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The Ultrastructure of the Nuclear Envelope of Amphibian Oocytes: A Reinvestigation

II. The Immature Oocyte and Dynamic Aspects

WERNER W. FRANKE AND ULRICH SCHEER

Division of Cell Biology, Institute of Biology II, University of Freiburg i. Br., Germany

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Different stages of oogenesis of the Alpine newt were compared with respect to the nuclear envelope structure, employing negative staining and sectional work: larval eggs, early and later lampbrush stages, and mature eggs. While in all the stages the general components of the nuclear pore complexes could be observed, differences were found concerning quantitative structural data. In particular the frequency of pores containing a central granule decreased from 61 % in the larval to 36 % in the mature egg. Some new information on the mode of nucleocytoplasmic transit of nucleolus-derived material could be obtained from the lampbrush stage eggs. In this stage, fibrillar strands spinning out from the nucleolar periphery can be seen in contact with the nuclear pore complex, expecially with the inner annular granules. Furthermore, dense spheres of nucleolar material migrate through the central channel of the pore in a rodlike configuration in the very same mode that has been described for salivary glands of *Chironomus thummi* (*69*). Alternative relationships of nucleocytoplasmic RNP transport to the constituents of the nuclear pore complex are discussed.

There exist drastic changes in quality and quantity of RNA synthesis during the oogenesis of the amphibian oocyte (e.g., *11–15*, *18–20*). Since the nuclear pore complexes are widely thought of as either regulating or being functionally correlated to the efflux of ribonucleoprotein-containing particles from the nucleoplasm into the cytoplasm, it appears reasonable to investigate whether any changes in nuclear envelope structure and composition occur during oogenesis. Merriam (*43*) has compared the structure of osmicated, air-dried nuclear envelopes after isolation from different stages of *Rana pipiens* oogenesis by using the Callan–Tomlin technique. It is the purpose of the present study to extend this work and to compare the structure of the mature amphibian oocyte which has been described in detail in a foregoing article (*23*) with that of the lampbrush and the larval stage by combining section and negative staining electron microscopy.

MATERIALS AND METHODS

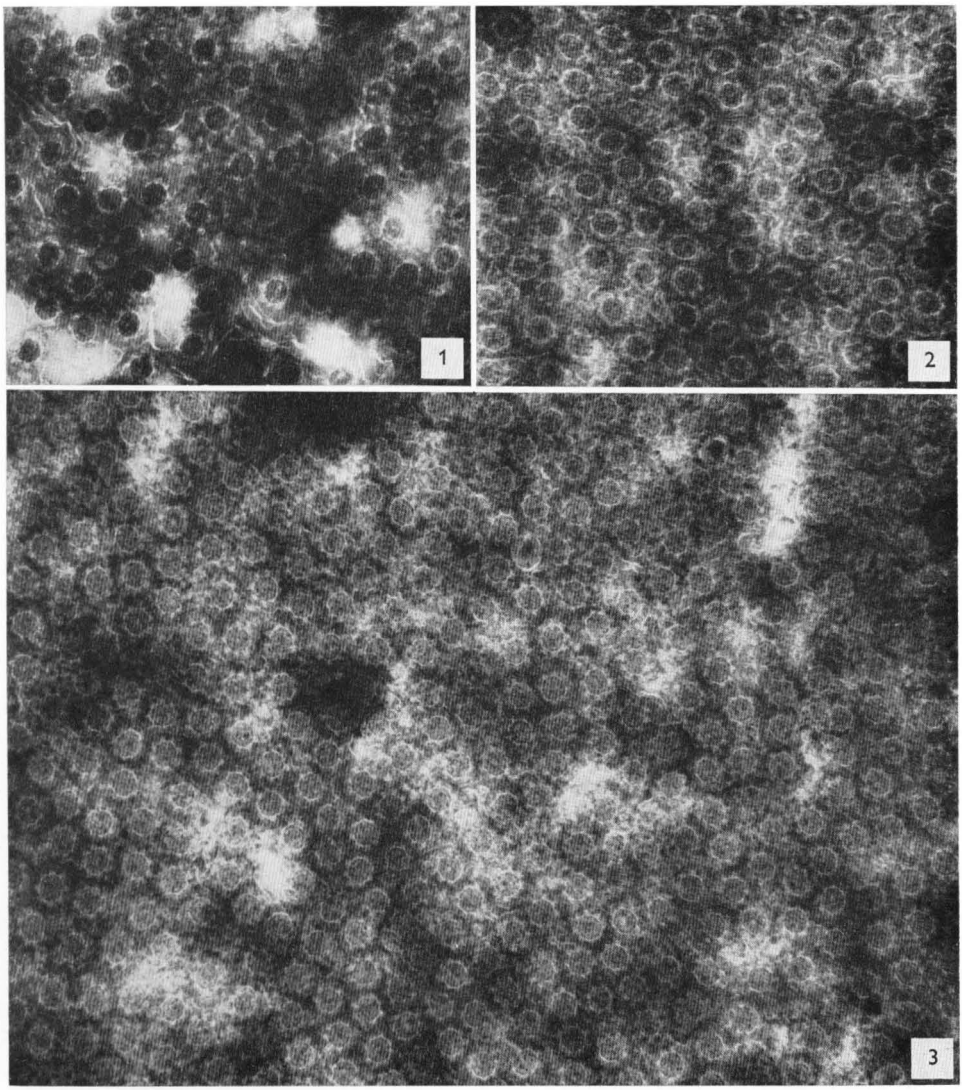
For negative staining preparations, nuclear envelopes were isolated from oocytes of the Alpine newt, *Triturus alpestris* Laur., according to the methods previously described (*23*). For section work the total isolated nuclei as well as intact oocytes were prepared as described in that article. Electron micrographs were made with Siemens Elmiskop IA and 101.

