AN ELECTRON-MICROSCOPE STUDY OF THE EXTRANUCLEOLAR BODIES DURING GROWTH OF THE OOCYTE IN THE PREPUBERTAL MOUSE

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SUMMARY

The dictyate nucleus of the growing mouse oocyte exhibits, besides the nucleolus, 3 ultrastructurally distinct types of smaller formed entities referred to as extranucleolar bodies. The extranucleolar bodies of the first type (fibrillogranular bodies) consist of intermingled masses made up of closely arranged convoluted fibrils, 6-10 nm in width, interspersed with electron-dense granules approximately 15 nm in diameter. The extranucleolar bodies of the second type (fibrillar bodies) are composed of an entanglement of loosely arranged convoluted fibrils 4-10 nm in diameter; such bodies are also characterized by the presence within their mass of irregularly shaped widely scattered islands of varying sizes made up of densely packed fibrillar material exhibiting a somewhat greater electron opacity. The extranucleolar bodies of the third type (coiled bodies) appear to consist of an aggregate of highly contorted threads, the thickness of which varies from 20 to 35 nm; the threads themselves are composed of bundles of fibrils 5 nm thick which are irregularly twisted along their axis.

An attempt is made to interpret these morphological findings in the light of current knowledge concerning the architectural and functional organization of the oocyte nucleus in general during the protracted dictyate stage of meiotic prophase. Our observations would be consistent with the view that the various types of extranucleolar bodies are morphological expression—like the puffs of the polytene and the loops or spheroids of the lampbrush chromosomes—of differential gene activity on the part of the localized regions of the chromosomes during oocyte growth.

INTRODUCTION

Previous observations have revealed that the dictyate nucleus of the growing mouse oocyte exhibits, besides the nucleolus, a number of smaller formed entities referred to as extranucleolar bodies (Chouinard, 1970, 1971). The occurrence of such extranucleolar bodies in the nucleus of the growing mammalian oocyte has been recorded only incidentally (Wartenberg & Stegner, 1960; Rhodin, 1963; Stegner, 1967; Baker & Franchi, 1967; Hertig & Adams, 1967).

It is the purpose of this paper, therefore, to provide a descriptive account of the occurrence, topographical distribution and ultrastructural features of the extranucleolar bodies in question during the entire growth period of the oocyte in the prepubertal mouse.
MATERIALS AND METHODS

Litters of ICR albino mice, obtained from Canadian Breeding Farm, St Constant, Province of Québec, were used. Preliminary observations had revealed that, in prepubertal mice of this strain, growth of a number of oocytes is initiated on the day of birth and completed by postnatal day 14. In order to secure all developmental stages of the growing oocyte in question – in principle, the largest oocytes to be observed in any given ovary at any given time post partum – neonatal mice were sacrificed at daily intervals from birth up to 14 days. All our observations have been made on these growing oocytes. It should be stressed at this point that, at each postnatal day, the oocytes and follicles selected for study were among the largest present in the ovary and that none showed any visible sign of involution or atresia. Thus it can be confidently assumed that these oocytes were, at the time of fixation, in a normal and active state of growth and differentiation.

The techniques employed for the study of the growing mouse oocyte by both light and electron microscopy were described previously (Chouinard, 1971).

OBSERVATIONS

For descriptive purposes, the growth period of the oocytes studied – i.e. those in which growth is initiated on the day of birth and completed by postnatal day 14 – will be divided into 3 successive stages depending on the extent of follicle development, and these will be referred to as the unilaminar (postnatal day 1–4), the bilaminar (postnatal day 5–8) and the plurilaminar follicle stages (postnatal day 9–14). A detailed description of the follicle and contained oocyte at each of these stages was presented in an earlier paper (Chouinard, 1971). Besides the nucleolus, the nucleus of the growing mouse oocyte exhibits a number of smaller formed bodies, stained with toluidine blue and Feulgen negative, which become especially conspicuous during the bilaminar and the plurilaminar follicle stages; 3 ultrastructurally distinct types of such extranucleolar bodies have been identified so far and, for the sake of brevity and convenience, these will be designated descriptively throughout this paper as the fibrillogranular, the fibrillar and the coiled bodies.

