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Morphology of the Nucleo-Cytoplasmic Interactions during the Development of *Acetabularia* Cells

I. The Vegetative Phase

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With 17 Figures

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Summary

The ultrastructure of the growing and maturing primary nucleus of *Acetabularia mediterranea* and *Acetabularia major* has been studied with the use of various fixation procedures. Particular interest has been focused on the details of the nuclear periphery and the perinuclear region. It is demonstrated that early in nuclear growth a characteristic perinuclear structural complex is formed which is, among the eukaryotic cells, unique to *Acetabularia* and related genera. This perinuclear system consists essentially of

a) the nuclear envelope with a very high pore frequency and various pore complex associations with granular and/or threadlike structures some of which are continuous with the nucleolus;

b) an approximately 100 nm thick intermediate zone densely filled with a filamentous material and occasional small membraneous structures from which the typical cytoplasmic and nuclear organelles and particles are excluded;

c) an adjacent lacunar labyrinthum which is interrupted by many plasmatic junction channels between the intermediate zone and the free cytoplasm;

d) numerous dense perinuclear bodies in the juxtanuclear cytoplasm which are especially frequent at the junction channels and reveal a composition of aggregated fibrillar and granular structures;

e) very dense exclusively fibrillar aggregates which occur either in association with the perinuclear region of the lacunar labyrinthum or, somewhat further out, in the cytoplasmic strands between the branches of the lacunar labyrinthum in the form of slender, characteristic rods or "sausages".

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A variety of other membraneous and non-membraneous structures characteristic of the juxtanuclear cytoplasm is described. The organization of the individual components in this special complex is summarized in a model drawing. The dynamic and transitory nature of this perinuclear complex apparatus is emphasized and its possible role in nuclear functions and in regulating nucleocytoplasmic interactions is discussed.

1. Introduction

Acetabularia is especially qualified for cell biological investigations because it is, in its vegetative phase, both unicellular and uninuclear (Hämmerling 1931). These properties together with its large size make possible operations like enucleation, isolation of the nucleus and implantation of a nucleus or other material into the cytoplasm (Schweiger 1969). Moreover, these properties make *Acetabularia* a favorable organism for studies on nucleocytoplasmic interrelationships. Earlier studies of *Acetabularia* have raised a number of questions as, *e.g.*, how is the genetic information released from the nucleus into the cytoplasm, how does the cytoplasm influence the nucleus, and which mechanism underlies the extraordinary stability of the genetic information in the cytoplasm.

In connection with all these problems it is most important to study in more detail the ultrastructure of the cell nucleus and the perinuclear region at different stages of development. A limited number of investigations on the morphology of the nucleus of *Acetabularia* have been carried out in the past decade (Crawley 1963, 1965, Werz 1964, Van Gansen and Boloukhère-Presburg 1965, Boloukhère 1969, 1970, Woodcock and Miller 1973, Zerban *et al.* 1973).

In this series of publications we will be reporting on the ultrastructure of the cell nucleus and the perinuclear region at different times in the vegetative and generative phases as well as during gametogenesis. We will also cytochemically characterize various structures inside and outside the nucleus, describe the structural differentiation of the nucleolus and, finally, provide an ultrastructural description of the consequences of nuclear transplantations.

The present study is confined to cells in the vegetative phase. After germination of the zygote the primary nucleus of *Acetabularia* enlarges dramatically over a period of about two months. While the giant nucleus is forming, an intricate and transient morphology develops in the vicinity of the nucleus. The organisation of the perinuclear region seems to be unique to the giant nuclei of some members of the siphoneous chlorophycean algae, *Bryopsis* and *Acetabularia* (Crawley 1963, 1965, Werz 1964, Boloukhère-Presburg 1969, Boloukhère 1970, Burr and West 1971, Woodcock and Miller 1973). This perinuclear organisation represents a special and exceptional situation among eukaryotic cells and, as we will show, provides a particular type of nucleocytoplasmic compartmentalization.

2. Materials and Methods

Acetabularia mediterranea and *Acetabularia major* were grown as described by HÄMMERLING (1944), BETH (1953), and SCHWEIGER *et al.* (1974). For the comparative studies of the developmental stages various fixation procedures were used.

A. Gametes, zygotes, germinating zygotes, whole vegetative cells at various stages of growth and cysts were fixed with 6% glutaraldehyde (0.1 M phosphate buffer, pH 7.4). Whole cells and cysts were fixed for 24 hours; gametes, zygotes and germinating zygotes were fixed for 2 hours. After 15 minutes of fixation whole cells were cut into pieces of 2 mm length. The glutaraldehyde was washed out six times with phosphate buffer. Postfixation was done with 2% osmium tetroxide (same buffer) for 2 hours. Before dehydration the specimens were washed extensively with buffer. All treatments were at room temperature.

B. Alternatively, the cells were fixed in 2.5% glutaraldehyde (0.05 M sodium cacodylate buffer, pH 7.2) in the cold (0–6 °C) for 2 hours. The fixed objects were thoroughly washed with ice-cold buffer, postfixed for 2 hours in 2% osmium tetroxide (same buffer) in the cold, and then washed with distilled water.

C. Other cells were fixed in the cold simultaneously with 2% glutaraldehyde and 1% osmium tetroxide (20 minutes; for details see FRANKE *et al.* 1969).

D. For cytochemical reactions some cells were fixed with 2.5% glutaraldehyde (0.05 M sodium cacodylate, pH 7.2) at 4 °C only.

E. Nuclei were isolated from dissected rhizoids at various developmental stages, with the use of micropipettes in a medium containing 0.083 N KCl, 0.017 N NaCl ("5 : 1 medium"; CALLAN and LLOYD 1960) and were fixed as described under B.

F. For positive and negative staining of isolated nuclear envelopes, isolated primary nuclei were washed two times in distilled H_2O (adjusted to pH 9 with a few drops of borate buffer) using micropipettes until all adhering cytoplasmic material was removed. Cleaned nuclei were opened with microneedles and the isolated envelopes were further processed either according to the positive staining technique of MILLER and BEATTY (1969) as modified by SCHEER *et al.* (1973) or, for negative staining, as originally described for amphibian oocyte nuclei (FRANKE and SCHEER 1970, see also FABERGÉ 1973).

Dehydration was carried out in a series of graded ethanol solutions, beginning at either 5% or 30%. After treatment with a 1 : 1 propylenoxide: ethanol mixture and pure propylenoxide, the specimens were embedded in Epon 812. Ultrathin sections were prepared with glass or diamond knives using Reichert Om U 2, Om U 3, or LKB ultramicrotomes. Sections were stained with uranyl acetate and lead citrate (REYNOLDS 1963) and examined with an electron microscope (Siemens Elmiskop I, I A or 101).

In addition BERNHARD's (1969) differential staining method was applied to sections of material which had been fixed with glutaraldehyde only (for details, see also FRANKE and FALK 1970).

3. Results and Discussion

The nucleus of the germinating zygote appears to be typical for a nuclear stage of low RNA synthetic activity. A considerable part of the nuclear interior is occupied by condensed chromatin which is especially pronounced in the nuclear periphery (Fig. 2 *a*). If a nucleolus is seen at all, it is very small. As usual, the cytoplasm directly borders the nucleus. In the early stages of nuclear growth a perinuclear membraneous apparatus and especially a lacunar labyrinthum gradually develop. The basic organization of the periphery of the fully developed primary nucleus in the rhizoids of *Acetabu-*

laria is shown schematically in Fig. 1 which also gives the nomenclature of the individual structural components used in the present article. The growth of the nucleus is attended by a tremendous enlargement and ramification of the nucleolus (Fig. 2 *b*). As the primary nucleus continues to grow and the nucleolus enlarges tremendously (*e.g.*, Fig. 3), the nuclear diameter increases from approximately 3 μm to as much as 150 μm before the onset of

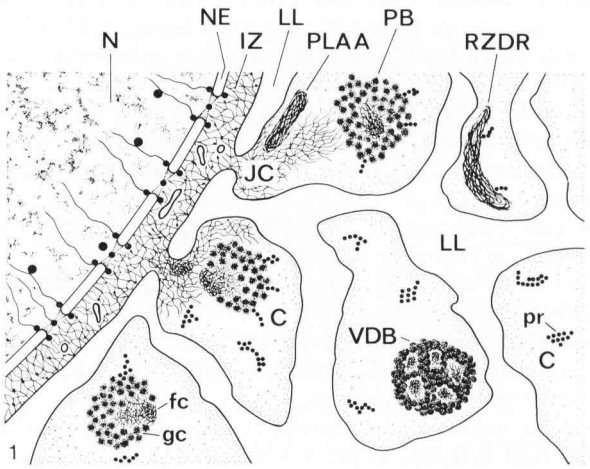
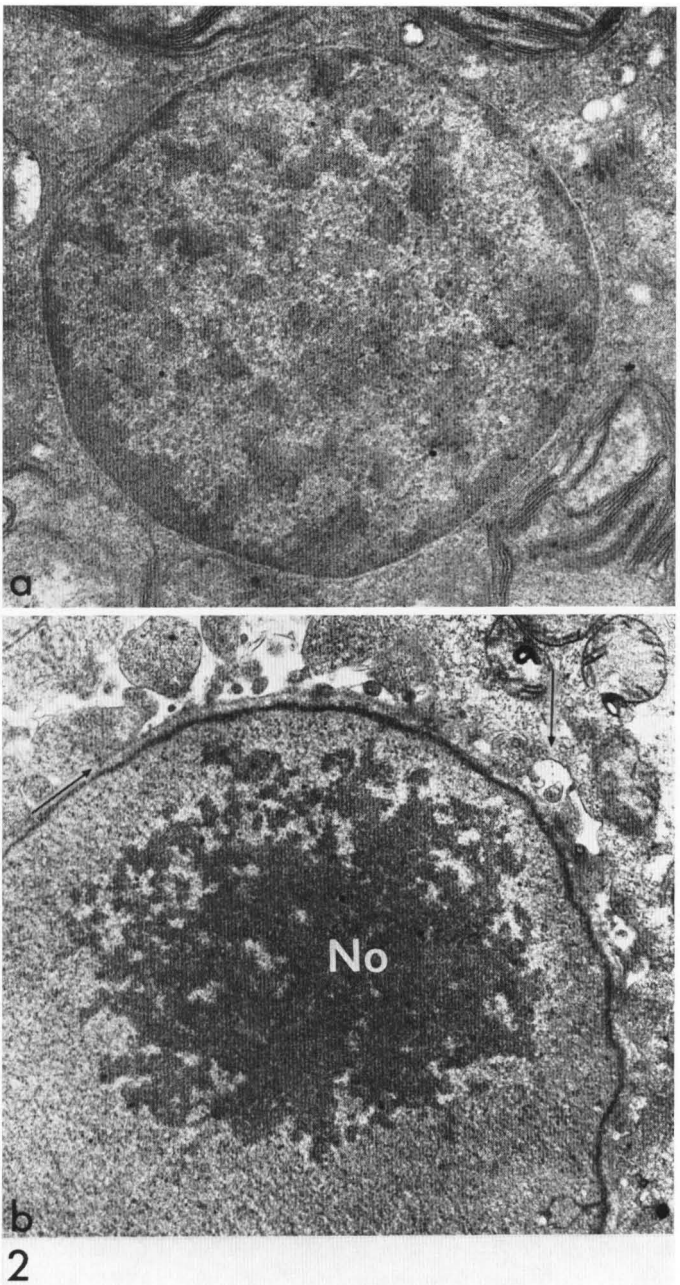


Fig. 1. Schematic drawing of the perinuclear zone of the giant primary nucleus of *Acetabularia*. *N* = nucleoplasm; *NE* = nuclear envelope; *IZ* = intermediate zone; *LL* = lacunar labyrinthum; *JC* = junction channel; *PLAA* = perinuclear lacuna associated aggregate; *PB* = perinuclear dense body; *RZDR* = reticulate zone dense rods; *C* = cytoplasm; *VDB* = vacuolated dense body; *fc* = fibrillar component; *gc* = granular component; *pr* = polyribosomes

cap formation, *i.e.*, measured at a time when the nuclei show still relatively few evaginations (see Fig. 3). Thus, the surface-to-volume ratio decreases about tenfold during nuclear development.

