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Morphology of chromatophores of the medaka*

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Introduction

The medaka *Oryzias latipes* is probably one of the most widely used teleosts in pigment cell research. Of five types of chromatophores that occur in lower vertebrates, the medaka has four: melanophore, xanthophore, leucophore and iridophore. A small scale taken from the dorsal part of the fish of the wild strain (*BIR*) contains numerous melanophores, xanthophores and leucophores all of which are clearly seen under a light microscope through the extremely thin epidermis or scale. These chromatophores, primarily under the control of adrenergic nerve fibers, are able to respond repetitively *in vitro* to exogenous signaling substances with vivid pigment translocation over a long period of time. The control mechanisms of pigmentary responses in this famed teleost fish medaka have long been studied extensively, and the findings discussed thoroughly in recent reviews (Fujii and Oshima, 1986; Fujii, 1993; Iwamatsu, 1993). On the other hand, morphological studies, especially those which are concerned with ultrastructure and cytochemistry, are relatively new and many fundamental questions remain unsettled. This article is an overview of the morphological studies on medaka chromatophores reported in the past three decades.

Melanophore

a) *Melanotic melanophore* Melanophores are the largest, highly dendritic cells which play a crucial role in the expression of integumental coloration in the wild type and other darkly pigmented varieties. The ultrastructure of the characteristic pigment organelle, melanosome, was studied in the wild type fish using conventional fixation methods (Nakajima and Obika, 1986) and recently, by the use of a rapid-freezing and freeze-substitution procedure (Obika, 1993). The profiles of melanophores observed by these two methods are essentially the same, although the latter method appears to give a better preservation of intracellular membranous structures such as Golgi apparatus and tubular endoplasmic reticulum. There exist two fundamental types of melanosome precursors (premelanosomes) distinguishable from each other by their matrix structure, namely, multi-vesicular and fibrillar types, the former being predominant in dermal melanophores at embryonic and larval stages. Occasionally, both vesicular and fibrillar structures are found together in a single organelle (mixed type). Melanosomes at various stages of development in adult melanophores of the wild strain are shown in Figs. 1 and 2.

Maintenance of melanophore morphology is primarily secured by adhesion of the cells to the ambient extracellular matrices (Obika, 1976a; Iwata *et al.*, 1985) and, internally, supported by the cytoskeleton. As a major cytoskeletal element, melanophores have an enormous number of cytoplasmic microtubules emanating from the center of the cell, and the bundles of microtubules run parallel to the long axes of the dendrites to prop up the stellate morphology. Microtubule bundles are particularly remarkable in highly dendritic melanophores such as those of the *BdmR* mutant (Obika, 1976b). When isolated and mounted on a cover slip, adjacent dendrites of a melanophore tend to coalesce so that the cell becomes a flat sheet with a simple outline (Obika, 1976a). Response of a chromatophores with such an altered, simplified structure are easy to monitor so that the cells *in vitro* have been frequently used in morphological and physiological experiments. Figs. 3 and 4 depict the distribution patterns of microtubules and vimentin filaments in a melanophore in culture.

Innervation of adrenergic nerve fibers into scale melanophores was visualized by autoradiography (Yamada *et al.*, 1984; Sugimoto and Oshima, 1995), while ultrastructurally, a number of nerve fibers with varicosities containing three morphologically different types of synaptic vesicles were observed in close contact with scale melanophores (Obika, 1988). However, histochemical identification of neurotransmitters in the synaptic vesicles has not yet been carried out at an ultrastructural level. Innervation of scale chromatophores was

