GROWTH OF THE NUCLEAR ENVELOPE IN THE VEGETATIVE PHASE OF THE GREEN ALGA *ACETABULARIA*

Evidence for Assembly from Membrane Components Synthesized in the Cytoplasm

WERNER W. FRANKE, HERBERT SPRING, ULRICH SCHEER, and HEIDE ZERBAN.
From the Division of Membrane Biology and Biochemistry, Institute of Experimental Pathology, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany, and the Institute of Plant Physiology and Cell Biology, Free University of Berlin, D-1000 West Berlin

In current cytology, the mechanisms of the formation of the various biomembranes in eukaryotic cells are subject to quite controversial hypotheses (1, 5, 11, 16, 17, 23–26, 30, 31, 33). For example, there is a continuing debate on questions such as: (*a*) whether constitutive membrane proteins are synthesized on free or membrane-bound polyribosomes or on both; (*b*) whether the proteins and lipids of membranes of the "rough" category including rough endoplasmic reticulum (rER), nuclear envelope (NE), and annulate lamellae (AL) are originally made and assembled *in situ*, i.e. at the specific membrane, or whether they are made elsewhere and incorporated into these membranes in a secondary step (see references above); (*c*) whether membranes grow by the integration of preformed membrane units as cisternae and vesicles (vesicular "membrane flow"), or of "free" individual molecules or morphologically ill-definable micelles; and (*d*) whether there exists a general mode of membrane biosynthesis and assembly at all.

In most cell types the nuclear envelope does not show considerable growth rates and special growth phenomena. It is very similar to the rER in most membrane properties such as morphology (e.g., thickness, polyribosome association at least in parts, formation of smooth-surfaced and coated vesicles), biochemical composition, and kinetics of labeling of its components (6–8, 12, 19–21). In some cell stages, however, there is an extensive nuclear envelope growth, particularly in cells characterized by the formation of giant nuclei such as during oogenesis of a great many animals, in the course of chromosome polytenizations, during micro- and macrospore formations in many plant organisms, and in the postgerminal vegetative growth phase of some dasycladacean green algae (14, 15, 36). Among such cell systems, the formation of the primary nucleus in the green alga genus *Acetabularia* provides a particularly interesting and unique demonstration of nuclear envelope growth by the incorporation of components which are synthesized in the cytoplasm and can reach the nuclear membranes only by flow processes.

MATERIALS AND METHODS

Algae (*Acetabularia mediterranea* and *Acetabularia* [*Polyphysa*] *cliftonii*) of various sizes, from the germinating zygote up to fully mature cells (ca. 4 cm large) at the beginning of cap formation, were fixed and processed for electron microscopy and morphometric evaluation as described earlier (9, 10, 32, 34). Freeze-cleave etch preparations were performed as previously described (18, 37, 38). For calculations of total nuclear membrane area and mass, the following parameters were determined: (*a*) the nuclear diameter (by phase-contrast light microscopy); (*b*) the "redundancy" (cf. reference 2), this is the increase of nuclear surface by the formation of evaginations, which occur especially in the very late stage of nuclear maturation (9), relative to the surface of the spherical central part of the nucleus (by membrane profile tracing in electron micrographs); (*c*) the mean nuclear membrane thickness which was consistently 6.0 nm ± 0.3 nm in all stages examined in ultrathin sections; (*d*) the nuclear pore frequency, i.e. number of pores per square micrometer, and the total number of pore complexes per nucleus (by electron microscopy of thin sections, freeze-cleave preparations, and whole mount preparations of isolated nuclear envelopes; with these nuclei, as with other giant nuclei such as in oocytes, almost identical values are found by the different preparation methods; cf. 8, 12, 18, 32, 37, 38); (*e*) the slight difference in membrane area between pore walls and the corresponding two inner areas (dimensions taken from electron micrographs: 64 nm mean inner pore diameter, 25 nm mean nuclear envelope width from cytoplasmic to nucleoplasmic surface). Membrane profile lengths were determined by projecting micrograph plates with a photographic enlarger onto tracing paper, tracing and drawing the contours of the classified membranes under controlled magnification, and measuring the individual membrane profiles with a map measurer. Inner pore diameters (for definition, see references 8, 12, 18, 21) of the nuclear pore complexes and in the paranuclear cisterna of the labyrinthum were determined in both tangential sections and in tangential freeze-fractures (see also reference 38).