3.1. The Nuclear Envelope

In the germinating zygote the envelope of the nucleus shows a "normal" structure (Fig. 2 *a*). The space between the two nuclear membranes, the perinuclear cisterna, is very thin, even in the fully developed primary nucleus in which case it is 10–20 nm thick. It exhibits only occasional local expansions of up to 35 nm maximal width. In the isolated nuclei the cisternae are outlined in more clarity and in general are more inflated (Figs. 6 *a–d*). Both nuclear membranes are also very thin (ca. 6 nm). Only very few nuclear pores are visible in the nuclear envelope in the germinating zygote. In contrast, in the fully developed cell the nuclear envelope is characterized by a high frequency of nuclear pore complexes (*e.g.*, Fig. 4) which, at least in the



Figs. 2 *a* and *b*. Nuclei of a zygote and of a germling. No special structures can be identified in the vicinity of the nucleus of the zygote (2 *a*) and of the young germling. The first indication of the formation of the complex perinuclear apparatus (arrows in Fig. 2 *b*) is accompanied by enlargement of the nucleus and nucleolus (*No*). *A. mediterranea*. 2 *a*, ×21,000. 2 *b*, ×12,250

later stages of giant nucleus development in *A. mediterranea*, has been determined to be 71 ± 5 pores per μm². The corresponding pore density for *A. major* is 83 ± 7. This value, determined in both tangential and transverse

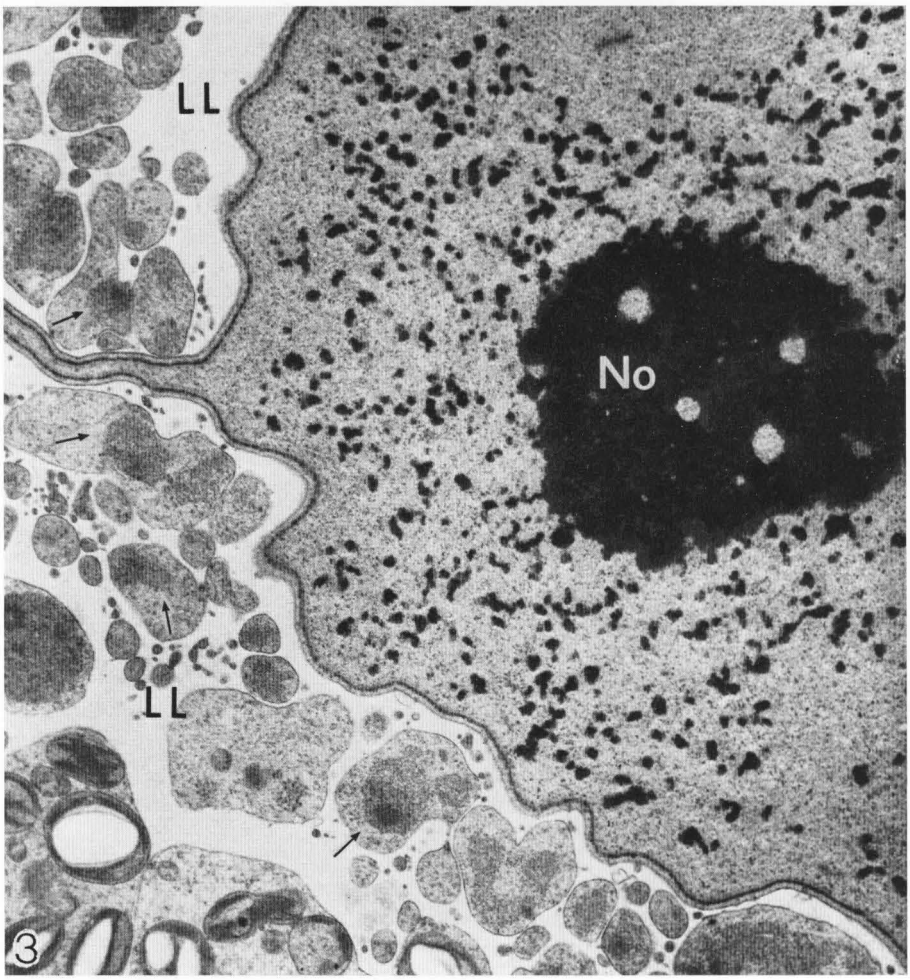


Fig. 3. Perinuclear zone of the giant nucleus. The nuclear surface exhibits some tortuosity and occasional small evaginations. The lacunar labyrinthum including the perinuclear lacuna is established; numerous perinuclear dense bodies can be identified (some of which are indicated by arrows). *LL* = lacunar labyrinthum, *No* = nucleolus. *A. mediterranea*. ×4,700

thin sections appears to be in agreement with corresponding data from freeze-etch preparations in *A. cliftonii* (ZERBAN *et al.* 1973). In the positively and negatively stained whole mount preparations (Figs. 5 *a* and *b*) the pore frequency was usually somewhat higher (up to 100) but more variable. It is interesting to note in this connection that the giant nucleus of *Acetabularia*

does not show dramatic differences in nuclear pore frequency depending on different electron microscope techniques as has been reported in various other cell systems (FRANKE 1970, SPETH and WUNDERLICH 1970, KARTENBECK *et al.* 1971). In this respect it resembles the giant nucleus of amphibian oocytes (SCHEER 1970, 1973, KARTENBECK *et al.* 1971). The absolute value

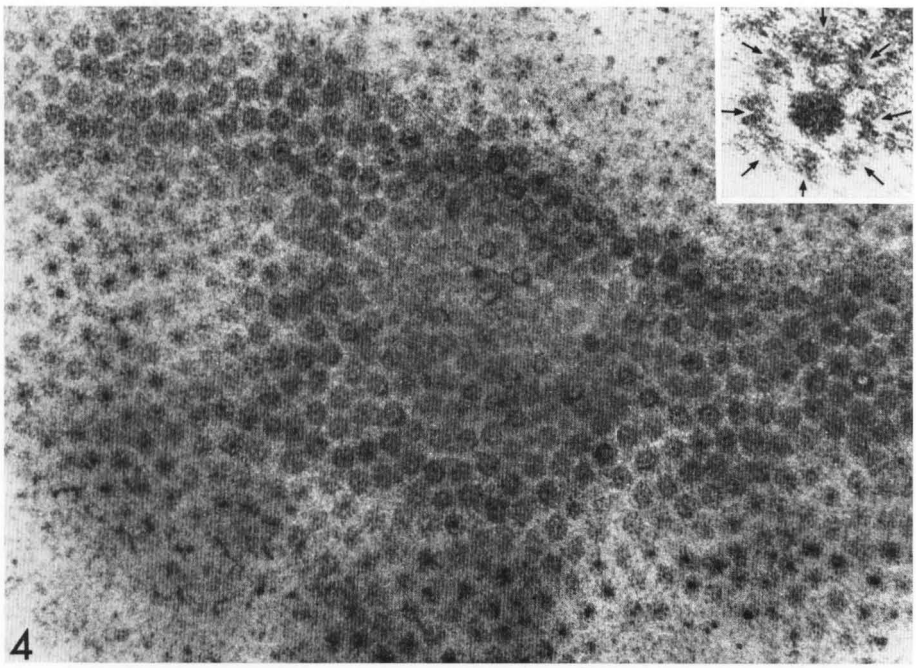


Fig. 4. Section tangential to the nuclear envelope of the fully developed nucleus demonstrating the very high pore frequency. The insert shows the typical appearance of the tangentionally sectioned nuclear pore complex in which the eight annular granules (arrows) are distributed in a radial symmetry and frequently appear to be connected to the large central granule by radial threads. *A. mediterranea.* ×34,000, Insert, ×191,000

of the pore frequency of *Acetabularia* is one of the highest so far reported and is close to that of the amphibian oocytes and the macronuclei of ciliates (for comparative data see, *e.g.*, MERRIAM 1961, 1962, WIENER *et al.* 1965, FRANKE 1967, 1970, COMES and FRANKE 1970, SCHEER 1970, 1973, THAIR and WARDROP 1971, FELDHERR 1972, WUNDERLICH and SPETH 1972, KESSEL 1973, FRANKE and SCHEER 1974). A rough calculation shows that during the growth of the primary nucleus, *i.e.*, in a two month period, the total number of pore complexes per nucleus increases from about 2,500 in the zygote to 2.2 millions (compare also the *A. cliftonii* data of ZERBAN *et al.* 1973), corresponding to a net pore complex formation rate of about 23 pores per minute. This value is significantly below the amphibian oocyte in which

SCHEER (1973) found, as an average for the whole period of *Xenopus laevis* lampbrush stage, a net nuclear pore formation rate of 480 pores per minute. The value for *Acetabularia* is, however, in the range of those reported for the activation of human lymphocytes and in HeLa cells (MAUL *et al.* 1971, 1972, 1973). It is important to keep in mind, however, that this dramatic (900fold) increase in the number of nuclear pores is less than the simultaneous increase of nuclear volume (about 40,000fold; compare also the earlier data of HÄMMERLING 1957, 1963, and HÄMMERLING *et al.* 1958). This has also been noted during the growth of other giant nucleus types, in particular during amphibian oogenesis (MERRIAM 1962, FRANKE and SCHEER 1970 b, SCHEER 1973).

In sections which graze the nucleus one gains the impression that the pore complexes are densely packed in an ordered pattern which approaches hexagonality (Figs. 4 and 5 *b*). Such ordered dense packing is not uncommon in other cell systems and is particularly frequent in nuclei active in transcription (for references, see KESSEL 1973, and FRANKE and SCHEER 1974). The mean inner pore diameter (for definition, see GALL 1967) is 63 nm ± 5 nm, a value close to those of most other cell types (*e.g.*, GALL 1964, 1967, FRANKE 1967, 1974, FRANKE and SCHEER 1970 a, 1974, WUNDERLICH and SPETH 1972, KESSEL 1973). The outer annulus diameter as determined in the whole mount spread preparations (*e.g.*, Fig. 5 *b*) is from 68 to 80 nm. The nuclear pore complexes of the *Acetabularia* primary nucleus reveal the subarchitectural organization which is known from other eukaryotes (reviews: GALL 1964, FRANKE 1966, 1970, FRANKE and SCHEER 1970 a, 1974, ROBERTS and NORTHCOTE 1970, 1971, LA COUR and WELLS 1972, FABERGÉ 1973). The eight annular granules are symmetrically arranged on both sides of the pore rim (Figs. 4 and 6 *a* and *b*; compare also WERZ 1964, and BURR and WEST 1971). Likewise, one can identify in many pores the radial fibers (about 3 nm thick) which extend between the centrale granule and the pore perimeter and the annular granules, respectively (*e.g.*, inset in Fig. 4; for references, see VIVIER 1967, YOO and BAYLEY 1967, WUNDERLICH and FRANKE 1968, DANIELS *et al.* 1969, KESSEL 1969, 1973, FRANKE 1970, 1974, FRANKE and SCHEER 1974). The frequency of pores showing a central dense granule is rather high (about 95% of pores). The size of the central granule varies between 4 and 35 nm (Figs. 4, 6 *a–d*, 8 *a* and *b*, and 10 *a* and *b*). Most of the pore interior is occupied by the electron opaque masses which project from the pore wall

