GENERAL AND COMPARATIVE ENDOCRINOLOGY 99, 137–144 (1995)

The Appearance of Proopiomelanocortin Early in Vertebrate Evolution: Cloning and Sequencing of POMC from a Lamprey Pituitary cDNA Library

JULIE A. HEINIG,* FRED W. KEELEY,† PAUL ROBSON,† STACIA A. SOWER,‡ AND JOHN H. YOUSON*,[1]

*Department of Anatomy and Cell Biology, University of Toronto, Toronto, Ontario, Canada; †Division of Cardiovascular Research, Research Institute, Hospital for Sick Children and the Departments of Biochemistry and Clinical Biochemistry, University of Toronto, Ontario, Canada; and ‡Department of Biochemistry and Molecular Biology, University of New Hampshire, Durham, New Hampshire

Accepted April 3, 1995

A proopiomelanocortin (POMC)-like hormone has been cloned and sequenced from a pituitary cDNA library of upstream migrant (prespawning) sea lamprey, *Petromyzon marinus*. The clone, designated LPP-1, consisted of 986 nucleotides, with an open reading frame of 277 amino acids, including a signal peptide of 22 amino acids. Like POMCs from more recently evolved vertebrates, lamprey POMC contained domains which corresponded to α-MSH, ACTH, and β-endorphin. However, sequences corresponding to γ- and β-MSH are absent or likely nonfunctional, respectively, in this cDNA. Northern blot analyses showed low but detectable expression levels of LPP-1 in larvae and strong expression in parasitic adults and prespawning animals. These observations indicate that a recognizable POMC, distinct from proenkephalin, has an ancient lineage within subphylum Vertebrata, likely dating back to the last common ancestor of the lamprey and gnathostome lines.

Proopiomelanocortin (POMC) is the common precursor of adrenocorticotropin (ACTH)-related peptides, β-endorphin (Eipper and Mains, 1980), and multiple copies of the melanotropins (Nakanishi *et al.*, 1979). The sequences of POMC mRNAs have been determined in several species of mammals (Douglass *et al.*, 1984), amphibians (Martens *et al.*, 1985; Hilario *et al.*, 1990), and teleosts (Soma *et al.*, 1984). All POMC sequences from gnathostomes (jawed vertebrates) show the same general organization; the ACTH domain is positioned in the middle of the precursor protein, and the β-MSH and β-endorphin sequences are located toward the C terminus. In the gnathostome anterior pituitary, the major POMC end products within corticotropic cells are ACTH, β-lipotropin, and β-endorphin, whereas α-MSH and β-endorphin are the major products in melanotropes of the intermediate lobe of the pituitary. While the general organization of pituitary POMC sequences has been remarkably conserved, gnathostome POMCs do vary with respect to the presence or absence of the γ-MSH sequence, the degree of post-translational N-acetylation of MSH and β-endorphin, the degree of glycosylation of ACTH, and the proteolytic processing strategies for the N-terminal and the β-MSH region of the precursor (for review see Baker, 1979; Eipper and Mains, 1980; Dores, 1988).

The recent hypothesis that POMC and another pituitary hormone precursor, proenkephalin, evolved from a common ancestral gene (Dores *et al.*, 1993) has raised our interest in the molecular evolution of POMC. Although POMC sequences are distinct from proenkephalin sequences in mammalian and nonmammalian vertebrates, sequence comparisons between POMCs of gnathostomes and agnathans (jawless fishes) have not been reported. Agnathans are the most ancient of vertebrates, with a lineage which can be traced back approximately

[1] To whom reprint requests should be addressed at Division of Life Sciences, Scarborough Campus, University of Toronto, Scarborough, Ontario M1C 1A4 Canada.

0016-6480/95 $12.00

550 million years (Forey and Janvier, 1993). Because of their ancient origins and conservative evolution of at least 350 million years, lampreys, one of the two living representatives of the agnathans, are of particular interest in studies of molecular evolution and could provide important insights into the evolutionary history of POMC and other pituitary hormones. Here we report the sequence of a cDNA encoding a POMC-like hormone from the sea lamprey (*Petromyzon marinus*), the first pituitary hormone to be cloned and sequenced from lampreys.

