# The Nuclear Envelope and the Architecture of the Nuclear Periphery

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By definition, the eukaryotic cell is characterized by a compartmentation structure that divides the intracellular space into two different regions: (*a*) the nucleus, which contains the genome and the structures involved in transcription and processing of transcription products; and (*b*) the cytoplasm, which contains the translational apparatus, the cell organelles, the endomembranes, and a variety of other particles. This nucleocytoplasmic compartmentation is maintained by a specific eukaryotic membrane structure, the "nuclear envelope" (exceptions are some special situations, such as some forms of nuclear division, certain sperm cells, and some pathological conditions; see below). The existence of a membranelike structure between nucleus and cytoplasm had been indicated in early light microscope studies.

In his description of the cell nucleus in 1833, Brown (1) had already mentioned the possibility that it might be surrounded by a membranelike structure. After decades of lively discussion of the existence, real or artifact of preparation, of a distinct boundary layer between nucleus and cytoplasm, it was Flemming (2), who, in 1882 in a thorough review, summarized the accumulated evidence for "the existence of a special achromatinous lamella, that is a real—though in most types of nuclei very thin—layer of substance, which ... is not merely the expression of the region of contact between nuclear substance and cytoplasmic substance." Extending earlier observations of Hertwig (3) in nuclei of certain protozoa and of Soltwedel (4) in plant cell nuclei, Flemming (2) also clearly distinguished between the nuclear membrane proper as the "outer, achromatinous layer" and an inner "usually interrupted layer of peripheral chromatin" (the "nuclear cortex layer" sensu Hertwig and Soltwedel). Hertwig (3) also described certain fine punctate interruptions in cross sections of the achromationus nuclear membrane structures and discussed the possibility that these might represent pores, which allow exchange between nucleus and cytoplasm, an idea critically discussed by Flemming (2), who correctly pointed to the lack of evidence for the existence of such pores ("Poren in der Kernmembran," 1882; in reference 2). Several authors (2, 5) also noted the plasticity and viscosity of the nuclear membrane, properties that were then demonstrated with special clarity in the nuclear microdissection experiments of Kite (6).

Further support for the existence of a true nuclear membrane structure, which was profoundly different in composition and molecular organization from the nuclear and cytoplasmic zones, was obtained by polarization microscopy. In these studies the nuclear membrane showed negative spherite birefringence, indicative of lamellar arrays parallel to the nuclear surface (7, 8). Disappearance and finally reversion of the character of this birefringence in imbibition series reported by Schmitt (9) then was interpreted to show that the lamellar structures responsible for the negative spherite appearance was a result of form birefringence and that the intrinsic birefringence of the nuclear membrane resulted from layers of molecules, probably lipids, oriented perpendicularly to the plane of the nuclear membrane.

However, the demonstration of the significance and the unique mode of organization of the nuclear membrane has been made possible only by the development of electron microscopic preparation techniques (10–20; for more complete and detailed treatment of literature see reviews in references 21–23). The nuclear envelope as the structure of the nucleocytoplasmic borderland has always been suggestive of being biologically important and, also because of the distinct and intriguingly regular morphology of the nuclear pore complex, has attracted many electron microscopists. Biochemical work on the nuclear envelope has begun relatively late, i.e., after methods were developed that allowed the isolation of nuclear membrane material in sufficient amount and purity from various cells and tissues (18, 20, 21, 23–34).

In the present article we shall discuss some major findings on the organization and composition of the nuclear envelope. In particular we shall focus on those aspects which are specific to the nuclear envelope, and only in passing will we mention properties which this membrane system has in common with other cytoplasmic membranes.

## *The Nuclear Envelope Is a Membrane Cisterna Interrupted by Pore Complexes*

The nuclear envelope is a special perinuclear cisterna of the endomembrane system and is constituted by the inner and outer nuclear membrane enclosing a lumen ("perinuclear space" [14]). The typical structure of the nuclear envelope as

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THE JOURNAL OF CELL BIOLOGY · VOLUME 91 NO. 3 PT. 2 DECEMBER 1981 39s–50s

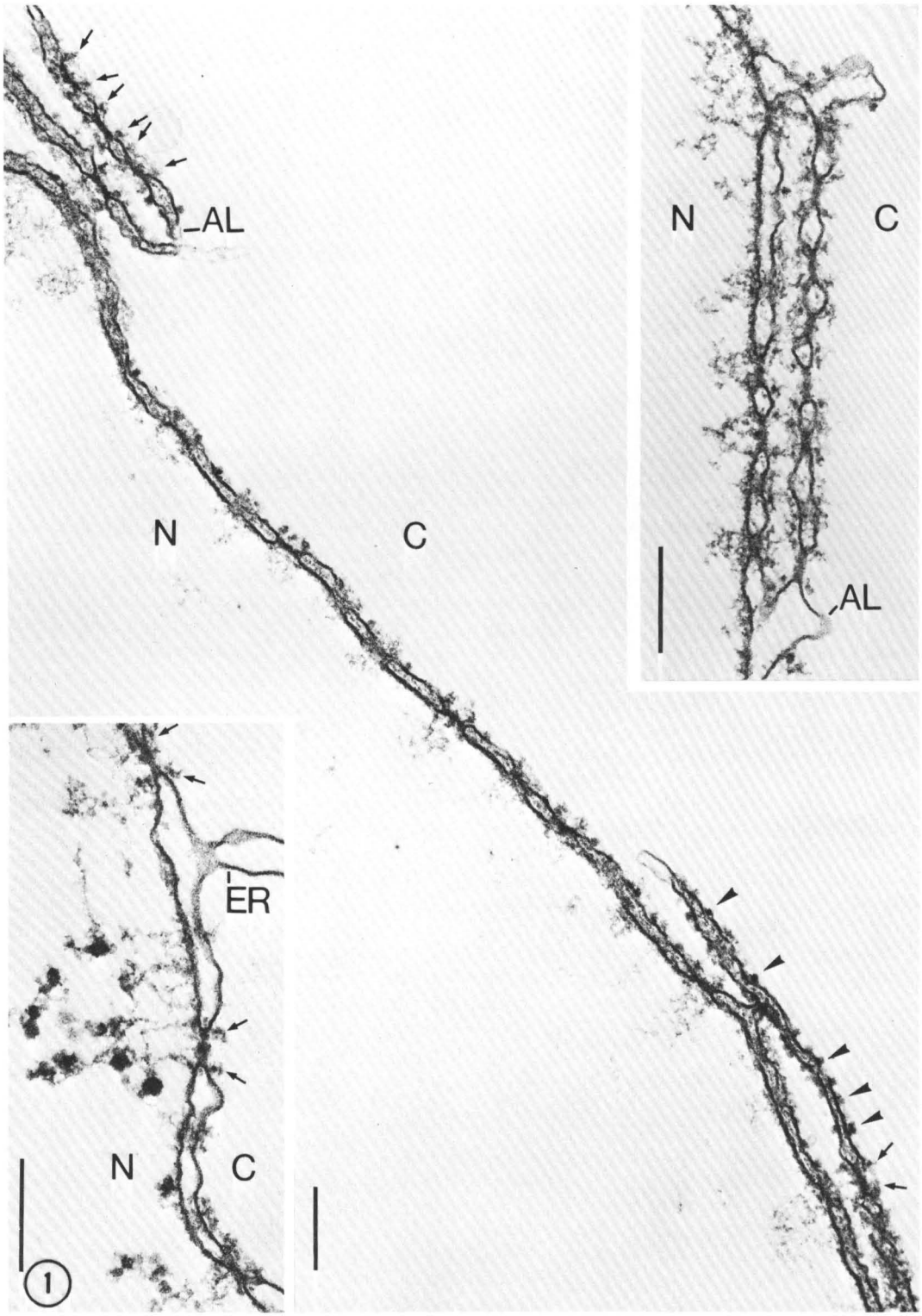


FIGURE 1 Transverse sections through isolated nuclear envelopes and attached cytoplasmic annulate lamellae (AL) from oocytes of various amphibian species (*Xenopus laevis, Pleurodeles waltlii,* lower left insert; *Bufo bufo,* upper right insert). Pore complexes are numerous in both membranes, the nuclear envelope and AL, and have identical ultrastructure: annular granules lie on either pore margin and cones of dense material protrude from the membranous walls of the pore into the pore lumen; often a centrally located granule or rod-like element is recognized (some annulus subunits are denoted by the arrows). Nucleoplasmic filaments terminate at the inner annulus and the central granule of the nuclear pore complex. These annulus-attached fibrils are often arranged in cylindrical arrays and are associated with densely stained granules, probably including ribonucleoprotein material (lower left insert). The juxtanuclear cytoplasmic AL are seen to be in luminal continuity with the perinuclear cisterna (e.g., upper right insert). Direct luminal interconnections with endoplasmic reticulum (ER) and with AL are also frequent (some membrane-associated ribosomes are denoted by arrowheads). C and N represent cytoplasmic and nucleoplasmic side of the nuclear envelope, respectively. Bars, 0.2 $\mu$m. × 65,000; upper insert, × 74,000; lower insert, × 85,000.

