# EXPERIMENTAL DISINTEGRATION OF THE NUCLEAR ENVELOPE

## Evidence for Pore-Connecting Fibrils

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ABSTRACT

The disintegration of the nuclear envelope has been examined in nuclei and nuclear envelopes isolated from amphibian oocytes and rat liver tissue, using different electron microscope techniques (ultrathin sections and negatively or positively stained spread preparations). Various treatments were studied, including disruption by surface tension forces, very low salt concentrations, and nonionic detergents such as Triton X-100 and Nonidet P-40. The high local stability of the cylinders of nonmembranous pore complex material is emphasized. As progressive disintegration occurred in the membrane regions, a network of fibrils became apparent which interconnects the pore complexes and is distinguished from the pore complex-associated intranuclear fibrils. This network might correspond to an indistinct lamella, about 15–20 nm thick, located at the level of the inner nuclear membrane, which is recognized in thin sections to bridge the interpore distances. With all disintegration treatments a somewhat higher susceptibility of the outer nuclear membrane is notable, but a selective removal does not take place. Final stages of disintegration are generally characterized by the absence of identifiable, membrane-like structures. Analysis of detergent-treated nuclei and nuclear membrane fractions shows almost complete absence of lipid components but retention of significant amount of glycoproteins with a typical endomembrane-type carbohydrate pattern. Various alternative interpretations of these observations are discussed. From the present observations and those of Aaronson and Blobel (1,2), we favor the notion that threadlike intrinsic membrane components are stabilized by their attachment to the pore complexes, and perhaps also to peripheral nuclear structures, and constitute a detergent-resistant, interpore skeleton meshwork.

While numerous studies of the nuclear envelope organization have focused on the attractive pore complex, which has led to the construction of a generally accepted model of its architecture (for reviews, see 19, 21, 25, 42, 49, 62), relatively few authors have investigated the interpore sections of the envelopes. In the past decade a special controversy has arisen as to the effects of detergents on

the nuclear envelope. Earlier reports have claimed that preferential or even selective removal of the outer nuclear membrane is effected by nonionic detergents as well as by mixtures of nonionic and ionic detergents, suggesting the maintenance of an intact inner nuclear membrane (e.g. 9, 10, 35, 36, 52, 64; for further references see 1; c.f., however, also 29). This notion, which has become a very popular a priori assumption in preparative strategies for isolating nuclei and nuclear components (e.g. 6, 9, 40, 48, 65, 67), has been questioned by other authors in a series of recent structural and biochemical studies. These investigators have come to the conclusion that nonionic detergents, particularly Triton X-100 and Nonidet P-40, (a) progressively remove material from both membranes, sometimes with a slight preference for the outer membrane which is generally more susceptible to various sorts of stresses (e.g. 1, 3, 38), and (b) solubilize different membrane components at different rates, which results, for example, in the removal of phospholipids from often still identifiable, apparently proteinaceous, membrane-derived structures (e.g. 1, 3, 38, see also 43, 66). The outermost layer of nuclear material, however, remains relatively stable after these treatments, and it is obviously this stability that maintains the specific nuclear morphology (e.g. 3, 27, 30, 38, 43, 66) and that sometimes might give the impression of preservation of an inner nuclear "membrane" (see some of the references quoted above). Aaronson and Blobel (1,2) using sections through detergent-treated isolated rat liver nuclei and nuclear membranes, have recently described the preservation of the nuclear pore complexes and their attachment to intranuclear structures, particularly to a distinct peripheral layer of nuclear material which they have interpreted as equivalent to the "fibrous lamina" described in a variety of cell types (e.g. 18, 47, 61, 62; for similar observations, see also 13, 51). The nuclear envelope is a structure particularly suitable for studying the processes of membrane disintegration because of the increased mechanical stability and cohesion at the multiple interspersed sites of nonmembranous material of the pore complexes (for review, see 25). Using different electron microscope techniques, we have examined the progressive disintegration of the nuclear envelope in (a) a cell type with marked blocks of peripheral condensed chromatin, namely the mammalian hepatocyte, and (b) a cell type in the later diplotene phase of meiosis with apparently no

permanent nuclear envelope-chromosome associations (for references, see 53).

## MATERIAL AND METHODS

### Materials

The newts *Triturus alpestris*, *Triturus cristatus*, *Pleurodeles waltlii* and the clawed toad *Xenopus laevis* were kept in aquaria at 14°C (*Triturus*) and 20°C (*Pleurodeles and Xenopus*), respectively. Albino Sprague-Dawley rats with a body weight of 100–150 g were used. The anesthetic MS-222 and the nonionic detergent Triton X-100 were obtained from Serva Feinbiochemica (Heidelberg, Federal Republic of Germany); Nonidet P-40 was a gift (Deutsche Shell Chemie Gesellschaft m.b.H., Karlsruhe, Federal Republic of Germany).

### Ultrathin Sectioning of Isolated Nuclei and Nuclear Membranes of Amphibian Oocytes

Pieces of ovary were removed from amphibia anesthesized with 0.1% MS-222 and were placed in Eagle's minimal essential medium diluted 1:1 with distilled water. Nuclei of lampbrush-stage or mature oocytes were manually isolated in the "5:1-medium" (83 mM KCl, 17 mM NaCl; c.f. 12) buffered to pH 7.2 with 10 mM Tris-HCl. They were then briefly cleaned of adherent cytoplasmic material and were either incubated for 5 min at room temperature in the same medium or transferred to "pH 9 water" (twice-distilled water adjusted to pH 9.0 with sodium borate buffer to a final concentration of about 0.1 mM) and incubated for 30 s. The nuclei were then fixed in 2.5% glutaraldehyde (buffered with 0.05 M Na-cacodylate to pH 7.2) for 1 h at 4°C, washed several times in the same buffer in the cold, and postfixed in 2% $OsO_4$ for 2 h at 4°C. Dehydration was carried out by the acetone vapor method (59), and embedding was done in Epon 812.

In order to preserve some juxtanuclear cytoplasmic material adhering to the outer nuclear membrane, nuclei were isolated in 5:1-medium containing 5 mM $MgCl_2$. Such nuclei were then incubated in the same medium containing 0.5% Triton X-100 for 5–30 min at room temperature before fixation. Nuclear membranes were prepared manually and fixed as previously described (53).

### Positive and Negative Staining of Spread Preparations of Nuclear Membranes from Amphibian Oocytes

Nuclei from lampbrush-stage oocytes were manually isolated as described above. For spreading the nuclear envelopes or fragments thereof, a single nucleus was first washed in pH 9 water and transferred to a drop of the same solution placed on a wax plate. The nucleus was

then lifted towards the surface of the water drop with a fine needle, whereupon it rapidly burst. With appropriate point source illumination (cold-light source KL B, Schott & Gen., Mainz, Federal Republic of Germany), the position of the spread nuclear membrane material could be observed under a binocular at magnification of 50. After 1,2,3 or 5 min, the material was then picked up on a carbon-coated grid (300 mesh) that had been freshly glow-discharged, by touching the drop surface. In another series of experiments, the nuclear envelope was first manually separated from the nucleoplasm (see 53), and then spread by the same method. Positive staining was carried out essentially according to Miller and Bakken (45). The grid was first immersed in a 3.5% formaldehyde solution (adjusted to pH 8.5 with 0.1 M Na-borate buffer) for 5 min, then dipped into diluted Kodak-Photoflo, air dried, and positively stained with 1% ethanolic phosphotungstic acid (PTA). For negative staining of such preparations, a drop of 2% aqueous PTA (adjusted to pH 7.2 with NaOH) was placed onto the still wet grid, immediately after the spread membrane was picked up from the drop surface, and was removed after 1 min by blotting with filter paper (c.f. 54). In some experiments the spread material was fixed in cold 1% $OsO_4$ (10 min) and washed in twice-distilled water before staining.

Isolated oocyte nuclei and nuclear envelopes were treated with detergents, using a modification of our previously described procedure (26), by incubating them for 10–15 min at room temperature in buffered "5:1-medium" containing 5 mM $MgCl_2$ or in pH 9 water, both made 0.1, 0.5 or 1% with respect to Triton X-100 or Nonidet P-40 (NP-40). Positive staining of such preparations was performed according to Miller and Bakken (45), i.e. the drop with the dispersed nuclear material was transferred to a centrifuge chamber and processed as described (55). Negative staining was carried out as described above, using grids that were coated with Formvar or with freshly glow-discharged carbon. For controls, isolated nuclei or nuclear envelopes were processed in parallel in the same media without Triton or NP-40.