MATERIALS AND METHODS

Landlocked, upstream migrant (prespawning) lampreys, *P. marinus*, were obtained in June from Duffins Creek and the Humber River, near Toronto, Canada. Pituitaries and, in some cases, abdominal muscle were removed and immediately frozen in liquid nitrogen.

Purification of RNA and Production of cDNA Library

In general, standard procedures were followed, using commercially available kits. Approximately 100 frozen pituitaries were suspended in ice-cold GTC buffer (25 m*M* sodium citrate, pH 7.0, containing 4 *M* guanidine thiocyanate, 0.2% sarcosyl, and 0.2 *M* β-mercaptoethanol) and homogenized using a Polytron homogenizer. Total RNA was prepared by CsCl gradient centrifugation (Sambrook *et al.*, 1989). Pellets containing RNA were extracted once with phenol:chloroform:isoamyl alcohol (25:24:1) and once with chloroform:isoamyl alcohol (24:1). After addition of 0.1 vol of 3 *M* sodium acetate and 2.5 vol of 100% ethanol, the RNA was allowed to precipitate overnight at −20°. The pellet was collected by centrifugation and resuspended in 100 μl TE buffer (10 m*M* Tris/HCl, pH 7.4, 1 m*M* EDTA).

Poly(A)$^+$ RNA was selected from total RNA using an oligo(dT)-cellulose column (Pharmacia, Baie d'Urfé, Quebec). Production of the cDNA library used a Lambda ZAP cDNA synthesis kit (Stratagene, La Jolla, CA). The cDNA was directionally cloned into the Lambda ZAP vector, with the *Eco*RI site at the 5′ end and *Xho*I site at the 3′ end of the cDNAs. Ligation products were packaged using Gigapack extracts (Stratagene).

PCR Amplification

A lamprey POMC cDNA fragment was amplified from the pituitary cDNA library by PCR, using as primers a degenerate 25-mer corresponding to a 3′ region of growth hormone (STH) conserved among human, teleost, and eel (MHKVETYL) and a primer corresponding to a sequence present in the arm of the Lambda ZAP vector just 5′ to the insert. Template DNA (10^5 to 10^6 phage) and 20 pmol of each primer were used in a final volume of 100 μl containing 50 μ*M* of each deoxynucleotide, 2 m*M* of $MgCl_2$, and 2 units of *Taq* DNA polymerase (Promega, Madison, WI). After an initial denaturing step at 94° for 5 min, the subsequent cycles consisted of denaturing at 94° for 1 min, annealing at 50° for 1 min, and elongation at 72° for 1.5 min for 35 cycles. A final elongation step was carried out at 75° for 5 min. Following DNA purification the PCR product was cloned into a PCR II vector using a TA cloning kit (Invitrogen, San Diego, CA).

Screening of the cDNA Library

The PCR-amplified cDNA fragment was labeled with [α-^{32}P]dCTP by random priming and used to screen the cDNA library. Hybridization was performed at 65° for 20 hr in 6× SSC (Sambrook *et al.*, 1989) containing 0.4% SDS, 20 m*M* sodium phosphate, and denatured salmon sperm DNA (500 μg/ml). Filters were washed once in prewarmed (65°) 2× SSC containing 0.1% SDS for 15 min, twice in 1× SSC containing 0.1% SDS at 65° for 15 min and 30 min, respectively, and once in 0.1× SSC containing 0.1% SDS at 65° for 20 min. Subsequent secondary and tertiary screens were done to ensure isolation of positive clones.

DNA Sequence Analysis

DNA was sequenced using a Pharmacia ALF Automated DNA Sequencer (HSC Biotechnology Service Center, Toronto, ON). The reported sequence represents consistent results of sequencing in both directions.

Northern Blot Analysis

Total RNA was obtained as described above from pituitary or abdominal muscle. RNA (10 μg) was suspended in 7.5 μl of deionized formamide for 15 min. To this suspension 3.75 μl of 37% formaldehyde and 3.75 μl of 0.02% MOPS, pH 7.0, containing 8 m*M* sodium acetate and 1 m*M* EDTA were added. The RNA was incubated at 60° for 15 min, electrophoresed on 1.2% agarose, and transferred to a Hybond-N membrane (Amersham Corp., Oakville, ON). Probes were labeled with [^{32}P]dCTP by random priming. Membranes were hybridized for 16 hr at 42° and washed twice at 55° in 2× SSC containing 0.1% SDS for 20 min, followed by two washings at 55° for 20 and 60 min, respectively, with 0.1× SSC containing 0.1% SDS. After the final washing the filter was exposed to X-ray film.

