Available online at www.sciencedirect.com
SCIENCE DIRECT
General and Comparative Endocrinology 148 (2006) 72–78
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

Occurrence of two functionally distinct proopiomelanocortin genes in all modern lampreys

Akiyoshi Takahashi [a,*], Osamu Nakata [a], Shunsuke Moriyama [a], Masumi Nozaki [b], Jean M.P. Joss [c], Stacia A. Sower [d], Hiroshi Kawauchi [a]

[a] *Laboratory of Molecular Endocrinology, School of Fisheries Sciences, Kitasato University, Sanriku, Ofunato, Iwate 022-0101, Japan*
[b] *Sado Marine Biological Station, Niigata University, Tassha, Sado, Niigata 952-2135, Japan*
[c] *Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia*
[d] *Department of Biochemistry and Molecular Biology, University of New Hampshire, NH 03824, USA*

Received 3 August 2005; revised 26 August 2005; accepted 1 September 2005
Available online 20 October 2005

Abstract

The lampreys (family Petromyzontidae) are divided into three subfamilies, the Petromyzontinae in the Northern Hemisphere and the Geotriinae and Mordaciinae in the Southern Hemisphere. We previously found two proopiomelanocortin subtypes, proopiocortin (POC) and proopiomelanotropin (POM) in sea lamprey, *Petromyzon marinus* (Petromyzontinae). *POC* encoding adrenocorticotropic hormone (ACTH) and β-endorphin (β-END) is expressed in the pars distalis of the pituitary, while *POM* encoding melanophore-stimulating hormone (MSH)-A and B together with a different β-END is expressed in the pars intermedia of the pituitary. All these hormonal segments are encoded on the third exon in both *POC* and *POM*. Here, we demonstrate the presence of both *POC* and *POM* genes in *Geotria australis* (Geotriinae) and *Mordacia mordax* (Mordaciinae) by molecular cloning of the third exons with the polymerase chain reaction using genomic DNA or pituitary cDNA. Molecular phylogenetic analysis showed that the POC and POM are distinctly different for the Southern Hemisphere lampreys as they are for *P. marinus*. Moreover, the relationship of each hormonal segments in POC and POM between *Geotria*, *Mordacia*, and *Petromyzon* is inconsistent. Immunocytochemical studies showed that the distribution of POC and POM in the pituitaries of the Southern Hemisphere lampreys is the same as that in the Northern Hemisphere. Taken together, these findings suggest that the duplication event which generated the two genes may have occurred in a common ancestor of the three extant lamprey subfamilies.

Keywords: Adrenocorticotropic hormone; β-Endorphin; Geotria; Lampreys; Melanophore-stimulating hormone; Mordacia; Petromyzon; Proopiocortin; Proopiomelanocortin; Proopiomelanotropin

1. Introduction

Lampreys of the class Cephalaspidomorphi are included in the superclass Agnatha together with hagfish, class Myxini (Nelson, 1994). The adenohypophysis of sea lampreys consists of a pars distalis (PD), which is further divided into the rostral PD and the proximal PD, and a pars intermedia (PI), the morphological organization of which is similar to that in ray-finned fish (Gorbman et al., 1983). We showed previously that adrenocorticotropic hormone (ACTH) and melanophore-stimulating hormone (MSH) are generated in the PD and PI, respectively, in the pituitary of sea lamprey, *Petromyzon marinus*, as they are in ray-finned fish pituitaries (Nozaki et al., 1995; Takahashi et al., 1995a).

In ray-finned fish, a common *proopiomelanocortin* (*POMC*) gene encoding ACTH and MSH together with β-endorphin (β-END) is expressed in both the PD and PI of the pituitary (Salbert et al., 1992; Takahashi and Kawauchi, 2005). In sea lamprey, however, two genes of the POMC family, *proopiocortin* (*POC*) and *proopiomelanotropin* (*POM*),

* 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.2005.09.003

are expressed in the PD and PI, respectively (Takahashi et al., 1995b). In addition to differences in tissue distribution, these genes differ in the hormonal segments they encode; the *POC* gene encodes ACTH, β-END, and nasophypophysial factor, whereas the *POM* gene encodes two kinds of MSH, a different β-END, and N-terminal segment (Heinig et al., 1995; Takahashi et al., 1995b). Recently, we revealed that both *POC* and *POM* contain two introns at homologous positions to gnathostome *POMC* genes (Takahashi et al., 2005). These genes are thought to have differentiated after the duplication of an ancestral gene and evolved in concert with the functional differentiation of the pituitary (Takahashi and Kawauchi, 2005; Takahashi et al., 2001).

