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Unusual light-reflecting pigment cells appear in the *Xenopus* neural tube culture system in the presence of guanosine

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ABSTRACT

Isolation and culture of *Xenopus laevis* neural tubes resulted in differentiation of melanophores and iridophores from neural crest cells; the differentiated melanophores and iridophores were then maintained in culture for more than 6 months. Guanosine has been reported to promote reflecting platelet formation in melanin-producing pigment cells; however, the process of pigment organellogenesis is still unclear. In the present study, unusual light-reflecting pigment cells were observed upon addition of guanosine to the neural tube culture system, which contained melanosomes specific to melanophores, and reflecting platelets specific to iridophores. Ultrastructural studies suggested that irregularly shaped reflecting platelets were formed from stage II melanosomes (the early stage of melanosome formation) in these unusual pigment cells.

1. Introduction

A wide variety of pigment cells exist in vertebrates, and are known to be derived from neural crest cells (Bagnara and Hadley, 1973). In poikilotherms, melanophores and iridophores are characterized by the presence of melanosomes and reflecting platelets, respectively (Bagnara and Hadley, 1973). Molecules and genes involved in melanosome biogenesis, and the process itself, have been intensively studied in mammalian melanocytes (Raposo and Marks, 2007; Schiaffino, 2010). In contrast, little is known about the mechanism controlling reflecting platelet formation in iridophores.

Xenopus laevis is useful to study the differentiation and organellogenesis of pigment cells, as three types of pigment cells (melanophores, iridophores, and xanthophores) are known to exist in *Xenopus*. Furthermore, the pigmentation mutant of *Xenopus laevis*: periodic albino, can be utilized to investigate the development of pigment cells, because unusual white pigment cells showing the characteristic features of both melanophores and iridophores arise from melanophore precursors, and reflecting platelets are formed from stage II melanosomes (Fukuzawa, 2010, 2015).

We have reported in previous studies, that melanophores and iridophores differentiated from neural crest cells in the neural tube culture system, wherein the processes of melanosome formation and reflecting platelet formation were investigated (Fukuzawa, 2010, 2015). Previous studies have shown that guanosine stimulates reflecting platelet formation in cultured bullfrog melanophores (Ide, 1979), and enhance iridophore development in axolotls (Frost et al., 1987). However, the process of reflecting platelet formation in the presence of guanosine is unclear.

In the present study, pigment organellogenesis in the presence of guanosine was investigated in the neural tube culture system using wild-type *Xenopus* embryos. The study revealed that unusual light-reflecting pigment cells, which showed characteristic features of both melanophores and iridophores, were observed in the presence of guanosine. It was suggested that irregularly shaped reflecting platelets were formed from stage II melanosomes in these unusual pigment cells. The organellogenesis in unusual light-reflecting pigment cells is described and discussed further.

2. Materials and methods

2.1. Experimental animals

Animal experiments were approved by The Keio University Institutional Animal Care and Use Committee (Permit Number: 16080). Wild-type *Xenopus laevis* were used in the present study. *Xenopus* eggs were obtained by gonadotropin stimulation, and the developmental stages were determined according to Nieuwkoop and Faber (1967).

