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A comparative ultrastructural and physiological study on melanophores of wild-type and periodic albino mutants of *Xenopus laevis*

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Summary. Pigment of tail-fin melanophores in periodic albino *Xenopus laevis* tadpoles is dispersed in response to darkness and to α-MSH in a manner similar to wild-type melanophores. However, periodic albino tadpoles lack the response to different background conditions and the melatonin-induced aggregation in darkness. The tyrosinase activity in cells of the latter type tadpoles is weak compared to the wild-type cells. Ultrastructural examination of melanophores from periodic albino mutants and cells from wild-type tadpoles shows similar organelles at corresponding sites. A morphological difference can be observed in the fine structure of the melanosomes, which in albinos resembles an earlier stage of development. It is postulated that periodic albino *Xenopus laevis* possess the cellular mechanism to disperse pigment in the melanophores, but that under physiological conditions the release of α-MSH appears to be absent or scarce.

Key words: Melanophores – Periodic albinism – Ultrastructure – Physiology – *Xenopus laevis*, tadpoles

Albinism in poikilothermic animals has frequently been studied in an attempt to obtain more insight into the development and function of pigment cells. In true albinos, the pigment cells of the skin or the retinal epithelium never contain pigment granules (Bowers and Carver 1978). In periodic albino mutants of *Xenopus laevis*, however, periods of albinism alternate with periods of pigmentation. From stage 45/46 melanin appears in the pigmented eye epithelium and in the dermis of the skin of the tadpoles; in the skin the amount of melanin gradually increases up to

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approximately stage 56. According to Hoperskaya (1975) and Bluemink and Hoperskaya (1975), this mutation is to be attributed to a recessive gene that affects melanin synthesis.

To study the difference in structure and function of melanophores in periodic albino *Xenopus laevis* (stage 50–54) and wild-type tadpoles, the responses of the melanophores to different background conditions and to conditions of darkness and illumination were examined. In addition, attention was paid to the role of the pigment-dispersing agent α-melanotropin (α-MSH) and of the pigment-aggregating substance melatonin in pigment migration and to the activity of the enzyme tyrosinase (essential in the biosynthesis of melanin pigments). In addition, an ultrastructural comparison is made between ventral tail-fin melanophores of periodic albino tadpoles and the corresponding melanophores of wild-types.

Materials and methods

Experiments were carried out with the tadpoles of *Xenopus laevis* in stage 50–54 (according to the Normal Table of Nieuwkoop and Faber 1956). The methods for hatching and breeding the animals were described by Seldenrijk et al. (1979, 1980). The periodic albino mutants were generously provided by Dr. R. Verhoeff-de Fremery, "Hubrecht Laboratorium", International Embryological Institute, Utrecht, The Netherlands.

The capacity of the melanophores to disperse the melanosomes was investigated in vivo in tadpoles adapted to an illuminated black background and in vitro in isolated pieces of ventral tail-fin in darkness or in a α-MSH-containing assay medium in the light. For in vitro physiological experiments, pieces of about 2 × 2 mm were excised from the ventral tail-fin and equilibrated in assay medium (modified Leibovitz L-15) according to the method of de Graan and Eberle (1980). The aggregation of the melanosomes was studied in vivo in tadpoles adapted to a white background in light, and in vitro in pieces of tail-fin in a melatonin-containing assay medium under conditions of darkness and also under light conditions. The movement of melanosomes was first examined under low magnification; the tail-fin pieces were then processed for electron microscopy. In the radiometric assay, the tyrosinase activity was determined by measuring the radioactivity of tritiated water released during melanin synthesis from L-[3.5-^{3}H]-tyrosine (45 mCi/mmol, Radiochemical Center, Amersham).

