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Matrix-Cytoskeletal Interactions in the Developing Eye

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The embryonic avian corneal epithelium in vitro responds to extracellular matrix (ECM) molecules in either soluble or polymerized form by flattening its basal surface, organizing the basal cortical actin cytoskeleton, and stepping up its production of corneal stroma twofold. Embryonic corneal epithelia, like hepatocytes and mammary gland cells, seem to contain heparan sulfate proteoglycan (HSPG) in their plasmalemma, which may interact with actin on the one hand or underlying collagen on the other. Work on the corneal epithelium suggests that, in addition to HSPG, specific glycoprotein receptors for laminin and collagen exist in the basal plasmalemma and play the critical role in actually organizing the basal epithelial cytoskeleton. As yet, uncharacterized proteins may link such receptors to actin. We suggest that ECM-dependent organization of the cytoskeleton is responsible for ECM enhancement of corneal epithelial differentiation. Cell shape and exogenous ECM also affect mesenchymal cell differentiation. In the case of the corneal fibroblast migrating in collagen gels, an actin cortex present around the elongate cell seems to interact with myosin in the cytosol to bring about pseudopodial extension. Both microtubules and actin microfilaments are involved in fibroblast elongation in collagen gels. It follows from the rules presented in this review that the mesenchymal cell surface is quite different from the epithelial cell surface in its organization. Nevertheless, epithelial cell surface-ECM interaction can be modified in the embryo at particular times to permit predesignated epithelial-mesenchymal transformations, as for example at the primitive streak. Though basal surfaces of definitive, nonmalignant epithelia adhere rather strictly to the rules of epithelium-ECM interaction and do not invade underlying ECM, the environment can be manipulated in vitro to cause these epithelia to send out pseudopodia and give rise aberrantly to mesenchymal cells in collagen gels. Further study of this phenomenon should cast light on the manner in which epithelial and mesenchymal cells organize receptors for matrix molecules on their cell surfaces and develop appropriate cytoskeletal responses to the extracellular matrix.

Key words: cell-matrix interaction, cytoskeleton, extracellular matrix, mesenchyme, epithelial-mesenchymal transformation

In this review, we develop the thesis that developmentally significant cell-matrix interaction takes place at the cell surface and that the subsequent effects of extracel-

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lular matrix (ECM) on cell metabolism and differentiation are mediated, at least in part, by the response of the cytoskeleton to plasmalemma-associated complexes with ECM receptors. Developmentally significant cell-matrix interactions occur between embryonic epithelia and between embryonic epithelia and mesenchyme [1] (Fig. 1). Both ECM deposition and ECM removal can be involved in such interactions. For example, removal of basal lamina components from specific areas of the distal lobule seems to cause simple epithelium (Fig. 1A) programmed to become a gland to branch. In these regions of outgrowth, the basal epithelial cytoskeleton becomes disorganized and epithelial cells contact the adjacent mesenchyme, which is producing enzymes that break down the basal lamina [2]. In the interlobular clefts, however, where ECM accumulates, the epithelial cytoskeleton is well organized and differentiated epithelial morphology is maintained.

ECM of dermal origin stimulates epidermal differentiation [3] (Fig. 1B) and floating collagen gels promote the differentiation of mammary gland cells [4]. Heparan sulfate proteoglycan (HSPG) and laminin have been reported to promote neurone outgrowth [5]. Schwann cells do not form basal laminae in vitro unless neurones (Fig. 1C) are present [6] and contact with collagen enhances their ability to ensheath the axones [7]. Collagen of fibroblast (Fig. 1E) origin promotes the differentiation of muscle (Fig. 1D) and ECM produced by retinal epithelium enhances chondrocyte (Fig. 1F) differentiation [8,9]. Collagens, glycosaminoglycans (GAG), and proteoglycans (PG) stimulate vertebral chondrogenesis [10–13]. Though the cytoskeleton has not yet been shown to be directly involved in all of these stimulatory effects of ECM, cell shape changes have been implicated, especially in the case of

