

Type I Collagen Gel Induces Madin–Darby Canine Kidney Cells to Become Fusiform in Shape and Lose Apical–Basal Polarity

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Abstract. In the embryo, epithelia give rise to mesenchyme at specific times and places. Recently, it has been reported (Greenburg, G., and E. D. Hay. 1986. *Dev. Biol.* 115:363–379; Greenberg, G., and E. D. Hay. 1988. *Development (Camb.)*. 102:605–622) that definitive epithelia can give rise to fibroblast-like cells when suspended within type I collagen gels. We wanted to know whether Madin–Darby canine kidney (MDCK) cells, an epithelial line, can form mesenchyme under similar conditions. Small explants of MDCK cells on basement membrane were suspended within or placed on top of extracellular matrix gels. MDCK cells on basement membrane gel are tall, columnar in shape, and ultrastructurally resemble epithelia transporting fluid and ions. MDCK explants cultured on type I collagen gel give rise to isolated fusiform-shaped cells that migrate over the gel surface. The fusiform cells extend pseudopodia and filopodia, lose cell membrane specializations, and develop an ac-

tin cortex around the entire cell. Unlike true mesenchymal cells, which express vimentin and type I collagen, fusiform cells produce both keratin and vimentin, continue to express laminin, and do not turn on type I collagen. Fusiform cells are not apically–basally polarized, but show mesenchymal cell polarity. Influenza hemagglutinin and virus budding localize to the front end or entire cell surface. Na,K-ATPase occurs intracellularly and also symmetrically distributes on the cell surface. Fodrin becomes diffusely distributed along the plasma membrane, ZO-1 cannot be detected, and desmoplakins distribute randomly in the cytoplasm. The loss of epithelial polarity and acquisition of mesenchymal cell polarity and shape by fusiform MDCK cells on type I collagen gel was previously unsuspected. The phenomenon may offer new opportunities for studying cytoplasmic and nuclear mechanisms regulating cell shape and polarity.

EPITHELIA form sheets of contiguous cells that reside in vivo on extracellular matrix (ECM).¹ The cells in a simple epithelium are usually cuboidal in shape, but may also be columnar or squamous. The tissue phenotype is defined by a high degree of apical–basal polarity. The apical cytoplasm contains the Golgi zone and the basal cytoplasm is usually rich in RER. Microvilli and a sialic acid-rich glycoprotein coat cover the apical cell surface, while the lateral surface contains intercellular junctions (zonula occludens, zonula adherens, desmosomes, and gap junctions). Membrane proteins may be polarized to the basolateral domain (e.g., Na,K-ATPase, Louvard, 1980; ECM receptors, Hay, 1989) or to the apical domain (e.g., leucine aminopeptidase, Louvard, 1980). In the commonly studied Madin–Darby canine kidney (MDCK) epithelial cell line, it has been shown that certain viruses, such as influenza, bud from the apical cell surface and others, such as vesic-

ular stomatitis, from the basolateral surface (Rodriguez-Boulan and Sabatini, 1978).

Mesenchymal cells are elongated or stellate in shape, and they give rise to fibroblasts, chondrocytes, and the like. A mesenchymal cell migrating through ECM is bipolar in shape (Tomasek and Hay, 1984), makes minimal intercellular contacts, and interacts with ECM on all sides. Apical–basal polarity of cell surface and cytoplasm is not present (Rodriguez-Boulan and Sabatini, 1978; Hay, 1984) and new membrane proteins are inserted into the polarized leading edge of the cell (Bergmann et al., 1983; Kupfer et al., 1987). Mesenchymal cells typically make collagen type I and other structural glycoproteins that make up the interstitial matrix, whereas epithelia usually synthesize basement membrane (basal lamina). Mesenchymal cells are characterized by vimentin intermediate filaments (Schmid et al., 1979). Epithelia produce intermediate filaments of the cytokeratin type with a few exceptions, such as the cultured MDCK cell line, that contains both vimentin and keratin (Virtanen et al., 1981).

A number of definitive epithelia have been shown, when

1. *Abbreviations used in this paper:* ECM, extracellular matrix; MDCK, Madin–Darby canine kidney.

suspended in type I collagen gels, to give rise to cells in vitro that express the mesenchymal phenotype (Greenburg and Hay, 1982, 1986, 1988). Individual elongated cells dissociate from either the basal (e.g., thyroid follicle) or apical surface (e.g., lens) of the tissue explant. They lose contact with the epithelium of origin and migrate individually through the surrounding collagen matrix. These migrating cells acquire the genetic program of mesenchymal cells, in the sense that they assume a bipolar shape, develop filopodia and pseudopodia, and exhibit the synthetic and ultrastructural features of mesenchymal cells in the embryo. Elongated RER becomes abundant, and type I collagen is synthesized instead of type IV (Greenburg and Hay, 1986). Epithelia which normally express cytokeratin (e.g., thyroid follicle) give rise to mesenchyme-like cells which produce vimentin and lose keratin (Greenburg and Hay, 1988).

To determine whether an established epithelial cell line is also capable of undergoing epithelial-mesenchymal transformation under these conditions, we cultured MDCK cells in type I collagen gels. MDCK cells are commonly grown on plastic, permeable substrata such as filters, or more recently on dried collagen (Simons and Fuller, 1985; Matlin, K., unpublished data). We cultured them to confluency on basement membrane gels to obtain explants that could be placed within the type I collagen gels. On basement membrane, we found that MDCK cells are much taller than their counterparts grown on plastic or dried collagen; they show the ultrastructural characteristics of cells actively engaged in transepithelial transport. When explants of MDCK cells cut from these basement membrane cultures are grown within collagen gels, they give rise to epithelial cysts and tubules, as has been reported for single MDCK cells grown in collagen gels (McAteer et al., 1987).

On the contrary, we found that when such MDCK explants on basement membrane are placed on top of hydrated type I collagen gels, cells at the periphery of the explant break cell-cell contact and migrate out onto the gel as elongated, bipolar-shaped cells. These cells, which we will refer to as fusiform cells, extend pseudopodia and filopodia and express both vimentin and cytokeratin. Fusiform cells do not exhibit the apical-basal polarity of the cell surface seen in the epithelium of origin. Na,K-ATPase uniformly distributes, instead of basolaterally, along the entire cell surface and the influenza virus buds symmetrically from fusiform cells. Other membrane-associated proteins also redistribute. Despite these dramatic changes, fusiform cells are not true mesenchymal cells. They do not lose keratin, develop mesenchymal RER, or acquire the ability to produce type I collagen. A likely interpretation seems to be that only part of the master gene program (Hay, 1989) for epithelial-mesenchymal transformation can be triggered in this epithelial cell line. The ability of the highly polarized MDCK epithelium to produce elongated, bipolar cells in response to hydrated type I collagen was previously unsuspected and offers new opportunities for investigating the supramolecular control of cell polarity and shape.

Materials and Methods

Stock Cell Culture

MDCK cells, strain II (low transmonolayer resistance), were originally cloned by Louvard (1980; Matlin and Simons, 1983) for high growth rate and dome formation.

For routine culture, MDCK II (passages 7-37) were grown in 75-cm² flasks (Falcon Labware, Oxnard, CA) in MEM supplemented with Earle's salts (Gibco Laboratories, Grand Island, NY), 5% FCS (Gibco Laboratories), 10 mM Hepes (Sigma Chemical Co., St. Louis, MO), pH 7.3, at 37°C in 95% air, 5% CO₂, and 98% humidity. MDCK cells were harvested with trypsin-EDTA (Gibco Laboratories) and subcultured at a 1:5 dilution twice weekly. In some experiments, a strain of MDCK II cells called MDCK II 9.1.1.3 was used. These were derived by subcloning the parental MDCK cell line four times, and selecting each time for high growth rate and dome formation.

Explant Culture

For experimentation, MDCK cells were seeded onto tissue culture dishes (Falcon Labware) or Millipore Corp. filters (type HA; 0.45 μm pore size; Bedford, MA) coated with basement membrane gel and fed with Ham's F12 medium (Gibco Laboratories) containing 10% FCS, 10 mM glutamine (Gibco Laboratories), 2.5 μg/ml fungizone (Gibco Laboratories), 50 μg/ml gentamycin (Sigma Chemical Co.), and 50 μg/ml ascorbic acid. 1 d after confluency, 1-mm² pieces of Millipore Corp. filter containing the monolayer attached to basement membrane gel (explants) were cut and suspended perpendicularly within solutions of hydrated type I collagen or basement membrane gels, and the gels allowed to polymerize.

MDCK explants were also prepared by cutting 1-mm² pieces of confluent cells on basement membrane from tissue culture plastic without the intervening filter. These explants were placed flat with apical end up on the top surface of polymerized basement membrane or hydrated type I collagen gels and incubated for 1-2 h in 500 μl of culture medium so as to facilitate attachment of the explant to the gel. The remainder of the growth medium was then added and the MDCK cell culture incubated from 3-22 d. Cultures were observed with an E. Leitz, Inc. (Rockleigh, NJ) Diavert microscope and photographed with Kodak Tri-X film, ASA 800.

Type I Collagen and Basement Membrane Gel Preparation

Type I collagen was extracted from adult rat tail tendon with 0.5 M acetic acid, according to a modification of the method of Ealsdale and Bard (1972). The collagen (2.5 mg/ml) was first extensively dialyzed against 1/10 Ham's F-12 medium containing 0.1 N HCl and then 0.7 ml was precipitated at 0°C with 0.1 ml 10× Ham's F12 (101 mg/ml), 0.1 ml 10× sodium bicarbonate (11.76 mg/ml), and 0.1 ml FCS. Droplets of collagen (0.3-1.0 ml) were then pipetted onto tissue-culture dishes and the MDCK explant quickly suspended within the gel or the collagen gel was allowed to polymerize at room temperature for 20 min before placing the MDCK explant on its upper surface.

Extracts of basement membrane were kindly provided by Dr. Hynda Kleinman (National Institute of Dental Research [NIDR], Bethesda, MD). This material was extracted from the Engelbreth-Holm swarm tumor and stored at -20°C. Extracts were thawed in ice water and tissue-culture dishes

Table 1. Summary of Conditions for Immunofluorescence Microscopy

| Antigen | Fixative | Permeabilize | Primary antibody dilution |
|---------------------|----------------------------|--------------|---------------------------|
| Vimentin | 100% methanol | + | 1:10 |
| Cytokeratin* | 1.75% paraformaldehyde† | - | 1:30 |
| Laminin | 1% acetic acid-99% ethanol | - | 1:50 |
| Type I procollagen | 1% acetic acid-99% ethanol | - | 1:20 |
| Hemagglutinin | 4% paraformaldehyde† | ± | undiluted |
| NaKATPase | 1.75% paraformaldehyde† | ± | 1:150 |
| Fodrin | 1.75% paraformaldehyde† | ± | 1:300 |
| Desmoplakin I & II* | 1.75% paraformaldehyde† | - | 1:100 |
| ZO-1 | 100% methanol | + | undiluted |

* Cells were extracted before fixation with 300 mM sucrose, 50 mM NaCl, 10 mM Pipes, pH 6.8, 3 mM MgCl₂, 1.2 mM PMSF, 1 mM EGTA, 0.1 mM DTT, 0.5% (vol/vol) Triton X-100 for 20 min at room temperature (Pasdar and Nelson, 1988b).

† Paraformaldehyde solutions were made in PBS, pH 7.4, containing 0.1 mM CaCl₂, 0.1 mM MgCl₂. Aldehyde groups were quenched (10 min) with 50 mM NH₄Cl in PBS, pH 7.4.