The investigated stages were (a) larval stage (transparent oocytes with a diameter of 140 ± 25 μm); (b) lampbrush stage (slightly pigmented oocytes with a diameter of 750 ± 30 μm; comparable to stage 4–5 *sensu* Duryee (*22*), to stage C *sensu* Grant (*25*), to stage Y_3 *sensu* Kemp (*33*, *34*), to stage 3 *sensu* Schäffner (*59*), to stage IIa *sensu* Wartenberg (*75*)); and (c) the mature stage (oocyte diameter about 1600 μm). In some cases late lampbrush stages (oocyte diameters about 900 μm) were also examined. Since the structure of the nuclear envelope depends strongly on the special isolation conditions used, particular care was taken to evaluate comparatively only material that had been prepared in the same way. For quantitative evaluation of central granule frequency, every distinct centrally located particle larger than 30 Å has been considered.

RESULTS AND DISCUSSION

As can be seen from Figs. 1–3 no remarkable differences in the structural properties of the nuclear pore complex of the isolated envelopes could be observed between the different stages of oogenesis. All the granular and fibrillar structures known from the nuclear pore complex of the mature newt egg (*23*) occur also in the earlier stages of oogenesis.

Comparative sectional studies on different aspects of egg development are numerous (*1*, *3*, *5*, *7*, *8*, *10*, *16*, *29*, *30*, *34*, *36*, *45*, *51*, *52*, *67*, *71*, *74–78*). With respect to nuclear pore complex structure and function, however, a new interesting finding could be obtained from the lampbrush stage. In this stage, in which exceedingly high RNA-synthesis takes place (e.g., *19*), the nucleoli are preferentially located in the periphery of the nucleus beneath the nuclear envelope (e.g., *39*, *78*). Strands consisting of finely filamentous (about 30 Å) and granular material of various size can be seen extending from the nucleolar periphery toward the nuclear pore complexes (Figs. 5 and 6). While some of these filaments appear to be associated with granules of diameters of about 30–60 Å, arrangements of the filaments with dense spheres with diameters from 200 to 700 Å also can be observed (Figs. 7–10; cf. "the streams of granules" of *39*). Similar "spinning out" processes of nucleolar fibrils in the direction of the nuclear envelope have been reported from other oocytes (*36–38*). Observations suggesting a structural transformation of coarse RNP granules into rods or fibrils when these approach the nuclear pore complex were recently reported by Monneron and Bernhard (*47*). Images like that shown, e.g., in Figs. 5 and 7, suggest that at least some of these nucleolar-derived filaments might be continuous with and identical to



