EVIDENCE FOR GRANULOLYSIS IN THE RETINULA CELLS OF A STOMATOPOD CRUSTACEAN, *SQUILLA MANTIS*

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INTRODUCTION

In different endocrine cells of the rat, Smith and Farquhar (1) and Orci et al. (2) described the incorporation of secretory granules within lysosomal bodies. This process, called granulolysis (2) or crinophagy,[1] occurs through fusion of the granule's

[1] de Duve, C. 1969. The Claude Bernard Lecture. 5th Annual Meeting of the European Association for the study of Diabetes. Montpellier.

membrane with that of the lysosome and has been interpreted as a cellular mechanism for disposing of excess secretory product under certain experimental (1) or metabolic conditions (2, 3). Granulolysis, at a low rate, is also present under normal conditions, as a means for the catabolism of superfluous secretory granules (1).

In the course of a study of the eye of a stomatopod crustacean *Squilla mantis*, it was found that
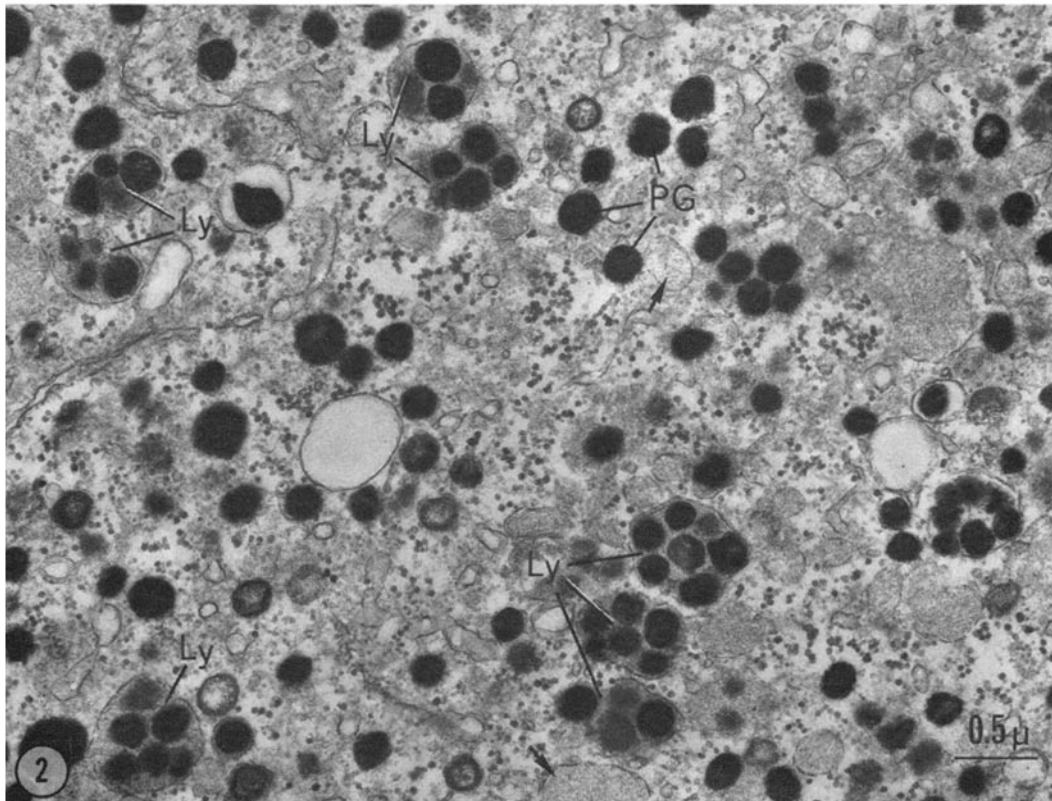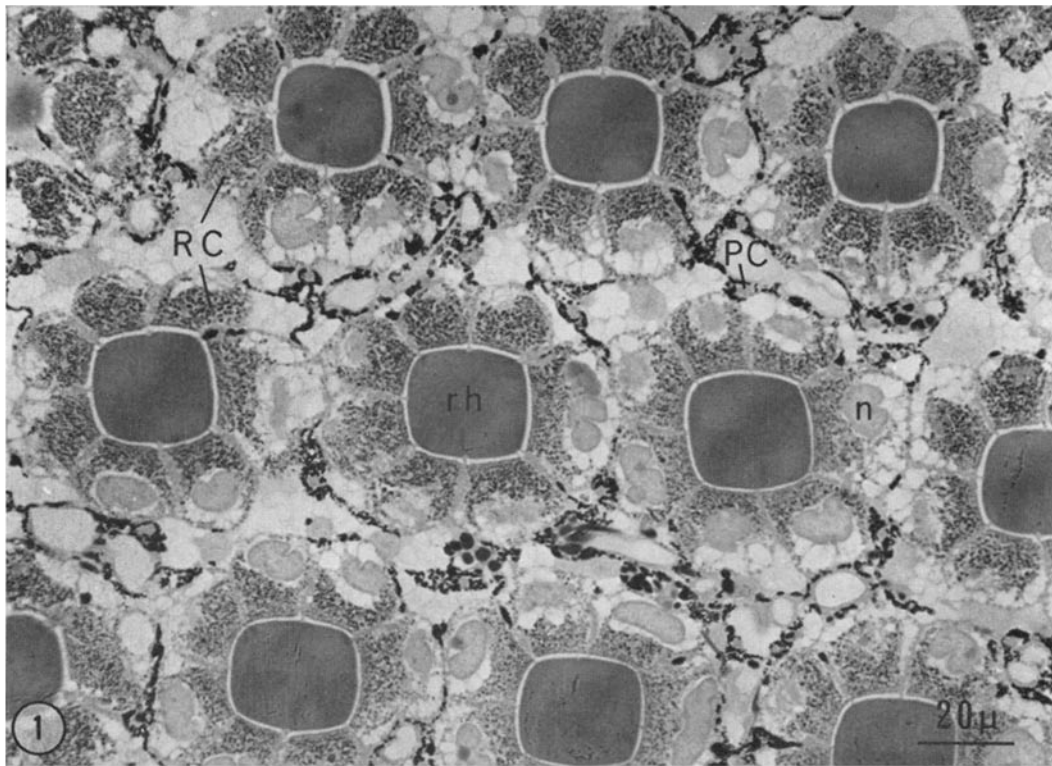
FIGURE 1 Thick section of a retina of *Squilla mantis*, stained with toluidine blue and seen in the light microscope. Each ommatidium is formed of seven retinula cells (*RC*) surrounding the central rhabdome (*rh*). The cytoplasm of the retinula cells appears filled with small dark granules (accessory pigment). Some cells show a lobulated nucleus (*n*). Between the ommatidia, sleeves of elongated pigment cells (*PC*) containing large black granules can be seen. × 600.