RESULTS

PCR from the lamprey pituitary cDNA library resulted in a product of approximately 0.9 kb. Partial sequencing of this PCR product revealed a 5′ sequence corresponding to that reported for nasohypophysial factor (NHF), a polypeptide previously isolated from lamprey

pituitary (Sower *et al.*, 1995), and a 3′ sequence corresponding to met–enkephalin. The PCR fragment was therefore used to screen the lamprey pituitary cDNA library, yielding several positive clones.

The strategy for complete sequencing of one of these positive clones, designated LPP-1, is shown in Fig. 1. Nucleotide and deduced amino acid sequences for LPP-1 are shown in Fig. 2. LPP-1 is a total of 986 nucleotides in length to the polyadenylation signal and includes an open reading frame encoding 277 amino acids. The derived protein has a molecular weight of 30 kDa. A signal peptide of 22 amino acids was suggested using methodology described by von Heijne (1986). Cleavage at this site predicted an N-terminal sequence corresponding to the NHF polypeptide (Sower *et al.*, 1995). With the exception of one amino acid residue (ile 32), numbering from the predicted signal peptide cleavage site, the NHF peptide sequence matches residues +1 to +122 of the derived amino acid sequence of LPP-1.

Alignment and comparison of the amino acid sequence of LPP-1 with POMC sequences from rainbow trout (A and B), chum salmon, frog, and human, as well as ACTH sequences from chicken and dogfish are shown in Fig. 3, demonstrating recognizable domains flanked by conserved dibasic cleavage sites. As is the case for teleosts, the N-terminal region of lamprey POMC lacks a sequence corresponding to γ-MSH, which is present in this domain in other vertebrate species (Fig. 3). Consistent in all species, the ACTH and the N-terminal domains in this lamprey POMC are separated by a dibasic (KR) cleavage site. However, ACTH in LPP-1 begins with an SVS rather than an SYS sequence found in other species and has a 5-amino-acid insert preceding the α-MSH core sequence. This MSH core sequence (MXH-FRWG) is strongly conserved in the lamprey, except for glycine replacing glutamine in the second position. Further areas of homology are also evident throughout the ACTH region, including the dibasic cleavage site (RR) at the C-terminal end of this domain. The C-terminal domain of other POMCs all contain a β-MSH core sequence which is less well conserved across species compared to the α-MSH core sequence. This lamprey cDNA, however, appears to have lost the dibasic cleavage site (KK is replaced by NK) which occurs 8–10 amino acids upstream from the β-MSH core sequence. In addition, VFRV appears to be a remnant of the core sequence of β-MSH in LPP-1. The dibasic cleavage site preceding the β-endorphin domain and the first five residues of this β-endorphin domain (YGGFM) are strongly conserved among all these species.

Northern blotting using this LPP-1 (Fig. 4) showed low but detectable expression of this form of lamprey POMC in larvae, but very strong expression in parasitic adults and upstream migrants. Expression was undetectable in abdominal muscle of upstream migrants, which was used as a negative control.

DISCUSSION

Sequencing of LPP-1 clearly demonstrates that this cDNA encodes a POMC-like prohormone in the pituitary of the lamprey (*P. marinus*), which includes recognizable domains corresponding to ACTH, α-MSH, and β-endorphin. As is the case for teleosts, the N-terminal region of lamprey POMC also lacks a sequence corresponding to γ-MSH, which is present in