The family Petromyzontidae (living lampreys) is composed of three subfamilies: one Northern Hemisphere subfamily, the Petromyzontinae, and the two Southern Hemisphere subfamilies, the Geotriinae, and Mordaciinae (Nelson, 1994). However, the occurrence of POC and POM and their tissue-specific distribution have been characterized only in the sea lamprey of subfamily Petromyzontinae (Ficele et al., 1998; Heinig et al., 1999; Nozaki et al., 1995; Sower et al., 1995; Takahashi et al., 1995a; Takahashi et al., 1995b). Therefore, the present study was undertaken to investigate the POMC family in the Southern Hemisphere subfamilies to determine whether the duality and tissue distribution observed in *Petromyzon* is common to all lampreys.

2. Materials and methods

2.1. Lampreys

Sampling of lampreys and collection of tissues were done in accordance with the UNH IACUC animal care guidelines. Southern Hemisphere lampreys were collected in Tasmania, Australia, during the period from January 2000 to October 2004. Larvae (ammocoetes) of the pouched lamprey, *Geotria australis*, and the short-headed lamprey, *Mordacia mordax*, 8–12 cm in total length, were collected by electroshocking in creeks of the Derwent River system near Hobart in late January 2000. Both species included specimens at several different stages of metamorphosis: from the pre-metamorphic to early metamorphic stage (presumably stage 3 of Potter et al., 1982). For molecular analysis, the entire body was submerged in ethanol and stored at −20 °C. For immunocytochemistry, tissue was prepared as described in Section 2.5. Adult *Geotria* and *Mordacia* were collected from a fish ladder in the Derwent. They were anesthetized in MS222 prior to dissecting tissues. For molecular analysis, pituitaries were immersed in RNA later (Ambion, Austin, TX). Liver was first frozen on dry ice and later stored at −80 °C.

2.2. Nucleic acid preparation

Genomic DNA was prepared from adult liver or a part of ammocoete body using Isotissue (Nippon Gene, Tokyo, Japan). Total RNA was prepared from pituitaries using Isogen (Nippon Gene). cDNA was synthesized using a First-Strand cDNA Synthesis Kit (Amersham Pharmacia Biotech, Buckinghamshire, UK) or a Smart RACE cDNA Amplification Kit (BD Biosciences Clontech, Franklin Lakes, NJ).

2.3. Polymerase chain reaction

Custom-made primers for PCR are listed in Table 1. The design of primers for POC-1 and 4, and POM-1, 2, 7, and 8 were based on the nucleotide sequence of *Petromyzon* POC and POM cDNA (Takahashi et al., 1995b). Other primers were gene-specific, based on the present study. *Hin*dIII Cassette DNA and cassette-specific primers were purchased from Takara (Tokyo, Japan). Templates for inverse PCR were prepared according to Ochman et al. (1990). DNA was amplified using AmpliTaq Gold Master Mix (Applied Biosystems, Foster City, CA), Takara LA Taq, or HotStar Taq Master Mix (Qiagen, Hilden, Germany). PCR was conducted using a thermal cycler (PC-808, Astec, Fukuoka, Japan). The reaction conditions are shown in Table 2.

The 3′ region of *Geotria* POC cDNA was amplified from cDNA using AmpliTaq Gold Master Mix with primers POC-1 and *Not*I adaptor in condition A. The 5′ region of *Geotria* POC cDNA was amplified from cDNA using HotStar Taq Master Mix with primers SMART Short and POC-2 in condition C after prePCR with SMART Long in condition B, followed by nested PCR with primers SMART Nested and POC-3 in condition C. *Geotria* POM DNA was first amplified from genomic DNA using AmpliTaq Gold Master Mix with POM-1 and POM-2 in condition A. To amplify the 3′ region of *Geotria* POM cDNA, a reaction mixture containing the pituitary cDNA, primers SMART Long, SMART Short and POM-3, and Takara

