ULTRASTRUCTURE OF LATE OOCYTE NUCLEI IN RANA TEMPORARIA

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SUMMARY

The ultrastructural organization of the nucleus during late oogenesis in Rana temporaria was examined in normal and in hormone-stimulated maturing oocytes in vitro. At this stage numerous nucleoli are assembled around a knot of highly contracted chromosomes (the karyosphere) making up a karyosphere capsule. The nucleoli are fibrillar. They bear no pro-ribosomal granules and do not synthesize RNA. This period is characterized by segregation of nucleolar material into core and cortex. The nucleoli are of irregular shape. The presence of micronucleoli and nuclear bodies indicates an intensive fragmentation of nucleolar material.

In the central fibrous zone of the capsule separating the chromosomes from the nucleoli, abundant accumulations of annuli lacking any membranous component occur. The annuli are connected by fibrous material and are regularly packed, forming peculiar pseudomembranes which are connected directly to the chromatin. Pseudomembranes, though less abundant and less regularly packed, are also found in the middle zone of the capsule. Along with annuli, membranous areas of various sizes and shapes are found in the pseudomembranes. Pseudomembranes are linked to micronuclei by granular filaments 20-30 nm thick.

A variety of membranous structures in the form of intranuclear annulate lamellae or membranous tubular formations was detected in the peripheral zone of the capsule and outside it.

The conclusion is drawn that the fibrous component of the karyosphere capsule consists of different membranous and pseudomembranous structures. It is suggested that chromatin participates directly in the formation of pseudomembranes and intranuclear membranous structures.

INTRODUCTION

The formation of a peculiar capsule around the centrally assembled chromosomes, or karyosphere, is typical of the oocytes of some invertebrates and vertebrates (see review by Gruzova, 1975). This phenomenon was described by some authors as the formation of 'a nucleus within the nucleus', since the chromosomes are separated from the rest of the nucleus by a kind of a 'sheath'. The nature and origin of these 'envelopes' or capsules were not known until recently. Recent electron-microscopic investigations of oocyte nuclei in insects have shown that either the material and derivatives of the synaptonemal complex in combination with elements of the nuclear membranes (Fiil & Moens, 1973), or peculiar products of the nucleoli and nucleolar extrachromosomal DNA (Zaichikova & Gruzova, 1973), are used for the formation of the capsule. In amphibians such isolation of chromosomes from the rest of the nuclear contents was described during oogenesis in frogs (Wagner, 1923). The findings of Wagner have been re-examined using modern cytological and cytochemical methods. It has been shown that the capsule (150 μm wide) appears around the knot of lamp-
brush chromosomes in the vitellogenic oocytes of *Rana temporaria* during the autumn–winter season. The capsule consists of numerous nucleoli, nucleolar threads, ribonucleoprotein granules and protein fibrous material of unknown nature (Gruzova & Parfenov, 1973; Parfenov & Gruzova, 1975a). At this time, chromosomes and nucleoli synthesize scarcely any RNA (Parfenov & Gruzova, 1975b). The capsule with its chromosomes was isolated from the nucleus by Callan’s method (Callan, 1966). In the process of isolation it was discovered that all components of the karyosphere are rather firmly bound together. Only the nucleoli may be detached by centrifugation, whereas the chromosomes remain in a sac of elastic material which is hard to tear with needles (Fig. 3) (Parfenov, 1974).

The light-microscopic level of investigation, however, does not reveal the nature of the nucleolar filaments and elastic fibrous material of the capsule. The purpose of this work was to describe the karyosphere capsule in *Rana* at the ultrastructural level and to try to elucidate the nature and sources of its fibrous material. Another aim was to compare the results with information on the karyosphere capsule in some insects, so that common features in the structure and origin of the capsule in invertebrates and vertebrates might be revealed.

**MATERIALS AND METHODS**

Oocytes with yolk (1200–1400 μm in diameter) from December gonads of *Rana temporaria* were used for electron-microscopic examination. In many cases maturation of these oocytes was stimulated by treatment in *vitro* with hypophyseal hormone (for methods see Parfenov & Gruzova, 1975a). Nuclei were isolated from oocytes fixed in 3-5% glutaraldehyde diluted in phosphate buffer at pH 7-4 and postfixed in 2% OsO₄ in phosphate buffer at pH 7-4. The material was embedded in Araldite and sectioned on a OM-J2 Ultratome (Reichert). The sections were stained with a saturated ethanolic solution of uranyl acetate and with lead citrate (Reynolds, 1963). Micrographs were taken with a JEM-7A electron microscope. Some of the grids with ultrathin sections, pretreated with 3% H₂O₂, were placed in 0.1% RNase solution (‘Reanal’, Hungary) for 2 h at 38 °C and then for 12 h at room temperature (Monneron & Bernhard, 1966).

