., 1996. Ptx1, a bicoid-related homeo box transcription factor involved in transcription of the pro-opiomelanocortin gene. Genes Dev. 10, 1284–1295.

Mierendorf, R.C., Pfeffer, D., 1987. Direct sequencing of denatured plasmid DNA. In: Berger, A.I., Kimmel, A.R. (Eds.), Methods in Enzymology, vol. 152. Academic Press, San Diego, pp. 556–562.

Nakanishi, S., Teranishi, Y., Watanabe, Y., Notake, M., Noda, M., Kakidani, H., Jingami, H., Numa, S., 1981. Isolation and characterization of the bovine corticotropin/β-lipotropin precursor gene. Eur. J. Biochem. 115, 429–438.

Notake, M., Tobimatsu, T., Watanabe, Y., Takahashi, H., Mishina, M., Numa, S., 1983. Isolation and characterization of the mouse corticotropin-β-lipotropin precursor gene and a related pseudogene. FEBS Lett. 156, 67–71.

Nozaki, M., Takahashi, A., Amemiya, Y., Kawauchi, H., Sower, S.A., 1995. Distribution of lamprey adrenocorticotropin and melanotropins in the pituitary of the adult sea lamprey, *Petromyzon marinus*. Gen. Comp. Endocrinol. 98, 147–156.

Ochman, H., Medhora, M.M., Garza, D., Hartl, D.L., 1990. Amplification of flanking sequences by inverse PCR. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols. Academic press, San Diego, pp. 219–227.

Ohtake, H., Ohtoko, K., Ishimaru, Y., Kato, S., 2004. Determination of the capped site sequence of mRNA based on the detection of cap-dependent nucleotide addition using an anchor ligation method. DNA Res. 11, 305–309.

Okuta, A., Ando, H., Ueda, H., Urano, A., 1996. Two types of cDNAs encoding proopiomelanocortin of sockeye salmon, *Oncorhynchus nerka*. Zool. Sci. 13, 421–427.

Ono, M., Harigai, T., Kaneko, T., Sato, Y., Ihara, S., Kawauchi, H., 1994. Pit-1/GH factor-1 involvement in the gene expression of somatolactin. Mol. Endocrinol. 8, 109–115.

Pulichino, A.M., Vallette-Kasic, S., Tsai, J.P., Couture, C., Gauthier, Y., Drouin, J., 2003. Tpit determines alternate fates during pituitary cell differentiation. Genes Dev. 17, 738–747.

Salbert, G., Chauveau, I., Bonnec, G., Valotaire, Y., Jego, P., 1992. One of the two trout proopiomelanocortin messenger RNAs potentially encodes new peptides. Mol. Endocrinol. 6, 1605–1613.

Smith, A.I., Funder, J.W., 1988. Proopiomelanocortin processing in the pituitary, central nervous system, and peripheral tissues. Endocr. Rev. 9, 159–179.

Takahashi, A., Kawauchi, H., 2005. Diverse structures and functions of melanocortin, endorphin and melanin-concentrating hormone in fish. In: Zaccone, G., Reinecke, M., Kapoor, B.G. (Eds.), Fish Endocrinology. Science Publishers. Enfield, in press.

Takahashi, A., Amemiya, Y., Nozaki, M., Sower, S.A., Joss, J., Gorbman, A., Kawauchi, H., 1995a. Isolation and characterization of melanotropins from lamprey pituitary glands. Int. J. Pept. Protein Res. 46, 197–204.

Takahashi, A., Amemiya, Y., Sarashi, M., Sower, S.A., Kawauchi, H., 1995b. Melanotropin and corticotropin are encoded on two distinct genes in the lamprey, the earliest evolved extant vertebrate. Biochem. Biophys. Res. Commun. 213, 490–498.

Takahashi, A., Takasaka, T., Yasuda, A., Amemiya, Y., Sakai, M., Kawauchi, H., 2000. Identification of carp proopiomelanocortin-related peptides and their effects on phagocytes. Fish Shellfish Immunol. 10, 273–284.

Takahashi, A., Amemiya, Y., Nozaki, M., Sower, S.A., Kawauchi, H., 2001. Evolutionary significance of proopiomelanocortin in agnatha and chondrichthyes. Comp. Biochem. Physiol. 129, 283–289.

Takahashi, A., Amano, M., Itoh, T., Yasuda, A., Yamanome, T., Amemiya, Y., Sasaki, K., Sakai, M., Yamamori, K., Kawauchi, H., 2005. Nucleotide sequence and expression of three subtypes of proopiomelanocortin mRNA in barfin flounder. Gen. Comp. Endocrinol. 141, 291–303.

Takeuchi, S., Teshigawara, K., Takahashi, S., 1999. Molecular cloning and characterization of the chicken pro-opiomelanocortin (POMC) gene. Biochim. Biophys. Acta 1450, 452–459.

Therrien, M., Drouin, J., 1991. Pituitary pro-opiomelanocortin gene expression requires synergistic interactions of several regulatory elements. Mol. Cell. Biol. 11, 3492–3503.