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Motility of Cultured Iridophores from the Freshwater Goby *Odontobutis obscura*

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ABSTRACT—Iridophores in the integument of the freshwater goby, *Odontobutis obscura,* are motile. Iridophores isolated from scales of the goby were cultured in L-15 medium. The primary cultured cells were motile. Their movements involved aggregation and dispersion of platelets within the cells, which were not caused by a reversible retraction of cellular processes. Alpha-MSH induced aggregation of platelets, while melatonin and norepinephrine, separately, induced dispersion of the platelets. These responses of the cultured iridophores to drugs were the same with as those of iridophores in preparations of isolated scales. The speed of migration of platelets in cultured iridophores was very slow, and it appeared to be the same as that in iridophores in intact scales. Most of the cultured iridophores exhibited sensitivity to light; they assumed a dispersed state in the light and an aggregated state in darkness.

INTRODUCTION

Iridophores are light-reflecting chromatophores commonly found in the dermis of many poikilothermal vertebrates, and they are known to play a predominant role in the generation of skin coloration [1]. Electron-microscopic observations have revealed that iridophores in fishes contain a large number of platelets, which run parallel to each other and form stacks [2–5]. These platelets are mainly composed of guanine and have a very high reflective index, so that, when stacked, they generate various colors. The phenomenon is called physical or structural coloration [6, 7]. Until recently, these iridophores in fishes were not thought to play an active part in changes of color via phenomena that involved motility.

Quite recently, we found that iridophores in the integument of the freshwater goby, *Odontobutis obscura,* respond to neural and hormonal stimulation via changes in the reflective surface of the cells [8]. Light- and electron-microscopic observation suggested that the motility of the iridophores involved the translocation of reflecting platelets within the cells [8, 9]. At present, however, information on the movements of the reflecting platelets within the iridophores is very scanty. Studies with cultured iridophores may provide us with much useful information about such movements.

The purpose of the present experiments was to present the motility of cultured iridophores from the freshwater goby, *Odontobutis obscura.*

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MATERIALS AND METHODS

Culture of iridophores

Scales isolated from the dorso-lateral side of the freshwater goby, *Odontobutis obscura,* were immersed in a solution composed of a mixture of equal volumes of physiological saline (128 mM NaCl, 2.6 mM KCl, 1.8 mM $CaCl_2$, 5 mM HEPES-NaOH buffer, pH 7.2) and an isotonic solution of KCl for 40 min. This solution, with in its high level of K^+, induced dispersion of platelets in the iridophores [8]. The epidermis was removed with fine forceps from the scales, after they had been immersed in physiological saline supplemented with 2.5 mg/ml collagenase (Type II, Worthington Bochemical Co., Freehold) for 20 min. The epidermis-free scales were then transferred into a vial filled with a dissociation medium which consisted of 2.5 mg/ml collagenase and 1.5 mg/ml trypsin (Sigma Chemical Co., St Louis). The vials

were gently stirred for 40–60 min at room temperature. Dissociated cells were collected with a fine pipette under a dissecting microscope and cultured in plastic dishes coated with collagen (Type A, Nitta Gelatin, Osaka). The culture medium was Leibovitz L-15 medium (Gibco Lab., New York) supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin (Gibco