**Abbreviations on figures**

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<td>ch</td>
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<td>fb</td>
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<td>tal</td>
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<td>interchromatin granules</td>
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Fig. 1. General appearance of the oocyte nucleus of *Rana temporaria* (an early stage of karyosphere formation). The fibrous central zone (arrows) is developed between the nucleoli and the chromosomes. ×240.

Fig. 2. Central part of the karyosphere of a stimulated oocyte. Note the fibrous component ×900.

Fig. 3. Central part of the karyosphere isolated from the oocyte nucleus by Callan’s method. Note the fibrous material (arrows) around the chromosomes. ×600.

Fig. 4. Irregular-shaped nucleolus from a winter oocyte. ×1000.
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RESULTS

The nuclei of both unstimulated (Fig. 1) and stimulated winter oocytes were subjected to electron-microscopic analysis. In oocytes stimulated in vitro the karyosphere retains its ordinary morphology but in this case the fibrillar material of the capsule is more readily visible. The ultrastructure of the karyosphere will be described moving from the centre to the capsule periphery.

The central zone of the karyosphere in stimulated oocytes is occupied by short diakineti c chromosomes. The chromosomes are surrounded by a rather wide fibrous zone separating them from the nucleoli. Electron-microscopic examination of this region revealed dense chromatin material and various structures associated with it (Fig. 5). Among these we distinguish the following. (1) Large accumulations of 25-nm interchromatin granules connected by fibrils (Fig. 6). Dense areas, probably of chromatin nature, occur frequently among the granules. Accumulations of granules are often seen in contact with chromatin, on the one hand, and with pseudomembranes (see below) on the other (Fig. 5). (2) Single or anastomosing fibrils about 20 nm thick are scattered in the karyosphere (Fig. 5). (3) Ring bodies, ranging from 0.1 to 1.5 μm in diameter are present (Figs. 5, 13). They bear a network of granules at the surface similar to interchromatin granules (Fig. 13). The ring consists of densely packed strands. The inner region of the ring body contains 1–3 thin fibrillar dense blocks (Fig. 13). The latter show a resemblance to the material of the nucleolar core (fibrillar centre). After RNase treatment the whole body becomes electron-transparent, whereas the peripheral granulo-fibrillar material retains its contrast. Similar ring bodies occur not only in the central karyosphere region but also among the nucleoli and outside the capsule in the karyolymph.

Examination of ultrathin sections showed that the fibrous barrier separating the chromosomes from the numerous nucleoli consists of a network of strands about 40–50 nm thick (Fig. 5). These strands often appear to join up, forming rings or hexagons. They appear superficially like a dashed line, due to alternating dark and light regions, 65–75 and 50–100 nm long, respectively. Higher magnification and the examination of sections in various planes convinced us that these strands are rows of annuli resembling those of the nuclear membrane. In transverse section, like the nuclear membrane annuli (Fig. 7), they appear as rings, 65–75 nm in diameter (Fig. 8). In longitudinal sections fibrillar material similar to that of the pore complexes in the nuclear membrane is located on both sides of the dense ring material. The annuli, however, are in this case connected by the same fibrillar material, not by a mem-

Figs. 5-9. Ultrastructure of the central zone of the karyosphere capsule.

Fig. 5. Accumulation of pseudomembranes (above) consisting of annuli; note rather numerous strands in the karyolymph (arrows). × 18,000.

Fig. 6. Intercrohamatin granules. × 46,000.

Fig. 7. Annuli of the nuclear membrane (transverse section). × 76,000.

Fig. 8. Annuli from the middle part of the karyosphere capsule. × 78,000.

Fig. 9. Chromatin directly connected with pseudomembranes. × 26,000.
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branous component. We have called them 'pseudomembranes', because they differ from the true nuclear membrane in the nature of the material connecting the annuli.

It is noteworthy that in many parts of the central region of the karyosphere the chromatin material comes into direct contact with the pseudomembranes and penetrates them (Fig. 9).

The middle part of the capsule is largely occupied by nucleoli. The width of the nucleolar zone may attain 50–100 μm. In normal winter oocytes most of the nucleoli are irregular and twisted in shape (Figs. 4, 10), while in hormone-stimulated maturing oocytes the majority of the nucleoli are spherical.

