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Review

Evolutionary significance of proopiomelanocortin in agnatha and chondrichthyes

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1. Introduction

Proopiomelanocortin (POMC) is the precursor protein for adrenocorticotropin (ACTH), β-lipotropin (β-LPH), melanotropin (MSH) and β-endorphin (EP), which are associated with stress response and environmental adaptation. Since the first demonstration of the amino acid sequence of bovine POMC which was deduced from the nucleotide sequence of its cDNA by Nakanishi et al. (1979), knowledge of the primary structure of POMC has been obtained from representatives of most vertebrate classes. Tetrapod POMC is composed of pro-γ-MSH, ACTH, and β-LPH. Characteristically, each domain has one MSH; γ-MSH in pro-γ-MSH, α-MSH in ACTH and β-MSH in β-LPH. Recently, POMCs of lungfish of dipnoans were also found to contain pro-γ-MSH, ACTH and β-LPH (Dores et al., 1999; Lee et al., 1999a; Amemiya et al., 1999b).

In contrast to sarcopterygian POMC, fish POMCs contain 1–4 MSHs, e.g. primitive actinopterygian POMCs contain three MSHs, as in sarcopterygians (Amemiya et al., 1997; Dores et al., 1997; Alrubaian et al., 1999; Danielson et al., 1999). However, teleost POMCs lack γ-MSH (Kitahara et al., 1988; Salbert et al., 1992; Okuta et al., 1996; Arends et al., 1998; Lee et al., 1999b; Takahashi et al., 2000). Chondricthyan POMCs contain four MSHs, in which δ-MSH is unique in this class (Amemiya et al., 1999a, 2000). Furthermore, in lamprey, two POMCs are produced; one contains a single MSH and the other contains two MSHs (Heinig et al., 1995; Takahashi et al., 1995b). Although POMCs show variation in the number of MSH, they consistently contain one β-EP at the C-termini throughout vertebrates. It is, therefore, suggested that POMC has evolved by internal duplication and deletion of MSH. The present review focuses on the diversity of POMC in chondrichthyans and agnathans and describes

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the scenario for the molecular evolution of POMC.

2. POMC in chondrichthyan

Chondricthyans consist of elasmobranchs, including sharks and rays, and holocephalans, including chimaera or ratfish. A recent molecular cloning study showed that dogfish POMC cDNA encodes the fourth MSH (δ-MSH) in addition to α-, β- and γ-MSH (Amemiya et al., 1999a) (Fig. 1). The newly identified δ-MSH was located between α- and β-MSH. Among the previously identified dogfish POMC-related peptides, the amino acid sequences of α-MSH, β-MSH and β-EP (Bennett et al., 1974; Lorenz et al., 1986) were identical to those deduced from the POMC cDNA, whereas the third MSH, which was first identified as γ-MSH (McLean and Lowry, 1981), was assigned to δ-MSH. Thus, this cDNA study demonstrated not only the occurrence of δ-MSH, but also the presence of γ-MSH in dogfish POMC as a sarcopterygian homologue. The presence of δ-MSH was also demonstrated in stingray (Amemiya et al., 2000) and ratfish (Takahashi et al., unpublished).

The four MSHs in the elasmobranch POMCs can be classified into two groups based on the amino acid sequence identity and the length between the N-terminus and the MSH-core sequence, His–Phe–Arg–Trp, which is a minimum essential sequence for MSH activity (Amemiya et al., 1999a, 2000). One group consisted of α-MSH and γ-MSH, with 57% sequence identity in both the dogfish and the stingray, and the other consisted of β-MSH and δ-MSH with 50% in the dogfish and 56% in the stingray. The sequence identity between the two groups was 28–50% in the dogfish and 33–50% in the stingray. These observations suggested that δ-MSH was derived from β-MSH during the course of chondrichthyan evolution by internal gene duplication (Fig. 2).