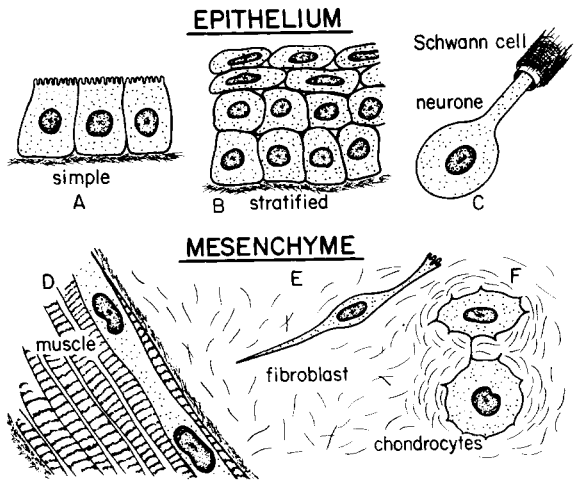

Fig. 1. Epithelium is the tissue that covers the body and lines internal cavities. The cells rest on a basal lamina, are joined by junctions, and present a free surface to the outside world (A,B). Neurones and Schwann cells (C) derive from neural epithelium and neural crest. Mesenchyme is an embryonic tissue composed of freely inwandering cells surrounded by ECM. It gives rise to muscle (D), fibroblasts (E), chondrocytes (F), and other cells of the fibroblast family. From Hay [1].

cartilage [14,15] and glandular epithelium [4]. It seems likely, moreover, that these cell-matrix interactions are mediated at the cell surface via transmembrane receptors for particular matrix molecules [1,16,17].

In the case of the developing eye, compelling evidence is accumulating for the idea that matrix molecules interact across the plasmalemma with the cytoskeleton. For example, corneal epithelium, which will be discussed here in detail, flattens its basal surface when grown on top of a collagen gel [18], steps up production of corneal stroma [19], and organizes its basal cortical actin mat to resemble that of the corneal epithelium in situ [20]. When fibroblasts are grown on gels, however, they seem programmed to invade the ECM and to become elongate in shape once within the collagen lattice [21–24]. In this review, we will explore this difference in response to ECM of the cell surface and cytoskeleton and attempt to define the rules that govern the distinctive interactions of epithelial and mesenchymal cells with ECM. Finally, we explore the stability of the epithelial and mesenchymal states and describe the remarkable effects on cell surface, cytoskeleton, and phenotype brought about by suspending epithelia within collagen gels.

EPITHELIUM-MATRIX INTERACTION

General Rules

The typical epithelial cell is characterized by polarized cytoskeletal and cell surface organization. In simple epithelia, the apical cell surface is enriched with molecules that do not occur on other surfaces [25–27] and the apical cytoskeleton takes the form of a highly differentiated terminal web [26]. Extracellular matrix molecules seem only to interact with the basal surface, and lateral surfaces display numerous cell junctions. Though some aspects of epithelial polarity may be promoted by the cell junctions [1,25], attachment to the substratum is an important factor in maintaining polarity [25]. It has been suggested that when epithelial cells attach to collagen, receptors for collagen, dispersed over the cell surfaces after cell suspension, circulate to the basal plasmalemma [28]. If epithelial cells are placed on ECM, moreover, they remain on top of the lattice, whereas, as noted above [18–24], mesenchymal cells invade such lattices. The general rule that epithelial cells follow is to flatten the basal surface and develop apical-basal polarity when placed on ECM.

Several recent studies have explored the nature of epithelial binding sites or receptors for ECM molecules. A membrane-intercalated HSPG has been characterized in hepatocytes [29], mammary gland cells [30], and corneal epithelium [31] that could bind collagen or stabilize the association of collagen with other cell surface receptors (Fig. 2A). Other possible binding sites have been suggested [32–41]. Glycoproteins (molecular weight 65–70 Kd) have been isolated from muscle, carcinoma, fibrosarcoma, and corneal epithelium that appear to be receptors for laminin [33–38]. Collagen binding protein(s) may be smaller, in the neighborhood of 47 Kd [38,39]. Both the laminin-binding glycoprotein [40] and the membrane-intercalated HSPG [41] interact with actin in vitro, but further evidence is needed before it can be concluded that they bind directly to actin in situ. Interestingly, the laminin-binding protein from a tumor [40] aggregates isolated actin into parallel bundles like those in the basal cortical cytoskeleton of corneal epithelium to be described in the next section.