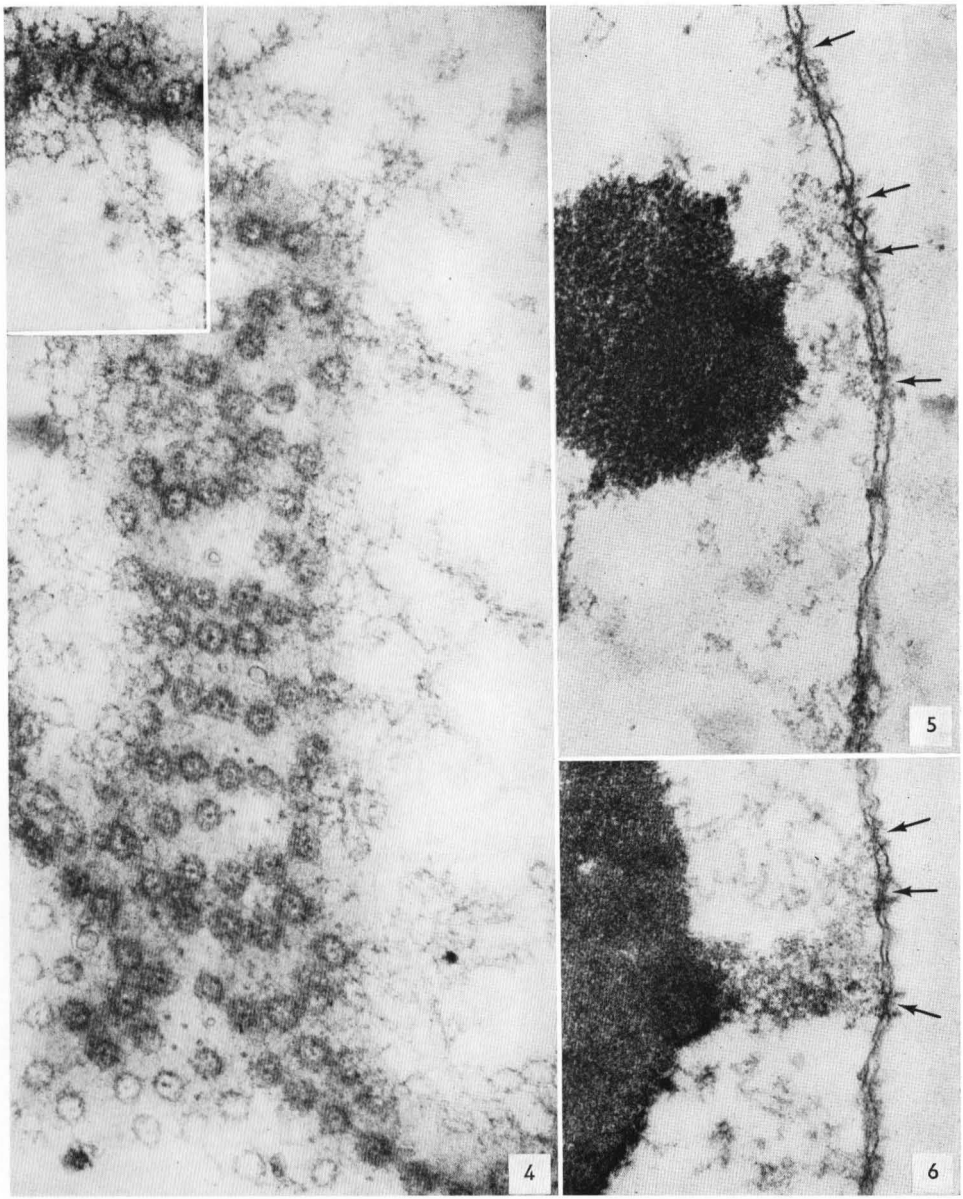
FIGS. 1–3. Nuclear envelopes isolated by Callan–Tomlin technique from different stages of oogenesis of *Triturus alpestris* (basal NaCl/KCl medium, 3 min duration), fixed with OsO_4 and negatively stained with 2 % phosphotungstic acid, adjusted to pH 7.2. Fig. 1 shows an envelope from a larval egg, Fig. 2 from a mid lampbrush stage, and Fig. 3 from a late lampbrush egg. Figs. 1 and 2, × 50,000; Fig. 3, × 44,000.

