Epithelial Structure Revealed by Chemical Dissection and Unembedded Electron Microscopy

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ABSTRACT  Cytoskeletal structures obtained after extraction of Madin-Darby canine kidney epithelial cell monolayers with Triton X-100 were examined in transmission electron micrographs of cell whole mounts and unembedded thick sections. The cytoskeleton, an ordered structure consisting of a peripheral plasma lamina, a complex network of filaments, and chromatin-containing nuclei, was revealed after extraction of intact cells with a nearly physiological buffer containing Triton X-100. The cytoskeleton was further fractionated by extraction with (NH₄)₂SO₄, which left a structure enriched in intermediate filaments and desmosomes around the nuclei. A further digestion with nuclease and elution with (NH₄)₂SO₄ removed the chromatin. The stable structure that remained after this procedure retained much of the epithelial morphology and contained essentially all of the cytokeratin filaments and desmosomes and the chromatin-depleted nuclear matrices. This structural network may serve as a scaffold for epithelial organization. The cytoskeleton and the underlying nuclear matrix-intermediate filament scaffold, when examined in both conventional embedded thin sections and in unembedded whole mounts and thick sections, showed the retention of many of the detailed morphological aspects of the intact cells, which suggests a structural continuum linking the nuclear matrix, the intermediate filament network, and the intercellular desmosomal junctions. Most importantly, the protein composition of each of the four fractions obtained by this sequential procedure was essentially unique. Thus, the proteins constituting the soluble fraction, the cytoskeleton, the chromatin fraction, and the underlying nuclear matrix-intermediate filament scaffold are biochemically distinct.

Sequential Extraction of Cytoskeletal Elements

Whole mounts of detergent-extracted cells have proved to be well suited to the study of cytoskeletal organization (1-9). In the absence of embedding plastic, the cytoskeletal filaments remaining after the removal of phospholipid and soluble proteins form clear images in transmission electron micrographs without the need for heavy metal staining. These images provide insights into the composition and organization of cytoplasmic filament networks that appear intimately associated with both the nucleus (9, 10) and the plasma lamina (11).

In initial extraction, Triton X-100 in a buffer designed to best preserve the architectural elements of the cell is used. However, further extraction of the cytoskeleton reveals both biochemically and morphologically important substructures of the cytoskeleton and nuclear matrix. Most of the cytoskeleton is removed by extraction with (NH₄)₂SO₄, leaving the network of intermediate filaments anchored to the nucleus. The nucleus itself is subfractionated by removal of the chromatin, a procedure that reveals the dense fibers of the nuclear lamina and the internal matrix (unpublished observations).

The nuclear matrix–intermediate filament structure is resistant to high salt and is clearly defined with respect to protein composition and morphology (4). We have suggested that it
be designated the nuclear matrix–intermediate filament (NM-IF) scaffold. The extraction of chromatin from the nucleus was pioneered by Berezney and Coffey (12) and others (13–17). The procedures that we use are considerably modified. We use a much lower salt concentration to effect extraction, with consequent better preservation of morphology (7–9, 18).

The protocol depicted in Fig. 1 shows the sequential extractions that divide the cellular components into the four protein fractions. The proteins of the soluble fraction (removed in the initial Triton extraction), represent 65% of the cellular proteins. These display a complex pattern in a two-dimensional gel electropherogram (Fig. 2). Although many proteins appear to be unique to the soluble fraction, the density of protein spots in the gel pattern precludes a detailed comparison of soluble proteins with those in other fractions. More specific techniques of protein identification are required for further comparison. The cytoskeleton (removed by either Tween 40-deoxycholate or 0.25 M (NH₄)₂SO₄), chromatin (removed after nuclease digestion and salt extraction), and the NM-IF scaffold (those proteins unextracted in this procedure) are characteristic fractions of the total cellular protein, i.e., there is relatively little overlap between the proteins in each of the three fractions. Thus, each of these fractions, when analyzed in two-dimensional gel electropherograms (Fig. 2), shows a unique pattern in which many major proteins are localized in one of the three fractions. Some proteins, most notably actin, are observed as major components of more than one fraction.

**Examination of Epithelial Cytoskeletons**

Differentiated epithelial cells are characterized by their ability to form complex intercellular junctions (19, 20), a property that appears to be fundamental to the development and maintenance of epithelial tissues (21, 22). Cells organized into epithelial tissues display a marked morphological and biochemical polarity in which the apical surface is composed of microvilli and in some cases cilia, while the basal surface is specialized for interaction with the extracellular matrix. Also, cell-cell contacts have highly specialized structures. These properties are clearly displayed by cells of the Madin-Darby canine kidney (MDCK) line (23–26).