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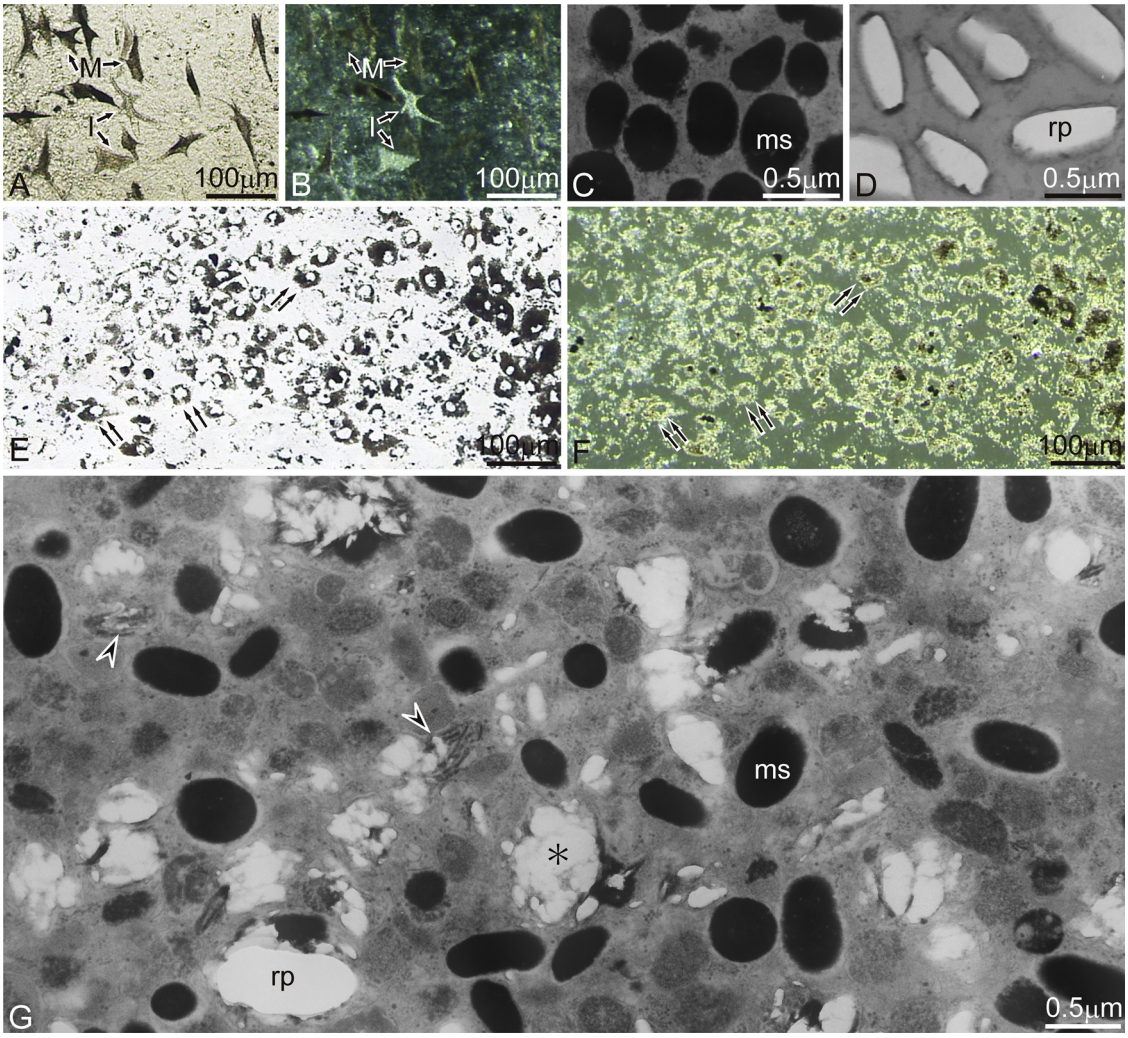

Fig. 1. Pigment cells in the absence (**A-D**) or presence (**E-G**) of guanosine after 1 month in culture. Melanophores (**A-B**, M) and iridophores (**A-B,** I) were observed under transmitted light (**A**) or incident light (**B**). Melanophores and iridophores were filled with melanosomes (**C,** ms) and reflecting platelets (**D,** rp), respectively. Unusual light-reflecting pigment cells observed under transmitted light (**E**) or incident light (**F**) indicated by double arrows. These unusual pigment cells contain both melanosomes (**G,** ms) and reflecting platelets (**G,** rp). Stage II melanosomes with "partial holes" (**G**, arrowheads), and peculiarly shaped reflecting platelets (**G,** asterisk) were observed in these cells.

2.2. Culture of neural tubes

Neural tubes of stage 22 embryos were used as the source of neural crest cells (Fukuzawa and Ide, 1988; Fukuzawa, 2004, 2006, 2010). For isolation and culture of neural tubes, we used the method described previously (Fukuzawa and Ide, 1988). Briefly, the epidermis, somites, and the notochord were removed from embryos after collagenase treatment, and the isolated neural tube was cultured in a sitting drop culture, using 70 μl of culture medium on a tissue culture dish (Falcon 3001; Becton Dickinson, Franklin Lakes, NJ, USA) at 25°C. On days 1 and 4 of culture, 1 ml of medium was added. Then, one half of the culture medium was changed every 4 days. The culture medium consisted of 5 parts of Leibovitz's L-15 (Gibco, Grand Island, NY, USA), 3 parts of Milli-Q ultrapure water (Millipore, Tokyo, Japan), and 2 parts of fetal bovine serum (Gibco) (Fukuzawa, 2004). To promote reflecting platelet formation, guanosine (0.2 mM) was added to the culture medium. The effect of α-melanocyte stimulating hormone (αMSH) (1μg/ml) was examined in pigment cells. In some experiments, pigment organelles were observed in pigment cells lysed after trypsin treatment.

2.3. Electron microscopy

Pigment organelles from melanophores, iridophores, and unusual light-reflecting pigment cells were examined using electron microscopy. We used the method described previously (Fukuzawa, 2015). Briefly, cultured cells were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), post-fixed in 2% O_sO_4, dehydrated using a graded series of ethanol, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed using the JEOL JEM-1010 electron microscope.

3. Results and discussion

When neural tubes were cultured in the absence of guanosine, melanophores and iridophores differentiated from neural crest cells after 1 day and 5 days, respectively. In the culture medium without guanosine, dendritic melanophores appeared black (Fig. 1A, B), and contained many mature melanosomes (Figs. 1C, 2 A). On the other hand, dendritic iridophores were identified by the presence of many reflecting platelets (Figs. 1D, 2B), which reflected light in culture (Fig. 1A, B). Mature reflecting platelets in iridophores were characterized by "empty holes" (Fig. 1D), because accumulated crystals from