Title	神経管培養系により示された、アフリカツメガエル色素突然変異（periodic albino）虹色素胞と黄色素胞における色素顆粒形成異常
Sub Title	Abnormal pigment organellogenesis in iridophores and xanthophores of the periodic albino mutant of xenopus laevis as shown in the neural tube culture system
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Abstract	Periodic albino mutant (ap/ap) of Xenopus laevis is a pigmentationmutation affecting pigment cells in the skin and pigment epithelium of the eye.It has been described that pigment epithelium, melanophores and iridophoresare abnormal, and that unusual leucophore-like cells develop as a part ofmelanophore lineage in this mutant. In the present study, differentiation ofiridophores and xanthophores was investigated using the neural tube culturesystem in various culture conditions, and the ultrastructures of differentiatedpigment cells were compared between the wild-type and the mutant. It is shownthat iridophores and xanthophores require different culture conditions fordifferentiation. In the periodic albino mutant, reflecting platelets of iridophoresare abnormal in size and shape. Pterinosomes are also abnormal in mutantxanthophores, and the pterinosome size is extremely large in the mutant. Thisis the first demonstration that detrimental effects on pigment organellogenesisare found in xanthophores, in addition to melanophores, iridophores, pigmentepithelium, and leucophore-like cells in the periodic albino. These findingsstrongly suggest that pigment organellogenesis is affected in all types of pigmentcells including unusual leucophore-like cells in the periodic albino. This mutantwould provide some insight for organellogenesis and development of variouspigment cells.
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Abnormal Pigment Organellogenesis in Iridophores and Xanthophores of the Periodic Albino Mutant of *Xenopus Laevis* as Shown in the Neural Tube Culture System

Toshihiko Fukuzawa

Summary —— Periodic albino mutant (a^p/a^p) of *Xenopus laevis* is a pigmentation mutation affecting pigment cells in the skin and pigment epithelium of the eye. It has been described that pigment epithelium, melanophores and iridophores are abnormal, and that unusual leucophore-like cells develop as a part of melanophore lineage in this mutant. In the present study, differentiation of iridophores and xanthophores was investigated using the neural tube culture system in various culture conditions, and the ultrastructures of differentiated pigment cells were compared between the wild-type and the mutant. It is shown that iridophores and xanthophores require different culture conditions for differentiation. In the periodic albino mutant, reflecting platelets of iridophores are abnormal in size and shape. Pterinosomes are also abnormal in mutant xanthophores, and the pterinosome size is extremely large in the mutant. This is the first demonstration that detrimental effects on pigment organellogenesis are found in xanthophores, in addition to melanophores, iridophores, pigment epithelium, and leucophore-like cells in the periodic albino. These findings strongly suggest that pigment organellogenesis is affected in all types of pigment cells including unusual leucophore-like cells in the periodic albino. This mutant would provide some insight for organellogenesis and development of various pigment cells.

Key words: Periodic albino, Xenopus laevis, Xanthophore, Iridophore, Pterinosome, Reflecting platelet

Introduction

A wide variety of pigment cells are known in poikilotherms. They are melanophores (brown or black due to melanosomes), iridophores (silver or gold due to reflecting platelets), leucophores (white due to leucosomes), xanthophores (yellow to orange

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due to pterinosomes and/or carotenoid vesicles), erythrophores (orange to red due to pterinosomes and/or carotenoid vesicles), and cyanophores (blue due to cyanosomes) (Bagnara, 1998; Bagnara and Hadley, 1973). Mutations on various types of pigment cells have been reported in poikilotherms (Armstrong, 1985; Browder, 1968; Frost et al., 1984; Kelsh et al., 1996; Krotoski et al., 1985; Lamoreux et al., 2005; Nishioka and Ueda, 1985b,c). However, the mechanisms controlling pigment synthesis, organelle biogenesis, and cell differentiation of these nonmammalian pigment cells are largely unknown, although genes regulating a variety of pigment cells have been recently analyzed in zebrafish and medaka (Fukamachi et al., 2006; Kelsh, 2004; Lister et al., 2006; Parichy et al., 2000a,b; Quigley et al., 2005). On the other hand, a great number of genes regulating melanin synthesis, melanosome biogenesis, melanocyte development and differentiation have been studied and identified using coat color mutations in mammals (Bennett and Lamoreux, 2003; Setaluri, 2003; Wei, 2006). Further research on mutations of non-melanophore pigment cells will provide clues to answer how a variety of pigment cells are formed and regulated.