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Figs. 5 *a* and *b*. Isolated nuclear envelope spread and positively stained with the Miller and Beatty technique. Note the attachment to the envelope of aggregated fibrils (arrows in Fig. 5 *a*) presumably representing the threads associated with the pore complex. In Fig. 5 *b* a spread and a partially folded piece of nuclear envelope is shown. Note the high pore frequency and the appearance of the heavily stained annuli (in the insert). *A. mediterranea*. 5 *a*, ×15,000. 5 *b*, ×22,800. Insert, ×37,500

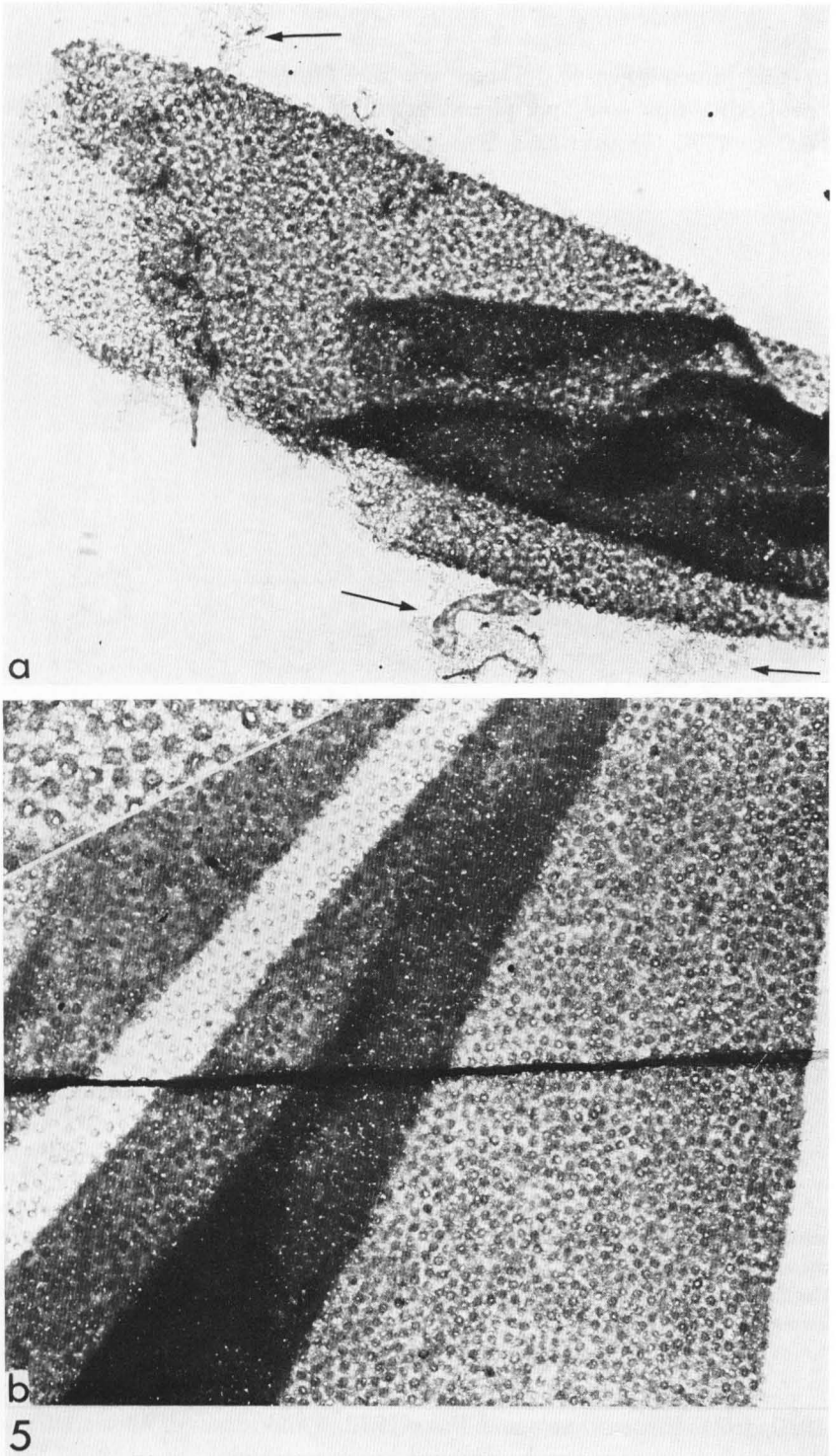
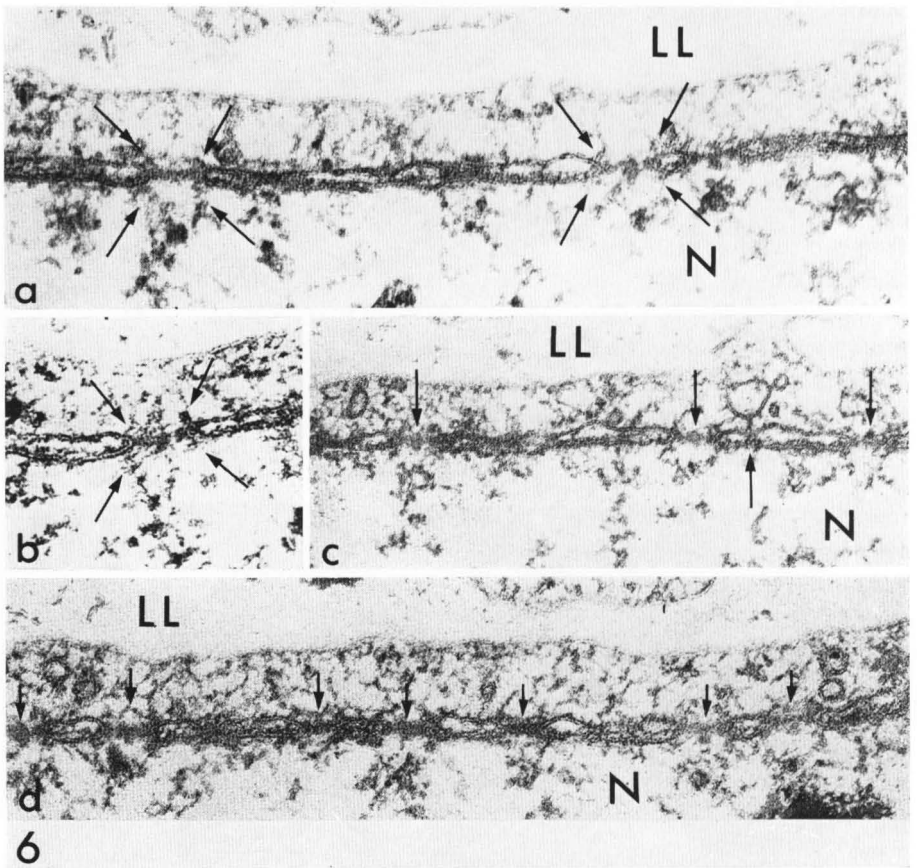


Fig. 5

to the lumen (Fig. 4). These are the "finger-like projections", "claws", "projecting tips", or "peripheral granules" of the literature (Watson 1959, Franke 1970, Franke and Scheer 1970 a, 1974, Roberts and Northcote

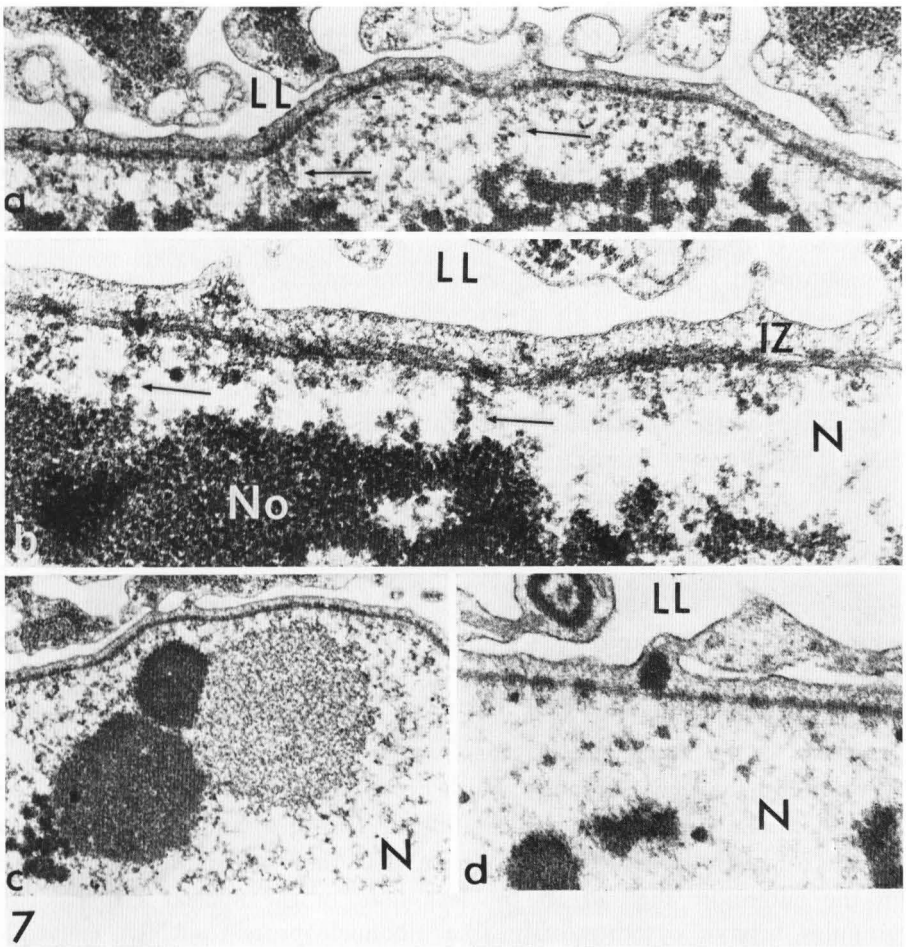


Figs. 6 *a–d*. Cross sections through the nuclear envelope and the intermediate zone of an isolated primary nucleus. Note the annular granules on the pore margins (arrows in Figs. 6 *a* and *b*), the thread-like extensions from both inner and outer annuli, the occurrence of dense granules in the pore center, and the pore-attached fibrils. Note also the predominance of fibrillar material in the intermediate zone as well as the appearance of occasional small vesicles (Figs. 6 *c* and *d*) which sometimes appear to be connected to either the outer membrane of the nuclear envelope (arrow in Fig. 6 *c*) or to the innermost membrane of the lacunar labyrinthum. The frequency of nuclear pores is indicated in Fig. 6 *d* by the arrowheads. *LL* = lacunar labyrinthum. *A. mediterranea*. 6 *a*, ×105,000. 6 *b*, ×110,000. 6 *c*, ×80,000. 6 *d*, ×95,000

1970, 1971, Engelhardt and Pusa 1972, La Cour and Wells 1972). Often the whole pore seems to be plugged by this material.