The C-terminal extension of δ-MSH is another unique segment in chondrichthyan POMC (Fig. 1). This extension showed relatively high nucleotide sequence identity with a segment corresponding to β-EP; 51% between dogfish POMC cDNA (745–831) and (910–996) and 42% between the stingray POMC cDNA (723–782) and (885–944) (Amemiya et al., 1999a, 2000). Thus, we proposed that during evolution of the chondrichthyans, there was a duplication of the segment between β-MSH and β-EP (Amemiya et al., 1999a, 2000) (Fig. 2). Subsequently, a mutation occurred in the duplicated region to give rise to δ-MSH. However, because the remainder of the duplicated region had a less stringent functional constraints than δ-MSH, the sequence corresponding to the β-EP-core sequence was deleted (Amemiya et al., 2000).

3. POMC in agnatha

Recently, we isolated three MSH-related peptides, MSH-A (19-amino acid peptide), MSH-B (20-amino acid peptide) and ACTH (60-amino acid peptide) (Takahashi et al., 1995a). In this study, we thoroughly fractionated the lamprey pituitary extract and determined the amino acid sequence of each isolated peptide to find peptides containing the MSH-core sequence. This was done because the lamprey pituitary glands showed little cross-reactivity to antisera prepared against mammalian POMC-related peptides (Baker and Buckingham, 1983; Nozaki and Gorbman, 1984). From these studies, MSH-A and B differed significantly from gnathostome MSHs and could not be assigned as α-MSH, β-MSH, γ-MSH or δ-MSH based on sequence similarity (Fig. 1). ACTH contained a MSH (22-amino acid peptide) at the N-terminal region which was separated by C-terminal region by four consecutive basic amino acids (Arg–Lys–Arg–Arg), and exhibited corticotropic activity in lamprey kidney (Takahashi et al., 1995a) (Fig. 1). This MSH-sequence in the ACTH did not correspond to either MSH-A or B and was significantly different from α-MSH.

Nozaki et al. (1995) showed that antisera prepared against synthesized lamprey ACTH (1–16) and MSH-B stained the pars distalis and the pars intermedia of lamprey pituitary, respectively. Using these antisera, we cloned two distinct POMC cDNAs, proopiocortin (POC) cDNA and proopiomelanotropin (POM) cDNA, from the λgt 11 cDNA library for lamprey pituitary (Fig. 1). POC cDNA encoded ACTH and β-EP, and POM cDNA encoded MSH-A, MSH-B and a different β-EP (Heinig et al., 1995; Takahashi et al., 1995b). Thus lamprey is different from gnathostomes in which ACTH, MSH and β-EP are encoded on a common POMC gene. The amino acid sequence of POC shows low sequence identity (approx.