## Electron Microscopy of Rat Liver Nuclei and Nuclear Envelopes

Small pieces of freshly prepared rat liver tissue were gently homogenized with a Teflon-homogenizer in a medium containing 0.4 M sucrose, 70 mM KCl, 2mM $MgCl_2$ and 1% Triton X-100, buffered with Tris-HCl (pH 7.2), and incubated at room temperature for 10 min. The detergent treatment was stopped by fixation of the homogenates with cold 2.5% glutaraldehyde (containing 2 mM $MgCl_2$, 70 mM KCl, 50 mM Na-cacodylate, pH 7.2) for 30 min. Fixation was either done immediately or after washing twice in detergent-free medium by centrifugation at ca. 1,000 $g$ for 5 min and resuspension. After extensive washing in cold medium, the samples were postfixed in 2% $OsO_4$ buffered with 50 mM Na-cacodylate (pH 7.2) for 2 h. Isolated rat liver nuclei (for isolation procedure, see 22, 38, footnote 1) were incubated in twice-distilled water or in 0.1 mM Tris-HCl (pH 7.5) at a concentration of about $3 \times 10^7$ nuclei/ml. After 5–10 min of incubation, the disruption of the swollen nuclei was completed by gentle homogenization, and large, "ghostlike" nuclear envelope fragments were enriched in the fraction which sedimented at 3,500 $g$ for 10 min from the supernate of an initial 3 min at 200 $g$ centrifugation. For thin section studies, the nuclear envelope ghosts (see also 32, 41) were suspended in an aqueous solution of 50 mM Tris-HCl (pH 7.2), 2 mM $MgCl_2$, and 0.1% or 0.5% Triton X-100 for 10 min and then fixed as described above for the homogenates. For negative staining, the nuclear envelope fraction either was stained immediately after pelleting from the incubation in Triton X-100 as described above or was suspended in 0.1 mM Tris-HCl (pH 7.5), and one drop of this suspension was mixed on a Formvar-coated grid with one drop of an aqueous medium containing 0.5% Triton X-100 and 2 mM $MgCl_2$. After 30–60 s, most of the fluid was blotted with filter paper, and the remaining material was negatively stained with aqueous PTA solution (see above). In addition, purified nuclear membranes were isolated as previously described (22, 38).

## Determination of Lipids and Carbohydrates

Isolated rat liver nuclei were incubated at 20°C for 10 min in 10 mM Tris-HCl (pH 7.2), 5 mM $MgCl_2$, containing 1% Triton X-100 at a resulting ratio of about 0.1 mg nuclear protein per mg detergent, and were washed once with the detergent-containing medium by centrifugation at 3,500 $g$ for 10 min and resuspension. Nuclear envelope "ghosts" and nuclear membrane fractions were similarly treated, except that all centrifugations were carried out for 40 min at 60,000 and 110,000 $g$. Then the nuclei and nuclear envelope fractions were washed twice by centrifugation in detergent-free medium and were used for determinations of cholesterol, phospholipids, and total and protein-bound carbohydrates (for procedures, see 38, footnote 1). Neutral sugars and hexosamines were determined by gas chromatography of their alditol acetates, after complete hydrolysis and thin-layer chromatographic separation, with preparative recoveries monitored by labeled compounds added (for details, see footnote 1). Nuclei and nuclear membranes which were processed in parallel in the same media but without detergent were analyzed for comparison.

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[1] Franke, W.W., T.W. Keenan, J. Stadler, E.D. Jarasch, R. Genz, and J. Kartenbeck. 1976. Nuclear membranes from mammalian liver. VII. Characteristics of highly purified nuclear membranes in comparison with other membranes. Manuscript submitted for publication.

## RESULTS

We have examined the effects of several solutions, with and without detergents added, and of mechanical tension on the integrity of the nuclear envelope of nuclei isolated from both cell types, the amphibian oocyte and the rat hepatocyte, with particular emphasis on the pore complex structures.