TABLE I

Stage in Oogenesis (oocyte diameter in μ)	Nuclear Diameter (μ)	Pore Diameter (Å)	Pore Frequency (No. of pores per μ^2)	Percentage of Nuclear Surface Occupied by Pore Area	Central Granule Frequency (% pores containing a central granule	Total number of Pores per Nucleus
Larval stage (140)	100	626 ± 21	55 ± 4.6	16.9	61	1.7×10^6
Lampbrush stage (800)	400	630 ± 35	68 ± 6.6	21.2	55[a]	34×10^6
Mature (1600)	600	737 ± 67	50 ± 6.4	21.3	36[a]	57×10^6

[a] From thin sections of the same material 60 % (lampbrush) and 37 % (mature) central granule-containing pores were calculated.

the annulus-attached fibrils of the pore complex as have been described for the mature egg (*23*). Those fibrils which are associated with the larger granules are generally directed more to the pore complex centers (e.g., Figs. 9, 10, 14, 15). Still coarser clumps of electron-dense material, which seems also to emanate from the nucleolar periphery, can be observed to approach the more central part of the pore complexes during their nucleocytoplasmic migration. Such dense clumps of material appear to produce slender projections which are directed toward the pore complex center. A next stage of their migration through the pore produces a dumbbell-shaped configuration with one half of the clump lying on the cytoplasmic side of the pore and the other on the nucleoplasmic side. Both are connected by a rod about 100–150 Å wide which occupies the "central channel" (*23*, *60*, *69*) of the pore (Figs. 11, 12, and 16). In later stages of extrusion the majority of such dense spheres lie on the cytoplasmic side of the pore complex (Figs. 13–15). In Figs. 11–14, a sequence of micrographs is arranged which represents the kinetic interpretation of the nucleocytoplasmic passage of the nucleolus-derived dense masses. This situation resembles very closely that shown by B. J. Stevens and Swift (*69*) for the Balbiani ring derived material in *Chironomus* salivary gland nuclei and the micrographs presented by A. R. Stevens (*68*) on the passage of the RNP-helices of *Amoeba proteus* through the nuclear pores. One is tempted to interpret such "material-in-passage" through the central part of the pore as being a type of nucleocytoplasmic RNP transport characteristic for cell states of high RNA synthesis activity. Furthermore this material is structurally related to the central granules, which are particularly abundant in the earlier stages of oogenesis (Table I; cf. also *43*). A series of the diverse structures that could lead to such an image of a particulate plug in the center of the pore is given in the cross sections of Figs. 16–21 in which different arrangements of electron-dense material pertaining to the central granule are summarized.