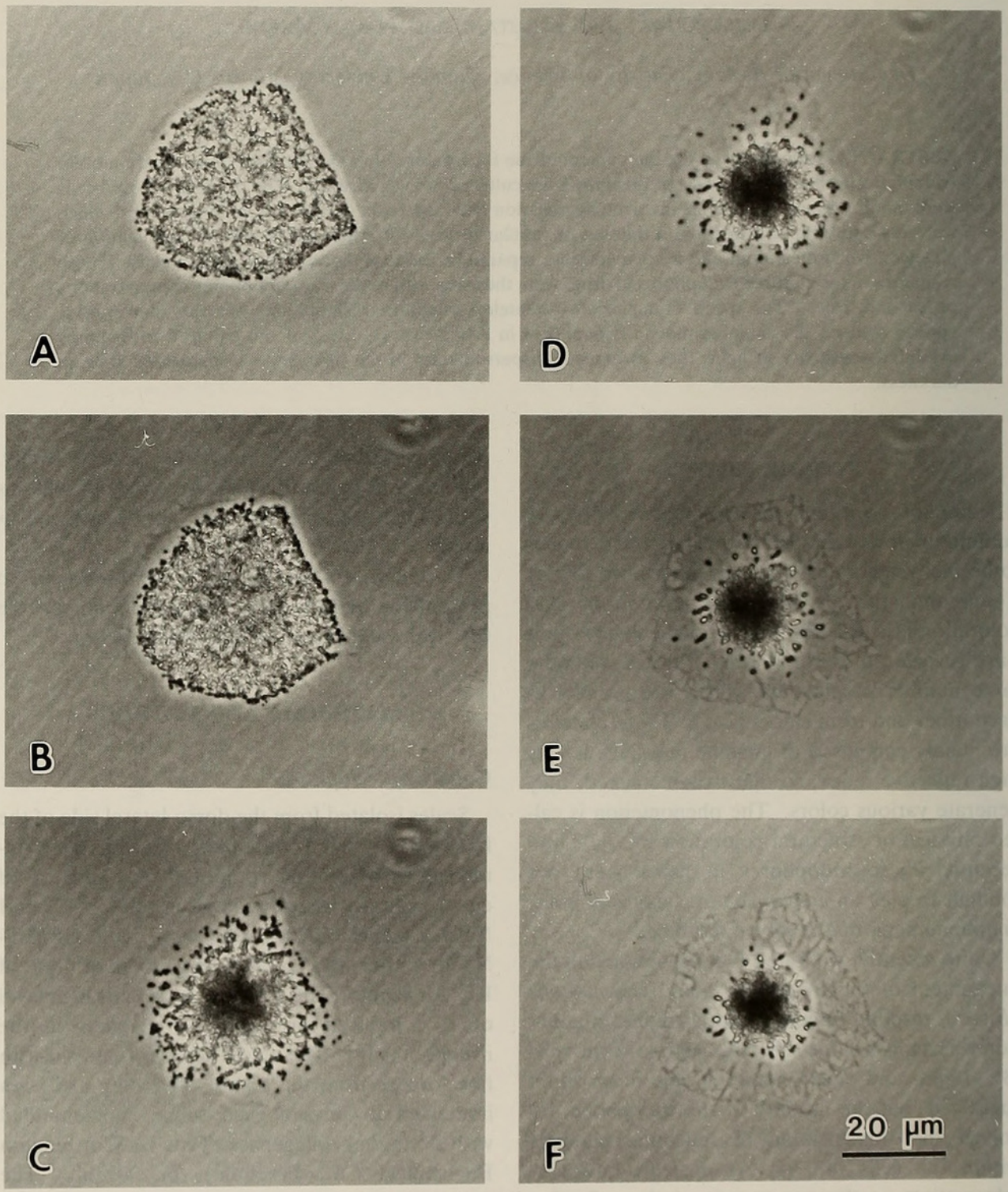

Fig. 1. Micrographs showing responses of cultured iridophores from *Odontobutis obscura* to alpha-MSH and melatonin. A, In culture medium. B, In saline. C, D, E, F and G, 10, 30, 60, 90 and 120 min, respectively, after treatment with 100 nM alpha-MSH. H, I, J, K and L, 20, 40, 60, 90 and 180 min, respectively, after treatment with 1 μM melatonin.

Lab.), and 10% DW. The dishes were incubated at 26°C in air.

Recording of responses of cultured iridophores

Cultures were observed under an inverted phase-contrast microscope (Olympus CK-2) and their responses were photographed for analysis of cellular motility.

Drugs

Alpha-MSH, melatonin and norepinephrine hydrochloride were obtained from Sigma Chemical Co. These drugs were dissolved in physiological saline.