The characteristic zonulae occludens and desmosomal junctions, as well as the apical microvilli, can be seen in conventional Epon-embedded thin sections of intact (i.e., unextracted) MDCK epithelial cells (Fig. 3a). When these cell colonies are extracted with Triton X-100 and viewed in embedded thin sections (Fig. 3b), all of these epithelial structures are retained in a recognizable form, with little apparent perturbation of the polarized morphology observed in the intact cell. Using a recently developed technique for preparing unembedded sections of cytoskeletal preparations (27) and sectioning through the longitudinal plane, we observe the junctional filament complexes in the absence of embedding resin or heavy metal stains (Fig. 3c). The unembedded section of the cytoskeleton, devoid of phospholipid and soluble proteins, also shows, with much greater clarity, the retention of the polarized epithelial morphology. In this preparation the organization of filaments in the apical microvilli and the filament association with desmosomes are particularly apparent. These images suggest that the cytoskeletal filaments form a structural continuum, through the junctional complexes, throughout the epithelial monolayer.

The structural continuity of cytoskeletal elements observed in unembedded preparations is further demonstrated in Fig. 4. Fig. 4a is a micrograph of an MDCK cytoskeletal preparation observed in a laterally cut (i.e., parallel to the substrate) conventional Epon-embedded thin section. Although the junctional complexes are visible, the cytoskeletal elements are masked and difficult to interpret. When an identical cytoskeletal preparation is viewed in an unembedded whole mount (Fig. 4b), the complexity and density of the cyto-

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1. **Abbreviations used in this paper.** MDCK, Madin-Darby canine kidney; NM-IF, nuclear matrix–intermediate filament.
FIGURE 2 Two-dimensional gel profiles of proteins obtained after fractionation of MDCK colonies. Two-dimensional protein gels were run according to the method of O'Farrell (39). The first dimension ranges from pH 10 to pH 3 (left to right), and the location of actin (A) and vimentin (V) is indicated for each gel. The patterns of the cytoskeleton, chromatin, and NM-IF fractions are characteristic, with little overlap of major proteins from one fraction to another.

FIGURE 3 Embedded and unembedded sections of MDCK intercellular junctional complexes. Whole MDCK colonies (a) and cytoskeletal preparations (b) were embedded in Epon-Araldite, sectioned, and stained with lead citrate and uranyl acetate. The characteristic zonula occludens (ZO) and desmosomes (D) observed in intact epithelial junctions (a) are retained in cytoskeletal preparations (b). Cytoskeletons prepared as unembedded sections as described (27) (c) again retain the structural elements observed in the embedded cytoskeletal preparation (b). The view afforded by the unembedded section reveals much of the detailed filament organization that is masked by embedding resins. Bars, 1.0 μm.
skeleton is clearly imaged. A higher magnification of the intercellular junction in this whole mount preparation reveals the desmosomes, unextracted by Triton, intimately associated with anastomosing filaments (Fig. 4c). The unembedded section of the same cytoskeletal preparation (Fig. 4d) shows that the nucleus is associated with numerous filaments and is virtually undistorted by the fractionation process.

**Nuclear Matrix–Intermediate Filament Scaffold**

Most cytoskeletal proteins can be removed using suitably buffered 0.25 M (NH₄)₂SO₄. The major structures remaining after this extraction are the intermediate filaments, desmosomes, and nuclei. The chromatin is extracted with nuclease digestion followed by extraction with 0.25 M (NH₄)₂SO₄ (described in the legend to Fig. 1), which removes most DNA and associated proteins (7). When this technique is applied to MDCK epithelial colonies, the structure that remains after this rigorous extraction is composed of only 5% of the total cellular protein. This stable structure is composed of the chromatin-depleted nuclear matrices, cytokeratin, and vimentin intermediate filaments and residual desmosomal cores (9). For this structure we have suggested the term nuclear matrix–intermediate filament (NM-IF) scaffold. A whole mount transmission electron micrograph of the NM-IF scaffold is shown in Fig. 5a. When this image is compared with that
FIGURE 5  NM-IF scaffold of an MDCK epithelium. The fractionation protocol described in Fig. 1 removes the majority of cellular components, leaving a stable structure composed of 5% of the cellular protein. When this NM-IF scaffold is viewed by whole mount electron microscopy (a), the retention of many epithelial characteristics is observed. The chromatin-depleted nuclear matrices (NM) are observed in association with cytoplasmic filaments. These filaments often terminate in residual desmosomal structures (D). Immunofluorescence microscopy of NM-IF scaffold structures after staining with antibodies derived to keratin (b), desmosomal proteins (c), and a 52-kdalton nuclear matrix protein (d) shows that each of these proteins is specifically localized within the NM-IF scaffold (9). Bar in a, 1.0 μm. Bar in b, 10 μm.