### Studies with Nuclei from Amphibian Oocytes

Ultrathin sections of nuclear envelopes that are manually isolated from amphibian oocytes in the presence of divalent cations exhibit a clear trilaminar "unit membrane" structure in both the inner and outer nuclear membrane (Fig. 1 a-c; c. f. 23, 25). The pore complexes reveal typical subarchitecture with globular annulus subunits, the centripetal tiplike projections ("peripheral granules") from the pore wall, the internal pore fibrils, and the central elements (for nomenclature and detailed descriptions, see 19, 21, 23, 25, 42, 49). Distinct, extended fibrillar strands are intimately attached to the inner (nuclear) annulus and to the central elements of pore complexes, thus resulting in an asymmetrical distribution of the electron-dense material attached to the nuclear envelope (Fig. 1 a-c). In addition, we frequently note somewhat less-defined fibrous material apposed to the nuclear surface of the inner nuclear membrane in interpore sections (e.g. Fig. 1 b; c.f. reference 23). When isolated nuclei are incubated for various times in media without divalent cations, such as the 5:1-medium (see Materials and Methods), the outer nuclear membrane seems to bleb and to detach in certain regions and, finally, to disappear completely (Fig. 1 d, e). This is in contrast to the higher stability of the material of the pore complexes (for partial losses of pore complex material under these conditions, see also 23) and of the inner nuclear membrane. The profile of the inner nuclear membrane, or what has remained thereof, appears to have an increased stainability and is markedly thickened (Fig. 1 d,e). During the detachment of the outer nuclear membrane, the pore complex material apparently remains associated with the inner nuclear membrane (Fig. 1 d, e), and it is intriguing that the profile of the detached outer membrane no longer shows interruptions that should correspond to the specific pore complex orifices (Fig. 1 e). This might suggest that rearrangements of outer membrane components have taken place. When isolated nuclei or nuclear envelopes are incubated in pH 9 water, a change in the nuclear membrane appearance, together with some progressive nuclear envelope disintegration, is also noted. This is again coincident with the appearance of a layer of electron-dense material (15–20 nm in width) at the inner nuclear membrane. The appearance of such a marked, nuclear envelope-apposed layer is frequently recognized after incubation of nuclei in media of low salt concentrations (see, for example, Figs. 15–21 in reference 54). This layer is clearly distinguishable in stainability and fine texture from the swollen nuclear pore complex material and does not occur in the adjacent juxtanuclear annulate lamellae (c.f. also reference 54). After incubation of isolated nuclei or nuclear envelopes in nonionic detergents, a similar progressive removal of nuclear membrane components is seen (Fig. 2 a-d), apparently also beginning with bleb formation of the outer nuclear membrane (Fig. 2 e). Again, the pore complexes remain relatively intact, although with less distinct fine structure, and appear to be attached to and embedded in a densely stained, 10–20 nm thick layer in which a membrane-like organization is only rarely resolved (Fig. 2 c,d). The aforedescribed attachment of nuclear fibrils to the pore complexes likewise survive the treatment (Fig. 2 a). It is interesting to note that in earlier stages of detergent action the nuclear membrane seems to be more readily dissolved than the adjacent membranes of the annulate lamellae and endoplasmic reticulum (ER; Fig. 2 b,c). Nuclear envelopes that have been disrupted on an aqueous surface show a progressive fragmentation with a conspicuous tendency of the rupture lines to delineate the pore complexes (39, 54). In such preparations, either fixed or unfixed, fibrils connect still intact envelope regions (e.g. Fig. 3 a; c.f. also reference 23). In addition, fenestrations ("holes") are produced which are well distinguishable from nuclear pores in that the fenestrations have widely variable diameters and pore complex-specific structures are absent (see also references 23, 25, 54). The dimensions of the membrane-fibrils are somewhat variable, ranging from 5 to 10 nm. Essentially identical observations are made with isolated nuclear envelopes obtained after spreading on aqueous surfaces and after prolonged incubation in pH 9 water. The interpore network of membrane-derived fibrils is perhaps best visualized in nuclear envelopes which have been spread on the surface of drops of pH 9 water for a short time (1–5 min), then fixed with formaldehyde and
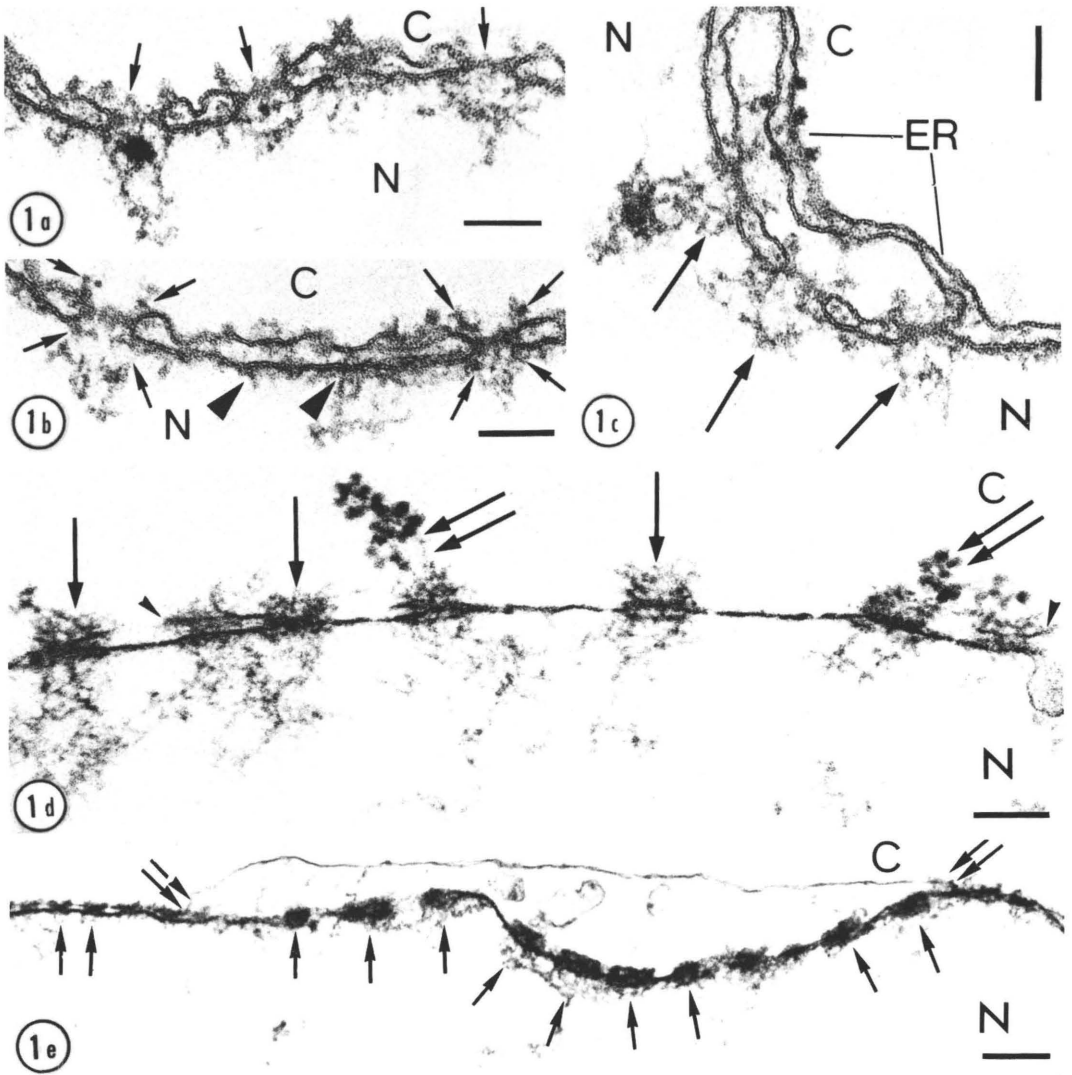