For ultrastructural studies, the material was fixed (for 90 min) in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at room temperature after brief washing. Then, an equal volume of 2% osmium tetroxide in cacodylate was added to this buffer at 0° C. Postfixation was carried out after 30 min in 2% buffered osmium tetroxide at 0° C for another 45 min. Subsequently, the specimens were dehydrated in graded ethanol, placed in pure propylene oxide and embedded in low viscosity Spurr embedding medium (Polysciences, Inc, Warrington). Ultrathin sections were examined in a transmission electron microscope (Philips EM 300) at 60 kV after improving the contrast with uranyl acetate – and lead citrate solutions.

Results

Physiological properties of the melanophores

In tail-fin melanophores of wild-type *Xenopus laevis* examined in vivo, the pigment disperses in darkness and the melanosomes aggregate in bright light. In tadpoles adapted to a white or black background, the melanophores are in an aggregated or in a dispersed state, respectively. In isolated tail-fins, the wild-type melanophores are in an aggregated state under incident light, and the pigment disperses upon adaptation to dark surroundings or upon stimulation with α-MSH (10^{-10} M). Melatonin (10^{-8} M) induces aggregation in melanophores dispersed in darkness.

These observations are in agreement with previously published data (for review Burgers and Van Oordt 1962; Seldenrijk et al. 1979, submitted for publication).

Periodic albino tadpoles of *Xenopus laevis* are able to adapt their melanophores to various conditions of illumination in a manner similar to wild-type tadpoles. These albino tadpoles, however, lack the background adaptation of wild-type specimens. On a white but also on a black background the melanosomes are aggregated in vivo; however, different concentrations of α-MSH (as low as 10^{-10} M) in the assay medium induce a complete dispersion in tail-fin melanophores in vitro. This response resembles the reactivity of wild-type melanophores. However, dark-adapted periodic albino melanophores in vitro do not aggregate upon addition of melatonin (up to 10^{-6} M) to the assay medium.

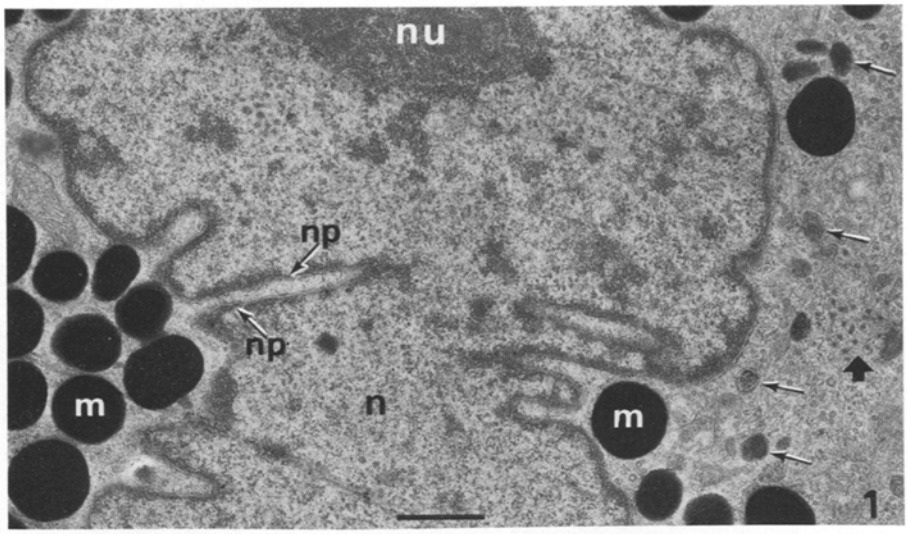
Table 1. Levels of tyrosinase activity in tail-fins of *Xenopus laevis*. Tyrosinase activity was measured after 20 h at 30° C in homogenates of three tail-fins. Values are an average ± S.E.M. of four separate duplicated measurements (after subtracting heat-inactivated control values that are ≤1.6% of total value)

	nmol of oxidized tyrosine/ mg protein/h ± S.E.M.
Wild-type tail-fin pigmented caudal portion	622.58 ± 44.27
Wild-type tail-fin nonpigmented rostral portion	52.04 ± 5.80
Periodic albino tail-fin caudal portion	136.45 ± 14.40

The pigmented caudal portion of the ventral tail-fin in wild-type tadpoles shows tyrosinase activity. In the nonpigmented rostral side of the ventral tail-fin the presence of a very weak tyrosinase activity can be established; this weak activity differs significantly from the activity in heat-inactivated controls ($p < 0.001$, Student t-test). In comparison to the wild-type caudal tail-fin, the tyrosinase activity in the corresponding caudal part of periodic albino tail-fins is about five times weaker (Table 1).