RESULTS AND DISCUSSION

Upon germination, the nucleus of the zygote of *Acetabularia* increases dramatically from 3.5 to 5 μm to a maximum diameter of 100 μm, in some cases even 150 μm. From a certain early germling stage onward (with about 10 μm nuclear diameter), a special membrane complex is continuously present in the form of a paranuclear cisternal system that obviously is derived from—and is at many sites continuous with—the large vacuolar cavity system of the rhizoid, the "lacunar labyrinthum" (LL) (3, 4, 9, 35). This perinuclear cisternal system which completely ensheathes the nucleus in a conspicuous parallelism (90–100 nm mean distance from the nuclear envelope surface, Fig. 1) and, so to say, constitutes a secondary nuclear envelope, has recently been described in detail, together with a variety of associated structures (9). In the context of the present note, it is worth emphasizing the following observations. (*a*) The nuclear envelope is not continuous with any part of the rER. (*b*) The outer nuclear membrane is not

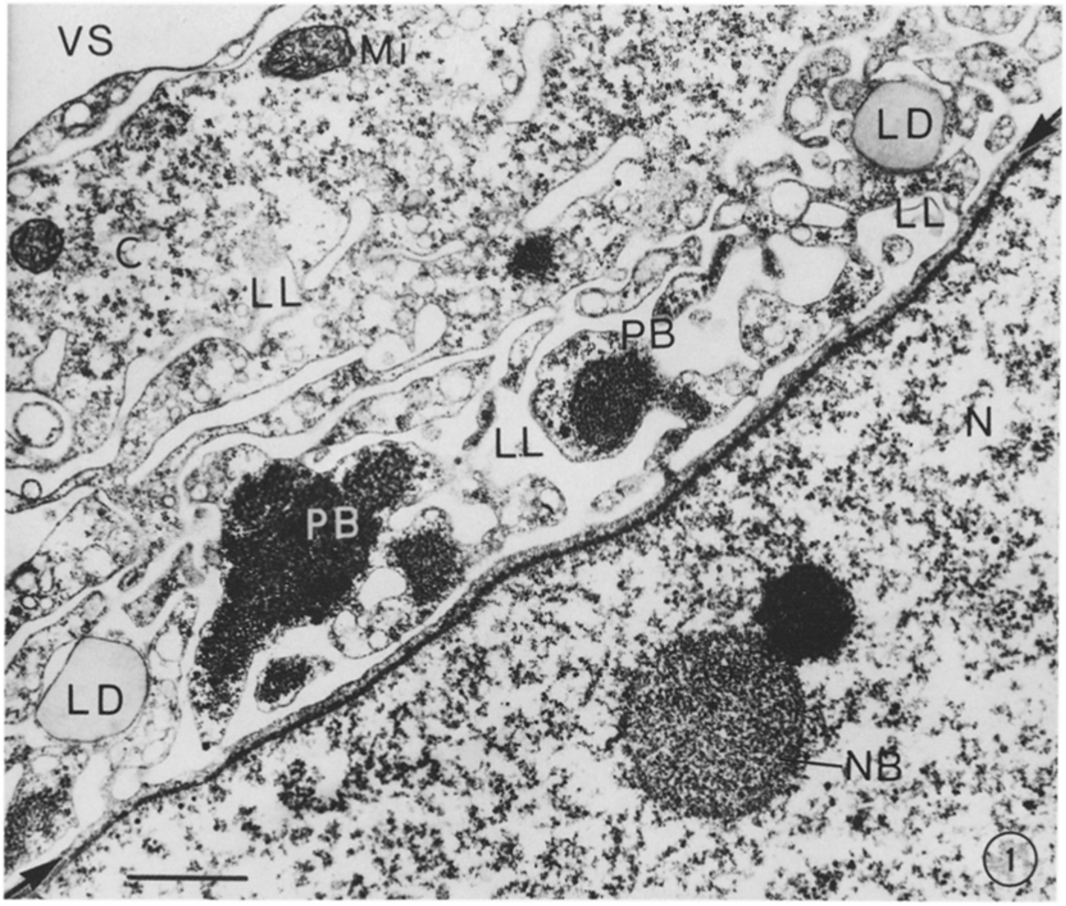


FIGURE 1 Survey electron micrograph of the perinuclear zone of *Acetabularia mediterranea* as revealed in ultrathin sections. The nucleus (*N*) is surrounded by the nuclear envelope, the intermediate zone (denoted by the two arrows), and the paranuclear cisterna of the lacunar labyrinthum (*LL*). Only very few junction channels connect the intermediate zone with the cytoplasm (*C*). *NB*, nuclear body; *LD*, lipid droplet; *VS*, vacuolar space; *Mi*, mitochondrion; *PB*, perinuclear body. Magnification, × 16,000; the scale = 1 μm.

associated with polyribosomes, nor are ribosomes recognized in the "intermediate zone" between the nuclear envelope and the innermost cisterna of the LL (Figs. 2 and 3). (*c*) Other distinct cytoplasmic structures and organelles such as mitochondria, plastids, dictyosomes, large vesicles, ER, and the characteristic "perinuclear bodies" of this stage (see above-quoted references) are also excluded