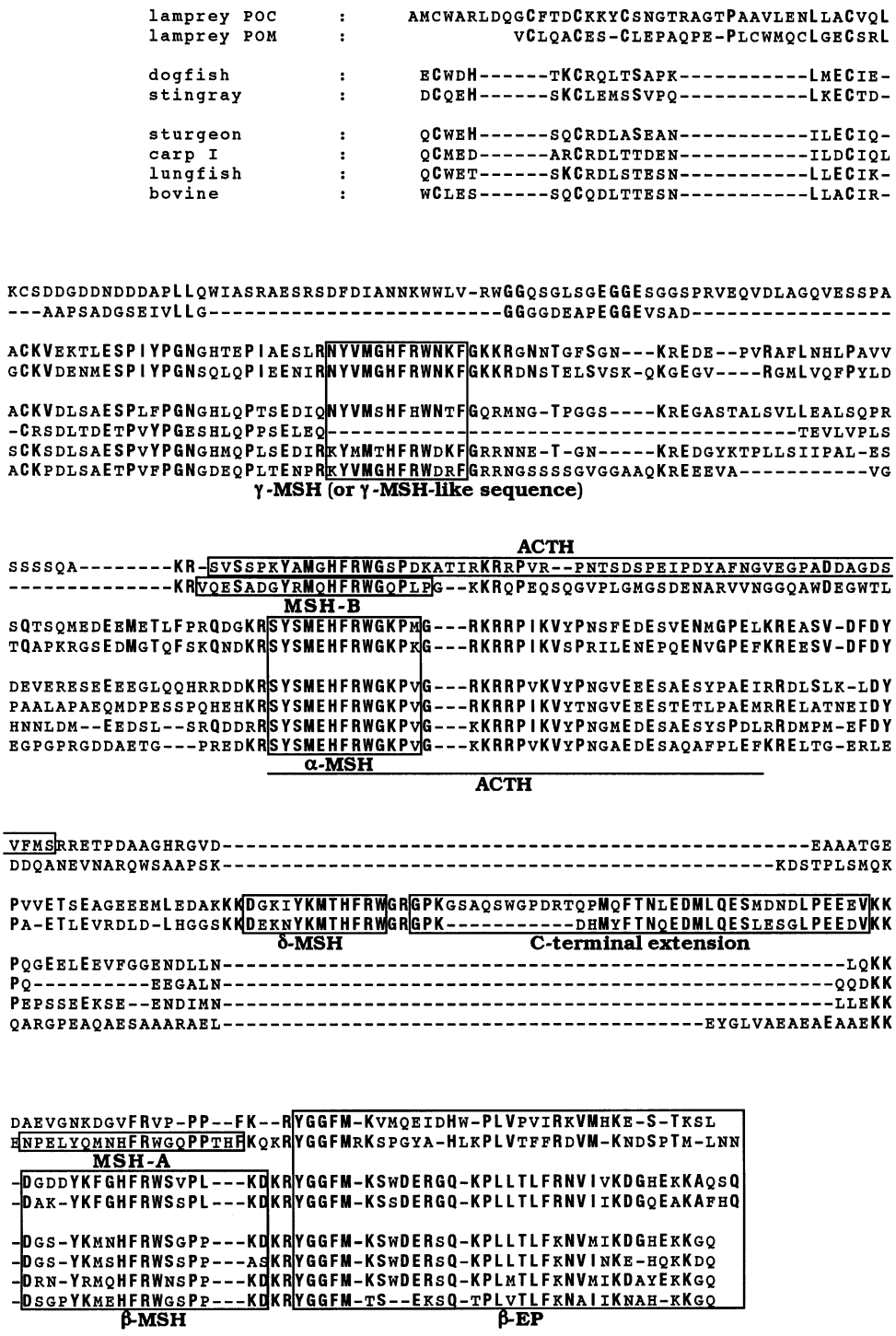

Fig. 1. Amino acid sequences of POMCs from lamprey (Takahashi et al., 1995b), dogfish (Amemiya et al., 1999a), stingray (Amemiya et al., 2000), sturgeon (Amemiya et al., 1997), carp (Arends et al., 1998; Takahashi et al., 2000), lungfish (Amemiya et al., 1999b) and bovine (Nakanishi et al., 1979). Common amino acids between lamprey POC and POM or dogfish and stingray are shown in bold letters. Amino acids in actinopterygians and sarcopterygians which identical to common amino acids in chondrichthyans are also shown in bold letters. Dashes show gaps.

