Evolution of the nucleolar apparatus during oogenesis in Acipenseridae

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SUMMARY

Evolution of the nucleoli has been followed during oogenesis in the Acipenserid fishes, *Acipenser ruthenus* (the sterlet) and *A. güldenstädti* (the sturgeon) using light and electron microscopes. In the ovaries of adults, the oogonial nuclei usually have a single nucleolus with an adjacent mass of paranucleolar fibrillar material. The cytoplasm of the oogonia contains two dense bodies peculiar only to gonocytes, one being electron dense and containing RNA and the other being electron-lucent and lacking RNA. Neither is surrounded by membrane. The fine structure of the electron-lucent body is identical to that of the paranucleolar material, while the RNA-containing body structurally resembles the nucleolus. A nuclear origin for both cytoplasmic bodies is likely.

Leptotene-stage oocytes usually still have a single nucleolus. During zygotene, it is adjacent to the nuclear envelope and opposite to the chromosomes contracted in synizesis. At pachytene, a ‘cap’ of extrachromosomal chromatin is formed under the nuclear envelope and around the nucleolus. Bivalents also contact this cap. In early diplotene, the primary nucleolus still persists. The material of the cap is dispersed beneath the entire nuclear envelope in the form of granules of extra DNA; each granule then produces a peripheral (secondary) nucleolus. These become typical amphinucleoli with differentially developed granular parts, depending on the age of the nucleolus and the stage of meiosis. Their fibrillar parts always face the nuclear envelope. New peripheral nucleoli continue to form as long as granules of extra DNA persist under the nuclear envelope, i.e. approximately until vitellogenesis.

In early vitellogenesis, the peripheral nucleoli become transformed, by re-distribution of their fine structural components, into circular threads trailing towards the centre of the nucleus. The axis of the thread consists of fibres and is coated with granules. In late vitellogenesis, the nucleoli round up and become vacuolized; they are then peripheral again.

Proteinaceous RNA-lacking structures which are also produced in the nuclei during oogenesis in the Acipenseridae, are the ‘nuclear bodies’ and ‘spheres’. The former are adjacent to peripheral nucleoli, the latter form on lampbrush chromosomes. Both are ultrastructurally alike. The loops of lampbrush chromosomes produce also RNP bodies (‘granules’ of Callan & Lloyd, 1960) which are ultrastructurally similar to nucleoli but lack segregation of granules from fibres into spatially distinct parts.

The evolution of the nucleolar apparatus during oogenesis in the Acipenseridae closely resembles that in amphibians.

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INTRODUCTION

The Acipenseridae, a fish family including sturgeons and sterlets, is a very ancient and primitive group among the Osteichthyes. The Acipenseridae are rather widely distributed in the rivers of the USSR and present a double interest to investigators: first, a purely economic one, being a national wealth by themselves and as producers of caviar; second, a scientific one, as the group is both primitive and successful (Gerbilsky, 1962). They also resemble the Amphibia in many developmental and cytological aspects, including oogenesis. Cytologically, the Acipenseridae seem to be even closer to Amphibia than to Teleostei.

Unfortunately, these fishes present problems for cytological studies: they cannot be reared in the laboratory until maturity because this would take too many years, and also they have too many chromosomes to be favourable for cytogenetic research. According to Serebryakova (1964), the diploid chromosome number \((2n)\) is about 60 in *Huso huso*, *Acipenser ruthenus* (the sterlet), and *A. stellatus*, and double that number (about 120) in *Acipenser gűldenstädti* (the sturgeon). According to Fontana & Colombo (1974), the respective chromosome numbers are even higher (approximately 2 times), due to the presence of microchromosomes not taken into account by Serebryakova.

All Acipenserids except the sterlet are migrators: they ascend rivers for hundreds of kilometres for spawning, which occurs in most species in spring, and thereafter they re-descend to the sea. Their larvae remain in the river for about a year after hatching; then, they also descend to the sea and return into rivers only after several years for their first spawning. The immaturity period is 13 to 15 years in the sturgeon, and only in the non-migratory fresh-water species, the sterlet (*A. ruthenus*), it is as short as 6 to 8 years (Berg, 1948).

The present research deals with only two species of Acipenseridae of the Volga: *A. gűldenstädti* Brandt, the sturgeon, and *A. ruthenus* L., the sterlet. Both spawn in late April–early May. The interval between two successive spawnings is about 2–3 years in the sterlet and about 5 years or more in the sturgeon (Shilov, 1964).