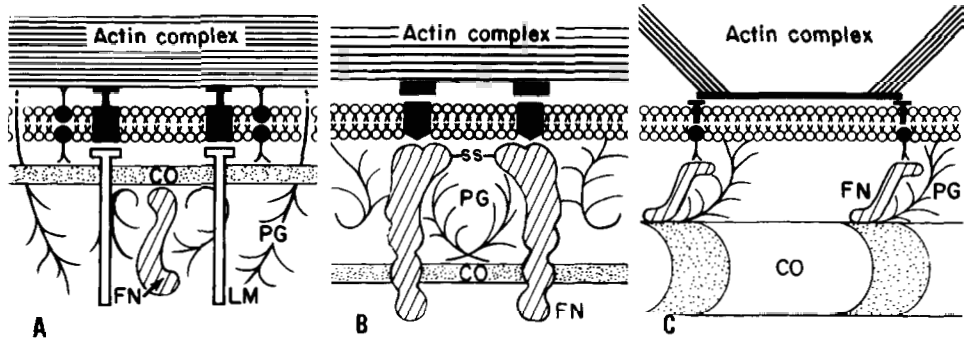

Fig. 2. Diagrams depicting the interaction of the cell surface with ECM. A) model of basal epithelial surface showing receptors and actin-binding proteins associated with the plasmalemma, based on Sugrue and Hay [20,31,38]. B) model of cell-ECM interaction that might describe a fibroblast cell surface, based on Hynes and Yamada [16]. C) model that might describe a cell interacting with ECM on a planar substrata, based on Kleinman et al [32]. Actin is depicted ending in a cell-ECM junction as stress fibers might do. In vivo it is likely that actin filaments run parallel to the plasmalemma in both epithelia [20] and fibroblasts [24]. CO, collagen; FN, fibronectin; LM, laminin; PG, proteoglycan. Modified from Hay [17].

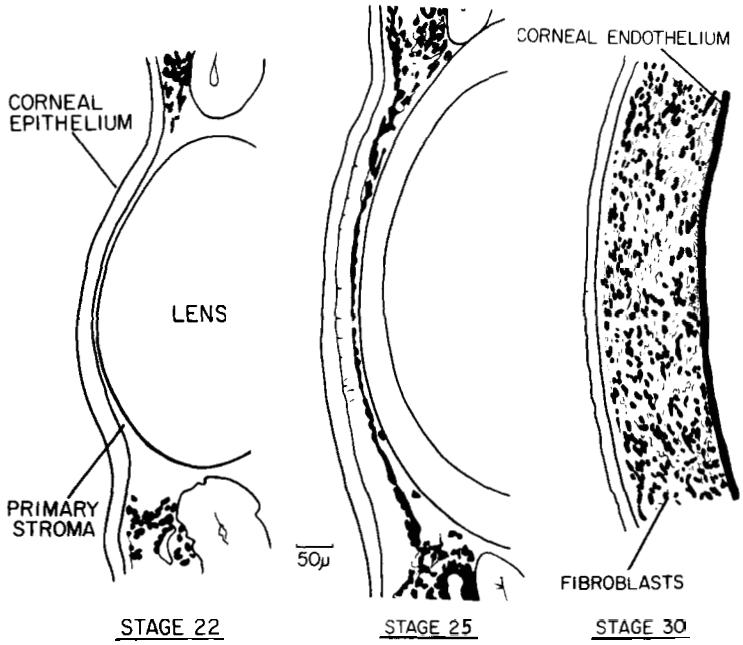

Fig. 3. Camera lucida drawings showing several stages in development of the avian cornea. At stage 22 (4 days), the corneal epithelium has secreted the primary corneal stroma and mesenchymal cells in the periphery are preparing to move in to coat the back of the cornea to form the corneal endothelium. At stage 25 (5 days), endothelial formation is completed and shortly thereafter (stage 27, 5½ days) the presumptive corneal fibroblasts begin to move in. The fibroblasts fill the cornea by stage 30 (7 days). From Hay and Revel [42].

