SMALL GRANULES IN THE AMPHIBIAN OOCYTE NUCLEUS AND THEIR RELATIONSHIP TO RNA*

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Plate 132

Recent electron microscopic studies on the structure of the cytoplasm have demonstrated the presence of small granules in many types of basophilic cells (1, 2, 3). These small granules average about 150 A in diameter, though in some tissues they may range up to 200 A or more. They are probably ribonucleoprotein in nature (1, 4). Similar particles are abundant in nucleoli (1, 5), again suggesting a correlation with the presence of ribonucleoproteins. The present note briefly describes small granules on the nuclear envelope and in the chromosomes of salamander oocytes.

Materials and Methods

Nuclear envelopes were removed from unfixed isolated oocyte nuclei of the salamander, *Triturus viridescens*, fixed in 1 per cent OsO_4 buffered to pH 7.4, and dried in air or by Anderson's (6) critical-point method (details in 7). Oocyte chromosomes were isolated in 0.1 M KCl, fixed in formaldehyde vapor followed by buffered 1 per cent OsO_4, embedded in *n*-butyl methacrylate, and sectioned (details in 8).

Nuclear Envelope

As shown in Fig. 1, the nuclear envelope is covered rather uniformly with annular structures 1000 to 1200 A in outside diameter. Such annuli, first described by Callan and Tomlin (9) in salamander oocyte nuclear envelopes, have now been seen in many different tissues by a number of workers. The individual annuli are composed of a ring of small particles, best seen in envelopes dehydrated according to Anderson's critical-point method (Fig. 2). The diameter of the small particles of Fig. 2 is 187 ± 5 A ($n = 30$), although the particles appear somewhat larger in air-dried, shadowed preparations. The small granules are probably closely related to the similarly sized particles of the cytoplasm for the following reasons. The particles of the cytoplasm are frequently associated with the membranes of the endoplasmic reticulum (1, 2, 3). These membranes and the outer membrane of the nuclear envelope are sometimes continuous and seem to constitute a single system (10). The cyto-

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plasmic particles are occasionally arranged in whorls or rosettes resembling loosely aggregated annuli (2, 11), and at times the cytoplasmic membranes display annuli disposed with all the regularity and clarity of the annuli of the nuclear envelope (12). It is possible, therefore, that the annuli of the nuclear envelope are composed of small ribonucleoprotein particulates similar to those of the cytoplasm.

Chromosomes

It is known that ribonucleoproteins occur within the nucleus not only in the nucleolus but also in the chromosomes proper (e.g. 13, 14, 15). RNA has been demonstrated in the lateral loops of the lampbrush chromosomes of salamander oocyte nuclei (8). These loops, moreover, contain no DNA demonstrable by the Feulgen reaction. Fig. 3 shows the structure of the loop regions as seen in a thin section of an isolated lampbrush chromosome. Apparently these RNA-containing regions of the chromosomes consist of fine particles, which here have a diameter of 294 $\pm$ 10 A (n = 78). Lafontaine and Ris (16) have suggested that these "particles" are actually sections through numerous microfibrils, a view which can perhaps be tested by examination of serial sections. The particles vary in diameter, however, and these variations I interpret as due in part to differences in the plane of sectioning through fairly uniform particles.

The giant chromosomes of dipteran salivary glands also contain RNA, particularly in the so-called Balbiani-ring regions (Gall, unpublished data; *cf.* reference 17). Beermann and Bahr (18) have described accumulations of dense particles about 300 A in diameter in these regions of sectioned chromosomes of *Chironomus*.

SUMMARY

Small particles (100 to 300 A in diameter) are seen in sections of nucleoli, the loops of the amphibian lampbrush chromosomes, and the Balbiani-ring regions of dipteran salivary-gland chromosomes. All of these structures contain cytochemically demonstrable RNA. Furthermore, the annuli seen on the nuclear envelope are composed of small particles which are similar to or identical with those commonly associated with the endoplasmic reticulum. It seems likely that ribonucleoproteins are organized as small particulates in the nucleus as well as in the cytoplasm.

