GRANULAR EXTENSIONS OF THE NUCLEOLI IN GIANT NEURONS OF *APLYSIA CALIFORNICA*

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INTRODUCTION

The nervous systems of many gastropods contain unusually large neurons. The cell bodies of these giant neurons can be more than 1 mm in diameter, as in the case of the colossal cells of *Aplysia* (Coggeshall, 1967). The nucleus represents approximately 30 per cent of the volume of the giant cell body, and in *Aplysia californica* the amount of nuclear DNA is many thousands of times greater than the haploid amount. It has been suggested that the entire genome in these cells undergoes repeated endoreduplication, resulting in a high level of polyploidy (Coggeshall et al., 1970; Lasek and Dower, 1971). The possibility that the entire genome is replicated synchronously many times is supported by the finding that the number of nucleoli in the giant cells of *Aplysia californica* increase in a fixed ratio to the DNA content of the nucleus (Garner and Lasek, unpublished observation). As many as 16,000 nucleoli have been estimated in cells which contain 0.067 μg of nuclear DNA. An unusual structural element is found to extend from the nucleoli of the giant cells. The prominence of this nucleolus-associated structure and its general distribution in giant neurons of *Aplysia californica* led us to make the following descriptive report.

MATERIALS AND METHODS

Specimens of *Aplysia californica* weighing from 25 to 400 g were maintained in Instant Ocean artificial sea water at 15–18°C. Giant neurons from the pleural and abdominal ganglia were preserved for electron microscopy by fixation *in situ* or immediately after removal from the organism. Individual giant neurons were dissected from the ganglia in fixative to reduce the time required for penetration and to facilitate the indentification of individual cells. These giant cells were fixed at room temperature in a 0.5% paraformaldehyde, 4.0% glutaraldehyde fixative (Karnovsky, 1965) buffered with 0.2 M cacodylate at pH 8.0, and were postfixed with 1% OsO_4 in 0.05 M Sorenson's phosphate buffer. Alcohol dehydration was followed by embedding in Epon (Luft, 1961). Sections were stained with uranyl acetate and lead citrate and photographed with RCA-EMU-3C, Philips 200 and 300 electron microscopes.

RESULTS AND DISCUSSION

Individual giant neurons in the nervous system of *Aplysia californica* can be identified on the basis of their pigmentation and topographical location in the ganglia. We have examined the ultrastructure of a number of these ganglion cells including the cells designated R-2, L-7, L-11, R-15 by Frazier et al. (1967). We also examined the pleural giant cell and several unidentified large cells of the abdominal ganglion. The following observations apply to all of these cells; however, it is unclear whether these observations apply to neurons less than 50 μ in diameter.

The nuclei of the ganglion cells present an open-faced appearance due to widely dispersed chromatin, nucleoli, and nucleoplasmic components (Figs. 1 and 2) as Coggeshall (1967) has previously described. The primary structure of interest in our study is the nucleolus. The nucleoli

increase in number as the organism and ganglion cells increase in size. As many as 16,000 nucleoli have been estimated in a single R-2 nucleus (Garner and Lasek, unpublished observation). Fig. 2 illustrates the ultrastructural features of the nucleoli, which approximate those found generally in other cells (reviewed in Busch and Smetana, 1970). The nucleoli typically contained a fibrillar region composed of 20–50 A filaments which existed either as a loosely intermeshed network or in a more compact configuration. The fibrillar region also contained areas having a granular appearance. The granular region of the nucleoli was compacted. The granules in this region of the nucleolus varied in size (50–150 A) and gave the impression of transition stages from fibrillar to granular components.

A unique feature of these nucleoli was the presence of elongate cylindrical structures which emanated from both the granular and the fibrillar zones (Figs. 1–4). These elongated structures occurred singly or in aggregates which numbered 10–20 in some cases. They were composed of a central continuous core to which distinct granules were attached in a regular array. The core approximated 250 A in diameter and has been seen to extend up to 5 μ in length. The electron opacity of the core was equal to that of the attached granules or slightly less. It is unclear whether this core is composed of tightly packed filamentous or amorphous particular material. The granules were located at the periphery of this core and were arranged in what appeared to be a helical configuration. Their size was approximately 175 A, or 75% of the size of cytoplasmic ribosomes which measured 236 × 267 A (Figs. 5–7). The granules were composed of a fine particulate material and were generally spherical in shape. The size of the granules in the nucleolar extrusions is comparable to that of the particles in the granular zone of the nucleolus in these and other cells (Hay, 1968).