Table 1
Custom oligonucleotide primers used for PCR to amplify DNA fragments of Southern Hemisphere lampreys

Primer	Nucleotide sequence
POC-1	5′-AGTACGCCATGGGGCATTTCCGCTG-3′
POC-2	5′-TGGCCAGTGGTCAATCTCCTGCAACACTTT-3′
POC-3	5′-GTCTCCCTGCGACTCAGCAACACAAGCTC-3′
POC-4	5′-GCTTCACCGACTGCAAGAAATACTG-3′
POC-5	5′-CAGAGCGACTTTGTGCTCTCCTTGT-3′
POC-6	5′-ATTGGCGAGGGTAGAGAGGATGGCCAGAGT-3′
POC-7	5′-GACGGCGGAGGAAGACGAAGATGATAATGG-3′
POM-1	5′-GCTACCGGATGCAACACTTCCGCTG-3′
POM-2	5′-TGCGCATGAAGCCGCCGTAGCGCTT-3′
POM-3	5′-TCGGGGCCCCTGAGCATTCAGAAG-3′
POM-4	5′-TGAGTCACTTTCGGTGGGGGCAACC-3′
POM-5	5′-TCTTCATGACGTCTCGGAAGAAGGTGACCA
POM-6	5′-AGCTCCGGATTCTCCTTCTGAATGCTCAGG-3′
POM-7	5′-TGCTGACGGCTCCGAGATTGTGCTCCT-3′
POM-8	5′-CATGACGTCCCGGAAGAACGTGACCA-3′
POM-9	5′-GCCAAGAAAGACCCGATGTCTCTGAACGTG-3′
POM-10	5′-ACCCGGAGCTCTACCAGATGAACCACTTCC-3′

Synthesis of primers was performed by Nihon Gene Research Laboratories (Sendai, Japan).

Table 2
Conditions of PCR to amplify DNA fragments of Southern Hemisphere lampreys

Condition	Activation of enzyme or preheating for hot start	Denaturation	Annealing	Extension	Final extension	Cycle
A	95/10	95 /1	55 /1	72 /1.5	72/10	35
B	96/5	96/1	70/2			35
C	94/5	95/1	60/1	72/2	72/5	35
D1	96/3	96/1	70/1		72/2	5
D2	96/3	96/1	57/1	72/2	72/5	35
E	95/10	95/1	55/1	72/1	72/10	35
F	95/5	95/1	70/1	72/3	72/5	35
G	95/5	95/1	70/0.67	72/1	72/5	35

Numbers show temperature and time (°C/min) excluding cycle. D1 was immediately followed by D2.

LA with GC buffer was prepared. After heating at 96 °C for one min, LA Taq was added for a hot start, and then PCR was performed in condition D1, and then immediately followed by condition D2. Subsequently, nested PCR was conducted with the primers SMART Nested and POM-4 in condition D2. The 5′ region of *Geotria* POM cDNA was amplified from cDNA using HotStar Taq Master Mix with primers SMART Short and POM-5 in condition C after prePCR with SMART Long in condition B, followed by nested PCR with primers SMART Nested and POM-6 in condition C.

Mordacia POC DNA was amplified from genomic DNA using AmpliTaq Gold Master Mix with primers POC-4 and 5 in condition E, or HotStarTaq Master Mix with primers POC-6 and cassette C1 in condition F, followed by nested PCR with primers POC-7 and cassette C2 in condition F. *Mordacia* POM DNA was amplified from genomic DNA using AmpliTaq Gold Master Mix with primers POM-7 and POM-8 in condition A, or HotStarTaq Master Mix with primers POM-9 and cassette C1 in condition G, followed by nested PCR with primers POM-10 and cassette C2 in condition G.

2.4. Nucleic acid sequence determination

PCR-amplified cDNA, purified using a QIAEX II Gel Extraction kit (Qiagen, Hilden, Germany) after electrophoresis on Nusieve GTG agarose (Cambrex BioScience Rockland, Rockland, ME), was cloned using the vector pT7 Blue T (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). DNASIS-Pro (Hitachi Software Engineering, Yokohama, Japan) was used for processing the sequence, for sequence alignment, and for phylogenetic analysis.