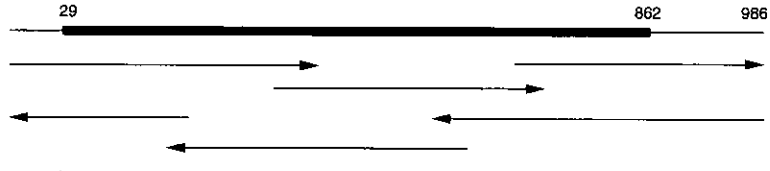

FIG. 1. Map of the nucleotide sequencing strategy for LPP-1. The filled bar represents the coding sequence and the lines represent the 5′ and 3′ untranslated regions of the cDNA. The arrows indicate the direction of sequencing and the sizes of the overlapping sequences.

```
LPP-1 gaaaaaggggaaaagtgctgctggaatg                                          28

LPP-1 ATG GGA AAC TGC TCT CGA CTG CTG CTT CTG CTG GAG ATG CTG TCA ATC ATC TCC CCG TCT  88
      met gly asn cys ser arg leu leu leu leu leu glu met leu ser ile ile ser pro ser

LPP-1 GCC AGT GCC ATG TGC TGG GCA CGG CTG GAC CAG GGG TGC TTC ACC GAC TGC AAG AAA TAC 148
      ala ser ala met cys trp ala arg leu asp gln gly cys phe thr asp cys lys lys tyr  18
NHF           ala met cys trp ala arg leu asp gln gly cys phe thr asp cys lys lys tyr

LPP-1 TGC AGC AAT GGG ACA CGG GCA GGC ACG CCG GCG GCG GTG CTG GAG AAT CTG CTG GCA TGC 208
      cys ser asn gly thr arg ala gly thr pro ala ala val leu glu asn leu leu ala cys  38
NHF   cys ser asn gly thr arg ala gly thr pro ala ala val ile glu asn leu leu ala cys

LPP-1 GTG CAG CTC AAA TGC AGC GAC GAC GGT GAT GAC AAC GAC GAC GAC GCT CCC CTG CTG CAG 268
      val gln leu lys cys ser asp asp gly asp asp asn asp asp asp ala pro leu leu gln  58
NHF   val gln leu lys cys ser asp asp gly asp asp asn asp asp asp ala pro leu leu gln

LPP-1 TGG ATC GCA AGC AGA GCC GAA TCC CGC AGC GAT TTC GAC ATC GCC AAC AAC AAG TGG TGG 328
      trp ile ala ser arg ala glu ser arg ser asp phe asp ile ala asn asn lys trp trp  78
NHF   trp ile ala ser arg ala glu ser arg ser asp phe asp ilu ala asn asn lys trp trp

LPP-1 CTC GTC CGC TGG GGT GGA CAG AGT GGC CTG AGT GGC GAG GGT GGC GAG AGT GGT GGA AGT 388
      leu val arg trp gly gly gln ser gly leu ser gly glu gly gly glu ser gly gly ser  98
NHF   leu val arg trp gly gly gln ser gly leu ser gly glu gly gly glu ser gly gly ser

LPP-1 CCG AGG GTG GAG CAG GTG GAT TTG GCG GGG CAG GTT GAG TCC TCC CCG GCG AGT AGT TCC 448
      pro arg val glu gln val asp leu ala gly gln val glu ser ser pro ala ser ser ser 118
NHF   pro arg val glu gln val asp leu ala gly gln val glu ser ser pro ala thr ser ser
                                          ACTH
LPP-1 AGC CAG GCC AAG CGT TCC GTG TCC TCC CCC AAG TAC GCC ATG GGG CAT TTC CGC TGG GGG 508
      ser gln ala lys arg ser val ser ser pro lys tyr ala met gly his phe arg trp gly 138
NHF   ser gln ala
ACTH                    ser val ser ser pro lys tyr ala met gly his phe arg trp gly

LPP-1 AGC CCC GAT AAG GCC ACC ATC CGC AAG CGC AGA CCG GTG CGA CCC AAC ACG TCC GAC AGC 568
      ser pro asp lys ala thr ile arg lys arg arg pro val arg pro asn thr ser asp ser 158
ACTH  ser pro asp lys ala thr ile arg lys arg arg pro val arg pro asn thr ser asp ser

LPP-1 CCC GAG ATC CCA GAC TAC GCC TTC AAT GGG GTG GAA GGC CCG GCA GAC GAC GCG GGC GAC 628
      pro glu ile pro asp tyr ala phe asn gly val glu gly pro ala asp asp ala gly asp 178
ACTH  pro glu ile pro asp tyr ala phe
                                                β-Lipotropin
LPP-1 TCC GTG TTC ATG AGC CGC AGG GAG ACG CCG GAC GCG GCC GGG CAC CGT GGA GTG GAC GAG 688
      ser val phe met ser arg arg glu thr pro asp ala ala gly his arg gly val asp glu 198

LPP-1 GCG GCG GCG ACG GGG GAA GAT GCC GAG GTT GGA AAT AAA GAC GGG GTC TTC CGC GTG CCT 748
      ala ala ala thr gly glu asp ala glu val gly asn lys asp gly val phe arg val pro 218
                                          β-Endorphin
LPP-1 CCG CCA TTC AAA CGC TAC GGT GGC TTC ATG AAA GTG ATG CAA GAG ATT GAC CAT TGG CCA 808
      pro pro phe lys arg tyr gly gly phe met lys val met gln glu ile asp his trp pro 238

LPP-1 CTG GTG CCA GTA ATC CGC AAG GTC ATG CAC AAG GAG AGC ACA AAG TCG CTC TGA 862
      leu val pro val ile arg lys val met his lys glu ser thr lys ser leu STP 255

LPP-1 gtgcctcgcgtgttgaggcaacgcagtgctttgtttaagtcggttaatgaaacgtcgatttgatttgcatgcattgatg
      tcaggatgcatttgtaggtgtcgtttagttaaattcaaaaataaa 986
```