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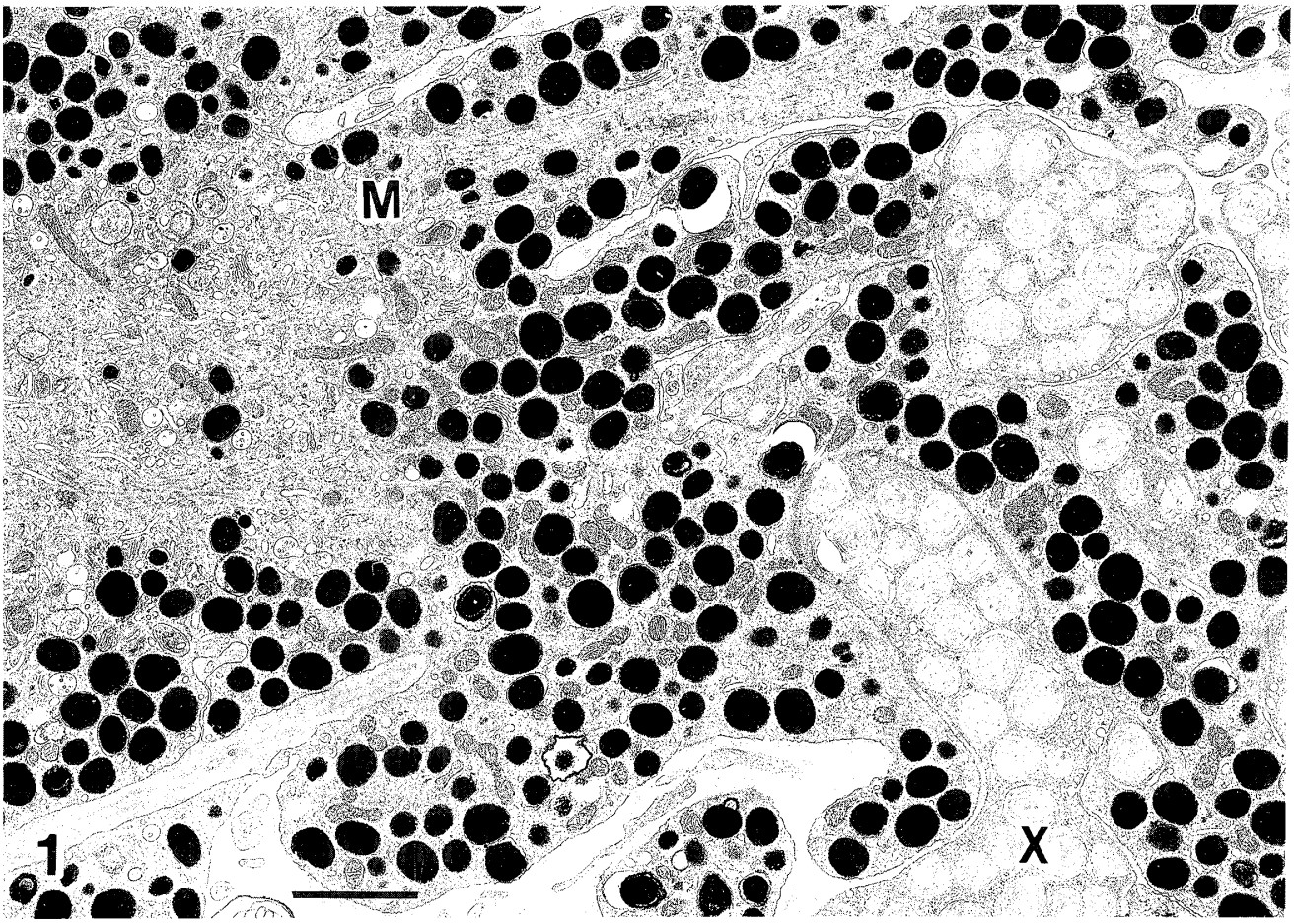