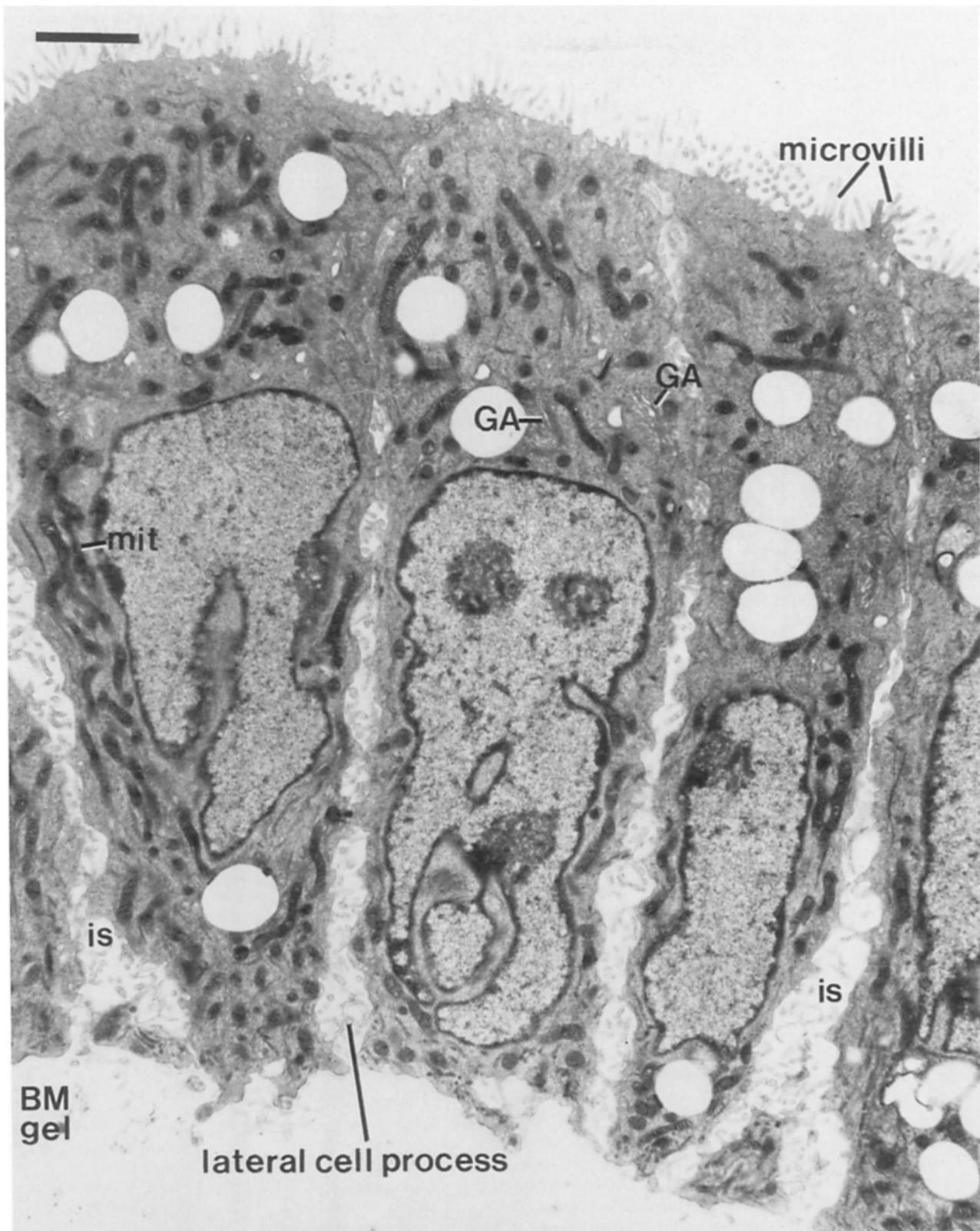


Figure 1. Electron micrograph of MDCK epithelial cells growing on the surface of basement membrane (BM) gel for 11 d. The cells are tall, columnar in shape, and contain numerous apical microvilli. Elongated mitochondria (*mit*) are frequent and tend to lie perpendicular to the substratum. The Golgi zone (*GA*) is in the apical cytoplasm. The lateral intercellular space (*is*) is quite wide and contains numerous cytoplasmic processes. Nuclei are large and highly folded. Lipid droplets appear empty in this preparation. Bar, 2.0 μm .

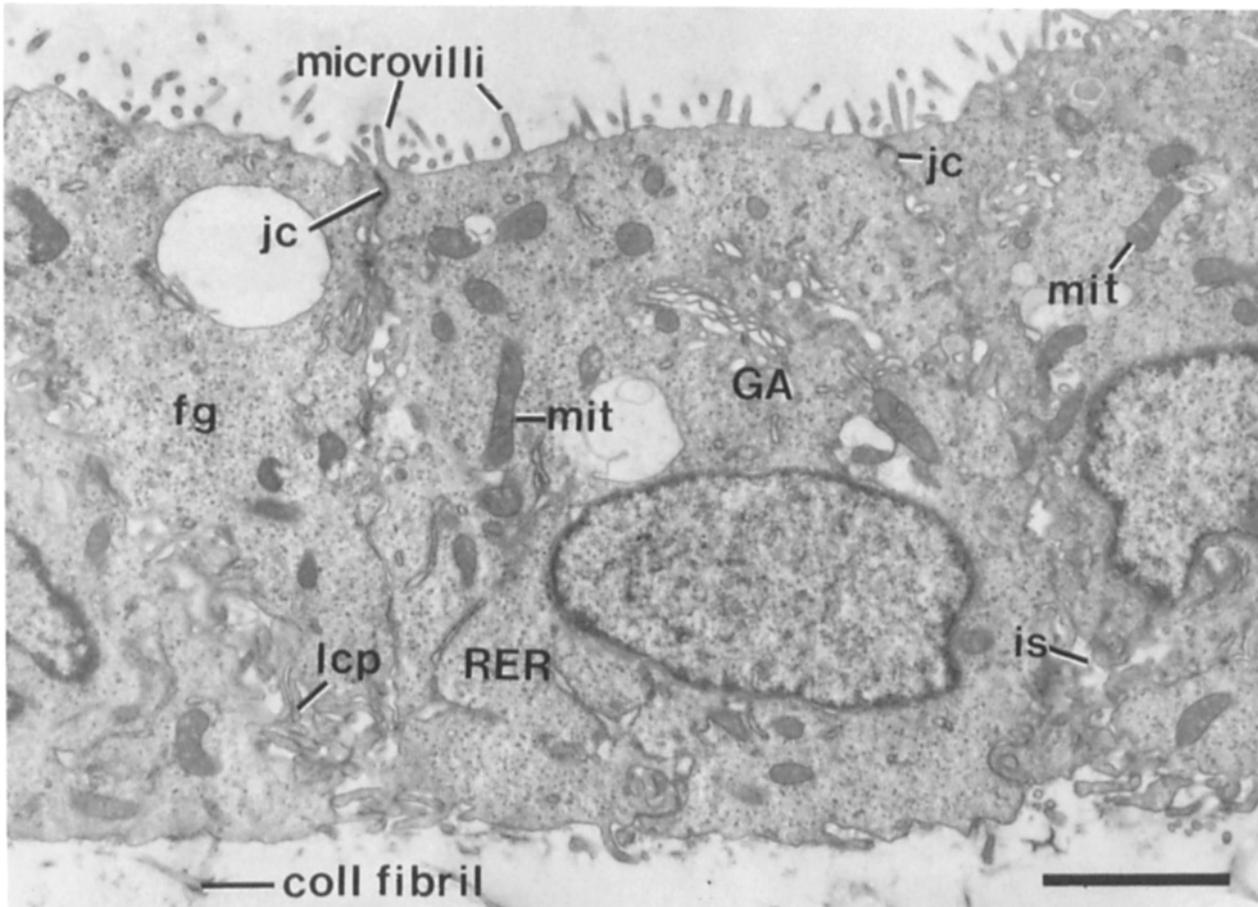


Figure 2. Electron micrograph of MDCK epithelial cell growing on the surface of hydrated type I collagen gel for 9 d. The cells are cuboidal in shape and contain only a few mitochondria (*mit*) oriented randomly in the cytoplasm. Fewer microvilli are present than in cells grown on basement membrane. RER profiles are thin and sparse and the cytoplasm is fibrillogranular (*fg*). The intercellular space (*is*) is small and may contain lateral cell processes (*lcp*). *Coll*, collagen; *GA*, Golgi zone; *jc*, junctional complex. Bar, 3.0 μ m.

were then coated with 300 μ l extract or small droplets of basement membrane gel were formed with 150 μ l extract. The gel was polymerized by incubation at 37°C for 20 min and MDCK cells were either seeded onto the tissue-culture dishes or MDCK explants were placed on top of the basement membrane drop. In other cases, explants were quickly suspended within the basement membrane gel before its polymerization.

