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HETEROGENEOUS DISTRIBUTION OF FILIPIN–STEROL COMPLEXES IN NUCLEAR MEMBRANES

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Filipin, a sterol-specific polyene antibiotic, has been shown by electron microscopy to form complexes in membranes of mouse urinary bladder cells. Following instillation of a glutaraldehyde-filipin-dimethylsulfoxide solution into the bladder lumen, filipin-cholesterol complexes appear as membrane corrugations in thin sections and as 20–25 nm protuberances and depressions on PF and EF faces in freeze-fracture replicas. The complexes are observed in plasmalemma, Golgi membrane, rough endoplasmic reticulum and nuclear membrane of five different cell types (urothelial, endothelial, mesothelial, smooth muscle and fibroblasts). In the present report, we direct particular attention to the localization of numerous filipin-cholesterol complexes present in the nuclear envelopes of these cells. Our results suggest that enrichment of cell membranes with cholesterol occurs at an earlier stage in the flow-differentiation process than previously suspected. In addition, the unequal distribution of complexes in favor of the outer nuclear membrane suggests that it has a higher cholesterol content than the inner membrane.

Introduction

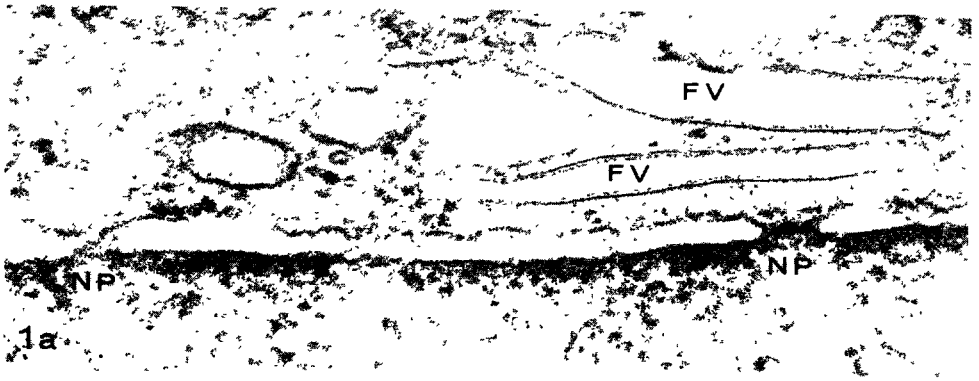
The nuclear envelope consists of two membranes which are the inner and outer aspects of flattened cisternae. Nuclear envelope is the least differentiated component of the 'endomembrane system' [1,2]. The 'endomembrane system' is defined as the structural and developmental continuum of internal membrane in the cytoplasm of eukaryotic cells, including the nuclear envelope, rough and smooth endoplasmic reticulum and Golgi apparatus [1,2]. The morphological features of nuclear membrane have been studied by thin section, negative staining and freeze-fracture electron microscopy [3,4]. The biochemical properties of nuclear membrane from diverse sources have been determined in isolated preparations [5]. Cholesterol, a major lipid component of the plasma membrane [6], is found only in small amounts in the nuclear membrane [5]. At present there is little morphological description of cholesterol distribution in the membranes of the nuclear envelope.

Filipin, a pentaene macrolide, is an antibiotic that binds specifically to sterols in biological and artificial membranes [7]. It forms ultrastructurally recognizable complexes with sterols in the membranes and thus serves as a useful cytochemical probe for cholesterol [8]. In the current study, we have observed a heterogenous distribution of filipin-cholesterol complexes in the nuclear envelopes of five different types of cells located in the walls of mouse urinary bladders.

Materials and Methods

Urinary bladders of five adult female DBA mice were examined. The animals were anesthetized with phenobarbital. The urinary bladder of each animal was inflated in situ with a fixative-cytochemical probe solution until fully distended. The solution contained 1% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4) and 50 μg/ml filipin which was dis-