During spawning, it is the senior generation of oocytes which mature and are discharged. The junior generation cells remain in a resting state (in previtellogenesis) until preparation for the next spawning begins, about a year before actual spawning. The oocytes then start vitellogenesis. Among resting-state oocytes, the larger ones are in diplotene of meiosis, the smaller ones, at earlier stages of the meiotic prophase.

The aim of this study is to make a general survey of the evolution of the nucleolar apparatus during oogenesis of the Acipenseridae. Some material included in this report has previously been published in Russian (Raikova, 1968, 1970, 1972, 1974a).
MATERIAL AND METHODS

Oocytes of the sterlet (A. ruthenus) and of the sturgeon (A. gūldenstädtii) were obtained from females caught during the last several years in the Kama (near Rybnaya Sloboda) and in the Volga (below the Kuybyshev dam, near Saratov, near Volgograd and above Astrakhan).

For light microscopy, pieces of the ovary were fixed with Bouin's, Champy's, Serra's, Carnoy's, Navaschin's, Sanfelice's, and Zenker's (with formalin) mixtures as well as with sublimate - acetic acid (19:1) and with 10% formalin. The material, dehydrated in ethyl alcohol, was embedded in paraffin. Sections (7 to 10 μm thick) were stained with iron haematoxylin, Heidenhain's 'azan', Mayer's haemalum, or azur-eosine after Nocht-Maximoff. For cytochemistry of the nucleic acids, Feulgen's reaction (with deoxyribonuclease-treated control), methyl green–pyronin staining and toluidine blue staining (both after Brachet, 1953), and gallocyanin staining (after Einarsson) were used. Ribonuclease-treated controls were made with the last three methods. Polysaccharides were detected by the PAS reaction, with salivary amylase-treated control. Lipids were osmicated according to Champy and subsequently treated with Na₂S. Proteins were stained with a sublimate solution of bromphenol blue (after Mazia, Brewer & Alfert, 1953). Danielli's tetrazonium coupling reaction and Serra's staining for arginin were also used, as well as staining with Fast green at pH 8.2 for basic proteins.

For electron microscopy, the material was fixed with 6% glutaraldehyde in phosphate buffer (pH 7.4) for 2 h at 4 °C, and postfixed with Caulfield's 1% osmium tetroxide in acetate–veronal buffer with 4.5% saccharose (also 2 h at 4 °C). The fixed pieces were dehydrated in acetone and embedded in Araldite. Sections were collected on uncoated grids and stained with saturated aqueous uranyl acetate (4 h) and then with Reynolds' lead citrate (10 min). Observations were made with a JEM-7A electron microscope at 50 kV.

OBSERVATIONS

Oogonia

In adult acipenserids, oogonia are the youngest stages of oogenesis available. Oogonia (Figs. 1–3) are small rounded cells with cytoplasm clearer than that of surrounding cells. Each oogonium is accompanied by another small cell which adheres to it (the future follicle cell, Fig. 3). Mitoses of oogonia are rare in adult fish (Fig. 1). It is not known how many generations of oogonia follow each other before oocytes are differentiated.

Oogonia have a large nucleus with a single nucleolus which is generally surrounded by a clear, apparently structureless space (Fig. 2). Their cytoplasm consistently contains two different bodies (Fig. 2). One of these stains lightly with haematoxylin, appears oval or spherical, contains no cytochemically
detectable RNA (Raikova, 1968, 1970), but stains for proteins. The other body is dark, nearly always irregular in shape, and contains RNA and protein.

Electron microscopy shows that a vacuole, filled with finely fibrillar material of low electron density, exists in the oogonial nucleus where it is adjacent to the nucleolus (Fig. 3). The content of this intranuclear vacuole is morphologically identical with that of the clear cytoplasmic (proteinaceous) body (Figs. 3, 6). Both have no surrounding membrane. This suggests that the clear cytoplasmic body has a nuclear origin and actually represents the paranucleolar vacuole material extruded into the cytoplasm. Inclusions of cytoplasmic origin are, in contrast to this, usually surrounded by a membrane.