Electron Microscopy

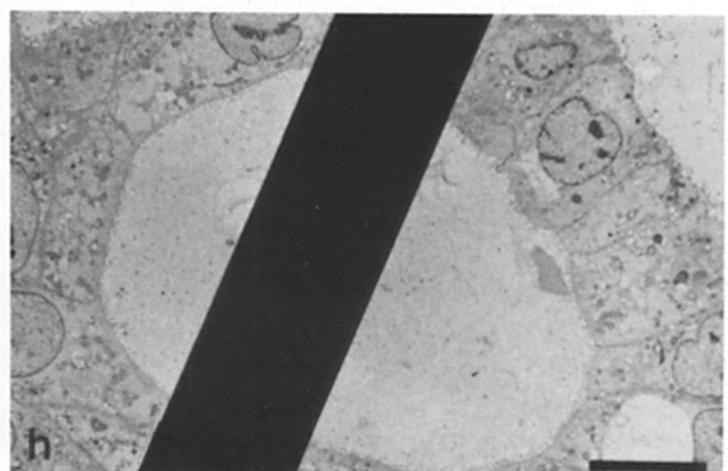
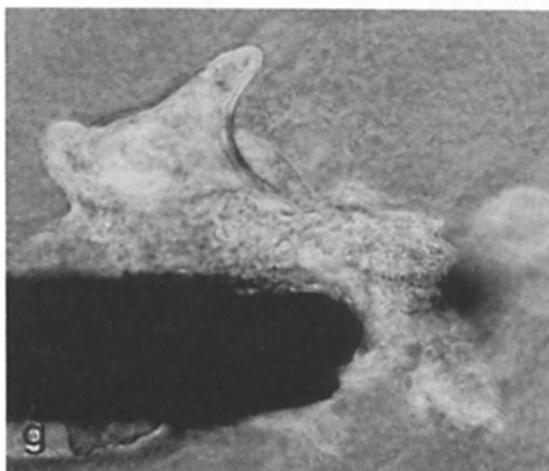
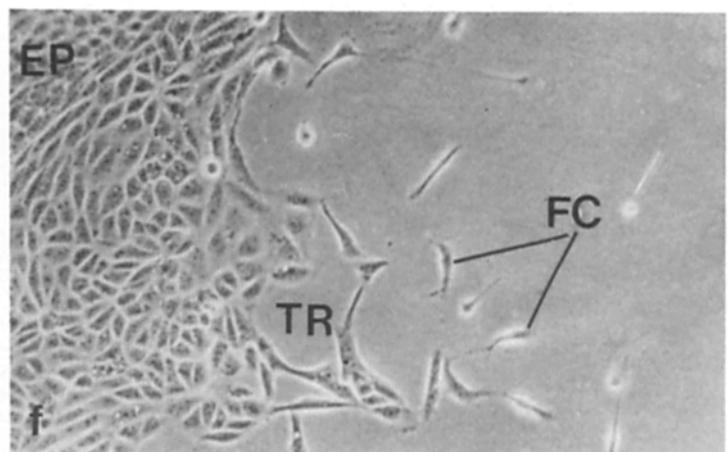
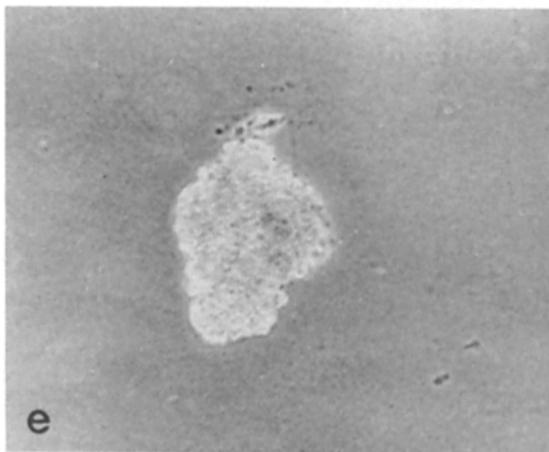
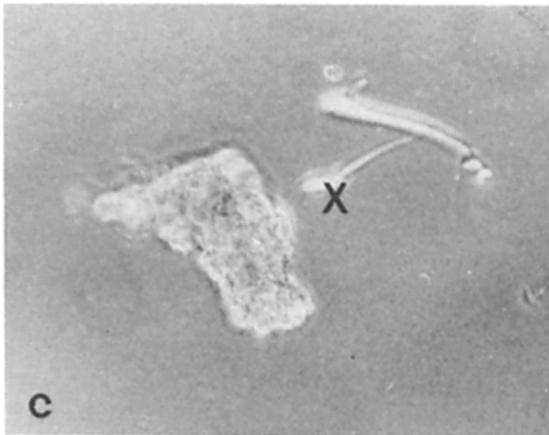
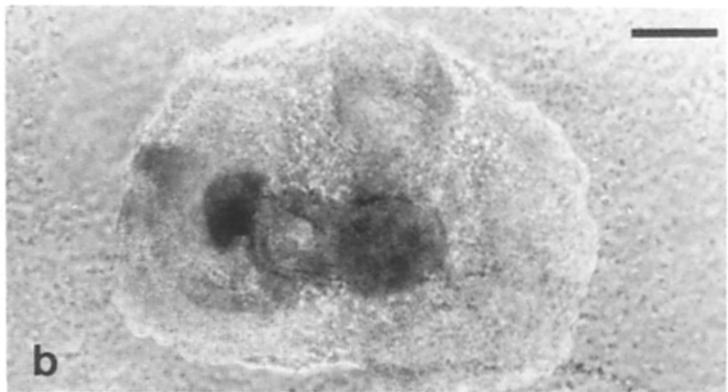
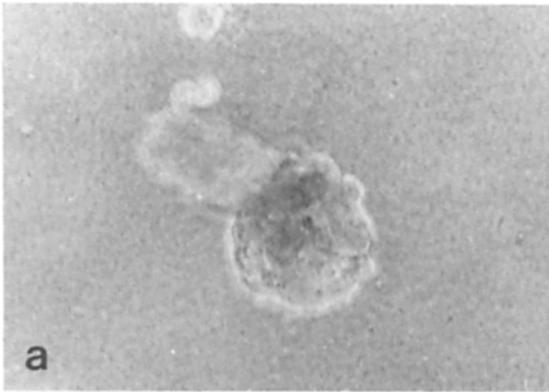
MDCK cultures were rinsed in 100 mM cacodylate buffer, pH 7.4, and fixed for 1 h at room temperature in 2.5% glutaraldehyde and 2% paraformaldehyde in 100 mM cacodylate buffer. After rinsing, specimens were osmicated

(1% OsO₄ in 100 mM cacodylate buffer) for 60 min at 0°C, rinsed, and stained en bloc with 1% uranyl acetate (1 h at room temperature). Specimens were then rinsed, trimmed, and dehydrated in a graded series of alcohol (15 min each). Specimens were embedded in Epon-Araldite and thin sections were cut on a Sorvall MT2-B ultramicrotome (DuPont Instruments-Sorvall BioMedical Division, Newton, CT). Sections were then stained with 0.2% lead citrate and observed on a JEOL model 100S, 100CX, or 1200CX electron microscope (JEOL USA, Cranford, NJ).

Virus Infection

Stocks of influenza virus PR8 (A/PR/8/34;H1) serotype were grown in 10-d-

Figure 3. Phase-contrast light micrographs of MDCK explants placed on top of basement membrane (*a* and *b*), plastic (*c* and *d*), or hydrated type I collagen gels (*e* and *f*), or suspended within hydrated gels of type I collagen (*g* and *h*). The explant consists of a 1-mm² piece of MDCK epithelium on basement membrane. (*a-d*) On top of basement membrane and plastic, MDCK cells from the explant spread over the gel or plastic surface as a closely apposed epithelial sheet. On plastic, the cells are flatter and more well spread than on basement membrane. *a* and *b* are the same culture at 0 and 72 h. *c* and *d* are the same culture at 0 and 72 h (the X labels a scratch in the plastic for orientation). (*e* and *f*) An MDCK explant placed on top of type I collagen gel gives rise to single, fusiform-shaped cells (*FC*) that form the leading edge of migration. Fusiform cells persist as long as a collagen gel substratum is available. Cuboidal epithelial cells (*EP*) are located near the explant of origin. Elongated cells that contact the cuboidal epithelial cells are located in a transition zone (*TR*) behind the leading edge. *e* and *f* are the same culture at 0 and 72 h. (*g* and *h*) Within type I collagen gel, the MDCK explant retains cell-cell contact and forms tubule and cystlike structures. In the culture shown here (*g*), the dark structure is the Millipore filter carrying the explant into the gel perpendicular to the surface. An electron micrograph of a cross section through a tubule (*h*) reveals the apical cell surface facing the tubule lumen and the basal surface contacting the hydrated fibrils of the collagen gel. The black structure is a grid bar. *g*, 19 d; *h*, 12 d. Bars: (*a-g*) 100 μ m; (*h*) 10 μ m.



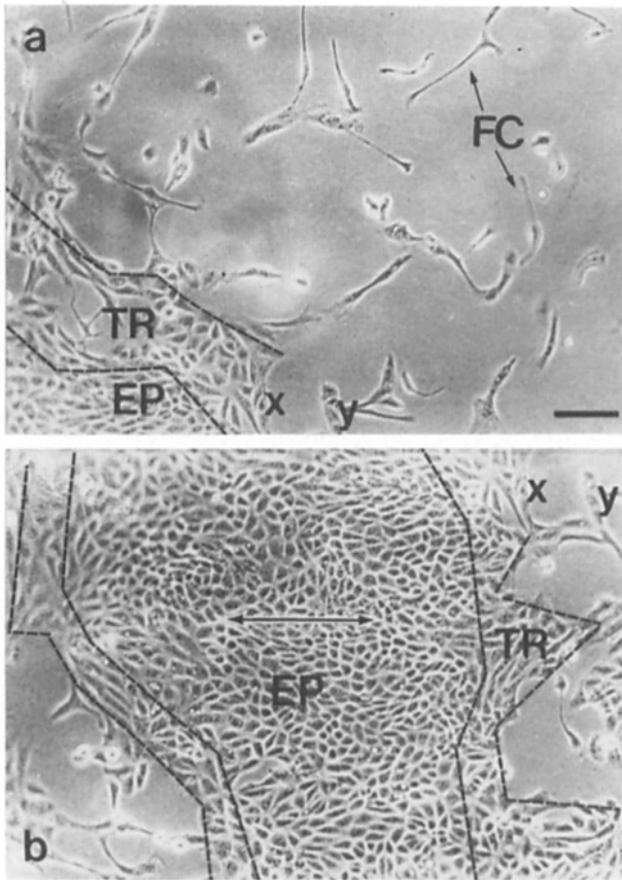


Figure 4. Phase-contrast micrographs of an MDCK explant cultured for 72 h on top of type I collagen gel. *x* and *y* represent the same cells in both panels *a* and *b*. The dotted lines (*a* and *b*) demarcate the boundaries between the epithelial (*EP*) zone, transition (*TR*) zone, and zone containing fusiform cells (*FC*). The double-headed arrow (*b*) represents the location of the original MDCK explant. Photographic reconstruction of two MDCK (72-h) cultures followed by area analysis of each zone using a Zeiss MOP III digitizer indicates the epithelial and transition zones occupy $4.6 \pm 0.1 \times 10^5 \mu\text{m}^2$ and $3.1 \pm 1.3 \times 10^5 \mu\text{m}^2$, respectively. Fusiform cells form the entire periphery of the culture and occupy $35.4 \pm 0.2 \times 10^5 \mu\text{m}^2$. Fusiform cells always form in this manner on top of type I collagen gel and they migrate away from the transition zone for a distance $\leq 740 \mu\text{m}$. *a* represents approximately 1/7 of the fusiform cell outgrowth for this culture. Bar, 100 μm .

old embryonated chicken eggs, concentrated, and plaque purified three times on MDCK cells, as previously described (Matlin et al., 1981; Matlin and Simons, 1983). Titers were typically 10^{10} - 10^{11} plaque-forming units/ml.

For virus infection, MDCK cultures were rinsed three times, 15 min each with infection medium, consisting of MEM containing 0.2% bovine serum albumin (Sigma Chemical Co.), 1% penicillin-streptomycin (Gibco Laboratories), and 10 mM HEPES, pH 7.3. A cloning ring, 10 mm in diameter, was placed on top of the collagen gel and influenza virus (1.7×10^8 plaque-

forming units/ml) was then added. Cultures were kept at 37°C for 1 h and then rinsed three times for 15 min each with the infection medium. The medium was then replaced with Ham's F12 containing the previously mentioned supplements and incubated for 3-10 h. Cultures were then processed for electron microscopy or indirect immunofluorescence of hemagglutinin.

Indirect Immunofluorescence

The precise fixation and antibody incubations used varied with the particular antigen in question and are summarized in Table I. After rinsing three times in PBS for 5 min each, MDCK cultures were fixed for 5-15 min at room temperature. If paraformaldehyde fix was used, aldehyde groups were quenched for 10 min with 50 mM NH_4Cl in PBS, pH 7.4. For permeabilization, samples were incubated in 0.1% Triton X-100 in PBS for 4 min. In some cases, excess collagen gel was trimmed from the MDCK culture. After further rinsing in PBS, nonspecific binding sites were blocked with 10% normal goat serum for 20-45 min. The goat serum and primary and secondary antibodies were diluted in 1% gelatin-PBS and centrifuged for 5 min at 15,000 *g* before use. All incubations were in a humidified atmosphere at room temperature. After blocking, samples were transferred to the primary antibody and incubated for 30-45 min followed by extensive washing in PBS or PBS-gelatin. Samples were then incubated in the secondary antibody (goat anti-rabbit IgG-FITC, goat anti-mouse IgG-FITC [Boehringer-Mannheim Biochemicals, Indianapolis, IN] or goat anti-mouse IgG-Rhodamine [Tago Inc., Burlingame, CA]) diluted 1:150-250 for 30-45 min. Samples were rinsed in PBS, mounted in 90% glycerol in PBS, and observed with a Zeiss photomicroscope III equipped with 25 \times and 63 \times oil-immersion objectives under epiillumination. Photographs were recorded on Kodak Tri-X film (ASA 800) or T-Max film (Eastman Kodak Co., Rochester, NY). Controls and experimentals were photographed and printed at identical exposure settings, unless otherwise noted.