2.5. Tissue preparation and immunocytochemistry

Both species of larval lampreys, 12 animals each, were killed by decapitation following anesthetization by immersion in MS222. After rapid removal of the dorsal fibrocranium and exposure of the dorsal surface of the brain, the brain, and the attached pituitary were immersed in Bouin-Hollande sublimate fixative (Romeis, 1948) for about 24 h, following which they were dehydrated through a series of increasing concentrations of ethanol. Deposited mercuric chloride was removed by treatment with iodine–potassium iodide in 90% ethanol for 1–2 days. Tissues were embedded in Paraplast, and serial sagittal sections of 6 μm were mounted on gelatin-coated glass slides.

Sections of lamprey pituitaries were immunohistochemically stained using a Vectastain ABC Elite kit and specific antisera prepared against sea lamprey $ACTH_{1–16}$ (Lot No. 9308) and MSH-B (Lot No. 9311) as described previously (Nozaki et al., 1995). To test the specificity of the immunostaining, the following controls were employed: replacement of the primary antiserum with rabbit serum, and preabsorption of the primary antiserum with corresponding antigens. The replacement of primary antisera with normal rabbit serum yielded no staining in the pituitary gland of either species of lamprey. Furthermore, preabsorption of the primary antisera with their corresponding antigens blocked the immunostaining of the pituitary gland of both species of lampreys. Incidentally, anti-lamprey $ACTH_{1–16}$ (Lot No. 9308) has been previously shown to cross-react with sea lamprey MSH-A and MSH-B (Nozaki et al., 1995).

3. Results

3.1. Structures of Geotria POMC family genes

A clone gPOC-A consisting of 463 bp excluding the poly A tail was obtained by PCR from *Geotria* pituitary cDNA using the primers POC-1 and *Not*I (Fig. 1A). A clone gPOC-B consisting of 345 bp was obtained using a 5′ SMART RACE cDNA Amplification kit with the gene-specific primer POC-3 (Fig. 1A). The overlapping of the clones gPOC-A and -B provided a partial sequence of *Geotria* POC cDNA (Fig. 1A).

A clone gPOM-A consisting of 283 bp was obtained by PCR from *Geotria* genomic DNA using primers POM-1 and POM-2 (Fig. 1B). Clones gPOM-B consisting of 389 bp and gPOM-C consisting of 289 bp excluding the poly A tail were obtained by PCR from pituitary using a 5′ SMART RACE cDNA Amplification kit with the gene-specific