FIGURE 1   Ultrathin section of nuclear envelopes manually isolated from mature oocytes of *X. laevis* (*a-c*) and of *Triturus alpestris* (*d,e*) as revealed after isolation in the presence of divalent cations (see Materials and Methods) and after incubating the nuclei in the 5:1-medium for several minutes (*d* and *e*). The "unit membrane" pattern of both the inner and outer nuclear membrane is clearly resolved in *a-c* and is not different from that of the adjacent ER-membranes (*c*). The pore complexes (arrows in *a-c*) are well preserved, and the annulus subunits are identified (arrows in *b*). Fibrils attached to the annulus and the heavily stained, centrally located globule (arrows in *a* and *c*) are recognized, especially at the nucleoplasmic (*N*) side. Similar granulofibrillar material appears to be tightly attached to the inner nuclear membrane in interpore regions and at some sites it forms an amorphous layer apposed to the inner membrane (e.g. at the triangles in *b*). The appearance of such an apposed layer is somewhat more pronounced after incubation of the isolated nuclei in the various media before fixation (*d,e,*). Under these conditions, the outer nuclear membrane is partially removed, especially in the interpore regions (some resistant outer membrane portions are denoted at the small arrowheads in *d*), or is detached over longer distances (double arrows in *e*) from the remainder of the nuclear envelope, i.e., the inner membrane and the pore complex material. The pore complexes, however, still reveal their typical subarchitecture and are, occasionally, connected with cytoplasmic (*C*) polyribosomes (double arrows in *d*). (*a-d*) × 100,000; (*e*) × 45,000; scales indicate 0.1 μm (*a-d*) or 0.2 μm (*e*).
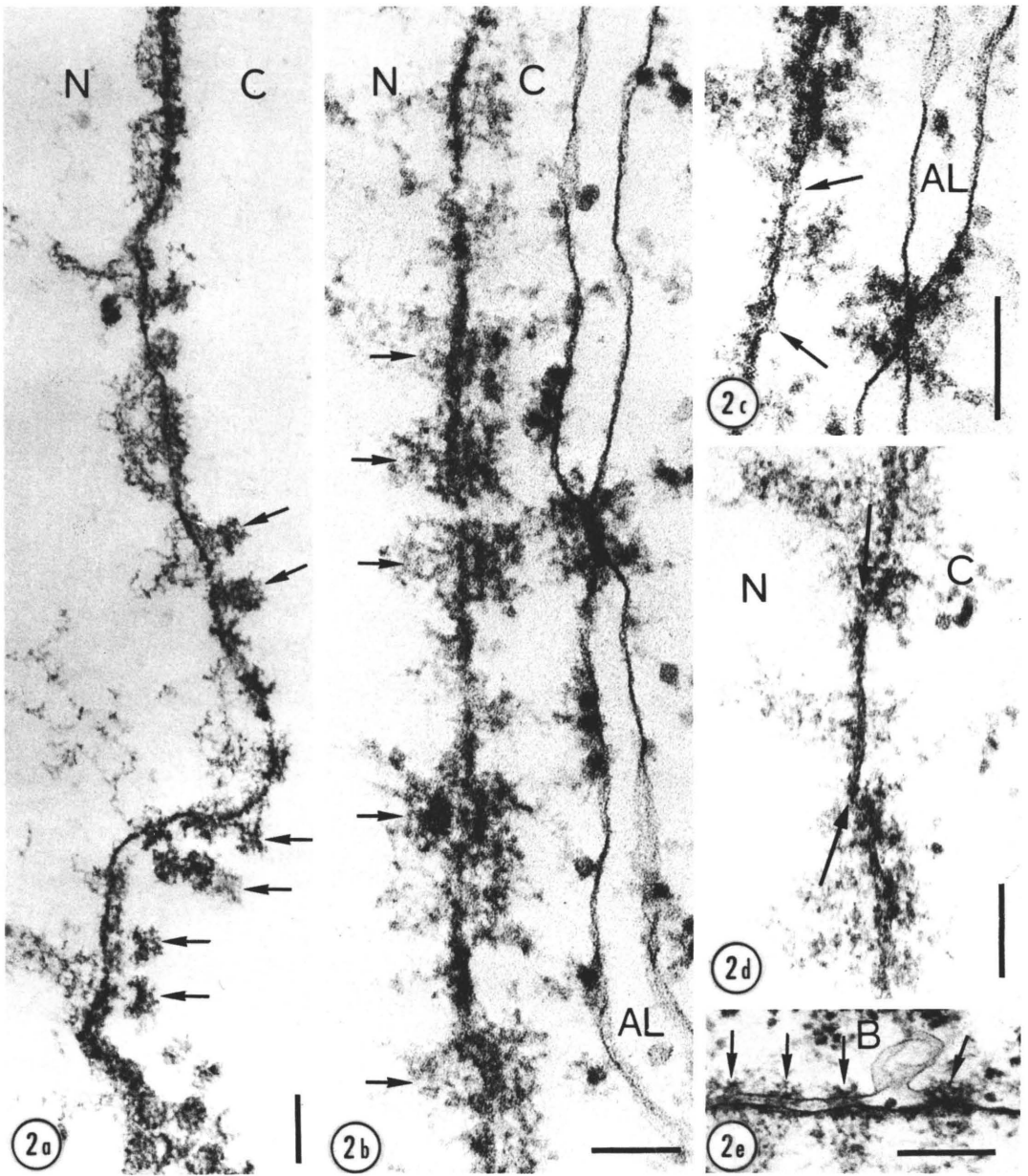
FIGURE 2  Nuclear envelopes and nuclear periphery in isolated nuclei from *P. waltlii* (*a*) and *X. laevis* (*b-e*) oocytes as revealed after treatment with solutions containing 0.5% Triton X-100. The nuclei were isolated in 5:1-medium (see Materials and Methods), with 5 mM $MgCl_2$ added in order to stabilize the nonmembranous components, and then incubated for 10 min with the detergent. Characteristically, the outer nuclear membrane has been lost in most nuclear envelope regions, whereas the inner membrane, or remnants thereof, is still frequently observed (see, e.g., the arrows in *c* and *d*). Usually, however, the nucleus is circumscribed by a layer of densely stained, relatively indistinct material of variable thickness to which the numerous pore complexes with well identifiable substructures and fully preserved pore complex-associated fibrils and granules are attached (*a;* pore complexes are denoted in *b* by arrows). Note that structural continuity of the individual pore complexes is maintained by the detergent-resistant dense layer derived from, or apposed to, the mostly disintegrated inner nuclear membrane. Note also that, under the same conditions, adjacent membranes of juxtanuclear ER-cisternae and annulate lamellae (*AL*) sometimes can still be recognized (*b* and *c*), although their ultrastructure looks somewhat altered. Upon disintegration of *AL*, isolated pore complexes, probably derived from *AL*, can frequently be found in the immediate vicinity of the nuclear envelope (arrows in *a*). Fig. 2 *e* presents the typical aspect of early stages of detergent-induced nuclear envelope disintegration, the formation and detachment of "blebs" (*B*) from the outer nuclear membrane. The arrows point to pore complexes. *N*, nucleoplasmic side; *C*, cytoplasmic side. (*a*) × 47,000; (*b*) × 120,000; (*c*) × 130,000; (*d*) × 85,000; (*e*) × 66,000; scales indicate 0.1 μm (*b*), 0.13 μm (*c*), 0.15 μm (*d*) or 0.2 μm (*a,e*).
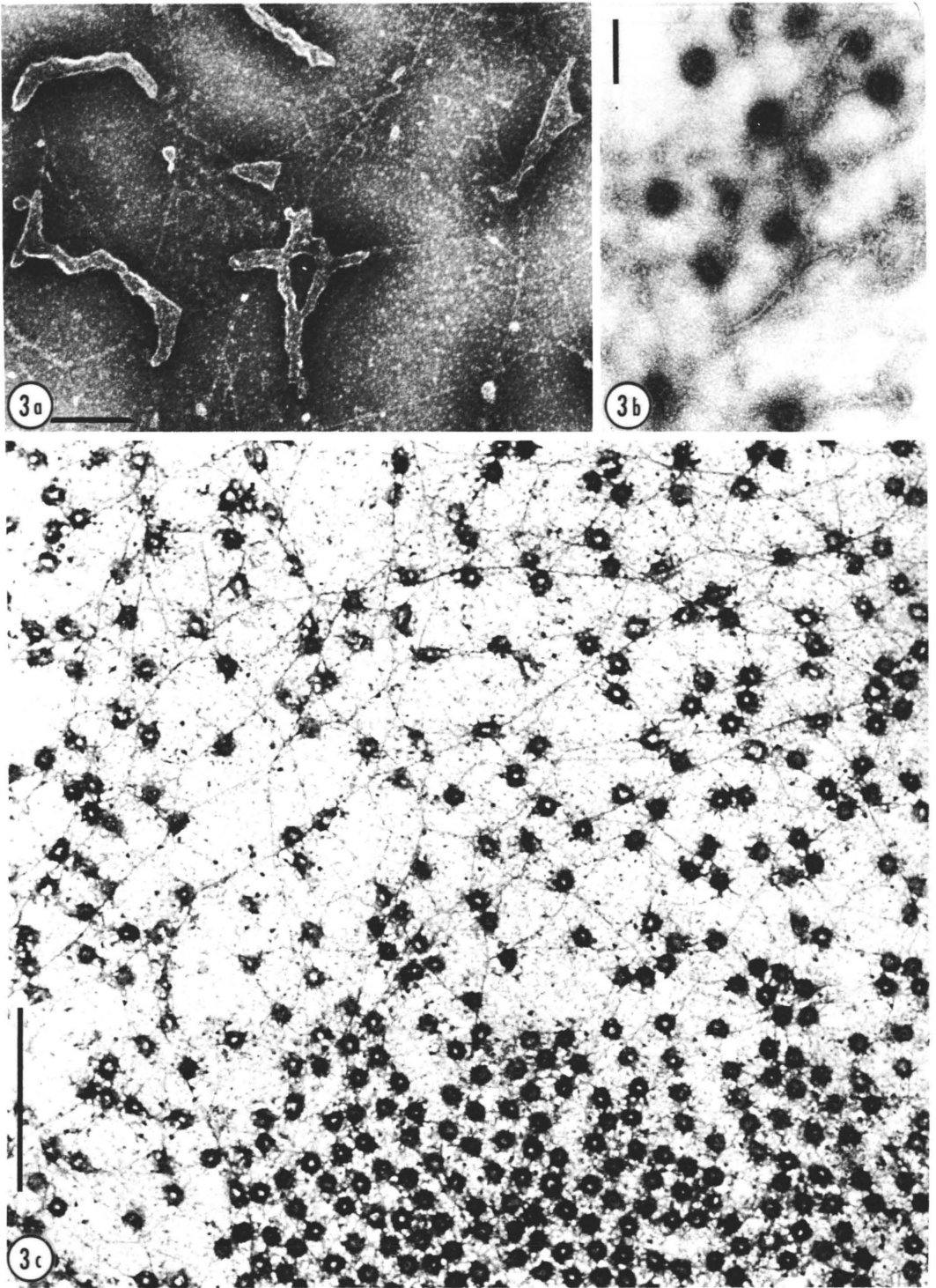
FIGURE 3  Nuclear envelopes isolated from lampbrush stage oocytes of *T. cristatus* (*a,b*) and *X. laevis* (*c*) spread on the surface of a drop of pH 9 water and either negatively stained with neutralized 2% PTA (*a,b*) or positively stained with ethanolic PTA (*c*). Stretching of the membrane by mechanical forces has resulted in the formation of extended fibrillar elements connecting small membrane fragments (*a*) or ringlike pore complex structures (*b* and *c*). Note the different aspects revealed by these structures after the different staining procedures. In the negatively stained preparation (*b*), heavy PTA-aggregates surround the individual pore complexes some of which appear to be in an almost isolated state.(*a*) × 63,000; (*b*) × 47,000; (*c*) × 27,500; scales indicate 0.2 μm (*a,b*) or 1 μm (*c*).
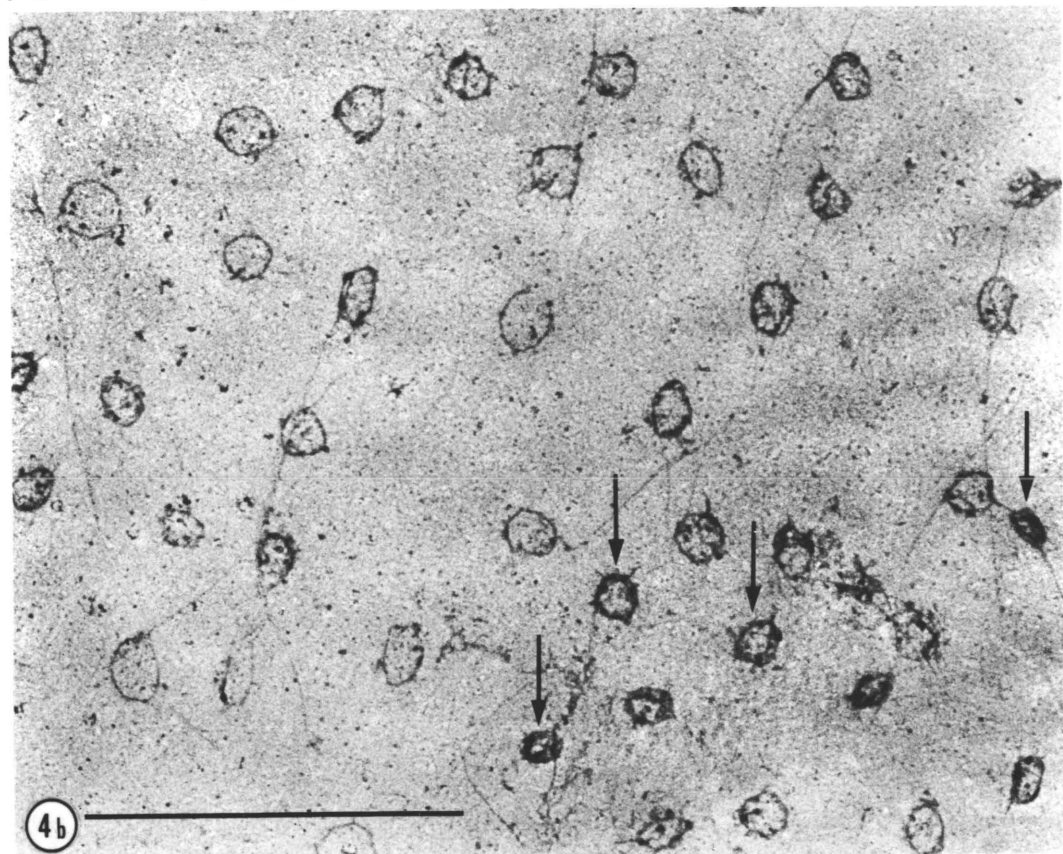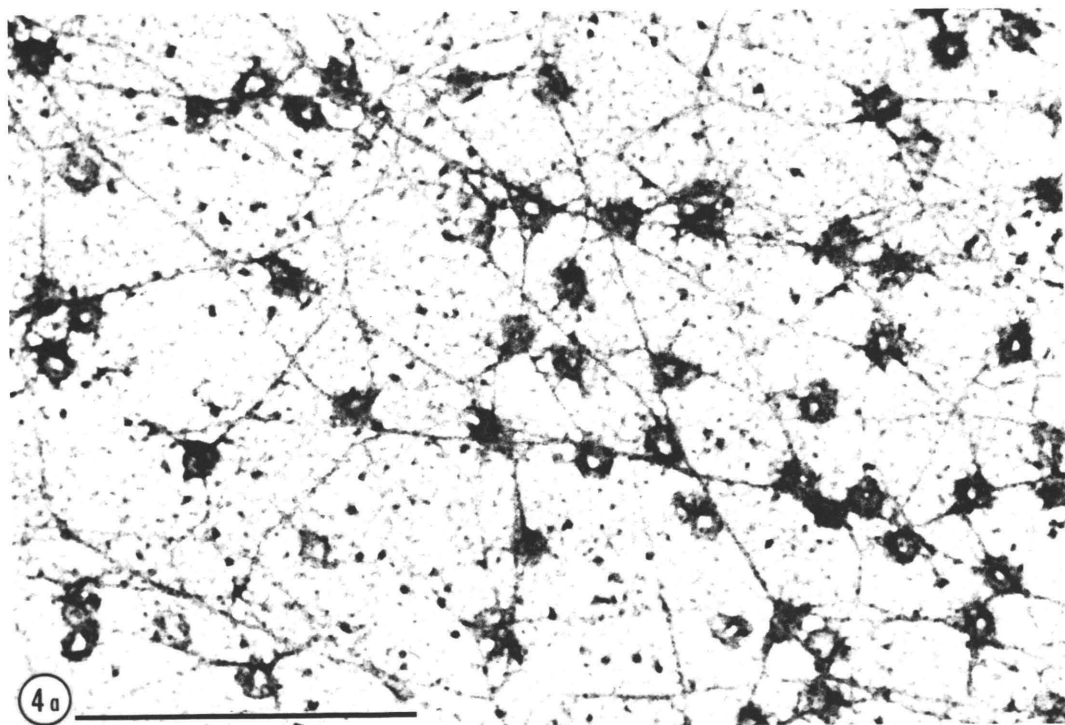
positively stained with ethanolic solutions of PTA (Figs. 3c and 4). In regions in which the dispersion forces are weaker, the pores remain in their normal close packing and the fibrils are relatively short and arranged in aster-like patterns around the annuli. In contrast, these fibrils appear stretched out in adjacent regions in which the pore complexes have been separated from each other (compare bottom and top part of Fig. 3 c). As is illustrated in Fig. 4 a, at higher magnification these membrane-derived fibrils might connect individual pore complexes over distances of up to 1 μm and seem to firmly insert at the pore complex periphery. Figs. 3 b and 4 b present what appears to be the final stage in the progressive disintegration of the nuclear envelope of amphibian oocytes. At this stage, one sees ringlike pore complex remnants which are often expanded and distorted; the remnants, however, are not completely isolated but are still connected to each other by fine filaments, which sometimes results in a spider-like appearance of the pore complexes (see, e.g., the pore in the upper right of Fig. 4 b). With negative staining, such pore complex-rings are accentuated by especially heavy PTA depositions similar to those described as "free" annuli in whole mount preparations of nuclear envelopes (28; c.f. also 17).