Annular and central granules are usually associated with fibrillar strands

which frequently insert at the inner annulus (Figs. 6 *a–d* and 7 *a* and *b*; compare FRANKE and SCHEER 1970 a, 1970 b, 1974). This relatively stable association of the nuclear envelope with nuclear fibrils is also demonstrable



Figs. 7 *a–d*. Sections through the nuclear periphery demonstrating the fibrillogranular strand connections between the nuclear pore complexes and the cortical parts of the nucleolus (*No*) (arrows in Figs. 7 *a* and *b*). Other types of bodies sometimes encountered in the nuclear periphery are a characteristic aggregate of spheroidal elements (Fig. 7 *c*) and groups of densely stained globules shown in Fig. 7 *c*. *LL* = lacunar labyrinthum; *IZ* = intermediate zone; *N* = nuclear interior; *No* = nucleolus. Figs. 7 *a–c*. *A. mediterranea;* Fig. 7 *d*. *A. major*. 7 *a*, ×22,500. 7 *b*, ×55,000. 7 *c*, ×14,000. 7 *d*, ×25,850

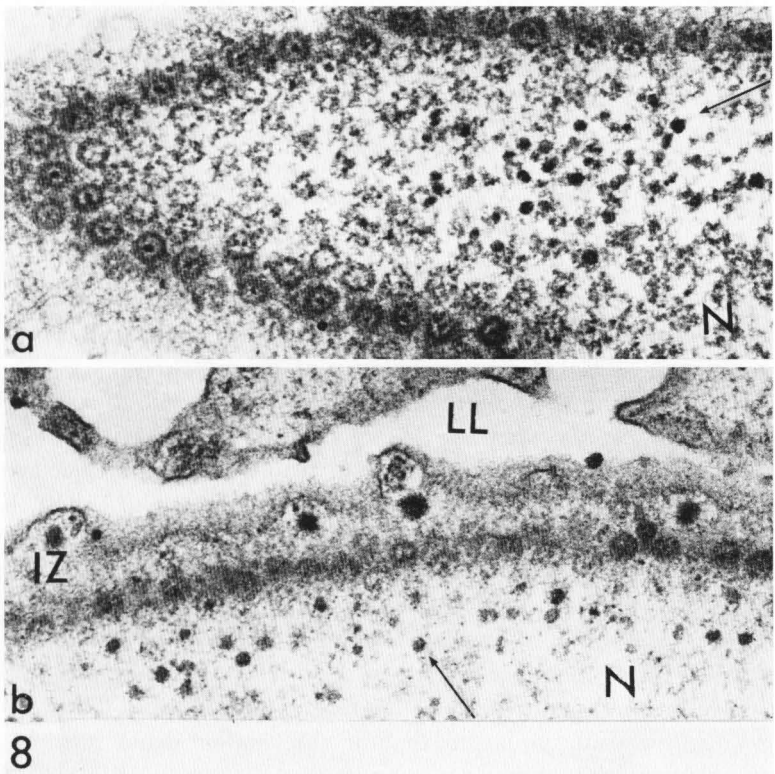
in the whole mount preparations of the isolated nuclear envelope fragments (arrows in Figs. 5 *a* and *b*). Some fibrils are also seen to extend from the outer annulus and connect the nuclear envelope with the membrane which

separates the intermediate zone and the lacunar labyrinthum (compare Figs. 1 and 6 *a–d*). Similar thin filaments, however, appear to span the "intermediate zone" in interpore regions as well. The central granule is usually the most conspicuous component of the pore complexes, due to its high density after the staining procedures employed (Figs. 4, 5, 6 *a–d*, 8 *a* and *b*, and 10 *a* and *b*). As a consequence of both the heavy accumulation of such electron opaque material and the high frequency of the pore complexes, as well as the low thickness of the perinuclear cisterna, the structural details of the nuclear membrane proper are relatively indistinct. Only in nuclei and nuclear envelopes isolated prior to fixation do the nuclear membrane outlines stand out clearly and reveal the trilaminar "unit membrane" appearance (Figs. 6 *a–d*).

We consistently noted various associations and direct continuities of the pore complex material with distinct intranuclear entities. Strand-like connections are seen between the nucleolar cortex and the pore complexes (Figs. 7 *a* and *b*), resembling the situation described in other cell systems, especially for the extrachromosomal nucleoli of the oocytes (Miller 1966, Lane 1967, Franke and Scheer 1970 b). Thin threads also span the nuclear sap between the pore complexes and a peculiar complex body which consists of a sphere of relatively loosely packed granulo-fibrillar material and an attached cap-shaped (usually smaller) electron dense aggregate (Fig. 7 *c*). Such "unidentified" nuclear bodies reveal sometimes, though not always, connections with parts of the giant nucleolar mass. The dense aggregates shown in Fig. 7 *d* are another type of structure which occasionally reveal filamentous connections with the nuclear pore complexes. In addition, fibers attached to the inner annulus can be seen in association with a class of distinct electron dense granules which occur in the vicinity of the nucleolus as well as elsewhere in the nucleus (*e.g.*, Figs. 7 *a*, *b*, and *d*, 8 *a*, and 10 *a*). Counts of these structures have shown that they are much more frequent in the outermost shell of the nucleus than in the nuclear center. These granules behave cytochemically like ribonucleoprotein (RNP) containing particles and, in general, resemble the "perichromatin granules" described in a variety of cell nuclei (Watson 1962, Monneron and Bernhard 1969, Vazquez-Nin and Bernhard 1971), the Balbiani ring-derived granules in *Chironomus* salivary glands (Beermann 1964, Stevens and Swift 1966), and the granules or granule aggregates in the juxtanucleolar regions in some amphibian oocytes (Lane 1967, Franke and Scheer 1970 b, Trendelenburg *et al.* 1974). Often the most peripheral granules are centered within the pore complexes in a way which suggests that they can replace or even be identical to the central granules (Figs. 8 *a* and *b*, 9 *a* and *b*, and 10 *a* and *b*). This suggestion comes from transverse sections and sections which graze the nuclear envelope.

3.2. The Perinuclear Zone

In a typical eukaryotic cell the nuclear envelope is surrounded by cytoplasm and its outer membrane is dotted with ribosomes. This is not the case in the developing nuclei of *Acetabularia* and *Bryopsis* (BURR and WEST 1971).



Figs. 8 *a* and *b*. Sections grazing the nuclear periphery showing the frequency of the distinct small dense granules in this zone (arrows in Figs. 8 *a* and *b*). These dense granules often are in close proximity to nuclear pores (Fig. 8 *a*) and are occasionally also aggregated into larger units within the intermediate zone (Fig. 8 *b*). *N* = nucleus, *IZ* = intermediate zone, *LL* = lacunar labyrinthum. Fig. 8 *a*. *A. mediterranea;* Fig. 8 *b*. *A. major*. 8 *a*, ×44,000. 8 *b*, ×31,000

These nuclei are ensheathed by an approximately 100 nm broad plasmatic zone which is sandwiched between the outer nuclear membrane and the "inner" membrane of the lacunar labyrinthum (see Figs. 1, 3, 7 *a–d*, and 9 *a–c*). This intermediate zone appears to be continuous, via the pores in the nuclear envelope and the cytoplasmic strands between the branches of the lacunar labyrinthum, respectively, with both the nucleoplasm and the cytoplasm. However, it represents a zone which does not contain organelles and components characteristic of both the cytoplasm and nucleoplasm

(Figs. 6–10) such as mitochondria, plastids, dictyosomes, secretory vesicles, and vacuoles, rough ER-components, ribosomes, microtubules, nucleoli, and chromatin bodies. The intermediate zone has a fibrillar matrix, in which only very occasionally small vesicular or tubular membrane profiles are recognized (Figs. 6 *c* and *d*, 9 *a* and *b*, and 10 *a*). These small membraneous vesicles sometimes appear to be linked to either the outer membrane or the "inner" membrane of the lacunar labyrinthum by fine 3.5 nm thick threads (*e.g.*, Fig. 6 *b*), similar to the membrane-to-membrane cross-bridges described in other membrane systems (*e.g.*, Franke *et al.* 1971 a, 1972). In a similar way the intermediate fibrils have been observed in isolated nuclei. They appear to link the membranes of the nuclear envelope and the perinuclear lacuna (see above). Observations made in our and other laboratories (*e.g.*, Werz 1964, Van Gansen and Boloukhère-Presburg 1965, Boloukhère 1969, Brändle and Zetsche 1973, Zerban *et al.* 1973) lead us to suggest that it is this dense meshwork of intermembraneous fibrils which is responsible for the fact that the perinuclear cytoplasmic layer tenaciously sticks to the isolated primary nucleus of *Acetabularia*.

It is worth emphasizing that the small vesicles, which are also found elsewhere in the cell, sometimes appear to be associated with the pore complexes (Figs. 6 *c* and 9 *a* and *b*; for observations of similar nuclear pore-vesicle relationships in other cell systems see Schjeide *et al.* 1970, Franke *et al.* 1971 b, Kiermeyer 1971, Franke 1974). It has not been possible yet to determine whether the tubular membrane profiles seen in this zone (*e.g.*, in Figs. 6 and 9 *a*) are in continuity with either the nuclear envelope or the "inner" membrane of the lacunar labyrinthum. The only other components which we occasionally observed in this perinuclear zone are 50–100 nm large, very electron-opaque granular aggregates (*e.g.*, Figs. 7 *d* and 8 *b*) which may contain material similar to that of the smaller dense intranuclear granules described in the previous section.

Figs. 9 *a–c*. Details of the perinuclear zone of the fully developed primary nucleus. The lacunar labyrinthum constitutes a special cisterna which surrounds the nucleus with the "inner" membrane parallel to the nuclear envelope. It is separated from the nucleus by the intermediate zone. This perinuclear lacunar or "secondary nuclear envelope" is interrupted at irregular intervals by junction channels between the intermediate zone and the cytoplasm (pairs of arrows). The interior of these channels is filled with a filamentous meshwork similar to that recognized in the intermediate zone. Distal to these junction channels one frequently sees small dense aggregates (denoted by the long arrows in Figs. 9 *a* and *c*. See also the insert of Fig. 9 *c*), rod- or sheetlike aggregates of the type denoted by the double arrow in Fig. 9 *b*, vesicles and the large perinuclear dense bodies. Note that the filaments of this region including the thicker ones (*e.g.*, denoted by the arrows in the insert of Fig. 9 *a*) are often in close association with the membranes of the lacunar labyrinthum. *LL* = lacunar labyrinthum, *IZ* = intermediate zone, *JC* = junction channels, *V* = vesicles, *PB* = perinuclear dense bodies. *A. mediterranea*. 9 *a*, ×70,000. Insert, ×107,000. 9 *b*, ×65,000. 9 *c*, ×47,000. Insert, ×67,000