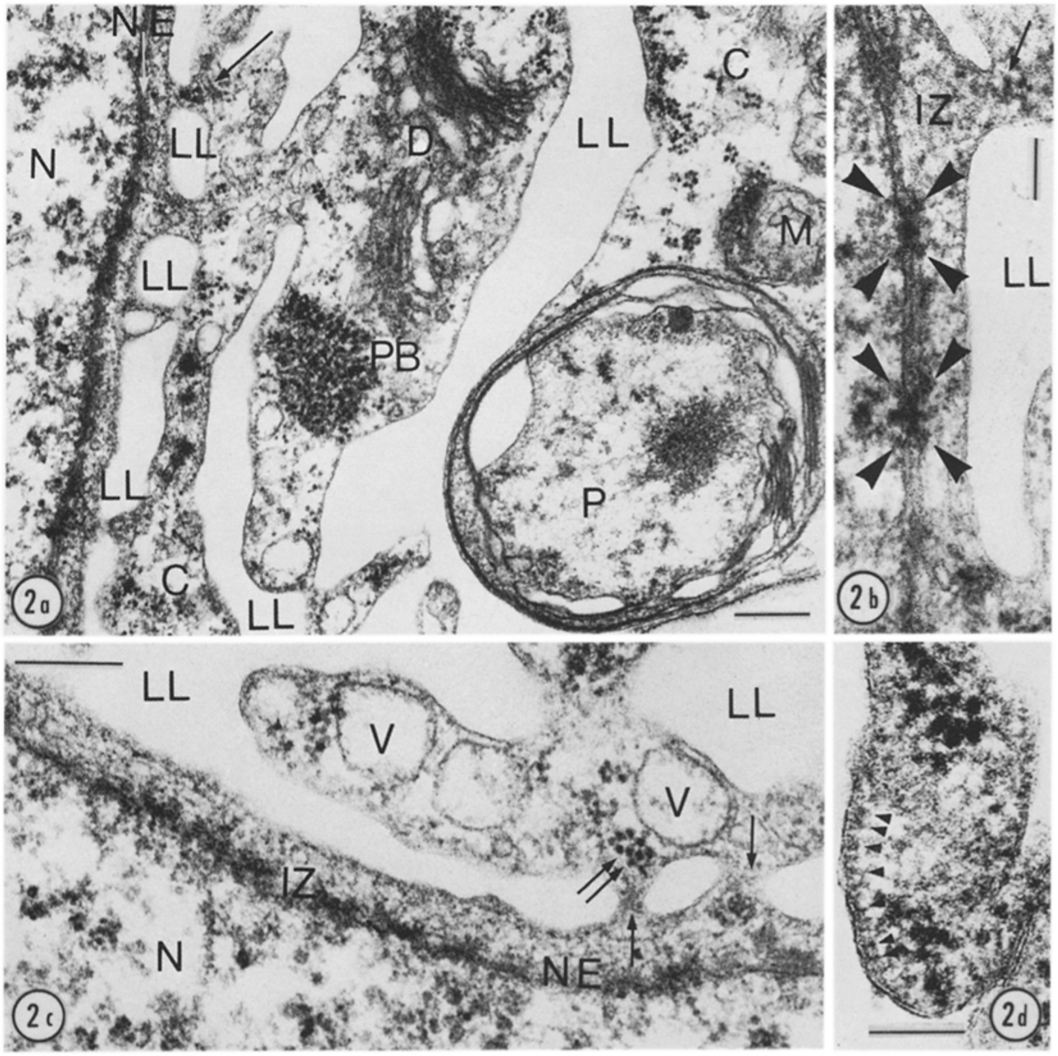
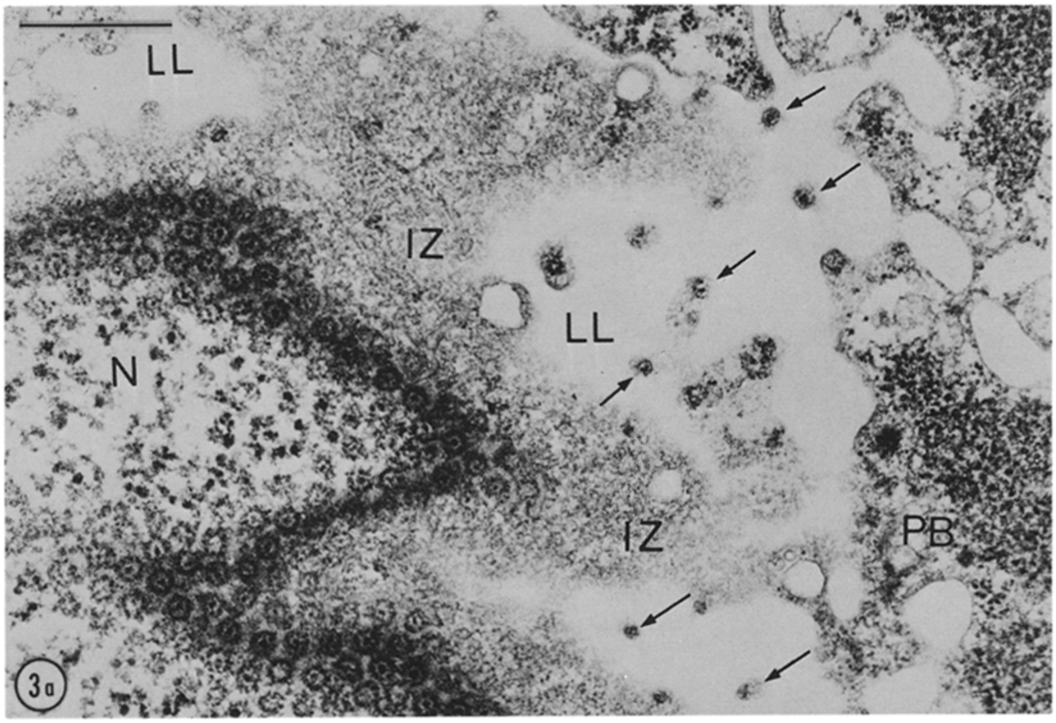
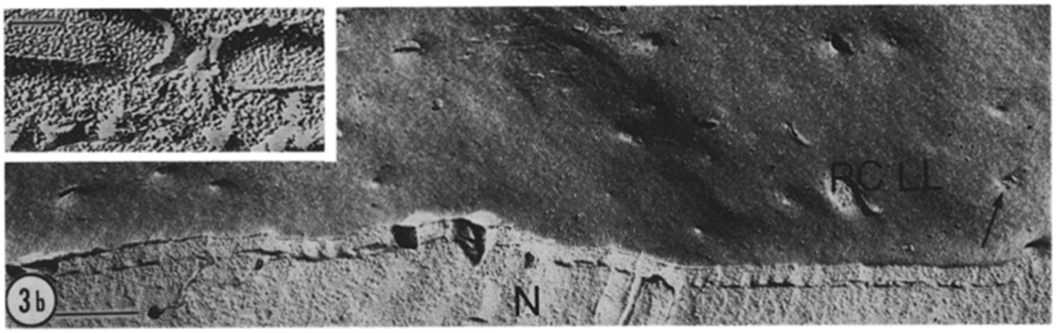