In both kinds of oocyte the nucleoli have a fibrillar structure and are practically devoid of granules (Fig. 10). The nucleolar structure is not homogeneous; each nucleolus consists of electron-dense fibrillar material in which the fibrils are 6–8 nm thick, with distinct less dense, though also fibrous, regions in which the fibrils are 3–4 nm thick (Figs. 10, 11). In the RNase-treated sections both zones look transparent, which indicates that they normally contain RNA. We suggest that these lighter regions correspond to the nucleolar core of amphibian nucleoli, containing DNP and RNP, while areas composed of electron-dense fibrils evidently correspond to the cortex and contain largely RNP. The absence of granules and low incorporation of [3H]uridine (Parfenov & Gruzova, 1975b) indicate the cessation of synthesis of pro-ribosomal particles in the nucleoli. This process is accompanied by segregation and fragmentation of the nucleolar material and production of numerous micronucleoli.

The appearance of micronucleoli has been described in *Xenopus* oocytes (Van Gansen & Schram, 1968). The micronucleoli are from 0.05–0.1 to 2–3 μm in diameter and they occur both along with the nucleoli and outside the karyosphere capsule (Figs. 12, 14). Micronucleoli (2–3 μm in diameter) are located at the periphery of the capsule. Most of the micronucleoli are fibrillar structures that cannot be distinguished from the electron-dense nucleolar cortex. It can often be seen that they are arranged near the nucleolar surface and sometimes linked to it through fibrils. It is probable that the twisted shape of nucleoli is accounted for by fragmentation of the nucleolar material and even promotes this process. Along with all these structures the middle zone of the capsule is also found to contain accumulations of pseudomembranes similar to those at the centre of the capsule. Unlike the latter, however, the pseudomembranes in this zone occupy smaller areas constituting 'islets' among nucleoli and micronucleoli (Fig. 16). They anastamose less freely, are more elongated and seldom convert into

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**Fig. 10.** Fibrillar nucleolus of irregular shape from a non-stimulated oocyte. Note the fibrillar centre of the nucleolus (arrow). × 6000.

**Fig. 11.** Part of the nucleolus outlined in Fig. 10 at higher magnification. Granular material is lacking. × 22000.

**Fig. 12.** Double fibrillar micronucleolus. × 24000.

**Fig. 13.** Nuclear ring body. Note the network of interchromatin granules in contact with it. × 53000.

**Fig. 14.** Micronucleolus with a halo of fibrils and granules. Note the ring nuclear body in contact with it (arrow). × 42500.
vesicles (Fig. 15). The analysis of such islets shows that small membranous regions occur among the annuli. They appear either as vesicles of various sizes, sometimes associated with a number of annuli, or as extended regions of double or single membrane lacking pore elements (Fig. 15). Membranous vesicles up to 0.5 μm in diameter and filled with electron-dense material are found here and there (Figs. 15, 18). Membranous regions as well as pseudomembranes are clad in fibrillar matrix. The pseudomembranes often merge with tubular membranous structures. Sometimes these have pore complexes and then resemble intranuclear annulate lamellae (Fig. 17). It was noted that micronucleoli are as a rule located in close proximity to pseudomembranes. Not infrequently it could be seen that a micronucleolus is attached to a pseudomembrane via a fine granular thread (Fig. 16).

Abundant membranous components are found in the peripheral zone of the karyosphere capsule. These are the membrane vesicles described before, together with tubular or lamellar structures (Figs. 17, 18). The inter-membrane distance in such structures varies from 80 to 130 nm. These structures contain pore complexes or their analogues but here they are not so regularly arranged as in the nuclear membrane or in pseudomembranes of the capsule central zone. The observed variations of intranuclear membranous structures in combination with annuli are diagrammed in Fig. 18.

**DISCUSSION**

*The fibrous component of the capsule.* The ultrastructural analysis shows that the fibrous component is represented by various derivatives of the nuclear envelope, such as pore complexes arranged as pseudomembranes, microfibrils (10–20 nm thick), membrane vesicles, intranuclear annulate lamellae and variations of membranous tubular structures. The latter are similar to the nucleolar ‘tails’ described in the oocytes of 2 amphibian species by Kezer, McGregor & Shabtach (1971), but unlike these authors we failed to observe membranous structures attached directly to nucleoli. We found, however, a number of structures intermediate between typical tubular structures ('nucleolar tails') devoid of pore complexes and annulate lamellae. Both of these can transform into pseudomembranes, consisting essentially of pore complexes (annuli). At the same time, there is a certain resemblance to intranuclear annulate lamellae (Kessel, 1968; Fiil & Moens, 1973) and narrow invaginations of the nuclear membrane (Fig. 18). It should be mentioned that at this stage of oocyte development the nuclear membrane is pleated (especially at the vegetative pole) and sends numerous long protrusions into the nucleus. Some of these protrusions are

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Figs. 15–17. Ultrastructure of peripheral region of the karyosphere capsule.