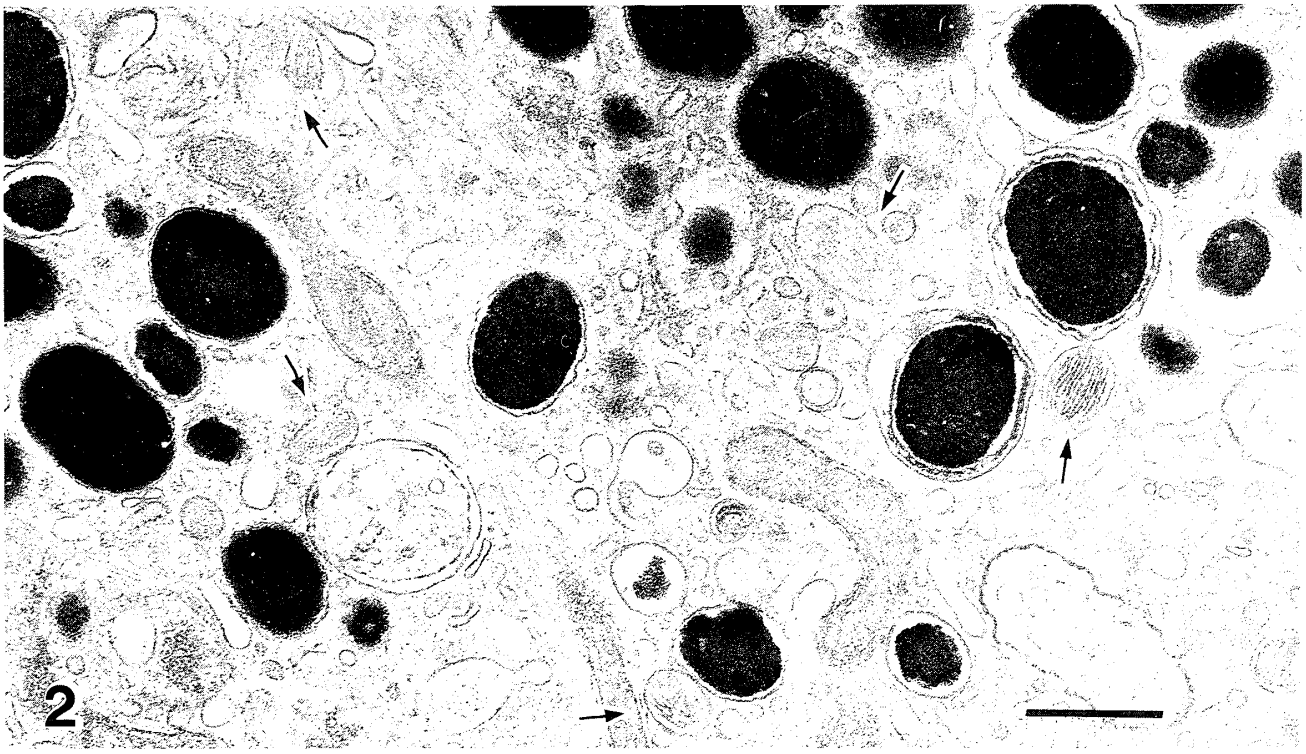


Fig. 1. Horizontal section of a melanophore of wild type (*BIR*) adult fish. Specimen was rapidly frozen by metal contact in liquid nitrogen, and freeze-substituted in acetone containing 4% osmium tetroxide. Numerous melanosomes are seen in the melanophore (M). Dendrites of a xanthophore (X) contain pterinosomes. Bar: 2 μm.

