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Review

Structural and functional diversity of proopiomelanocortin in fish with special reference to barfin flounder☆

Akiyoshi Takahashi [a,*], Yuki Kobayashi [a], Masafumi Amano [a], Takeshi Yamanome [b]

[a] School of Marine Biosciences, Kitasato University, Ofunato, Iwate 022-0101, Japan
[b] Iwate Inland Fisheries Technology Center, Hachimantai, Iwate 028-7302, Japan

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ABSTRACT

Proopiomelanocortin (POMC) is a precursor of adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormone (MSH), and endorphin (END). We have characterized POMC systems in barfin flounder. The results revealed unique aspects of POMC systems. Notable features in terms of pituitary functions are the occurrence of three functional POMC genes, the mutation of an essential sequence in the β-END in one of the genes, occurrence of α-MSH in addition to ACTH in the pars distalis of the pituitary, and expression of the three genes in a single cell. While MSHs stimulate pigment dispersion, expression of the POMC gene and plasma levels of MSH do not always respond to background color changes between black and white. The functions of MSHs in skin pigmentation are very unique, because acetylation at the N-terminal of α-MSH inhibits its pigment dispersing activity. This is in contrast to results from other teleosts and amphibians, in which acetylation increases the activity. In the skin, the POMC gene is expressed in the non-chromatophoric dermal cells, indicating that MSH produced in the skin *de novo* has a paracrine function. The detection of MSH peptides in skin extracts seems to show that the control of skin pigmentation by MSHs is twofold—endocrine control by the pituitary, and paracrine control by the skin itself. Thus, fish provide an interesting model to help understand the structural and functional diversity of POMC systems. In this review, we provide an overview of our recent studies on the characterization of molecules and biological significance of POMC systems in barfin flounder.

Contents

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* Corresponding author. Tel.: +81 192 44 1925; fax: +81 192 44 3934.
E-mail address: akiyoshi@kitasato-u.ac.jp (A. Takahashi).

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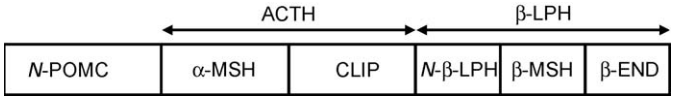
1. Introduction

Proopiomelanocortin (POMC) is a precursor of adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormone (MSH), and endorphin (END) [76]. POMC is unique in that it has a repetitive sequence of MSH, one of which is a part of ACTH, and a single END at the C-terminal (Scheme 1). The number of MSHs is rather class specific—two in teleosts, three in ancient teleosts, lobe-finned fish, and tetrapods, and four in cartilaginous fish [20,68,74,77]. Moreover, some species have two POMCs consisting of the same hormonal organization, while lampreys of agnathans have two distinct POMCs—one contains ACTH and END, and the other contains two MSHs and a different END [43,78,85]. These various structures of POMCs in vertebrates show that POMC may have diverged by the deletion and insertion of MSH segments and gene duplication of the entire POMC segment during fish radiation [77,80]. Namely, an ancestral POMC of a direct evolutionary antecedent of vertebrates is supposed to have three MSH segments and a single END. Thereafter, the number of MSH segments may have decreased in strains of teleosts and lampreys and increased in cartilaginous fish.

The major tissue generating POMC is the pituitary gland, where corticotrophs of the pars distalis (PD) and melanotrophs of the pars intermedia (PI, or neurointermediate lobe, NIL, in teleosts, because of the complex structure with the pars nervosa and PI [37]) produce distinct POMC-derived peptides depending on tissue-specific post-translational processing [20,47,61,68,74]. It is generally accepted in mammals that the major products of corticotrophs are ACTH, β-END, and N-terminal peptide of POMC (N-POMC), and those in melanotrophs are further processed forms such as α-MSH split from ACTH, N-terminally acetylated and C-terminally truncated β-END, and γ-MSH split from N-POMC. A similar processing path has been suggested in fish using several POMC-derived peptides identified in some species [76]; however, the precise mechanisms in both lobes have yet to be elucidated.