The Corneal Epithelial Cytoskeleton and Its Role in Differentiation

On the third day of development of the chick embryo (Fig. 3), the corneal epithelium produces the primary corneal stroma without the help of fibroblasts [42,43]. The primary stroma contains 20 or so orthogonally arranged layers of striated fibrils composed of type I and type II collagens [44] associated with proteoglycans [45]. The stroma itself is acellular until the sixth day of development, when it is invaded by corneal fibroblasts that secrete abundant type I collagen [43].

Corneal differentiation is induced, at least in part, by the adjacent lens [43]. Since production of the ECM that comprises the primary stroma is the main measure of epithelial differentiation in this system, it can be said that corneal epithelial differentiation, as measured by this parameter, is promoted by lens ECM [18,19]. Isolated corneal epithelium grown on frozen-killed lens capsule produces a facsimile of the primary stroma, but it does not do so on Millipore filters alone [18]. The basal surface of the epithelium starts to bleb when the epithelium is isolated with EDTA or trypsin-collagenase and continues to do so if the epithelia are cultured on Millipore filters. The basal surface of the isolated epithelium in contact with lens capsule or other collagens [18,19], however, flattens within 6 hr, and within 24 hr production of corneal stroma is detectable morphologically. Using ^{3}H-proline as a label, it can be determined that the epithelia are producing twice as much new collagen when grown on collagenous substrata as compared to Millipore filters or other non-ECM substrata [19].

Solubilized types I, II, or IV collagen, laminin, or fibronectin added to the medium under a blebbing epithelium on a Millipore filter, cause it to flatten in 6 hr [20]. Thus, ECM need not be polymerized to exert its effect on the epithelium. HSPG, GAG, and non-ECM proteins in solution do not affect the blebbing epithelial surface (Fig. 4). Epithelia treated with soluble collagen, laminin, or fibronectin

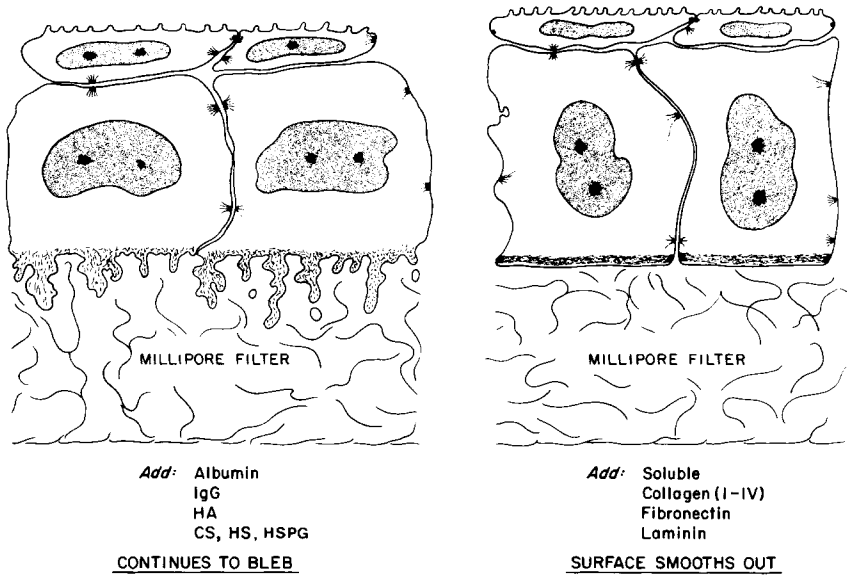

Fig. 4. Diagram summarizing the morphological effect of adding soluble collagens, fibronectin, and laminin to the medium under isolated corneal epithelium on Millipore filter (right), as opposed to adding GAG, PG, and nonECM proteins (left). HA, hyaluronic acid; IgG, immunoglobulin; CS, chondroitin sulfate; HS, heparan sulphate; HSPG, heparan sulfate proteoglycan. From Sugrue and Hay [20].