The other cytoplasmic body, which appears dark and contains RNA (Figs. 1–3, 6), is ultrastructurally an accumulation of small granules which very frequently are associated with several mitochondria. The granular material looks very much like the nucleolar cortex material and thus may also have nuclear origin, representing nucleolar granules extruded into the cytoplasm. This supposition is almost beyond doubt in the case of amphibians, as shown by Clérot (1968), Kessel (1969), and others. This makes it likely that both specific cytoplasmic bodies, which are markers of the sexual cells in Acipenseridae, have a nuclear origin.

In Acipenseridae, oogonia of various generations are morphologically difficult to distinguish from very young oocytes. Whereas in amphibians oogonia usually have irregular (lobulated) nuclei (Al-Mukhtar & Webb, 1971; Coggins, 1973), in acipenserids both oogonia and young oocytes have spherical nuclei with a single central nucleolus and sometimes with two or three additional small nucleoli.

**Figures 1–5**

Fig. 1. Three sturgeon oogonia. The top one is in metaphase of mitosis (pole view); the other two oogonia contain resting nuclei with single central nucleoli and dense cytoplasmic bodies (db); nuclei of future follicle cells (fen) are also seen. Bouin, iron haematoxylin. ×1800.

Fig. 2. Sturgeon oogonium showing both lucent (Ib) and dense (db) cytoplasmic bodies. A lucent zone (Iz) is seen inside the nucleus, adjacent to the nucleolus. Nava-uschin, iron haematoxylin. ×1800.

Fig. 3. Electron micrograph of a sturgeon oogonium showing nucleus with nucleolus (n) and paranucleolar material (pm). The cytoplasm contains both dense (db) and lucent (Ib) cytoplasmic bodies, lipid droplets (l), mitochondria (m), a Golgi body (g), and desmosomes (d). A follicular cell with lobulated nucleus (fen) is seen at top. OsO₄ fixation. ×12300.

Fig. 4. Young sturgeon oocyte with nucleus in leptotene, containing the nucleolus (n). fen, nucleus of a future follicle cell. Bouin, iron haematoxylin. ×1800.

Fig. 5. Three sturgeon oocytes in zygotene (synizesis). The nucleolus (n) is seen in the top cell opposite to the chromosome threadball. Bouin, iron haematoxylin. ×1800.
Oocytes in previtellogenesis

During the leptotene stage of meiosis, the nucleus of a young oocyte sometimes contains one nucleolus but sometimes shows no nucleoli at all (Fig. 4). On the other hand, a nucleolus always exists during the next (zygotene) stage. It generally lies at the pole of the nucleus opposite to the threadball of chromosomes (Fig. 5), the bouquet stage taking in Acipenserids the form of synizesis.

With the electron microscope, the nucleolus appears adjacent, during zygotene, to the inner side of the nuclear envelope, though separated from it by a layer of loose fibrillar material of unknown nature (Fig. 7).

During pachytene, chromosome bivalents are apparent, but there is also a cap of chromat in which appears inside the nucleus at one side of it and is very characteristic of this stage (Figs. 8, 9). The nucleolus is always enclosed in the cap material (Fig. 8). Direct connections between the chromatin cap and some bivalents are sometimes seen. The oocyte is already surrounded by small cells forming a kind of follicle (Figs. 8–11).

During diplotene, chiasmata are seen on the bivalents. The cap material becomes more discrete and spreads beneath the nuclear envelope; this material is now distinctly granular (Figs. 10–13). Beginning with this stage, new nucleoli are formed at the inner side of the nuclear envelope and remain attached to it (Figs. 10, 11). These are peripheral nucleoli, forming in close contact with the chromatin granules which earlier made up the cap. The cap chromatin certainly is extrachromosomal DNA synthesized by amplification of ribosomal genes, exactly as in amphibians. Schmantzar (1976) showed cytophotometrically that the DNA content of oocyte nuclei is at this stage 50 times higher than that of the spermatids in the sturgeon, and 30 times higher, in the sterlet. We also observed intense incorporation of [³H]thymidine into the chromatin cap (Chmilevsky & Raikova, 1976).

Figures 6–11

Fig. 6. Electron micrograph of two sturgeon oogonia interconnected with a cytoplasmic bridge (at arrow). Note the paranucleolar material (pm) and its similarity to that of the lucent cytoplasmic bodies (lb) in both cells. db, dense cytoplasmic body. OsO₄ fixation. × 9200.