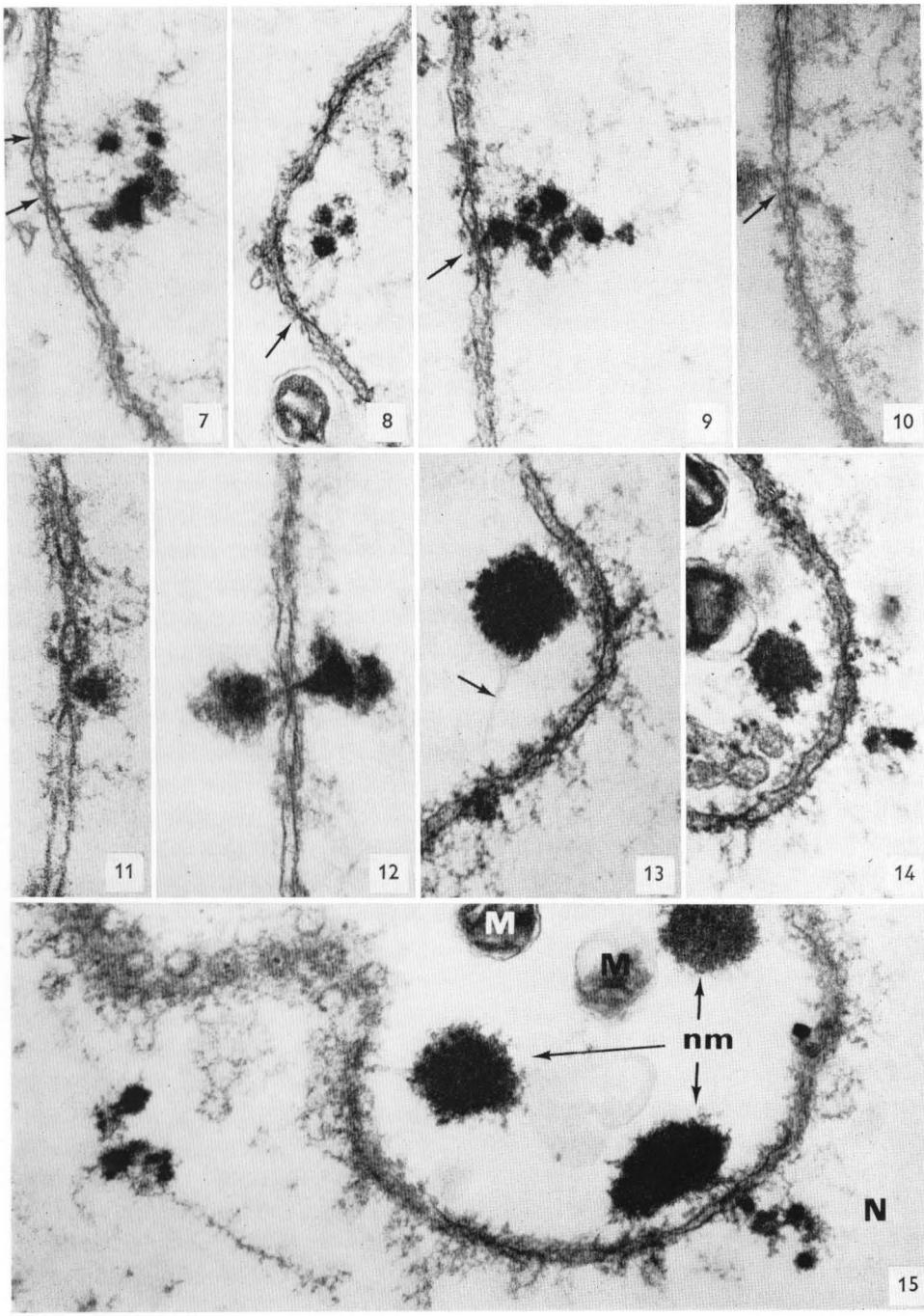
FIGS. 4–6. Sectioned nuclei of *T. alpestris* lampbrush oocytes. Fig. 4 illustrates the frequency of central granules in the pore complexes of a tangentially sectioned envelope and the intranuclear annulus-attached fibrils which can be discerned in more detail in the inset. ×45,000. In Figs. 5 and 6 the "spinning out" of finely fibrillar material, sometimes associated with about 30–50 Å granules, from the nucleolar periphery toward the nuclear envelope can be seen, particularly in the direction of the pore complexes (arrows). Fig. 5, ×65,000; Fig. 6, ×48,000.

In a few cases it could be recognized that slender strands of nucleolar-derived material extending through the pore center were as thin as 30–50 Å (Fig. 17). This corresponds to the class of "small" central granules occasionally found in negative staining preparations of lampbrush as well as of later oocytes (cf. *23*). Although there is evidently no doubt about the RNA-content of such nucleocytoplasmic migrating dense material (e.g., *4*, *7*, *9*, *35*, *44*, *49*, *53*, *63*, *68*, *69*), it is not clear what kind of RNA they contain, i.e., either mRNA (dRNA) or rRNA or both. While some authors have favored an interpretation of messenger ribonucleoprotein (*69*, *72*), the nucleolar origin of these particles seems to indicate a ribosomal nucleoprotein (cf. *2*, *6*, *49*, *74*). In this connection, however, it should be kept in mind that extrusion of nucleolar material into the cytoplasm need not necessarily to take place via the nuclear pores since other mechanisms have been reported: e.g., budding off of parts of the perinuclear cisterna into vesicles including the nucleolar material (e.g., *70*) or partial dissolution of nuclear envelope (*73*).