FIGURE 2 Part of a retinula cell cytoplasm showing a large number of accessory pigment granules (*PG*), as well as numerous bodies (*Ly*) containing granule cores. One also sees vacuoles with a pale and flocculent content (arrows). Glycogen particles are scattered throughout the cytoplasm. × 22,700.

numerous accessory (nonvisual or screening) pigment granules present in the retinula cells were involved in a process closely resembling granulolysis or crinophagy in endocrine cells. Granulolysis in retinula cells could represent the normal pathway for catabolizing the accessory pigment granules. The formation of accessory pigment granules has been studied by Shoup (4) in the eye of *Drosophila* and it has been shown that most granules first appear within Golgi vesicles. The cellular site where destruction of pigment granules possibly occurs has not been investigated so far.

MATERIALS AND METHODS

Living *Squilla mantis* were kept at room temperature in aerated artificial seawater. The stalked eyes were removed and sliced, then fixed overnight in a cold formaldehyde-glutaraldehyde mixture in 0.1 M phosphate buffer, pH 7.6, containing NaCl and sucrose (5). After brief washing in phosphate buffer, the slices were postfixed for 2 hr in 2% phosphate-

buffered osmium tetroxide, pH 7.6 (6), then dehydrated in alcohol and embedded in Epon (7). Ultrathin sections contrasted with lead citrate (8) were examined in a Philips EM300 electron microscope. Thick sections stained with toluidine blue in 1% Borax (9) were photographed on Agfa negative plates in a Zeiss Ultraphot microscope (Carl Zeiss, Oberkochen, Germany). The acid phosphatase was demonstrated with the method of Gomori (10). For this purpose fixation was carried out as described above for 3 hr but with Na-cacodylate 0.1 M, pH 7.4, as buffer. After an overnight washing in 0.1 M cacodylate buffer containing 7% sucrose, the eye slices were cut into tiny fragments with a razor blade and the fragments were incubated in Gomori's medium for 1 hr at room temperature. The blocks were then briefly rinsed in 2% acetic acid, washed several times in cacodylate buffer, pH 7.4, and postfixed for 45 min in 2% osmium tetroxide buffered with phosphate. Controls consisted of blocks incubated in Gomori's medium without glycerophosphate. The blocks were dehydrated and embedded as described above. Thin