Antibodies

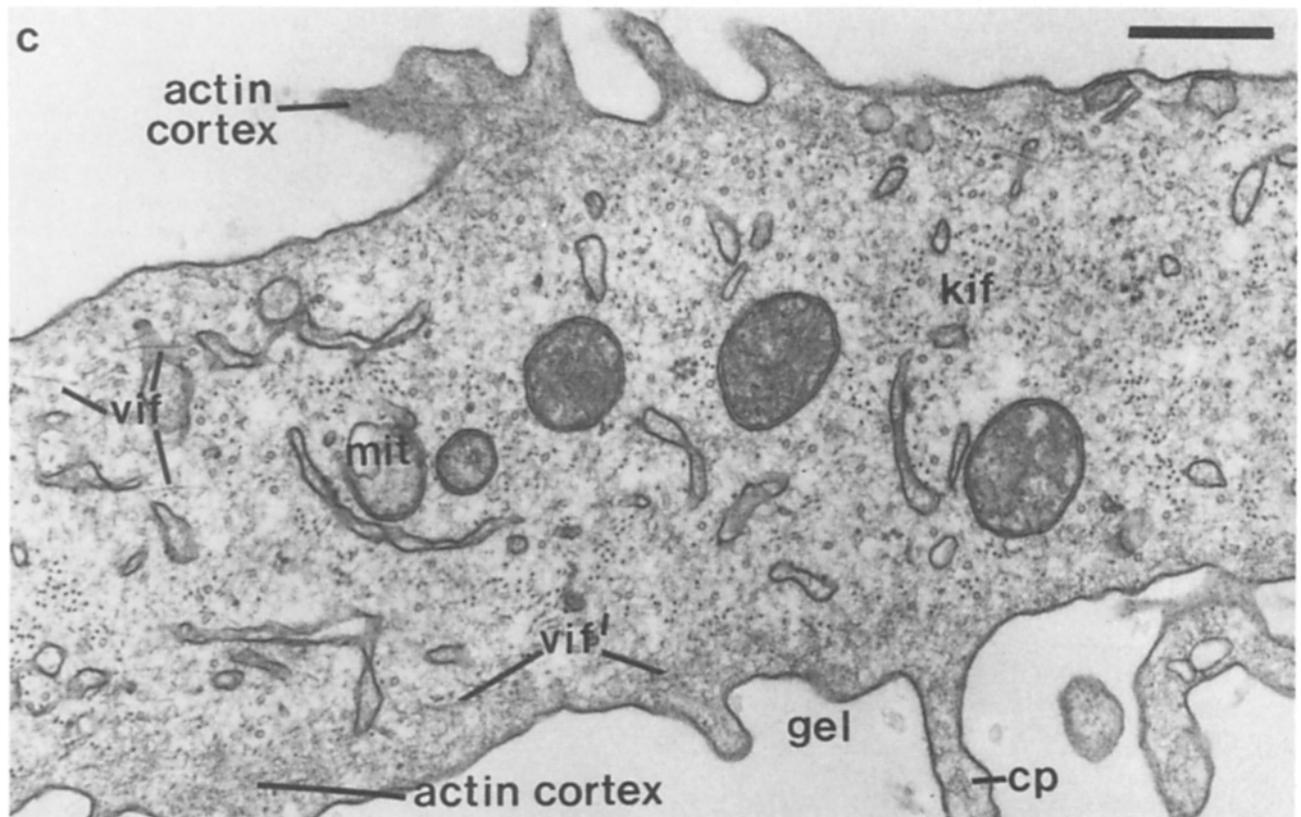
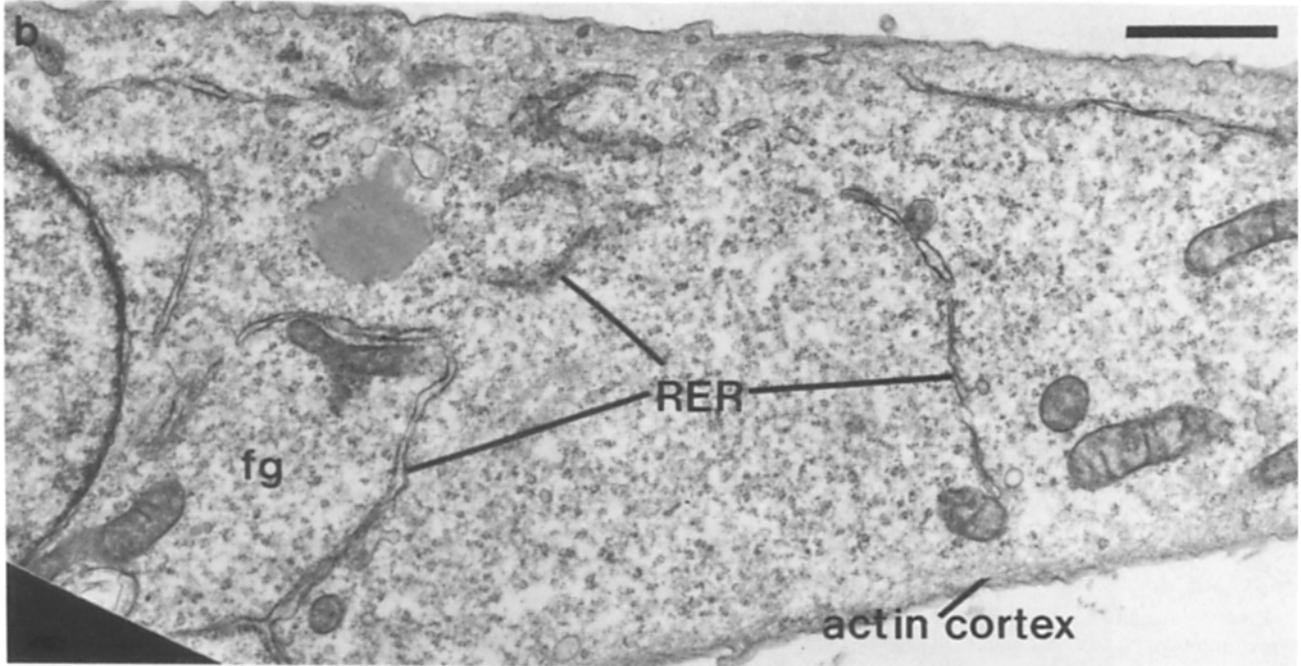
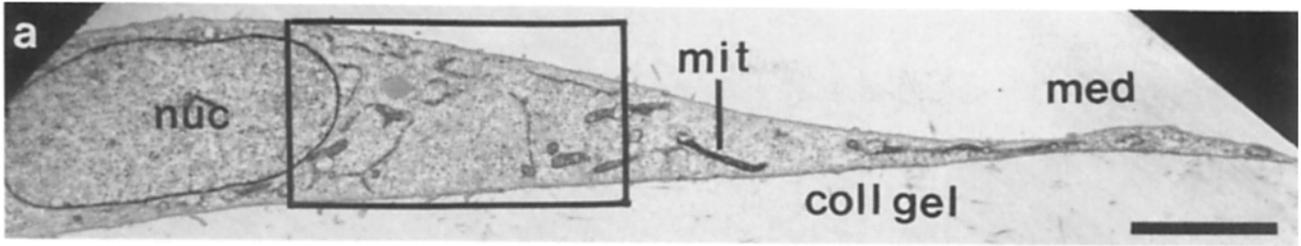
Mouse monoclonal antibodies to porcine vimentin and human cytokeratin were purchased from Boehringer-Mannheim Biochemicals and rabbit antiserum to laminin, isolated from the Engelbreth-Holm swarm tumor, was purchased from E-Y Laboratories, Inc. (San Mateo, CA). Monoclonal antibody to human procollagen type I (carboxy-terminal propeptide; McDonald et al., 1986) was kindly provided by Dr. John A. McDonald, Washington University School of Medicine (St. Louis, MO), and monoclonal culture supernatant against influenza virus hemagglutinin (H28-E23) was obtained from Drs. J. Yewdell and W. Gerhard of the Wistar Institute (Philadelphia, PA) (Matlin, 1986). The B subunit of Na,K-ATPase was stained by a rabbit polyclonal antibody (Louvard, 1980) provided by Dr. D. Louvard, the Pasteur Institute (Paris, France). Dr. A. Harris, Yale University School of Medicine (New Haven, CT), provided a rabbit polyclonal to brain spectrin (fodrin; Harris et al., 1986), and Dr. D. Goodenough, Harvard Medical School (Boston, MA), provided monoclonal culture supernatant against ZO-1 (Stevenson et al., 1986). Rabbit polyclonal antiserum raised against purified desmoplakin II from bovine epidermal desmosomes (Pasdar and Nelson, 1988a,b) was obtained from Dr. W. J. Nelson, Fox Chase Cancer Center (Philadelphia, PA). The antiserum reacts with both desmoplakin I and II in MDCK cells (Pasdar and Nelson, 1988a,b).

Results

Appearance of MDCK Cells Growing on Basement Membrane and Collagen Gels

For these experiments, single cell suspensions of MDCK cells were initially seeded onto tissue-culture dishes coated with basement membrane gel. Upon reaching confluency,

Figure 5. Electron micrographs of a fusiform MDCK cell migrating over the surface of type I collagen (*coll*) gel at 9 d. (*a*) The elongated cell contains none of the cell membrane specializations of the cuboidal epithelial MDCK cell. Microvilli are infrequent and morphologically identifiable junctional complexes are absent. (*b*) Higher power electron micrograph of the boxed area in *a*. The fibrillogranular cytoplasm (*fg*) is rich in ribosomes and contains a few, thin profiles of RER. A well-developed actin cortex lies under the plasma membrane. (*c*) Fusiform cells contain both keratin and vimentin intermediate filaments. Keratin filaments (*kif*) seem to be cut in cross section here and are present throughout the cytoplasm, as judged by immunohistochemistry (Fig. 6 *c*). Vimentin intermediate filaments (*vif*) run longitudinally in the cell as seen here and in Fig. 6 *a*. They frequently (*vif'*) contact the actin cell cortex. Cell processes (*cp*) extend from the lateral surfaces. *mit*, mitochondria; *med*, media. Bars: (*a*) 5.0 μm ; (*b* and *c*) 1.0 μm .