seen by electron microscopy of sections is presented in Fig. 1. Since the discovery of porelike discontinuities in nuclear envelopes by Callan and colleagues (10, 11), the nuclear pore complex (15) has received special attention as a distinct site for nucleocytoplasmic exchange (16–23). The architecture of the pore complex has been studied with various electron microscopic techniques (ultrathin sectioning, e.g. Fig. 1; metal shadowing, positive and negative staining, e.g. Fig. 2; freeze-cleavage, cf. references 19 and 35; high-resolution surface scanning, cf. references 36 and 37), and pore complex structure models proposed by several authors show remarkable agreement in the essentials (Fig. 3; e.g., 14–24, 38–40). Today it is clear that the pore complex, with its characteristic ultrastructure (see below), is a universal feature of the nuclear envelope in all cells, with the possible exception of late stages of spermiogenesis in some species (although definite proof of the existence of nuclear envelopes completely devoid of pore complexes has not been reported).

## *The Pore Complex Has a Unique Symmetrical Organization*

The pore complex is a highly symmetrical (bilaterally and radially) array of distinct, particulate, nonmembranous substructures associated with the transcisternal orifice of the nucleocytoplasmic pore (Figs. 1–3). It is profoundly different in organization from other similarly sized pore formations in membranes, such as in capillary endothelia, in cisternae of Golgi apparatus, and in the "secondary envelope" surrounding the giant primary nuclei of certain green algae (Fig. 4; for references see 20–22). The pore orifice is constituted by the locally fused transitions of inner and outer nuclear membrane and has an inner-pore width (membrane-to-membrane pore diameter) that appears to be rather sharply defined in a given type of cell or nucleus, but can show considerable variation (range: ~ 60–90 nm) when different types of nuclei and different electron microscopic methods are compared. The most prominent morphologically distinguishable components are recognized in Figs. 1 and 2 and are schematically illustrated in Fig. 3. They include (*a*) two rings (annuli; see references 10, 11, 13–17), located on either pore margin, each composed of eight 10- to 25-nm large granular particles (annular granules; see references 13, 18, 38–41) that are arranged in a precise eightfold symmetry (18); (*b*) eight radially distributed cones or tips projecting from the pore wall into the pore lumen (15, 18, 24, 39, 40) that some authors regard as locally aggregated fibrils

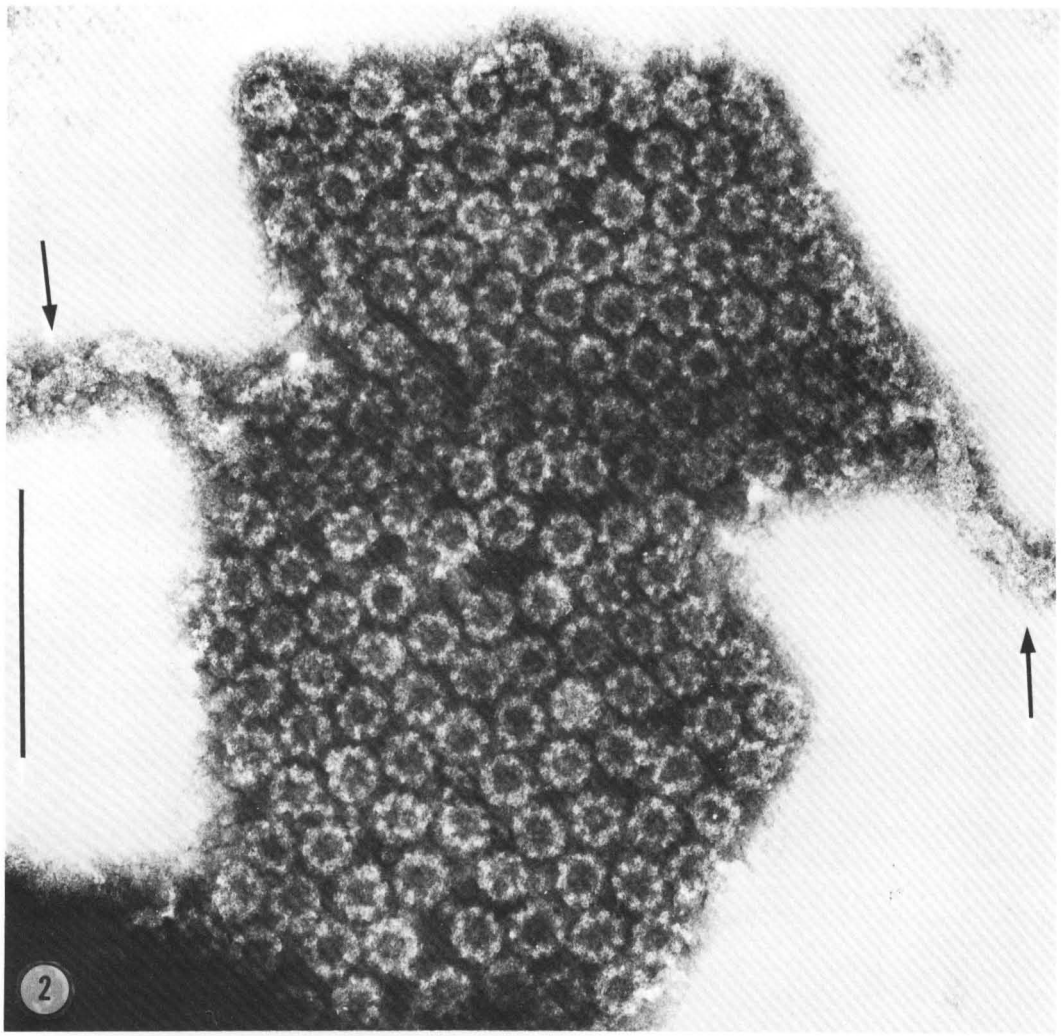


FIGURE 2 Negatively stained (phosphotungstic acid, PTA) cytoplasmic annulate lamella isolated from *Xenopus laevis* oocyte. The ring-like annulus material lying on each pore rim consists of eight symmetrically arranged granules. In the lumen of some pores a central granule is observed. Note the abundance and high packing density of pore complexes in the AL and structural continuities of AL with membranes of endoplasmic reticulum (arrows). Bar, 0.5 μm. × 70,000.