shown in Fig. 4b, it is evident that the overall morphological organization of the epithelium is conserved in this structure. Immunofluorescence micrographs of NM-IF scaffolds stained for cytokeratins (Fig. 5b), desmosomes (Fig. 5c), and a 52-kdalton nuclear matrix protein (Fig. 5d) demonstrate that each of these proteins is retained in the NM-IF scaffold with a spatial localization much the same as that observed in the intact cell (9). The desmosome antibodies stain in linear punctate patterns that correspond to the dense junctional complexes shown in Fig. 5a. The chromatin-depleted nuclear matrices retain a spheroid morphology and are in direct association with numerous intermediate filaments, many of which are also directly associated with desmosomes.

The image of desmosome and cytokeratin organization in epithelial tissues afforded by unembedded whole mount microscopy is compatible with models of epithelial structure derived from many studies. The association of intermediate filaments with nuclei has been observed (28, 29). The association of cytokeratins (or tonofilaments) with desmosomal complexes is a long-standing histological observation (19) that has been demonstrated in isolated desmosomes (30–32). The extensive distribution of keratin and cytokeratin filaments in sectioned tissue, cultured epithelial cells, and epithelial cytoskeletons has been amply demonstrated (33–38). Thus, the nuclear matrix, intermediate filaments, and desmosomal core structures can be isolated and purified as an intact structure of morphological relevance. The NM-IF scaffold shown in Fig. 5a reflects the morphology of intact epithelia. Profound
alterations of epithelial morphology by tumor promoters and by malignant transformation are retained in both cytoskeletal and NM-IF structures.  

Conclusions

In the fractionation method described here, the Triton-resistant cytoskeleton and the salt-resistant NM-IF scaffold are used as morphological end points. In the course of fractionation, four biochemically distinct populations of proteins are generated that account for all the proteins present in the cell. Both the cytoskeleton and NM-IF are visualized by unembellished microscopy of whole mounts or thick sections. The combination of the apical and longitudinal views of cytoskeletal organization permits clear images of epithelial structure in three dimensions under conditions under which differentiated cell polarity and intercellular junctions appear to remain virtually unaltered. These techniques can be applied to the study of more complex epithelial tissues, both in culture and in whole tissue, and are particularly well suited to the study of epithelial differentiation in embryogenesis.

