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Review

# Interrelation between melanocyte-stimulating hormone and melanin-concentrating hormone in physiological body color change: Roles emerging from barfin flounder *Verasper moseri*

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ABSTRACT

In teleosts, as their names suggest, the main target cells of melanocyte-stimulating hormone (MSH) and melanin-concentrating hormone (MCH) are the chromatophores in the skin, where these peptide hormones play opposing roles in regulating pigment migration. These effects are obvious especially when their activities are examined *in vitro*. On the contrary, while MCH also exhibits activity *in vivo*, MSH does not always stimulate pigment dispersion *in vivo* because of predominant sympathetic nervous system. A series of our investigations indicates that this is also the case in barfin flounder, *Verasper moseri*. Interestingly, we observed that *mch* expression and the tissue contents of MCH can be easily influenced by changes in environmental color conditions, while gene expression and tissue contents related to MSH scarcely respond to color changes. Transcripts of MSH and MCH receptor genes have been identified in a variety of tissues of this fish species, suggesting that these are multifunctional peptide hormones. Nevertheless, chromatophores in the skin still offer important clues in the efforts to elucidate the functions of melanotropic peptides. Herein, we review the most recent advancements of our studies on MSH and MCH and their receptors in the barfin flounder and discuss the interrelations between these peptides, focusing on their roles in influencing pigment migration in the skin.



## 1. Introduction

Melanocyte-stimulating hormone (MSH) and melanin-concentrating hormone (MCH) are the two major hormonal peptides associated with the physiological body color changes of teleost fish [42]. MSH is mainly produced in the neurointermediate lobe (NIL) of the pituitary gland, where MSH is derived from a precursor protein named proopiomelanocortin (POMC) by specific proteolytic cleavage [35,39]. MCH is produced in the hypothalamus and secreted by the neurohypophysis after migration through neural fibers [1]. As in the case of MSH, MCH is also derived from a precursor protein called proMCH [21]. The receptors of MSH and MCH are members of G protein-coupled receptors with seven transmembrane domains [33,38].

It is generally accepted that both MSH and MCH control pigment migration in the chromatophores of teleost fish [12–15,42]. MSH stimulates pigment dispersion that results in dark body color, while MCH induces pigment aggregation that results in light body color. These functions are apparent in *in vitro* conditions where these peptides are examined individually. Moreover, it was found that MSH and MCH control pigment migration in a competitive manner when their effects are examined simultaneously *in vitro* [5,10,11,32]. Thus, these two peptides are "melanotropic peptides" having opposing functions. *In vivo* treatment of MCH in the fish triggers similar color changes as in the corresponding *in vitro* treatment [19,48]; however, MSH does not always stimulate pigment dispersion because the pigment-aggregating activities of the sympathetic nervous system usually overcome the functions of MSH [12,48]. This suggests that interrelation between MSH and MCH is masked by the dominant neural effects.

The barfin flounder *Verasper moseri* is a member of the flatfish family, which inhabits the northeastern coast of Japan facing the Pacific Ocean. The body color of this fish species is regulated by coordinate migration of pigments in the melanophores and xanthophores [32]. We have been investigating the roles of the melanotropic peptides in influencing chromatophore migration using this species of fish as a model. After a series of investigations, some interesting results linked to the interrelation between MSH and

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MCH have been obtained. Herein, we review the effects of MSH and MCH and their receptors on body color changes in the barfin flounder.

## 2. Receptors

### 2.1. Melanocortin receptors in the skin of the barfin flounder

MSH and ACTH are collectively called melanocortin (MC). While five subtypes of MC receptor (MCR; MC1R to MC5R) have been identified in mammals, chicken, goldfish, and zebrafish, so far [16,31,33,46], only four MCRs excluding MC3R, have been identified in the barfin flounder [25,29,30]. In addition, *mc3r* has not been identified in the draft genome of fugu either [31]. Phylogenetically, flounder and fugu are closely related to each other [34], hence, it is conceivable that *mc3r* might have been lost in this lineage after divergence from the Cypriniformes including zebrafish and goldfish. Among the four MCRs, transcripts of *mc1r* and *mc5r* are predominantly present in the skin [30]. Studies using isolated chromatophoric cells revealed that *mc1r* and *mc5r* are expressed in melanophores, while *mc5r* is expressed in xanthophores [30].

The three molecular forms of α-MSH have different numbers of acetyl groups at the N-terminus [42]. In the barfin flounder, desacetyl (Des-Ac)-α-MSH (i.e., $ACTH_{1-13}$ amide) stimulates pigment dispersion in both melanophores and xanthophores, while α-MSH (i.e., acetylated $ACTH_{1-13}$ amide) is effective in xanthophores, though it exerts no effects on melanophores [28]. These results are inconsistent with pharmacological properties, which showed that α-MSH exhibits higher activities than Des-Ac-α-MSH on both MC1R and MC5R in the barfin flounder (Saito and Kobayashi, unpublished data). Therefore, we hypothesized that a heterodimer consisting of MC1R and MC5R is formed in the melanophores, and the affinity of α-MSH to this putative heterodimer is relatively weak; as a result, melanophores do not respond to α-MSH (Fig. 1) [27,30,43]. This assumption can be supported by evidence obtained from several lines of our investigations using Japanese flounder and goldfish [24,26].