FIGURE 2 *a–d* Details of the nuclear periphery and the juxtanuclear region of *Acetabularia mediterranea* as revealed in electron micrographs of transverse ultrathin sections. Note the exclusion of cytoplasmic organelles and constituents such as plastids (*P*), mitochondria (*M*), dictyosomes (*D*), vesicles (*V*), endoplasmic reticulum cisternae, perinuclear bodies (*PB*), and polyribosomes (e.g., at the pair of arrows in Fig. 2 *c*) from the intermediate zone (*IZ*) which is sandwiched between the nuclear envelope (*NE*) and the paranuclear cisterna of the lacunar labyrinthum (*LL*). *N*, nuclear interior. The arrowheads in (*b*) denote the annular granules of nuclear pore complexes which also reveal central elements. The fenestrae ("junction channels") in the paranuclear LL cisterna do not show such a complex organization but merely aggregated fibrillar material (e.g., arrows, *a–c*). The only structure identifiable in the intermediate zone is a fibrillar meshwork. Note also the regular spike pattern at the inner surface of the LL membrane (*d*), a feature which distinguishes this membrane from, e.g., ER-type membranes. (*a*) Bar = 0.25 μm; × 40,000. (*b*) Bar = 0.1 μm; × 85,000. (*c*) Bar = 0.25 μm; × 57,000. (*d*) Bar = 0.1 μm; × 130,000.