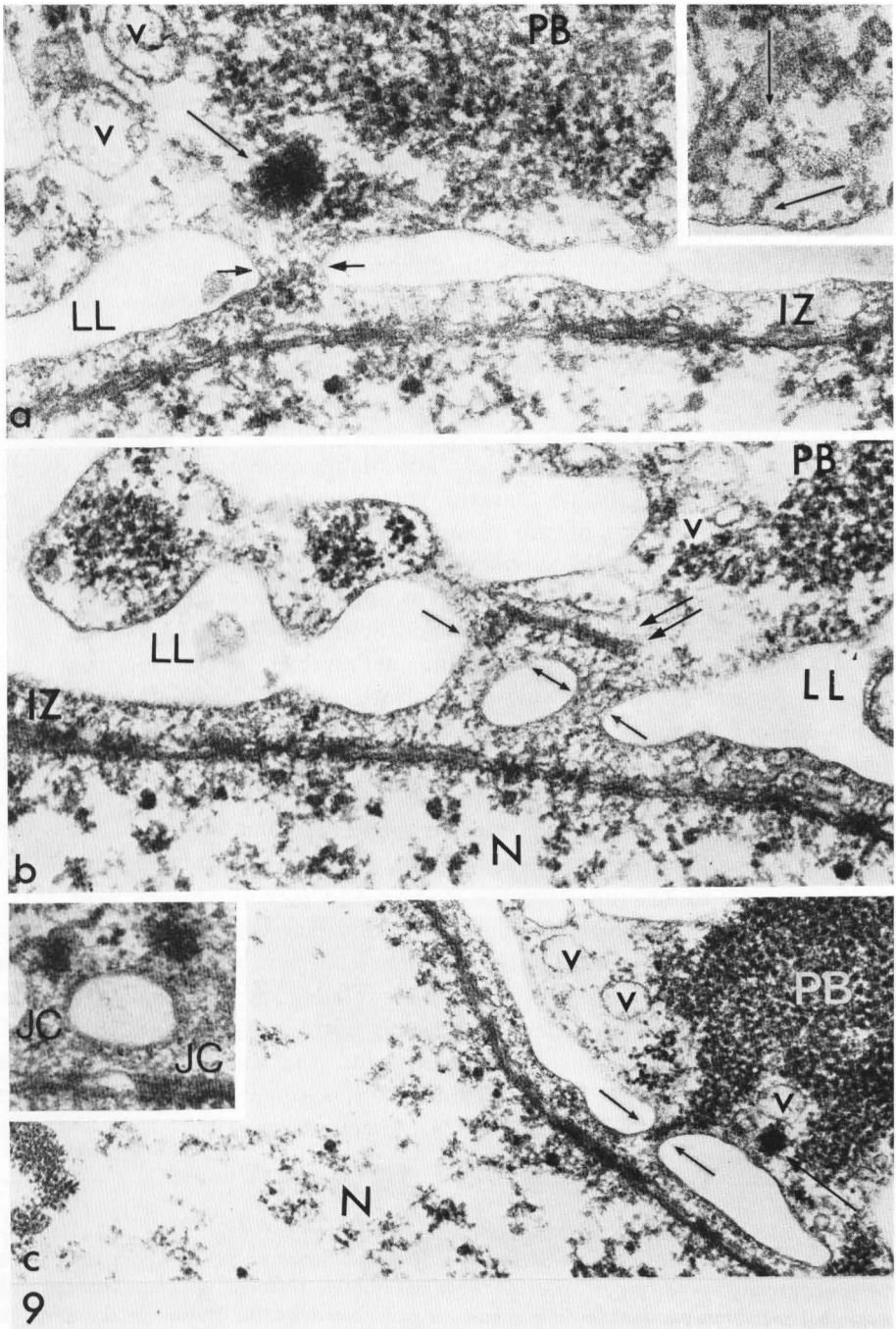


Fig. 9

3.3. The Lacunar Labyrinthum

On the cytoplasmic side the intermediate zone is bordered by the parallel "inner" membrane of the lacunar labyrinthum. This membrane is part of the membrane system of the lacunar labyrinthum (Fig. 1). The membrane ultrastructure of this additional "perinuclear envelope" can at many sites be resolved into the typical trilaminar "unit membrane" structure with a total thickness ob about 7 nm (Figs. 9 *a–c*). We were never able to find a clear association of these membranes with either cytoplasmic ribosomes or polysomes and thus consider it unlikely that they are similar to rough endoplasmic reticulum.

The lacunar labyrinthum is in continuity with the whole lacunar labyrinthum of the cell (Figs. 1, 3, 7 *a–d*, 9 *a–c*, and 11 *a*). Some authors have speculated that the lacunar labyrinthum is continuous with, or at least homologous to the large intracellular cell vacuole. Available evidence, however, cannot fully exclude the possibility that the corresponding membrane system represents deep infoldings of the plasma membrane, comparable to what is known, for example, in the sarcolemma, in developing bone marrow megakaryocytes, in gastric and mucosa cells, and in keratinocytes (see, *e.g.*, Behnke 1968, for review, see Franke and Scheer 1974).

We sometimes noted a somewhat regular arrangement of the electron dense components coating the cytoplasmic surface of this labyrinthum as well as non-regular arrays of filaments of various dimensions, from 3 to 12 nm in diameter (*e.g.*, Figs. 9 *a–c*). The perinuclear part of the lacunar labyrinthum is frequently interrupted by fenestrations. These are the sites at which the inner and outer membranes of the perinuclear lacuna are fused and thus form channels ("junction channels", Fig. 1), of inner diameter varying in the range from 40 to 120 nm (Figs. 9 *a–c*, 10 *a* and *b*, and 11 *a–c*). The mean frequency of these channels through the perinuclear lacuna is at an average of about 2–5 per μm^2, a number significantly less than the pore density of the subjacent nuclear envelope. It has to be made clear, though, that these junction channels between the nucleus and the cytoplasm are different from "true" pore complexes of the nuclear envelope and the annulate lamellae (for reviews, see Gall 1964, Kessel 1968, 1973, Gouranton 1969, Stevens and Andre 1969, Franke 1970, 1974, Wischnitzer 1970, 1973, Franke

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Figs. 10 *a* and *b*. Sections grazing the nuclear envelope and the perinuclear zone. Note the sequence of nuclear interior, nuclear envelope (identified by the numerous pore complexes), the intermediate zone which contains a filamentously textured groundsubstance with occasional membraneous vesicles and tubules (*e.g.*, denoted by the arrows in the insert of Fig. 10 *a*), the perinuclear lacuna of the lacunar labyrinthum, and the cytoplasmic zone. Note the difference in the frequencies of nuclear pores and junction channels demonstrated in Fig. 10 *b*. *N* = nuclear interior, *IZ* = intermediate zone, *LL* = lacunar labyrinthum, *C* = cytoplasmic zone. *A. mediterranea*. 10 *a*, ×21,000. Insert, ×51,000. 10 *b*, ×12,000

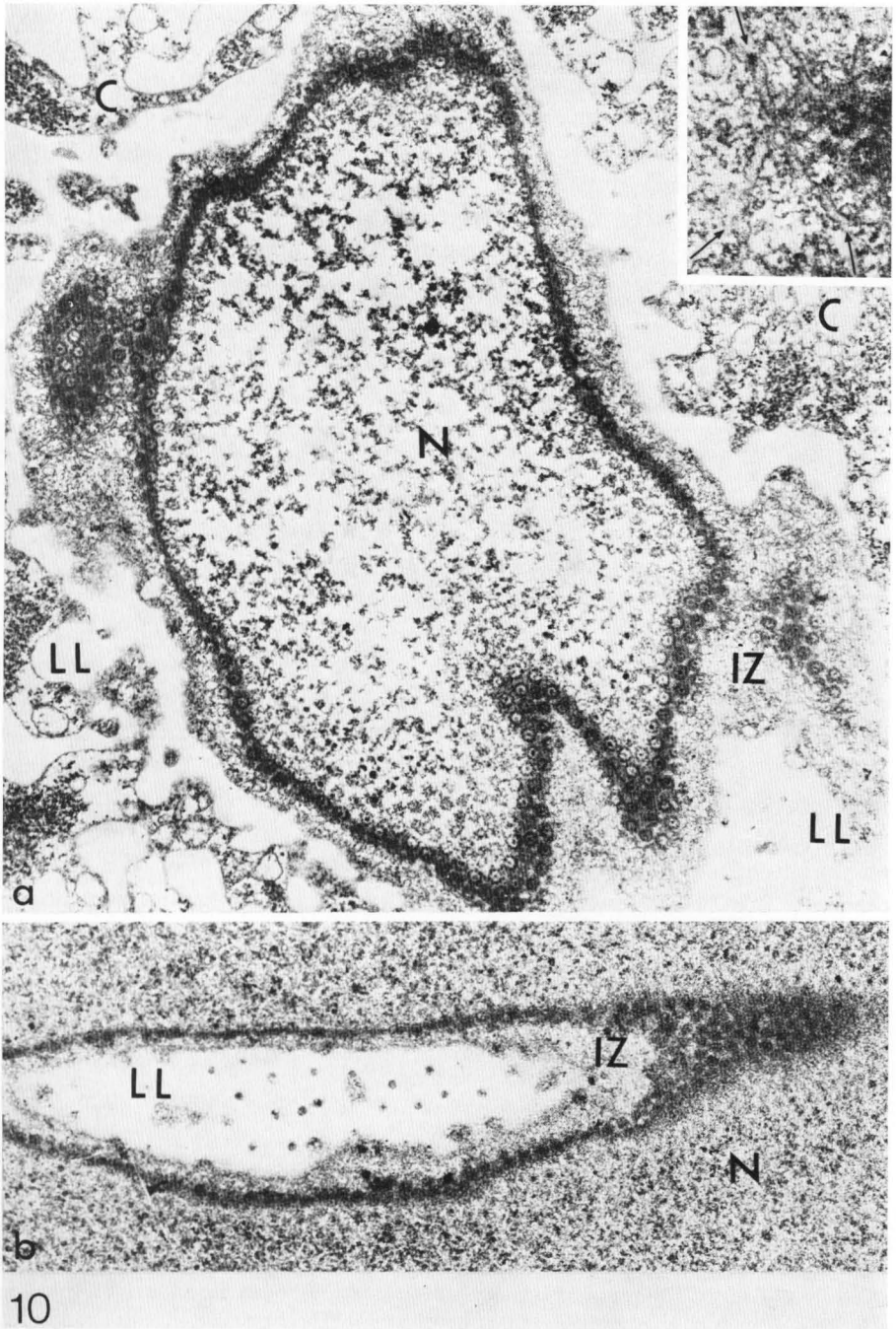

Fig. 10

and SCHEER 1974) by not having the typical symmetrical arrangement of non-membraneous components associated and are rather related to the less regular channel formations in the cisternae of the endoplasmic reticulum, dictyosomes, and the plasma membrane of, for instance, the blood epithelium (PALADE and BRUNS 1968, FRIEDERICI 1969, MAUL 1971, FRANKE *et al.* 1971 b, FRANKE and SCHEER 1972). These channels are filled with densely coiled fibrillar material but, not infrequently, they reveal associations with distinct, very electron opaque aggregates of granules or rods of the "perinuclear lacuna associated aggregates" (PLAA, Fig. 1), and a variety of small membraneous profiles (Figs. 9 *a* and *c*, and 11 *b* and *c*). Both types of structures, aggregates and small vesicles can also be found in the cytoplasm interspersed in the perinuclear lacuna.

3.4. The Perinuclear Dense Bodies

The cytoplasm of the distal or cytoplasmic side of the perinuclear lacuna is characterized by the occurrence of conspicuous spherical or ellipsoidal perinuclear dense bodies which vary in diameter from 200 to 2,000 nm (CRAWLEY 1963, WERZ 1964, VAN GANSEN and BOLOUKHÈRE 1965, BOLOUKHÈRE 1970, WOODCOCK and MILLER 1973, ZERBAN *et al.* 1973). These bodies (PB in Fig. 1) are visible with a light microscope and are heavily stained with the usual electron microscopical staining reagents such as uranyl, lead, and indium salts (for cytochemical details see below and SPRING *et al.* 1974). A list of synonyms from the literature for such "heavy body"-like structures in other cell types has been given in a previous article (FRANKE and SCHEER 1971). Such perinuclear dense bodies are situated, for instance, at the distal, *i.e.*, the cytoplasmic side of the majority of the "junction channels" (*e.g.*, Figs. 9 *a–c* and 11 *a–c*). They are not structurally homogeneous but consist of distinct components. One can distinguish aggregates of large (ca. 30 to 40 nm) dense granules (*gc* in Fig. 1; Figs. 9 *a–c*, 11 *a–c*, and 14) which are usually separated from each other by electron-transparent "halos" and, as a whole, are attached to or surrounded by large coils of densely packed fibrils (*fc* in Fig. 1; Figs. 11 *a–c*, 14, and *15 a*). The existence of these two distinct structural moieties within the perinuclear dense bodies