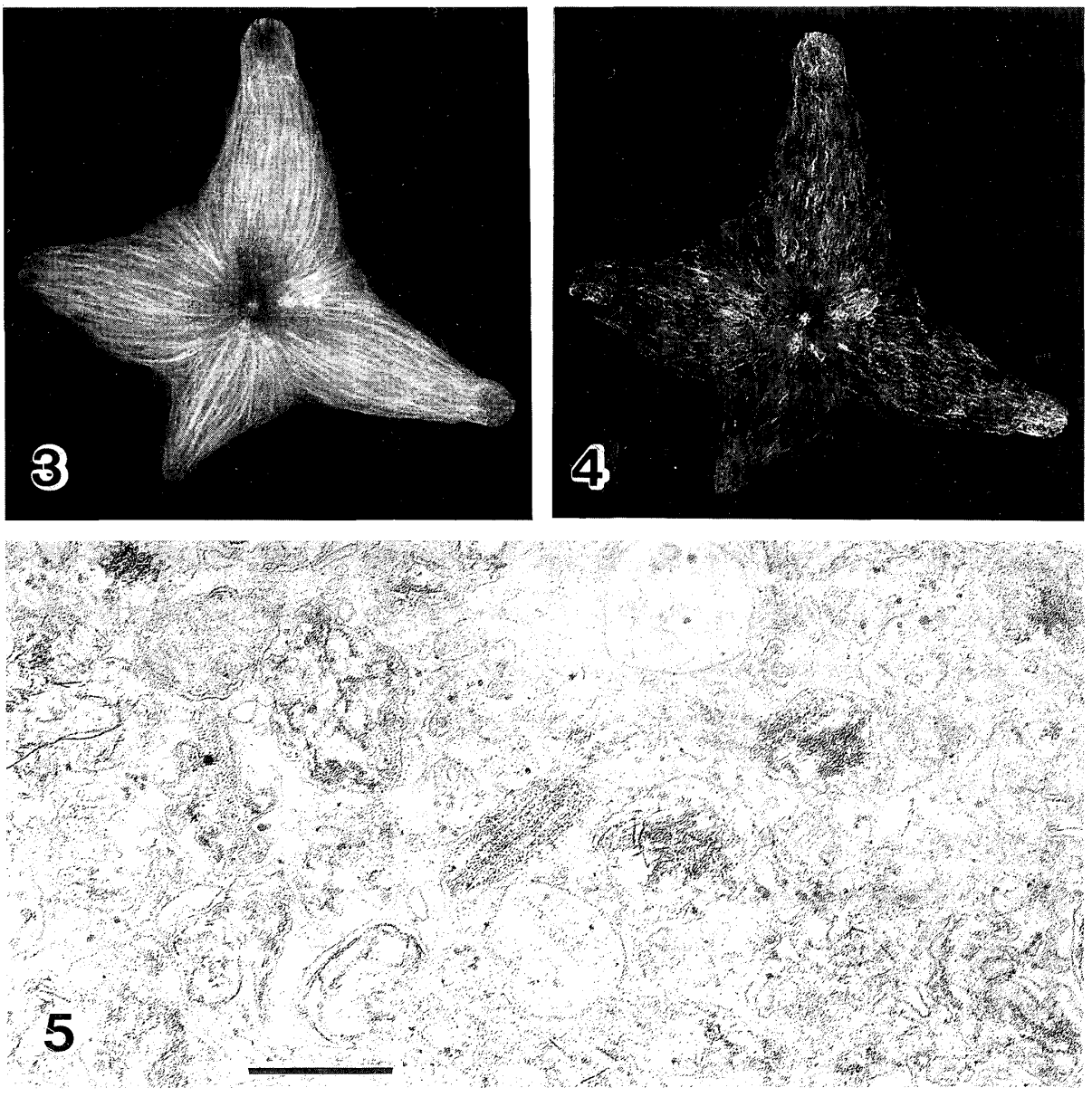
Fig. 2. Melanosomes and premelanosomes (arrows) of wild type adult fish. Rapid-frozen preparation. Some nascent melanosomes are also observed. Bar: 0.5 μm.

also demonstrated by immunofluorescence (Obika and Fukuzawa, 1993a) and scanning electron microscopy (unpublished observation).

b) *Amelanotic melanophore* Melanophores of the orange-red (*bIR*) or white (*bIr*) mutant possess high tyrosinase activity but exhibit a very low degree of pigmentation compared to those of the wild type (Hishida *et al.*, 1961; Tomita and Hishida,1961a, b). Because of their faint coloration, they are called "amelanotic" or "colorless" melanophores. Fig. 5 shows an ultrastructural profile of a portion of an amelanotic melanophore in the scale of the adult orange-red mutant. Melanosomes of fibrillar, vesicular and mixed types of premelanosomes are abundant, and various degrees of pigment deposition are evident. This observation is consistent with studies of Hori and his collaborators that the tyrosinase gene is in fact expressed in amelanotic melanophores of the *bIR* strain (Inagaki *et al.*, 1994; Koga *et al.*, 1994). Melanin deposition in larval amelanotic melanophores was also reported by Hama (1975).