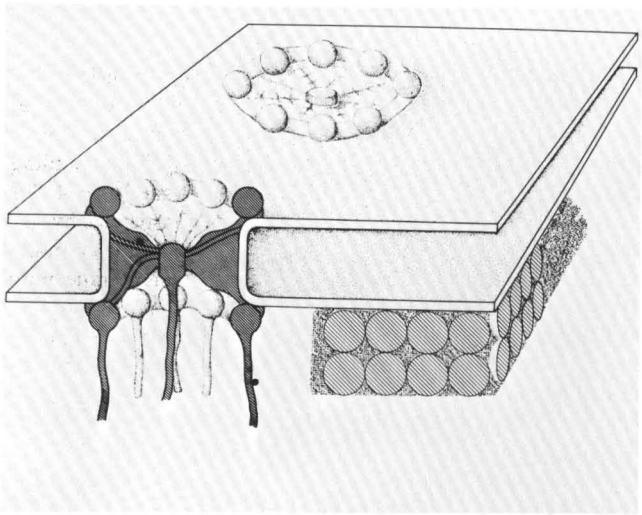

FIGURE 3 Schematic presentation of nuclear pore complex architecture and the association of peripheral condensed chromatin with inner nuclear membrane. The pore complex consists of (*a*) inner and outer annulus, each composed of eight granules of diameter 10–25 nm which are symmetrically distributed on either pore margin, (*b*) eight conical tips which project from the pore membrane wall and also are arranged in an eightfold symmetry, (*c*) a frequently present central granule or central rod, and (*d*) bundles of nuclear filaments which are attached to the granular components. In the interporous region of interphase nuclei (in the right) chromatin strands appear closely associated with the inner nuclear membrane, and this attachment of peripheral chromatin (20- to 30-nm large, higher order globular units are indicated by hatched circles) seems to be mediated by interchromatinous nonhistone protein material (dotted) containing the specific peripheral nuclear "skeletal" proteins (see text).

traversing the pore periphery (for references see 22); (*c*) a centrally located particle, granular or rodlike, of variable diameter and shape, which, however, is not recognized in all pore complexes; and (*d*) tangles of nucleoplasmic 4- to 8-nm filaments that terminate at the inner annulus, that often reveal eightfold radial symmetry, and that seem to be interconnected and to form cylindrical arrays ("channels," "funnels"; 39, for other references see 20–23) extending deep into the nucleus.

Great variations have been reported, in different types of nuclei, of both numbers of pore complexes per total nuclear surface (range from $10^2$ to $5 \times 10^7$) and pore complex frequency (i.e., pore complexes per/$\mu m^2$ nuclear surface; range from 1 to 3 pores/$\mu m^2$ to 50–60 pores/$\mu m^2$) as well as differences of pattern of distribution. Although correlations of pore numbers and pore frequencies with certain nuclear activities, e.g., transcription, are sometimes suggestive (16–22), the functional associations of pore morphology and number cannot be resolved at the moment.

Pore complexes of the same symmetrical ultrastructure have also been observed in nuclear envelope fragments during mitotic breakdown of the envelope and during reformation of nuclear envelope in anaphase and telophase stages (e.g., Fig. 9*c*; see 17–22).

## *Pore Complexes Are Not Exclusive to the Nuclear Envelope*

Transcisternal pore formations with essentially the same symmetrical architecture as that of the nuclear pore complex are also observed in cytoplasmic cisternae of the endoplasmic reticulum (ER), either as ordered stacks rich in pore complexes (annulate lamellae [AL]; 14, 42; for references see 22 and 43) or as single pore complexes in rough ER, as well as in "intranuclear AL" (for references see 20–22). The only difference from the pore complexes of the nuclear envelope appears to be the absence of polarity because both sides of the pore complex of AL are exposed to the same compartment. Typical cisternae of cytoplasmic AL are seen in Figs. 1 and 2. Such AL have been observed in a diversity of animal (22, 42, 43) and plant (44; cf. references 20–22) cells. Characteristically, the pore complex density can attain higher values in cytoplasmic AL than in the nuclear envelope of the same cell (16, 22, 44, 45). As in the case of nuclear pore complexes, neither the mode of formation of AL and their pore complexes nor their functions are known. Their mere occurrence, however, demonstrates that pore complexes are not exclusive to the nuclear envelope and are not formed only in association with nucleocytoplasmic compartmentation and exchange.

## *Nuclear Pore Complexes Contain Stable Architectural Components and Are Integrated into a Peripheral Karyoskeletal Framework*

When nuclear envelopes are exposed to mechanical stress or to rigorous extraction treatments involving solutions of high ionic strength or containing nondenaturing detergents, the basic structural elements of the pore complex are still identified, even under conditions that result in the disintegration and removal of most of the membrane material proper (for mechanical stability see references 20–23, 29, 35, 41, 46; for resistance to extractions see references 20–23, 36, 46–53). Nuclear envelopes treated with both detergent solutions and high salt concentrations show the persistence of the pore complex studs and interconnecting dense material (Figs. 5 and 6). The latter, located at about the level of the inner nuclear membrane, has been described as a continuous layer ("lamina"; 49, 50, 52) or as a meshwork of filaments (Fig. 5; 46, cf. references 22 and 23). The composition of such skeletal complexes including pore complex structures and interconnecting material (Figs. 5 and 6) has been examined, and a simple polypeptide pattern has been observed (49, 50, 52–55). Characteristic in such preparations ("pore complex-lamina-matrix," PC-L-M) made from mammalian liver (49–54) and from other cells (e.g., references 55 and 56) is the predominance of a triplet of three major polypeptides with apparent molecular weights in the range of 60,000–80,000 and three minor polypeptides of higher molecular weight (cf. Fig. 7, slot 7), which are also recognized as significant protein components in unextracted isolated nuclear membranes (32, 49, 57, 58). An even more simplified protein pattern has been found when such extractions were performed on manually isolated nuclear envelopes from amphibian oocytes (Figs. 6 and 7, slots 1–6; cf. reference 54): in such preparations, which are highly enriched in pore complex material, a protein of apparent moelcular weight of ~ 68,000 is prominent, together with only a few minor polypeptides of higher molecular weight (Fig. 7, slots 2–6). At the moment, it cannot be decided whether these polypeptides are exclusive to the pore complex or to the interconnecting material, or are common to both. Moreover, the possibility that residual nonhistone proteins of peripheral, nuclear membrane-associated structures (Figs. 8 and 9; for references see 20 and 21) contribute to this protein fraction has not been excluded. Complicating this characterization of the components of the nuclear periph-

