Egg Pigment: Early Elimination from the Developing Retina of the Frog

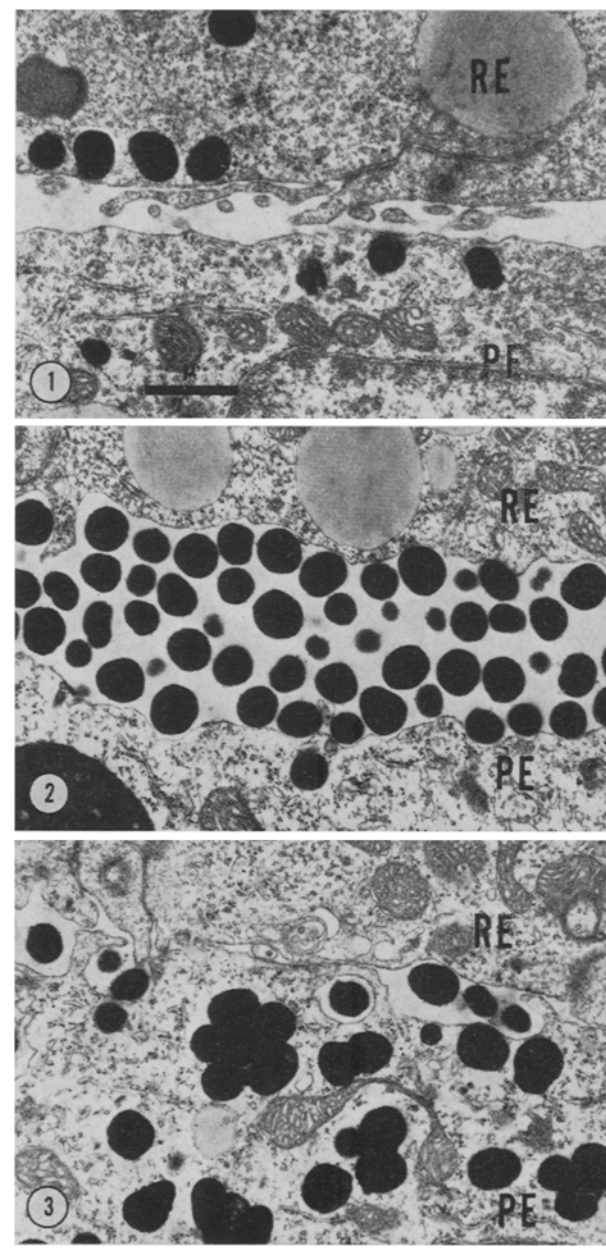
In the amphibians, melanin granules are produced in large quantities during oogenesis.[1-6] At the time of fertilization, this egg pigment is not uniformly distributed within the egg cytoplasm. More pigment is found in the animal hemisphere of the egg than in the vegetal hemisphere and more pigment resides in the cortical cytoplasm than in deeper regions. The cortical cytoplasm of the animal hemisphere with this large component of pigment is ultimately confined to the ectodermal cells of the embryo.

Since several tissues in the eye develop from pigment-laden ectoderm it is necessary to rid these tissues of pigment that will interfere with light transmission. In the vertebrate eye, light must pass through the cornea, lens and retina before reaching the photoreceptors. Excess light not absorbed by the photoreceptors is absorbed by melanin located behind the retina. The presence of egg melanin in cells of the cornea, lens and retina would cause these tissues to absorb light rather than allow the free transmission of light to the photoreceptor. Egg pigment is eliminated from the tissues associated with light transmission significantly earlier than it is removed from other tissues. This paper describes the unique mechanism by which egg pigment is removed from the neural retina of the frog, *Rana pipiens*.

Methods. Embryos were selected which conformed closely with the stages of development described by SHUMWAY[7]. They were fixed for 1 h in a mixture of 1% glutaraldehyde, 1% acrolein and 1% paraformaldehyde with 1% sucrose, 0.02% $CaCl_2$ and 0.0073 molar KCl in 0.067 molar cacodylate buffer at pH 7.5. After rinsing in 0.1 molar buffer with 6% sucrose they were post-fixed for 1 h in 1% OsO_4 with 1% sucrose in buffer as above. The material was then dehydrated and embedded in Epon. After polymerization, 1 µm sections were cut with glass knives. These were stained with toluidine blue 'O' and examined under oil immersion. The pigment granules are clearly visible at this magnification (× 1000). The blocks were then trimmed down and thin sections cut with a diamond knife. These were picked up on 300 mesh coated grids and examined in a Zeiss electron microscope.

Results. The ovarian pigment granules in this animal are nearly spherical particles (0.6–0.7 µm in diameter) of high electron density. Shortly before the appearance of the lens (stage 17), this pigment is randomly distributed within the cells of the retinal neuroepithelium and the developing pigment epithelium. Concomitant with the appearance of the lens (stage 18), pigment in the center of the retinal neuroepithelium begins to accumulate at the outer surface of the cells and some granules are eliminated into the space between the developing retina and pigment epithelium.