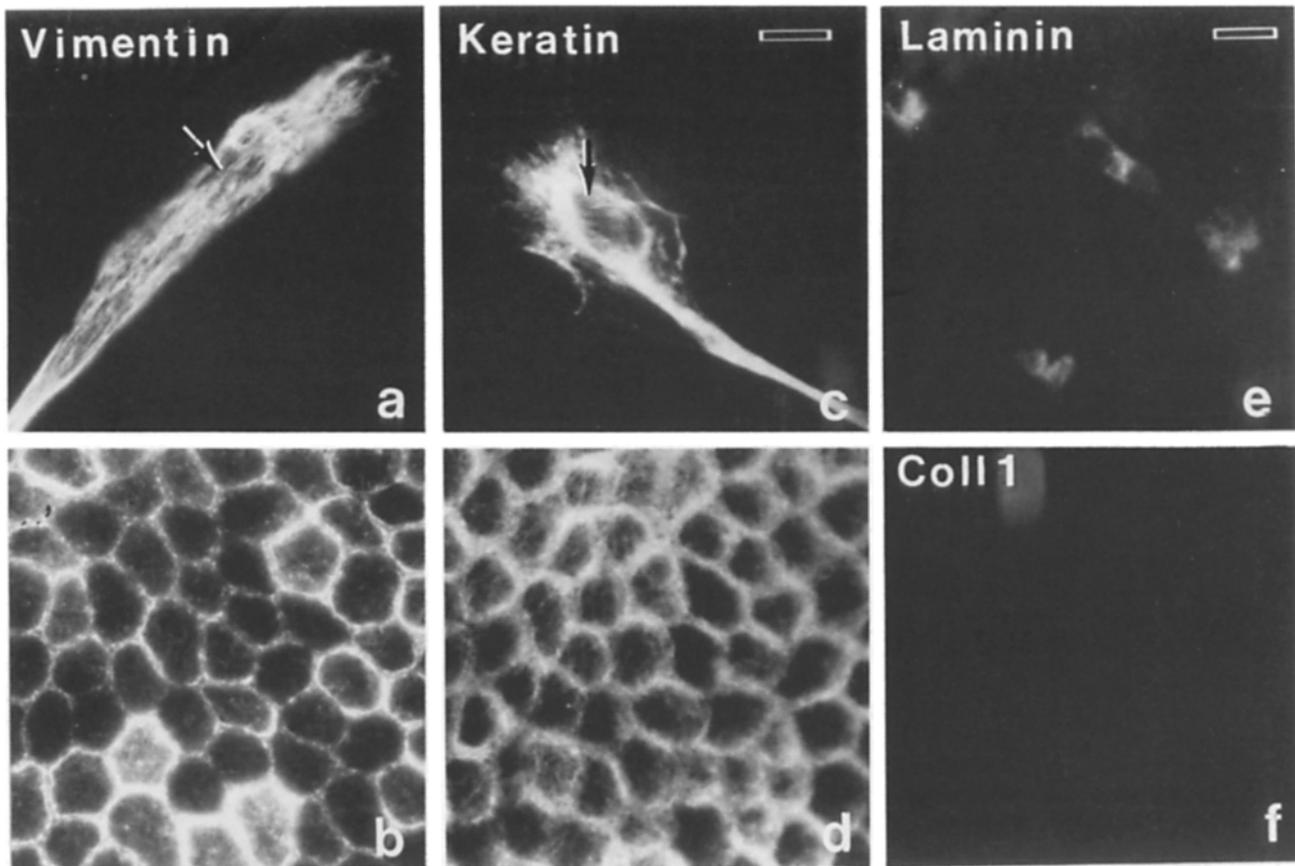


Figure 6. Immunofluorescence localization of vimentin (*a* and *b*), keratin (*c* and *d*), laminin (*e*), and type I procollagen (*f*) in MDCK cells cultured on top of type I collagen gels. Migrating fusiform cells express vimentin (*a*) as do MDCK epithelial cells (*b*). In the fusiform cell, clumps of vimentin filaments (*arrow, a*) run longitudinally in the cytoplasm. Fusiform cells express keratin (*c*), even after 17 d in culture as do MDCK epithelial cells (*d*). The filaments run across the cell (*arrow, c*) or randomly in the cytoplasm. Fusiform cells continue to express laminin, albeit somewhat weakly (*e*), but do not turn on type I procollagen (*f*). (*a* and *c*) 3 d; (*b* and *d*) 7 d; (*e*) 10 d in culture. Bars: (*a-d*) 10 μm ; (*e* and *f*) 20 μm .

MDCK cells on basement membrane gel are tall, columnar in shape, and are morphologically polarized (Fig. 1). The nucleus resides in the basal cytoplasm and the apical cytoplasm contains a well-developed Golgi zone (Fig. 1, *GA*) and other cell organelles. The numerous mitochondria are elongated in shape and tend to be oriented perpendicular to the substratum (Fig. 1, *mit*). The cytoplasm is fibrillogranular in appearance and the RER is thin and sparse, as in MDCK cells grown on plastic (Rodriguez-Boulant and Sabatini, 1978; Valentich, 1982) or hydrated collagen gels (Fig. 2, *RER*).

The apical surface of the tall cells on basement membrane gel exhibits many long microvilli. Microvilli are shorter and fewer on the apical surface of MDCK cells cultured on plastic (Rodriguez-Boulant and Sabatini, 1978; Valentich, 1982) or type I collagen gel (Fig. 2). Along the lateral surface of MDCK cells on basement membrane (Fig. 1) numerous cell processes extend into the intercellular space from the apex to the base of the cell; and the intercellular space (Fig. 1, *is*) is much more prominent than in confluent cells grown on type I collagen (Fig. 2, *is*). It appears that on basement membrane the cytoplasm narrows in an apical to basal direction and the intercellular space towards the base of the cell is wider than at the apex (Fig. 1). That is, the cells are shaped like upside down pyramids as in epithelia (Kaye et al., 1966)

actively transporting ions and water. On collagen gel, the intercellular space also contains individual lateral cell processes (Fig. 2, *lcp*), rather than the type of mitochondria-rich lateral interdigitations typical of kidney tubules. Desmosomes and apical junctional complexes are present in the MDCK epithelium on either basement membrane or collagen gel.

Explants on Basement Membrane Suspended in Collagen

1 d after confluency, explants (1 mm² in diameter) of MDCK cells on basement membrane gel were cut and were either placed on top of polymerized gels or suspended within ECM components that were then allowed to polymerize into a hydrated gel. When an explant of MDCK cells is placed on the surface of basement membrane gel (Fig. 3, *a* and *b*) or plastic (Fig. 3, *c* and *d*), a monolayer of contiguous epithelial cells moves over the gel or plastic surface. The cells that grow out of the explant have a closely apposed epithelial morphology complete with intercellular adhesions. The MDCK cells at the leading edges are flatter on plastic (Fig. 3 *d*) than on basement membrane (Fig. 3 *b*; Fig. 1). The cells on the leading edges of MDCK epithelium on hydrated colla-

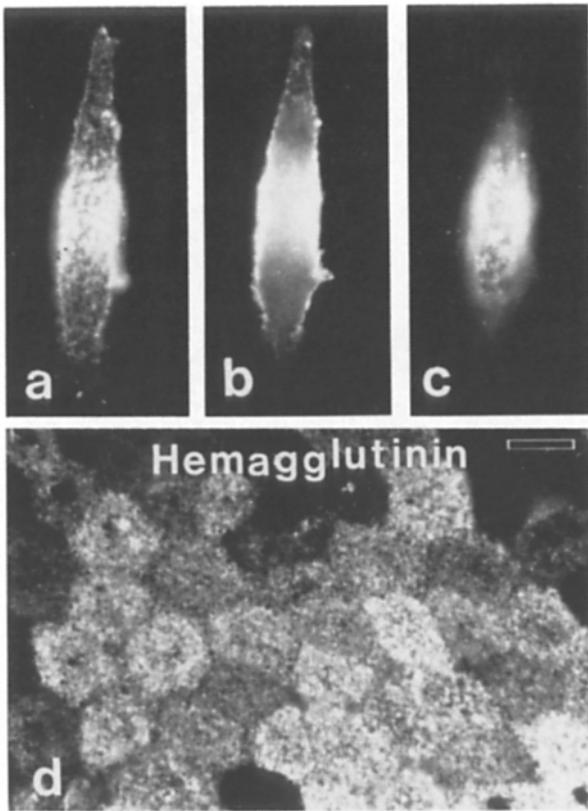


Figure 7. Immunofluorescence localization of hemagglutinin in MDCK cells cultured on top of type I collagen gel for 7 d and fixed 3 h after infection with influenza virus. Antihemagglutinin stains the top (a), sides (b), and bottom (c) cell surfaces of fusiform cells, as determined by through series focusing. In epithelial MDCK cells located behind the leading edge, only the apical cell surface fluoresces (d). Cells were not permeabilized. Exposure times, (a–c) 30 s; (d) 15 s. Bar, 10 μm .

gen gel (Fig. 3, e and f), however, are quite different in appearance.

When suspended within hydrated type I collagen or basement membrane gels, MDCK cells within the explant retain contact and with continued culture form multicellular tubules and cystlike structures (Fig. 3, g and h). The cells maintain apical contacts and the basolateral surface seems to move on the surrounding ECM to zipper the epithelium into a cyst. The final product is a simple cuboidal epithelium facing a central lumen (Fig. 3 h) into which project microvilli from the surrounding apical cell surfaces. Lateral cell surfaces are closely apposed and the basal surfaces contacting the collagen gel are flat (Fig. 3 h).

MDCK Cells Migrate onto Type I Collagen as Individual, Fusiform Cells

Thus, when a confluent explant of MDCK cells is placed on a basement membrane or within a basement membrane or collagen gel, cells migrating from the cut edges maintain cell–cell contact and epithelial morphology. However, within 24 h of culture on top of type I collagen gel, single MDCK cells on the cut edges of the explant migrate out onto the hydrated fibrils of the collagen gel as fusiform-shaped cells.

Fusiform cells make few contacts with other cells. Within 72 h, numerous fusiform cells are seen (FC, Figs. 3 f and 4 a). These cells persist on the leading edge as long as a collagen gel substratum is available for migration. They occupy an area of $35.4 \pm 0.2 \times 10^5 \mu\text{m}^2$ out of a total culture area of $43.0 \pm 0.7 \times 10^5 \mu\text{m}^2$ (Fig. 4). Behind the leading edge, MDCK cells that have migrated onto the collagen gel from the explant of origin (Fig. 4 b, double-ended arrow) form a pavement of closely apposed cells (Fig. 4 b, EP). MDCK cells on the collagen gel near the explant (Figs. 3 f and 4 b, EP) have the epithelial ultrastructure shown in Fig. 2. In the transition zone, MDCK cells behind the leading edge retain some cell–cell contact but are elongated, rather than cuboidal in shape (Figs. 3 f, 4, a and b, TR). For the extent of the culture period, cuboidal-shaped MDCK cells and the elongated, attached cells of the epithelial sheet coexist with single, isolated fusiform cells located at the leading edge of migration. These results are obtained with all passages of MDCK II, as well as with the subclone MDCK II 9.1.1.3.