Fig. 7. Electron micrograph of the nucleolus of a sterlet oocyte in synizesis. Note the fibrillar material attaching the nucleolus to the nuclear membrane (at arrow). Glutaraldehyde – OsO₄ fixation. × 45000.

Figs. 8 and 9. Two focal planes of a sturgeon oocyte nucleus in pachytene. Note the dense chromatin cap with the primary nucleolus in central position inside it (in Fig. 8), and edges of the cap and the bivalents (in Fig. 9). Navaschin, iron haematoxylin. × 1800.

Figs. 10 and 11. Sturgeon oocyte nuclei in early diplotene. The chromatin cap with several peripheral nucleoli forming inside it is shown in side view (in Fig. 10) and in pole view (in Fig. 11). The cap is now loose and granular; its granules begin to spread beneath the nuclear envelope. Navaschin, iron haematoxylin. × 1800.
Nucleolar apparatus in Acipenseridae

Granules of extrachromosomal DNA, regularly interspaced at the inner side of the nuclear envelope, can be shown very well (Figs. 12, 13) by a Feulgen-type fluorescent reaction specific for DNA, the auramine-SO$_2$ staining after HCl hydrolysis (Rosanov & Kudriavtsev, 1967). Some chromosomal bivalents are seen to contact certain peripheral chromatin granules (Fig. 12).

Diplotene-stage oocytes enter the period of rapid growth, the bivalents transforming into lampbrush form. Many peripheral nucleoli are already present, as are several central intranuclear bodies which stain very intensely with iron haematoxylin but not with pyronine or toluidine blue (Fig. 14). These are ‘spheres’, resembling those described by Callan & Lloyd (1960) in *Triturus*. They seem to form on the loops of the lampbrush chromosomes.

Electron microscopy reveals both the young peripheral nucleoli and the granules of extrachromosomal chromatin adhering to the nuclear envelope (Fig. 15). At first, the nucleoli contain only fibres, but later (with the start of rapid growth) each nucleolus develops a granular part in the form of a knob, always turned towards the centre of the nucleus (Fig. 16). Each nucleolus thus becomes an amphinucleolus (Figs. 16, 17). The granular part of such an amphinucleolus is more basophilic than its basal (fibrillar) part, but, inversely, the latter stains more strongly for proteins. The fibrillar part thus seems to contain more protein, and the granular part, more RNA. The granular knob can completely detach from the base of the nucleolus. This has also been observed in *Xenopus* by Van Gansen & Schram (1968). Two neighbour nucleoli sometimes have a common granular knob (Fig. 18).

The spheres are less numerous than the nucleoli. In previtellogenesis the former are always attached to lampbrush chromosomes (Fig. 14). Sometimes a sphere is connected with two or three bivalents at once, which seems to indicate that several spheres can fuse. Large spheres may be as large as nucleoli.

**Figures 12-16**

Fig. 12. Fluorescence photograph of a sterlet oocyte nucleus in diplotene, stained Auramine 00 for DNA (after Carnoy fixation). Note the chromatin granules dispersed all around the nuclear periphery, and many bivalents and three spheres in the interior of the nucleus. × 1800.

Fig. 13. The same, showing granules of extra DNA regularly spaced under the nuclear membrane, as seen in a tangential section of a diplotene nucleus. × 2900.

Fig. 14. Sterlet oocyte nucleus at the beginning of rapid growth, showing peripheral nucleoli (*pn*) at different stages of formation, and a sphere (*s*) attached to several bivalents. Sanfelice, iron haematoxylin. × 630.

Fig. 15. Electron micrograph of a young peripheral nucleolus (*pn*), of mainly fibrillar structure, in a sturgeon oocyte nucleus. Chromatin granules (*eg*) under the nuclear envelope (*ne*) are also seen. OsO$_4$ fixation. × 51000.

Fig. 16. Electron micrograph of a typical peripheral amphinucleolus in a sterlet oocyte nucleus, showing differentiation into a fibrillar part (*fp*) and a granular knob (*gk*). *ne*, nuclear envelope, *m*, mitochondria. OsO$_4$. × 19500.
Their fine structure is fibrillar; the fibres are loosely packed and of low electron density (Fig. 19).