At stage 19, all phases in the mobilization and elimination of pigment from the retina can be observed. At the margin of the retina, pigment granules are randomly distributed in the cell cytoplasm in a manner similar to that observed throughout the retinal neuroepithelium at stage 17 (Figure 1). No free pigment granules are found between the epithelial layers at the margin of the retina. Midway between the margin and center of the retina, pigment is accumulated near the apical ends of the neuroepithelial cells and occasionally pigment granules can be found in the space between the epithelial layers. At the center of the retina, large numbers of pigment granules are found between the pigment epithelium and the retinal neuroepithelium (Figure 2). Only rarely are pigment granules found in the neuropithelial cells in the center of the developing retina at this stage.



Figs. 1–3. Electron micrographs of the retinal neuroepithelium (RE)-pigment epithelium (PE) interface in the developing frog eye illustrating different stages in the elimination and removal of egg pigment. 1. Pigment granules are present in both these tissues. 2. Pigment is eliminated from the retinal neuroepithelium into the intraepithelial space. 3. The pigment granules are engulfed by the pigment epithelium.

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In the central region of the retina at stage 20, many more pigment granules are within the cells of the pigment epithelium than were observed in this tissue at the previous stage and only a few granules remain in the space between the epithelial layers. Most of these extracellular granules appear to be actively engulfed by cytoplasmic processes from the pigment epithelium (Figure 3). Inside the pigment epithelium, some of the pigment granules aggregate to form a larger unit. Up to 18 granules have been observed in some sections but usually only 3 to 6 granule profiles are observed. Never were aggregates of egg pigment observed in the retinal neuroepithelial cells or in the space between the epithelial layers.

The rod shaped melanosome (2.0–2.5 μm long, 0.6–0.7 μm wide) typically found in the pigment epithelium of this animal[8–10] first appears during late stage 20. At this time there are 2 types of melanin granules in the pigment epithelium: one produced during the development of the egg and the other synthesized de novo by the pigment epithelium. These are the melanosomes of 2 different morphologies that have been observed in the pigment epithelium of the tadpole[11].

A complete electroretinogram (ERG) can be recorded from the developing eye of this animal only when both receptor outer segments and receptor synapses have developed[10]. In my material both these structures are present at stage 24. Also at this stage the differentiating retina and intraepithelial space are free of pigment except for a few granules which persist in the cells at the margin where the retina merges with the pigment epithelium. Thus, by the time the retina has differentiated to the degree that it responds to light with the usual pattern of electrical activity, most of the egg melanin has been eliminated from its cells.

The early elimination of egg pigment from the neural retina is in sharp contrast to the time this pigment is eliminated from neurons of the brain and spinal cord. In the central nervous system of young tadpoles I have found large amounts of pigment within neurons 2 to 3 weeks after the retina is free of pigment. In some species of frogs, egg pigment is present in the central nervous system well after metamorphosis[12].

The role of the pigment epithelium in removal of egg pigment eliminated by the embryonic retina offers a preview to a role of this tissue in the adult. In the mature retina, protein is assembled in the cell body and added at the base of the rod outer segments. The material lost from the outer segment tips is engulfed and destroyed by the pigment epithelium[8, 9, 13, 14].

Zusammenfassung. Die während der Oogenese entstandenen Melaningranula werden aus dem neuralen Netzhautanteil ausgeschieden und vom Pigmentepithel aufgenommen. Dies geschieht, bevor das Zentralnervensystem pigmentfrei geworden ist, und ermöglicht wirkungsvolle Lichtaufnahme in den Photorezeptoren.

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Scanning Electron Microscopy of Golden Hamster Spermatozoa Before and During Fertilization

Recent development of scanning electron microscopy has made possible the investigation of details of cellular surface topography, which would be very difficult or impossible to examine by conventional light microscopy or transmission electron microscopy. This relatively new technique has apparently not been fully applied for studies of morphology of mammalian gametes[1–4]. This paper reports the result of our scanning electron microscopic observations on golden hamster spermatozoa before and during fertilization. The details of the internal anatomy of the golden hamster spermatozoon and its changes during fertilization have been published[5–10].

Fresh spermatozoa were obtained from the caudae epididymides of fertile golden hamster males. Capacitated spermatozoa were prepared as described by Yanagimachi[11]. The spermatozoa were spread over clean slides, quickly air-dried, and fixed 1 h in cold 2.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.4). After thorough rinsing in 0.1 *M* phosphate buffer (pH 7.4), the sperm smears were dehydrated with a graded acetone series. After complete dehydration with 100% acetone, the smears were allowed to dry in air. The smear-holding slides were then cut into small pieces (1 × 1 cm^2) and with smear surface up each piece was glued to an aluminum stub using an electron conductive cement (silpaint; Fansteel Electric Material Lab.). The specimen stubs were placed in a vacuum evaporator (JEOLCO, model 4B) and a thin film of gold was evaporated at a high vacuum (10^{-4} mm Hg) onto the specimens, which were rotated constantly so that the gold coated all parts of the specimens. The coating process was stopped when the slides turned slightly bluish-green. Observations and photography were

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