LL
IZ
LL
N
IZ
PB
3a


RC LL
N
3b


PC LL
IZ
NE
N
3c

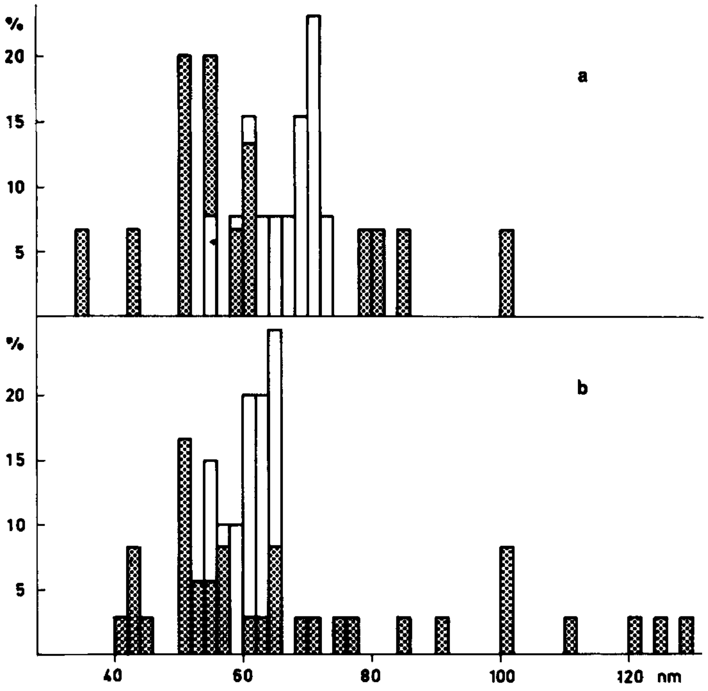


FIGURE 4 Distribution (percent) of inner pore diameters (nanometers measured as membrane to membrane) of nuclear pores (open blocks) and the fenestrae in the lacunar labyrinthum (dotted blocks) as determined in freeze-fractures tangential to the nuclear surface (panel *a*) or in tangential ultrathin sections (panel *b*). The total number of nuclear pores evaluated was 230 (thin sections) and 65 (freeze-etch), respectively; the number of LL fenestrae evaluated was 136 (thin sections) and 42 (freeze-etch), respectively.

from this intermediate zone (Figs. 2, 3). The only structures regularly recognized in this zone are densely interwoven fine filaments (ca. 3.5 nm in width) and occasionally, densely stained globular aggregates (40–100 nm in diameter) of the kind commonly observed in the peripheral zones of the nuclear interior (Figs. 2 and 3; reference 9). (*d*) The intermediate zone is—to some extent—continuous with the nucleoplasm via the pore complexes, and with the cytoplasm via specific fenestrae, the "junction channels," in the paranuclear cisterna of the LL system (Figs. 2 and 4; reference 9). The size distribution of the "junction channels" is given in Fig. 4, in comparison with that of the nuclear pore complexes. Both the pore complexes and the junction channels are associated with some granulofibrillar structures but the organization of the membrane-associated components is clearly different in both kinds of pores. The pores in the LL cisterna do not reveal symmetrically arranged annular and internal granules but appear to be rather homogeneously filled with some finely filamentous tufts (Figs. 2, 3). (*e*) The nuclear pore frequency is about 20 pores per μm^2 in the germlings but then increases dramatically and is fairly constant at between 70 and 80 pores per μm^2

FIGURE 3 *a–c* The periphery of the primary nucleus of *Acetabularia mediterranea* (*a*, *b*) and *A. cliftonii* (*c*) as revealed in grazing sections (*a*) or in tangentially (*b*, *c*) and transversely fractured preparations of frozen cells. Note the high pore frequency of the nuclear envelope (e.g., in *a* and *c*) compared to a low pore frequency in the paranuclear LL cisterna (*PCLL*) (some of these fenestrae are denoted by the small arrows in *a–c*). Note the absence of typical cytoplasmic structures in the intermediate zone (*IZ*). *N*, nucleus; *PB*, perinuclear body. The arrowheads in the inset of (*b*) denote the rims of a fenestra in the paranuclear LL cisterna as revealed in cross fracture. (*a*) Bar = 0.5 μm; × 40,000. (*b*) Bar = 0.5 μm; × 22,000. Inset, bar = 0.1 μm; × 64,000. (*c*) Bar = 0.25 μm; × 59,000.

during the phase of maximal nuclear growth (see also reference 38); the frequency of the junction channel pores in the secondary envelope is much lower (two to six per square micrometer) and they are much less regular in pattern (e.g., Fig. 3). The total number of pore complexes per nucleus attains values of 2-8 × 10^6 (see also references 9, 37, 38), and the total number of junction channels per fully mature nucleus is between 70,000 and ca. 300,000. Maximally, only 1.0-1.5% of the surface of the paranuclear cisterna of the LL is represented by pore area, in contrast to about 23% pore area in the nuclear envelope. (*f*) The growth rate of both the nuclear envelope and the paranuclear LL cisterna is very impressive; during maximal growth the nuclear membrane surface doubles within about 10 days (Table I). The mean increase of nuclear envelope between the 50th and the 90th day after germination would correspond to an average input rate of approximately 1 μm^2 membrane area per minute. (*g*) Nuclear growth and, correspondingly, nuclear envelope enlargement (Table I) takes place within this "cage" constituted by the lacunar labyrinthum cisterna. (*h*) Apart from the occasional occurrence of some (smooth-surfaced) vesicular or tubular profiles (30-120 nm in size) in the intermediate zone of some nuclei (Table II) and, infrequently, of some membranous structures located at the junction channels (Figs. 2, 3; see reference 9), there is no morphological indication of an ongoing flow of vesicles across the LL pores described. These vesicles in the intermediate zone represent only very little membrane material and are not ubiquitous structures; in a great many cells they were not noted at all (Table II; compare also their absence in references 3, 4, and 35 and other earlier studies on *Acetabularia*). Neither at the nuclear envelope nor at the lacunar labyrinthum have bleb formations or vesicle fusions been noted. (*i*) After full maturation of the nucleus, and after the cap formation of the cell has been completed, the LL cisterna is disintegrated, and the strict separation becomes progressively looser (9). Although the function of this accessory envelope of the LL system is not known (similar structures have hitherto been noted only with the macronuclei of some marine ciliates; see, e.g., references 27-29) its strictly transitory existence only during the phase of giant growth is remarkable.

