Melanogenesis in amphibians

II. Electron microscope studies of the normal and PTU-treated pigmented epithelium of developing Notophthalmus viridescens eyes

By JOHN J. EPPIG, JR.

Molecular Anatomy (MAN) Program, Oak Ridge National Laboratory

SUMMARY

Electron microscopy of 11-day-old Notophthalmus viridescens retinal pigmented epithelium reveals particulate premelanosomes which are identical to the melanosomes found in the oocyte. These organelles, when found in the pigmented epithelium, are called premelanosomes because they undergo further maturation to form relatively homogeneous, spherical melanosomes. At this stage, oocyte melanosomes found in cells other than melanocytes have not undergone this subsequent maturation.

Elongated melanosomes which develop from fibrillar premelanosomes are also found in the pigmented epithelial cells.

Treatment with phenylthiourea blocks the maturation of both the fibrillar and particulate premelanosomes.

INTRODUCTION

Electron microscopic studies on the retinal pigmented epithelium of Rana pipiens tadpoles have revealed the presence of elongated melanosomes which develop from organized fibrillar premelanosomes (Eppig, 1970). Also present are smaller spherical melanosomes, some of which become incorporated into complex melanosomes. Some evidence indicated that these spherical melanosomes may originate in the oocyte; however, no truly distinctive morphological characteristics could link them with melanosomes of maternal origin.

Melanogenesis in anuran oocytes (Wartenberg, 1962; Balinksy & Devis, 1963) involves deposition of melanin on a particulate premelanosome which forms an electron-dense, somewhat homogeneous, membrane-bound organelle. In the oocyte of Notophthalmus viridescens (formerly Triturus viridescens), however, the particulate premelanosome is not completely filled in, i.e. melanin deposition terminates while the individual particles are still clearly separated (Hope, Humphries & Bourne, 1964; Wischnitzer, 1965). Melanosomes identical to these are the only pigment granules found in the ectodermal cells during early development in Triturus pyrrhogaster before melanocyte differentiation begins.

1 Author's address: Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A. 37830.
Melanogenesis in amphibians. II

(Karasaki, 1959). The studies reported here were initiated in order to ascertain whether these oocytic melanosomes could be located in the early retinal pigmented epithelium and to investigate their fate.

MATERIALS AND METHODS

Adult female Notophthalmus viridescens were collected locally in April and fertilized eggs were obtained by induction with implanted Rana pipiens pituitaries. Some early cleavage stage embryos were placed in tap water containing Versene (to counteract its high copper content) while others were reared in Versene solution containing 0.005% phenylthiourea (PTU), an antimelanogenic, tyrosinase inhibitor. After 11 days (when eye pigmentation first appears macroscopically) or 20 days some animals from each group were decapitated and the whole heads were fixed for 2 h in 1% osmium tetroxide at 4°C in veronal acetate buffer at pH 7.4. The tissues were then dehydrated and embedded in Epon. Silver sections were cut on a Porter Blum MT-2 ultramicrotome and mounted on 200-mesh copper grids coated with Parlodion and carbon films. The sections were stained with uranyl acetate and lead citrate and examined with an RCA EMU-3G electron microscope operated at 50 kV.

RESULTS

Cell organelles commonly found in the normal retinal pigmented epithelium of 11- (Fig. 1) or 20-day-old N. viridescens included: nucleus, mitochondria, rough and smooth endoplasmic reticulum and free ribosomes. Occasionally, a Golgi system is encountered. The 11-day-old cells contain many structures that are identical to the melanosomes found in the oocyte; these are membrane-bound organelles containing electron-dense particles. Sometimes more than one melanosome is found bound within the same membrane (Fig. 2). The oocyte-like melanosomes are seen in stages of further maturation (Figs. 3-5), i.e. more melanin is subsequently deposited on the particles so that they become filled in with more electron-dense material to form a relatively homogeneous spherical melanosome. In later stages of maturation the periphery appears to be completed before the central area (Fig. 5). Most of these particulate structures are filled in by 20 days. The 11- and 20-day cells also contain elongated melanosomes which develop from fibrillar premelanosomes (Fig. 1, inset). The fibres are generally oriented parallel to the long axis of this membrane-bound organelle.

---

Fig. 1. Electron micrograph of the normal retinal pigmented epithelium in an 11-day-old N. viridescens. In addition to the nucleus (N), mitochondria (Mi), rough (R) and smooth (S) endoplasmic reticulum, also present are approximately spherical melanosomes (M), which arise from particulate premelanosomes similar to (P), and elongated melanosomes (E) which mature from fibrillar premelanosomes. Arrows point to oblique sections through early fibrillar premelanosomes. The inset shows a fibrillar premelanosome with electron dense material, presumably melanin, deposited on the fibres.
Fig. 2. Electron micrograph from an 11-day-old eye shows three particulate premelanosomes (P) enclosed within the same membrane (arrow).

Figs. 3, 4, 5. A series of micrographs demonstrates the deposition of additional dense material (melanin) in the particulate premelanosome (P) to form a spherical melanosome (M). The periphery of the organelle is confluent before the central area (Fig. 5).