The interpore fibrillar network is particularly clear after treatment of the nuclear envelopes with detergents (Fig. 5). While the remnants of the pore complexes appear with an especially high contrast in positively stained preparations (Fig. 5 a, c–e), they are less defined after negative staining (Fig. 5 b) because they are again somewhat obscured by heavy accumulations of the staining material. Besides the somewhat aster-like pore-connecting fibrils, which occasionally reveal the characteristic eightfold radial symmetry (Fig. 5 c,e; for references see introductory paragraph), there are long, thicker (about 15 nm) and more tortuous fibrils which seem to insert at the annuli and are sometimes associated with granular (20–30 nm) components (Fig. 5 d, e). Most probably, these fibrils

represent the intranuclear annulus-attached fibrils described above in thin sections and in previous reports (for reviews, see 23–26).

The mechanical stability of the fibrillar pore-to-pore connections was also demonstrable in those preparations in which we regularly noted that, even after prolonged disintegrative treatments (see above), the nuclear envelope ghosts still exhibited structural cohesion and resistance to tension forces such as pulling with microforceps and tearing with forceps and needles (see also Materials and Methods). Electron microscopy showed an oriented distortion of the pore arrays in such stretched-out nuclear envelope regions; the pore complexes are still interconnected by fibrillar elements, also apparently somewhat stretched.

### Studies with Nuclei from Rat Liver

When isolated rat liver nuclei are exposed to intense mechanical stress, a progressive detachment of parts of the outer nuclear membrane takes place, resulting in a relative enrichment of structurally intact inner membrane (Fig. 6 a; c.f. 31, 38, 69, footnote 1). The nonmembranous pore complex material, however, is notably stable and is frequently retained (Fig. 6 a). During treatment of nuclei with very low salt solutions and/or nonionic detergents, the outer nuclear membrane tends to rupture and is more readily lost than the material of the inner nuclear membrane (Fig. 6 b, c). Advanced stages of detergent-induced disintegration of the nuclear envelope are characterized by still well-preserved pore complexes (Fig. 6 b, c) and the absence of outer membrane. Most of the inner membrane is also lost, except for tiny regions of nuclear surface with still identifiable trilaminar membrane-like elements (Fig. 6 c). In addition, we observed small, obviously membrane-derived fragments, without membrane substructure resolved (see 38). A marked stability of the nuclear pore complexes to mechanical forces and detergent action is also noted with isolated nuclear envelope ghosts (Fig. 6 d–f), a finding that confirms earlier