Periodic albino of *Xenopus laevis* is an interesting mutant on pigmentation and pigment cell differentiation (Fukuzawa, 2004; Fukuzawa and Ide, 1986, 1987; Hoperskaya, 1975, 1981; MacMillan, 1979, 1981). This mutant (a^p/a^p), inherited as a typical recessive trait, was first characterized by the absence of melanin in oocytes, the appearance of melanin in the pigment epithelium of the eye and in skin melanophores at larval stages, and the almost complete disappearance of melanin in metamorphosed animals (Hoperskaya, 1975). Mutant melanophores contain many abnormal melanosomes with granular internal structures (Fukuzawa and Ide, 1986, Hoperskaya, 1981; Seldenrijk et al., 1982). The culture of mutant melanophores made previously for clarification of the mechanism of depigmentation during larval development has revealed that mutant melanophores depigmented gradually during proliferation *in vitro*, and that the depigmented cells were filled with premelanosomes with typical lamellar structures instead of mature melanosomes (Fukuzawa and Ide, 1986). It has been shown that not only melanophores but also iridophores are affected in the periodic albino (MacMillan, 1979; MacMillan and Gordon, 1981). However, development of xanthophores and their ultrastructure have not been yet investigated in the periodic albino mutant. Recently, unusual leucophore-like cells have been reported to appear specifically in the lineage of melanophores in this mutant (Fukuzawa, 2004). Thus far, little is known about the function of the a^p gene.

In the present experiment, differentiation of iridophores and xanthophores was thoroughly examined using the neural tube culture system in various culture conditions. Differentiated iridophores and xanthophores *in vitro* were examined by electron

microscopy, and their ultrastructures were compared between the wild-type (+/+) and the mutant (a^p/a^p). It was demonstrated that iridophores and xanthophores, which require different conditions for differentiation, are abnormal in the periodic albino mutant. Reflecting platelets of mutant iridophores are abnormal in size and shape. Pterinosomes in mutant xanthophores are much larger than those in the wild-type. The present results, together with previous reports, show that all types of pigment cells are abnormal including unusual leucophore-like cells in the periodic albino mutant of *Xenopus laevis*. Pigment organellogenesis and development of various pigment cells are discussed in the present paper.

Materials and Methods

The wild-type (+/+) and periodic albino mutant (a^p/a^p) of *Xenopus laevis* were used in the present experiments. *Xenopus* eggs were obtained by gonadotropin stimulation, and developmental stages were determined according to Nieuwkoop and Faber (1967).

Isolation and Culture of Neural Tubes

Neural tubes of *Xenopus* embryos (stage 22) were used as the source of neural crest cells as described previously (Fukuzawa and Ide, 1988; Fukuzawa and Bagnara, 1989). The epidermis, somites, and notochord were removed from *Xenopus* embryos after 0.1 % collagenase treatment for 30 min. The neural tube was then cut with a tungsten needle at the level of the seventh somite. The caudal half of the neural tube was cultured at 25 ℃ in a sitting drop of 70 µl of culture medium on a tissue culture dish (Falcon 3001; Becton Dickinson, Franklin Lakes, NJ, USA). After 2 days, 2 ml of the medium was added to the culture. Subsequently, the medium was changed every 5 days.

Culture Medium

To culture isolated neural tubes, L-15 medium was basically used, which consisted of 5 parts of Leibovitz's L-15 (Gibco, Grand Island, NY, USA), 3 parts of Mili-Q ultrapure water (Millipore, Tokyo, Japan), and 2 parts of fetal bovine serum (Gibco, Grand Island, NY, USA). In some experiments, L-15 medium conditioned with embryonic *Xenopus* cells was used. To prepare conditioned medium (CM), whole neural tubes from *Xenopus* embryos (a^p/a^p, stage 22) were cultured in the method described above until the cells became confluent. Then waste medium was collected and used as CM. For this purpose, the medium was changed every 2 days.

Examination on Iridophore Differentiation

Wild-type or mutant neural tubes were isolated and cultured using L-15 medium with or without alpha-melanocyte stimulating hormone (α-MSH; Sigma, St. Louis, MO, USA) (1 µg/ml). In some experiments, attached neural tubes were removed after 3 days in culture to examine the effect of the presence of a neural tube on iridophore differentiation. Newly differentiated iridophores *in vitro* were identified by reflecting light, and were examined by electron microscopy. Iridophore differentiation *in vitro* was compared between the wild-type and the mutant.

Examination on Xanthophore Differentiation

Wild-type or mutant neural tubes were isolated and cultured using either L-15 medium or CM. For each medium, plain tissue culture dishes (Falcon 3001) or those coated with fibronectin (Sigma, St. Louis, MO, USA) (50 µg/ml) were utilized. In some experiments, attached neural tubes were removed after 3 days in culture to examine the effect of neural tube presence on xanthophore differentiation. Newly differentiated xanthophores were identified by their yellow color under transmitted light, and were investigated electron microscopically. Xanthophore differentiation *in vitro* was compared between the wild-type and the mutant.