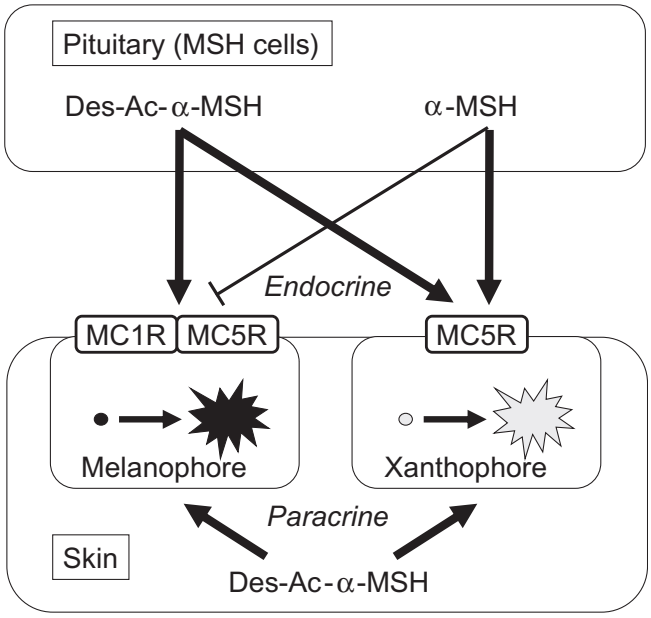


**Fig. 1.** A schematic diagram showing a 2-fold (endocrine and paracrine) control over skin pigmentation in the barfin flounder by α-MSH-rp (taken from Ref. 43 and modified). α-MSH has no effect on pigment dispersion in melanophores, where MC1R and MC5R may compose heterodimeric form of GPCR.

### 2.2. Melanin-concentrating hormone receptor in the skin of the barfin flounder

In the barfin flounder, MCH receptor genes, *mch-r1* and *mch-r2*, are expressed in the brain, indicating that both receptor subtypes are responsible for the central roles of MCH [44]. *Mch-r1* is exclusively expressed in the brain, whereas *mch-r2* is also expressed in several peripheral tissues, including the skin. The wide distribution of *mch-r2* transcripts in peripheral tissues coincides with the role of MCH as a neurohypophysial hormone as shown by the extensive neuronal projections protruding from the hypothalamus to the neurohypophysis in the barfin flounder [2]. Both *in vivo* and *in vitro* treatments of MCH result in pigment aggregation in both the melanophores and xanthophores, indicating that stimulus from MCH is mediated via MCH-R2 [32,48].

## 3. Background colors and profiles of *pomc* mRNA and α-MSH

Most cells in the NIL of the barfin flounder pituitary co-express the three POMC genes, i.e., *pomc-a*, *pomc-b*, and *pomc-c* [41]. The short term transferring experiment with the barfin flounder—transferring from black tank to white tank, and *vice versa*—indicated that expressions of the three POMC genes do not always show similar response with respect to background color changes [23]. Changes in the mRNA contents of NIL corresponding to the backgrounds were only observed for *pomc-c*, in fish which were transferred from a black tank to a white tank. Long-term acclimation to background color may exert no influence on the expression of each *pomc*. Rearing in either black or white tanks may not make much of a difference in affecting the levels of each *pomc* mRNA in the NIL, because no difference was observed in the mRNA contents of the three POMC genes in the NIL taken from the barfin flounder reared in a black tank and a white tank for 126 and 163 days, respectively. These results suggest that the transcription of *pomc* is regulated with multiple neuroendocrine controls and that the background color may have minor effects on the transcription of POMC genes in the NIL.

Despite the absence of changes in the *pomc* mRNAs in the NIL of the barfin flounder pituitary in response to changes in background color, remarkably, the plasma immunoreactive (ir)-α-MSH increased 1 day after transfer from a white tank to a black tank (Fig. 2B) [23]. *Pomc* mRNAs might be constitutively expressed in this particular experimental condition. Hence, the remarkable increase in plasma ir-α-MSH on day 1 in the black tank may indicate the release of accumulated α-MSH in response to a change in the background from white to black. Nevertheless, background color may not be the exclusive factor that affects α-MSH secretion because plasma ir-α-MSH levels are not always maintained at high levels in a black background and at low levels in white background, as shown by several lines of evidence. First, no changes were observed in those fish that were transferred from a black tank to a white tank (Fig. 2A). This is a typical example showing that the plasma MSH concentrations do not always synchronize with background color changes. Second, the translocation of fish from a white tank to another white tank increased plasma ir-α-MSH levels on day 7 (Fig. 2B). This result suggests that MSH release from the pituitary is influenced by factors other than background color change. The secretion of α-MSH may also be influenced by other environmental or artificial factors in addition to the changes in background color. Third, the ir-α-MSH levels of those fish reared in a black background for 7 days after having been transferred from white tank (Fig. 2B) were higher than those in fish reared in a black tank on day 0 (Fig. 2A), while on day 0, no differences were observed in the plasma levels of ir-α-MSH between fish reared in a black tank or a white tank. The fish were allowed to adapt in either