FIGURE 4  Positively stained nuclear envelope from a *X. laevis* oocyte after short (*a*) and prolonged (*b*) exposure to surface spreading forces on pH 9 water. Fig. 4 *a* reveals a dense or more dispersed network of fine fibrils of variable widths which extend between the heavily stained ringlike pore structures. Similar, relatively compact ring structures can still be found in preparations that have been exposed to mechanical stress (arrows in *b*). In such preparations most of the pore complex rings reveal a very thin annular wall, and some of the rings still show internal fibrils and even central elements (*b*). Note the very fine fibrils that interconnect most of the annular structures. Some of the pore complexes, however, appear to be completely isolated. (*a*) × 45,000; (*b*) × 50,000; scales indicate 1 μm.
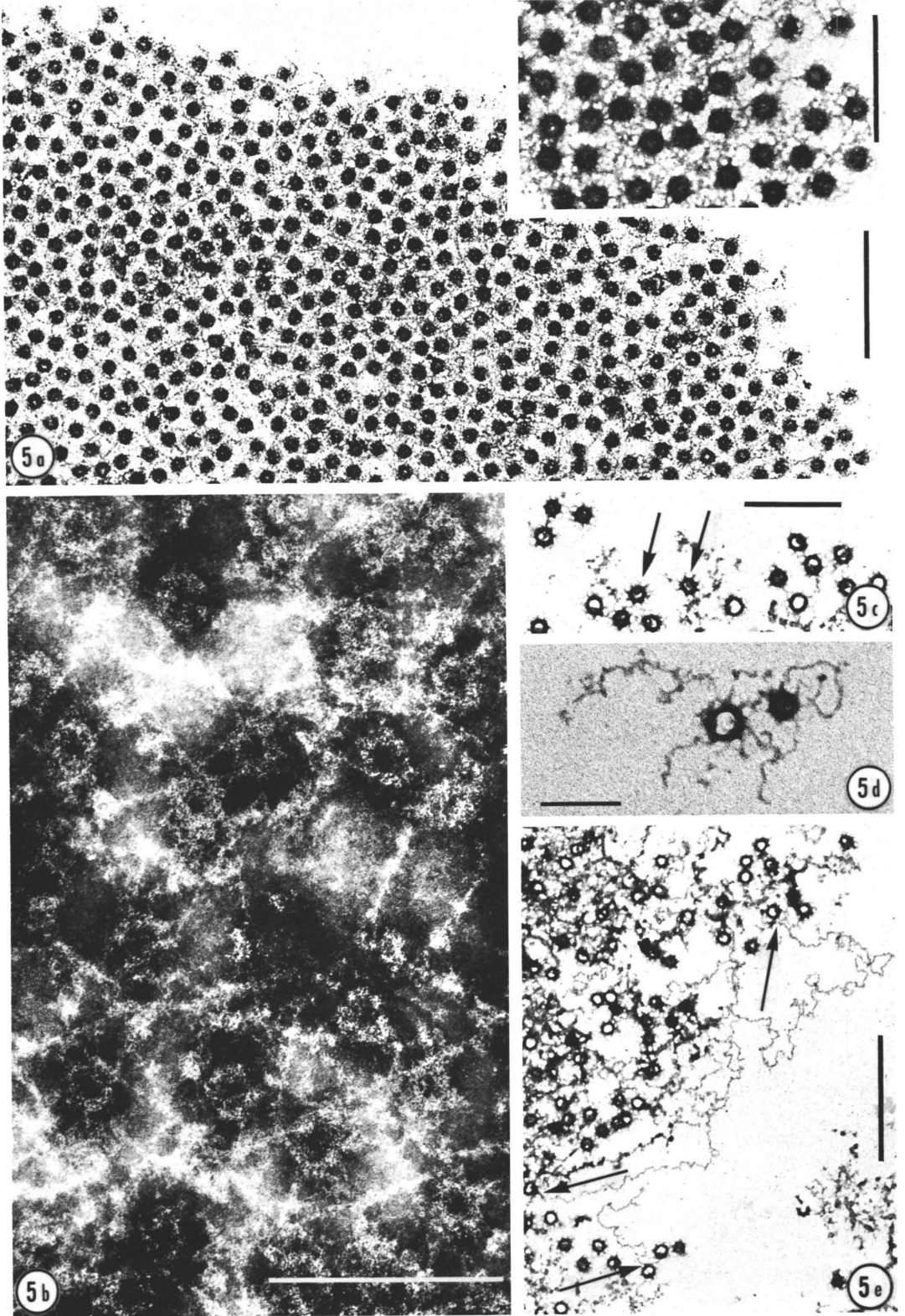
observations of other authors (1, 13, 51). In addition, we have repeatedly noted, in sections grazing the nuclear surfaces, the appearance of fine interpore filaments of variable widths (3–15 nm; Fig. 6 e). The existence of such interpore threads is more clearly recognized in negatively stained preparations of detergent-treated nuclear envelope fractions (Fig. 7).

When nuclei or nuclear membrane fractions from rat liver are incubated in solutions that stabilize chromatinous structures but that contain Triton X-100 or NP-40 at "saturating" concentrations, i.e. above the critical micellar concentration and above the maximal binding capacity of most proteins (see Materials and Methods; for comparative data, see 33, 50, 63), the remaining sedimentable material is almost completely devoid of phospholipids (1, 4, 38; c.f. also the data of reference 64) and cholesterol. There is, however, a small but significant moiety of glycoprotein retained, perhaps still attached to intranuclear structures and to the pore complexes (see above), as demonstrable by the gas chromatographic analysis of the sugar alditol acetates (Table I; for an extensive analysis of nuclear membrane carbohydrates, see footnote 1). The hexoses and hexosamines are retained at a considerable percentage of their initial concentrations, the proportions being somewhat variable for the specific sugar compound but fairly reproducible from experiment to experiment. The only significant exception was the relative enrichment of glucosamine, most probably derived from $N$-acetyl-glucosamine, in detergent-washed nuclei, which perhaps reflects the occurrence of this hexosamine compound in structures other than membranes (for detailed studies, see 40, footnote 1). The high mannose:galactose ratio was similar after the detergent treatments, and the whole carbohydrate pattern suggests the retention of typical nuclear membrane- and endoplasmic reticulum (ER)-type glycoproteins (compare also 8, 34, 40, 44, 46, footnote 1).

## DISCUSSION

Initially, we had attempted to use membrane solubilization by nonionic detergents, in the presence of stabilizing amounts of divalent cations, in order to isolate nuclear pore complexes from nuclear envelopes (see also 26). In such experiments, however, we noted that the residues from the detergent treatment were not released as isolated pore complex particles but that "the nonmembranous nuclear pore complex constituents are obtained in a form that is still sedimentable at low speed" (26). The present findings, together with those of Aaronson and Blobel (1, 2), make it clear that this inefficiency in separating the individual pore complexes is due to the connections between the columns of nonmembranous pore complex material that resist this treatment.

The nonmembranous components of the pore complexes of nuclear envelopes and annulate lamellae are markedly resistant to membrane disintegrating forces, be they mechanical (see, e.g. 17, 23, 28, 54) or induced by the interaction of membrane components with detergent molecules (e.g. 1, 2, 13, 51). With both kinds of forces, one notes a more or less preferential disintegration of the outer nuclear membrane, perhaps simply due to its higher accessibility and the absence of large associated supporting structures such as occur in the nuclear periphery. However, there is no evidence for the exclusive and complete removal of only the outer membrane by the action of deter-