After glutaraldehyde fixation, electron microscopy shows that oocyte nuclei with amphinucleoli contain also small fibrillar masses of loose structure and of low contrast, which lie near the peripheral nucleoli (Fig. 18). These masses are similar to the ‘nuclear bodies’ described by Kessel (1969) in amphibian oocytes. They might serve to fix nucleoli on the nuclear membrane (Lane, 1967). The nuclear bodies still cannot be seen in the light microscope at this stage. Their fine structure (Fig. 18A) is comparable to that of the paranucleolar material and to that of the clear cytoplasmic body found in oogonia.

The fate of the primary nucleolus, which persists in the previtellogenic oocyte nucleus from the pachytene stage, is difficult to ascertain, because it is rather similar to secondary (peripheral) nucleoli. However, one nucleolus is sometimes larger and more spherical than others. This nucleolus is usually vacuolized and can be found either near the nuclear envelope or near the centre of the nucleus (Fig. 20). With the electron microscope, its vacuole is seen to contain a finely fibrillar substance (Fig. 20) which again resembles the paranucleolar material of the oogonia. We suppose this to be the primary nucleolus which has grown and acquired a fibrillar vacuole since the pachytene stage.

Oocytes in vitellogenesis

At the time of beginning of vitellogenesis, the number of peripheral granules of extrachromosomal chromatin, which produce new nucleoli, rapidly decreases. Formation of peripheral nucleoli thus gradually ceases.

The start of vitellogenesis coincides with a spectacular transformation of the nuclear structure. This is also the time when an oocyte, which before belonged to the junior generation, differentiates to become member of the senior generation of oocytes destined to mature for the next spawn, the junior generation remaining blocked in previtellogenesis. During early vitellogenesis, the lampbrush

Figures 17–20

Fig. 17. Peripheral amphinucleoli (pn) in a sturgeon oocyte nucleus. Two of them are apparently extruded into the cytoplasm, if not lying in deep evaginations of the nuclear envelope. Two spheres (s) are also seen. Bouin, azan. × 900.

Fig. 18. Electron micrograph of two peripheral nucleoli having separate fibrillar parts (fp) but a common granular part (gp). A nuclear body (nb) is seen near the nuclear envelope (ne). Sterlet oocyte, OsO₄ fixation. × 9000.

Fig. 18A. Enlargement of a part of a nuclear body. Sterlet oocyte, OsO₄ fixation. ×45000.

Fig. 19. Electron micrograph of a sphere in a sterlet oocyte nucleus. Glutaraldehyde – OsO₄ fixation. × 24500.

Fig. 20. Electron micrograph of the primary nucleolus in a sterlet oocyte diplotene nucleus. Note the fibrillar material (fm) filling the vacuoles inside the nucleolus. Glutaraldehyde – OsO₄ fixation. × 7800.
Nucleolar apparatus in Acipenseridae

Chromosomes stretch to maximum; they are practically invisible in sections (Fig. 21). They are very active in synthesis, producing at their loops a large number of spheres and of RNP bodies ('granules' of Callan & Lloyd, 1960). The nucleus becomes filled with various small inclusions.

The peripheral nucleoli undergo a striking transformation at the time of appearance of the first yolk bodies in the cytoplasm (Fig. 22). At first, they round up (Figs. 21–22, 26), then they elongate into circular threads extending towards the centre of the nucleus (Figs. 22, 23). At that time, there is already much yolk (Fig. 23).

Electron microscopy shows that the secondary rounding up of the peripheral nucleoli is due not to loss of their granular knobs but to uniform re-distribution of the granular material all around the fibrillar part. Also, the nucleolus becomes vacuolized, and the granular component coats not only the outer surface of the nucleolus, but also the surfaces of the vacuoles (Fig. 26A).

The small fibrillar bodies ('nuclear bodies' of Kessel, 1969) also round up, become more compact and follow the movements of their corresponding nucleoli. They are no longer attached to the nuclear membrane (Fig. 26). These bodies can now be seen under the light microscope as well, especially in semi-thin Araldite sections. They do not stain with toluidine blue. At later stages they seem to disappear.

When nucleoli extend into circles formed by a more or less beaded thread (Fig. 23), electron microscopy shows that the axis of the circular thread is fibrillar and that granular material covers only the periphery of the thickenings occurring on the thread (Fig. 27).