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Figs. 11 *a–c*. Details of the perinuclear dense bodies (two long arrows in Fig. 11 *a*) which differ in appearance from their associated satellite bodies. The latter may be either dense aggregates of the junction channels (long arrows in Fig. 11 *b* and *c*), sheetlike formations (triangle in Fig. 11 *b*) or vesicles. The perinuclear dense bodies reveal a subdivision into two regions, a granular component and a "fibrillar" one. These two components are illustrated at higher magnification in Figs. 11 *b* and *c*. Note also the characteristic aggregates of dense granules usually located at the distal side of the perinuclear bodies (uppermost arrow in Fig. 11 *a*). *P* = plastid, *N* = nucleus, *LL* = lacunar labyrinthum, *gc* = granular component, *fc* = fibrillar component. *A. mediterranea*. 11 *a*, ×22,500. 11 *b*, ×79,000. 11 *c*, ×75,000

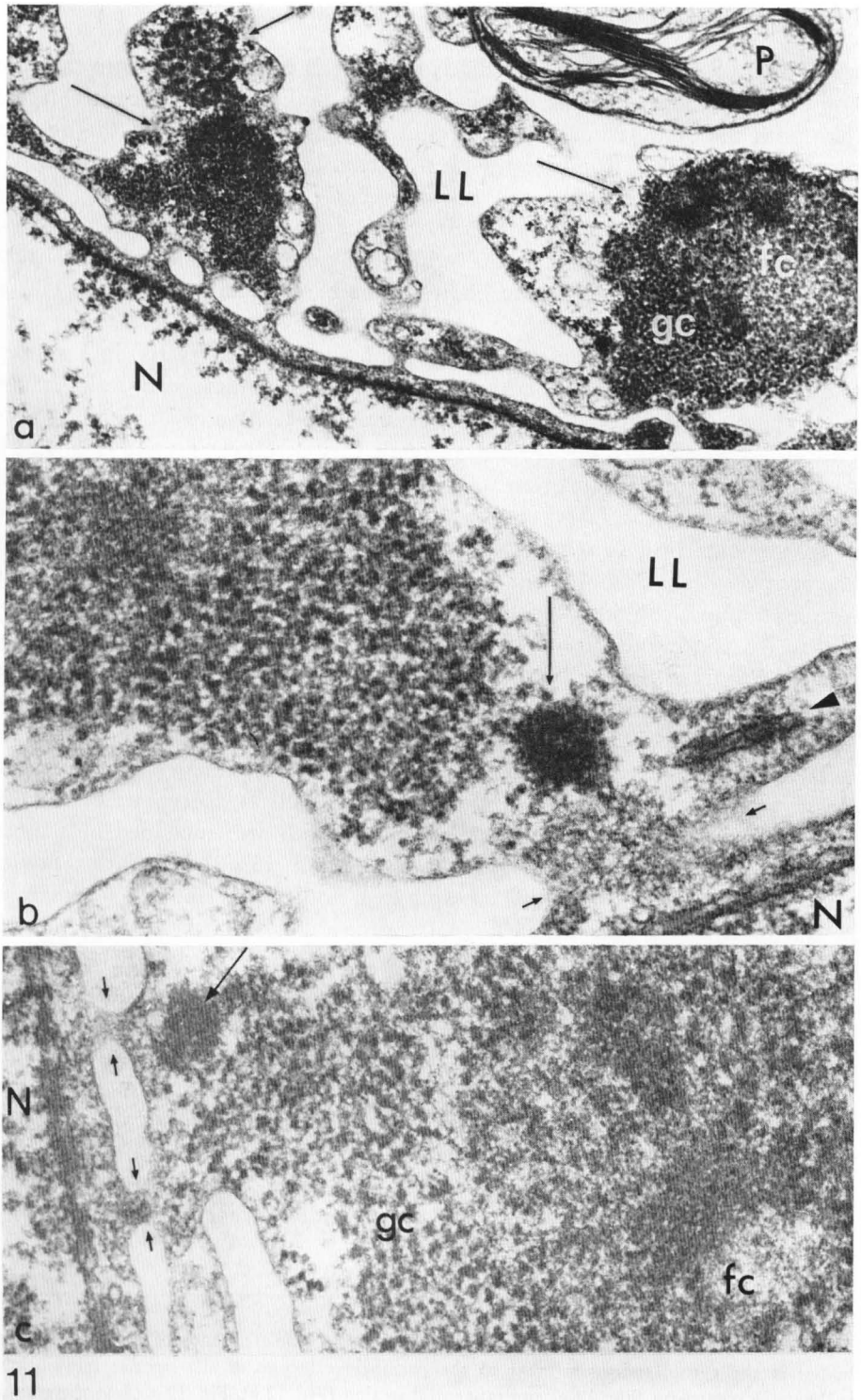

Fig. 11

was evident with all fixation methods used. It is important to note that the granular components of the perinuclear dense bodies are significantly larger than both the cytoplasmic ribosomes and the annular granules of the nuclear pore complex. This is especially clear in those situations where ribosomal chains, putatively representing polyribosomes (*pr* in Fig. 1; Fig. 15 *a*) are recognized in close proximity to, or even as emerging from, these bodies. Frequently, the fibrillar part seems to be clearly segregated and can constitute either a central core within the bodies or lie excentrically (*e.g.*, Figs. 11 *a–c* and 14).

The frequency of these perinuclear dense bodies is demonstrated in a survey micrograph in Fig. 3 and is perhaps best illustrated in micrographs of sections which barely cut through the nucleoplasm (Fig. 12 *a*) and in sections tangential to the nuclear surface but through this perinuclear zone (Fig. 12 *b*). A rough estimate indicates that up to 20,000 such bodies can surround a full size primary nucleus. At a greater distance from the nucleus where there is an intricate branching of the lacunar labyrinthum (Fig. 1), these bodies frequently show a somewhat altered structure with conspicuous lighter regions within them (the "vacuolated dense bodies" in Fig. 1; *e.g.*, Fig. 15 *b*) and a high packing density of the fibrillar components. However, occasionally this modification of the perinuclear dense bodies is also recognized in the immediate nuclear vicinity (*e.g.*, Fig. 7 *d*). In close association with the perinuclear dense bodies one frequently sees very tightly packed fibrillar coils of the kind described above as PLAA (Fig. 1), usually in the region closest to the nucleus (Figs. 9 *a* and 11 *a–c*), as well as aggregates of smaller (40–70 nm in diameter) very dense floccules which are embedded in a more loosely fibrillar matrix (*e.g.*, Figs. 11 *a*, 14, and 15 *a*). The latter bodies resemble the type of complex cytoplasmic bodies described, for example, in the pollen mother cells of *Canna* (SCHEER and FRANKE 1972) and the oocytes of the marine snail *Ilyanassa* (TAYLOR and ANDERSON 1971). Another component, frequently associated with both the perinuclear dense bodies and the previously described bodies are small membraneous elements which resemble flattened cisternal pieces and vesicles (Figs. 9 *a* and *b* and 11 *a* and *b*). It is not clear whether the dense aggregates occasionally encountered in the perinuclear zone (see section 3.2.) are related to the perinuclear dense bodies or one of their subcomponents. It is noteworthy that very few other cytoplasmic constituents such as plastids, mitochondria and dictyosomes occur in the perinuclear zone where the dense bodies are so characteristically found.

Figs. 12 *a* and *b*. Distribution of perinuclear dense bodies. The frequency of perinuclear dense bodies in the cytoplasmic zone next to the perinuclear lacuna of the lacunar labyrinthum is shown in transverse (Fig. 12 *a*) and tangential section (Fig. 12 *b*). Fig. 12 *a*. *A. mediterranea*; Fig. 12 *b*. *A. major*. 12 *a*, ×4,500. 12 *b*, ×9,500

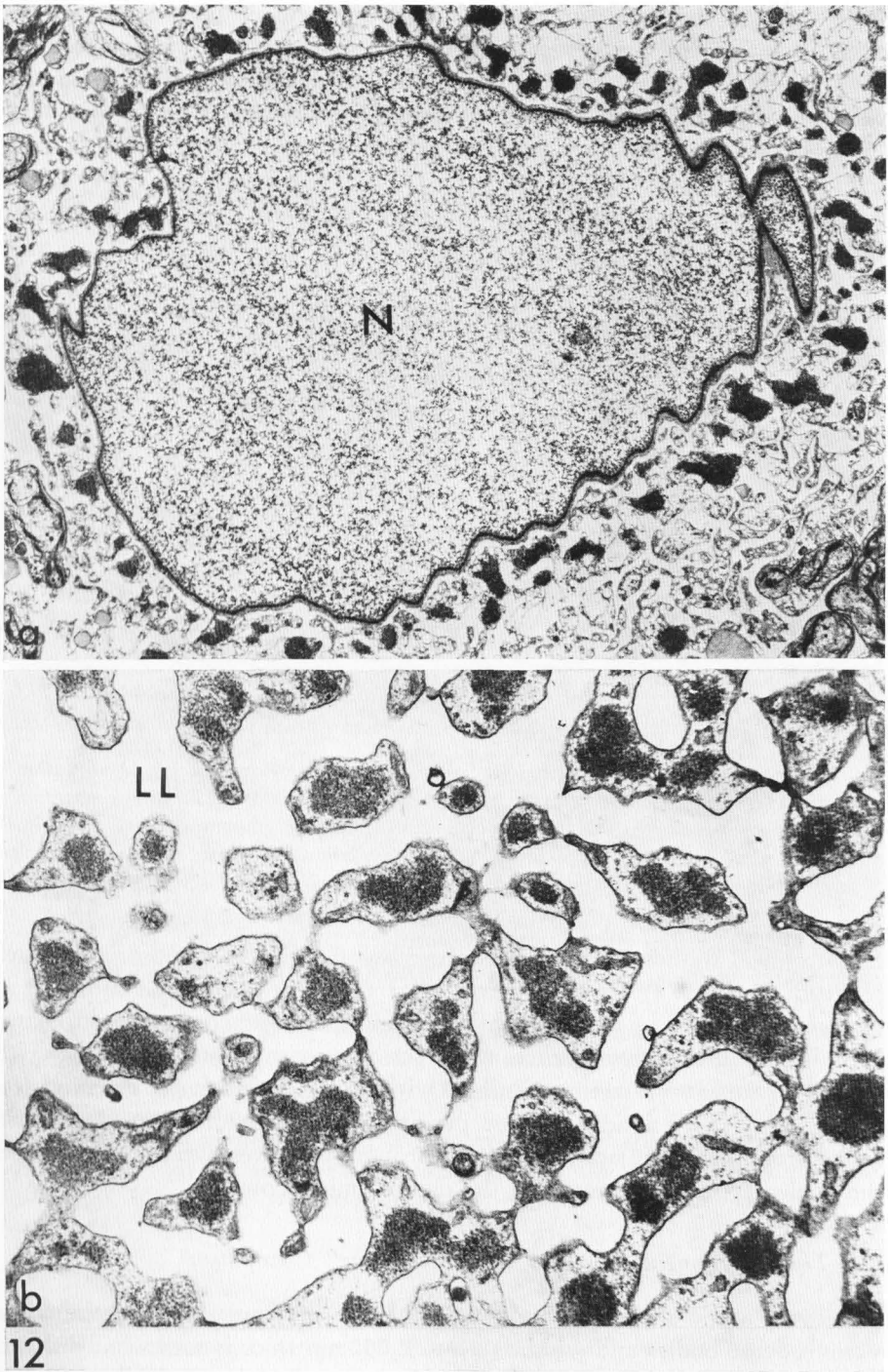

Fig. 12

As with all of the demonstrations of cytoplasmic heavy body-like aggregates cited for other cell types such as oocytes, spermatids, and tumor cells, one could argue that these structures represent a special assembly of ribonucleoproteins (for references, see Allen and Cave 1969, Conway and Metz 1970, Dhainaut 1970, Franke and Scheer 1970 b, 1971, 1974, Conway 1971, Eddy and Ito 1971, Mahowald 1971, Weakley 1971, Fawcett 1972) or are purely proteinaceous (*e.g.*, Clérot 1968, Eddy and Ito 1971, Gerin 1971).