Ultrastructure of wild-type melanophores

Melanophores are stellate cells with an almost spherical central part possessing radiating processes. They have an irregular nucleus (Fig. 1) and the nuclear envelope is provided with pores. The nucleoplasm contains mostly euchromatin interspaced with electron-dense clumps of heterochromatin. The Golgi complex and related vesicles are located mainly in the central part of the cell. The cytoplasm contains many melanosomes (Figs. 1, 2). The pigment content of the melanosomes varies; some of the melanosomes are granular (premelanosomes), but most of them are homogeneously electron dense (Fig. 1). Near the nucleus a cytocentrum with centrioles can be observed. In the aggregated and in the dispersed state the centrioles are surrounded by a number of electron-dense patches, microfilaments and microtubules; the microtubules are arranged radially (Fig. 2). Mitochondria are particularly abundant near the cytocentrum in the dispersed state and in the aggregated state in the transitional area between the nucleated part and the processes. Near the plasma membrane is a network of thin filaments (diameter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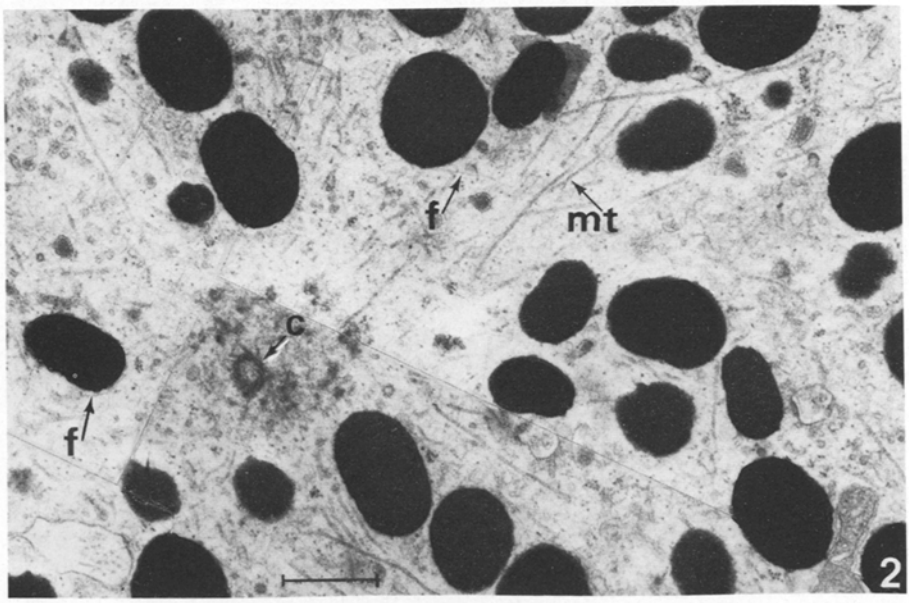
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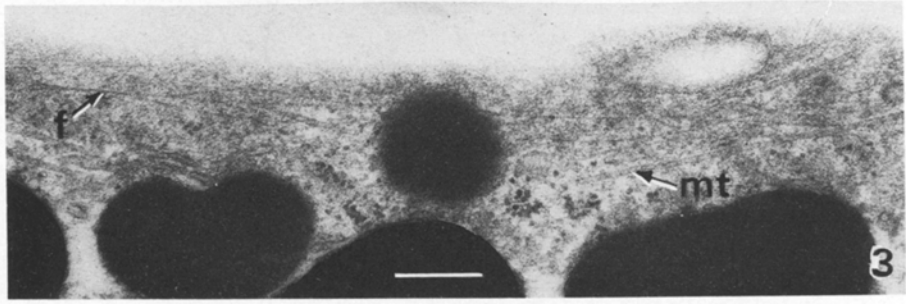
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± 6 nm), isolated filaments (diameter ± 11 nm), and microtubules (diameter ± 25 nm) oriented parallel to the cell surface (Fig. 3). Nerve fibres are present in the vicinity of the melanophores, but synaptic contacts have not been observed.