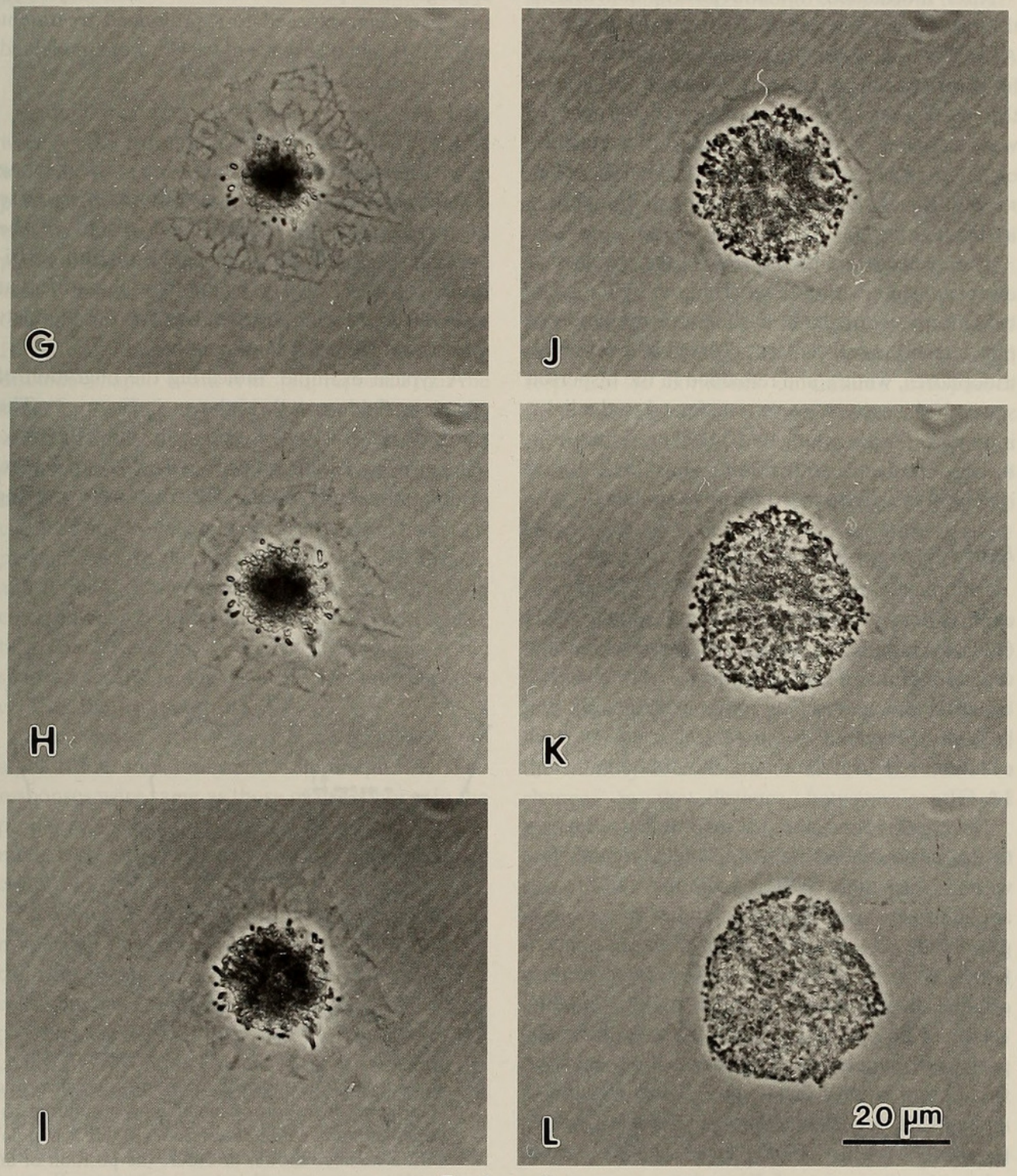

FIG. 1. —Cont.

All experiments were performed at room temperature (22.0–25.0°C).

RESULTS

Cultured iridophores

After inoculation, the iridophores attached to the substratum and began to spread within a day. After 2 to 3 days in culture most iridophores were fully spread and platelets were evenly dispersed throghout the cells. The shapes of iridophores were variable, with rod-like, dendritic, and discoidal iridophores being observed. The diameters ranged from 20 to 50 μm, like those of iridophores in intact scales.

The platelets in these iridophores assumed a dispersed state within the cells in the light. After being kept overnight in darkness, aggregation of platelets in the cell centers occurred in most of the iridophores, while some remained in the dispersed state. If the cultures were transferred to the light, iridophores with aggregated platelets returned to the dispersed state within 3 hr. Thus, the cultured iridophores appear to be sensitive to light.