In a previous article it was shown that there exist no interspecific differences with respect to the structural properties of the nuclear pore complex (*23*). Interesting quantitative intraspecific differences, however, were observed in the course of oocyte development (Table I). Thus, a comparison of the three stages of oogenesis in *Triturus alpestris* reveals that during egg growth the portion of central granule containing pore complexes declines from an average of 61 % in the larval eggs down to 36 % in the mature oocytes. This finding is in general agreement with the remark by Merriam (*43*) that the central dots were more frequent in immature frog oocytes than in mature ones. The fact that this author found in both stages an absolutely lower percentage of central granule-containing pores (16 % and 2 %) seems to be explained by the lower resolution and contrast difference of the preparation method he employed.

The relative pore area seems to keep stable during oogenesis. The importance of the question how pore complexes are formed may be emphasized by the calculation that

FIGS. 7–15. Details of the structural relationship of the nucleolus-derived dense material with the nuclear pore complexes. In Figs. 7 and 8 the fibrillar connections of such nucleolar clumps to the inner annulus of pore complexes (arrows) can be seen. In Fig. 9 a cluster of dense spheres and thin fibrils is in contact with a pore complex (arrow). Note also the outer annular granules. Fig. 10 presents a line of such spheres, which seemingly is in transit through the central part of a pore complex (arrow). In Fig. 11 a large clump of nucleolar material lies attached to the pore still on the nucleoplasmic side while in Fig. 12 such material reveals a dumbbell-shaped configuration with one half intranuclear and the other half lying already in the cytoplasm, both halves connected by a slender rod extending through the central channel of the pore. In Figs. 13 and 14 such clumps of nucleolar material lie in the cytoplasm adjacent to the nuclear envelope, but other smaller particulate material seems to be "en route." Note the thin 30 Å filament (Fig. 13, arrow) connecting two such dense spheres through a nuclear pore complex. Fig. 15 presents a survey which demonstrates how frequent such nucleocytoplasmic migration of nucleolus-derived dense material (*nm*) is in the lampbrush stage. *N*, nucleoplasm; *M*, mitochondrion. Figs. 7, 8, and 10, × 55,000; Figs. 9 and 12, × 70,000; Fig. 11, × 100,000; Figs. 13 and 14, × 62,000; Fig. 15, × 50,000.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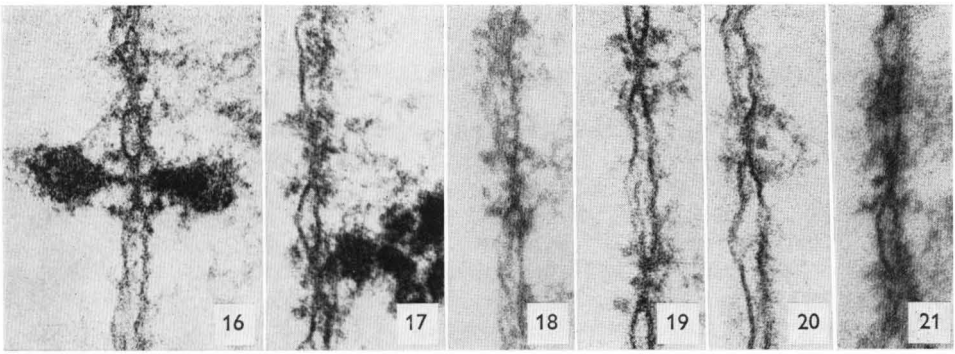
during the described interval of oogenesis of the Alpine newt the total number of pores, as a general consequence of nuclear growth, must increase from 1.7 million pores to 57 million pores per nucleus (Table I).