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FIGURE 5 Detergent-treated nuclear envelope as revealed after positive (*a, c-e*) or negative (*b*) staining. Nuclei and nuclear envelopes were isolated from *T. alpestris* or *T. cristatus* oocytes incubated in aqueous 1% NP-40 (pH 9), and positively stained (*a, c-e*). Fig. 5a presents a survey micrograph of the disintegrated nuclear envelope. The heavily stained pore complex structures are embedded in, and connected by, a network of fibrillar material (inset in *a*). Upon complete disintegration of the nuclear envelope, ringlike pore complex-derived structures can still be recognized (*d*). Adjacent to the short pore-connecting fibrils are seen extended (up to 2 µm long) fibrils with "free ends", which frequently seem to terminate at the pore structures (*d* and arrows in *e*). Often the pore complex-derived structures are accentuated by eight "spikes", presumably remnants of annular components or of annulus-attached fibrils (e.g., at the arrows in *c*). Note also the presence of a "central granule" within many of the pore structures (*c*). For the preparation shown in *b*, an isolated nucleus was incubated for 10 min in 5:1-medium containing 5 mM MgCl₂ and 0.1% Triton X-100 and then negatively stained with 2% PTA. The remnants of the pore complexes are recognized as relatively ill-defined ring structures composed of finely textured material and are interconnected by fibrillar strands.(a) × 20,000; *inset*, × 40,000; (b) × 75,000; (c) × 30,000; (d) × 60,000; (e) × 20,000. Scales indicate 1 µm (*a,e*), 0.5 µm (*inset* in *a* and *b,c*) or 0.2 µm (*d*).
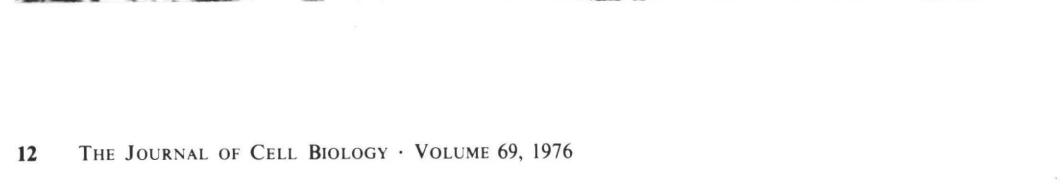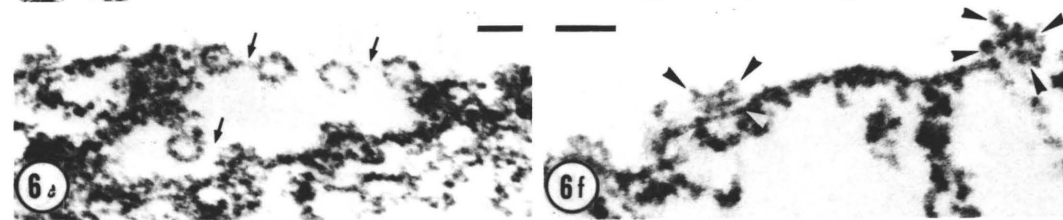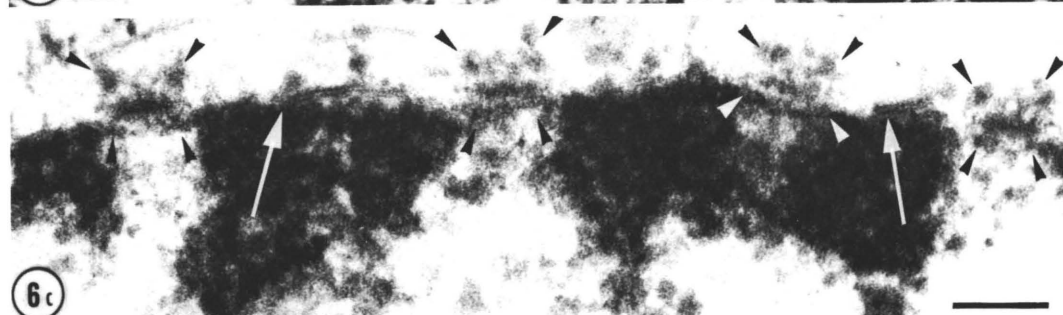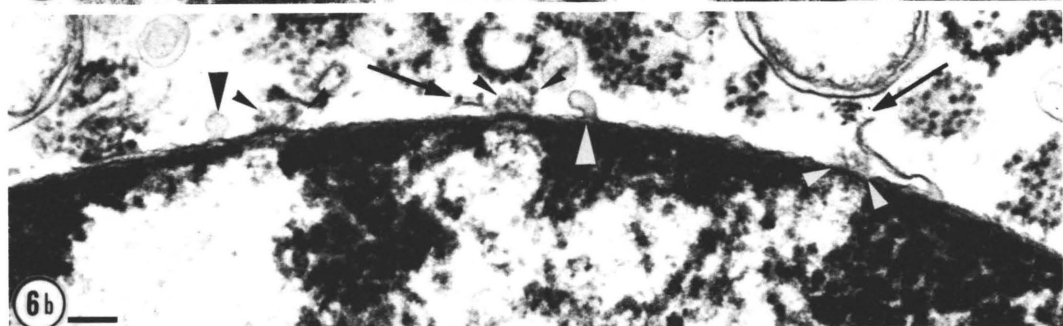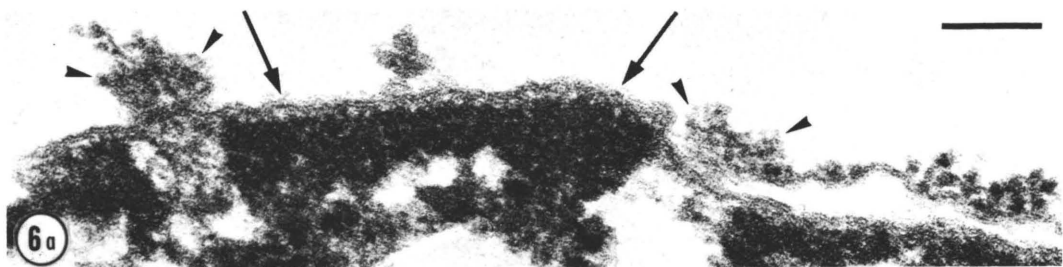
gents (see also 1, 3, 30, 38, 43). Provided an excess of unbound detergent molecules is maintained during the incubation (for detailed discussions of detergent effects on biological membranes, see the recent review in 33), both membranes are effectively solubilized with respect to the disappearance of lipid components (see also 1, 4, 38; see, however, 7), some glycoproteins and enzyme activities (e.g. also 38, 40), and the typical trilaminar membrane organization. A somewhat indistinct leaflet, however, is sometimes noted on the nuclear surface (3, 21, 25, 38) which may be equivalent to the "fibrous lamina" described in various cell types (2; for references, see introductory paragraph). It is conceivable that such inner nuclear membrane-apposed layers act as stabilizing "peripheral proteins" (for terminology, see 20, 58) that confer an enhanced resistance to mechanical stress and to the lipophilic action of detergents on the integral membrane proteins with which they are associated. The present demonstration of a fibrillar network as well as of some retained glycoproteins (c.f. also the data in 40) in detergent-treated nuclear envelopes might indicate that, indeed, some topological selection for certain membranous or membrane-associated proteins takes place, apart from the generally selective solubilization of membrane components, in the course of the detergent action

(c.f. 11, 15, 33, 63; for locally enhanced membrane stability to detergent by associated material, see also 68).

It is difficult to decide whether the indistinct layer at the level of the inner nuclear membrane is due to a change in the membrane material proper, or whether it reflects the more pronounced appearance of a dense layer of nuclear material attached to the inner nuclear membrane (see above). Aaronson and Blobel (2) have favored the notion that this structure reflects the in vivo existence in the rat liver nucleus of a layer apposed to the inner surface of the nuclear envelope that consists mainly of proteins assembled in a mode resistant to both high salt concentrations and treatments with detergents. While for normal interphase cells one would generally allow the argument that this layer represents condensed chromatin (5), the observations on amphibian oocytes in the present study exclude a chromatinous character of such material. On the other hand, untreated oocytes usually do not reveal the presence of an about 20-nm thick dense lamina at the inner nuclear membrane (for references, see introductory paragraph). Our study further shows that (a) such pore complex-connecting material can also be visualized after nuclear envelope disruption without detergents, for example, disintegration by mechanical forces and by very low salt