Fig. 15. Electron-dense body (arrow) near pseudomembranes consisting of annuli. Note the membranous structure (barred arrow). × 24,000.

Fig. 16. Accumulation of pseudomembranes among micronucleoli; the latter are connected with pseudomembranes by thin filaments (arrow). × 19,000.

Fig. 17. Intranuclear annulate lamellae. × 33,500.
very narrow, and extend into the nucleus for a distance of 30–40 μm. It is noteworthy that the karyosphere capsule starts its formation asymmetrically by accumulating nucleoli on the side facing the vegetative pole. It is at this pole that the nuclear envelope starts to disintegrate.

All our observations point to the conclusion that the formation of diverse mem-
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branous structures is related to translocation of marginal nucleoli, earlier attached to the nuclear membrane, to central regions of the nucleus. As a result, the karyosphere capsule is formed. Moving from the periphery to the centre of the germinal vesicle one may observe a gradual transition and replacement of membranous structures by pseudomembranes, the latter consisting exclusively of pore complexes lacking membranous components.

The observations on the formation of the karyosphere capsule of *Rana temporaria* demonstrate the autonomous existence of pore complexes outside the membranous component of the nuclear membranes. It seems surprising. Autonomous pore complexes in the nuclei of *R. temporaria* oocytes were also observed by Chentsov & Polyakov (1974). Free pore complexes, not infrequently associated with pseudomembranes, have also been described in mosquito oocyte nuclei (Fiil & Moens, 1973). These authors suggest that the fibrillar material lining the annuli may be a derivative of the central element of synaptonemal complexes. Autonomous pore complexes were also recently demonstrated in somatic cells by experiments in which the nuclear envelope was removed (Kirschner, Rusli & Martin, 1977; Sarah, Clawson, Rottman & Patterson, 1977).

What is the functional significance of the karyosphere capsule? It may play the role of a mechanical supporting structure, keeping the chromosomes together in the germinal vesicle and separating them from extrachromosomal nucleolar DNA. All this provides for normal reduction division. The capsule also performs a protective function in preserving the genetic apparatus after destruction of the oocyte nucleus. In this connexion the specific role of pseudomembrane pore complexes needs to be considered. The great number of pore complexes in the central regions of the capsule indicates convincingly that they are newly formed and not transferred from the nucleus periphery by means of membrane nucleolar structures. Thus a kind of ‘crystallization’ gradient of pseudomembranes is found between the periphery and the centre of the capsule. It is expressed as an increase of the number of pore complexes, their regular packing as closed vesicles, frequently of hexagonal form, and separation from the membranous component.

Micrographs showing pseudomembranes making contact with chromatin in the central part of the karyosphere occur so frequently that we cannot help suggesting a specific role of chromatin in the formation and arrangement of pseudomembrane material. A question arises as to what this chromatin material is: this has still to be clarified. We are inclined to think, however, that it is extrachromosomal DNA. Its source may be the numerous nucleoli which are one of the main components of the karyosphere capsule. ‘Staining’ of the *Rana temporaria* oocytes with $^3$H-actinomycin D reveals considerable amounts of DNA, not only in the nucleoli (Ebstein, 1969, and our unpublished data), but also in the internucleolar regions. The process of nucleolar segregation and fragmentation is likely to provide for release of this DNA from nucleoli in the form of numerous micronucleoli and nuclear bodies. In this connexion it is of interest that micronucleoli are frequently associated with pseudomembranes by thin fibrils.

Elimination of large amounts of nucleolar DNA during oocyte maturation has been
described in other amphibian species (Brachet et al. 1974). It should be also pointed out that formation of the capsule, both in insects (in Chrysopa perla according to Gruzova, Zaichikova & Sokolov, 1972; in Blaps lethifera – Gruzova, unpublished data) and in Rana temporaria (Parfenov & Gruzova, 1975b) proceeds where the level of RNA synthesis in the oocyte nucleus is low. It is not inconceivable that against the background of lowered RNA synthesis, proteins entering the nucleus (Gruzova, 1967; Parfenov & Gruzova, 1975b) assemble and arrange themselves into structures required by the oocyte for some reason. The nucleolar chromatin (in Chrysopa), or chromatin of unknown nature (in Rana and Blaps) evidently take a direct part in this process. In this connexion it is of interest that in Triturus oocytes crystalloid lamellar protein bodies arose in contact with nucleoli after inhibition of RNA synthesis with actinomycin D (Snow, 1972).

REFERENCES


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