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REFERENCES

1. Brown, S., W. Levinson, and J. Spudich. 1976. Cytoskeletal elements of chick embryo fibroblasts revealed by detergent extraction. J. Supramol. Struct. 5:119-130.
2. Webster, R. S., D. Henderson, M. Osborn, and K. Weber. 1978. Three-dimensional electron microscopical visualization of the cytoskeleton of animal cells: immunofluorescent identification of actin- and tubulin-containing structures. Proc. Natl. Acad. Sci. USA. 75:5511-5515.
3. Fulton, A. B., R. Wan, and S. Penman. 1980. The spatial distribution of polyamines in 3T3 cells and the associated assembly of proteins into the skeletal framework. Cell. 20:849-857.
4. Fulton, A. B., R. Prives, S. R. Farmer, and S. Penman. 1981. Developmental reorganization of the skeletal framework and its surface lamina in fusing muscle cells. J. Cell Biol. 91:103-112.
5. Schiwi, M., and J. Van Blerkom. 1981. Structural interactions of cytokeratin components. J. Cell Biol. 90:223-235.
6. Schiwi, M. 1982. Action of cytochalasin D on cytoskeletal network. J. Cell Biol. 92:79-91.
7. Capco, D. G., K. Wan, and S. Penman. 1982. The nuclear matrix: three-dimensional architecture and protein composition. Cell. 29:847-858.
8. Capco, D. G., and S. Penman. 1983. Mitotic architecture of the cell: the filament networks of the nucleus and cytoplasm. J. Cell Biol. 96:896-903.
9. Fey, E. G., and S. Penman. 1984. The epithelial cytoskeletal framework and nuclear matrix-intermediate filament scaffold: three-dimensional organization and protein composition. J. Cell Biol. 94:19-73. 1984.
10. Small, J. V., and J. E. Celis. 1978. Direct visualization of the 10-nm (100A) filament network in whole and enucleated cultured cells. J. Cell Sci. 31:393-409.
11. Ben-Ze'ev, A., F. Solomon, and S. Penman. 1979. The outer boundary of the cytoskeleton: a lamina derived from plasma membrane proteins. Cell. 17:859-865.
12. Beretsey, R., and D. S. Coffey. 1974. Identification of a nuclear protein matrix. Biochem. Biophys. Res. Commun. 60:1410-1417.
13. Aaronson, S. A., and M. P. Gottesman. 1975. Isolation of nuclear pore complexes in association with lamina. Proc. Natl. Acad. Sci. USA. 72:1017-1011.
14. Capco, D. G., and S. Penman. 1978. Heterogeneous nuclear RNA-protein fibers in chromatin-depleted nuclei. J. Cell Biol. 76:663-674.
15. Kaufmann, S. H., D. S. Coffey, and H. Shapiro. 1981. Interactions in the isolation of rat liver nuclear matrix, nuclear envelope, and pore complex lamina. Exp. Cell Res. 132:102-123.
16. Brockman, T., and S. Penman. 1983. "Prompts" heat-shock proteins translationally regulated synthesis of new proteins associated with the nuclear matrix-intermediate filaments as an early response to heat shock. Proc. Natl. Acad. Sci. USA. 80:4737-4741.
17. Fanarh, M. G., and G. E. Palade. 1963. Junctional complexes in various epithelia. J. Cell Biol. 17:375-412.
18. Weinstein, R. S., B. M. Kerker, and J. Alroy. 1976. The structure and function of the apical and lateral intercellular junctions in cancer. Adv. Cancer Res. 23:24-89.
19. Steinberg, M. S. 1978. Cell-cell recognition in multicellular assemblies: levels of specificity. Soc. Exp. Biol. Symp. 32:25-49.
20. Fey, E. G., R. Wan, and S. Penman. 1980. Triton-X-100 extraction of cultured kidney epithelial cells. Exp. Cell Res. 128:233-235.
21. Roussell, D. 1980. Hepatocellular carcinoma associated with hepatitis B virus. J. Cell Biol. 91:103-112.
22. Richardson, J. C. W., and N. L. Simmons. 1979. Demonstration of protein asymmetries in the plasma membrane of cultured renal (MDCK) epithelial cells by lactoperoxidase-mediated iodination. J. Cell Biol. 122:384-391.
23. Capco, D. G., G. Krochmalnic, and S. Penman. 1984. A new method of preparing enucleated-fiber sections for TEM: applications to the skeletal framework and other three-dimensional networks. J. Cell Biol. 98:1878-1885.
24. Lehto, V. P., I. Virtanen, and P. Kurih. 1978. Intermediate filaments anchor the nuclei in nuclear monolayers of cultured human fibroblasts. Nature (London) 272:175-177.
25. Woodcock, C. L. F. 1980. Nuclear-associated intermediate filaments from chicken erythrocytes. J. Cell Biol. 85:881-889.
26. Summertype, P. C. Freudentstein, J. C. Watson, L. Laurenzi, T. W. Krenan, J. Studler, R. Leupol, and W. F. Franke. 1979. Structure and biochemical composition of desmosomes and tonofilaments isolated from calf muzzle epidermis. J. Cell Biol. 79:427-434.
27. Gorbetsky, G., and M. S. Steinberg. 1981. Isolation of the intercellular glycoproteins of desmosomes. J. Cell Biol. 92:243-248.
28. Franke, W. W., Winter, C. A., and C. Schmid. 1981. Isolation and characterization of desmosome-associated tonofilaments from rat intestinal brush border. J. Cell Biol. 90:116-127.
29. Sun, T.-T., C. Shih, and H. Green. 1979. Keratin cytoskeletons in epithelial cells of internal organs. Proc. Natl. Acad. Sci. USA. 76:2813-2817.
30. Franke, W. W., K. Weber, M. Osborn, E. Schmid, and C. Freudentstein. 1978. Antibody to keratin: decoration of tonofilament-like arrays in various cells of epithelial character. Exp. Cell Res. 116:429-445.
31. Franke, W. W., E. Schmid, K. Weber, and M. Osborn. 1979. Hela cells contain intermediate-sized filaments of the keratin type. Exp. Cell Res. 118:505-509.
32. Summertime, I. C., Y.-S. Cheng, T.-T. Sun, and L. B. Chen. 1981. Expression of keratin and vimentin intermediate filaments in rabbit biliary epithelial cells at different stages of biliary/growth-induced neoplastic progression. J. Cell Biol. 90:63-69.
33. Sun, T.-T., and H. Green. 1978. Immunofluorescent staining of keratin fibers in cultured cells. Cell. 14:469-476.
34. Virtanen, I., V.-P. Lehto, E. Lehtonen, T. Varti, S. Steeman, P. Kurki, O. Wagner, V. J. Small, D. Dahl, and R. A. Bradley. 1981. Expression of intermediate filaments in cultured cells. J. Cell Sci. 50:65-63.
35. O'Farrell, P. H. 1975. High-resolution two-dimensional electrophoresis of proteins. J. Biochem. 250:4007-4021.