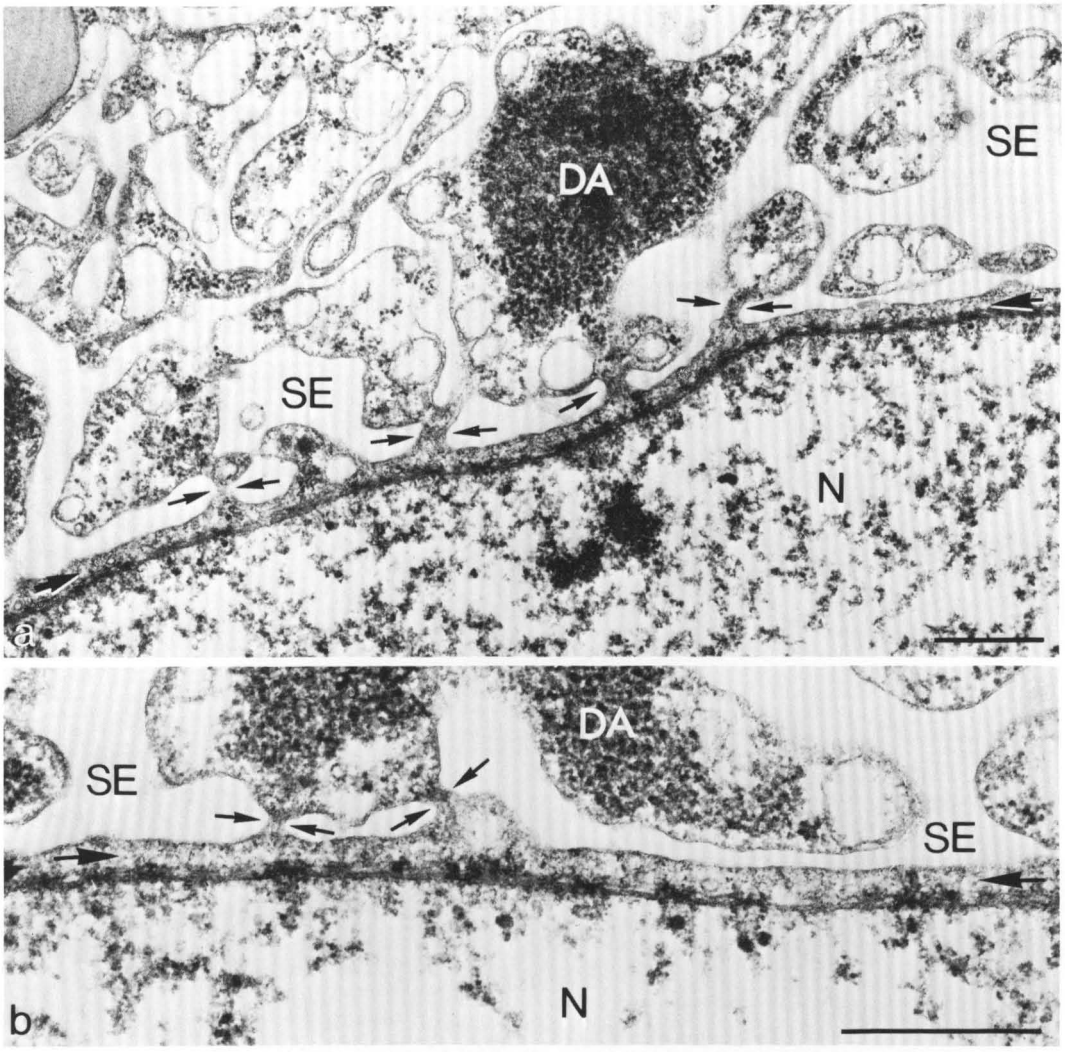


FIGURE 4 The primary nucleus of the green alga *Acetabularia mediterranea*, including the nuclear envelope, is separated from the cytoplasm by a special labyrinthine membrane system which constitutes a "secondary envelope" (SE). The thin intermediate zone sandwiched between the "true" nuclear envelope and this secondary envelope (thick arrows in *a* and *b*) opens into the cytoplasm via narrow channels (some are denoted by the pairs of arrows in *a* and *b*). Membrane continuities of the nuclear envelope with cytoplasmic ER via these channels has not been observed. Large, dense aggregates (DA) are frequently observed in the juxtanuclear cytoplasm. Bars, 0.5 μm. (*a*) × 27,000; (*b*) × 52,000.

ery is the observation that polypeptides of similar sizes, as the triplet proteins mentioned above, have also been reported to be predominant in other nonhistone protein fractions ("nuclear matrix" fractions; cf. reference 51) made from whole nuclei or chromatin (for reviews see references 59 and 60; for differences between PC-L fraction and internal matrix components see references 54 and 61).

On the other hand, in immunolocalization experiments, antibodies directed against the major triplet protein(s) of the PC-L fraction from rat liver have reacted with the periphery of interphase nuclei of different, although not all, mammalian cell types (Fig. 10; cf. references 52, 53, 62), but not with matrix structures of the nuclear interior. Interestingly, this protein of PC-L-M fractions is distributed throughout the cytoplasm during mitosis and has not been localized in metaphase chromosomes (52, 53, 62). Biochemical comparison of the major polypeptides of the nuclear envelope (58) has further indicated that the polypeptides of the triplet group are different, the middle-band polypeptide being a component with a distinct proteolytic cleavage pattern. Clearly, further experimental work is required to elucidate the nature of the skeletal components of the nuclear periphery and the pore complex and their topological relationships. However, the present data already permit the conclusion that the periphery of the interphase nucleus and the nuclear pore complex contain specific proteins that form structures of unusually high stability.

## *Chromosomes Interact in a Specific Mode with the Nuclear Envelope and the Nuclear Periphery*

It has been demonstrated in many cases that in the interphase nucleus the genomic material, i.e., chromosomes and extrachromosomal genes, is not distributed at random but that certain chromosomes or chromosomal regions are arranged in an ordered fashion with respect to the nuclear surface. Certain chromosomes and chromosomal regions are located regularly in the nuclear periphery; prominent examples in many cell types include centromeres and pericentromeric heterochromatin, telomeres and telomeric heterochromatin, perinucleolar heterochromatin, and sex chromosomes (e.g., X and Y chro-

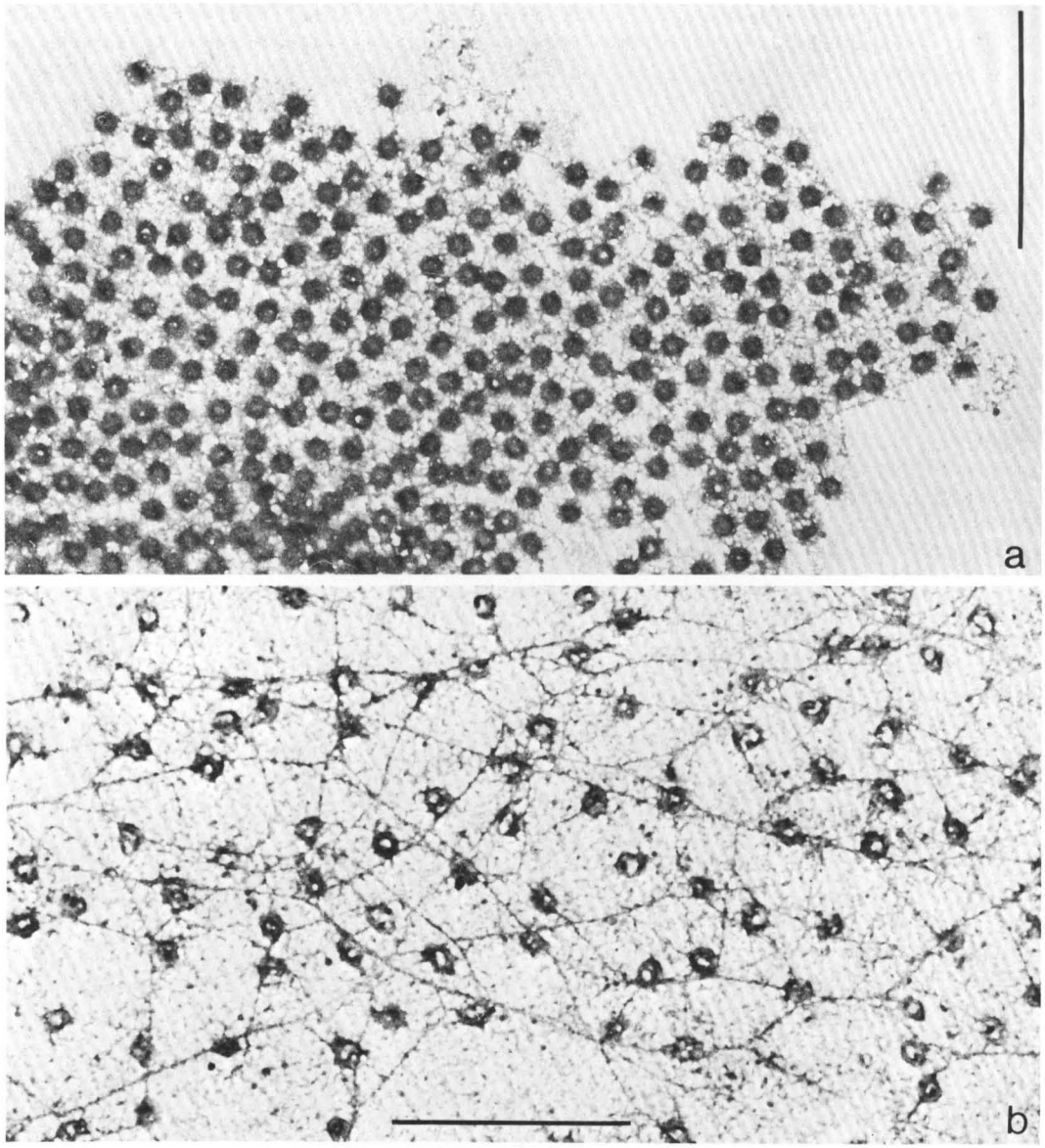


FIGURE 5 Different stages of experimental disintegration of the nuclear envelope isolated from oocytes of *Triturus alpestris* (*a*) and *Xenopus laevis* (*b*) revealing the abundance of pore-connecting fibrils. The preparation shown in (*a*) has been treated with 0.5% NP-40 in water and then centrifuged on an electron microscopic grid, whereas the nuclear envelope presented in (*b*) has been disrupted by spreading on the surface of a water droplet, which results in additional mechanical stretching. Both preparations were positively stained with ethanolic PTA. Bars, 1 μm. × 32,000.