Abbreviations EF, extracellular fracture; PF, protoplasmic fracture; RER, rough endoplasmic reticulum.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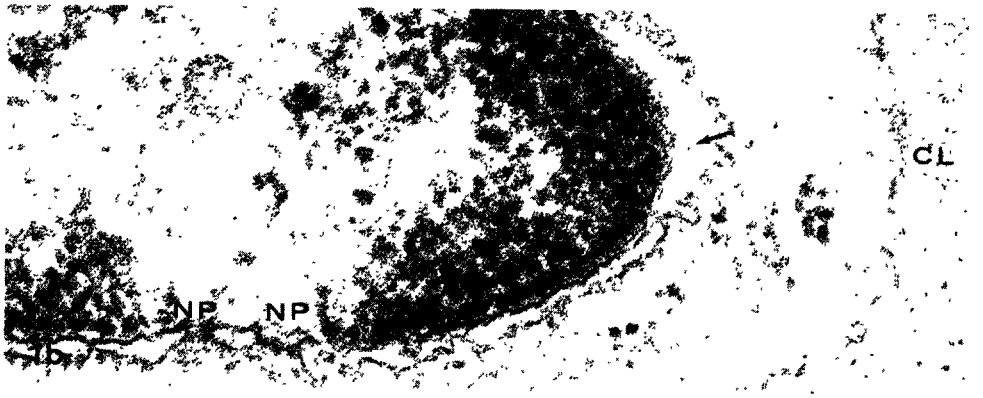
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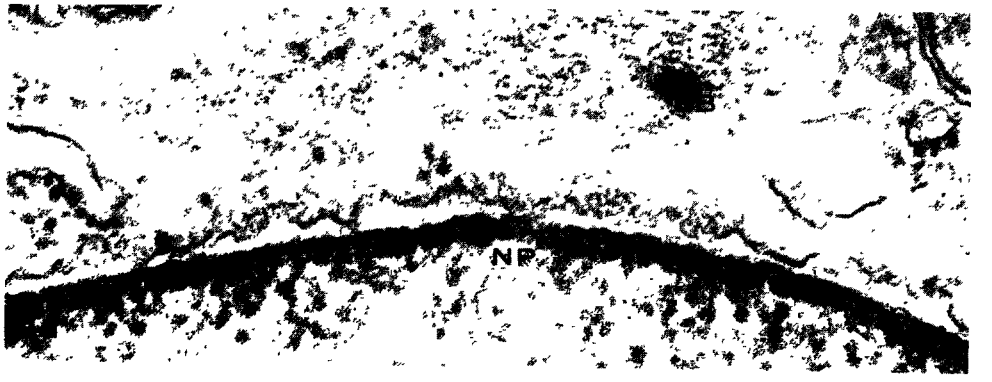
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solved in a small amount of dimethyl sulfoxide (DMSO). More DMSO was added to the final solution until it represented 1% of the total volume. When the urinary bladders were filled they were ligated, excised and immersed for one hour in the solution at room temperature. They were then hemisected and stored overnight in filipin cacodylate buffer solution at 4°C. Control bladders of three adult female DBA mice were fixed with the same glutaraldehyde-DMSO mixture, but in the absence of filipin.

For light microscopy and thin section electron microscopy, the tissue blocks were post-fixed in 1% OsO_4 in cacodylate buffer, stained en bloc with 5% uranyl acetate, dehydrated through graded ethanol solutions, and embedded in Epon 812. One μm thick sections of Epon-embedded tissue were stained with toluidine blue for light microscopy. Thin sections for electron microscopy were not stained.

For freeze-fracture electron microscopy 2–3 mm^3 slices of bladders, fixed in the filipin-glutaraldehyde-DMSO solution, were infiltrated for 4 h in 20% glycerol (v/v) in Millonig's phosphate buffer, pH 7.4. They were freeze-fractured and then etched for 60 s at −100°C in a Balzers model BAF 301 freeze-etch device, according to the method of Moor and Muhlethaler [9]. Platinum-carbon replicas were separated from the tissue with Clorox, washed with distilled water, and mounted on uncoated 300 mesh grids. Thin sections and freeze-fracture replicas were examined in a Philips EM 300 electron microscope.

Results

The nuclear envelopes of capillary endothelium, transitional cell epithelium (urothelium), mesothelium, smooth muscle cells and fibroblasts from specimens not exposed to filipin appear similar to those previously described [3,4]. In thin sections, the nuclear envelope consists of two roughly parallel membranes, one of which is in contact with the nucleoplasm, while the other is in contact with the cytoplasm and is continuous with the rough endoplasmic reticulum (RER). Each of these membranes is 7–8 nm thick. They are separated by a gap, the perinuclear space (i.e., perinuclear cisterna). Both membranes are united at the location of nuclear pores. In freeze-fracture replicas, the inner and the outer membranes display characteristic nuclear pores, which are 85–90 nm in replica diameter. Intramembrane particles, which measured 8–10 nm in diameter, are embedded in the nuclear membrane.