Well-known biological activities of POMC-derived peptides in fish are cortisol synthesis/release in interrenal glands [93] and pigment synthesis/dispersion in chromatophores [31]. In addition to these classical activities, MSH has been shown to be associated with food intake [21,73], lipolysis [94], and immunomodulation [41,79,92], and β-END has been shown to be associated with food intake [26,27] and immunomodulation [90,91]. Moreover, investigations of receptors for melanocortin (MC), a collective term for ACTH and MSH, have revealed the wide distribution of MC receptors (MC-R) in a variety of tissues [49,59,60]. These results show that POMC-derived peptides play multifunctional roles in fish.

The barfin flounder *Verasper moseri* is a member of flatfish, Pleuronectiformes, which is the most derived teleost order after Tetradontiformes containing *Fugu* [63]. This species, inhabiting the Pacific coast of northern Japan [9], has revealed some interesting features of POMC concerning the number of genes, post-translational processing, tissue distribution, pigment synthesis/dispersion, expression profiles in relation to background color change, and receptor functions. We review recent advances in POMC investigations regarding molecular and biological aspects, with special reference to the barfin flounder.



Scheme 1. Overview of teleost POMC. Teleost POMC is composed of three segments—N-POMC, ACTH, and β-LPH. ACTH is subdivided into α-MSH and CLIP, and β-LPH into N-β-LPH, β-MSH, and β-END. See Refs. [76] and [77] for details.

2. Amino acid sequences

The presence of two subtypes of POMC has been reported in ray-finned fish such as the sturgeon [2], paddlefish [25], zebrafish [36], carp [10,79], and sockeye salmon [66]. The amino acid sequence identity observed in ancient groups of sturgeon (90%) and paddlefish (88%) is comparable to that observed in the more advanced fish carp (91%) and is higher than that in zebrafish (35%) and sockeye salmon (43%). These results indicate that the pair of POMC genes (*Pomc*) in ancient and advanced groups might not have been generated by duplication events occurring in a common ancestor. *Pomc* may have duplicated independently after speciation events – perhaps, it is an order- or class-specific event – during the course of evolution in fish. The *Pomc* of carp, paddlefish, and sturgeon might have duplicated much later than those of salmonids. A phylogenetic tree of fish POMCs suggests that, in salmonids, POMC-A may have evolved as an invariant copy after duplication because it comprises a clade in a phylogenetic tree together with the single POMC of tuna [81], tilapia [58], and sea bass [89] (Fig. 1). Salmonid POMC-B [57,66,72] may have diverged as a variant copy, because it has numerous mutations in N-POMC and β-END segments, and comprises an independent clade. Additionally, the partial primary structure of a zebrafish POMC-like sequence indicates mutations of processing sites for ACTH and β-MSH to non-functional sequences [36].

Working with the barfin flounder, we identified for the first time the presence of three subtypes of *Pomc* [83]. Later, the presence of three functional *Pomc* types was also reported in rainbow trout [57]. Barfin flounder (bf) PrePOMC-A, -B, and -C are composed of 199, 214, and 220 aa, respectively. Protein sequence alignment of POMCs reveals that bfPOMC-A and -B are composed of an N-POMC, an ACTH segment containing α-MSH, and a β-lipotropic hormone (LPH) segment containing β-MSH and β-END, as in other teleost POMCs (Fig. 2). bfPOMC-C has a similar organization, whereas two out of four Cys residues in the N-POMC segment are mutated, and mutations from Tyr to Ser and Gly to Arg occur in the first and third residues of the segment, corresponding to the β-END essential sequence (*Tyr*-Gly-*Gly*-Phe-Met). Based on the phylogenetic tree, bfPOMC-A and -B retain the structural characteristics of invariant copies, because they make up a clade with the POMCs of tuna, tilapia, sea bass, sockeye salmon-A, and rainbow trout-A (Fig. 1). However, bfPOMC-C is considered to be a variant copy because it comprises a clade with POMC-B of sockeye salmon and rainbow trout.

A phylogenetic tree using lamprey proopiomelanotropin (POM) [78] as an outgroup suggests that the lineage leading to an ancestor of bfPOMC-A and -B and that leading to bfPOMC-C may have diverged from a common ancestor at the first duplication event, and then the lineage of bfPOMC-A may have diverged from that of bfPOMC-B at the second duplication event. Thereafter, bfPOMC-C may have accumulated a number of point mutations in the