Responses of cultured iridophores

Iridophores fully spread after 2 to 3 days in culture were used for experiments. Alpha-MSH (100 nM) induced aggregation of platelets into the central regions of the iridophores. The platelets began simultaneously to move centripetally and became aggregated in the central region of each cell after 90 to 120 min of the treatment (Fig. 1A-G).

During this time, the cell membranes attaching to the substratum remained in their original state without retraction. When melatonin (1 μM) was applied to iridophores with platelets in an aggregated state, the platelets began to disperse centrifugally from the aggregates and returned to their original dispersed state after 90 to 180 min (Fig. 1H-L). Norepinephrine (1 μM) also induced dispersion of platelets with the same time course as that observed with melatonin. If the solution of alpha-MSH was changed to physiological saline, the platelets remained aggregated for at least 60 min without any sign of dispersion.

Requirement for Ca^{++} in the action of MSH

The action of MSH was inhibited in Ca^{++}-free saline that contained 1 mM EDTA. If the vehicle for the peptide was changed to the standard saline, aggregation of the platelets was induced in standard fashion. It is noteworthy that a similar aggregation of platelets occurred when iridophores that had been exposed to a prolonged treatment with MSH, in an absence of Ca^{++}, were immersed in the standard saline.

Migration of reflecting platelets

For analysis of the migration of reflecting platelets within the iridophores during the course of the aggregation response, the results of the application of 100 nM alpha-MSH to cultured iridophores were followed by photographing them at intervals of 10 min, and the migration of platelets was traced from the micrographs.

A typical example, indicating the migration of platelets within a cell, is shown in Figure 2. The platelets did not always appear to move linearly. The profile of their velocity was also not linear. Among the bulk of platelets that were moving

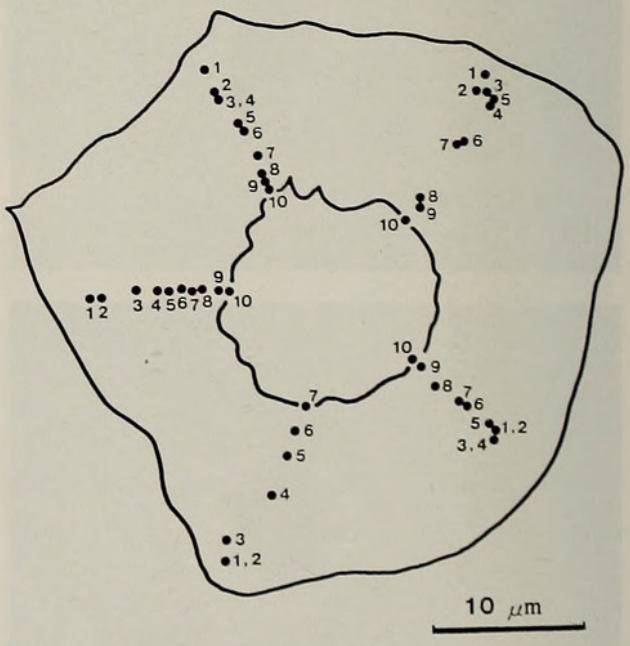

FIG. 2. A typical example of recordings that show the centripetal migration of platelets in a cultured iridophore from *Odontobutis obscura*. Each point with a number shows, in order, the position of an individual platelet at intervals of 10 min.

centripetally, there were some that were stationary and other that even moved in the reversal direction. From the distance of platelet migration measured every 10 min with 35 of platelets in 7 cells, the velocity of platelet migration was calculated. The maximum velocity of platelet migration was calculated as 0.5 μm/min, with a mean value of 0.1 μm/min.

DISCUSSION

Iridophores with platelets in a dispersed state were used for isolation of cells, because they gave well-spread cultured cells. Iridophores with platelets in the dispersed state may be more resistant to the isolation treatment than those with platelets in the aggregated state, which did not give such well-spread cells in culture. Iridophores of *Odontobutis obscura* appeared dendritic *in vivo*. In culture, however, most of the cells assumed a discoidal shape. Iwata *et al.* [10] reported that the shapes of cultured cells may be influenced by various factors, especially by the properties of the substratum in the case of melanophores from the medaka, *Olyzias latipes*, and that melanophores cultured on a collagen-coated substratum had complex shapes. In the present case, better adhesion and spreading of the cells were obtained on a collagen-coated substratum than on uncoated plastic. There were no observable differences in the shapes of cells in the well-spread state between cells on the two substrata.