matin; for a more detailed treatment of the literature see references 20 and 21). An especially striking example of this ordered interaction of chromosomes with the nuclear envelope is observed during meiotic prophase of many organisms in which the chromosomal ends are attached to interpore regions of the nuclear envelope; this is particularly well seen in the synaptinemal complexes of such chromosomes (Fig. 9 *d*; 63; for references see 20 and 21). Moreover, nucleic acid hybridization techniques have shown that certain subfractions of DNA (e.g. heterochromatin satellite DNAs) are often preferentially accumulated in the nuclear periphery (for references see 20 and 21). The molecular basis of this localization is not understood. Morphologically, two different situations must be distinguished: (*a*) in diverse types of nuclei, a distinct 15- to 80-nm thick layer of nonmembranous material is seen to be sandwiched between the inner nuclear membrane and the chromatin ("fibrous lamina," 64; for references see 20–23, 65). This laminar material, determined cytochemically to be proteinaceous, has been correlated with the "lamina" structure observed to interconnect pore complexes in isolated, extracted, and detergent-treated nuclear envelopes (49, 50, 52). Thus, generalized nuclear-structure models have been proposed in which the chromatin does not directly border on the inner nuclear membrane but rather is separated from it by a continuous layer containing the triplet polypeptides mentioned above (49, 52, 65). (*b*) On the other hand, chromatin has been shown in various types of plant and animal nuclei to border directly on the inner nuclear membrane, within the limits of resolution of the electron microscope thin-section technique (1–2 nm), with no identifiable fibrous lamina interspersed (Figs. 8 and 9). This absence of a nonchromatinous laminar structure between chromatin and inner nuclear membrane is also seen in cytochemical experiments that result in selective chromatin bleaching (Figs. 8 *c–e* and 9 *b*) and in isolated nuclei, in which the chromatin

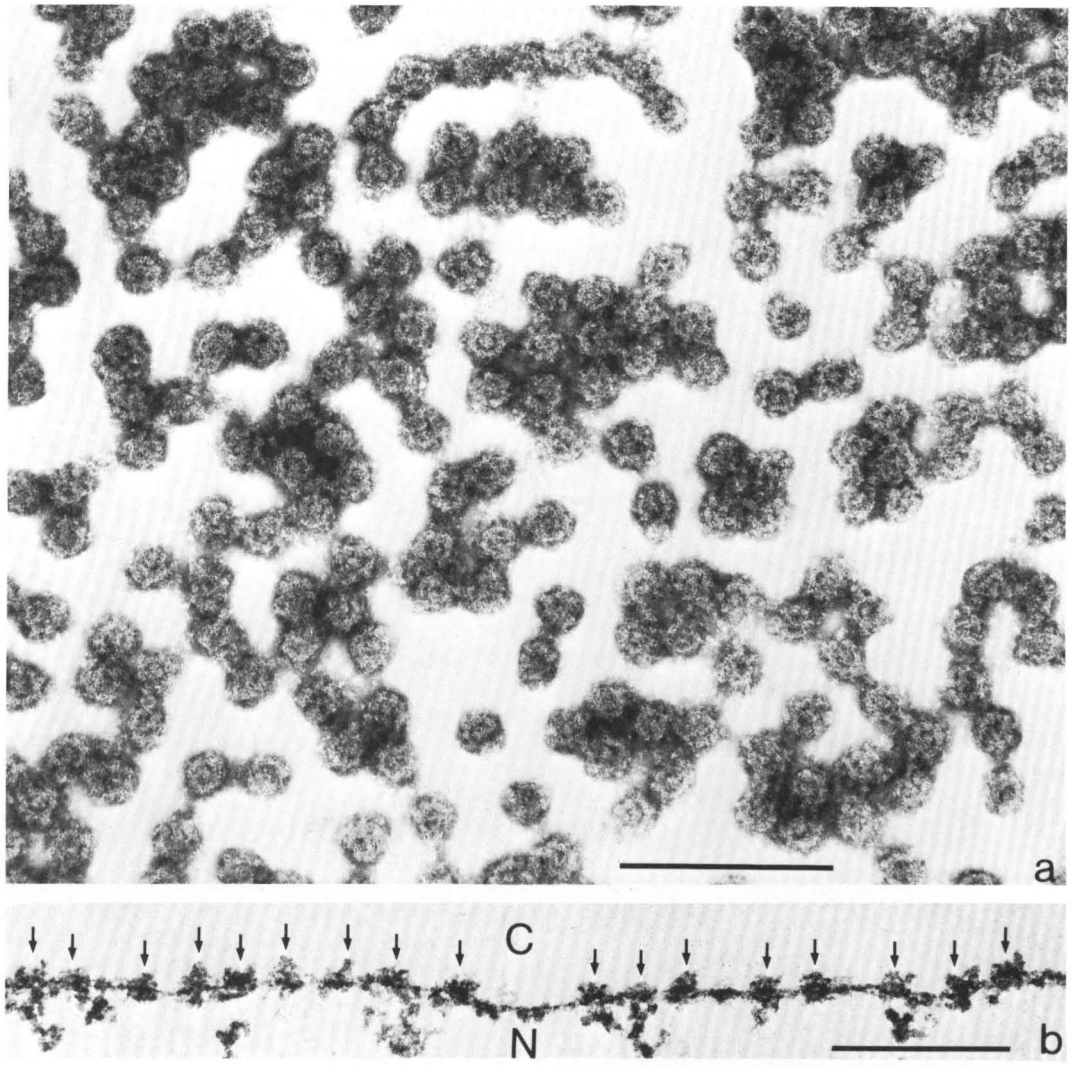


FIGURE 6 Nuclear membrane material from oocyte nuclei of *X. laevis* as obtained after treatment with high salt buffer and detergent and demonstrated by electron microscopy in negatively stained preparation (*a*) and ultrathin section (*b*). The sequential extraction with buffered 1.5 M KCl solution followed by 1% Triton X-100 (for details see text) removes most of the interporous membrane material (*b*) but leaves the basic organization of the pore complex intact (*a, b*); pore complexes in (*b*) are denoted by arrows. C, cytoplasmic side; N, nuclear side. Bars, 0.5 μm. (*a*) × 57,000; (*b*) × 47,000.

has shrunk slightly and separated from the inner nuclear membrane, with only a few thread connections left in an electron-translucent "gap" (Fig. 8 *b*). Moreover, DNA has been shown to be intimately associated with isolated nuclear membranes of various cells (for references, see 20 and 21). Therefore, an alternative explanation is proposed which emphasizes the existence of a proteinaceous, weblike material finely dispersed and associated with both the nuclear envelope and the peripheral chromatin (see right part of Fig. 3), which in certain cells accumulates or, upon chromatin extraction, collapses to form a fuzzy peripheral lamina (see also the left part of Fig. 3 of reference 49). Future experiments will doubtless help to clarify the relationship of chromatin and the nuclear membrane and its possible functional significance. Evidently, strong and specific forces exist in chromosomes to permit association with this type of membrane-attached, nonhistone proteins, and to promote formation of a continuous nuclear envelope. This is best demonstrated in the cycle of dispersion of both nuclear membrane and the PC-L-M proteins during nuclear divisions of the "open" form in which during anaphase-telophase stages elements of the reforming nuclear envelope are assembled on the chromosomal surfaces (Fig. 9 *c*; cf. references 20–23) and PC-L-M proteins are reaccumulated in the forming daughter nuclei.

There is no experimental evidence that pore complexes themselves contain chromatin (for references see 20–23).