From these observations, we draw the following alternative conclusions. Either (*a*) the nuclear membrane proteins are synthesized at the nuclear envelope or within the nucleus or in the intermediate zone, but without ribosomes being involved. Such a view is in obvious contrast to the current concepts of protein synthesis (for the continuing debate as to the existence of an intranuclear

TABLE I

Nuclear Membrane Growth during the Vegetative Phase of Acetabularia mediterranea

Stage of development	Days after germination	Nuclear diameter*	Nuclear membrane area	Nuclear membrane volume	Nuclear membrane mass‡
		μm	*μm²*	*μm³*	*pg*
Germling (1 mm cell size)	30	10	618	3.7	4.4
Medium-size cell (6-7 mm in length)	50	40	9,590	57	68
Maturing cell (ca. 30 mm in length, before cap formation)	90	100	59,660	357	424
Maximum-size cell (ca. 40 mm in length, at about onset of cap formation)	110	150	134,245	805	598
Mature cell (stage of lobed primary nucleus during cap formation)§	140	ND	ca. 300,000	ca. 1,800	ca. 2,156

* Values from slightly ellipsoidal nuclei were corrected.

‡ Assuming a density of 1.19 g/cm^{-3} (see references 7, 8).

§ It is not clear whether this stage is obligatory for nuclear maturation.

ND, not determined.

Table II

Quantities of Vesicular Structures Observed in the Intermediate Zone of the Perinuclear Region of Acetabularia mediterranea

Nucleus	Total nuclear envelope profile length evaluated in sections*	Total membrane profile length of vesicles in the intermediate zone‡
no.	*μm*	*μm*
1	179 (20)	4.7 (2.6)
2	152.5 (11)	0.0 (—)
3	89 (9)	3.3 (3.8)
4	181.5 (15)	11.4 (6.3)
5	33.5 (7)	0.0 (—)
6	138 (8)	0.0 (—)
7	42 (11)	0.0 (—)
8	116 (9)	0.0 (—)
9	108 (16)	0.3 (0.3)
10	49 (9)	0.17 (0.4)
11	121 (19)	0.0 (—)
12	127 (15)	7.2 (5.7)
13	76 (13)	0.26 (0.3)
14	81 (17)	0.0 (—)
15	88 (11)	0.05 (0.1)

* Ultrathin sections were routinely taken from each nucleus as series of three to five; each series, however, was at least 2 μm distant from the previous one since intermediate 2-μm thick sections were taken for light microscope work. Figures in parentheses give total number of sections evaluated per nucleus.

‡ Figures in parentheses indicate percent relative to nuclear membrane profile in the same section.

protein sythesis see the evaluations in references 13 and 22). Or (*b*) the nuclear membrane proteins are synthesized in the cytoplasm and migrate via the fenestrae ("junction channels") in the paranuclear LL cisterna to the sites of their incorporation into the growing nuclear envelope. This seems much more likely to us. As to the morphological form of their migration, there is a strong indication that they are not transported as defined vesicular elements ("membrane flow") but rather as individual molecules or as indistinct micellar aggregates since the rather few and narrow fenestrae in the secondary envelope are obviously very efficient in keeping even small cytoplasmic particles (including, for example, ribosomes) out of the intermediate zone. It cannot be excluded with certainty that membrane might be translocated at a high migration rate across the paranuclear LL cisterna and the intermediate zone in the form of vesicles of the type occasionally encountered in this region (see above), but the absence of such vesicles in many nuclei as well as their relatively small membrane area, compared to the nuclear envelope (Table II), and the absence of any fusion or pinching off of vesicles at all these membranes seem to provide strong arguments against a considerable contribution of vesicle flow to nuclear membrane growth. Therefore, we propose that these membrane proteins get into the intermediate zone in a nonmembrane-bound state and are then assembled into the nuclear envelope. As to the type of polyribosomes on which these nuclear membrane proteins are synthesized, it is interesting to note the conspicuous predominance of helically arranged, nonmembrane-associated polyribosomes in the whole juxtanuclear cytoplasm.

For obvious reasons, most morphological studies hitherto have emphasized contributions to membrane formation and growth by fusions with pre-existing membrane structures such as vesicles and cisternae. The unique situation of the growing *Acetabularia* primary nucleus now appears to provide an example indicative of a contribution of "free" molecular or micellar units to the growth of a membrane system.