Xanthophore

Xanthophores contain two types of orange and yellow pigment, carotenoids and pteridines (Hama, 1975), the former being solely responsible for the expression of body color in the adult. These pigments are presumably contained within two types of organelles, carotenoid vesicles and



Figs. 3 and 4. A melanophore of wild type fish in culture strained with monoclonal anti beta-tubulin antibody (3) and anti-vimentin antibody (4). Melanosomes dispersed in cytoplasm have strong quenching effect.

Fig. 5. A portion of amelanotic melanophore in a scale of adult orange-red (*bIR*) mutant. Almost all melanosomes are partially melanized. Bar: 0.5 μm.

pterinosomes. The ultrastructure of the carotenoid vesicles is somewhat different in specimens fixed by conventional methods from that obtained after fixation by rapid-freezing. In the conventionally fixed specimens, carotenoid vesicles are moderately electron-dense and appear to be independently separate particles while those observed in the quick frozen preparations are electron-lucent and frequently found in association with endoplasmic reticulum (Fig. 6). In both cases, the vesicles measure from 50 to 80 nm, and the vesicles lack typical membranous envelopes. In physiological responses, carotenoid vesicles or vesicles associated with endoplasmic reticulum aggregate or disperse within the cytoplasm, while the movement of another pigmentary organelle, pterinosome, is much slower and inconspicuous. The pterinosome is a large (about 1 μm) spherical body with a concentric lamellar inner structure, and distributes near the periphery of the cell or in the dendritic processes (Fig. 1). The development of this organelle was described by Kamei-Takeuchi and Hama (1971) and recently by Obika (1993). The microtubule system is well developed and vimentin filaments co-localize with microtubules (Obika and Fukuzawa, 1993b).

Having alpha type adrenoceptors and being innervated by the same nerve fibers as melanophores, xanthophores respond in the same way as melanophores (Iwata *et al.*, 1981), but synaptic contacts are rather infrequent on xanthophores as judged from immunofluorescence and ultrastructural observations.

Leucophore

In the wild type fish the population of leucophores is less than that of the other two types of light-absorbing chromatophores, and the cells generally sit underneath the melanophores, constructing a melanophore-leucophore complex in the dorsal integument. Characteristic organelles found in the cytoplasm are membrane-limited, roughly spherical vesicles of about 0.5 μm diameter for which the term leucosome is proposed (Fujii, 1993). To date, we do not have any reliable evidence to identify the chemical nature of the putative pigment within this organelle which probably reflects light under incident illumination. Working on larval chromatophores, Hama and his collaborators came to the conclusion that red-colored pigment cells of the larvae containing pteridine pigments are leucophore precursors, which later transform into light-reflecting leucophores that contain uric acid (Kamei-Takeuchi *et al.*, 1968; Hama, 1970; Hama, 1975). This raises a possibility that the medaka leucophore is a cell of xanthophore-erythrophore lineage. This problem, however, remains as yet unsolved. In adult wild

