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Differential expressions of melanocortin receptor subtypes in melanophores and xanthophores of barfin flounder

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ABSTRACT

α-Melanocyte-stimulating hormone (α-MSH) is a member of the melanocortin (MC) family, and the MC receptor (MCR) is a member of the G protein-coupled receptor (GPCR) superfamily. We previously found that in barfin flounder, a flatfish, α-MSH with an acetyl group at the N-terminus stimulated pigment dispersion in xanthophores; however, this effect was not observed in melanophores. Therefore, the present study was undertaken to find which MCR subtypes are expressed in these pigment cells in order to elucidate how acetylation regulates activities of α-MSH-related peptides. Here, we also report the cloning of *Mc1r* and *Mc5r* from barfin flounder. Three types of cells—melanophores, xanthophores, and nonchromatophoric dermal cells—were isolated from the skin samples collected from the dorsal fin. These cells were then tested for the expression of *Mc1r* and *Mc5r* as well as *Mc2r* and *Mc4r* that we had previously cloned. *Mc1r* and *Mc5r* transcripts were detected in melanophores, and a sole *Mc5r* transcript was detected in xanthophores. We had previously found that the efficiency of α-MSH was higher than that of desacetyl-α-MSH for pigment dispersion in xanthophores. Acetylated MSH peptide may have increased binding affinity to MC5R, whereas α-MSH lacks melanin-dispersing activity. Increasing evidences indicate that many GPCRs form heterodimers, and this may affect the affinity of the ligand for the corresponding GPCR. Taken together, the expression of two different *Mcr* subtypes in melanophores may suggest that a heterodimer consisting of MC1R and MC5R may have a low binding affinity toward α-MSH. The present results clarify the types of MCRs that are expressed in melanophores and xanthophores of barfin flounder; furthermore, the results provide important clues about the functional regulation of α-MSH-related peptides.

1. Introduction

In fish, skin pigmentation is associated with several biological processes, including camouflage, protection from ultraviolet rays, and nuptial coloration (Eberle, 1988; Fujii, 1993). Body colors and patterns displayed by many fish species depend on the presence of several types of pigment cells, including melanophores and xanthophores (Fujii and Oshima, 1986, 1994; Fujii, 1993). α-Melanocyte-stimulating hormone (α-MSH) is a classical peptide hormone that participates in physiological and morphological color changes by controlling fish chromatophores (Eberle, 1988; Fujii and Oshima, 1986, 1994). α-MSH is liberated from a precursor protein called proopiomelanocortin (POMC) by posttranslational processing in the intermediate lobe of the pituitary (Eberle, 2000; Takahashi and Kawauchi, 2006a; Takahashi et al., 2006, 2008). This peptide comprises 13 amino acids with an acetyl group and an amide group at the N- and C-termini, respectively (Eberle, 1988, 2000; Takahashi et al., 2006). Acetylation of the N-terminus significantly modifies the biological activity of the peptide, because the presence of an acetyl group enhances melanin-dispersing activity (Eberle, 1988; Kawauchi et al., 1984; Kobayashi et al., 2009; Mountjoy et al., 2003).

Barfin flounder is a promising flatfish for aquaculture along the Pacific coast of northern Japan. We reported that barfin flounder expresses three *Pomc* subtypes in the pituitary gland (Kobayashi et al., 2008b; Takahashi et al., 2005, 2006, 2009). *Pomc-c*, one of the three genes, is also expressed in a variety of tissues, including the skin, and the total amount of *Pomc-c* transcripts in the skin is comparable to the sum of *Pomc-a*, *-b*, and *-c* transcripts in the pituitary (Kobayashi et al., 2008b, 2009; Takahashi et al., 2005). Recently, we identified desacetyl-α-MSH derived from POMC-C in the skin extract and found that *Pomc-c* is expressed in nonchromatophoric dermal cells (Kobayashi et al., 2009; Takahashi et al., 2009). These results indicate that desacetyl-α-MSH produced in the skin plays a paracrine role in pigmentation. Moreover, acetylation of the N-terminus of α-MSH-related peptides resulted in opposite functions in melanophores and xanthophores in the skin

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of barfin flounder—presence of the acetyl group inhibited the pigment-dispersing activity in melanophores but enhanced this activity in xanthophores (Kobayashi et al., 2009; Takahashi et al., 2009). These different responses may suggest the presence of distinct α-MSH receptors in the two chromatophoric cell types.