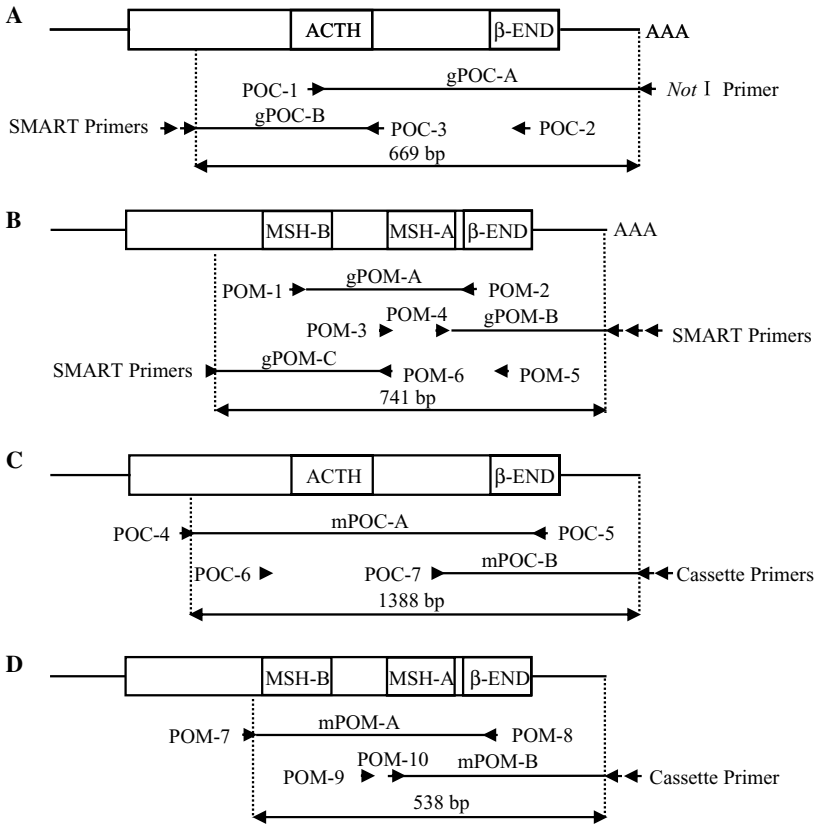

Fig. 1. Schematic diagram depicting the relative positions of DNA fragments of the *POC* and *POM* genes or cDNA amplified by PCR. (A) *Geotria* POC, (B) *Geotria* POM, (C) *Mordacia* POC, and (D) Mordacia POM. Numbers show positions on POMC cDNAs. Horizontal arrows show relative positions and direction of primers.

primers POM-4 and POM-6, respectively (Fig. 1B). The overlapping of the clones gPOM-A, -B, and -C provided a partial sequence of *Geotria* POM cDNA (Fig. 1B).

3.2. Structures of Mordacia POMC family genes

A clone mPOC-A consisting of 634 bp was obtained by PCR from *Mordacia* genomic DNA using primers POC-4 and POC-5 (Fig. 1C). mPOC-B consisting of 861 bp was obtained by PCR from genomic DNA ligated with the *Hin*dIII cassette using the gene-specific primer POC-7 and cassette primer C2 (Fig. 1C). The overlapping of the clones mPOC-A and -B provided a partial sequence of *Mordacia* POC cDNA (Fig. 1C).

A clone mPOM-A consisting of 406 bp was obtained by PCR from *Mordacia* genomic DNA using primers POM-7 and POM-8 (Fig. 1D). A clone mPOM-B consisting of 219 bp was obtained by PCR from genomic DNA ligated with the *Hin*dIII cassette using the gene-specific primer POM-10 and the cassette primer C-2 (Fig. 1D). The overlapping of the clones mPOM-A and -B provided a partial sequence of *Mordacia* POM cDNA (Fig. 1D).

Partial amino acid sequences of POC and POM deduced from cDNA sequences described above are shown in Fig. 2 together with those of sea lamprey POC and POM. In both *Geotria* and *Mordacia* POC, amino acid sequences of ACTH and β-END were obtained. Moreover, in both *Geotria* and *Mordacia* POM, amino acid sequence of MSH-A, MSH-B, and β-END were obtained. For details of nucleotide sequences, see Accession No. AB223047 for the *Geotria POC* gene, AB223048 for the *Geotria POM* gene, AB223049 for the *Mordacia* POC gene, and AB223050 for *Mordacia POM* gene in the DDBJ/EMBL/GenBank nucleotide sequence database.

3.3. Phylogenetic analysis

Fig. 3 shows phylogenetic tree of lamprey POMC family molecules constructed by neighbor-joining method using dogfish POMC (Amemiya et al., 1999), a representative of gnathostome POMC, as an outgroup. Segments used for sequence alignment were ACTH to β-END for lamprey POCs and dogfish POMC, and MSH-B to β-END for lamprey POMs. In lampreys, POC and POM are distinctly different from each other and both are approximately equally removed from dogfish POMC.

3.4. Distribution of POMC family-producing cells

An immunoreaction to antiserum prepared against *Petromyzon* $ACTH_{1–16}$ was found in most cells of RPD of both *Geotria* and *Mordacia* pituitaries (Figs. 4A and C). An immunoreaction to antiserum prepared against *Petromyzon* MSH-B was found in most cells of PI of both *Geotria* and *Morda-*

Fig. 2. Partial amino acid sequence of POC (A) and POM (B) for *Geotria* and *Mordacia* deduced from corresponding DNA nucleotide sequences. They are compared with the entire sequence of *Petromyzon* POC and POM. Squares with a broken line indicate hormonal segments. Underlines show amino acids identical to those in *Petromyzon* hormonal segments. Colons show amino acids identical between *Geotria* and *Mordacia* hormonal segments.