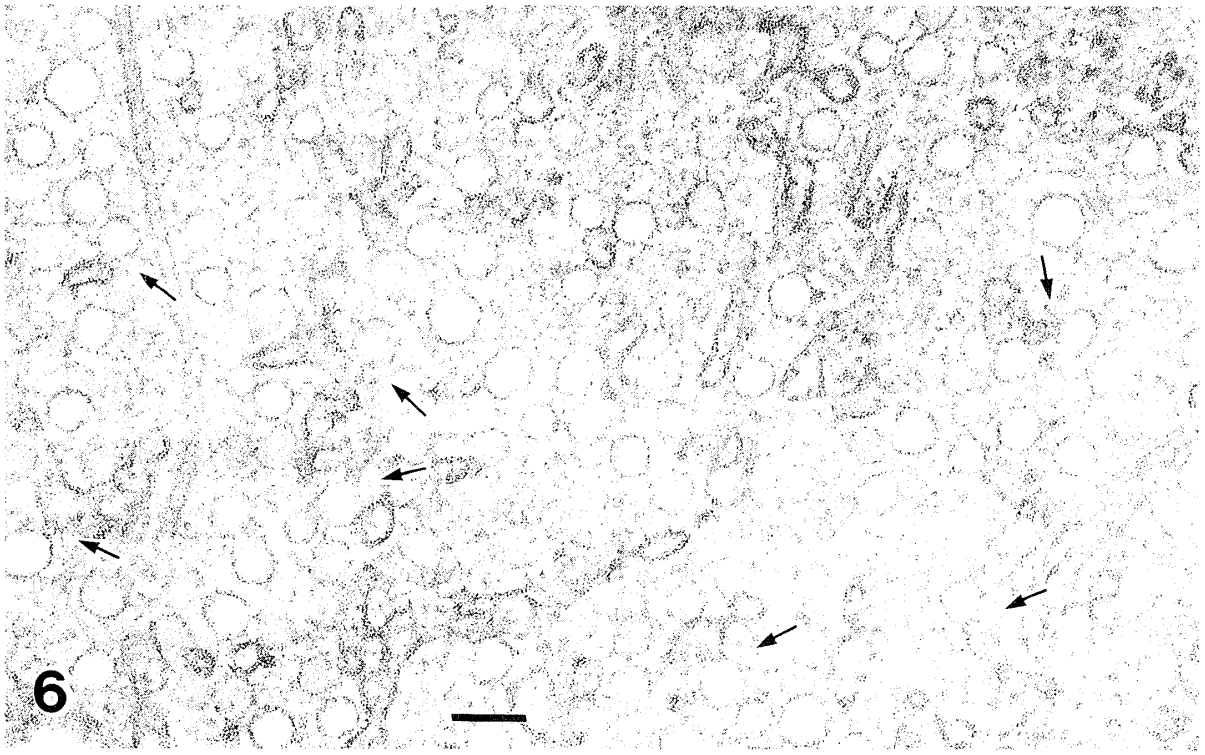
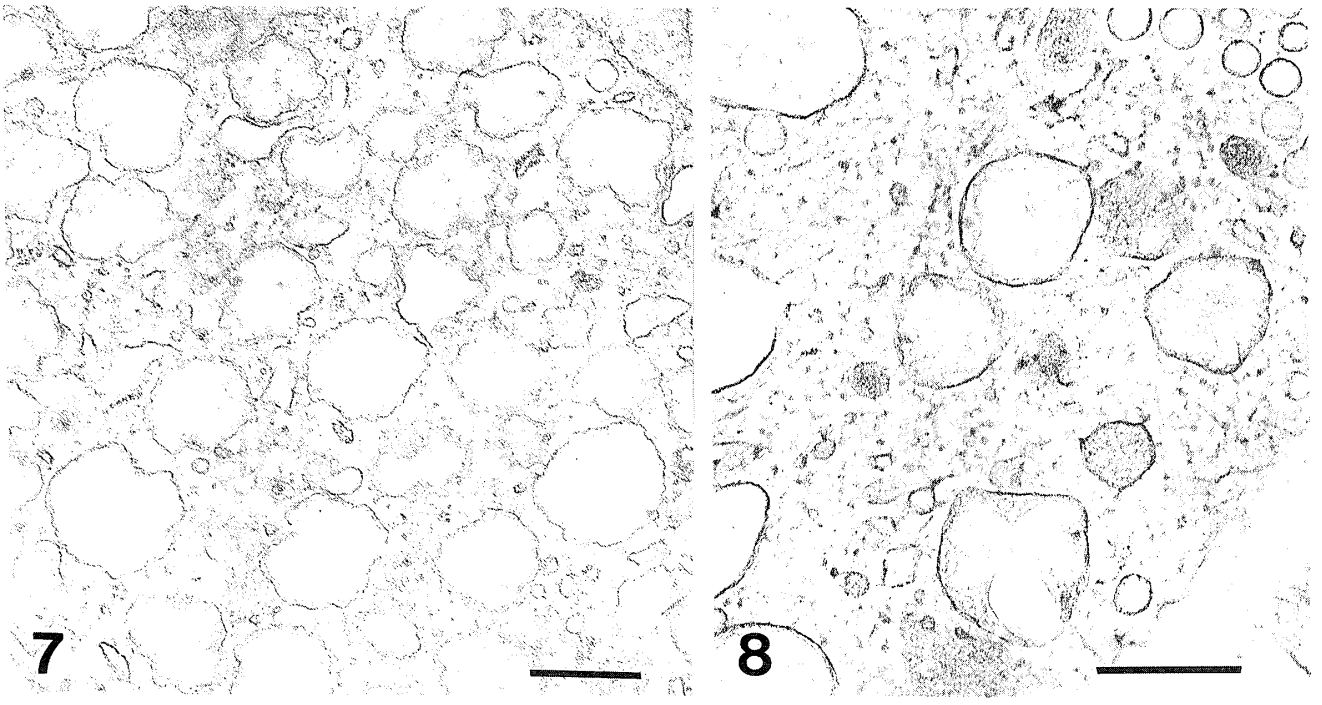