The fibrillogranular bodies

During almost the entire growth period of the oocyte, the nucleus is seen to contain a number of fibrillogranular bodies which usually exhibit a rounded profile. Such bodies, which first become clearly recognizable as such on or around postnatal day 2, undergo a gradual increase in size during the unilaminar, the bilaminar and the plurilaminar follicle stages; some of these bodies may reach up to 2·5 μm in diameter in the nucleus of the fully grown oocyte. From an examination of a number of serial 0·5-μm thick sections stained with toluidine blue, it appears that, at all stages of oocyte growth, the moderately stained fibrillogranular bodies are randomly distributed within the nuclear cavity; they usually lie widely separated from one another and at varying distances from the nucleolus and the nuclear envelope. Up to 4 such fibrillogranular bodies have been observed in a single nuclear profile thus indicating that their number per nucleus is relatively high. The fibrillogranular bodies bear no obvious topographical relationship with the other 2 types of extranucleolar bodies to be described below, namely the fibrillar and the coiled bodies. At the ultrastructural
level, the fibrillogranular bodies are seen, throughout the growth period of the oocyte, to consist of intermingled masses made up of closely arranged convoluted fibrils, 6-10 nm in width, interspersed with electron-dense granules approximately 15 nm in diameter; these constituent elements appear to be embedded in some sort of ill-defined matrical substance ultrastructurally similar to that of the surrounding nucleoplasm (Figs. 1-9).

The fibrillar bodies

The fibrillar bodies, which usually exhibit a round or oval profile, become recognizable as such within the oocyte nucleus during the transition from the unilaminar to the bilaminar follicle stage (postnatal days 5 and 6). From then on, the fibrillar bodies undergo a gradual increase in size until the later part of the plurilaminar follicle stage is reached (postnatal day 12 or 13); some of these fibrillar bodies may then reach up to 2-5 \( \mu m \) in diameter. Toward the end of the plurilaminar follicle stage, the fibrillar bodies become increasingly difficult to delineate and, as the oocyte completes its growth, they disappear completely from view. Fibrillar bodies are, indeed, not observed in the nucleus of the fully grown oocyte. Examination of a number of serial sections under light microscopy reveals that the lightly stained fibrillar bodies, during both the bilaminar and plurilaminar follicle stages, can be found anywhere in the nucleoplasm except in contiguity with the nuclear envelope; as a rule, they lie widely separated from one another and at varying distances from the nucleolus. Occasionally, a fibrillar body is seen in juxtaposition to the nucleolus or separated from that organelle by a thin zone of low-density material (Fig. 20). The fibrillar bodies show no obvious topographical relationship to the previously described fibrillogranular bodies, but in 7 out of 10 nuclear profiles examined, they are found in proximity to the third type of extranucleolar bodies, namely the coiled bodies (Figs. 13, 20). In sections for light microscopy, on average about 2 widely scattered fibrillar bodies are observed in a single nuclear profile during the bilaminar and plurilaminar follicle stages. At the fine-structural level, the fibrillar bodies are always seen to be made up of an entanglement of loosely arranged convoluted fibrils, 4-10 nm in diameter, embedded in a matrical substance which bears strong resemblance to that of the nucleoplasm; such agranular bodies are also characterized by the presence within their mass of irregularly shaped widely scattered islands of varying sizes consisting of closely packed fibrillar material exhibiting a somewhat greater electron opacity (Figs. 10-13, 20).

The coiled bodies

As in the case of the fibrillar bodies, the coiled bodies, which usually exhibit a round, oval or slightly angular contour, first become identifiable as such during the transition from the unilaminar to the bilaminar follicle stage (postnatal days 5 and 6). From then on, these coiled bodies undergo a gradual increase in size until the later part of the plurilaminar follicle stage is reached (postnatal day 12-13); some of the coiled bodies may then reach up to 0-9 \( \mu m \) in diameter. As the oocyte completes its growth, the coiled bodies become increasingly difficult to delineate as such and
eventually they disappear from view. The nucleus of the fully grown oocyte indeed contains no recognizable coiled bodies. Examination under light microscopy of serial sections of the oocyte nucleus reveals that the densely stained coiled bodies usually lie well separated from one another and may be located anywhere in the nucleoplasm except in close proximity or in contact with the nucleolus and the nuclear envelope. The coiled bodies show no obvious topographical relationship with the fibrillorgranular bodies, but, in 7 out of 10 nuclear profiles examined, they are seen in proximity to the fibrillar bodies (Figs. 13, 20). We have observed up to three coiled bodies per nuclear profile of the growing oocyte. From an ultrastructural standpoint, the coiled bodies are always seen to consist of an aggregate of dense masses, roughly rounded or elongated in contours, of comparable diameter and electron opacity and spaced at a rather uniform distance apart (Figs. 14–19). This characteristic appearance is probably best interpreted as resulting from the longitudinal, oblique and transverse sectioning of randomly disposed, highly contorted threads, 20–35 nm in diameter. Examination at high magnification indicates that the threads in question consist of bundles of fibrils 50 nm thick which are irregularly twisted along their axis. The interspaces among the threads are fairly uniform and appear to contain a matrical material matching that of the surrounding nucleoplasm in both texture and electron density. Occasionally, the coiled body is seen to contain one or two small eccentrically located rounded lacunar spaces; such spaces often display a centrally located core of more electron-dense chromatin-like material (Fig. 18).