FIG. 2. Nucleotide and deduced amino acid sequences of LPP-1. The predicted signal peptide (von Heijne, 1986) is designated as amino acid residues −22 to −1, with the cleavage site indicated by the arrow. NHF (nasohypophysial factor) is a polypeptide previously isolated and sequenced from lamprey pituitary (Sower *et al.*, 1995). ACTH is a polypeptide sequence previously reported from lamprey pituitary, and suggested to be a fragment of lamprey ACTH (Takahashi *et al.*, 1995). Boxed residues are potential convertase cleavage sites preceding the putative ACTH, β-lipotropin, and β-endorphin polypeptide sequences. Sequence corresponding to the core sequence of MSH is indicated by the single underline. Sequence corresponding to the met–enkephalin sequence at the N-terminal of the β-endorphin polypeptide is indicated by the double underline. The polyadenylation signal at the end of the 3′ untranslated region is indicated by boldface.

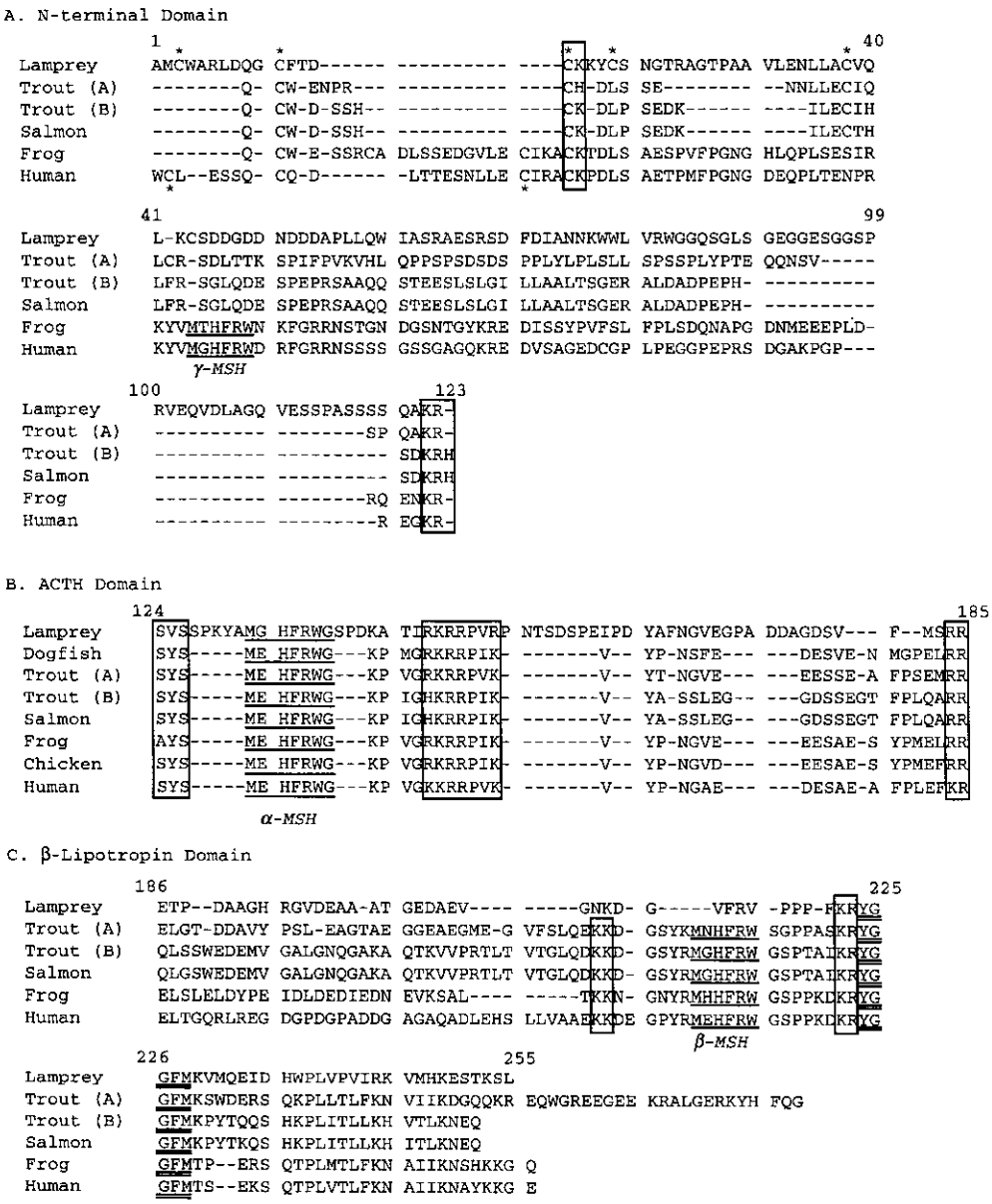

FIG. 3. Sequence comparison of the LPP-1 clone of lamprey POMC with POMC sequences previously deposited in the NCBI data base. Data base and Accession Numbers are: rainbow trout A (A45359, PIR), rainbow trout B (B45359, PIR), chum salmon (P01204, SWISS), African clawed frog (A01460, PIR), and human (P01189, SWISS). For convenience of comparison the sequences are separated into (A) N-terminal, (B) ACTH, and (C) β-lipotropin domains. The ACTH domain comparison includes polypeptide sequences reported for spiny dogfish (A01462, PIR) and chicken (A61127, PIR). Cysteine residues clustered near the N-terminal are indicated by *. Boxed residues point out regions of clear homology, including potential convertase cleavage sites. Single underlined regions indicate core sequences for α-, β-, and γ-MSH. Double underlined regions indicate the met-enkephalin sequence at the N-terminal of β-endorphin.

