[Pigment Cells and Novel Aspects of Researches in Animal Colors]

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Significance of Body Color and Its Changes and Complex Regulation of Chromatophore Motility in Teleost Fish

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Animals utilize conspicuous colors and patterns as "aposematic" coloration. Inconspicuous them are conversely exploited as "cryptic" coloration. For fishes lacking the ability of vocal communication, such colors, patterns and their changes represent strategies of the utmost importance for the survival of individuals or of species. For example, "protective" color, which constitutes part of the cryptic coloration, is useful for avoiding attacks by predators, while conspicuous displays function to frighten predators. On many occasions, delicate changes in hues and patterns are used for communication with conspecifics. Further, in colorful places such as coral reefs, the yellows and blues that adorn many fishes blend well with that average reef background, providing camouflage from predators. Therefore, we must understand how marine creatures' eyes perceive the light and see each other—far differently than humans see them. Colors of fishes are due to the presence of pigment cells (chromatophores) in the dermis of skin tissue, where many varieties of chromatophores are located. Rapid changes of hues and color patterns are caused by the motility of chromatophores, and aggregation of pigment granules in the perikaryon and dispersion throughout the cytoplasm are the characteristic of the cellular motility of an ordinary dendritic chromatophores. Generally, in addition to several hormones such as MSH, MCH and melatonin, a neurotransmitter, norepinephrine controls the motile activities. However, there are exceptional cases, in which acetylcholine (ACh) is liberated from sympathetic nerve fibers: Melanophores of catfishes belonging to the family Siluridae possess cholinoceptors of the muscarinic type and are responsive to ACh, leading to pigment aggregation. Recently, prolactin has been found to cause pigment dispersion only in erythrophores and xanthophores, suggesting the possible involvement of the peptide in nuptial coloration. Endothelins (ET-1, -2 and –3) also induce pigment aggregation (in melanophores, xanthophores and erythrophores) or dispersion (in leucophores). ETs might play a role in the delicate and exquisite control of hues and patterns. In addition to biogenic chemical substances, light is an important factor affecting the chromatic state of the skin. Light-sensitive chromatophores that respond directly to light have been found in some adult fishes, indicating the possible existence of visual pigment in the cells. We lately found that Nile tilapia erythrophores recognize differences in the peak wavelength of incident light, and suggested the presence of multiple cone-type visual pigments in the cells. These chromatophores may be responsible for generation of peculiar color that may ethologically signify much. Thus, the chromatic systems of fish among members of the class Osteichthyes have developed extraordinarily sophisticated properties during the evolution of this class of vertebrates over the course of more than 400 million years.

Thermoreceptive and Photoreceptive Pigment Cells as Sensors of Changing Environmental Conditions

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In addition to being constantly involved in the front line of a defense system against varied environmental stresses, animal body skin functions as a sensor of changing environmental factors. Pigment cells which are involved in body coloration are localized in the skin tissue. We are interested in understanding the possible function(s) of pigment cells as sensors of environmental factors. Many poikilothermic vertebrates are able to change body color to adapt to their ambient coloration. These changes depend upon the activities of chromatophores, which are the pigment cells in the body skin of poikilothermic animals. Generally, the motility of chro-

matophores is controlled by the nervous and/or hormonal system on the basis of visual information, but some of the chromatophores are able to control their motility directly in response to changing environmental conditions. We observed that the isolated tails from larvae of northern amphibians *Rana chensinensis* (an anuran) and *Hynobius retardatus* (an urodele) turned dark in cold physiological salt solution just as their tail fins in situ did. Microscopic observation revealed that exposure to cold caused reversible dispersion of melanin granules within integumental melanophores. This reaction is thought not to be mediated by MSH or adrenergic receptors. There was no darkening, however, in the tail of the *Xenopus* (South African clawed frog) tadpole at low temperatures. Currently, we have little evidence for molecular entities of thermoreception, but we do know that melanophore dispersion induced by cold is unique to northern amphibians.

On the other hand, photoreceptive chromatophores which respond directly to light were widely distributed in invertebrate and in poikilothermal vertebrate, and these pigment cells are considered to be one of the non-visual photo receptor systems. However, little is known about the mechanisms of photoreception.

We ascertained the expression of rhodopsin mRNA using RT-PCR with rhodopsin specific primers, in addition to melanopsin, in *Xenopus* tail fin, where photosensitive melanophores exist. We also detected several kinds of G proteins, including transducin(Gt), which are considered to be involved in the process of photo transduction. The presence of these molecules in pigment cells of poikilothermic vertebrates raises the possibility that pigment cells in animal skin function as photo-sensors generally. To explore this possibility in homeothermic vertebrates, we tried to detect photoreception molecules in mammalian melanocyte. The expression of opsin and transducin mRNA was detected using murine and human cultured melanocytes. The expression of these proteins in mammalian melanocytes suggests the possibility of the existence of photoreception systems in mammalian skin, and further experiments might provide important clues about mammalian physiological functions following photoreception to visible light.

Melanin-Concentrating Hormone and Melanophore-Stimulating Hormone: Diverse Structures and Functions in Fish

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Body color change in fish in response to background coloration is an important process for environmental adaptation in which peptide hormones with opposing effects play roles as mutual physiological antagonists. The melanin-concentrating hormone (MCH), which is produced in the hypothalamus area of the brain, turns the body a pale color by aggregating melanin granules, while α-melanophore-stimulating hormone (α-MSH) produced in the pituitary gland turns the body a dark color by stimulating melanin synthesis. Increasing evidence has shown the diverse functions of these hormones in both the central nervous system and peripheral tissues.

[Structure and function of MCH] MCH isolated from chum salmon pituitary extract for the first time is composed of 17 amino acids (aa) and possesses one disulfide bond. cDNA cloning studies have demonstrated that fish MCH consisting of 15 or 17 aa locates consistently on the C-terminal region of proMCH. Amino acid sequence of MCH is highly conserved among fish species with 76% sequence identity, of which the consensus sequence is DxMxCMVGRVYRPCW. Possible involvement of MCH in food intake in fish was suggested using barfin flounder on the basis of greater growth and higher MCH gene expression of fish reared with a white background than those reared with a black background, and higher MCH gene expression in fasted fish than in the fed control. The greater growth in the white-reared fish might be a result of enhanced feeding frequency, which was estimated using a self-feeding device. MCH is produced in cell bodies in hypothalamic areas such as the nucleus tuberis lateralis (NLT) and the area above the lateral ventricular recess (LVR). The number of cell bodies in the NLT increases in response to background color and that in the area above the LVR increased in response to fasting, indicating that MCH in the former area is associated with body color change and that in both areas is related to feeding behavior.

[Structure and function of MSH] The proopiomelanocortin (POMC) gene encodes melanocortins (MCs) such as MSH and adrenocorticotropic hormone (ACTH) which have a common core sequence, HFRW, together with an opioid peptide β-endorphin (β-END), of which core sequence is YGGFM. POMC is suggested to have evolved by recombination of MSH segment because, in jawed fish, the number of MSH segment varies depending on taxonomic classes, whereas a single β-END is consistently present. The MSH encoded are α- and β-MSH in derived ray-finned fish, α-, β- and γ-MSH in lobe-finned fish and primitive ray-finned fish, and α-, β-, γ- and δ-MSH in cartilaginous fish. γ-MSH in some fish has mutation in the core sequence. In fish pituitary, common POMC is