The occurrence of the nucleolar extrusions varied considerably from one cell to another. In some giant cells the extrusions were found associated with the vast majority of the nucleoli while in other cells only a few of the nucleoli contained the extrusions. A systematic attempt has not been made to correlate the frequency of occurrence of the extrusions with the activity or type of neuron. 1 μ plastic sections (Fig. 1) showed that the nucleoli were distributed throughout the interior of the nucleus and were located generally at distances greater than 5–10 μ from the nuclear envelope. This prediction was borne out by the electron microscope investigations.

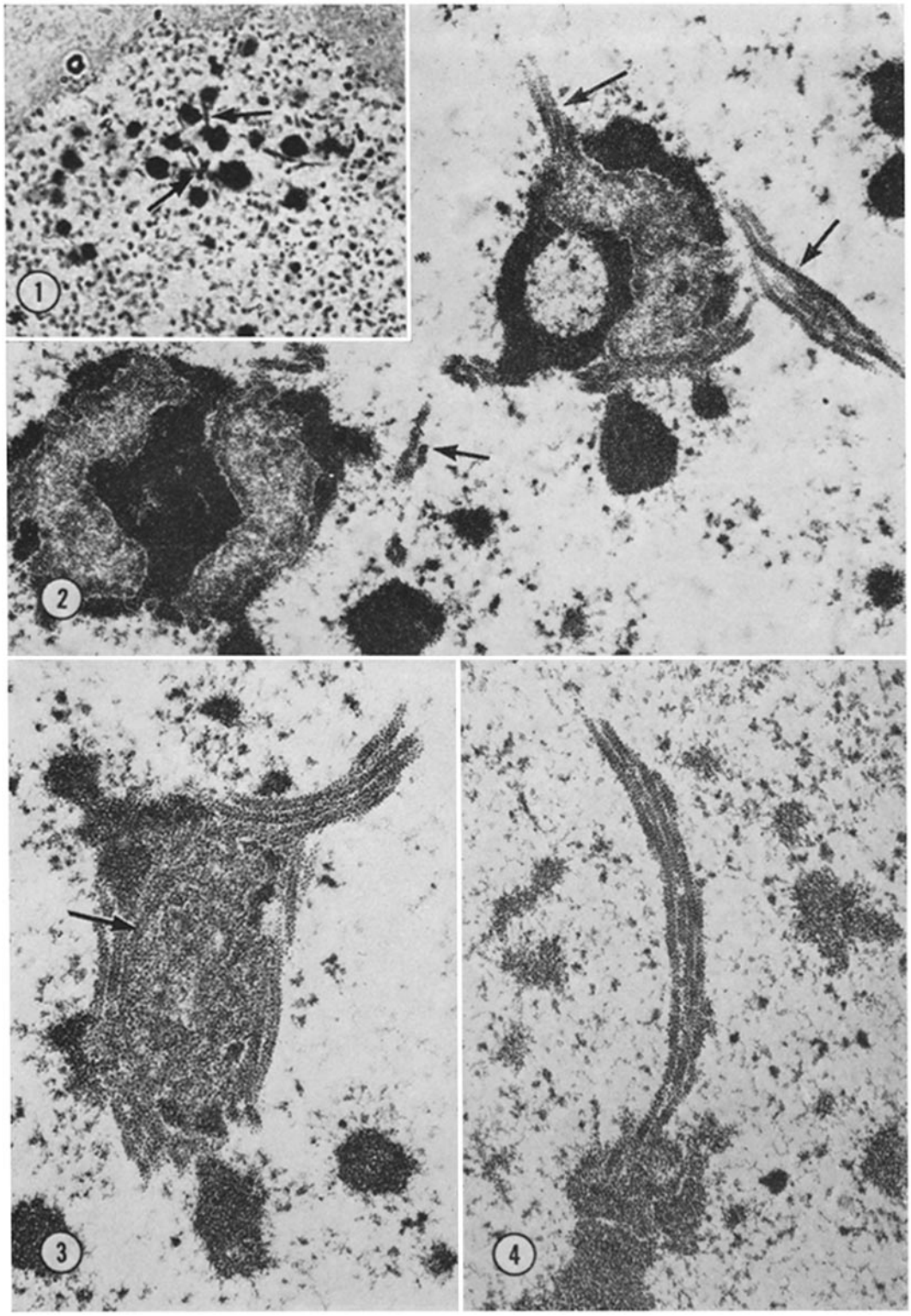
The nucleolar extrusions of the *Aplysia* giant neurons may represent an extremely specialized component peculiar to these unusual cells, or they could represent a more general phenomenon which is made visible by the tremendous synthetic demands placed on the giant cells. The latter possibility is made less remote by the demonstration of similar structures in other cells. Beams and Sekhon (1968) have described a lamellar structure extending from the nucleoli in oocytes of a centipede (*Scutigera forceps*). The lamellae are composed of granular elements which appear to be arranged in a helical configuration. Comparable structures which are less differentiated have been designated nucleonemata in striated muscle fibers of *Amblystoma* larva (Porter, 1954), in *Planaria* parenchymal neoblasts (MacRae, 1964), in oogonia of the third instar larva of *D. immigrans* (Mahowald, 1971), in cells of the flies *Glypotendipes*