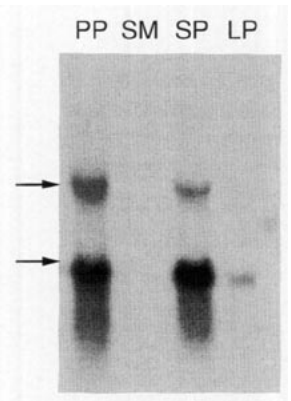

FIG. 4. Northern blot of total RNA isolated from pituitaries of larval (LP), parasitic (PP), and upstream migrant (prespawning, SP) phases of the life cycle of sea lamprey and probed with LPP-1 labeled by random priming. Total RNA from abdominal muscle of the prespawning (SM) was also used as a negative control. Arrows indicate the approximate migration positions of 28S and 18S RNA. Equal loading of lanes was confirmed by staining with ethidium bromide. The sizes of the mRNAs detected correspond to ~1.1 kb and ~4 kb.

this domain in many other vertebrate species. This N-terminal sequence is essentially identical to a polypeptide designated NHF previously isolated from lamprey pituitary. The N-terminal domain of POMC has generally been regarded as a spacer region with no intrinsic biological activity (Wolin and Walter, 1993), although conservation of a cysteine-rich region in all POMCs, including that of the lamprey, suggests that this domain may be important for proper folding and processing of the precursor. However, recent demonstration of NHF in the blood of lampreys has led to proposals that this polypeptide may have some peripheral biological function (Sower *et al.,* 1995). Furthermore, the N-terminal domain of rat POMC has been reported to have growth factor activity, affecting postnatal development of lactotrophic cells (Tilemans *et al.,* 1994).