In oocytes filled with yolk, the lampbrush chromosomes somewhat contract; their axes are now well seen in light microscopical sections (Fig. 24). The spheres are at this stage usually rounded and rather small (Fig. 24). No more

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**Figures 21–25**

Fig. 21. Sterlet oocyte in late previtellogenesis. The nucleus contains many peripheral nucleoli (pn) and several spheres (s); new peripheral nucleoli still form (at arrows). Bouin, mercuric bromphenol blue. × 420.

Fig. 22. Nucleus of a sterlet oocyte in early vitellogenesis. Some peripheral nucleoli begin to transform into circles (at arrows). The interior of the nucleus is filled with various bodies formed on lampbrush chromosomes (lcb). y, yolk. Bouin, iron haematoxylin. × 400.

Fig. 23. Nucleus of a sterlet oocyte in middle vitellogenesis. Circular nucleolar threads (at arrow) trail towards the centre of the nucleus, occupied by lampbrush chromosome products (lcb). y, yolk. Bouin, iron haematoxylin. × 280.

Fig. 24. Central part of the nucleus of a sterlet oocyte in vitellogenesis, showing spheres (s) and 'granules' (g) attached to lampbrush chromosomes. Zenker, iron haematoxylin. × 900.

Fig. 25. Electron micrograph of a lampbrush chromosome ‘granule’. Sterlet oocyte, glutaraldehyde – OsO₄ fixation. × 32 500.
fusion of spheres is observed. Their attachment to chromosomes becomes difficult to observe. They seem to be mostly detached, as in Reptilia (Agamidae) (Arronet, 1975). As before, the spheres contain no nucleic acids.

The small RNP bodies formed on the chromosome loops (the ‘granules’ of Callan & Lloyd, 1960) consist of both fibrils and submicroscopic granules, as true nucleoli do. But here the two components are mixed together (Fig. 25). These bodies can detach from the chromosomes without changing their aspect. Their RNA is generally supposed to be messenger RNA because they form on the loops of lampbrush chromosomes and not at nucleolus-organizing loci or on their extra copies. On the other hand, peripheral nucleoli obviously contain ribosomal RNA. This makes us consider misleading the use of the term ‘micronucleoli’ to designate these RNP bodies (Sterba, 1961; Sterba & Schäffner, 1965; Schäffner, 1968; Sanchez, 1969).

At the end of vitellogenesis, the nucleoli contract to the nuclear periphery and round up again (Fig. 28). They are now clearly vacuolized and remain in this condition until maturation of the oocyte.

Just before spawn, the oocyte nuclei generally show no nucleoli at all and look optically empty. The cytoplasmic RNA concentrates at the animal pole. According to Votinov (1947, 1963) and Kazansky (1953), the nucleoli gradually dissolve while moving towards the centre of the nucleus.

**DISCUSSION**

The data presented above demonstrate that the Acipenserid nucleolar apparatus undergoes a definite series of transformations during oogenesis. The primary nucleolus, which can be traced from oogonia to pachytene and, with some doubt, to diplotene, shows no budding or fragmentation. No continuity thus exists between the primary nucleolus and the peripheral nucleoli except that the former may become one among the latter. But all other

**Figures 26–28**

Fig. 26. Electron micrograph of a rounded-up peripheral nucleolus (pn) before its stretching into a circle, with its nuclear body (nb). Nucleus of a sterlet oocyte in early vitellogenesis. ne, nuclear envelope. Glutaraldehyde – OsO₄ fixation. × 15500.

Fig. 26A. Electron micrograph of a part of a rounded-up vacuolized peripheral nucleolus showing granules mainly at the surface of an intranucleolar vacuole (v); the fibrillar material is more peripheral (fm). Sterlet oocyte, glutaraldehyde – OsO₄ fixation. × 26000.

Fig. 27. Electron micrograph of parts of nucleolar circular threads, showing fibrillar cores (f) and granular cortexes (g). Sterlet oocyte, glutaraldehyde – OsO₄ fixation. × 56000.

Fig. 28. Periphery of sturgeon oocyte nucleus in late vitellogenesis, showing indented nuclear envelope (ne) and secondarily rounded peripheral nucleoli (pn). y, yolk. Bouin, azan. × 900.
peripheral nucleoli arise independently of it. In this respect, the oogenesis of Acipenseridae fits into the general pattern of alimentary type oogeneses (Raven, 1961).