## *The Nuclear Membranes Are Similar in Composition to Membranes of the Endoplasmic Reticulum But Represent an Independent Membrane System*

The biochemical composition of nuclear membranes from various plant and animal cells has been compared with that of other cellular membranes (for reviews see references 20–23, 32, 33, 57, 66). These studies have shown that, in cells that allow the direct comparison of membrane fractions, the nuclear membranes are similar to ER membranes in their lipid pattern, in a large number of proteins and enzymes, in the carbohydrate

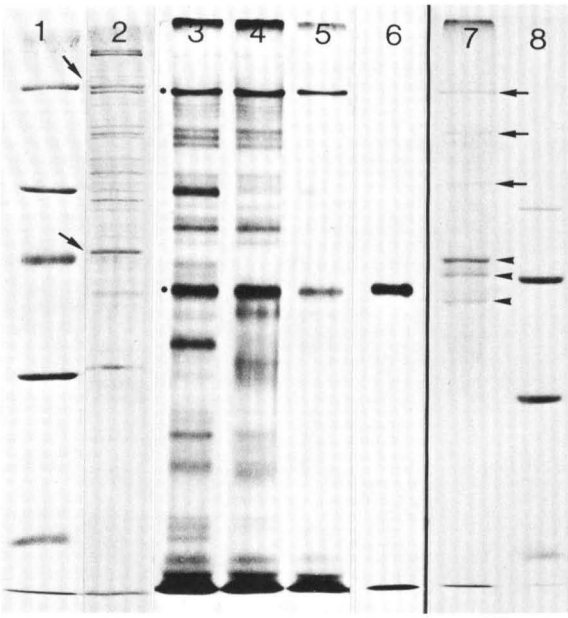


FIGURE 7 Polypeptide composition of unextracted and extracted nuclear membranes of *X. laevis* oocytes (slots 2–6) and rat liver (slot 7) separated by SDS-polyacrylamide slab gel electrophoresis (for references see text). Oocyte nuclear envelopes were manually isolated and cleaned under a stereomicroscope. Proteins of unextracted membranes were separated and stained with Coomassie Blue (slot 2; 170 nuclear membranes; two of the major polypeptides are denoted by arrows; reference proteins separated in the same gel are shown in slot 1, and are, from top to bottom myosin heavy chain (220,000 $M_r$), phosphorylase a (94,000 $M_r$), bovine serum albumin (67,000 $M_r$), actin from rabbit skeletal muscle (42,000 $M_r$), and chymotrypsinogen (25,000 $M_r$). Manually isolated nuclear membranes of oocytes were then extracted with various high salt and detergent solutions, resistant membrane components were sedimented, and proteins were radioactively labeled in vitro with [$^3$H]dansyl chloride and visualized by radiofluorography (slots 3–6; slot 3 represents the protein of 22 total nuclear envelopes, slots 4–6 contain the protein equivalent to 40–45 nuclear envelope residues). The polypeptide composition of the unextracted nuclear envelope is shown in slot 3; slot 4 demonstrates the effect of treatment with buffer containing Triton alone. The two major polypeptide bands resistant to sequential extractions with buffers containing 1.5 M KCl and 1% Triton are shown in slot 5. Only one major polypeptide band of the oocyte nuclear envelope has been found to be resistant to simultaneous extraction with 1.0 M KCl and 1% Triton (gel shown in slot 6). The two major resistant polypeptides observed in the pore complex material enriched fractions are denoted by asterisks (slots 3–6) and seem to be identical with the two polypeptides denoted by arrows in slot 2. Slot 7 shows for comparison, the polypeptide pattern of purified nuclear membranes from rat liver which then have been extracted simultaneously with 1.5 M KCl and 1% Triton X-100 (for details and references see text). The three major polypeptide bands reexamined (apparent $M_r$ values relative to those of the reference proteins mentioned below: 74,000; 72,000; 62,000) are marked by arrowheads, the minor components (apparent relative $M_r$ values: 200,000; 160,000; 125,000) of high molecular weights are denoted by short arrows. Reference proteins (slot 8) are, from top to bottom, phosphorylase a, bovine serum albumin, actin, and chymotrypsinogen (slots 7 and 8 have been stained with Coomassie Blue); in other slots of this gel (myosin heavy chain, clathrin (180,000) and $\beta$-galactosidase (125,000 $M_r$), transferrin (76,000 $M_r$), vimentin (57,000 $M_r$) and glutamate dehydrogenase (55,000 $M_r$) were run for comparison.

pattern of their glycoproteins, in their lectin-binding properties, and perhaps also in their pattern of hormone receptors and several components defined as antigens (as to the latter see the examples discussed in references 67 and 68; there is a continuing discrepancy as to the significance of determinations in nuclear membrane fractions of components widely assumed to be characteristic of mitochondria such as cardiolipin, cytochrome oxidase, and oxidative phosphorylation, cf. references 20, 21, 23, 27–29, 32, 33, 66, 69). Certain proteins (32, 57) and enzyme activities, however, seem to be specific for the nuclear envelope. In rat liver, for example, a special nuclear envelope-bound protein kinase system has been described (70, 71), as have differences in the substrate specificity, stability, and drug inducibility of the nuclear membrane monooxygenase system (cf. references 32, 72–74). If proven correct, the nuclear pore complex-bound ATPase (cf. references 20–23, 32) might also represent a speciality of the nuclear envelope. Like rough ER, the nuclear envelope in many, though not in all, cells appears to be a site of membrane-bound protein synthesis, as suggested by the attachment of ribosomes and polyribosomes to the outer nuclear membrane of interphase cells and to both sides of nuclear envelope fragments during mitosis (Fig. 9 *c*; for references see 20 and 21).

The close biochemical similarity of nuclear and ER membranes may well reflect the direct continuity between these two membranes via manifold, mostly tubelike connections (15, 17, 20–23). However, the nuclear envelope can also exist independently from the ER system, and cells have been described that do not show nuclear envelope-ER continuities, such as the vegetative cells of *A. mediterranea* and related green algae (Fig. 4), avian erythrocytes, and late spermiogenic stages of many species (20, 21). In many cell types, the nuclear envelope also shows "transitional elements," i.e., regions with intensive secretory vesicle blebbing from the outer nuclear membrane (20, 21). In cells that do not have an extended endomembrane system, it is obvious that the nuclear envelope makes an important contribution to the total endomembrane functions of the cells. Thus, it seems as if the nuclear envelope not only is a means to nucleocytoplasmic compartmentation, but also can provide the minimum function of the endomembrane system in the eukaryotic cell.

## *The Nuclear Envelope is Permeable to Small Molecules, But Directs Nucleocytoplasmic Exchange of Particles to the Nuclear Pore Complexes*

The nuclear envelope is readily permeable to ions and small molecules (for references see 20 and 21). Observations of dumbbell-shaped structures in the pore complexes (cf. references 20–23; 75) indicate that nucleocytoplasmic transport of particulate material of diameters larger than the pore interior lumen is via the pore complexes by a nonpassive process. The configuration of these particles, presumed to represent ribonucleoproteins, suggests that not the whole membrane-to-membrane diameter is used for such transport events, but only a central channel of a patent diameter of 10–20 nm. Microinjection experiments using various particles and proteins as probes and performed primarily in large cells such as the amoebas and oocytes, have also demonstrated the existence of a size exclusion limit of approximately 18 nm for migration from cytoplasm into the nucleus (76–78). That pore complexes are preferential, if not exclusive, sites for nucleocytoplasmic exchange of particles, and that size limitations exist for such exchange have also been recognized in studies of the infection of cells with certain DNA viruses. After infection, the virus particles are distributed in the cytoplasm, attach to the central