Fig. 6. These particulate melanosomes (M) are outside the retinal pigmented epithelium in the mesenchymal tissue. These melanosomes apparently do not undergo further maturation. Also present is a yolk platelet (Y).
Fig. 7. In retinal pigmented epithelium of a 20-day-old *N. viridescens*, which was reared in PTU, neither the particulate premelanosomes (P) nor the fibrilar premelanosomes (F) have undergone further maturation.
Sometimes particulate melanosomes (oocytic) are observed outside the pigmented epithelium in the extracellular space, in mesenchymal cells (Fig. 6), and even in blood cells. These melanosomes do not undergo further maturation even when most of those in the pigmented epithelium are completely matured.

In the PTU-treated pigmented epithelium, the oocyte-type melanosomes do not, however, undergo further maturation from the particulate form, even after 20 days (Fig. 7). The fibrillar premelanosomes also fail to mature in the PTU solution.

**DISCUSSION**

It is clear that melanosomes develop in the retinal pigmented epithelium of *N. viridescens* by two distinctly different mechanisms: melanin deposition on particulate premelanosomes and on fibrous premelanosomes. It would seem that the particulate premelanosomes found in the developing retinal pigmented epithelium are the particulate melanosomes of the egg since the eye contains no earlier stages of these organelles, as described by Hope *et al.* (1964) and Wischnitzer (1965) in the oocyte, and they are found in spite of PTU treatment.

There is some difficulty applying the accepted nomenclature (Fitzpatrick *et al.* 1966) in this situation. The term 'melanosome' applies only to the mature melanin granule while 'premelanosomes' are developmental stages of melanosome maturation. Thus, the particulate organelle of the oocyte is mature *in situ* and therefore would be called a melanosome. However, since these oocytic melanosomes undergo further maturation if incorporated into the pigmented epithelium, they should, in fact, when referred to in the eye, be termed premelanosomes.

Sometimes oocytic melanosomes are observed outside the pigmented epithelium in the extracellular space, in mesenchymal cells, and even in blood cells. These melanosomes do not become mature even when most of those (particulate premelanosomes) in the pigmented epithelium are completed. It seems likely, therefore, that the pigmented epithelial cells supply additional tyrosinase which is necessary for additional melanin synthesis. An alternative hypothesis might be that the non-melanocytes exert a tyrosinase suppressing effect on the oocytic melanosomes.

This report also confirms the finding of fibrillar premelanosomes in amphibians, as reported in the first of this series of studies which dealt with *Rana pipiens*. It also gives support to the hypothesis that mechanisms for premelanosome formation are different in the egg and in the larva. Development in the egg involves a particulate premelanosome which may mature completely *in situ* to the more homogeneous form, as in anurans, or remain in the particulate configuration, as in *N. viridescens*. The larval mechanism for melanosome development as observed in the pigmented epithelium, involves the formation of a fibrillar premelanosome. Although it cannot be stated unequivocally that particulate premelanosomes are not formed in tissue other than oocytes, there
Melanogenesis in amphibians. II

is no evidence as yet to show that they are. Melanogenesis in squid ink sack (Vogel & McGregor, 1964) and fish skin (Stolk, 1960) involves a mechanism which is apparently similar to that of the particulate premelanosome. Higher vertebrates such as reptiles (Breathnach & Poyntz, 1966) and mammals (Breathnach & Wyllie, 1966; Drochmans, 1967; Lerche & Wulle, 1967; Moyer, 1966) possess the organized fibrillar premelanosome for melanogenesis. It might be, therefore, that the ontogeny of melanosomes undergoes a transition at the amphibian level of the phylogenetic scale from particulate to fibrillar premelanosomes. Confirmation of this hypothesis, however, will have to await further studies on the lower forms as well as other amphibian melanocytes.

RESUME

Mélanogenèse chez les amphibiens. II. Etudes au microscope électronique, au cours du développement de l'œil de Notophthalmus viridescens, de l'épithélium pigmenté normal et traité par la phenylthiouuriée

L'étude au microscope électronique de l'épithélium rétinien pigmenté, chez l'Amphibien Notophthalmus viridescens âgé de 11 jours, révèle l'existence de prémélanosomes particu- laires, identiques aux mélanosomes trouvés dans l'oocyte. Ces organites, lorsqu'on les trouve dans l'épithélium pigmenté, sont appelés prémélanosomes car ils subissent une maturation ultérieure pour devenir des mélanosomes sphériques, relativement homogènes. A ce stade les mélanosomes de l'oocyte, trouvés dans des cellules autres que les mélanocytes, n'ont pas subi cette maturation ultérieure.

On trouve aussi dans les cellules de l'épithélium pigmenté des mélanosomes allongés qui se développent à partir de prémélanosomes fibrillaires.

Un traitement par la phénylthiouuriée bloque la maturation des prémélanosomes fibrillaires et des prémélanosomes particu- laires.

The author is an Oak Ridge Graduate Fellow from Catholic University of America, Washington, D.C., under appointment from Oak Ridge Associated Universities.

The Molecular Anatomy (MAN) Program is supported by the National Cancer Institute, the National Institute of General Medical Sciences, the National Institute of Allergy and Infectious Diseases, and the U.S. Atomic Energy Commission.

The Oak Ridge National Laboratory is operated by Union Carbide Corporation Nuclear Division, for the U.S. Atomic Energy Commission.

REFERENCES


(Manuscript received 30 December 1969)