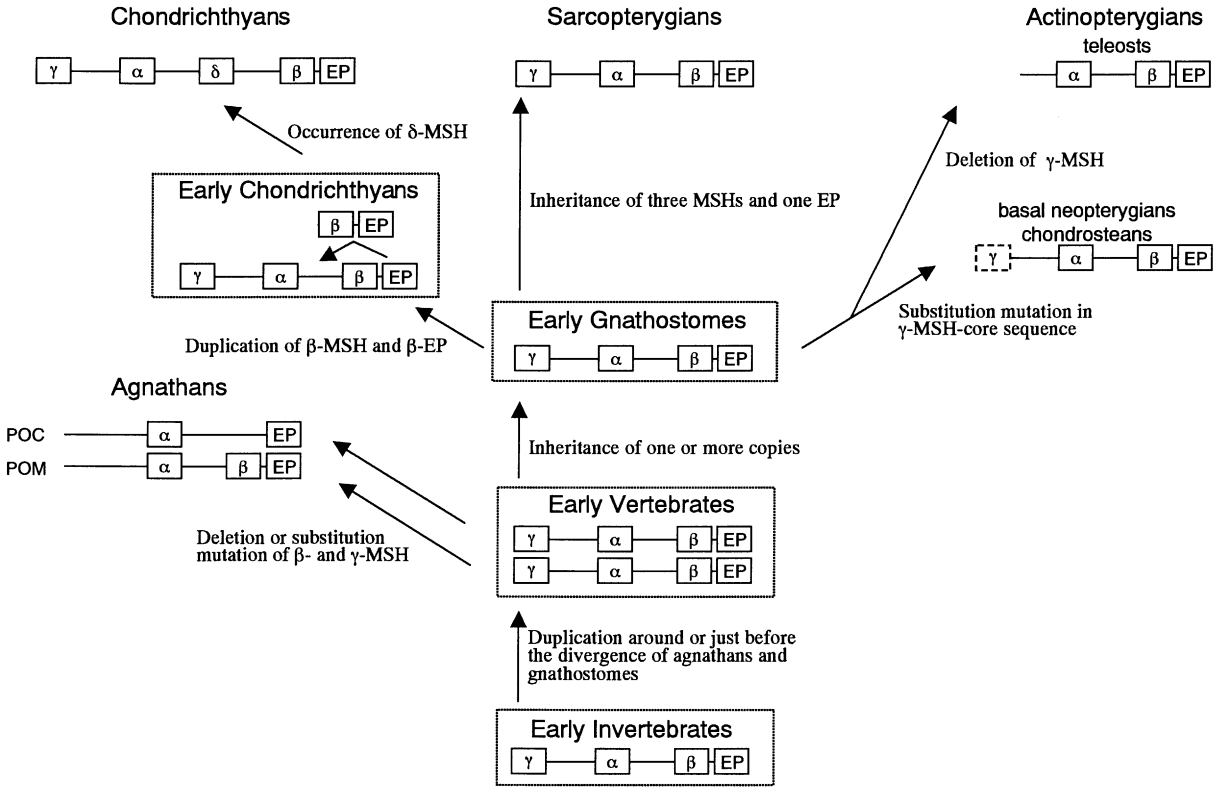

Fig. 2. Schematic diagram for molecular evolution of POMC. Boxes with dotted line show hypothetical ancestor and evolutional intermediates of POMC. α, β, γ and δ show α-, β-, γ- and δ-MSH, respectively. EP shows β-EP. Box with broken line shows remnant of MSH. See Amemiya et al. (1997) for the evolution of POMC in actinopterygians.

30%) with that of POM. However, the locations of β-EP sequences in the POC and POM are well conserved and MSH-B can be aligned with MSH in ACTH. It is, therefore, probable that POC and POM originated from a common ancestral gene by duplication (Fig. 2).

4. Tissue-specific expression of POMC genes in lamprey

It has been established that in gnathostomes, the pars distalis and the pars intermedia of the pituitary produce a common POMC, whereas the final products are different in each lobe depending on the tissue-specific processing (Castro and Morrison, 1997). Pro-γ-MSH, ACTH and β-LPH are produced in the pars distalis, while β-LPH is partially cleaved to β-EP. In the pars intermedia, these peptides are further cleaved to small peptides such as α-MSH and β-MSH.