FIGURE 1 A light micrograph of a Toluidine blue-stained 1 μ plastic section of a giant cell of the abdominal ganglion. The nucleoli in these cells are situated within the central portion of the "open-faced" nucleus and occur in large numbers. In this section, 20 nucleoli are evident as the densely stained structures. Several of the nucleoli bear extrusions at their periphery (arrows). × 1300.

FIGURE 2 An electron micrograph of a portion of the nucleus showing the sparse distribution of componponents in the nucleoplasm and two nucleoli containing granular and fibrillar regions. Both nucleoli have extrusions emanating from them (arrows) consisting of particles attached to a central core. × 21,000.

FIGURE 3 An electron micrograph showing the nucleolar extrusions emanating from a nucleolus. In one region (arrow), these extrusions appear to be associated with the granular region of the nucleolus. × 22,700.

FIGURE 4 This electron micrograph demonstrates the parallel orientation of the extrusions and the length which they achieve. This aggregate approximates 3 μ in length. × 23,400.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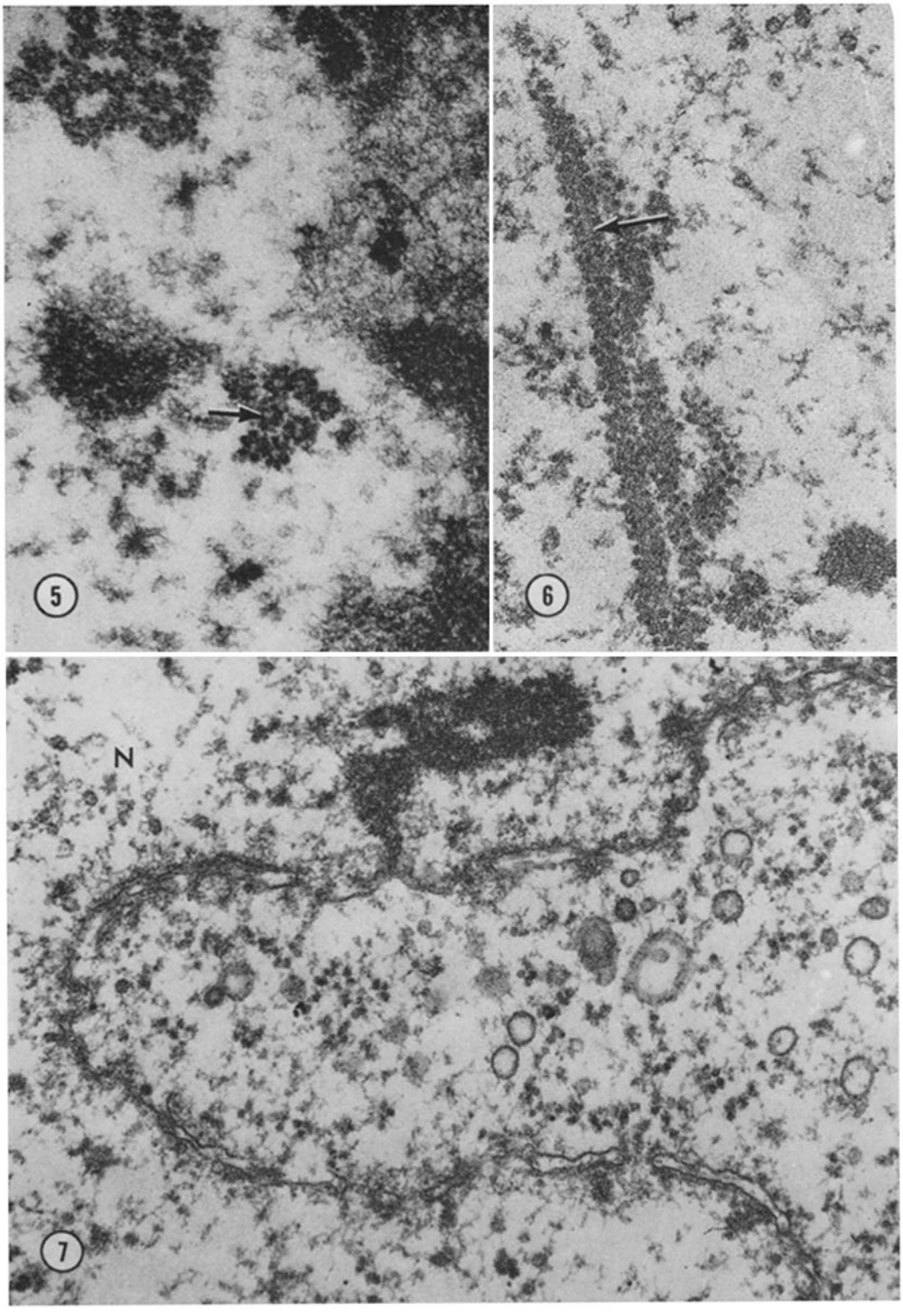
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lobiferus and *G. barbipes* (Busch and Smetana, 1970), in the dinoflagellate *Gyrodinium kohni* (Kubai and Ris, 1969), and in the neurons of another mollusc, *Aplysia vaccaria* (unpublished observation). Many reports have been made of ribosomes or ribosome-like elements attached in helical arrays (for review see Monneron et al., 1971). However, these varied helical structures show differences from the nucleolar extrusions described here in that, in most cases, they are found in the cytoplasm and do not contain a central matrix.