Electron Microscopy

Iridophores and xanthophores in culture were fixed in 2.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 60 min at 4 ℃ , post-fixed in 2 % O_SO_4 in the same buffer for 60 min at 4℃ , dehydrated through a graded series of ethanol and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed on a JEOL JEM-1010 electron microscopy.

Results

Iridophores Differentiate Specifically on Neural Tube Explants.

Neural tubes attach to the culture dishes within 10 hr, and neural crest cells begin to migrate from them. Melanophores differentiate from migrated neural crest cells after 1 day (+/+) or 2 days (a^p/a^p) in culture as described previously (Fukuzawa, 2004). Melanophores also appear on the neural tube explants. It is after 7 days in culture that iridophores appear on the neural tube explants (Fig. 1). Wild-type and mutant iridophores specifically differentiate on neural tube explants (Figs. 1A and D), however, iridophores do not appear among neural crest cells migrating from neural tube explants.

Iridophore differentiation did not occur among migrated neural crest cells when neural tube explants were removed after 3 days in culture (Fig. 2). Iridophores proliferated over months in the cell sheet derived from neural tube explants (Figs. 1B and E). When alpha-melanocyte stimulating hormone (α-MSH) (1 µg/ml) was added to the culture medium, the appearance of iridophores on neural tube explants was delayed extremely, however, iridophore differentiation was not inhibited completely in the presence of attached neural tubes in both the wild-type and the mutant (Fig. 2). The color, morphology, and ultrastructural characteristics of wild-type and mutant iridophores which differentiated on neural tube explants in the presence of α-MSH did not differ from those of wild-type and mutant iridophores which differentiated in the absence of α-MSH, respectively. On the other hand, α-MSH promoted melanophore differentiation and proliferation in the present culture as described previously (Fukuzawa and Ide, 1983; Fukuzawa and Bagnara, 1989). Xanthophores never appeared in the neural tube culture system using L-15 medium, regardless of the presence or absence of α-MSH.

Reflecting Platelets of Iridophores Are Abnormal in the Periodic Albino Mutant.

When the color of the differentiated iridophore was compared between the wild-type and the mutant by reflecting light, a clear difference was observed between the two. The color of the wild-type iridophore is iridescent gold (Fig. 1B), while that of the mutant iridophore is silver or whitish-grey (Fig. 1E). This difference can be attributed to the reflecting platelets contained in iridophores. In the wild-type, iridophores are filled with typical reflecting platelets in iridophores (Fig. 3A). However, reflecting platelets of mutant iridophores are abnormal in size and shape (Fig. 3B). The ultrastructures of reflecting platelets of wild-type and mutant iridophores which differentiated on neural tube explants in the presence α-MSH (Figs. 3A and B) did not differ from those which differentiated in the absence of α-MSH.

Xanthophores Differentiate in Migrated Neural Crest Cells Especially in Medium Conditioned with *Xenopus* Cells.

Neural tubes attach to the culture dishes, and neural crest cells migrate from them in L-15 medium or in conditioned medium (CM). In fibronectin coated dishes, neural crest cells extend dendrites, and migrate much further than those in non-coated dishes. Xanthophores differentiate specifically in CM, but not in L-15 medium (Table 1). It does not affect xanthophore differentiation whether fibronectin is coated or not in the culture dishes (Table. 1). Likewise, it does not influence xanthophore differentiation whether attached neural tube explants are removed after 3 days in culture or not (Table 1).

Table 1 Effect of the medium, neural tube, and fibronectin on xanthophore differentiation.

Culture Conditions			Xanthophore Differentiation*	
Medium	Neural tube	Fibronectin	+/+	a^p/a^p
L-15 medium	not removed	coated	0/4 (0 %)	0/4 (0 %)
	not removed	not coated	0/4 (0 %)	0/4 (0 %)
	removed	coated	0/4 (0 %)	0/4 (0 %)
	removed	not coated	0/4 (0 %)	0/4 (0 %)
Conditioned medium (CM)	not removed	coated	4/4 (100 %)	4/4 (100 %)
	not removed	not coated	4/4 (100 %)	4/4 (100 %)
	removed	coated	4/4 (100 %)	4/4 (100 %)
	removed	not coated	4/4 (100 %)	4/4 (100 %)

*the number of the explants in which xanthophores differentiated per total number of neural tube explants

Indeed, xanthophore differentiation occurs among migrated neural crest cells in CM even if neural tube explants are removed. Wild-type xanthophores differentiate after 5 days in culture, while mutant xanthophores appear after 7 days in culture. Xanthophores usually appear in the peripheral region of migrated neural crest cells, and proliferate in CM (Figs. 1C and F). In contrast with xanthophores, iridophore differentiation did not occur among migrated neural crest cells in CM when neural tube explants are removed after 3 days in culture.