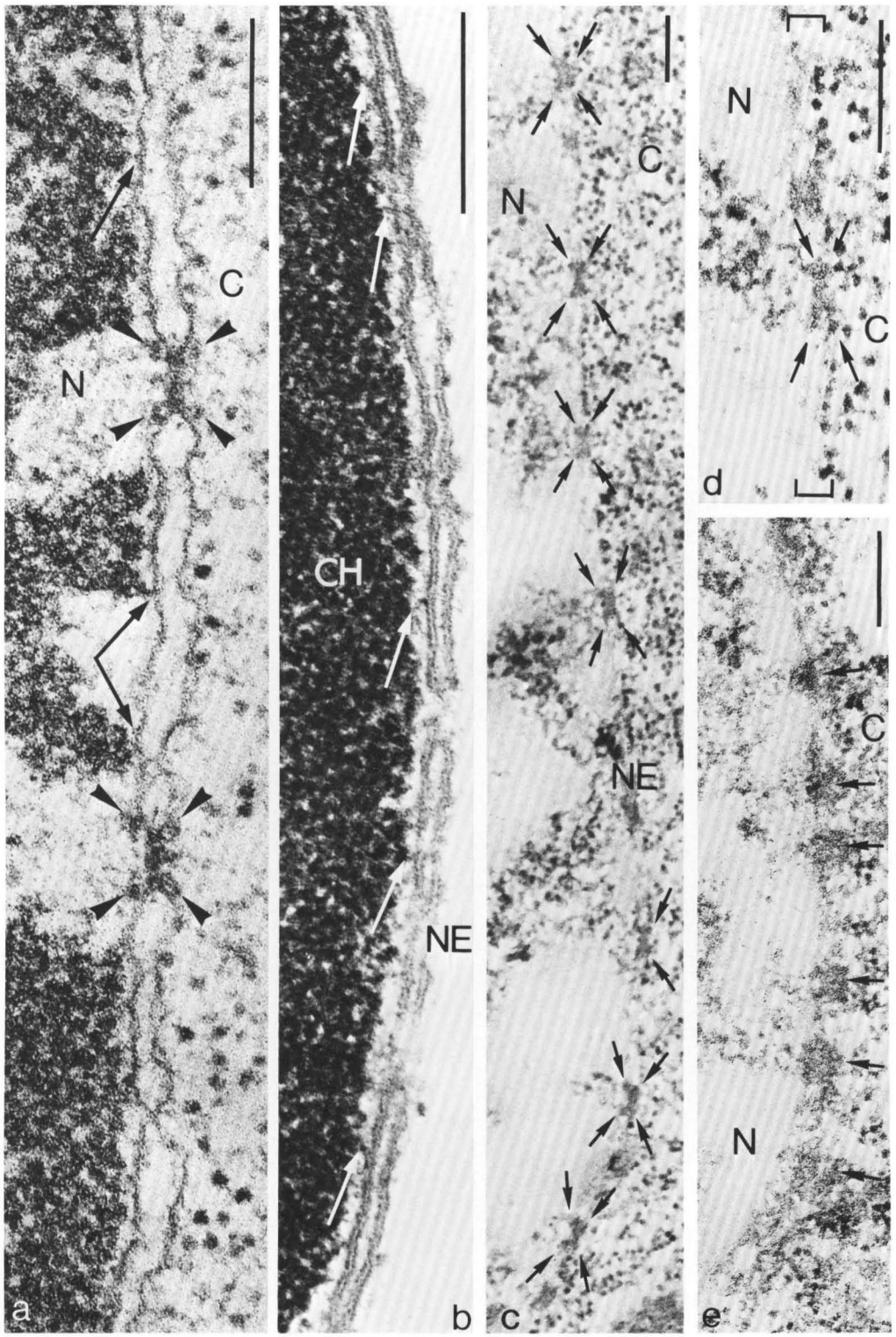


FIGURE 8 Association of the inner nuclear membrane with condensed chromatin as seen in conventionally double-stained (*a*, *b*) or EDTA-treated (*c*, *d*) transverse sections through onion root tip cells fixed *in situ* (*a*, *c*, *d*) or after isolation (*b*). Note the close apposition of peripheral condensed chromatin with the inner nuclear membrane, which often appears to be mediated by short and thin (7–12 nm) thread connections (some are denoted by arrows in *a* and *b*). This peripheral chromatin, which often reveals a composition by tightly packed granular units (see Fig. 3), is regularly interrupted at the pore complexes (arrowheads in *a* and arrows in *c* and *d*) thus forming interchromatinic "channels" which lead to the pores (*a*). Note the absence of a densely stained nonchromatinous layer (*lamina densa*) separating the peripheral chromatin from the inner nuclear membrane; this is especially well seen in isolated nuclei in which some chromatin shrinkage has been occurred (*b*). When the selective staining method of Bernhard is used (*c–e*), chromatin is "bleached" whereas ribonucleoproteinaceous and proteinaceous structures retain the staining. The pore complexes (some are denoted by arrows in *c–e*) as well as the annulus-associated nuclear fibrils and the ribosomes are positively stained. A distinct lamina structure located between the inner face of the nuclear membrane (the contours of the nuclear envelope are indicated by brackets in *d*) and the bleached heterochromatin is not seen, both in onion root tips (*c*, *d*) and in HeLa cells (*e*). NE, nuclear envelope; N, nucleoplasm; C, cytoplasm; CH, chromatin. Bars, 0.2 μm. (*a*) × 115,000; (*b*) × 150,000; (*c*) × 48,000; (*d*) × 100,000; (*e*) × 70,000.