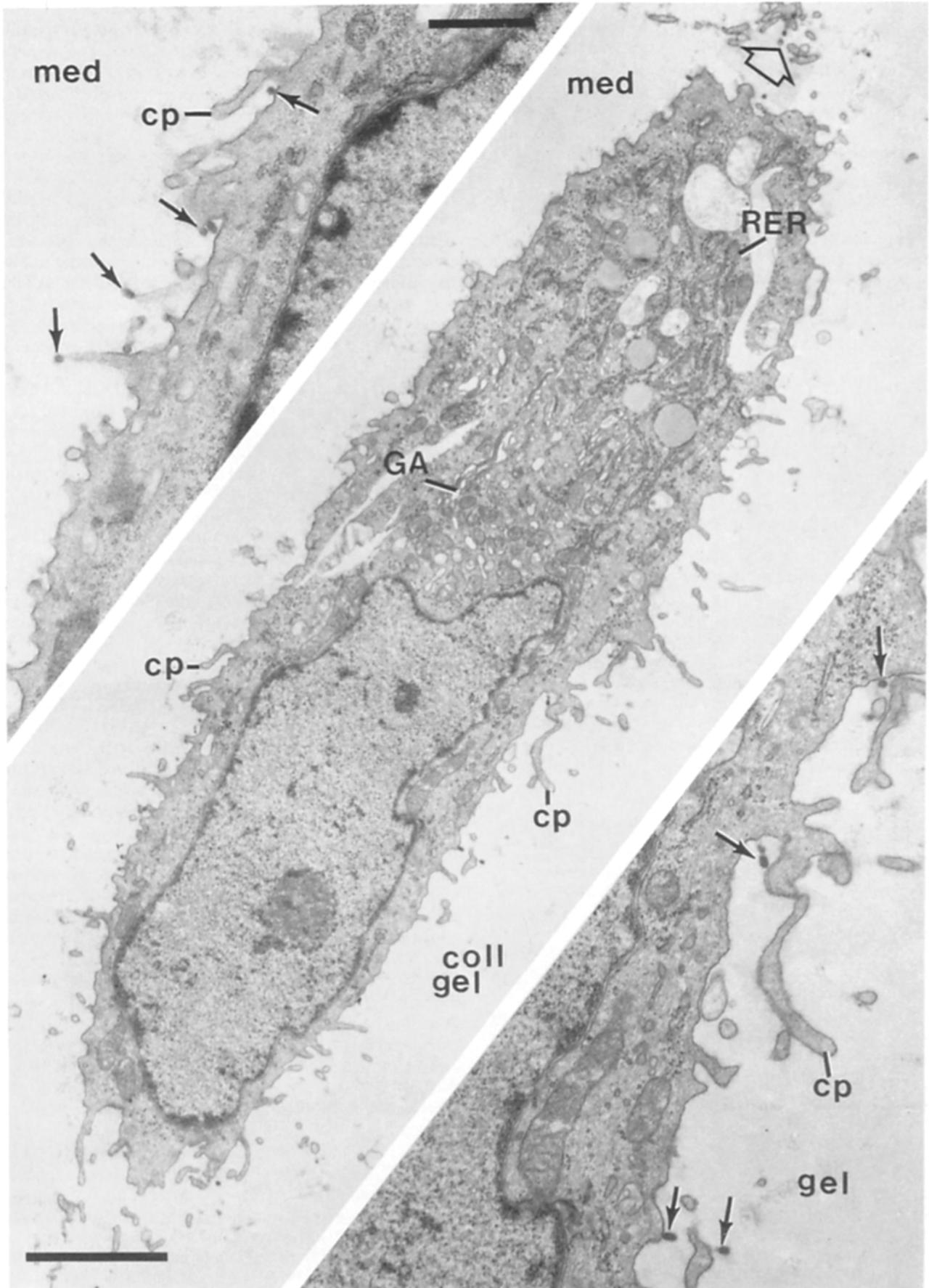
The fusiform cells are bipolar and highly elongated (Fig. 5 a) in contrast to the tall, columnar, MDCK cells within the explant (Fig. 1), and the cuboidal epithelial cells on the type I collagen gel (Fig. 2). The cell shape resembles that of a migrating mesenchymal cell. The nucleus (Fig. 5 a, nuc) tends to be near the center of the elongated cell, organizing the cytoplasm into leading and trailing ends. Pseudopodia extend from opposite poles and filopodia are best developed at the leading edge. The Golgi zone is usually in the cytoplasm of the trailing end. Other cytoplasmic organelles, such as mitochondria (Fig. 5 a, mit), lysosomes, and ribosomes are distributed among the leading and trailing compartments. A well-developed actin cortex is present under the plasmalemma all around the cell (Fig. 5, b and c), as in mesenchymal cells (Tomasek et al., 1982; Tomasek and Hay, 1984).

Like the MDCK cell of origin (see Fig. 2), however, RER profiles are thin and not abundant in fusiform cells (Fig. 5 b, RER), and the cytoplasmic ground substance appears fibrillogranular (Fig. 5 b, fg). Fusiform cells contain lateral cell processes that do not appear to be microvilli (Fig. 5 c, cp), and morphologically identifiable junctional complexes are absent. The transitional cells behind the leading edge exhibit junctional complexes where they attach to the epithelial cells, but the elongated ends of these cells have the ultrastructure of fusiform cells (Fig. 9 d).

Fusiform Cells Retain MDCK Intermediate Filaments and Do Not Express Type I Collagen

Thus, the fusiform cells develop the shape and cell-surface configuration of mesenchymal cells, but the cytoplasmic ground substance and RER resemble that of the epithelial MDCK cell. Mesenchymal cells would be expected to express only vimentin intermediate filaments and to produce type I collagen (see Introduction). Therefore, we next examined the intermediate filament profile and production of matrix by the mesenchyme-like fusiform cells.

With regard to the cytoskeleton, vimentin (Fig. 6, a and b) and keratin (Fig. 6, c and d) intermediate filaments are expressed by MDCK cells regardless of their shape. Vimentin expression appears to be somewhat reduced in confluent MDCK cells (Fig. 6 b), in agreement with Ben-Ze'ev (1984), but vimentin does not disappear in these cultured epithelial



cells. In the fusiform cells, vimentin is well developed (Fig. 6 *a*). Keratin is turned off in true mesenchymal cells (Franke et al., 1982), but this intermediate filament persists in the fusiform cells through the longest period observed (17 d, Fig. 6 *c*).

At the ultrastructural level, the intermediate filaments that appear to be vimentin (Fig. 5 *c*, *vif*) because they run parallel to the cell surface (Fig. 6 *a*, *arrow*) are frequently found in the vicinity of the actin cell cortex (Fig. 5 *c*, *vif'*), as well as running longitudinally through the cytoplasm (Fig. 5 *c*, *vif*). Keratin, on the other hand, may appear ultrastructurally as groups of filaments cut in cross section (Fig. 5 *c*, *kif*). This organization and distribution agrees with the immunolocalization data that reveals a meshwork of keratin staining the perinuclear region and ramifying throughout the cytoplasm (Fig. 6 *c*).

ECM expression also does not seem to be affected qualitatively by the dramatic cell-shape change. Laminin is expressed by cuboidal MDCK cells (data not shown; Caplan et al., 1987) and continues to be expressed by fusiform cells (Fig. 6 *e*). Type I collagen is not turned on even when the MDCK cell shape becomes mesenchyme-like (Fig. 6 *f*).

Basolateral and Apical Membrane Proteins Redistribute in Fusiform Cells

We also examined the effect of cell-shape change on the distribution of membrane proteins that are targeted to either the apical or basolateral surface in polarized MDCK cells. Cultures of MDCK explants grown for 6–7 d on collagen gel were infected with influenza virus, which buds from the apical epithelial surface (Rodriguez-Boulan and Sabatini, 1978). Virus budding was analyzed by transmission electron microscopy and hemagglutinin, the major viral membrane glycoprotein (Maltin et al., 1981), was immunolocalized.

Immunostaining with a mouse monoclonal antibody to influenza hemagglutinin reveals that 3 h after infection, hemagglutinin is distributed uniformly along the plasma membrane of fusiform cells migrating over the type I collagen gel. Antihemagglutinin stains the top, sides, and bottom of the cell as determined by a focal series (Fig. 7, *a*, *b*, and *c*). The staining pattern is the same regardless of whether or not the cells are permeabilized before incubating with the primary antibody. In MDCK epithelial cells in or near the explant, antihemagglutinin stains only the apical surface (Fig. 7 *d*). Focusing on the lateral and basal surfaces of the epithelial cells reveals only background fluorescence (data not shown).

Ultrastructural analysis confirms the immunofluorescent findings. 6 h after infection, influenza virus buds from the top (Fig. 8, upper left inset), sides, and bottom (Fig. 8, lower right inset) surfaces of the fusiform cell. Similar results are obtained if budding is analyzed 8 h after infection. The rela-

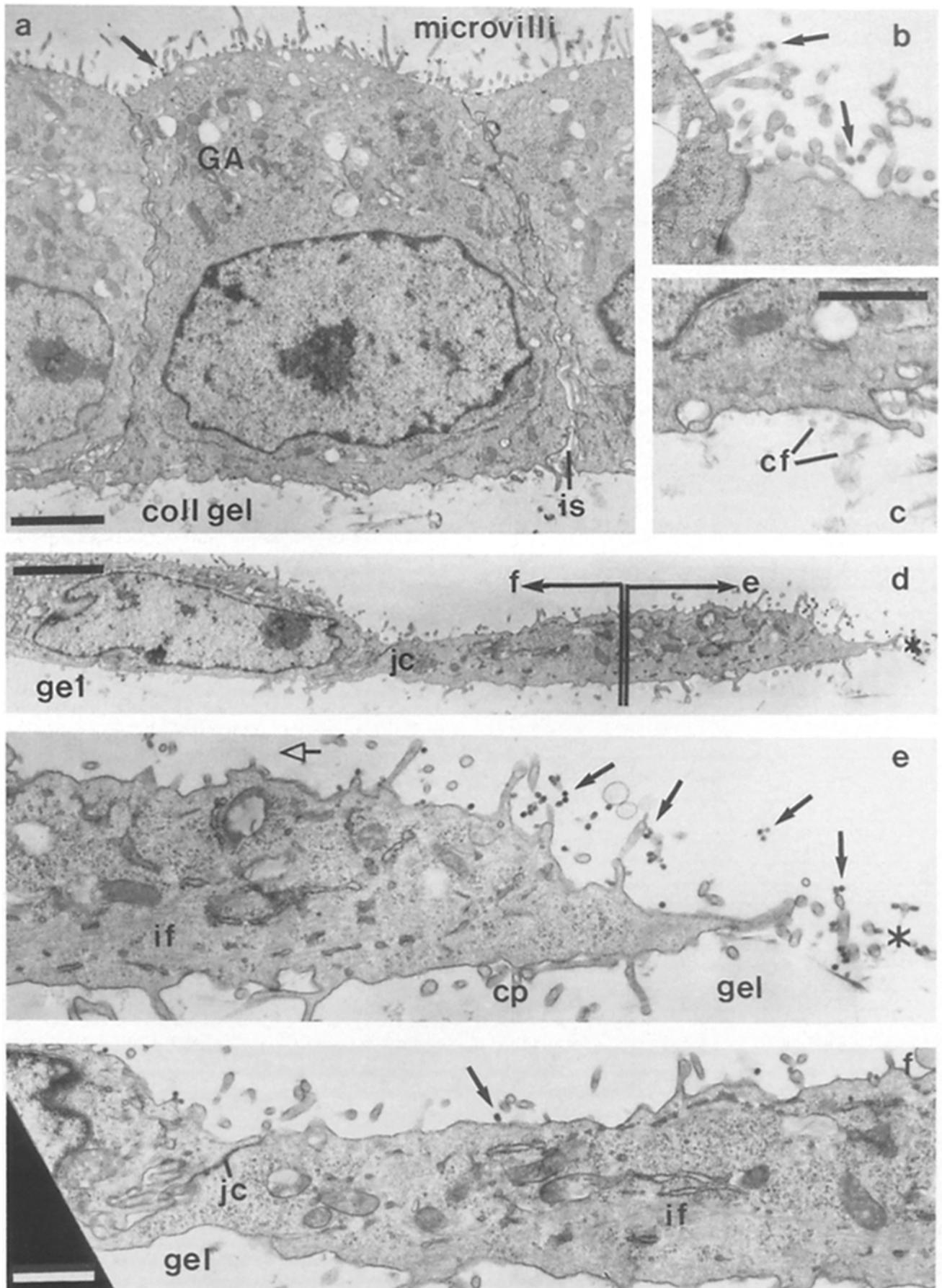
tive number of virions budding from the top vs. the bottom cell surface varies. As determined by semiserial sections of four cells (17 sections) sectioned lengthwise perfectly through the nucleus (as in Fig. 8), the average number of viruses on the top vs. the bottom was 5.7 ± 4.7 vs. 5.3 ± 3.2 , 16.0 ± 7.3 vs. 6.5 ± 2.4 , 7.3 ± 3.0 vs. 4.8 ± 2.6 , and 4.0 ± 0.0 vs. 3.5 ± 0.7 . Interestingly, fusiform cells infected with virus seem to have more RER and a better developed Golgi apparatus (Fig. 8) than uninfected fusiform cells (Fig. 5).