Ultrastructure of periodic albino melanophores

The central part of albino melanophores is smaller than that of the wild-type melanophores. The nucleus is less irregular, and contains more heterochromatin than euchromatin and fewer nuclear pores. The cells also contain less mitochondria. The structure and the distribution of ribosomes, endoplasmic reticulum, electron-dense patches, microtubules, microfilaments, and the Golgi complex are not markedly different from the same features in the wild-type melanophores. The mitochondria are distributed throughout the cytoplasm with a higher concentration in the pericentriolar region; a translocation during pigment migration was not observed. The number of melanosomes and their degree of melanization are significantly lower in the albino than in the wild-type melanophores. Only a few melanosomes contain homogeneous electron-dense material (Figs. 4, 5). Most of the melanosomes remain in a premelanosomal stage and are spherical with a predominantly granular internal structure of punctate electron-dense material. Several ovoid premelanosomes contain a fibrillar network of loosely coiled fibres (Fig. 6).

Discussion

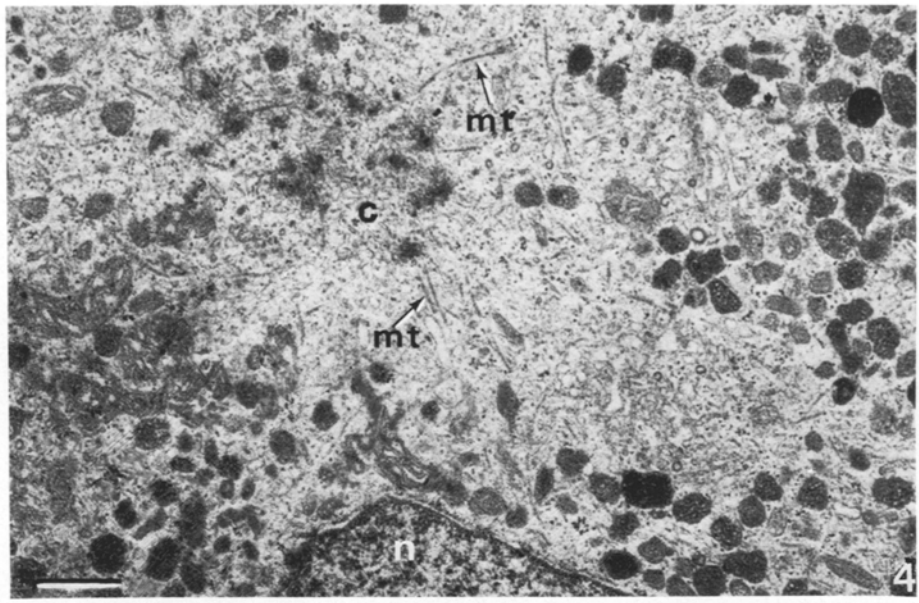
The ultrastructural organization of melanophores in periodic albino mutants is not distinctly different from that in melanophores of wild-type tadpoles, except for the degree of melanization in the melanosomes, the shape of the nucleus and its heterochromatin content, as well as the location of mitochondria in the aggregated and in the dispersed state.