SUMMARY

The primary nucleus of the green alga *Acetabularia* grows about 25,000-fold in volume while it is separated from the endoplasmic reticulum and the whole cytoplasm by a special paranuclear cisterna of a vacuolar labyrinthum system which shows only very few (two to six per square micrometer) and small (ca. 40–120 nm in diameter) fenestrations. The nuclear envelope does not bear polyribosomes, nor do they occur in the entire zone intermediate between the nuclear envelope and the paranuclear cisterna. It is suggested that this special form of nuclear envelope growth takes place by assembly from cytoplasmically synthesized proteins that are translocated across the paranuclear cisterna in a nonmembrane-structured form.

We are indebted to Drs. H. Falk of the Institute of Biology II, University of Freiburg i. Br., Federal Republic of Germany, H. G. Schweiger of the Max Planck Institute for Cell Biology. Wilhelmshaven, F.R.G., G. Werz of the Institute of Plant Physiology and Cell Biology, Free University of West Berlin, and J. Kartenbeck of the German Cancer Research Center for helpful discussions. We also thank Miss Marianne Winter for skilful technical assistance.

The work has been supported by the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, grants Fr. 308/8 and 308/9.

Received for publication 16 January 1975, and in revised form 13 March 1975.

REFERENCES

1. Amsterdam, A., M. Schramm, I. Ohad, Y. Salomon, and Z. Selinger. 1971. Concomitant synthesis of membrane protein and exportable protein of the secretory granule in rat parotid gland. *J. Cell Biol.* **50:**187–200.
2. Bloom, S., and P. A. Cancilla. 1969. Conformational changes in myocardial nuclei of rats. *Circ. Res.* **24:**189–196.
3. Bouloukhere-Presburg, M. 1969. Ultrastructure de l'algue *Acetabularia mediterranea* au cours du cycle biologique et dans différentes conditions expérimentales. Ph.D. Thesis, Université Libre de Bruxelles.
4. Crawley, J. C. W. 1963. The fine structure of *Acetabularia mediterranea. Exp. Cell Res.* **32:**368–378.
5. Eriksson, L. C. 1973. Studies on the biogenesis of endoplasmic reticulum in the rat liver. *Acta Pathol. Microbiol. Scand.* **239:**1–72.
6. Feldherr, C. M. 1972. Structure and function of the nuclear envelope. *In* Advances in Cell and Molecular Biology. E. J. DuPraw, editor. Academic Press, Inc., London and New York. **2:**273–307.
7. Franke, W. W. 1974. Structure and biochemistry of the nuclear envelope. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **268:**67–93.
8. Franke, W. W. 1974. Structure, biochemistry, and function of the nuclear envelope. *Int. Rev. Cytol.* (Suppl.). **4:**71–236.
9. Franke, W. W., S. Berger, H. Falk, H. Spring, U. Scheer, W. Herth, M. F. Trendelenburg, and H. G. Schweiger. 1974. Morphology of the nucleo-cytoplasmic interactions during the development of *Acetabularia* cells. I. The vegetative phase. *Protoplasma.* **82:**249–282.
10. Franke, W. W., K. Kartenbeck, H. W. Zentgraf, U. Scheer, and H. Falk. 1971. Membrane-to-membrane crossbridges. *J. Cell Biol.* **51:**881–888.
11. Franke, W. W., D. J. Morré, B. Deumling, R. D. Cheetham, J. Kartenbeck, E. D. Jarasch, and H. W. Zentgraf. 1971. Synthesis and turnover of membrane proteins in rat liver: an examination of the membrane flow hypothesis. *Z. Naturforsch.* **26b:**1031–1039.
12. Franke, W. W., and U. Scheer. 1974. Structures and functions of the nuclear envelope. *In* The Cell Nucleus. H. Busch, editor. Academic Press, Inc., New York. **1:**219–347.
13. Goldstein, L. 1970. On the question of protein synthesis by cell nuclei. *In* Advances in Cell Biology. D. M. Prescott, L. Goldstein, and E. McConkey, editors. North Holland Publishing, Amsterdam. **1:**187–210.
14. Hämmerling, J. 1963. Nucleo-cytoplasmic interactions in *Acetabularia* and other cells. *Annu. Rev. Plant Physiol.* **14:**65–92.
15. Hämmerling, J., H. Clauss, K. Keck, G. Richter, and G. Werz. 1958. Growth and protein synthesis in nucleated and enucleated cells. *Exp. Cell Res.* (Suppl.). **6:**210–226.
16. Hirano, H., B. Parkhouse, G. L. Nicolson, E. S. Lennox, and S. J. Singer. 1972. Distribution of saccharide residues on membrane fragments from a myeloma cell homogenate: its implication for membrane biogenesis. *Proc. Natl. Acad. Sci. U. S. A.* **69:**2945–2949.
17. Ichikawa, Y., and H. S. Mason. 1974. Cytochrome P450 associated with free hepatic polyribosomes. *J. Mol. Biol.* **86:**559–575.
18. Kartenbeck, J., H. W. Zentgraf, U. Scheer, and W. W. Franke. 1971. The nuclear envelope in freeze-etching. *Ergeb. Anat. Entwicklungsgesch.* **45:**1–55.
19. Kasper, C. B. 1974. Chemical and biochemical properties of the nuclear envelope. *In* The Cell Nucleus. H. Busch, editor. Academic Press, Inc., New York. **1:**349–384.
20. Kay, R. R., and J. R. Johnston. 1973. The nuclear envelope: current problems of structure and function. *Sub-Cell. Biochem.* **2:**127–167.
21. Kessel, R. G. 1973. Structure and function of the nuclear envelope and related cytomembranes. *Progr. Surf. Membr. Sci.* **6:**243–329.
22. Kuehl, L. 1974. Nuclear protein synthesis. *In* The Cell Nucleus. H. Busch, editor. Academic Press, Inc., New York. **3:**345–375.
23. Lodish, H. F. 1973. Biosynthesis of reticulocyte membrane proteins by membrane-free polyribosomes. *Proc. Natl. Acad. Sci. U. S. A.* **70:**1526–1530.
24. Lowe, D., and T. Hallinan. 1973. Preferential synthesis of a membrane-associated protein by free polyribosomes. *Biochem. J.* **136:**825–828.
25. Meldolesi, J. 1974. Dynamics of cytoplasmic membranes in guinea pig pancreatic acinar cells. I. Synthesis and turnover of membrane proteins. *J. Cell Biol.* **61:**1–13.
26. Morré, D. J., T. W. Keenan, and C. M. Huang. 1974. Membrane flow and differentiation: origin of Golgi apparatus membranes from endoplasmic reticulum. *In* Advances in Cytopharmacology. B. Ceccarelli, F. Clementi and J. Meldolesi, editors. Raven Press, New York. **2:**107–125.