Apart from the central granule which seemingly represents a variable structure (Figs. 16–21; cf. *23*), it is apparent that two basic structural components chiefly make up the particulate pore complex material, namely the 30 Å-broad fibrils, sometimes associated with about 40–60 Å granules, and the 100–200 Å large annular granules. Considering the present background of biochemical knowledge in this field, the following alternative speculations on the possible biological meaning of the pore complex seem to be reasonable and might serve as stimuli for further experiments:

1. The inner annulus-attached fibrils are identical with the 30 Å ribonucleoprotein (RNP) strands described by Spirin and his colleagues (review *64*). Thus, nascent ribosomal RNP strands coil up at the pore margin into granules equivalent to ribosomal subunits. This would be consistent with the general predominance of rRNA synthesis in such oocytes (e.g., *20*), with their origin from the nucleolar periphery as can be seen particularly well in the lampbrush stage, with their high stainability with lead and uranyl stainings, with the existence of an electron-transparent core in the annular subunits and with the coiling tendency of these fibrils (*23*). This evidence fits into the general concept of the pore complex as a site where ribosomal and/or polysomal constituents are accumulated and assembled. Then the final maturation of the ribosomal precursor particles to the effective ribosome subunits, e.g., the completing addition of ribosomal protein, could also occur within the pore complex region.

2. The annulus-attached fibrils represent initiator complexes (recent review, *27*), i.e., mRNA plus smaller ribosomal subunits (*17*, *28*, *32*, *40*). This could explain the small particles observed as attached to these fibrils within the pore complex and studded along the fibrils extending into the nucleoplasm. In this concept the pore complex would act as a gateway in which the polysome is completed ready to translation by adding simply the large subunits. Thus, in this case the rate of polysome formation within the pore complex could control the time of the messenger's stay at the pore complex. This means that under conditions of slow polysome formation, as is the case in the fully mature oocyte, the pore complex functions by piling up informational RNA in the mode of a structure-bound pool, thus providing a storage of "stable messengers" known to be present in egg cells (e.g., *18*, *26*, *66*). In this concept the annulate lamellae could serve as a greatly enhanced structural basis of pore complex-bound messenger capacity (cf. *60*).

3. The annulus-attached fibrils represent "informator complexes" ("informosomes" *sensu 58*, *65*; review, *24*), i.e., mRNA bound by nonribosomal "informofer" protein (*31*, *46*, *54*, *57;* cf., however, *50*). The dimensions of such complexes as have been reported by Samarina *et al.* (*58*) and by Monneron and Moulé (*48*), however,



FIGS. 16–21. Sectional study on the diversity of structures which can produce the image of a central granule in tangential sections or in isolated envelopes: A central about 100 Å thick rodlike waist of the nucleolar material penetrating the pore complex (Fig. 16), an about 30 Å thin filament directed to the pore center (Fig. 17, upper pore), a 100–150 Å large plug in the pore center (Fig. 18), two 100–150 Å granules lying on either side of the pore equator (Fig. 19, both pores), one 100–150 Å large granule lying on the nucleoplasmic (Fig. 20) or on the cytoplasmic side of the pore equator (Fig. 21). All, ×110,000.

exceed those of the 30 Å fibrils by far and could otherwise rather correspond to the central dot material.

4. The pore complex itself is some sort of a special polysome in that the 16 annular granules, and possibly also the central granule, represent the ribosomes of one or several sets of nuclear pore complex polysomes at which the start of cytoplasmic protein synthesis takes place (cf. *42*, *56*).

5. The nuclear pore complex is a site of special ribonucleotidase activity thus cleaving either the polycistronic 40 S–30 S RNA containing RNP strands (*55*) to the smaller RNA's (reviewed, e.g., *11*) of the ribosomal subunits or cleaving a polycistronic messenger as known from bird erythroblastic cells (*61*, *62*) into the smaller effective messengers.

6. Despite some unlikelihood it can nevertheless so far not be excluded that the annulus-attached fibrils are highly disperse chromatin strands in a relatively active state (*21*). DNA-containing masses associated with nuclear pores were recently described for an insect ovarian nurse cell (*41*).

Further experiments including labeling, cytochemical, and autoradiographic techniques are in progress in our laboratory to determine which of these functional alternatives approach reality.

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