**DISCUSSION**

My observations reveal the occurrence of 3 ultrastructurally distinct types of extranucleolar bodies within the dictyate nucleus of the growing mouse oocyte. In the following discussion, an attempt will be made to interpret these morphological findings in the light of current knowledge concerning the architectural and functional organization of the oocyte nucleus in general during the protracted dictyate stage of meiotic prophase.

In most female mammals which have been investigated, germ cells enter the prophase of meiosis during foetal life and reach the diplotene stage shortly before or immediately after birth (Franchi, Mandl & Zuckerman, 1962; Franchi & Mandl, 1962; Maulcon, 1967; Peters, 1970). After rapidly traversing diplotene, the oocytes enter the lengthy dictyate stage during which most of the oocyte growth occurs. During the diplotene stage, which is probably only a few hours in duration (at least in the case of rodents), the bivalents are seen to exhibit a whiskery appearance with each chromosomal axis provided laterally with fine lateral loop-shaped projections (Tsuda, 1965). As the oocyte reaches the dictyate stage, the chromosomes lose their whiskery appearance and, from then onwards, can no longer be visualized as such either under light or electron microscopy. Although the exact configuration of the chromosomes in the dictyate nucleus is not yet known with certainty, most workers agree, however, that the bivalents are in a highly diffuse state resulting from an unravelling of both the chromosomal axes and their lateral projections throughout the nuclear cavity (Franchi & Mandl,
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From an organizational standpoint, it is generally believed that the dictyate chromosomes observed in the growing oocytes of eutherian mammals are homologous to the so-called lampbrush chromosomes seen in the growing oocyte of non-mammalian vertebrates. Both the dictyate and the lampbrush chromosomes rapidly incorporate radioactive precursors of RNA and are thus considered to be the sites of intense transcriptive activity during oocyte growth (Gall & Callan, 1962; Izawa, Allfrey & Mirsky, 1963; Davidson, Crippa, Allfrey & Mirsky, 1966; Callan, 1969; Oakberg, 1967, 1968; Baker, Beaumont & Franchi, 1969). In the growing oocyte of non-mammalian vertebrates, morphologic expression of these transcriptive activities is provided by the presence, in addition to numerous nucleoli, of several types of loop-shaped and spheroidal bodies, each with highly specific morphologies. Such bodies, which are closely associated with the lampbrush chromosomes, are thought to represent the accumulation products of specific gene activities consisting primarily of RNA and associated proteins (Davidson, 1968; Callan, 1969; Davidson & Hough, 1969). In the mouse, the observations presented in a previous paper have been shown to be consistent with the view that the nucleolus is also the site of massive synthesis and accumulation of nucleolar material during growth of the oocyte (Chouinard, 1971).

In the light of the above findings and considerations, it might not be too farfetched to postulate that the 3 ultrastructurally distinct types of extranucleolar bodies present in the growing mouse oocyte also represent accumulation products of specific gene activities. The relevant observational evidence in favour of the view that genomic sites, other than those associated with the nucleolus, are also capable of synthesis and accretion of gene products, during growth of the mouse oocyte, can be summarized as follows. (1) Throughout the growth period of the oocyte, the extranucleolar bodies are seen to bear no structural or topographical relationships with the nucleolus or any of its components. Thus the possibility that the nucleolus as such may be somehow instrumental in the formation of the extranucleolar bodies in question must be excluded. (2) Throughout the growth period of the oocyte, the extranucleolar bodies are classified into 3 ultrastructurally distinct types with no occurrence of transitional forms suggesting conversion of one type into another. Thus it can be surmised that each of the 3 types of extranucleolar bodies has a separate chromosomal origin. (3) During growth of the oocyte, the extranucleolar bodies undergo a gradual increase in size as would be expected if these bodies were sites of synthesis and accretion of genic and associated materials. (4) The occurrence of the extranucleolar bodies can be correlated with the stage of differentiation and development of the mouse oocyte. The fibrillogranular bodies, for instance, form during the unilaminar follicle stage but the fibrillar and coiled bodies make their appearance only during the transition from the unilaminar to the bilaminar follicle stage. Also the fibrillogranular bodies are still present in the fully grown oocyte but the fibrillar and coiled bodies disappear toward the end of the plurilaminar follicle stage. As in the case of the puffs on the polytene and the loops or spheroids on the lampbrush chromosomes (cf. Callan 1963; Berendes, 1968; Hess & Meyer, 1968; Callan, 1969; Hess, 1970; Berendes & Thijsse, 1971), the foregoing observations are probably best interpreted by assuming that the extra-
nucleolar bodies of the growing mouse oocyte are also the phenotypic manifestation of stage-specific chromosomal activities. (5) The extranucleolar bodies of the growing mouse oocyte bear some resemblance to other chromosomally associated RNP-containing structures, distinct from the nucleolus, which have been described by a number of authors as normal constituent of nucleoplasm in oocytes of lower forms (cf. Das & Alfert, 1966; Allen & Cave, 1969; Halkka & Halkka, 1968; Cave & Allen, 1971) as well as in a variety of mammalian somatic cells (cf. Toro & Rohlich, 1966; Monneron & Bernhard, 1969; Hardin, Spicer & Greene, 1969; Grillo, 1970; Le Beux, 1971). It is of interest to note that the extranucleolar structures in question have usually been observed in functionally hyperactive cells.