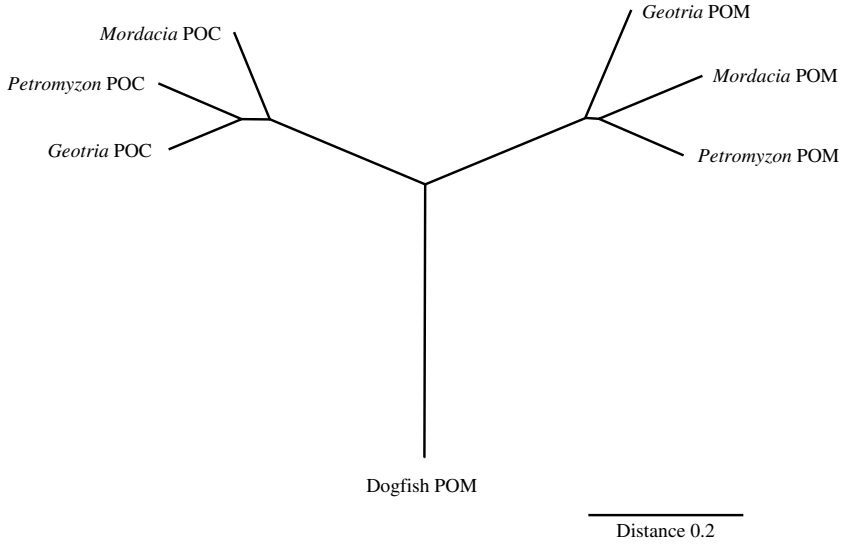

Fig. 3. Phylogenetic tree for lamprey POMC family molecules constructed by neighbor-joining method using dogfish POMC as an outgroup.

cia pituitaries (Figs. 4B and D). Stainability with MSH-B antisera was weaker in the *Geotria* pituitary than those in *Mordacia* pituitary. An ACTH-positive reaction was also observed in most cells of the pars intermedia of the *Geotria* pituitary. This ACTH-positive reaction in the pars intermedia was most likely due to a cross-reaction with lamprey MSHs (see Section 2).

4. Discussion

Here, we determined partial nucleotide sequences of *POC* and *POM* in *Geotria* and *Mordacia* to obtain necessary and sufficient sequence data for the identification of *POC* and *POM* genes already described for *Petromyzon* (Takahashi et al., 1995b). The nucleotide sequences

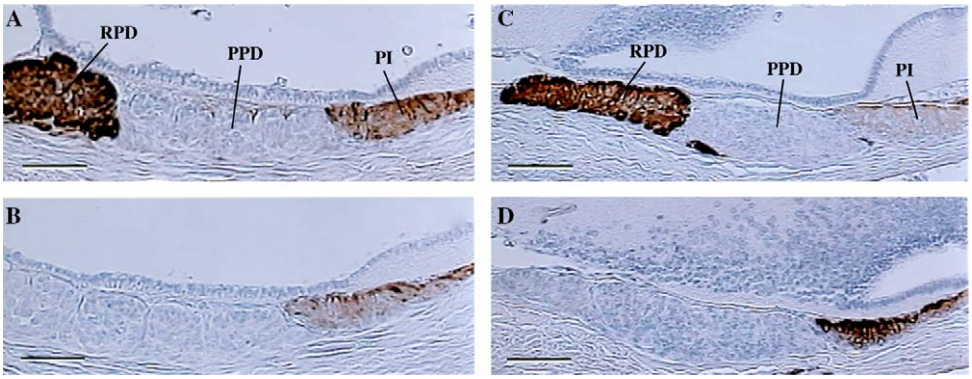

Fig. 4. Immunocytochemical staining of the pituitary gland of larval *Geotria* and *Mordacia*. A, *Geotria* pituitary stained with antiserum prepared against *Petromyzon* $ACTH_{1–16}$. B, *Geotria* pituitary stained with antiserum prepared against *Petromyzon* MSH-B. C, *Mordacia* pituitary stained with antiserum prepared against *Petromyzon* $ACTH_{1–16}$. D, *Mordacia* pituitary stained with antiserum prepared against *Petromyzon* MSH-B. PI, pars intermedia; PPD, proximal pars distalis; RPD, rostral pars distalis. Scale bars: 50 μm (x165).

determined in the present study corresponded to exon 3 of *Petromyzon POC* or *POM* which encodes all hormonal segments such as ACTH, MSH, and β-END as reported recently (Takahashi et al., 2005).