FIGURE 6    Peripheral regions in disintegrated tissue (b), in isolated nuclei (a and c), and in nuclear envelope ghosts (d-f). During the isolation, the outer nuclear membrane is frequently detached and removed by the mechanical forces (e.g. at the site between the arrows in a), but in such regions the nonmembranous pore complex structures (arrowheads) still remain associated with the nuclear material. After homogenization of tissue pieces in 1% Triton X-100 (b), the outer nuclear membrane is somewhat preferentially fragmented and removed (e.g., as denoted by the arrows in b); but, at many sites, the remaining inner nuclear membrane pieces also show interruptions and membrane disintegration, frequently in the form of small membranous blebs (triangles). Nuclear pore complex structure, however, is clearly retained (see, e.g., at the arrowheads). After prolonged exposure of nuclei or nuclear membrane fractions to the detergent (c), both nuclear membranes are removed, except for tiny portions of the inner membrane (arrows), whereas the pore complexes appear to be largely intact (arrowheads). These observations suggest that it is the outermost layer of condensed chromatin that essentially contributes to the relatively higher resistance of the inner nuclear membrane (as is especially suggestive with isolated nuclear ghosts, d and e). Fig. 6d demonstrates the removal of portions of the outer nuclear membrane, but not of the nuclear pore complexes (arrowheads), during a "normal" nuclear envelope isolation without the use of detergents. Fig. 6e and f show the appearance of these nuclear envelope ghosts after treatment with 0.1% Triton X-100. After application of the detergent, membranous profiles are no longer visible. The ghostlike structure, however, is maintained by a persisting layer of densely stained, proteinaceous or nucleoproteinaceous material. The nuclear pore complexes (arrowheads in f) can be identified in association with this layer and still show the triple organization in outer annulus, peripheral granules, and inner annulus (for nomenclature see Franke and Scheer, 25). Sometimes, especially in grazing sections, the persistence of pore-connecting fibrils is indicated (e.g., at the arrows in Fig. e).(a) × 130,000; (b) × 65,000; (c) × 125,000; (d) × 81,000; (e) × 60,000; (f) × 80,000; scales indicate 0.1 μm.
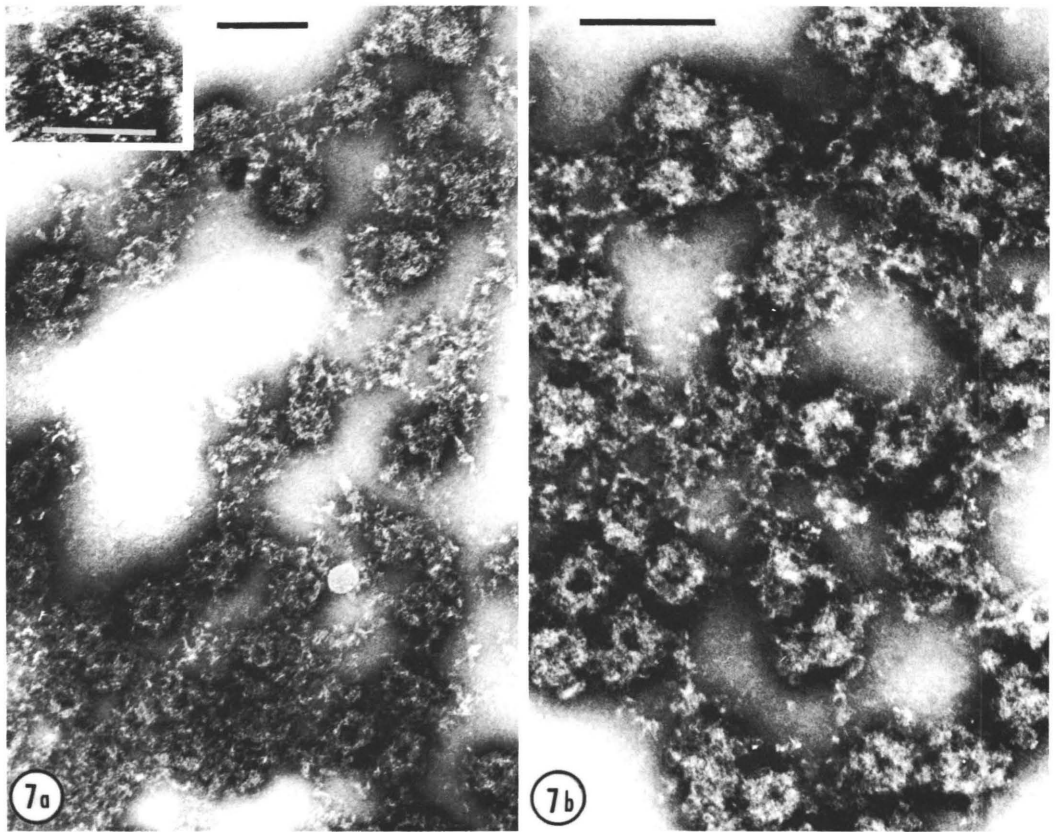
FIGURE 7 Negatively stained preparations of isolated rat liver nuclear membranes as revealed after treatment with Triton X-100 (for details see Materials and Methods). While the membrane is largely disintegrated and most of the membrane material is dissolved, the nuclear pore complexes have resisted the detergent treatment (details of the pore complex material as revealed after this treatment are presented in the inset in *a*). The pore complex-derived rings are connected to each other via threadlike fibrils that sometimes reveal more or less irregular associations with fine granules. (*a*) × 60,000; *inset*, × 150,000; (*b*) × 90,000; scales indicate 0.2 μm and 0.1 μm (*inset in a*).

TABLE I

*Carbohydrate Composition of Nuclei and Nuclear Membranes from Rat Liver with and without Detergent Treatment*

|  | Mannose | Galactose | Glucosamine | Mannose: galactose |
|---|---|---|---|---|
|  | nmol/mg protein (parentheses indicate % retained) | | | |
| Nuclei |  |  |  |  |
| Untreated | 9.0 | 3.2 | 2.9 | 2.8 |
| Washed with Triton | 2.2 (24) | 1.1 (36) | 2.2 (75) | 2.0 |
| Nuclear Membranes |  |  |  |  |
| Untreated | 49.0 | 14.8 | 14.5 | 3.3 |
| Washed with Triton | 18.5 (38) | 8.0 (54) | 5.0 (34) | 2.3 |

concentrations, and (b) the pore complexes are attached to a network of fibrils rather than to a continuous lamina. Similarly structured fibrillar pore complex connections have also been noted in the partially disrupted nuclear envelopes from a variety of cells (16, 17, 31) as well as in detergent-extracted nuclear envelope residues from *Physarum* plasmodia (56), and from human amnion cells (14). Previous authors, however, have interpreted such pore complex-associated fibrillar arrays as representing pore complex-associated chromatin (e.g. 14, 16, 56; for detailed discussions, see the reviews mentioned in the introductory paragraph).

In this study, as well as in previous ones (23, 24), we have emphasized the abundance of tangled fibrils, which most likely contain ribonucleo-proteins, attached to the pore complexes, especially at the inner annuli. This class of fibrils that apparently correspond to the thicker, annulus-attached fibrils noted in our whole mount preparations (see, e.g., Fig. 5 d, e) is also very unlikely to be identical with the interpore fibril reticulum. First, the fibrils run more or less radially and in individual tangles from the pore complex into the nucleus and are distinguished structurally from the pore-to-pore fibrils by several properties that allow their identification even in the flattened state, such as in whole mount preparations (e.g. Fig. 5 d, e): they are thicker and usually longer, appear more flexible, and reveal "free ends". Secondly, they could hardly connect the pore complexes to each other within the plane of the nuclear envelope, as demonstrated in sections through detergent-treated nuclear envelope ghosts (Fig. 2; see also references 1, 2), and maintain the relatively tight interpore cohesion noted during the experimental manipulations (see above). Therefore, the remaining alternatives that seem plausible to us are that the pore-connecting fibrils represent either (a) skeletal structures which in vivo are apposed to the inner nuclear membrane in a way equivalent to, albeit much thinner than, the "honeycomb layer" or the "fibrous lamina" described in some cell types (2; for references, see introductory paragraph), or (b) intrinsic membranous structures that are exposed after, or rearranged during, the disintegration and the removal of other membrane moieties. From our observation, we favor the second interpretation, which also finds some support in the descriptions of intramembranous threads that connect adjacent pores as seen in

tangential sections and freeze-cleavage preparations (23, 39, 60; for reviews, see 21, 25). This interpretation is also supported by the occurrence of carbohydrates with a typical membrane-like pattern in such residual thread material (the possible alternative that the nonmembranous structures of the pore complexes contain carbohydrates of the same composition as ER-membranes seems very unlikely to us, especially since such carbohydrates were not found in high salt extracts from isolated nuclear envelopes and nuclei; for references, see 21, footnote 1) as well as by the finding that some of the membrane cytochromes, particularly cytochrome $b_5$, are retained in detergent-treated nuclei and nuclear membrane preparations (Jarasch and Franke, unpublished data; for discussion and references, see also 21, 37, 65). On the other hand, the apparent absence of such interpore thread structures in the residues from juxtanuclear annulate lamellae indicates that a special association with some nuclear material is required for the stabilization of the whole fibrous reticulum.

Although irregular holes similar to those noted in partially disintegrated or damaged nuclear envelopes (see also 23, 54) are also common after limited damage in other membranes, including those of erythrocytes (57), the extensive network described in the nuclear envelope has not been noted in other membrane systems. This, however, does not necessarily indicate that such structures do not exist in these membranes, but might simply be consequence of the absence of such stable and relatively compact membrane-intercalated non-membranous components. Thus, due to its stable pore complexes, the nuclear envelope might be a particularly useful membrane type in which to differentially examine the processes of membrane disintegration. The evidence presented for the exposure or formation of membrane-derived, horizontally arranged fibrils in partly disintegrated nuclear membranes points to the possibly more common existence of certain integral membrane (glyco) protein molecules that contain large, probably hydrophobic, regions horizontally arranged. Such fibrillar membrane core components, then, would represent strong reinforcement structures, especially in concert with the well-known class of the vertically oriented membrane-spanning proteins.

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