In summary, our observations would be consistent with the view that the various types of extranucleolar bodies are morphological expression – like the puffs of the polytene and the loops or spheroids of the lampbrush chromosomes – of differential gene activity on the part of localized regions of the dictyate chromosomes during oocyte growth. The extranucleolar bodies – like the nucleolus – would then correspond to specialized structural devices allowing for the formation, stabilization and packaging of essential gene products possibly derived from redundant DNA sequences. In relation to this problem, it should be recalled that, according to current thinking, the early development of the embryo is mainly directed by gene products which have been presynthesized and stored during the protracted dictyate or diplotene stage of first meiotic prophase (Davidson et al. 1966; Crippa, Davidson & Mirsky, 1967; Davidson, 1968).

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REFERENCES


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Figs. 1–9 on pages 64 and 65. Electron micrographs depicting the ultrastructural organization of the extranucleolar bodies of the first type (fibrillogranular bodies) during the unilaminar (Fig. 1, postnatal day 2; Fig. 2, postnatal day 3; Fig. 3, postnatal day 4), the bilaminar (Fig. 4, postnatal day 6; Figs. 5 and 6, postnatal day 8), and the plurilaminar follicle stages (Figs. 7 and 8, postnatal day 12; Fig. 9, postnatal day 14). Throughout the growth period of the oocyte, the fibrillogranular bodies are seen to consist of intermingled masses made up of closely arranged convoluted fibrils, 6–10 nm in width, interspersed with electron-dense granules approximately 15 nm in diameter; these constituent elements appear to be embedded in some sort of ill-defined matrical substance ultrastructurally similar to that of the surrounding nucleoplasm. × 38,000.
Figs. 10-13. Electron micrographs depicting the ultrastructural features of the extranucleolar bodies of the second type (fibrillar bodies) during the bilaminar (Fig. 10, postnatal day 5; Fig. 11, postnatal day 6) and the plurilaminar follicle stages (Fig. 12, postnatal day 9; Fig. 13, postnatal day 11). During these 2 stages, the fibrillar bodies are seen to consist of an entanglement of loosely arranged convoluted fibrils 4-10 nm in diameter; such bodies are also characterized by the presence within their mass of irregularly shaped widely scattered islands of varying sizes made up of closely packed fibrillar material exhibiting a somewhat greater electron opacity. An extranucleolar body of the third type (coiled body, cb) is seen in the vicinity of the fibrillar body (fb) depicted in Fig. 13. × 38000.
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Figs. 14–20. Electron micrographs depicting the ultrastructural organization of the extranucleolar bodies of the third type (coiled bodies) during the bilaminar (Figs. 14 and 15, postnatal day 6; Figs. 16 and 17, postnatal day 8) and the plurilaminar follicle stages (Figs. 18–20, postnatal day 11). During these 2 stages, the coiled bodies are seen to consist of an aggregate of dense masses, roughly rounded or elongated in contours, of comparable diameter and electron opacity, and spaced at a rather uniform distance apart. This characteristic appearance is probably best interpreted as resulting from the longitudinal, oblique and transverse sectioning of randomly disposed, highly contorted threads, 20–50 nm in diameter. Fig. 20 shows 2 coiled bodies (cb) in the vicinity of a fibrillar body (fb), which is itself in contact with the surface of the densely stained nucleolus (n). $\times 38,000$. 
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