The functional implications of the nucleolar extrusions remain problematic and require physiological experiments for clarification. However, the morphology of the nucleolar extrusions is consistent with the interpretation that the extrusions are dynamic structures. Several lines of evidence indicate that the 45s precursor molecule of ribosomal RNA is synthesized in the fibrillar zone of the nucleolus and that cleavage products representing processed RNA are subsequently found in the more peripheral granular zone (Das et al., 1970). Narayan and Birnstiel (1969) have isolated ribonucleoprotein particles from nucleoli of liver cells. These particles contain the 35s intermediate of ribosomal RNA processing and morphologically resemble the granular elements in the nucleolus. Extrapolating from these and other studies which support the centrifugal flow of RNA from nucleolar core to cortex (Karasaki, 1965; Granboulan and Granboulan, 1967; Gueskens and Bernhard, 1966), it seems reasonable that the nucleolar extrusions in *Aplysia* neurons may represent the passage of nucleolar products from the fibrous zone into the nucleoplasm. Given this assumption, the nucleolar extrusions appear to originate in the fibrillar regions of the nucleolus and terminate in the nucleoplasm where the complex probably dissociates.

The movement of RNA precursors from the site of synthesis in the fibrillar zone to the granular region of the nucleolus is well documented. However, the detailed sequence of morphological events underlying this process have escaped analysis. Furthermore, a morphological gap exists between the granular zone of the nucleolus where 28s and 18s RNA have been identified and the appearance of ribosomal subunits in the cytoplasm. The highly ordered structure of the nucleolar extrusions of *Aplysia* may allow us to assess some of these transitional events, particularly those which occur between the synthesis of the ribosomal precursor and its movement into the nucleoplasm.

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FIGURES 5 and 6 Higher magnification of the nucleolar extrusions sectioned transversely (Fig. 5, × 81,500) and longitudinally, (Fig. 6, × 56,300). A central core (arrow) is present which approximates 250 A, and attached to this core in an apparent helical array are spherical electron-opaque particles. These spherical particles approximate 175 A in diameter, which is 75% of the size of cytoplasmic ribosomes.

FIGURE 7 An electron micrograph showing the nuclear-cytoplasmic boundary of the ganglion cell with an intervening nuclear envelope. The nucleus (*N*) contains fibrillar and particulate material and a portion of a chromosome attached to the nuclear envelope. Fibrillar material is present on the nuclear and cytoplasmic faces of the nuclear envelope and within the nuclear pores. The nucleolar extrusions have never been observed in contact with the nuclear envelope or its pores. × 55,700.

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