Lamprey shows a different expression pattern of POMC genes compared to gnathostomes. The mRNA of POC was detected only in the pars distalis and that of POM in the pars intermedia in lamprey by northern blot analysis (Takahashi et al., 1995b). In an in situ hybridization study, POC mRNA was shown to be distributed throughout most cells of the rostral pars distalis during the entire life cycle (Ficele et al., 1998). POC mRNA was also detected in scattered cells of the proximal pars distalis. The presence of POM mRNA was completely confined to most cells of the pars intermedia. The distribution of POC mRNA and POM mRNA corresponded to immunohistochemical localization of ACTH and those of MSH-A and MSH-B, respectively (Nozaki et al., 1995). These results indicated that the expression of POC gene is specific in the pars distalis, where ACTH is processed from POC, and the expression of POM gene is specific in the pars intermedia where MSHs are processed from POM. Thus, the POC-producing cells of the pars distalis in lamprey is functionally similar to the POMC-pro-

ducing cells in gnathostome pars distalis because ACTH is a common major product in these cells. Moreover, the POM-producing cells of the pars intermedia in lamprey is functionally similar to the POMC-producing cells in gnathostome pars intermedia because MSH is the common product in these cells.

The tissue-specific expression of POC and POM gene in lamprey showed that after the duplication of the ancestral POMC gene, each copy evolved in concert with a specialization process of tissue function during the course of lamprey evolution. However, gnathostomes have evolved a tissue-specific processing system to generate different POMC-related peptides from a single POMC in each lobe.

5. Molecular evolution of POMC

The recent characterization of POMC in invertebrates has revealed that all of the hormonal segments in sarcopterygian POMC are present in the same sequential order in POMCs of leech and a marine bivalve molluscan (Salzet et al., 1997; Stefano et al., 1999). These findings suggested that α-, β- and γ-MSH appeared by duplication of an ancestral MSH at an early stage of invertebrate evolution. Thus, POMC in early vertebrates might have three MSHs. Sequence comparisons between lamprey and gnathostome POMC suggest that β-MSH and γ-MSH in ancestral lamprey POC were mutated mainly by the accumulation of amino acid substitutions (Fig. 1), whereas in the case of lamprey POM, the domain for γ-MSH in the ancestral molecule might have been deleted.

Suga et al. (1999) proposed that after the separation from cephalochordates, vertebrates evolved a variety of tissue-specific genes of identical structure in each gene subfamily around or just before the divergence of agnathans and gnathostomes by gene duplication and possibly chromosomal duplication. The lamprey POC and POM might have been derived from the copies which were generated by the gene duplication early in the origin of vertebrates (Fig. 2).

In addition to lamprey, duplication of the POMC gene was observed in primitive (sturgeon and paddelfish) and advanced actinopterygians (carp and salmonids), and amphibians (Martens, 1986; Salbert et al., 1992; Okuta et al., 1996; Arends et al., 1998; Alrubaian et al., 1999; Danielson et al., 1999; Takahashi et al., 2000). Unlike lamprey, two POMCs in these species have the same number of MSHs. These duplications seem to correspond with genome duplication in their own strains. The POMCs of the extant gnathostomes might have originated from one of the copies generated from their ancestors (Fig. 2).

The extant sarcopterygians and primitive actinopterygians might have inherited the molecular architecture of POMC containing three MSHs and one EP from early invertebrates (Fig. 2). Chondrichthyans are only one class of vertebrates having δ-MSH in addition to α-, β- and γ-MSH. It is, therefore, suggested that δ-MSH appeared after the divergence of chondrichthyans from the ancestral vertebrate lineage and before the divergence of elasmobranchs and holocephalans.

6. Concluding remarks

The present review suggests that POMC evolved by gene duplication which increased the number of copies of POMC, and by internal gene duplication and deletion of the MSH domain that prompted the diversity of POMC as to the number of MSHs. The POMC gene is the first gene of the adenohypophysial hormones whose presence was demonstrated throughout all classes of vertebrates. Moreover, this is the first adenophypophysial hormone gene homologue detected in invertebrates. Thus, characterization of the POMC gene in urocordates and cephalocordates, which are considered to be the immediate evolutionary antecedents of vertebrates, may provide insight into not only the molecular evolution of POMC but also the phylogenetic origin of the pituitary.

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