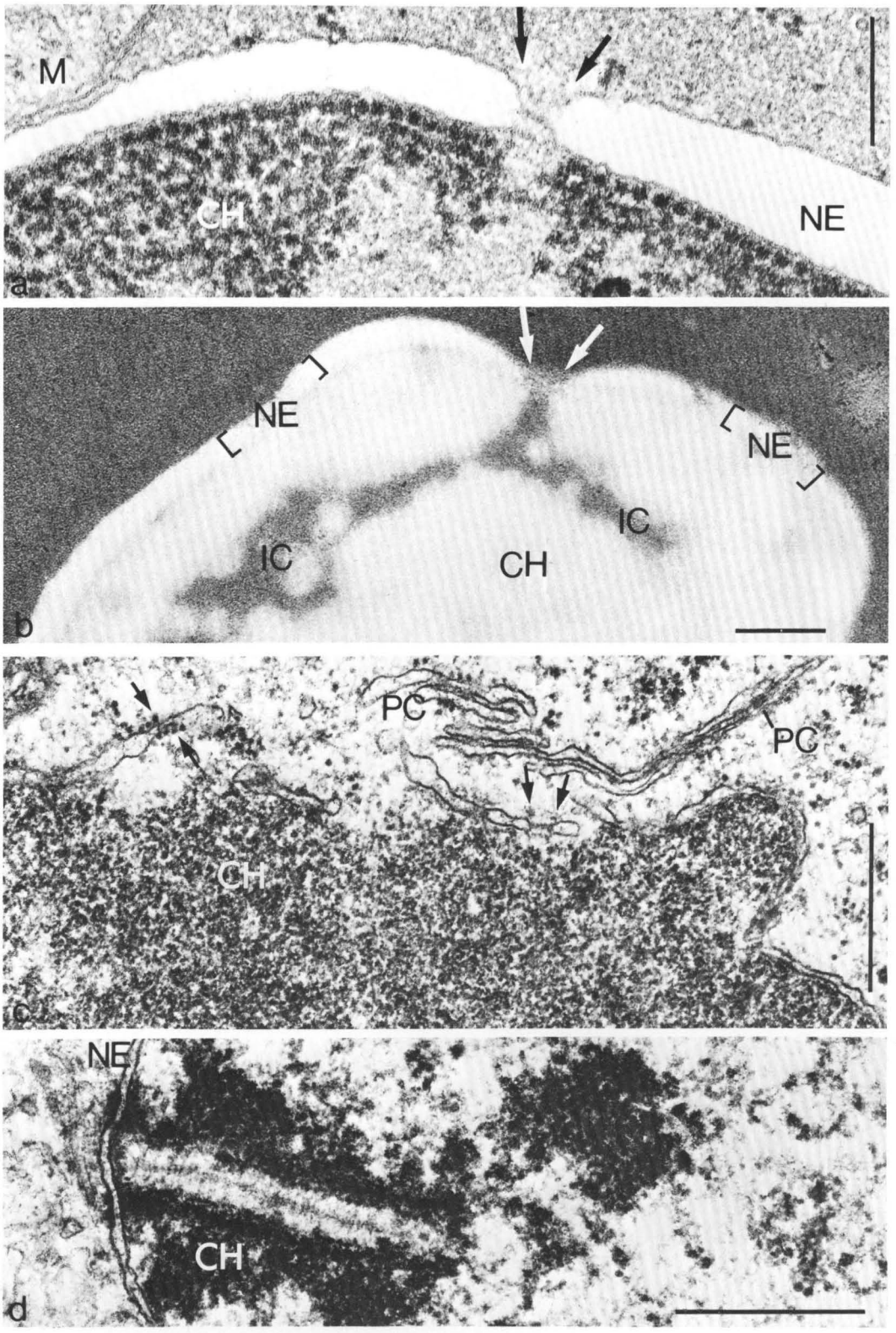


FIGURE 9 Ultrathin sections showing various aspects of chromatin (CH)-to-nuclear envelope (NE) association as seen (*i*) in nuclei of late stages of avian erythropoiesis (*a*, late erythroblasts in chick bone marrow; *b*, erythrocyte in chick leg muscle capillary), after conventional double-staining (*a*) and after treatment with EDTA according to Bernhard's regressive staining method (*b*), (*ii*) during envelope reconstitution on chromosome surfaces of late anaphase stages of mitosis in an experimentally (using dimethylbenzanthracene) induced adenocarcinoma cell in lactating rat mammary gland (*c*), and (*iii*) in form of the specific situation of chromosomal telomeres in termini of synaptinemal complexes in meiotic spermatocytes of rat testis (*d*). The peripheral condensed chromatin is intimately associated with the inner nuclear membrane, and no distinct "lamina" structure is seen in stained and chromatin-bleached nuclei (*a*, *b*). Intranuclear channels (IC in *b*) leading to the pore complexes (arrows) have retained intense uranly staining (*b*). Contours of the two nuclear membranes in (*b*) are demarcated by brackets. During mitosis chromatin and nuclear membrane material are dissociated but begin to reassociate in anaphase-to-telophase on the chromatin of the chromosomal surfaces (*c*). Such fragment units of NE often show ribosomes on both sides (arrows in the left) and typical pore complexes (pair of arrows in the center). In the vicinity of such mitotic configurations often "paired cisternae" (PC) are observed that may include membrane of nuclear envelope fragments. A demonstration of the topological specificity of chromatin-nuclear membrane interaction is presented in the example of the synaptinemal complex (*d*). M, mitochondrion. Bars, 2.0 μm (*a*, *b*) and 0.5 μm (*c*, *d*). (*a*) × 100,000; (*b*) × 70,000; (*c*) × 52,000; (*d*) × 58,000.

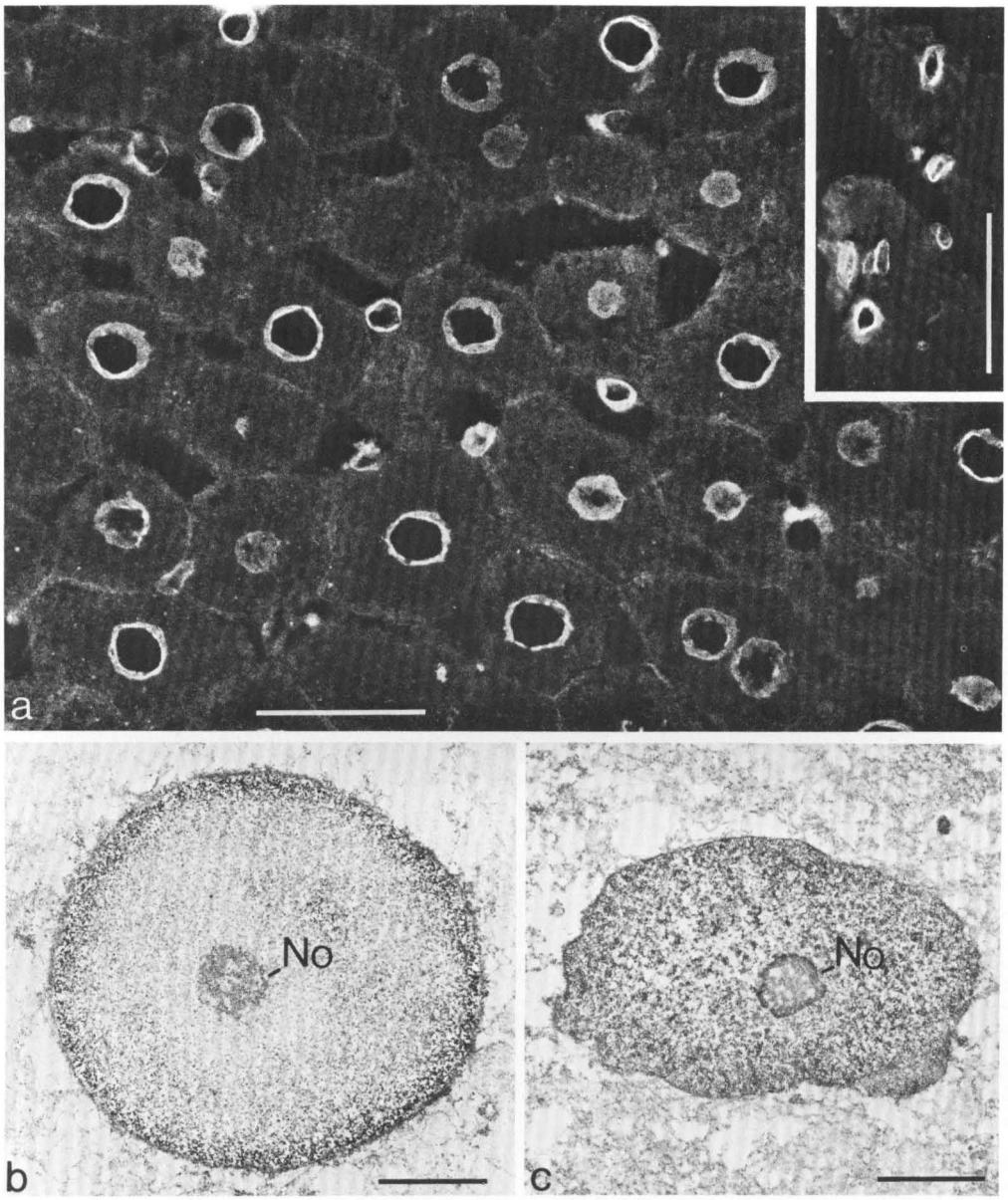


FIGURE 10 Localization of a nuclear envelope-associated karyoskeletal nonhistone protein by indirect immunofluoresce microscopy (*a*) and by immunoelectron microscopy using the peroxidase method (*b*, for details see text) using an antibody against one of the major polypeptides (i.e., the middle band polypeptide of the triplet denoted by arrowheads in Fig. 7, slot 7) from a rat liver fraction enriched in nuclear-envelope-associated material. Frozen sections of rat liver (*a*; for ultrathin section made therefrom see *b*) and rat myocardium (insert in *a*) show a strong peripheral staining of the nuclei. The intense immunostaining is restricted to a relatively thin peripheral nuclear zone corresponding to some layers of granules of the peripheral chromatin (*b*) but does not appear restricted to a thin layer (lamina) interposed between the nuclear envelope and the chromatin. By contrast, sections treated with antibodies against histones (*c*, antihistone H2b) show a uniform staining of the chromatin of the nucleus (*c*). No, nucleolus. Bars, 30 μm (*a*, and insert in *a*) and 2 μm (*b*, *c*). (*a*, and insert in *a*) × 730; (*b*, *c*) × 7,000.

portion of nuclear pore complexes, and release their nucleic acid content into the nucleus, leaving the emptied capsids on the cytoplasmic side of the pore complexes (78–80). This shows that the passage of particles across the nuclear envelope is confined to pore complexes and may involve profound changes in the shape of structures in transit. However, whether the pore complex material itself can exert some control on the nucleocytoplasmic exchange, e.g., influence selectivity and rate, awaits further experimental evidence. In fact, there is not even direct demonstration that the nuclear envelope itself is critical for the maintenance of ordered pathways of biological significance, such as nuclear uptake of certain proteins and cytoplasmic transport of transcription products. Experiments which have shown, in living amphibian oocytes, that neither nuclear accumulation of proteins nor nucleocytoplasmic transfer of ribosomal RNAs are markedly affected when the nuclear envelope is experimentally disrupted (81, 82) point to our fundamental ignorance of the true biological function of the nuclear envelope in intracellular compartmentation.

## ACKNOWLEDGMENTS

The authors thank Doctors M. C. Dabauvalle, M. Gerhard, J. Kartenbeck, S. Moreno Diaz de la Espina, H. Spring, H. Zentgraf (all of this

Center), S. Ely, E. Jost (EMBL, Heidelberg), H. Falk (University of Freiburg im Breisgau), and T. Martin (University of Chicago) for valuable contributions and discussions.

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