Interaction between filipin and cholesterol is detected in thin sections by the characteristic corrugated appearance of affected membranes. Focal corrugations of the nuclear membrane are often noted (Fig. 1a, b, c, d). There are distinct differences in the amount of membrane corrugation between the outer and inner membrane. The outer membrane has numerous foci with prominent corrugations (Fig. 1a–d), while very small foci are associated with the inner membrane (Fig. 1 b and c).

The filipin-cholesterol interaction is detected in freeze-fracture replicas by the appearance of 20–25 nm protuberances and slightly smaller depressions on the same membrane face. Numerous protuberances and depressions are observed on the protoplasmic fracture (PF) and extracellular fracture (EF) faces of the outer nuclear membrane, while only a few are associated with the inner nuclear membrane (Fig. 2 a and b).

Discussion

We elected to study the distribution of filipin-cholesterol complexes in the 'endomembrane system' of cells in the walls of mouse urinary bladders for the

Fig. 1. Thin-section electron micrographs of four different cell types in filipin-treated urinary bladder. (a) Portion of urothelial cell, with the characteristic fusiform vesicles (FV), which display non-corrugated asymmetric unit membrane. The nuclear envelope contains nuclear pores (NP), and its outer membrane is corrugated (arrows), whereas the inner one is not. Magnification × 89 000. (b) Portion of endothelial cell and capillary lumen (CL), illustrating extensive corrugation of the plasmalemma and the nuclear envelope's outer membrane. Only slight corrugation (arrows) of the nuclear envelope's inner membrane is noted. Magnification × 89 000. (c) Portion of submucosal fibroblast, with extensive corrugation of the plasmalemma, rough endoplasmic reticulum (RER), and nuclear envelope's outer membrane. The inner membrane is slightly corrugated (arrow). Magnification × 89 000. (d) Smooth muscle cell with characteristic cytoplasmic filaments and dense body. Extensive corrugation of the outer membrane of the nuclear envelope is apparent. Magnification × 89 000.