In epithelial MDCK cells near the explant, virus budding was observed only at the apical cell surface in these experiments (Fig. 9, *a*, *b*, and *c*). Virions are mainly associated with the prominent microvilli (Fig. 9 *b*). Quantitation of virus budding of five cells sectioned longitudinally through the nucleus (five sections) gave an average number of 39.5 ± 14.0 vs. 0.3 ± 0.5 for apical vs. basolateral budding. The epithelial cells on the collagen gel near the explant are similar in appearance whether infected with virus (Fig. 9 *a*) or not (Fig. 2). RER and Golgi apparatus do not appear to be hypertrophied in epithelial cells (Fig. 9 *a*) or transitional cells (Fig. 9 *d*).

Where the front edge of a migrating MDCK cell can be identified, as in a transitional cell still connected to neighbors behind it (Fig. 3 *f*, *TR*), influenza virus can sometimes be seen to bud primarily from the leading edge (Fig. 9, *d*, *e*, and *f*). The number of virions these elongated cells bud is greater at the leading edge (Fig. 9 *e*), when compared to the remainder of the cell surface (Fig. 9 *e*, *open arrow*; Fig. 9 *f*, *solid arrow*), perhaps indicating that this transitional cell was infected just before fixation (see Discussion). Virus budding is not usually observed at the bottom surface of the transitional cells (Fig. 9 *f*).

The distribution of a basolateral membrane marker was studied next. Immunostaining with a rabbit polyclonal antibody to the B-subunit of Na,K-ATPase reveals localization to an internal vesicular pool, identified as cytoplasmic vacuoles (Fig. 10, *a* and *b*). Immunofluorescence of fusiform cells processed for surface staining shows a uniform distribution of Na,K-ATPase on the plasma membrane (Fig. 10 *f*). Na,K-ATPase, however, in polarized MDCK cells is localized only to the basal and lateral plasma surface either after fixation with permeabilization (Fig. 10, *d* and *e*) or without (data not shown; Louvard, 1980; Nelson and Veshnock, 1986). Cytoplasmic vacuoles containing Na,K-ATPase are absent in permeabilized epithelial MDCK cells, consistent with previous observations that, upon polarization, Na,K-ATPase distributes to the basolateral domain (Nelson and Veshnock, 1986) and is no longer cytoplasmic. It appears that in fusiform cells, the majority of Na,K-ATPase is intracellular rather than cell-surface associated. Interestingly enough, MDCK cells in the transitional zone distribute Na,K-ATPase to points of lateral cell contact and to an intracellular pool (data not shown).

Figure 8. Electron micrograph of fusiform MDCK cell cultured on type I collagen (*coll*) gel for 6 d, fixed 6 h after infection with influenza virus. Virions bud symmetrically from the fusiform cell surface. In the micrographs shown here, a fusiform cell appears at low magnification across the center. The areas at high magnification in the upper and lower insets are from the upper and lower surfaces of this cell in the regions containing the label *cp* (cell process). The upper inset is from an adjacent serial section. Virions (*black arrows*) are produced from both cell surfaces. Note the hypertrophy of RER and Golgi apparatus (*GA*) in this infected fusiform cell. The open arrow calls attention to virus budding from filopodia at the leading edge of the cell. *med*, medium. Bars: (center) 3.0 μm ; (insets) 1.0 μm .



Other Membrane-associated Proteins Also Redistribute

Other membrane-associated proteins redistribute as MDCK cells acquire a fusiform cell shape on hydrated type I collagen gels. One of these is fodrin, the functional and structural homolog of spectrin in nonerythroid cells (for review see Glenney and Glenney, 1984; Nelson and Veshnock, 1986). In polarized MDCK cells, fodrin localizes to regions of lateral cell contact (Fig. 10 g) and to the basal cell surface. In fusiform cells, fodrin antiserum recognizes the cytoplasmic face of the entire plasma membrane and produces a diffuse staining pattern throughout the cytoplasm (Fig. 10 c).

Immunostaining of ZO-1, a tight junction-associated polypeptide, reveals a continuous apical band of staining around epithelial MDCK cells (Fig. 10 h; Stevenson et al., 1987). In fusiform cells, ZO-1 cannot be detected (Fig. 10 i). In regions of transition, ZO-1 staining becomes discontinuous along points of lateral cell contact and cannot be detected at the cell's free edge (data not shown).

Immunolocalization with antisera directed against desmoplakin I and II, components of the desmosomal plaque (Mueller and Franke, 1983), reveals distinct lines of punctate staining at points of cell-cell contact in epithelial MDCK cells (Fig. 10 j). In fusiform cells, which lack morphologically identifiable cell junctions, desmoplakins exhibit a random, intracytoplasmic pattern (Fig. 10 k) evident after permeabilization.

Discussion

When explants of differentiated MDCK cells on basement membrane gel are placed on a hydrated type I collagen gel, the cells that migrate out onto the hydrated fibrils of the gel lose apical-basal polarity and intercellular adhesions and acquire fusiform cell shape, whereas these cells spread as cohesive epithelial sheets over other substrata. This change is induced by type I collagen fibrils, because the MDCK cells migrating on plastic or basement membrane gels retain cell-cell contacts and epithelial morphology. The fusiform cells on type I collagen symmetrically redistribute apical and basolateral membrane markers (hemagglutinin and influenza virus, Na,K-ATPase). Other membrane-associated proteins assume a punctate (desmoplakin I and II) or diffuse distribution (fodrin) or cannot be detected (ZO-1). Despite the dramatic change in cell surface and shape, fusiform MDCK cells on collagen display the MDCK intermediate filament phenotype (keratin and vimentin) and they do not synthesize type I collagen, as would be expected if they were true mesenchymal cells.

The MDCK cells in the explant on basement membrane

that give rise to the fusiform cells comprise a highly differentiated epithelium. They are much taller than MDCK cells grown on plastic, glass, or permeable filters, which may or may not be coated with type I collagen (Misfeldt et al., 1976; Cerejido et al., 1978; Richardson et al., 1981; Fuller et al., 1984). The tall, columnar, MDCK cells on basement membrane gel exhibit more apical microvilli than do epithelia on collagen or plastic, and project many cellular processes into a lateral compartment that may be quite wide. As compared to MDCK on other substrata, MDCK on basement membrane appear to be more actively pumping fluid into the distended intercellular space. The morphology of these cells resembles that of gall bladder epithelium actively involved in ion and water transport (Kaye et al., 1966), rather than that typical of kidney tubules or ducts. Assuming that basement membrane promotes only existing genetic programs, one might raise the question of the true origin of these cells. MDCK cells are presumed to have originated from distal tubule or collecting duct (Rindler et al., 1979; Herzlinger et al., 1982; Garcia-Perez and Smith, 1983). Distal tubule cells are characterized by interdigitations of the basal cytoplasm, whereas collecting duct cells typically infold the basal cell membrane. Mitochondria lie either within the interdigitations (distal tubule) or the infoldings (collecting duct). The basal cytoplasm and membrane of MDCK cells do not show these specializations on any substratum. It is possible that in adapting to long-term culture, the genetic program of the cells of origin was modified.

Most epithelia migrating on top of type I collagen gels move as cohesive epithelial sheets (Dodson and Hay, 1974; Greenburg and Hay, 1988). The one exception that has been noted heretofore involves explants of lens epithelia cultured on top of type I collagen gel, which give rise to isolated stellate-shaped cells at their leading edge (Greenburg and Hay, 1986). We report here that when small explants of MDCK cells on basement membrane are placed on top of hydrated collagen gel, the cells that migrate out onto the collagen gel do not maintain cell-cell contact and are fusiform in shape.

One might speculate that the reason for the difference in migratory behavior among these different epithelia cultured on top of hydrated collagen gels is the relative amount of vimentin vs. keratin in the cytoskeleton. Most epithelia contain keratin intermediate filaments and no vimentin (Osborn and Weber, 1983; Greenburg and Hay, 1988). Lens is one of the few epithelia in situ that contains abundant vimentin and no keratin (Ramaekers et al., 1980). Vimentin has been postulated to play a role in cell-shape changes and keratin in cell-cell contact (Ben-Ze'ev, 1984). Epithelia that form mesenchyme in the embryo have a vimentin cytoskeleton at

Figure 9. Electron micrographs of polarized, epithelial MDCK cells (a-c) and of transitional MDCK cells (d-f) after 6 d of culture on type I collagen (coll) gel. They were fixed 6 h after infection with influenza virus. Influenza virus (arrow) buds only from the apical surface of polarized cells (a). No virions were seen on the basal (a and c) or lateral (a) surface in this study. Virions are associated with the numerous apical microvilli (a and b). The elongated MDCK cell in d is identified as a transitional cell because of the close contact with the MDCK cell behind it. Higher magnification micrographs e and f are from the areas indicated (e and f) in d. Virions (arrows) bud primarily from filopodia on the leading cytoplasmic edge (d and e, asterisks) and from the upper cell surface immediately proximal to the leading edge. Near the center (open arrow, e) and trailing part of the cell (f), viruses are rare. GA, Golgi apparatus; is, intercellular space; cf, collagen fibril; cp, cell processes negative for virus on the bottom of the cell; if, intermediate filaments; jc, junctional complex. Bars: (a and d) 3.0 μ m; (b, c, e, and f) 1 μ m.