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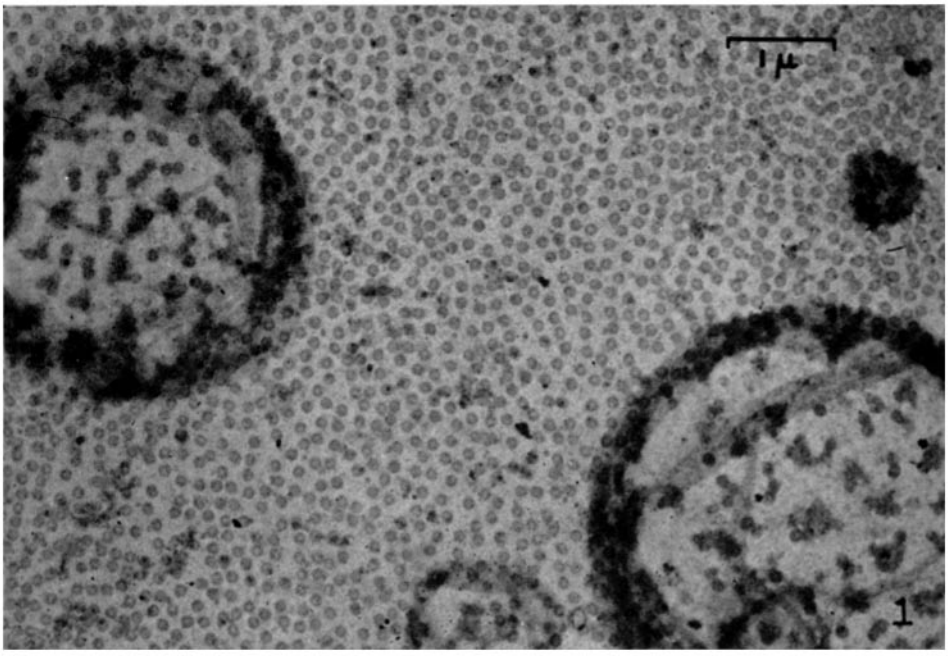
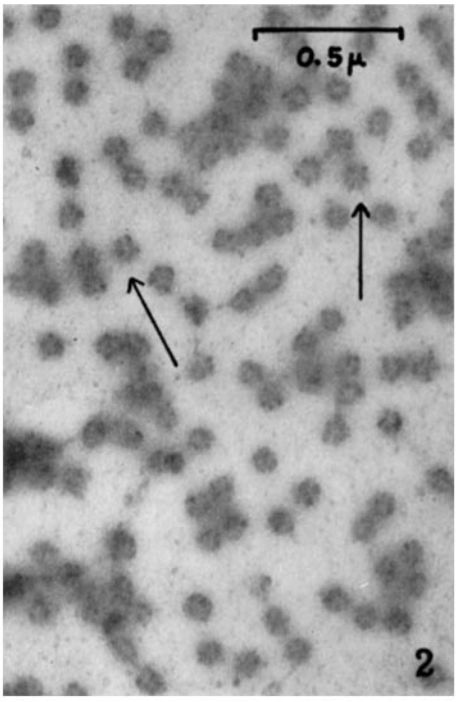
EXPLANATION OF PLATE 132

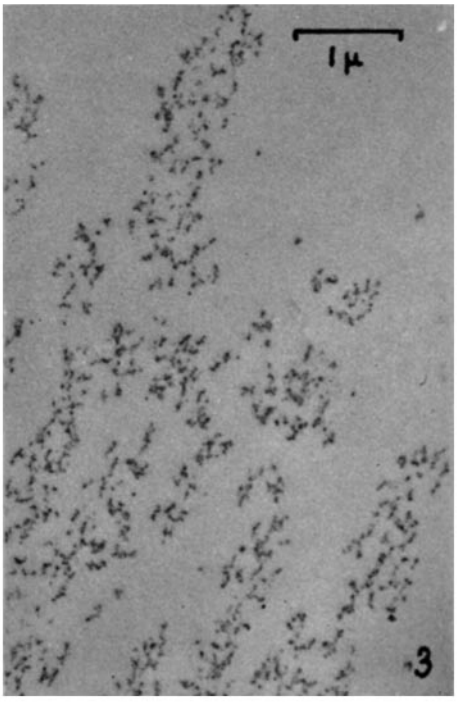
FIG. 1. Surface view of the nuclear envelope from an oocyte of the salamander, *Triturus viridescens*; isolated in 0.1 M KCl from an unfixed nucleus, fixed in 1 per cent OsO_4, pH 7.4, and air-dried. Note the numerous annuli identical to those often seen in sectioned material. The very large spheres may be similar to the "nuclear blebs" described by Anderson (19) on isolated rat liver nuclei. × 13,900.

FIG. 2. Higher magnification of the nuclear envelope surface from an oocyte of *T. viridescens*. In this specimen, dried by Anderson's critical-point method, the annuli are seen to consist of a ring of small dense particulates about 190 A in diameter. × 38,600.

FIG. 3. Thin section through the loop regions of an isolated lampbrush chromosome from an oocyte of *T. viridescens*. The chromosome was first isolated from the unfixed nucleus, fixed in formaldehyde vapor followed by 1 per cent OsO_4, pH 7.4, and sectioned in methacrylate. Note the numerous fine particulates averaging about 290 A in diameter. The region of the chromosome shown here is Feulgen-negative, but contains cytochemically demonstrable RNA. × 14,100.







(Gall: Small granules in amphibian oocyte nucleus)