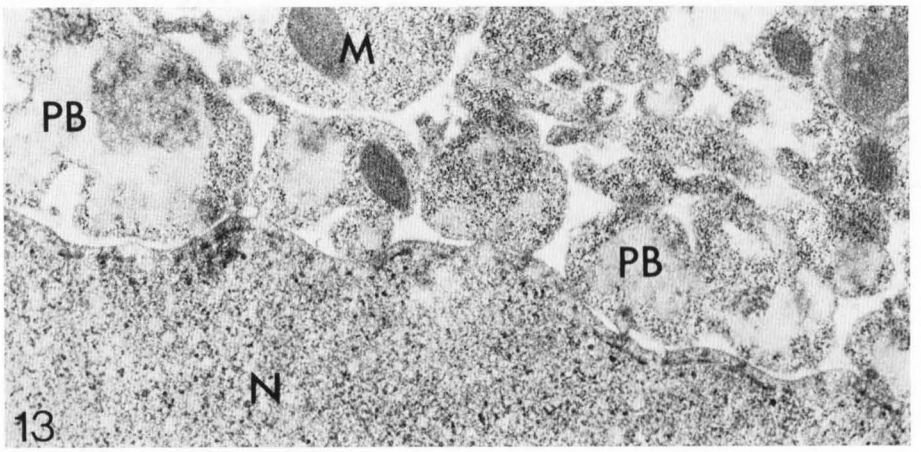

Fig. 13. Cytochemistry of the perinuclear zone. Sections were stained for identification of nucleoproteins according to Bernhard (1969) using EDTA for bleaching. Note retention of stain in the nucleus, in the nuclear pore complexes, in the cytoplasmic ribosomes, and in the mitochondria, in contrast to the bleaching of the perinuclear dense bodies. *N* = nucleus, *M* = mitochondrion, *PB* = perinuclear dense bodies. *A. mediterranea*. ×19,200

Cytochemical studies, which will be described in detail in a subsequent article (Spring *et al.* 1974) show, however, that these perinuclear dense bodies in *Acetabularia* react in a way usually considered characteristic for chromatin, including a green fluorescence with acridine orange and bleaching after EDTA-treatment of sections stained with uranyl acetate (as demonstrated in Fig. 13). The reaction of these perinuclear dense bodies was clearly different from that of adjacent ribonucleoprotein structures such as the nucleolus, the nuclear pore complexes and the cytoplasmic ribosomes.

3.5. The Components of the "Reticulate Zone" Cytoplasm

The cytoplasmic shell which surrounds the perinuclear zone containing the various dense bodies and which is up to 2,000 nm thick is somewhat different from average rhizoid cytoplasm (Figs. 3, 14, and 15 *b*). It is characterized by an intricate branching of the lacunar labyrinthum (*c.f.*, Burr and West

1971, ZERBAN *et al.* 1973) and the occurrence of rod- or sausage-like dense fibrillar aggregates ("reticulate zone dense rods", Fig. 1), which are 80–110 nm broad, and the "vacuolated dense bodies" described in the previous section. The ultrastructure suggests that these bodies consist mainly of fibrillar mate-

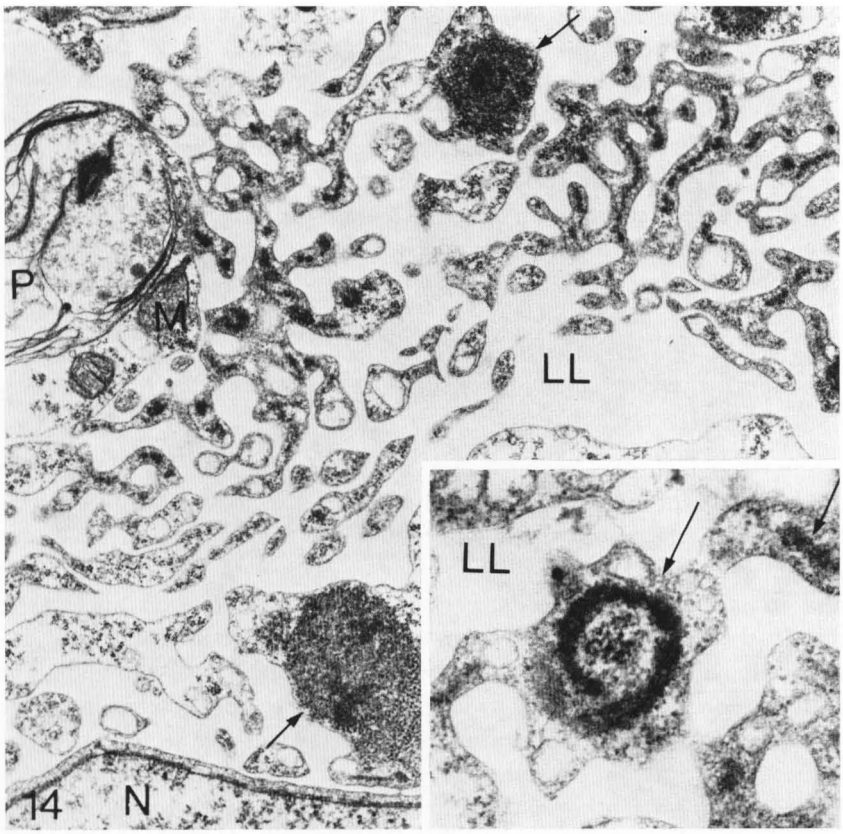

Fig. 14. Radial cross-section of the "reticulate zone" of the perinuclear cytoplasm around the primary nucleus. Note the intricate branching of the lacunar labyrinthum which is traversed by only slender cytoplasmic strands. These cytoplasmic strands contain some perinuclear dense bodies (arrows), but also mitochondria, plastids, and the sausage- or rod-like dense aggregates which are characteristic for this zone ("reticulate zone dense rods"; recognized, *e.g.*, in the upper right and, at higher magnification, at the arrows of the insert). *N* = nucleus, *LL* = lacunar labyrinthum, *M* = mitochondrion, *P* = plastid. *A. mediterranea.* ×14,500. Insert, ×36,500

rial whereas the relatively few granular components appear to be confined to the more transparent regions ("vacuolizations") within these bodies (Fig. 15 *b*). The normal cytoplasmic organelles and components are more frequent in this zone than in the perinuclear zone.

3.6. Changes of the Perinuclear Complex during Nuclear Maturation

The complex perinuclear structures described above are confined to, and characteristic for, the nucleus of the developing vegetative cell. They do not occur in the germinating zygote nor in the nuclei of the very young germling (Figs. 2 *a* and *b*). Their formation seems to coincide with the dramatic growth of the nucleolus and is then maintained up to the stage when the nucleus differentiates into a mature state which eventually gives rise to secondary nuclei. This maturation is indicated by the development of large and bizarre-shaped nuclear evaginations which take place during cap formation (*e.g.*, Fig. 16). Thereupon the perinuclear region changes so that less vacuolized cytoplasm borders the fully mature nucleus. This stage is also characterized by a supercondensation of the nucleolus (see Fig. 17 b). The perinuclear zone is no longer different from the normal rhizoidal cytoplasm and the perinuclear dense bodies seem to become more dispersed throughout the rhizoid (Figs. 17 *a* and *b*).

3.7. Conclusions and Speculations as to the Possible Functions of the Perinuclear Complex Components

Although a complex perinuclear apparatus is characteristic for the developmental stage of the primary nucleus, there appears to be no direct clue as to its role in the subsequent formation of the secondary nuclei (BERGER *et al.* 1974). Perhaps, the whole perinuclear apparatus simply is an expression of, and functions in, the specific metabolic roles of the giant nucleus, *i.e.*, primarily the production of large amounts of RNA of all categories, especially of ribosomal RNAs as suggested by the development of the giant nucleolus. In this respect, it is puzzling to see that the nuclear pores which have increased in frequency as well as in total number per nucleus (see section 3.1.) obviously do not constitute a critical spatial barrier for any translocation of ribonucleoproteins or other particulate materials because of the much smaller cross-sectional area of junction channels between the indermediate zone and the cytoplasm (for general reviews on nucleo-cytoplasmic transportation of particulate matter see, *e.g.*, GALL 1964, STEVENS and ANDRE 1969, FRANKE

Figs. 15 *a* and *b*. Details of the perinuclear dense bodies found in the reticulate zone. Note dense small islets within a fibrillarly textured matrix of intermediate packing density (in Fig. 15 *a*) as well as granular clusters (arrowheads in Fig. 15 *a*). Note also the occurrence of ribosomes and polyribosomes at the periphery of these perinuclear dense bodies (small arrow in the left of Fig. 15 *a*) and the frequency of a specific reticulate type of aggregated granules and flocculent filamentous material (denoted by the upper and lower arrow in the left of Fig. 15 *a*). Fig. 15 *b* presents the typical appearance of a vacuolated dense body. *LL* = lacunar labyrinthum, *M* = mitochondrion. *A. mediterranea*. 15 *a*, ×54,000. 15 *b*, ×27,500

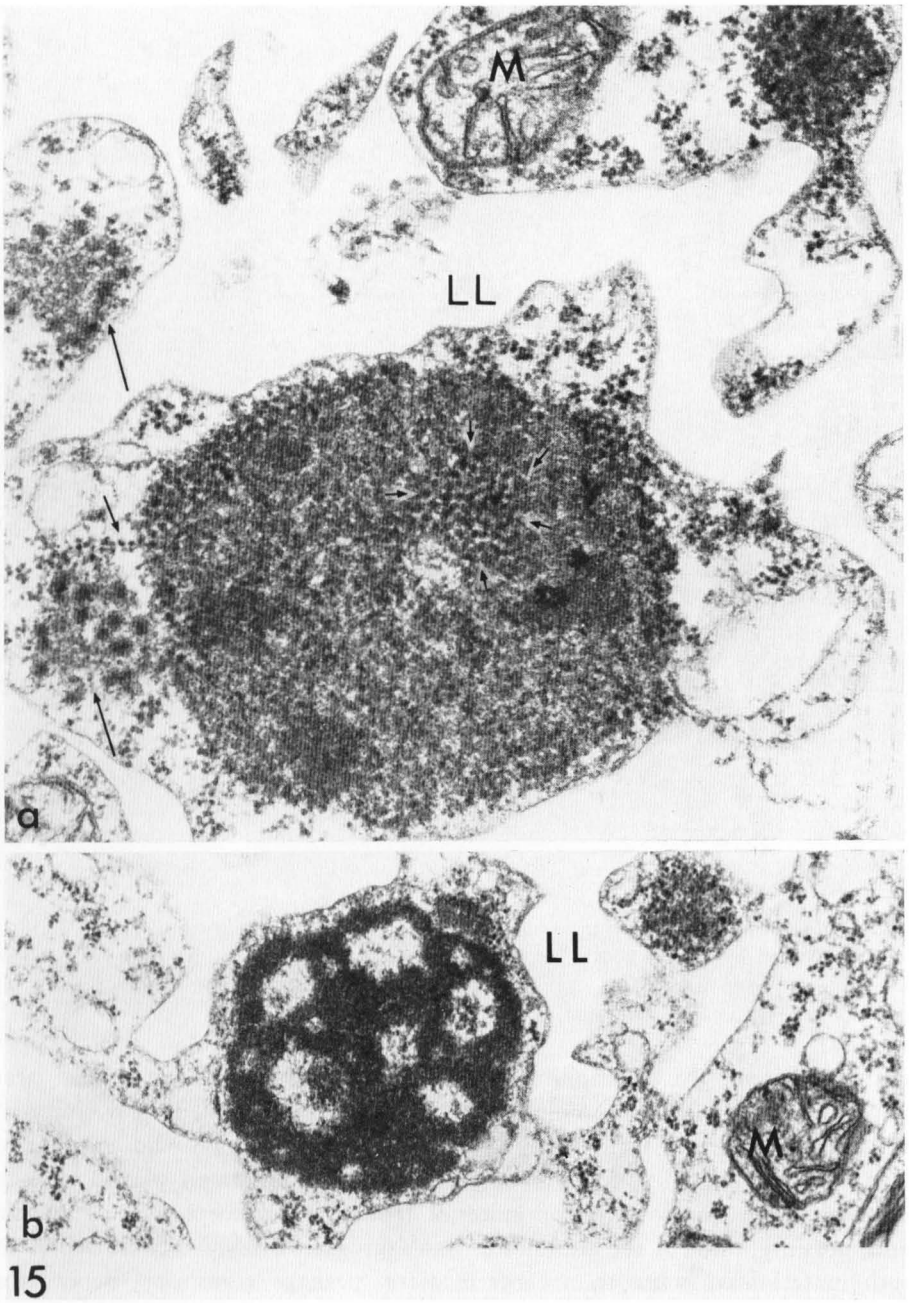

Fig. 15

and Scheer 1970 b, 1974, Feldherr 1972, Kay and Johnston 1973, Kessel 1973).