These data disagree, however, with Olschwang's (1936) description, according to which the primary nucleolus of the oocyte of _A. ruthenus_ would grow, become polymorphous and then fragment into many small peripheral nucleoli. Such polymorphous intranuclear bodies can be seen also in some figures of Ionescu-Varo & Grigoriu (1963) concerning the same species, though no interpretation of them is given in the text. Some of our preparations also show large irregular nucleoli at one side of the oocyte nucleus, but these are atypical and likely to be stages of oocyte degeneration or fixation artifacts. At any rate, they appear only in diplotene, when peripheral nucleoli are already present and oocytes are surrounded by follicle cells.

A cap of extrachromosomal DNA is produced in pachytene nuclei. Its component DNA granules progressively spread beneath the nuclear membrane in diplotene, assuming a very regular distribution. Each granule is a centre of neoformation of a peripheral nucleolus (Raikova, 1968). Nucleologenesis continues until the start of yolk formation. It is now beyond doubt that the chromatin cap consists of amplified copies of the ribosomal RNA gene: the cap material is strongly Feulgen-positive, incorporates [3H]thymidine (Chmilevsky & Raikova, 1976), the total DNA content of the oocyte nucleus reaching in result 30–50 times that of a spermatid (Schmantzar, 1976). We also obtained good hybridization _in situ_ of Acipenserid (sterlet and sturgeon) oocyte DNA with iodinated rRNA isolated from oocytes of _Xenopus laevis_. The chromatin cap in pachytene oocyte nuclei was strongly marked (unpublished results obtained during the author's stay in the Laboratory of Molecular Cytology and Embryology of the Free University of Brussels, in collaboration with C. Thomas, G. Steinert, and A. Schram).

These observations are in good agreement with Arndt's (1960) and Chmilevsky's (1970) data on oogenesis of some Teleosts. Arndt reported that, in some Cyprinidae, a substance beneath the nuclear envelope remained Feulgen-positive much longer than the bivalents did, and supposed this 'heterochromatin', localized in the folds of the nuclear envelope, to participate in formation of peripheral nucleoli. Chmilevsky (1970) also found a chromatin cap in oocyte nuclei of _Acerina cernua_ and demonstrated that incorporation of [3H]thymidine into it, indicating synthesis of extra DNA, was limited to pachytene.

The mode of formation of peripheral nucleoli in the oocytes of Acipenseridae is also very similar to that in _Xenopus laevis_ (Gall, 1968; Macgregor, 1968), although the primary nucleolus in _Acipenser_ oocytes, unlike that of _Xenopus_ oocytes (Van Gansen & Schram, 1972) shows no budding. On the other hand the 'supernumerary' nucleoli were reported to arise in oocytes of _Salvelinus fontinalis_ (Salmonidae) in succession, on a single heterochromatin segment of a bivalent (Chouinard, 1963).
Nucleolar apparatus in Acipenseridae

In spite of the fact that lampbrush chromosomes produce in Acipenseridae an enormous quantity of ‘granules’, most of which are of ribonucleoprotein nature and some of which are rather large and nucleolus-like, these ‘granules’ are unlikely to become peripheral nucleoli as has been said in Triturus (Macgregor, 1965). In Acipenser at least, the ‘granules’ differ from true peripheral nucleoli in size, fine structure, and mode of formation.

The peripheral nucleoli of the Acipenseridae undergo a definite sequence of changes: at first they develop into amphinucleoli carrying a granular ‘knob’, later, during early vitellogenesis, they round up and then pull into circles. At the end of vitellogenesis, they return to the nuclear periphery and become spherical vacuolized bodies.

Amphinucleoli are common in oocytes of fishes and other animals. They have been observed in Acipenser stellatus (Votinov, 1947), in Salmonidae (Persov, 1966), some Cyprinidae (Arndt, 1960), and in Amphibia (Brown & Ris, 1959). In all these cases, both cytochemical (Guyénot & Danon, 1953; Tandler, 1958) and ultrastructural differences (Miller, 1962; Raikova, 1972; Van Gansen & Schram, 1972) exist between the two parts of an amphinucleolus. Amphinucleoli are most typical of molluscan oogenesis (Häcker, 1899); many authors report differential staining properties and density of their two parts (Bolognari, 1959; Romanova, 1963, 1964), as well as differential incorporation of precursors of nucleic acids and proteins into them (Romanova & Gazarian, 1966).