27. RAIKOV, I. B. 1968. Macronucleus of ciliates. *In* Research in Protozoology. Tze-Tuan Chen, editor. Pergamon Press, Oxford. **3**:1–128.

28. RAIKOV, I. B. 1972. Ultrastructures macronucléaires et micronucléaires de *Tracheloraphis dogieli*, Cilié marin à macronoyaux diploides. *C. R. Soc. Biol. Fil.* **166**:608–612.

29. RAIKOV, I. B. 1974. Fine structure of the nuclear apparatus of a lower psammobiotic ciliate, *Tracheloraphis dogieli*. *Acta Protozool.* **13**:85–102.

30. SCHIMKE, R. T., and P. J. DEHLINGER. 1971. Turnover of protein constituents of rat liver membranes. *In* Drugs and Cell Regulation. E. Mibich, editor. Academic Press, Inc., New York, London. 121–143.

31. SIEKEVITZ, P., G. E. PALADE, G. DALLNER, I. OHAD, and T. OMURA. 1967. The biogenesis of intracellular membranes. *In* Organizational Biosynthesis. H. J. Vogel, J. O. Lampen, and U. Bryson, editors. Academic Press, Inc., New York. 331–362.

32. SPRING, H., M. F. TRENDELENBURG, U. SCHEER, W. W. FRANKE, and W. HERTH. 1974. Structural and biochemical studies of the primary nucleus of two green algal species, *Acetabularia mediterranea* and *Acetabularia major*. *Cytobiologie.* **10**:1–65.

33. SUBBAIAH, P. V., and G. A. THOMPSON. 1974. Studies of membrane formation in *Tetrahymena pyriformis*. *J. Biol. Chem.* **249**:1302–1310.

34. TRENDELENBURG, M. F., H. SPRING, U. SCHEER, and W. W. FRANKE. 1974. Morphology of nucleolar cistrons in a plant cell, *Acetabularia mediterranea*. *Proc. Natl. Acad. Sci. U. S. A.* **71**:3626–3630.

35. WERZ, G. 1964. Untersuchungen zur Feinstruktur des Zellkernes und des perinukleären Plasmas von *Acetabularia*. *Planta* (*Berl.*). **62**:255–271.

36. WERZ, G. 1974. Fine structural aspects of morphogenesis in *Acetabularia*. *Int. Rev. Cytol.* **38**:319–367.

37. ZERBAN, H., M. WEHNER, and G. WERZ. 1973. Über die Feinstruktur des Zellkerns von *Acetabularia* nach Gefrierätzung. *Planta* (*Berl.*). **114**:239–250.

38. ZERBAN, H., and G. WERZ. 1975. Changes in frequency and total number of nuclear pores in the life cycle of *Acetabularia*. *Exp. Cell Res.* In press.