Pterinosomes of Xanthophores Are Abnormal in the Periodic Albino Mutant.

Both wild-type and mutant xanthophores appear yellow, however, the yellow color is much lighter in the former (Figs. 1C and F). Pigment granules in mutant xanthophores are large enough to see under an optical microscope (Fig. 1F). Electron microscopic observation reveals that pterinosomes of mutant xanthophores (Figs. 4B and C) are extremely large when compared to those of wild-type xanthophores (Fig. 4A). Indeed, giant pterinosomes are found in mutant xanthophores (Fig. 4C). Amorphous materials or concentric lamellar structures are found in pterinosomes. The short and long diameters of pterinosomes in xanthophores were measured to compare the pterinosome size between the wild-type and the mutant. The data on size distribution of the pterinosomes are shown in Fig. 5, and the statistics are summarized in Table 2. It is demonstrated that wild-type xanthophores are filled with pterinosomes of similar size, while the size of the pterinosome varies greatly within mutant xanthophores (Figs. 4 and 5). Statistical analysis demonstrates that a^p/a^p pterinosomes are much larger than +/+ pterinosomes (Table 2). The difference of the size between a^p/a^p pterinosomes and +/+ pterinosomes is

Table 2 Comparison of the pterinosome size between the wild-type and the mutant.

Size of the pterinosome	+/+ pterinosomes (n=500)	a^p/a^p pterinosomes (n=491)
Mean size of the short diameter examined by electron microscopy	0.52 μm	0.93 μm (P<0.001)*
Mean size of the long diameter examined by electron microscopy	0.67 μm	1.26 μm (P<0.001)*

*statistically significant (t-test)

statistically significant ($P < 0.001$) (Table 2). The largest pterinosome found in the mutant xanthophore was 3.7 μm in short diameter and 5.7 μm in long diameter.

Discussion

Iridophores and Xanthophores Require Different Culture Conditions for Differentiation.

Among the three types of pigment cells (melanophores, xanthophores, and iridophores), melanophores differentiate first in *Xenopus* embryos (Hoperskaya, 1975). Xanthophores and iridophores appear later in *Xenopus* development (MacMillan, 1979; Yasutomi and Hama, 1972). Contrary to melanophores which differentiate from neural crest cells even in a salt solution (Fukuzawa and Ide, 1988), xanthophores and iridophores do not appear in such a simple culture condition in *Xenopus laevis*. Akira and Ide (1987) performed clonal culture of neural crest cells and cultured xanthophores and iridophores in *Xenopus laevis*, however, culture conditions required for development of specific pigment cells were not assessed. So far, little is known about factors required for differentiation of xanthophores or iridophores in *Xenopus laevis*, although *Mitf* has been recently suggested to regulate melanoblast and retinal pigment epithelium development (Kumasaka et al., 2005). On the other hand, we have previously demonstrated that xanthophores differ from melanophores and iridophores in the expression of cell adhesion molecules in *Xenopus laevis* (Fukuzawa and Obika, 1995).

Unusual leucophore-like cells have been recently reported to appear in the lineage of melanophores in the periodic albino mutant of *Xenopus laevis* (Fukuzawa, 2004). Although various culture conditions were tested for pigment cell differentiation in the present experiment, leucophore-like cells did not appear in culture. It is possible that leucophore-like cells specifically appear in the tissue environment of the periodic albino mutant.

The present study demonstrates that iridophores and xanthophores require different culture conditions for differentiation. In both the wild-type and the mutant, iridophore

differentiation occurs on neural tubes in L-15 medium, while xanthophores do not appear in the same culture condition using L-15 medium. Based upon the fact that iridophores do not appear among migrated neural crest cells when neural tube explants are removed (Fig. 2), contact between neural crest cells and neural tubes may be needed for iridophore differentiation and/or proliferation. Although neural tubes have been reported to influence neural crest differentiation, little is known about factor(s) affecting pigment cell differentiation (Derby and Newgreen, 1982; Glimelius and Weston, 1981; Norr, 1973; Perris and Lofberg, 1986).

In the present experiments, iridophore differentiation was extremely delayed in the presence of α-MSH. However, differentiation of iridophores on neural tube explants was not inhibited completely as long as attached neural tube explants remained in the culture. It is possible that the effects of neural tubes on iridophore precursor cells and/or iridophores may override those of MSH, which is known to stimulate melanophores but have inhibitory effects on iridophores (Bagnara, 1958, 1998; Taylor, 1969).