Fig. 6. Carotenoid vesicles in a xanthophore of adult wild type fish. Some of the vesicles (arrows) are closely associated with endoplasmic reticulum. Rapid-frozen preparation. Bar: 0.1 μm.



Figs. 7 and 8. Profiles of leucosomes in conventionally fixed (7) and rapid-frozen (8) preparations of wild type fish. Bar: 0.5 μm

type fish, a large number of leucophores are induced *de novo* by adaptation to prolonged light background (Sugimoto, 1993). So far we have not been able to show the appearance of red-colored pigment cells or the marked increase in pteridine content during the period of leucophore induction (unpublished observation). Nevertheless, from its physiological and morphological traits, the leucophore seems more like a xanthophore than an iridophore. Light reflection by leucophores gradually diminished when the cells are transferred to aqueous fixatives. In sections of conventionally fixed specimens, leucosomes appear to contain only a small amount of amorphous inclusions or look almost empty (Kamei-Takeuchi *et al.*, 1968; Obika, 1988). Hollows, which generally occur in thin sections of the material containing purine crystals such as iridophores (Kawaguti and Takeuchi, 1968), are more frequently encountered in rapidly frozen specimens (Figs. 7, 8). The microtubule system is the least developed among the three motile dermal chromatophores, but numerous microtubules are still found in dendritic processes. As predicted by physiological observations (Iwata *et al.*, 1981), electron microscopy verified that leucophores and melanophores are innervated by the same nerve fibers (Obika, 1988). Since leucophores have beta adrenoceptors, adrenergic drugs induce leucophores to disperse while melanophores and xanthophores respond with pigment aggregation (Obika, 1976b; Iga, 1977; Yamada, 1980).