The function of the perinuclear lacunar labyrinthum, together with the fibrillar intermediate zone on which it borders, is still enigmatic. It might be that the perinuclear membrane system of the lacunar labyrinthum provides the nucleus with ions and small molecules by establishing a cisternal supply

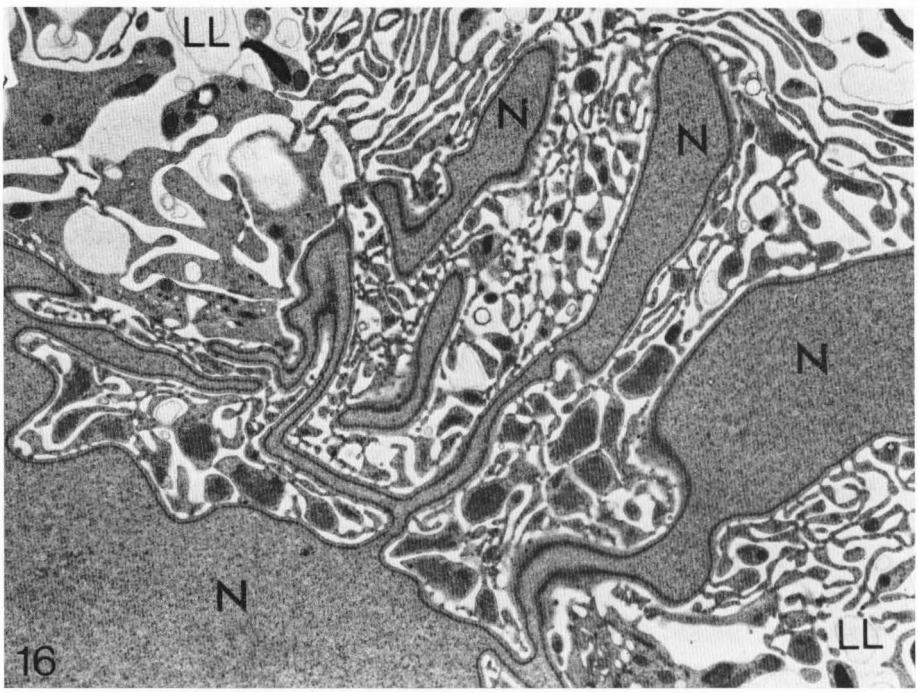

Fig. 16. Perinuclear zone in a maturing primary nucleus (during cap formation). This stage is characterized by an increase in nuclear surface in the form of extensive evagination and nuclear lobe formation. Note the preserved highly branched lacunar labyrinthum. *N* = nucleus, *LL* = lacunar labyrinthum. *A. mediterranea*. ×2,300

channel system. In addition, it represents a large membrane surface area which could be active in molecular transport in the immediate vicinity of the giant nucleus. On the other hand, the perinuclear zone whose organization is determined by the lacunar labyrinthum could well play a role in regulating the translocation of material from the nucleus to the cytoplasm and, possibly, some of the granulofibrillar aggregates described could represent such material en route to an intermediate, perhaps transitory, depot (for similar discussion as to the juxtanuclear bodies in the other cell systems mentioned above see Clérot 1968, Harris 1967, Franke and Scheer 1970 b, 1974, Eddy and Ito 1971, Mahowald 1971, Fawcett 1972). It is therefore possible, that these aggregations are either perinuclear storage of such material

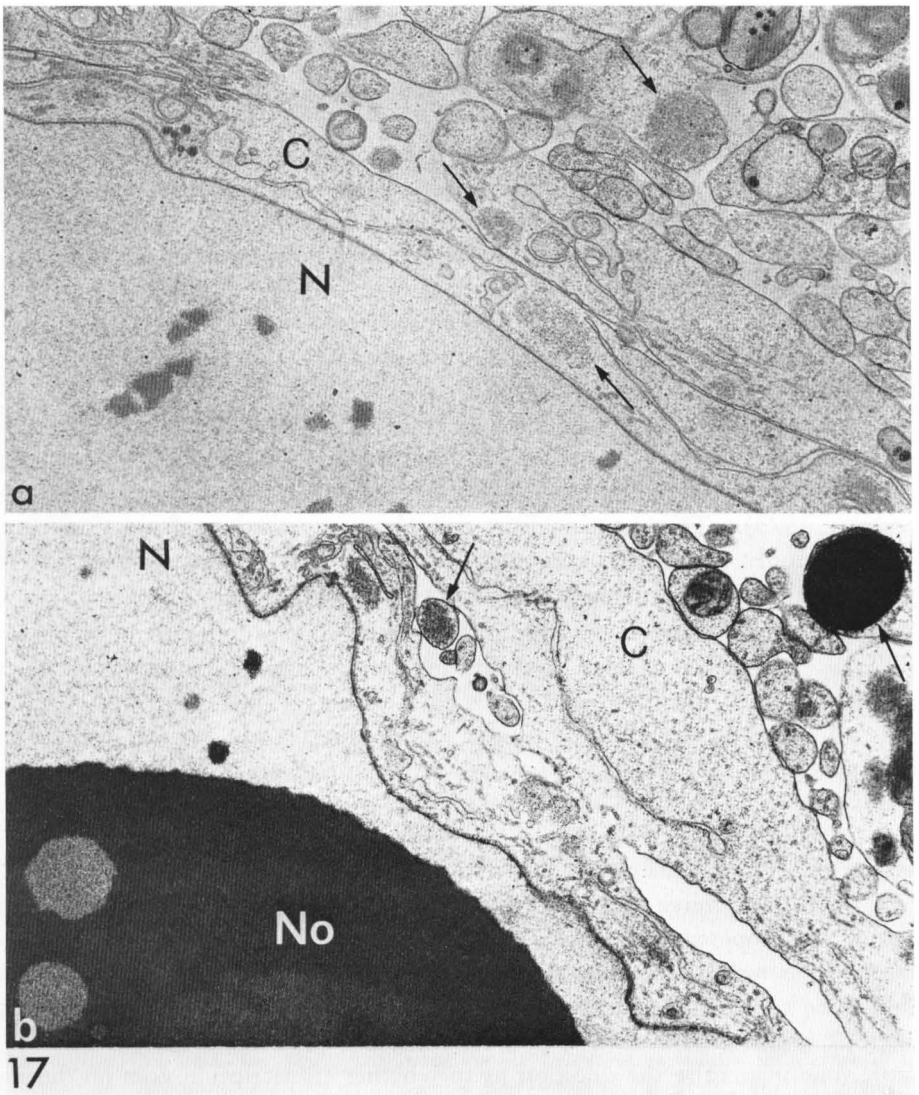

Fig. 17 *a* and *b*. Perinuclear zone in a mature nucleus (maximal cap size). Such a nucleus is characterized by extreme nucleolar condensation (Fig. 17 *b*) and the disappearance of the perinuclear lacuna and the perinuclear cytoplasmic organization characteristic of earlier stages. Note the preservation of the perinuclear dense bodies (some are denoted by arrows) which sometimes appear extremely condensed. *N* = nucleus, *No* = nucleolus, *C* = cytoplasm. *A. mediterranea*. 17 *a*, ×10,125. 17 *b*, ×10,125

or are related to the relatively rapid nucleoprotein elimination phenomena, in response to cell irritations, described by WERZ (1962).

Another possibility, and not necessarily an exclusive one, is that the perinuclear lacunar apparatus with the dense filamentous web of the inter-

mediate zone serves as some sort of exoskeleton for the giant nucleus and fixes it in its position relative to the other components of the rhizoidal cytoplasm. Such a skeletal supportive role has likewise been discussed for other structural formations in specific giant nuclei, in particular for the intranuclear dense laminae or "honeycomb-layers" associated with the inner nuclear membranes of some amoebae (Harris and James 1952, Greider *et al.* 1956, Pappas 1956, Mercer 1959, Daniels and Breyer 1967, Stevens 1967, Daniels *et al.* 1969, Flickinger 1970) of neuronal and other animal tissue cells (Gray and Guillery 1963, Coggeshall and Fawcett 1964, Fawcett 1966, Stelly *et al.* 1970) and, interestingly, also of the siphoneous green alga *Bryopsis* (Burr and West 1971). In this connection one is also reminded of the endoplasmic reticulum cisternae which have been described to surround the dividing nuclei of various algae (*e.g.*, Marchant and Pickett-Heaps 1970, Pickett-Heaps 1972, McDonald 1972) and, for example, also the young spermatids of the fruitfly, *Drosophila melanogaster* (Rasmussen 1973). These perinuclear endoplasmic reticulum formations are, however, only (perhaps functionally) analogous but not homologous to the perinuclear lacunar system of *Acetabularia.*

The perinuclear bodies exhibit some morphological details reminiscent of the normal nucleolar composition, including a division into a granular and fibrillar part (review: Busch and Smetana 1970), a similar size of their granular particles (same review; see also Simard *et al.* 1973), and associations with groups of vesicles (compare, *e.g.*, Miller 1966, Kezer *et al.* 1971). In view of this structural similarity as well as of the cytochemical indications that these bodies contain, in addition to proteins and RNA, significant amounts of a substance reacting like DNA (see above and Spring *et al.* 1974), and because they are found during developmental stages which are characterized by nucleolar hypertrophy, we would like also to suggest a rather unusual hypothesis, namely, that these bodies include amplified DNA molecules, perhaps containing rRNA cistrons, which are not held within the nucleus but are translocated. Furthermore, one may raise the question as to whether transcription goes on during and even after this translocation. A nucleocytoplasmic translocation of a body containing amplified DNA with rRNA cistrons, though apparently after transcription and via nuclear pocket formation, has recently been described in the maturing oocytes of the house cricket by Jaworska and Lima-de-Faria (1973, see also Jaworska *et al.* 1973).

The discussion about the structural and functional organization of the perinuclear region in *Acetabularia* indicates that the definition of the nucleus as an organelle which is circumscribed by the nuclear envelope is not sufficient to characterize the nucleus in functional terms. It appears that some nuclear functions which might even include transcription of parts of the nuclear genome can take place outside the nucleus *sensu stricto* in the perinuclear

region during certain stages of cell development. In fact, we would not exclude the possibility that some nuclear functions have a dynamically variable compartmentalization. This means that certain functions, which in some cell stages are localized within the nucleus, in other stages may occur in juxtanuclear regions of the cytoplasm as well.

The question as to whether the perinuclear structures described here for the primary nucleus contain any determinants for the further development of the alga will be dealt with in a subsequent article (BERGER *et al.* 1974).

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