Contrary to iridophores, xanthophores appear among migrated neural crest cells in CM, even if neural tube explants are removed. Because xanthophores do not differentiate in L-15 medium, factor(s) in *Xenopus* CM may be required for xanthophore differentiation and/or proliferation. So far, proteins with high molecular weight were specifically detected in *Xenopus* CM (data not shown). Further study is going on to investigate factor(s) to stimulate differentiation and/or proliferation of xanthophores.

In zebrafish, mutations affecting xanthophore pigmentation have been described (Odenthal et al., 1996). It was reported that two genes, *fms* and *foxd3*, are required for development of xanthophore precursors and iridophore precursors, respectively in zebrafish (Parichy et al., 2000b; Lister et al., 2006). On the other hand, somatolactin has been recently suggested to play a role in chromatophore development in medaka (Fukamachi et al., 2004, 2006). However, it is not known whether somatolactin affects xanthophore differentiation and proliferation in other species.

Pigment Organellogenesis Is Abnormal in All Types of Pigment Cells in the Periodic Albino Mutant.

It has been already reported that melanosomes are abnormal in melanophores of the periodic albino mutant (Fukuzawa and Ide, 1986, Hoperskaya, 1981; Seldenrijk et al., 1982). In the present experiment where iridophore differentiation was studied *in vitro*, it was shown that the size and shape of reflecting platelets are irregular in the periodic albino mutant (Fig. 3). This observation is consistent with the previous reports on mutant iridophores (MacMillan and Gordon, 1981). Although reflecting platelets in iridophores

have been reported to become thinner in frogs receiving intermedin injections (Taylor, 1969), ultrastructures of reflecting platelets of *Xenopus* iridophores were not affected by α-MSH in the present experiment in both the wild-type and the mutant. The effects of neural tubes on iridophores may override those of MSH in the present culture condition as discussed above.

Leucophore-like cells in the periodic albino mutant have been reported to contain peculiar reflecting platelet-like organelles (Fukuzawa, 2004). Pigment organelles found in leucophore-like cells are also irregular both in size and shape. Although pigment organelles in leucophore-like cells resemble those of mutant iridophores, leucophore-like cells differ from iridophores in manner of response to α-MSH (Fukuzawa, 2004).

This is the first report to show that pterinosomes are also abnormal in xanthophores in the periodic albino mutant. Pterinosomes in mutant xanthophores are much larger than those in wild-type xanthophores (Fig. 4 and Table 2). While pterinosomes are almost similar in size in wild-type xanthophores, the size of the pterinosome varies greatly in mutant xanthophores (Fig. 5). Since Matsumoto (1965) discovered pterinosomes in swordtail erythrophores, ultrastructures of pterinosomes have been studied in many species (Bagnara et al., 1979b; Ichikawa et al., 1998; Obika, 1993; Yasutomi and Hama, 1971, 1976) including *Xenopus laevis* (Yasutomi and Hama, 1972). However, there is no literature describing such giant pterinosomes that are found in xanthophores of the periodic albino (Fig. 4), except for abnormal pterinosomes described in the *yellow* mutation of *Rana rugosa* (Ichikawa et al., 2001).

Many genes controlling the pathway of melanosome biogenesis have been identified in mammals (Setaluri, 2003; Wei, 2006), however, the mechanism of organellogenesis in non-melanophore pigment cells is largely unknown. Chediak-Higashi syndrome (CHS) gene in humans and *beige* gene in mice have been described to cause giant melanosomes in melanocytes (Spritz, 1998; Ward et al., 2000). It is not known whether the appearance of giant pterinosomes in xanthophores of the periodic albino relates to the defect in secretory lysosome function regulated by CHS/*beige* gene.

Periodic Albino Gene Affects Organellogenesis and Development of Pigment Cells.

In the albinistic *Rana catesbeiana* and the certain albino stock of *Rana nigromaculata*, melanophores and iridophores have been reported to be abnormal, however, xanthophores are normal (Bowers and Carver, 1978; Nishioka and Ueda, 1985a). On the other hand, it was described that xanthophores and melanophores, but not iridophores, are affected in the *pale* mutation in *Bombina orientalis* (Frost et al., 1982). Recently, mutations affecting three types of pigment cells have been described in zebrafish (Kelsh

et al., 1996), medaka (Kelsh et al, 2004), *Xenopus laevis* (Droin, 1992), and *Rana rugosa* (Ichikawa et al., 2001). Although ultrastructures of pigmentary organelles have not been described in those pigmentation mutants in fish (Kelsh et al., 1996, 2004) and in *white lethal* of *Xenopus laevis* (Droin, 1992), electron microscopic observation has been made in the *yellow* mutation of *Rana rugosa* (Ichikawa et al., 2001). Interestingly, the pigment organelles in the three types of pigment cells are abnormal in the *yellow* mutation, and the ultrastructures of these abnormal pigment organelles resemble those in the periodic albino mutant. It is not known whether the *yellow* gene is related to the a^p gene in *Xenopus laevis*. Although three types of pigment cells (melanophores, iridophores, and xanthophores) are abnormal in both the *yellow* mutant of *Rana rugosa* and the periodic albino mutant of *Xenopus laevis*, the periodic albino mutant is unique in that unusual leucophore-like cells develop as a part of melanophore lineage (Fukuzawa, 2004). Therefore, it is possible that the a^p gene affects not only organellogenesis but also development of pigment cells.