FIGURE 3   Area of a retinula cell cytoplasm showing several vacuoles (v) with a pale content, together with numerous bodies containing granule cores (Ly). Granule cores frequently gather in rosette disposition within the inclusion. The possible fusion of two small bodies may give rise to a larger inclusion (arrows). The inset displays the close contact between the limiting membranes of a pigment granule and of a vacuole (v). × 18,000; inset, × 30,200.
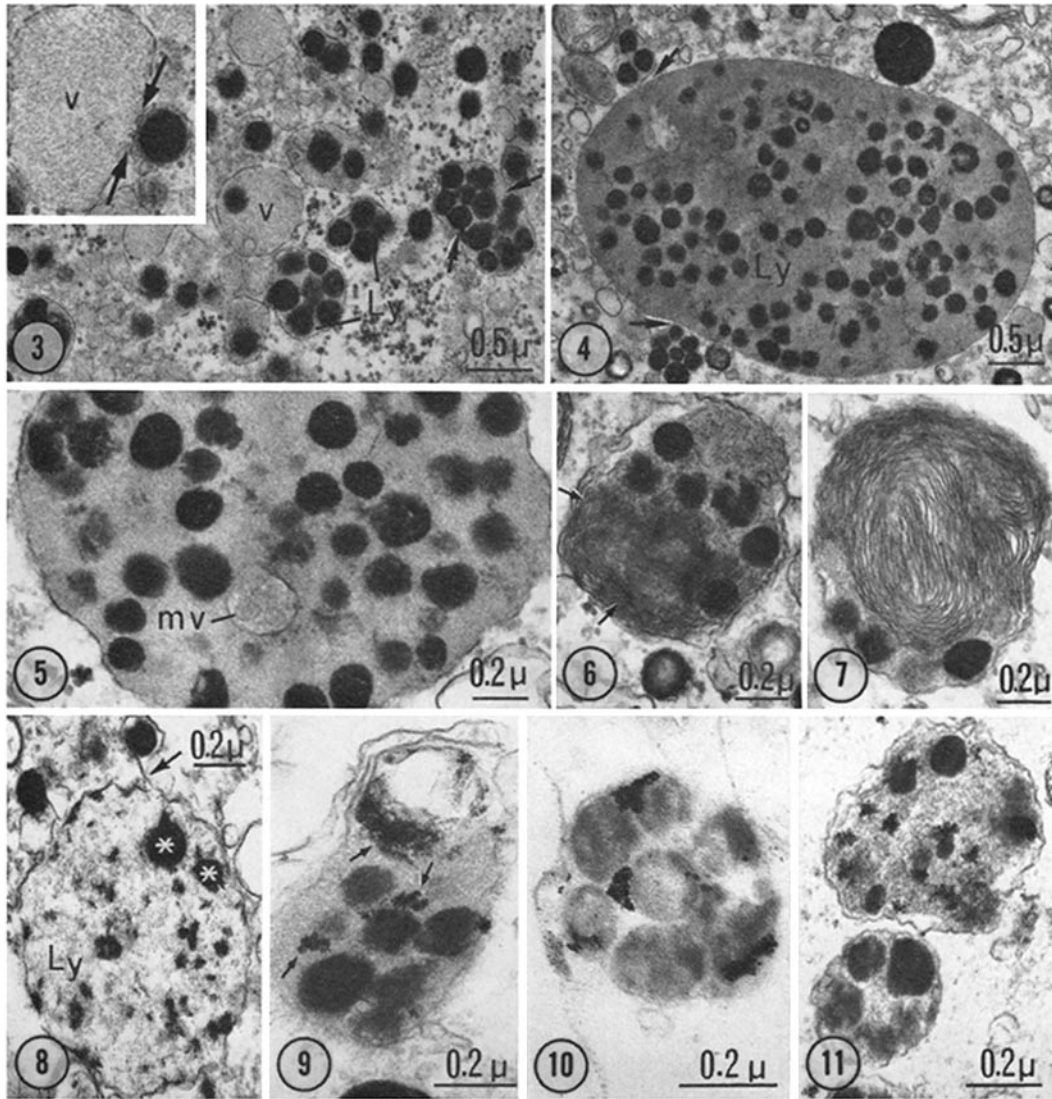
FIGURE 4   Giant cytoplasmic body (Ly) containing numerous granule cores. Two small inclusions (arrows) are seen in close proximity to the giant one by which they might be incorporated, contributing to its increase in size. × 15,000.