Stretching of nucleoli into threads, especially during vitellogenesis and before the meiotic divisions, is a rather common phenomenon during oogenesis in various animals, such as Hydrozoa, Mollusca, Aranei, Insecta, and Vertebrata (Jörgensen, 1913; Kedrovsky, 1959; Sokolov, 1960). This phenomenon occurs also among fishes, e.g. during vitellogenesis in sharks (Maréchal, 1907) and some deep sea fishes (Jörgensen, 1913; Nusbaum, 1913), and before meiotic divisions in some teleosts (Yamamoto, 1956; Sakun, 1961). The nucleoli of the newt oocyte also transform into thick strands (Guyénot & Danon, 1953). In axolotl oocyte nuclei, the peripheral nucleoli transform into circular threads which later condense again into spherical nucleoli (Callan, 1966; Lane, 1967). These phenomena are very similar to those in Acipenseridae. It is quite possible that in other animals also, where nucleoli are reported to transform into ‘strands’ or ‘threads’, these structures are in reality circular.

It is now known that an axis of extrachromosomal DNA exists inside each peripheral nucleolus (Miller, 1964) and that the transcription activity of this DNA axis affects both the morphology and the metabolism of the nucleolus (Miller, 1964, 1966, 1967; Macgregor, 1967; Lane, 1967; Ebstein, 1969, Miller & Beatty, 1969; and others). According to Ebstein, the cycle of changes of this DNA axis can be compared to that of a chromomere which transforms into a loop of a lampbrush chromosome. Transformation of the DNA axis from chromomere state into loop state may bring about a corresponding change of the shape of the entire nucleolus which becomes circular and beaded. Lane
(1967) demonstrated that this transformation is correlated with a change in the pattern of incorporation of \[^3H\]uridine into the nucleolar RNA: while previously the label was incorporated into the fibrillar core only, it now becomes distributed all along the circle.

No experiments with uridine incorporation have yet been made with Acipenser oocytes. However, the morphological transformations of their nucleoli being very similar to those of Amphibia, we have reasons to believe that the intrinsic mechanism of these transformations is the same in both groups.

Apart from both primary and peripheral nucleoli, RNA-lacking proteinaceous structures, topographically related to nucleoli, are consistently observed during oogenesis in Acipenseridae. These structures are: (1) the paranucleolar 'vacuole' in the oogonial nucleus, which probably gives rise to the less stainable of the two cytoplasmic bodies considered as markers of germ line cells; (2) the 'nuclear bodies' of Kessel (1969), which are satellites of the peripheral nucleoli in oocytes; (3) the spheres, which are formed on lampbrush chromosomes in oocyte nuclei.

The first two types of structure undergo a somewhat similar evolution, during development of the respective nucleoli: they grow, become more compact and transform into well delimited bodies visible even with the light microscope and accompanying their respective nucleoli. The last type of structure is the spheres; these are never in contact with nucleoli and are produced on lampbrush chromosomes. They consist of thicker fibrils (10–11 nm) than those of the 'nuclear bodies' (9 nm) and of the paranucleolar 'vacuoles' in oogonia (7 nm).

The paranucleolar fibrillar material is also reported to exist in oogonal nuclei of Amphibia (Coggins, 1973). Cytoplasmic bodies similar to those in Acipenser oogonia have been found in gonocytes and oogonia of the teleost fish Oryzias (Satoh, 1974). Finally, nuclear fibrillar inclusions, closely resembling those of Acipenseridae, are known to exist in oogonia of Lacerta (Hubert, 1970a, b).

Spheres are rather common in oocytes where much yolk is produced; in the nuclei of such oocytes, spheres often occur in enormous quantities, e.g. in some fishes, amphibians, and especially reptiles (Arronet, 1975) and birds (Gaginskaya, 1972). The function of the spheres remains, however, obscure.

In conclusion, we can state that a great cytological resemblance exists between oogenesis in the Acipenseridae on one hand, and in the Amphibia on the other. This includes not only nucleolar and other nuclear structures, which we have discussed, but also the cytoplasmic organelles of the oocytes. Oocytes of the two groups show, in particular, the same structure of yolk plates, the same presence of cortical granules, the same absence of ribosomes in previtellogenesis, the same extrusions of nucleolar material into the cytoplasm, and the same dense material cementing mitochondria (Raikova, 1973, 1974b).
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REFERENCES


Nucleolar apparatus in Acipenseridae


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