The most characteristic ultrastructural difference is observed in the structure of melanosomes. Bowers and Carver (1978) reported similar observations in albino *Rana catesbeiana*, although the melanophores of this species had another ultrastructural appearance. In the present observations on the tail-fin melanophores of periodic albino *Xenopus laevis*, most of the membrane-bounded premelanosomes (with granular internal structure) are in a late phase of development rather than in an intermediate premelanosomal stage (with laminar structure). This suggests an incomplete melanogenesis in tadpoles of periodic albino *Xenopus laevis*. It is

Fig. 1. Portion of the irregular nucleus (*n*) of a wild-type melanophore showing a nucleolus (*nu*), nuclear pores (*np*), melanosomes (*m*), premelanosomes (*thin arrows*) and a cytocentrum (*thick arrow*) in the nuclear region. Scale bar = 0.75 µm

Fig. 2. Portion of the cytocentrum of a wild-type melanophore (photomontage). The centriole (*c*) is surrounded by electron-dense patches and fibrous material. The microtubular system (*mt*) shows a radial arrangement in contrast to the filaments (*f*). Scale bar = 0.5 µm

Fig. 3. Microtubules (*mt*) and filaments (*f*) in the cortical region of a wild-type melanophore. Scale bar = 0.25 µm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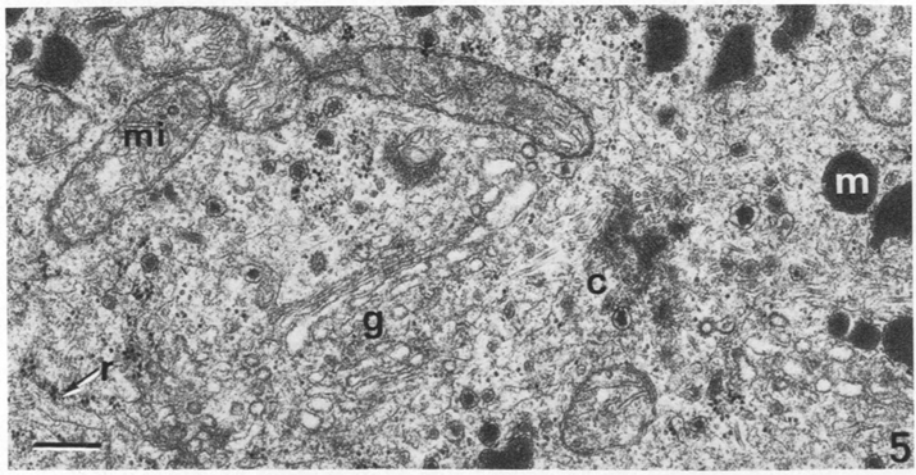
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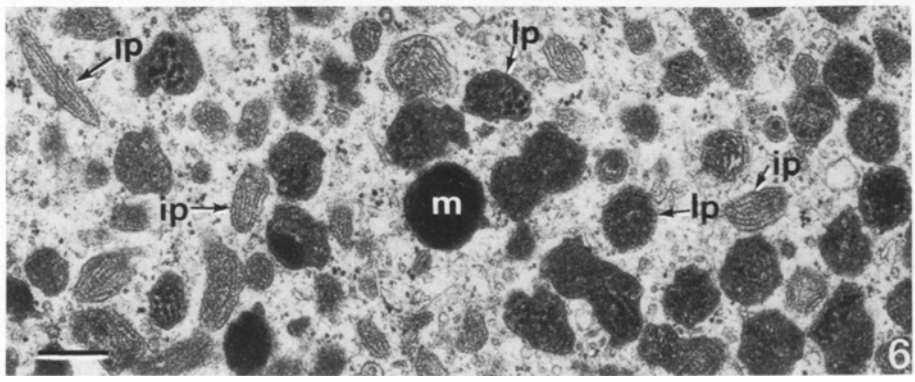
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possible that enzymes necessary for the maturation of melanosomes are absent or do not function properly.

The structure of the nucleus (fewer nuclear pores, less folded) and the lower number of mitochondria in albinos also suggest a decreased metabolic activity of these cells compared to wild-type melanophores. In other respects periodic albino and wild-type melanophores show similar ultrastructural features. On the basis of the ultrastructural results it is concluded that periodic albino melanophores have the cellular organization to induce migration of pigment granules, however, the process of melanogenesis may be disturbed.