The cultured iridophores were responsive to alpha-MSH, melatonin and norepinephrine. The responses were same as those of iridophores in the preparations of intact scales. Properties of hormone receptors appeared to be unchanged by culture *in vitro*.

The present experiments clearly showed that motility of the iridophores involves centripetal or centrifugal migration of the platelets within the cells, but no retraction or elongation of cellular processes. Most of the cultured cells did not change their contours during their responses to the agents applied. Some cells did change their shapes during their responses, perhapes as a result of a weakness of adhesion of the cells to the substratum. Responses of such cells were not so clear-cut. In iridophores in preparations of isolated scales, the shapes of cells were unchanged after repeated responses. Electron-microscopic observations have suggested that dendrites of the iridophores are firmly anchored in the connective tissues and that the platelets move within the cells [9].

The iridophores of the integument of some species of amphibian undergo conspicuous changes in shape, from a dendritic to a punctate appearance, and such changes are regulated by MSH from the hypophysis [11, 12]. However, whether movement of platelets results passively from dendritic retraction, or whether it is a selective movement independent of changes in dendritic morphology remains to be determined [13]. Cultured iridophores from the tail skin of tadpoles of the bullfrog, *Rane catesbeiana,* have been shown to attach to dishes and form dendritic structures; such iridophores respond to ACTH with contraction of cells [14]. Recently, Butman *et al.* [15] studied responses to hormones of cultured iridophores from the integument of the Mexican leaf frog, *Pachymedusa dacnicolor*. These iridophores were of two distinct types which differed with respect to both morphological and physiological features. One type (type I) of iridophore responded to some hormones by a reversible retraction of cellular processes and rounding up of cells.

Physiologically active iridophores are also known to be present in the integument of some species of fish, namely the neon tetra, *Paracheirodon innesi* [16], and the blue damselfish, *Chrysiptera cyanea* [17]. In iridophores of the damselfish, the motility is assumed to involve simultaneous changes in the distance between contiguous platelets in all the piles of platelets within the cells, and such changes cause a shift in the spectral reflectance from the cells [18]. The situation may be similar to that in iridophores of the neon tetra.

Thus, motile iridophores in fishes can be classified into two types, in terms of motility: the damselfish type, where changes occur in the distance between adjoining platelets; and the goby type, where the intracellular migration of platelets occurs. Quite recently, we found that iridophores in the integument of some species of Gobiidae are motile. The motility appears to be of the goby type [19, Honma and Iga, unpubl.].

In the centripetal migration of platelets, the course and the velocity of the migration are not always linear. The movement is very slow, as in iridophores in preparations of scales [8, 20]. As far as we know, the movements may be the slowest of those examined in fish chromatophores [21–25]. The present findings are important for elucidation of the mechanisms of movement of the iridophores.

MSH possesses strong pigment-dispersing action in melanophores of poikilothermal vertebrates [1, 26], and Ca^{++} is indispensable for its action [27–29]. Such a dependence of the action of MSH on Ca^{++} was also shown in some non-melanophoral, motile chromatophores of fishes, namely in platyfish erythrophores, and in xanthophores as well as leucophores of the medaka, *Olyzias latipes* [30]. The involvement of Ca^{++} may be important for signal transduction from the receptor to the catalytic unit of adenylate cyclase and/or for the activation of the catalytic unit [31–33]. MSH acts to induce "aggregation" of platelets in the iridophores of *Odontobutis obscura,* as it does in preparations of isolated scales [8], and Ca^{++} is indispensable for its action. Upon subsequent application of standard saline, in the absence of the peptide, after prolonged treatment with MSH in a Ca^{++}-free medium, the platelets in the iridophores began to aggregate. This result suggests a role for Ca^{++} in transduction of signal generated by MSH.

Iridophores in the integument of the neon tetra were light-sensitive and changed in color from deep violet to blue-green in response to illumination [16]. Most of the cultured iridophores from the integument of the *Odontobutis* goby appear to be light-sensitive. Studies on the light sensitivity of chromatophores in fishes are now in progress.

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