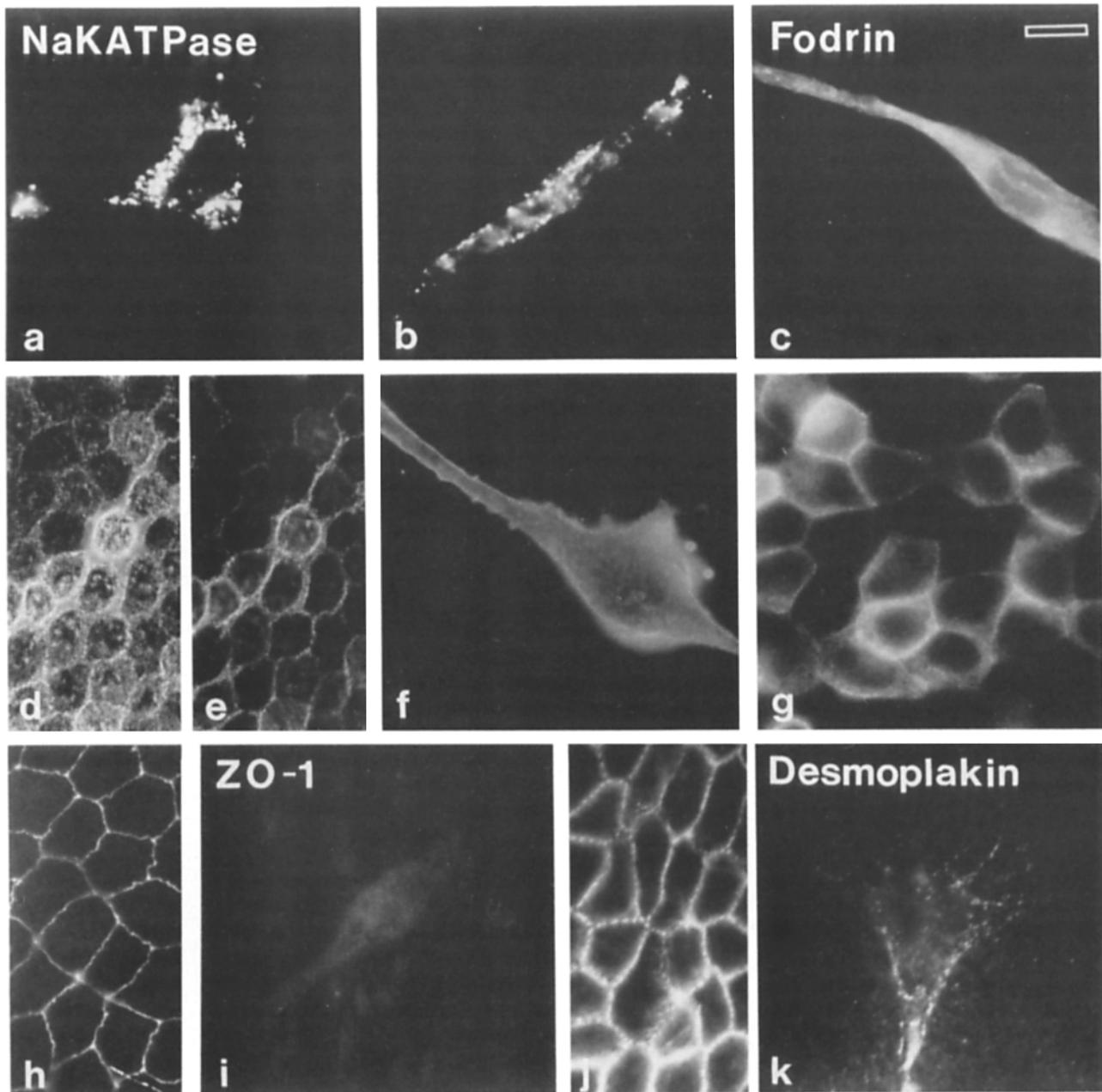


Figure 10. Immunofluorescence localization of Na,K-ATPase (*a, b, and d-f*), fodrin (*c and g*), ZO-1 (*h and i*), and desmoplakin I and II (*j and k*) in fusiform (*a-c, f, i, and k*) and cuboidal (*d, e, g, h, and j*) MDCK cells. Because the surface of the gel is uneven, several different focal planes are seen in one field of view (*d, e, and g*). Fusiform cells express an internal, cytoplasmic pool (*a and b*) of Na,K-ATPase that is predominantly perinuclear in location. In unpermeabilized fusiform cells (*f*), Na,K-ATPase symmetrically distributes on the cell surface. Unpermeabilized epithelial MDCK cells located behind the leading edge localize Na,K-ATPase to the basal (*d*) and lateral (*e*) plasma membranes. In permeabilized fusiform MDCK cells, fodrin has a diffuse cytoplasmic distribution (*c*), whereas in epithelial MDCK cells behind the leading edge, fodrin localizes to the basal and lateral cell surfaces (*g*). ZO-1 is diffuse in fusiform cells (*i*), whereas in polarized MDCK cells (*h*), ZO-1 localizes to the lateral plasma membrane near the cell apex. Fusiform cells continue to express desmoplakin I and II (*k*), even though desmosomes are not present. Epithelial MDCK cells localize this antigen to points of cell-cell contact (*j*). Time in culture: (*a, b, d, and e*) 10 d; (*c and g*) 11 d; (*f*) 13 d; (*h and i*) 8 d; (*j and k*) 9 d. Exposure: *a and d* (basal), 30 s; *b and f*, 120 s; *e* (lateral), 60 s. Bar, 10 μm .

the time they give rise to mesenchyme (Franke et al., 1982; Fitchett and Hay, 1989). As mentioned in the Introduction, it is possible to induce a number of definitive, keratin-rich epithelia to give rise to mesenchymal cells by suspending them within collagen gels; such cells acquire vimentin and

lose keratin (Greenburg and Hay, 1982, 1986, 1988). Interaction of the hydrated collagen gel with the vimentin cytoskeleton might occur via the actin cortex (Tomasek et al., 1982; Tomasek and Hay, 1984) to bring about the bipolar cell shape. Intermediate filaments of fusiform MDCK cells are

associated with a well-developed actin cortex. It would be interesting to explore the possibility that other long term epithelial cell lines that have acquired vimentin (Franke et al., 1979; Virtanen et al., 1981) would also be prone to fusiform cell shape changes if cultured on hydrated collagen gels.

Despite a vimentin cytoskeleton, however, MDCK cells do not transform into fusiform cells when explants are suspended in type I collagen gels. In this case, the apical epithelial surface faces the gel. When thyroid follicles are suspended in type I collagen gel, the basal surface faces ECM and it is the cells proliferating from the basal surface that are induced to turn on vimentin, lose desmosomes, and elongate (Greenburg and Hay, 1988). When lens epithelium on lens capsule is suspended within type I collagen gel, the elongated cells arise from the apical surface (Greenburg and Hay, 1986). This may be possible because the lens has such poorly developed cell junctions that cells proliferating from the apical surface easily lose cell-cell contact. It seems likely that the tight adherence of MDCK cells to one another, imposed by numerous junctional complexes, precludes transformation of cells on the free surface into fusiform cells, because on top of collagen gels only the basal surface of cells leaving the explant contacts ECM. In other experiments showing formation of ducts by epithelia suspended in type I gel (Yang et al., 1979; Haeuptle et al., 1983), it was not determined whether the apical or basal cell surface faced the surrounding collagen fibrils or whether or not these epithelia were actually immersed in the gel.

Upon loss of cell-cell contact and acquisition of an elongated cell shape, fusiform cells on type I collagen gel lose apical-basal polarity. The change in polarity involves more than loss of intercellular adhesions since MDCK cells cultured on collagen in the absence of cell contact (Rodriguez-Boulan et al., 1983; Vega-Salas et al., 1987) are not completely depolarized. Basolateral proteins distribute homogeneously over the entire cell surface, but an apical 184-kD protein is still polarized. The observation that fusiform cells symmetrically distribute influenza, thus, requires an explanation involving more than lack of cell contact. The physical interaction of collagen fibrils with the basal surface of MDCK cells leaving the explant on top of the gel may play a role, for isolated MDCK cells merely seeded onto collagen regain apical-basal polarity.

Fusiform cells may symmetrically distribute apical and basolateral markers (hemagglutinin, influenza budding, Na,K-ATPase) because they assume the motile activity that has been described for mesenchymal cells. Migrating mesenchymal cells, like fusiform cells, develop pseudopodia and filopodia and organize the cytoplasm into leading and trailing compartments. The cell thus has two poles. New membrane mass seems to be delivered preferentially to the polarized front end (Abercrombie et al., 1970). Some elegant recent evidence for this idea has recently been obtained by Bergmann et al. (1983) and Kupfer et al. (1987), who showed that the G-glycoprotein of the temperature-sensitive mutant of vesicular stomatitis virus preferentially polarizes to the leading edge of motile fibroblasts. This distribution is evident at the earliest time point after shifting to the permissive temperature for passage of G-protein from the RER through the Golgi complex to the cell surface.

In our experiments, we were not able to produce a pulse for new membrane protein in this way. However, in selected

cells we clearly observed virion budding only at the leading edge, which could be explained if these cells became infected late, just before they were fixed. The symmetrical distribution of virions over the cell surface of most fusiform cells could result from the movement of new membrane proteins with time from the front end to the rest of the cell surface, as was observed for G-protein (Kupfer et al., 1987). Thus, the remarkable polarity of the front end of moving cells might only be noticed when new membrane proteins can be pulse-labeled. Polarity of other cytoplasmic components can be shown by special techniques. mRNA for actin is found in the front end of migrating mesenchymal cells (Lawrence and Singer, 1986), as is the Golgi apparatus (Kupfer et al., 1982; Bergmann et al., 1983; but see Trelstad, 1977). It is erroneous to say that fibroblasts exhibit nonpolarized budding (Rodriguez-Boulan and Sabatini, 1978) just because symmetrical virus budding is observed after the cell has been infected for some time.

In the transport of new membrane proteins to the front end of a migrating fusiform or mesenchymal cell, it is possible that the underlying cytoskeleton is involved. Microtubules have been implicated in the sorting and transport of hemagglutinin to the apical surface of MDCK cells (Salas et al., 1986; Rindler et al., 1987). Delivery of membrane proteins in fibroblasts is also believed to be microtubule mediated (Rogalski et al., 1984). In migrating mesenchymal cells, microtubules are organized lengthwise in the cytoplasm (Tomasek and Hay, 1984) and might be in a position to direct vesicles containing membrane proteins from the Golgi apparatus to the front end. Surprisingly, the front end of a moving cell can change quickly in location to another part of the cell when contact inhibition occurs (Bard and Hay, 1975), suggesting that polarity in a mesenchymal cell is a very dynamic process.

Although Na,K-ATPase distributes symmetrically on the cell surface of the fusiform cell, as do the other membrane markers studied here, more of this protein is intracellular than on the cell surface. Intracellular Na,K-ATPase has been reported in MDCK cells plated onto Cytodex beads, but when the cells reach confluency the internal pool decreases, presumably because the enzyme can now be transported basolaterally (Smith et al., 1988). The intracellular pool of fusiform cells, which appears to be contained in vacuoles, could reflect newly synthesized enzyme that cannot be transported or enzyme that was endocytosed during change to the fusiform cell shape.

It has recently been hypothesized that Na,K-ATPase is held in the basolateral domain through interaction with the submembraneous fodrin cytoskeleton (Nelson and Veshnock, 1987). The assembly of fodrin on the plasma membrane coincides with extensive cell-cell contact and with the development of the apical-basal polarized distribution of Na,K-ATPase (Nelson and Veshnock, 1986). In fusiform cells, fodrin diffusely distributes in the cytoplasm and ZO-1 cannot be detected. Desmoplakin I and II assume a random cytoplasmic distribution. It is possible that during conversion from epithelial to fusiform cell shape, as cell-cell contact is lost, fodrin disassembles. The appearance of cytoplasmic Na,K-ATPase in fusiform cells could indicate that the enzyme cannot be held on the plasma membrane because of disassembly of the fodrin cytoskeleton. Future work is needed to clarify this point.

Finally, it must be emphasized that even though fusiform cells have the shape, motility, and polarity of mesenchymal cells, they have not acquired the complete mesenchymal phenotype in the sense that they do not exhibit mesenchymal ultrastructure or synthetic activity. The cytoplasm of the fusiform cell, like that of the parent epithelial cell, is fibrillogranular in appearance and contains only a few thin strands of RER. In a mesenchymal cell, many elongated profiles of RER fill the cytoplasm and type I collagen is synthesized (Hay, 1984). Both fusiform cells and the parent epithelium produce laminin and keratin. Unlike true mesenchymal cells, fusiform cells do not invade the collagen gel. Thus, the potential to acquire the complete mesenchymal phenotype, which is retained by lens, thyroid, and other epithelia (Greenburg and Hay, 1982, 1986, 1988), seems to be partially lost in this long-standing cell line. In becoming immortal, MDCK cells acquired vimentin intermediate filaments, which may predispose them to switching to fusiform cell shape and polarity on top of type I collagen gels, but in adapting to long term culture, they seem now to be unable to switch off keratin and laminin and to turn on type I collagen. As noted earlier, this cell line no longer even resembles kidney epithelium.

In conclusion, we have in this discussion considered possible mechanisms underlying the switch in cell polarity observed when MDCK epithelium gives rise to fusiform cells. We emphasize the point that migrating mesenchymal cells express a form of cell polarity that is quite different from the kind of polarity exhibited by epithelial cells. The vimentin cytoskeleton may predispose vimentin-rich epithelia, like MDCK and lens, to form elongated cells on top of type I collagen gel. Further study of the phenomenon can be expected to shed new light on the factors involved in the creation and maintenance of epithelial vs. mesenchymal cell shape and polarity.

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