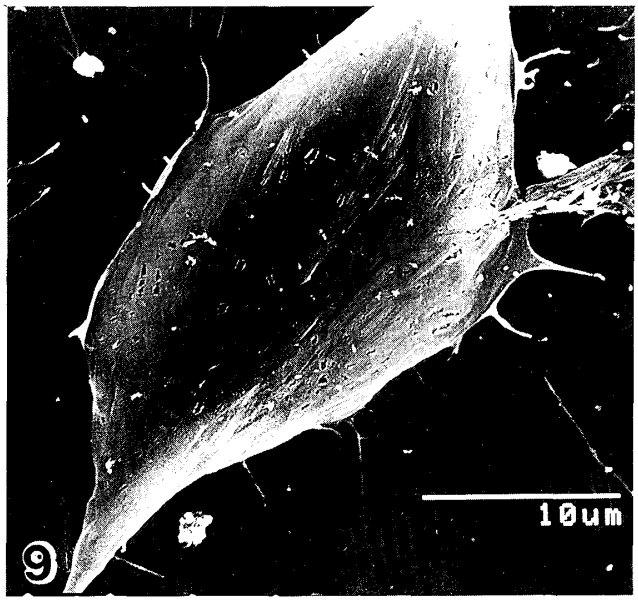
Iridophore

In the adult wild type fish, iridophores are largely localized in the lateral to ventral side of the body, and are most abundant on the peritoneum. Fig. 9 shows a peritoneal iridophore in culture in which purine crystals (reflecting platelets) are stacked parallel to the long axis of the bipolar cell. Because of the extremely low solubility of the crystals in water, reflecting platelets are easily isolated by centrifugation of lysed cell suspension. Isolated reflecting platelets are very thin, elongated hexagonal crystals sometimes more than 10 μm in length (Fig. 10). Absorption spectra of the material prepared from peritoneal iridophores coincide perfectly with those of authentic guanine (Obika and Fukuzawa, 1993a). The microtubules system in iridophores is very poor, and the reflecting platelets are probably immotile.

Perspectives

So far, pigment cell biologists have learned a lot from this tiny fish, but there are many things which are still unknown. The following are only few examples that should be considered hereafter.

To understand the chromatophore lineage tree, we have to find key enzymes or lineage-specific molecules involved in the process of cell differentiation. This line of research has started only recently in the mammalian melanocytes (Adema *et al.*, 1994) but at this moment, we do not have a reliable method to distinguish the four types of chromatoblasts in the early phase of teleost development.



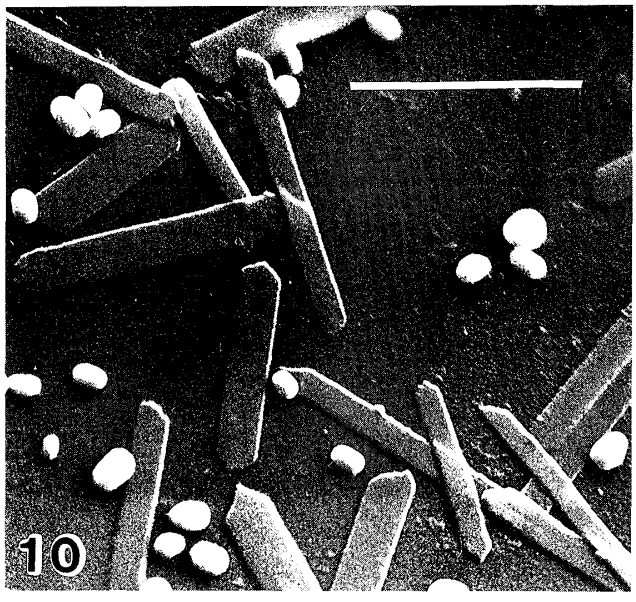


Fig. 9. Scanning electron micrograph of an isolated peritoneal iridophore of wild type fish.

Fig. 10. Scanning electron micrograph of isolated reflecting platelets. Small particles are melanosomes. Bar: 5 μm.

Regarding the motility of chromatophores, we know that the rapid pigment translocation is dependent on microtubules, probably in the same way as in other cell types where dyneins transport cargoes toward the minus end of microtubule while kinesins do the job in the opposite direction (Endow and Titus, 1992). The involvement of dynein in pigment aggregation (Ogawa *et al.*, 1987) and of kinesin in pigment dispersion (Rodionov *et al.*, 1991) has been implicated in fish chromatophores. Very recently, Nilsson *et al.* (1996) studied the localization of kinesin and dynein in cultured melanophores of the Atlantic cod by immunofluorescence microscopy. The result of this experiment strongly suggests that the two motor molecules are directly bound to pigment granules. An attempt to localize kinesins on the melanosomes of retinal pigment epithelium of a teleost turned out to be negative (King-Smith *et al.*, 1995).

Furthermore, it may be the right time to go one step further, beyond the classical cytochemistry and morphology, in order to inaugurate a new era of pigment cell biology in the quest for the answers to very basic, yet ultimate questions, such as, "Why should fish accumulate a large quantity of guanine in the integument?"

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