FIGURE 5   High-power micrograph showing the internal organization of a cytoplasmic body containing pigment granules. The numerous granule cores are embedded in a homogeneous matrix of moderate electron opacity; some granules appear fuzzy and of irregular size, which might indicate that they are undergoing digestion within the inclusion. Several microvesicles enclosed within a membrane (mv) can also be seen. × 37,000.

FIGURE 6   Inclusion containing a few whorls of membranes (arrows) in addition to normal and partially "digested" granule cores. × 33,300.

FIGURE 7   Image suggestive of a transformed cytoplasmic body; its matrix is filled with membrane whorls, except for a peripheral zone which still contains a few granule cores. This transformed inclusion can be regarded as a residual body. × 35,200.

FIGURE 8–11   Blocks incubated for the demonstration of acid phosphatase. FIG. 8, Vacuole (Ly) containing two granule cores (∗) and showing on its matrix numerous patches of lead deposit which reveal the presence of acid phosphatase. Close to the vacuole a single membrane-bounded pigment granule (arrow) can be seen. Section stained with lead citrate. × 27,500. FIG. 9, Cytoplasmic body containing several granule cores. Acid phosphatase activity (arrows) is revealed by the lead deposits on the homogeneous matrix of the inclusion. Section stained with lead citrate. × 55,200. FIG. 10, Typical cytoplasmic body with seven granule cores assuming a rosette disposition. In between the granules, a heavy deposit of lead can be seen. Unstained section. × 65,300. FIG. 11, Cytoplasmic bodies containing a few granule cores. One of the bodies shows lead deposits on its matrix, whereas the other appears unreactive. Section stained with lead. × 47,500.

Figures 3–11. Electron micrographs.

3 — v, v, Ly, 0.5 μ

4 — Ly, 0.5 μ

5 — mv, 0.2 μ

6 — 0.2 μ

7 — 0.2 μ

8 — Ly, 0.2 μ

9 — 0.2 μ

10 — 0.2 μ

11 — 0.2 μ

sections were examined either unstained or after contrasting with lead citrate.

RESULTS AND DISCUSSION

The retinula cells of *Squilla mantis* contain a large number of accessory pigment granules dispersed throughout the cytoplasm (Figs. 1 and 2). Most of the granules are of small diameter, averaging 0.2 μ. Some granules, however, are of larger size, up to 0.5 μ. In cell areas not occupied by pigment granules (periphery of the retinula cell, opposite the rhabdome) numerous cisternae of granular endoplasmic reticulum and Golgi stacks can be seen. The cytoplasm of the retinula cells also contains abundant glycogen particles (Fig. 2). Besides the single, scattered pigment granules described above, several granules were found forming clusters enclosed within a membrane. These bodies are dispersed within the retinula cell cytoplasm without apparent preferential localization. They contain from two to several dozen pigment granules, embedded in a matrix of variable electron opacity (Figs. 2, 3, and 4). In areas of large accumulation of bodies containing pigment granules, several vacuoles of varying diameter are seen (Fig. 3); the vacuoles are lined by a single membrane and show a pale and flocculent content which is suggestive of that of primary lysosomes (11). Single membrane-bounded granules sometimes appear in contact with these vacuoles (inset Fig. 3); the fusion of their respective membranes could lead to the incorporation of the granule within the vacuole (as a granule core, the limiting membrane of the granule being lost during incorporation). The successive incorporation of several granules might produce the typical bodies containing five to seven granule cores. These bodies could later fuse with others and give rise to giant inclusions as seen in Fig. 4. Morphological evidence suggests that the pigment granules undergo progressive degradation within the inclusion (Figs. 4 and 5) which finally resembles a residual body (Figs. 6 and 7). The digestion of the granules could be aided by the incorporation into the vacuole of microvesicles (Fig. 5), as these were shown to be involved in the transport of lytic enzymes (12). In blocks incubated for the demonstration of acid phosphatase, activity was observed in the vacuoles

with a pale content (Figs. 8 and 11), as well as in the bodies containing granule cores (Figs. 9 and 10). This localization of acid phosphatase supports the morphological evidence presented above and which suggested that both the vacuoles and the bodies containing pigment granules are of lysosomal nature. The process of incorporation of pigment granules by fusion with lysosomes appears quite different from that reported by Fahrenbach in *Limulus* ommatidium (5) and consisting of the sequestration of pigment granule together with other cytoplasmic components within autophagic vacuoles. At present we ignore the mechanisms capable of initiating or modifying granulolysis in *Squilla* retinula cells.

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