In periodic albino melanophores, changes in illumination and addition of α-MSH to the assay medium cause melanosome migration. This leads to the conclusion that these melanophores actually possess a light/dark receptive system, MSH-receptors, and a mechanism required for pigment migration. However, the response to background adaptation in vivo (normally caused by MSH-release) is absent, and in vitro a melatonin-induced melanosome aggregation has not been observed.

The lack of response to melatonin in vitro is difficult to explain. Obviously, the albino melanophores are without specific melatonin-binding sites as present in wild-type melanophores (Seldenrijk et al., submitted for publication). If melatonin is involved in the process of pigment migration by controlling the MSH-production in the pars intermedia of the hypophysis (as suggested by Charlton 1966, Oshima and Gorbman 1969 and van Eys 1980), a possible additional defect at the level of the hypothalamus cannot be excluded.

Tyrosinase is the essential enzyme for the synthesis of melanin pigments. The present findings for the ventral tail-fin of wild-type *Xenopus laevis* indicate that tyrosinase activity is mainly found in melanophore-containing tissue; this is contradictory to the statement of Lee and Lee (1971) that tyrosinase activity is evenly distributed in pigmented and nonpigmented skin of adult *Rana pipiens*. Tail-fin tissue of periodic albino *Xenopus laevis* shows tyrosinase activity (described earlier in oocytes by Wyllie and de Robertis 1976), but weaker than in wild-type tail-fins. Since MSH has been found to stimulate tyrosinase activity in melanoma cells (Eberle et al. 1979), the low tyrosinase activity might be attributed to the absence or scarcity of MSH. On the other hand, it should be emphasized that the periodic

Fig. 4. Survey micrograph of a periodic albino melanophore showing premelanosomes with electron-dense material. The cytocentrum (*c*) shows electron-dense patches in combination with microtubules (*mt*). The nucleus (*n*) of the periodic albino melanophores contains more heterochromatin than that of the wild-type cells. Scale bar = 0.5 μm

Fig. 5. The central portion of a periodic albino melanophore with mitochondria (*mi*), a Golgi complex (*g*) and related vesicles, free ribosomes (*r*), almost mature melanosomes (*m*) and some electron-dense patches and microtubules adjacent to the centriole (*c*). Scale bar = 0.25 μm

Fig. 6. Periodic albino melanosomes in different phases of development: ovoid intermediate premelanosomal stage with a fibrillar network of loosely coiled fibres (*ip*); spherical premelanosomes with a granular internal structure in a late phase of development (*lp*); and only a few almost mature melanosomes with homogeneous electron-dense material (*m*). Scale bar = 0.25 μm

albino tail-fins contain smaller and fewer melanophores ($\pm$ 1,200 against $\pm$ 4,000 in wild-types), and this leads to a lower tyrosinase activity expressed per mg tail-fin protein. Therefore, a weak specific activity of tyrosinase in periodic albinos is not self-evident.

The fewer and smaller albino melanophores as such might also point to an insufficiency in MSH in periodic albino tadpoles; this is based on the experiments of Hadley and Quevedo (1967), suggesting a stimulating effect of MSH on the development of *Xenopus laevis* melanophores; however, MSH levels in periodic albino tadpoles should be measured under various conditions to substantiate this assumption. Furthermore, periodic albino tadpoles do not adapt to a black background notwithstanding the fact that their melanophores possess MSH receptors. Apparently, these tadpoles do not respond to stimuli for MSH release (or synthesis) in this stage of development. There are several possible explanations for the lack of background adaptation in the periodic albinos: a) absence of perception of background changes; b) impairment of hypothalamic control of the pars intermedia in the hypophysis; c) disturbance of the biosynthesis and/or release of α-MSH by the pars intermedia.

From the present results it may be excluded that the periodic albino mutation affects only melanin synthesis. Despite the conclusion of Tompkins (1977), Hoperskaya (1978) and MacMillan (1979), (based on neural crest transplantations and parabiotic tadpole combinations), that the hormonal control of melanophore differentiation is not affected, our results indicate that under physiological conditions the release of α-MSH is absent or scarce in tadpoles of periodic albino *Xenopus laevis*.

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