This study clearly demonstrates the presence of both *POC* and *POM* genes in Geotriinae and Mordaciinae in the Southern Hemisphere as in Petromyzontinae in the Northern Hemisphere. Moreover, the immunocytochemical analysis of the products from these two genes showed the localization of ACTH-like and MSH-B-like immunoreactivity in the PD and PI, respectively, for all three subfamilies of lampreys. These facts strongly suggests that the *POC* and *POM* genes have evolved from a common ancestor in concert with the functional differentiation of the pituitary gland prior to separation of the three groups of modern lampreys (Takahashi and Kawauchi, 2005; Takahashi et al., 2001).

The phylogenetic tree for POMC family molecules shows that the relationship of POC among *Petromyzon*, *Geotria*, and *Mordacia* is different from that of POM. For the POC, *Petromyzon* is closer to *Geotria* than to *Mordacia*, whereas for the POM, *Petromyzon* is closer to *Mordacia* than to *Geotria*. The relationship of each hormonal segment in POC and POM among the three lamprey species is also inconsistent (Fig. 2 and Table 3). For POC, ACTH of the two Southern Hemisphere species consists of 59 amino acids, which is one residue shorter than *Petromyzon* ACTH. Also, *Geotria* and *Mordacia* ACTHs have higher sequence identity, than either has with *Petromyzon*. β-END of POC in both Southern Hemisphere species and *Petromyzon* is composed of 32 amino acids but this time, *Geotria* versus *Petromyzon*, and *Mordacia* versus *Petromyzon* show higher sequence identity than *Geotria* versus *Mordacia*. For POM, MSH-A of both Southern Hemisphere species and *Petromyzon* is composed of 19 amino acids. *Mordacia* MSH-A shows identical amino acid sequence to *Petromyzon* and differs from *Geotria* in one residue. MSH-B of all three lamprey subfamilies is composed of 20 amino acids, and *Geotria* versus *Mordacia*, and *Geotria* versus *Petromyzon* show higher sequence identity than *Mordacia* versus *Petromyzon*. β-END of POM in all three lampreys is composed of 35 amino acids and the highest sequence identity is observed between *Geotria* and *Petromyzon*.

Table 3
Sequence identity among lamprey melanocortins or β-END (%)

Compared species	ACTH	ACTH (MSH segment)	β-END (POC)	MSH-A	MSH-B	β-END (POM)
G vs M	75	83	75	95	85	80
G vs P	70	78	84	95	85	86
M vs P	67	74	84	100	70	77
Mean	71	78	81	97	80	81

G, *Geotria australis*; P, *Petromyzon marinus*; M, *Mordacia mordax*.

In the gnathostome *POMC* gene, the number of MSH segments encoded is more or less class-specific. *POMC* of lobe-finned fish and primitive ray-finned fish encodes three (α, β, and γ) MSHs, while *POMC* of derived ray-finned fish encodes two (α and β) MSHs, and *POMC* of chondrichthian encodes four (α, β, γ, and δ) MSHs (Takahashi and Kawauchi, 2005;Takahashi et al., 2001). A comparison of the location of hormonal segments in the POMC family indicates that an MSH segment in ACTH of POC and MSH-B of POM correspond to α-MSH of gnathostome POMC, and that MSH-A of POM corresponds to β-MSH of gnathostome POMC (Takahashi et al., 1995b) (Fig. 2). Among the four molecular types of MSH in gnathostomes, α-MSH consisting of 13 amino acids, has the most conserved amino acid sequence (Dores and Lecaude, 2005; Eberle, 2000; Shen et al., 2003; Takahashi and Kawauchi, 2005). Indeed, the same amino acid sequence excluding the C-terminus is observed throughout gnathostomes including lobe-finned fish, ray-finned fish and cartilaginous fish. In gnathostomes, ACTH is produced in the PD, while it is cleaved to α-MSH and C-terminal peptide in the PI (Castro and Morrison, 1997; Smith and Funder, 1988). Thus, the α-MSH segment has bifunctional roles, as α-MSH itself and as an N-terminal segment of ACTH. This property may have provided a strong functional constraint on the α-MSH segment to conserve the amino acid sequence. However, mean sequence identity of the MSH segment derived from ACTH among the three lamprey species is 78% and that in MSH-B

is 80% (Table 3). Both these values are lower than that of MSH-A (97%). In lampreys, the MSH segment in ACTH and MSH-B may have evolved independently under a functional constraint weaker than that for α-MSH in gnathostomes, because ACTH and α-MSH (MSH-B of lamprey), are generated from distinct precursors, POC in the PD, and POM in the PI, respectively. In this context, MSH-A having the highest mean sequence identity might be responsible for important biological functions in lampreys.