The data obtained so far show that organellogenesis is abnormal in all kinds of pigment cells including unusual leucophore-like cells in the periodic albino mutant. This mutant is very interesting in the organelle biogenesis and pigment cell development, since various pigment cells have been thought to derive from a stem cell that contains a primordial organelle of endoplasmic reticular origin (Bagnara, 1987; Bagnara et al., 1979a). Analysis on the function of the a^p gene in *Xenopus laevis* would provide some insight for organellogenesis and development of a variety of pigment cells.

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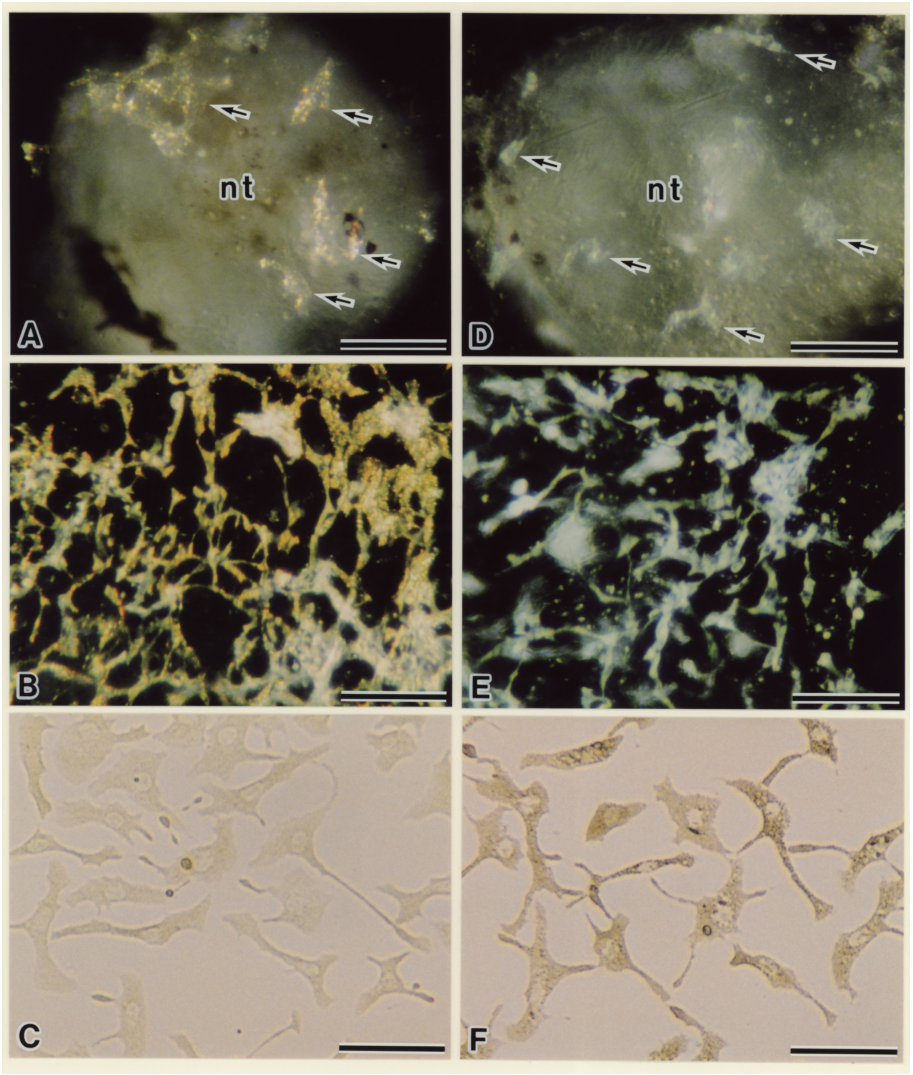

Fig. 1 Differentiation of iridophores and xanthophores in the wild-type (A–C) and the periodic albino mutant (D–F). Iridophores (A, B, D, and E; reflecting light) and xanthophores (C and F; transmitted light) which differentiated *in vitro* are shown. Iridophores appear on the neural tube explants after 7 days in culture (A and D, arrows), and then proliferate over months in the cell sheet derived from neural tubes (B and E; after 2 months in culture). Note that the color of the wild-type iridophore is iridescent gold while that of the mutant iridophore is silver to whitish-grey. In contrast, xanthophores usually appear in the peripheral region of migrated neural crest cells, and proliferate in culture (C and F; after 1 month in culture). Note that the yellow color is much lighter in wild-type xanthophores than in mutant xanthophores in which pigment granules are so large as to be visible with the optical microscope. nt: neural tube. Bar = 100 μm.