The loss of the dibasic cleavage site upstream from the expected position of the β-MSH sequence and the presence of a VFRV sequence which may be a remnant of the β-MSH core suggest that, unlike all other known vertebrate POMCs, LPP-1 does not include a functional β-MSH. Melanotropic hormones do, however, appear to be physiologically important in lampreys since hypophysectomy has been shown to result in melanin concentration and pallor in *Geotria australis* and *Lampetra fluviatilis* (Larson, 1965; Eddy and Strahan, 1968). While the presence of the VFRV remnant suggests that this lamprey cDNA has lost the β-MSH sequence over its evolutionary history, we cannot discount the alternative possibility that the ancestral POMC contained only a single MSH sequence which was reduplicated over evolution to result in the multiple copies now present in higher vertebrates.

It appears likely that, as in other species such as the rainbow trout, more than one isoform of POMC may be expressed in the lamprey. Recently, three peptides containing the MSH core sequence (MXHFRWG) were isolated from pituitary glands of adult sea lampreys (*P. marinus*) (Takahashi *et al.,* 1995). They reported an ACTH-like sequence identical to the sequence of the ACTH domain of LPP-1. In addition, they isolated a MSH sequence not identical either to the α-MSH sequence shared by their ACTH peptide and LPP-1 or to the remnant of the β-MSH region seen in LPP-1. Immunocytochemical studies indicated nonuniform distribution of these peptides in cells from various regions of the lamprey pituitary (Nozaki *et al.,* 1995). Together these data strongly suggest the presence of two or more isoforms of POMC in the lamprey, at least one of which may possess a functional β-MSH.

The original detection of this POMC as a PCR product using a degenerate primer directed toward a conserved sequence in STH appears to be due to unexpected partial sequence homology between LPP-1 (residues 247–253) and the degenerate STH primer (MHKVETYL), together with the very strong expression of LPP-1 in the pituitaries of upstream migrant lampreys from which the cDNA library was made. Expression of LPP-1 in lamprey pituitary appears to be developmentally regulated, with low expression in premetamorphic, larval animals, but very strong expression in parasitic adults and upstream migrants. Detailed analyses now underway of expression during development may

therefore provide insights into the physiological role of POMC in the transforming and sexually maturing lamprey. As well as the ~1.1-kb band predicted for LPP-1, a second mRNA of 3–4 kb was also evident on Northern blotting. This larger mRNA may represent the second POMC isoform alluded to previously, although its possible origination through multiple polyadenylation signals cannot be ruled out at the present time.

A previous study using antibodies to mammalian POMC-related peptides in another species of lamprey (Dores *et al.,* 1984) had led to the hypothesis that, in the intermediate region of the lamprey pituitary, met-enkephalin may be derived from the lamprey equivalent of POMC, reflecting a common origin for proenkephalin and POMC (Dores *et al.,* 1993). These two hormone precursors do, in fact, share some structural similarities, including an N-terminal cysteine-rich region, and the pentapeptide sequence of met-enkephalin, which is represented in the first five residues of the β-endorphin sequence in the C-terminal region of POMC. However, the ACTH and MSH domains of POMC have no equivalents in proenkephalin (Dores *et al.,* 1993). The similarity of the structural organization of LPP-1 reported here to that of POMCs from other vertebrates suggests that any common ancestral prohormone linking POMC and proenkephalin must be considerably more ancient, although, in the absence of evidence for a distinct proenkephalin in this species, the alternative possibility of generation of met-enkephalin from POMC in the lamprey cannot be ruled out.

Prior to the present study, POMC had only been recognized in vertebrates with a lineage that can be traced back ~250 million years. Considering the conservative evolutionary history attributed to agnathans, and indications that the divergence of the lamprey and gnathostome lines occurred more than 500 million years ago (Forey and Janvier, 1993), the presence of a distinct and recognizable POMC in lamprey pituitary with structural organization similar to that in teleosts and higher vertebrates strongly suggests that POMC was present in the common ancestor of lampreys and gnathostomes, adding a further ~250 million years to the lineage of this hormone precursor.