Melanocortin receptor (MCR; MC is a collective term for MSH-related peptides) is a G protein-coupled receptor (GPCR) with seven transmembrane domains and short extracellular and intracellular domains at the N- and C-termini, respectively (Metz et al., 2006; Schiöth et al., 2005; Takahashi and Kawauchi, 2006a,b). Molecular cloning studies have revealed the presence of at least five subtypes of MCR (MC1R to MC5R) in fish, as in the case of tetrapods (Aluru and Vihayan, 2008; Cerdá-Reverter et al., 2003a,b; Haitina et al., 2004, 2007; Klovins et al., 2004a,b; Kobayashi et al., 2008a; Logan et al., 2003; Metz et al., 2005; Ringholm et al., 2002, 2003; Sánchez et al., 2009a,b, 2010; Selz et al., 2007; van der Salm et al., 2005). Among the five types of *Mcr*, *Mc1r*, and *Mc5r* are profoundly expressed in the skin of tilapia and goldfish, respectively (Cerdá-Reverter et al., 2003a; van der Salm et al., 2005). Currently, only *Mc4r* has been reported to be present in barfin flounder (Kobayashi et al., 2008a). Signals indicating the presence of this receptor transcripts were detected in the skin, but their intensities were weaker than those in the brain, liver, testis, and ovary. The present study was performed to investigate the type of *Mcr* expressed in isolated

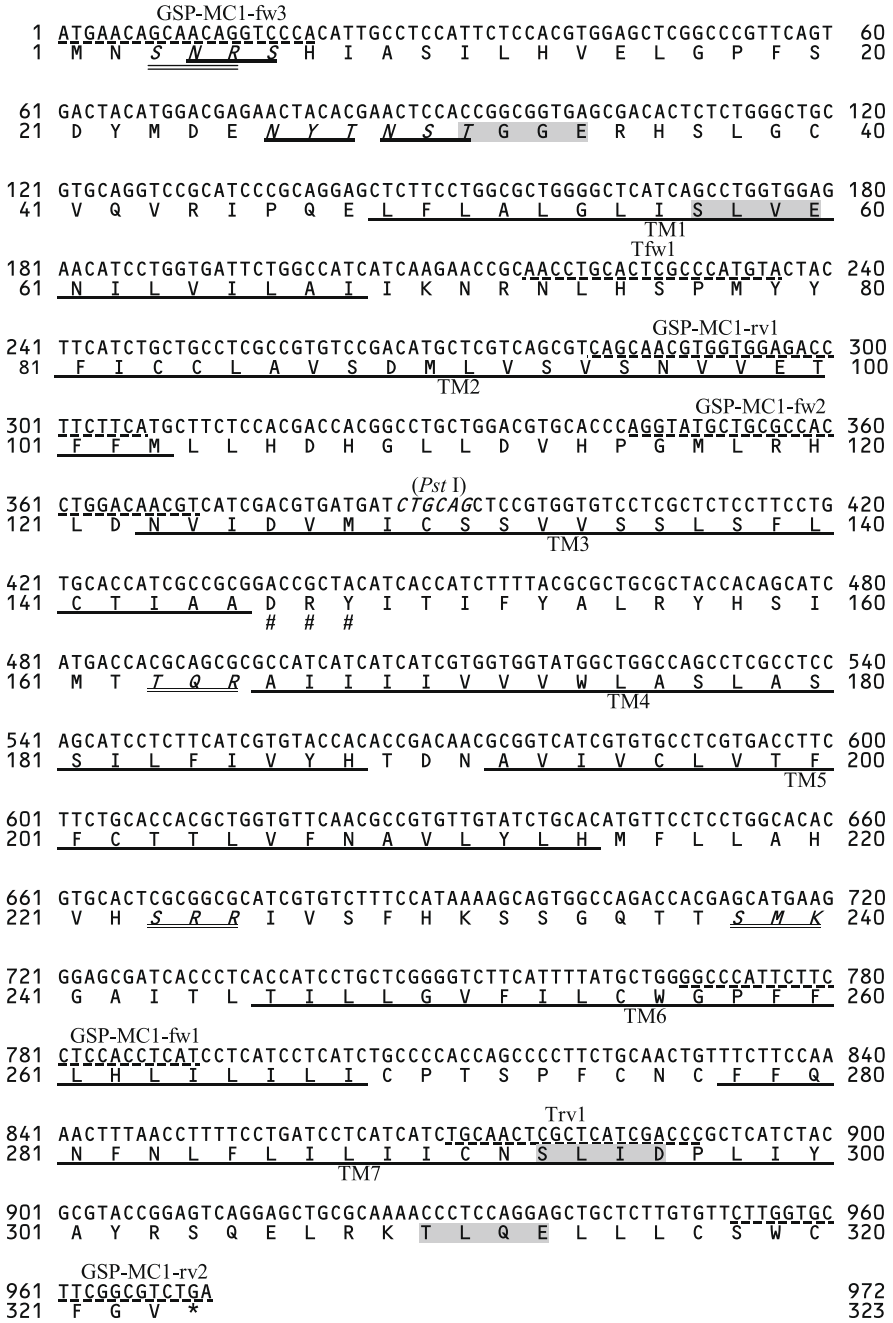

Fig. 1. The nucleic acid sequence of the reading frame and the deduced amino acid sequence of barfin flounder MC1R (Accession No. AB287974). DNA was amplified from the genomic DNA or the brain cDNA. Positions of nucleic acid and amino acid sequences are indicated on both the sides. Broken lines indicate primer sites mentioned in Section 2.2. Italicized bases indicate restriction sites. Underlined regions of amino acid (aa) sequence are the transmembrane domains (TM). Italicized aa along with underlines indicate potential *N*-glycosylation motifs. Italicized aa along with double-underlines indicate potential protein kinase C phosphorylation motifs. Shadows indicate potential casein kinase 2 phosphorylation motifs. *Stop codon. ###DRY motif.