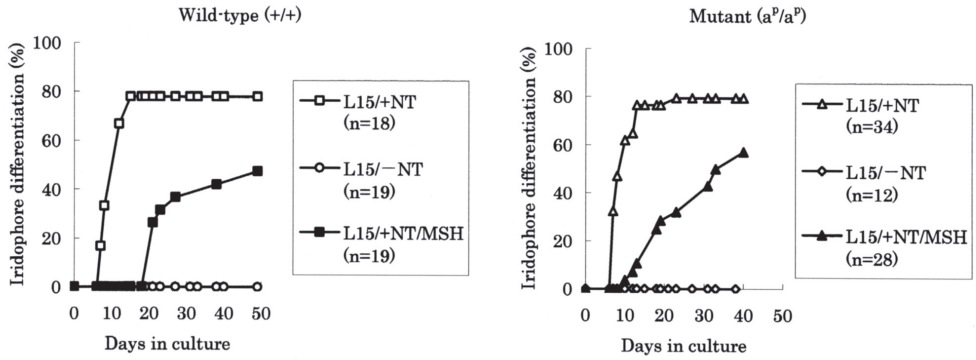

Fig. 2 Effect of neural tube and MSH on iridophore differentiation. Neural tubes were explanted and cultured in L-15 medium without removing attached neural tubes (L15/+NT). In some experiments, attached neural tubes were removed after 3 days in culture to see the effect of neural tube on iridophore differentiation (L15/−NT). To examine the effect of MSH, neural tube explants were cultured in the presence of α-MSH (1 μg/ml) without removing attached neural tubes (L15/+NT/MSH). The vertical axis of the graph represents the percentage of the explants in which iridophores differentiated among total neural tube explants. Wild-type and mutant iridophores differentiated on neural tube explants, however, they did not appear when attached neural tubes were removed in culture. Iridophore differentiation extremely delayed in the presence of α-MSH in both the wild-type and the mutant.

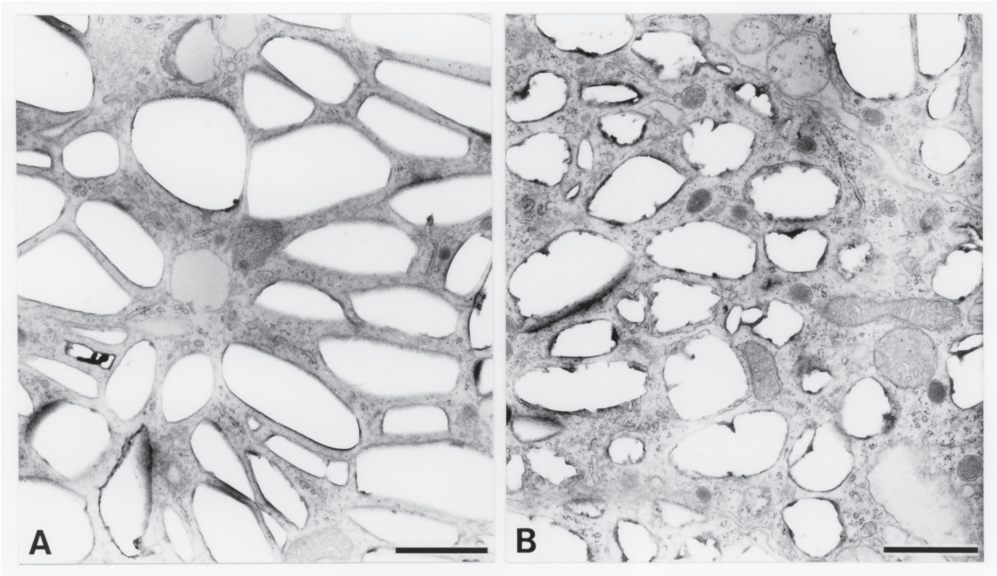

Fig. 3. Ultrastructures of iridophores after 2 months in culture. In the wild-type, iridophores are filled with typical reflecting platelets (A). In contrast, irregular and abnormal shaped reflecting platelets are present in mutant iridophores (B). Ultrastructures of wild-type and mutant iridophores which differentiated on neural tube explants in the presence of α-MSH (A and B) did not differ from those of wild-type and mutant iridophores which differentiated in the absence of α-MSH, respectively. Bar = 1 μm.