In conclusion, we have demonstrated the occurrence of *POC* and *POM* in the Southern Hemisphere lampreys, *Geotria* and *Mordacia*, as in the Northern Hemisphere lamprey, *Petromyzon*. Immunocytochemical analysis showed that POC and POM are generated in the PD and PI, respectively, in *Geotria* and *Mordacia* as they are in *Petromyzon*. These results indicated that the *POC* and *POM* genes may have occurred by gene duplication in a common ancestor of the extant three lamprey lineages which has subsequently led to changes in the genes of each.

Acknowledgments

We thank Mr. Kazue Nagasawa, Kitasato University, for technical assistance. This study was supported in part by a Grant-in-Aid for Scientific Research (B) (15370029) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, to H.K.

References

Amemiya, Y., Takahashi, A., Suzuki, N., Sasayama, Y., Kawauchi, H., 1999. A newly characterized melanotropin in proopiomelanocortin in pituitaries of an elasmobranch, *Squalus acanthias*. Gen. Comp. Endocrinol. 114, 387–395.

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

Dores, R.M., Lecaude, S., 2005. Trends in the evolution of the proopiomelanocortin gene. Gen. Comp. Endocrinol. 142, 81–93.

Eberle, A.N., 2000. Proopiomelanocortin and the melanocortin peptides. In: Cone, R.D. (Ed.), The Melanocortin Receptors. Humana Press, Totowa, pp. 3–67.

Ficele, G., Heinig, J.A., Kawauchi, H., Youson, J.H., Keeley, F.W., Wright, G.M., 1998. Spatial and temporal distribution of proopiomelanotropin and proopiocortin mRNA during the life cycle of the sea lamprey: a qualitative and quantitative in situ hybridization study. Gen. Comp. Endocrinol. 110, 212–225.

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

Heinig, J.A., Keeley, F.W., Robson, P., Sower, S.A., Youson, J.H., 1995. The appearance of proopiomelanocortin early in vertebrate evolution: cloning and sequencing of POMC from a lamprey pituitary cDNA library. Gen. Comp. Endocrinol. 99, 137–144.

Heinig, J.A., Keeley, F.W., Kawauchi, H., Youson, J.H., 1999. Expression of proopiocortin and proopiomelanotropin during the life cycle of the sea lamprey (*Petromyzon marinus*). J. Exp. Zool. 283, 95–101.

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.

Nelson, J.S., 1994. Fishes of the World. John Wiley, New York.

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.

Potter, I.C., Hilliard, R.W., Bird, D.J., 1982. Stages in metamorphosis. In: Hardisty, R.W., Potter, I.C. (Eds.), The Biology of Lampreys 4B. Academic Press, London, pp. 137–164.

Romeis, B., 1948. Microskopische Technik 15 verb. Aufl. Leibnit Verlag, München.

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.

Shen, S.-T., Lu, L.-M., Chen, J.-R., Chien, J.-T., Yu, J.-Y., 2003. Molecular cloning of proopiomelanocortin (POMC) cDNA from mud turtle, *Pelodiscus sinensis*. Gen. Comp. Endocrinol. 131, 192–201.

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

Sower, S.A., Takahashi, A., Nozaki, M., Gorbman, A., Youson, J.H., Joss, J., Kawauchi, H., 1995. A novel glycoprotein in the olfactory and pituitary systems of larval and adult lampreys. Endocrinology 136, 349–356.

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., 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., Nakata, O., Kasahara, M., Sower, S.A., Kawauchi, H., 2005. Structures for the proopiomelanocortin family genes *proopiocortin* and *proopiomelanotropin* in the sea lamprey *Petromyzon marinus*. Gen. Comp. Endocrinol. (in press).