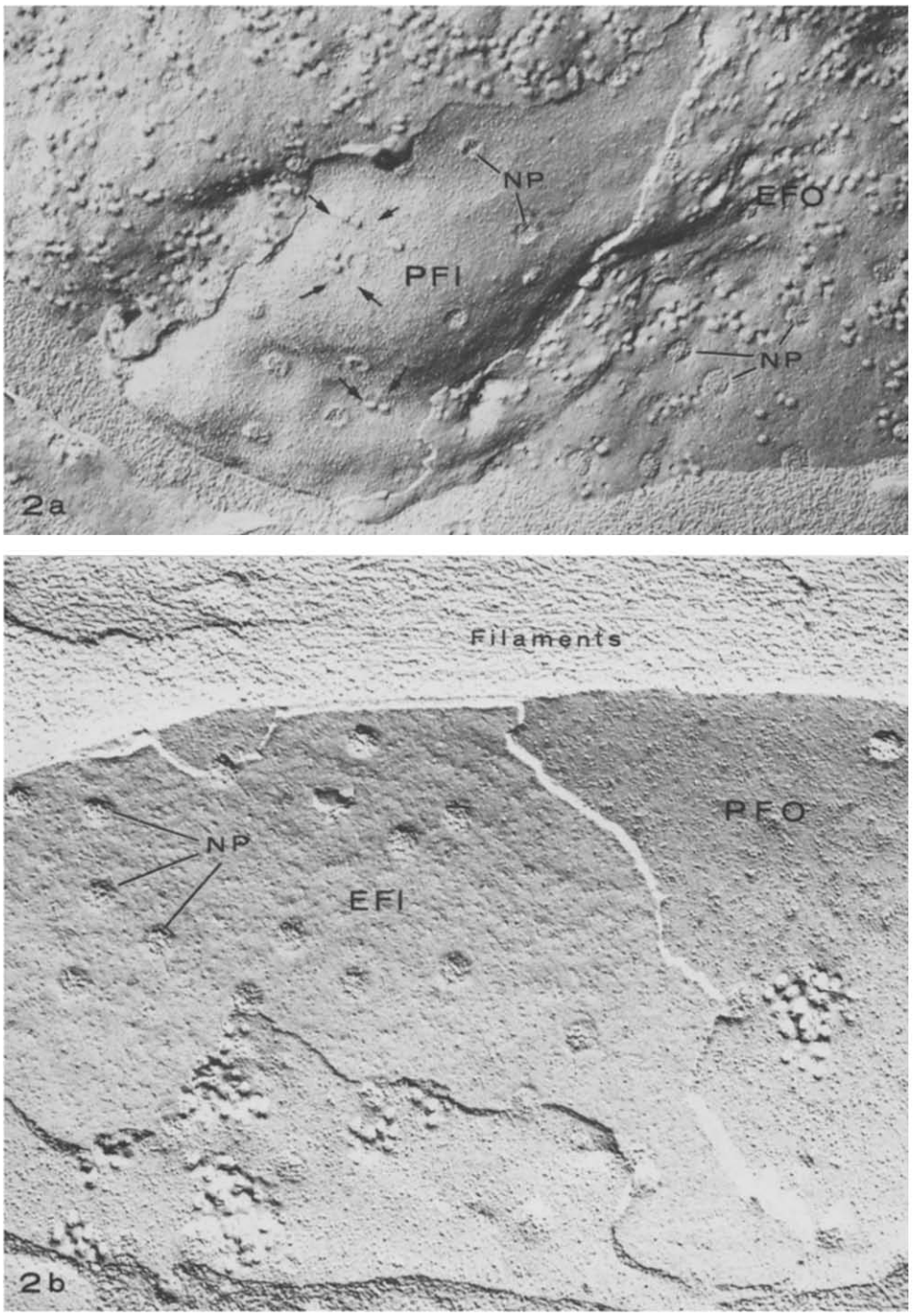

Fig. 2. Freeze-fracture replicas of two different cell types in filipin-treated urinary bladder. (a) Nuclear envelope of urothelial cell with characteristic nuclear pores (NP). Numerous protuberances and depressions are present on the extracellular fracture face of the outer nuclear membrane (EFO). Only a few protuberances (arrows) are noted on the protoplasmic fracture face of the inner nuclear membrane (PFI). Magnification × 35 000. (b) Smooth muscle cells is rich in cytoplasmic filaments. Nuclear pores (NP) are evident on fracture faces of the inner and outer nuclear membranes. Protuberances and depressions are found on the protoplasmic fracture face of the outer nuclear membrane (PFO), but not on the extracellular fracture face of the inner membrane (EFI). Magnification × 53 000. The shadow angle is: (a) from lower right, (b) from lower left.

following reasons: (1) The bladder wall contains five different cell types (i.e., urothelial, endothelial, mesothelial, smooth muscle cells and fibroblasts). (2) By exposing the inner and outer aspects of thin-walled bladder simultaneously to the solution, we were able to secure even and rapid fixation and penetration of filipin to the five cell types with minimal disruption of tissue organization. We treated our specimens with filipin and fixative together to minimize ultrastructural damage of the cell membranes [10].

The formation of filipin-cholesterol complexes is specific for planar steroids that have an unesterified 3-β-hydroxyl group and an apolar side chain at C-17 [7]. Lateral mobility of sterol molecules is thought to be required for the formation of complexes [10]. In addition, a threshold level of sterol concentration in the membrane must be exceeded in order for the complexes to form. Corrugations, protuberances and depressions indicate the presence of filipin-cholesterol complexes in membranes [8,10].

In the current study, we confirm reports regarding the presence of these complexes in plasmalemma and membranes of cytoplasmic organelles [8,11,12], and describe for the first time their presence in membranes of the nuclear envelope. It is noteworthy that the filipin-cholesterol complexes are much more numerous in the outer membrane of the nuclear envelope than in the inner. Although it is possible that: (1) cholesterol has preferentially migrated from fixed [10] inner membrane, (2) complex formation is inhibited by the closeness of chromatin to the inner membrane, and/or (3) low concentration of filipin is available at the inner membrane due to limited penetration, the most likely explanation for our observation is that cholesterol enrichment is greater in the outer membrane. Our interpretation is strengthened by the fact that this phenomenon is observed in five cell types, regardless of their location in the tissue.

Our findings are consistent with the theory which states that there are variations in major classes of lipids in the endomembranes that range from the nuclear envelope to the plasmalemma [1]. Structural and chemical changes occur during maturation of the 'endomembrane system' [1,2]. They include alterations in membrane thickness, lipid and carbohydrate composition [1,2] and increases in density of intramembrane particles [13]. Orci et al. [12] have utilized filipin as an ultrastructural probe for the presence of membrane cholesterol and found numerous filipin-cholesterol complexes at the level of the Golgi apparatus. They have concluded that in secretory cells the Golgi apparatus is the site where the endomembrane system is enriched by cholesterol [12]. We have found in five non-secretory cell types that enrichment of endomembrane by cholesterol occurs at an earlier level of membrane development, i.e., at the nuclear envelope.

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