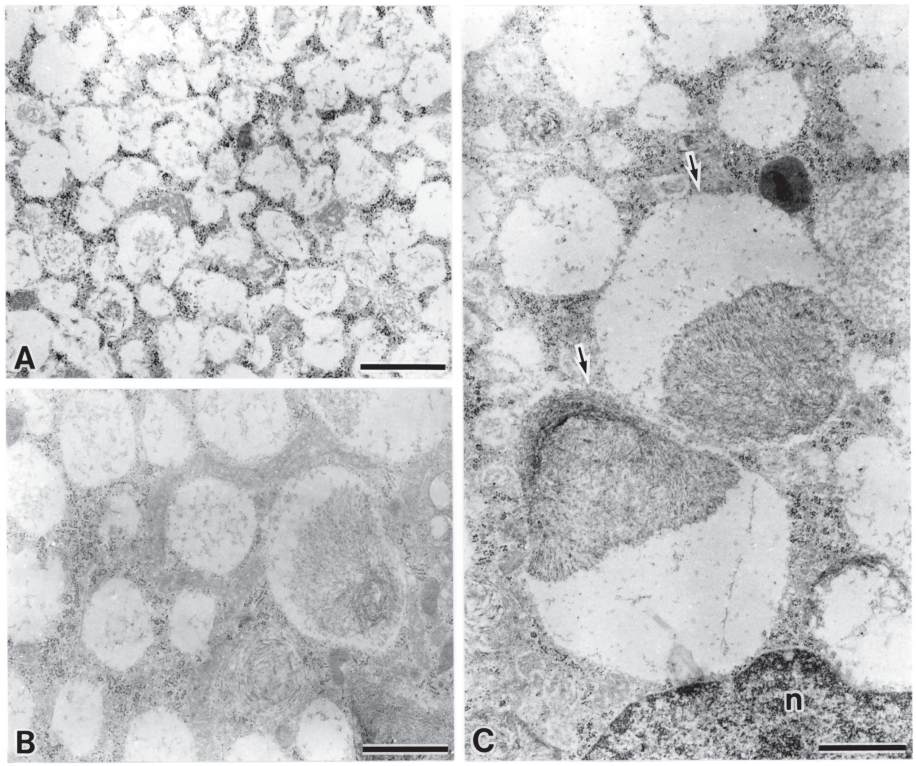

Fig. 4 Ultrastructures of xanthophores after 1 month in culture. Wild-type xanthophores contain typical pterinosomes, which are almost uniform in size (A). In contrast, mutant xanthophores are filled with large pterinosomes which vary in size (B and C). Note giant pterinosomes in mutant xanthophores (C, arrows). Amorphous materials or concentric lamellar structures are seen in the pterinosomes. n: nucleus. Bar = 1 μm.

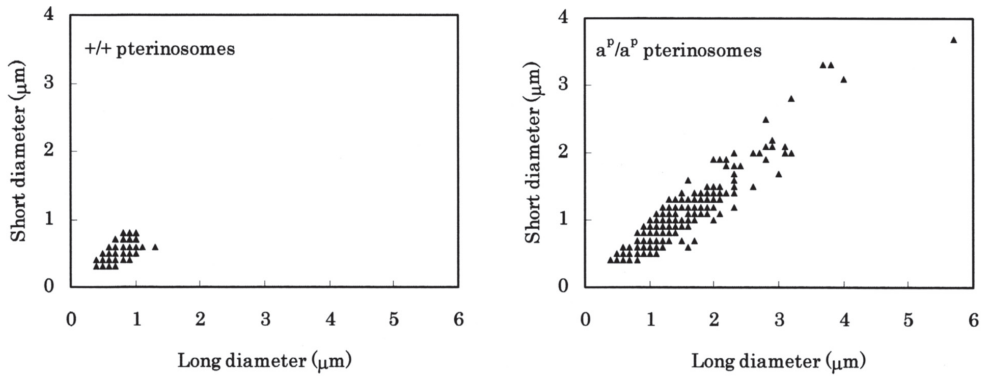

Fig. 5 Size distribution of the pterinosomes in wild-type and mutant xanthophores. By observing dozens of xanthophores electron microscopically, the short and long diameters of pterinosomes were measured in the wild-type (n = 500) and the mutant (n = 491). Pterinosomes in the mutant are much larger than those in the wild-type. The size of the pterinosome varies tremendously in mutant xanthophores, while pterinosomes are almost similar in size in wild-type xanthophores. The statistical data are shown in Table 2.