ACKNOWLEDGMENTS

This work was supported by operating grants from the Natural Sciences and Engineering Research Council of Canada, the National Science Foundation, and the Great Lakes Fishery Commission. The authors thank Dr. H. Kawauchi and his colleagues at the School of Fisheries, University of Kitasato, Sanriku, Iwate, Japan, for providing peptide sequences and preprints of manuscripts during the course of these investigations. Dr. R. M. Dores provided helpful comments during the course of our investigation.

REFERENCES

Baker, B. (1979). "Hormone Evolution" (E. J. W. Barrington, Ed.), pp. 643–722. Academic Press, London.

Dores, R. M., Finger, T. E., and Gold, M. R. (1984). Immunohistochemical localization of enkephalin- and ACTH-related substances in the pituitary of the lamprey. *Cell Tissue Res.* **235,** 107–115.

Dores, R. M. (1988). "The Melanotropic Peptides" (M. E. Hadley, Ed.), Vol. 1, pp. 26–38. CRC Press, Boca Raton, FL.

Dores, R. M., McDonald, L. K., Goldsmith, A., Deviche, P., and Rubin, D. A. (1993). The phylogeny of enkephalins: Speculations on the origins of opioid precursors. *Cell Physiol. Biochem.* **3,** 231–244.

Douglass, J., Civelli, O., and Herbert, E. (1984). Polyprotein gene expression: Generation of diversity of neuroendocrine peptides. *Annu. Rev. Biochem.* **53,** 665–715.

Eddy, J. P., and Strahan, R. (1968). The role of the pineal complex in the pigmentary effector system of the lampreys, *Mordacia mordax* (Richardson) and *Geotria australis* Gray. *Gen. Comp. Endocrinol.* **11,** 528–534.

Eipper, B. A., and Mains, R. E. (1980). Structure and function of pro-adrenocorticotropin/endorphin and related peptides. *Endocr. Rev.* **1,** 1–27.

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

Hilario, E., Lihrmann, I., and Vaudry, H. (1990). Characterization of the cDNA encoding proopiomelanocortin in the frog *Rana ridibunda*. *Biochem. Biophys. Res. Commun.* **173,** 653–659.

Larsen, L. O. (1965). Effects of hypophysectomy in the cyclostome, *Lampetra fluviatilis* (L.) Gray. *Gen. Comp. Endocrinol.* **5,** 16–30.

Martens, G. J. M., Civelli, O., and Herbert, E. (1985). Nucleotide sequence of a cloned cDNA for pro-opiomelanocortin in the amphibian *Xenopus laevis*. *J. Biol. Chem.* **260,** 13685–13689.

Nakanishi, S., Inoue, A., Kita, T., Nakamura, M., Chang, A. C. Y., Cohen, S. N., and Numa, S. (1979). Nucle-

otide sequence of cloned cDNA for bovine corticotropin-β-lipotropin precursor. *Nature* **278,** 423–427.

Nozaki, M., Takahashi, A., Amemiya, Y., Kawauchi, H., and 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.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual," 2nd ed., Vol. 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Soma, G. I., Kitahara, N., Nishizawa, T., Nanami, H., Kotake, C., Okazaki, H., and Andoh, T. (1984). Nucleotide sequence of a cloned cDNA for proopiomelanocortin precursor of chum salmon, *Oncorhynchus keta. Nucleic Acids Res.* **1,** 8029–8041.

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

Takahashi, A., Amemiya, Y., Nozaki, M., Sower, S. A., Joss, J., Gorbman, A., and Kawauchi, H. (1995). Isolation and characterization of melanotropins from the lamprey pituitary gland. *Int. J. Pep. Protein Res.,* in press.

Tilemans, D., Andries, M., Proost, P., Debreese, B., Beeumen, J. V., and Denef, C. (1994). In vitro evidence that an 11-kilodalton N-terminal fragment of proopiomelanocortin is a growth factor specifically stimulating the development of lactotrophs in rat pituitary during postnatal life. *Endocrinology* **135,** 168–174.

von Heijne, G. (1986). A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res.* **14,** 4683–4690.

Wolin, S. L., and Walter, P. (1993). Discrete nascent chain lengths are required for the insertion of presecretory proteins into microsomal membranes. *J. Cell Biol.* **121,** 1211–1219.