produce twice as much collagen as blebbing epithelia [1], and visible new stroma is present but, at the early time point shown here (Fig. 4), no ECM is visible by electron microscopy on the basal epithelial surface. Fibronectin does not flatten the cell surface in the presence of cycloheximide and so we believe its action is indirect (Fig. 2A). Collagen and laminin, however, interact with the basal surface without an endogenous source of proteins [46].

The flattening of the basal surface involves reorganization of the basal epithelial cytoskeleton as well as withdrawal of the blebs. When the basal surface smooths out, the disorganized microfilaments in the blebs are drawn up into a dense cortical mat that can be shown by S1 labeling to contain actin [20]. In the organized cortical mat, bundles of actin filaments run parallel to the basal plasmalemma, whereas in the blebs individual filaments run in various directions and may point toward the plasmalemma (Fig. 5).

Because previous work indicates that ECM is not internalized during its interaction with corneal epithelium [47], it seems likely that ECM molecules bind to the basal plasmalemma and exert their effect on the cytoskeleton and epithelial metabolism across the plasmalemma. To determine that the molecules do bind to the cell surface, we labeled laminin and heat-denatured type I collagen with fluorescein, or bound them to fluorescein-containing Covaspheres, before adding them to isolated epithelia floating in culture medium [1,38]. We found that laminin and collagen bind to the basal cell surface but not to the apical surface of the epithelium and that the

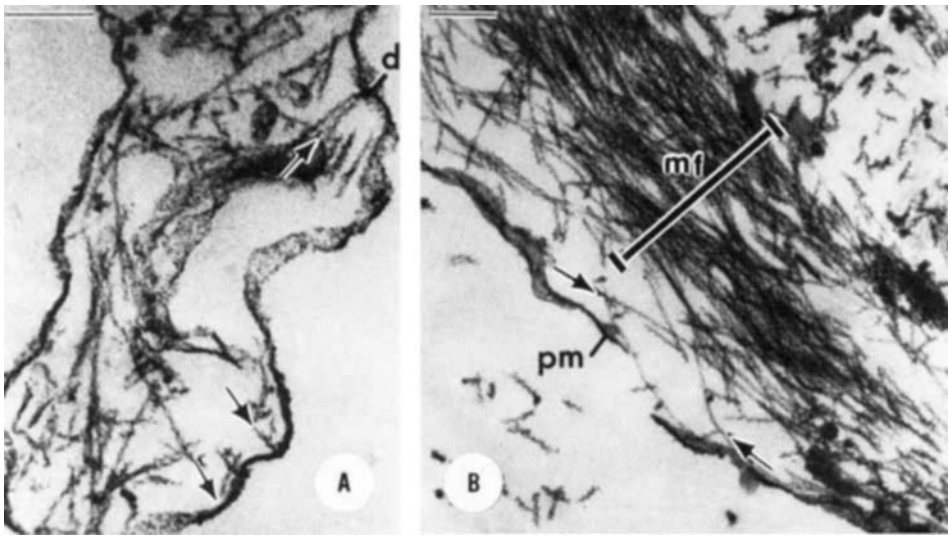

Fig. 5. Electron micrographs of corneal basal cytoplasm after fixation, detergent extraction, and labeling with S1 fragments of heavy meromyosin. A) bleb on basal surface of an epithelium isolated by trypsin-collagenase treatment. S1 decorated actin filaments form a loose meshwork and may point (arrows) toward the plasmalemma. At d, two filaments insert in a dense area on the plasmalemma. B) isolated epithelium treated with 100 μg/ml type IV collagen flattens its basal surface and reorganizes its actin filaments (arrows) into a cortical microfilamentous mat (mf). The space between the plasmalemma (pm) and microfilamentous mat is exaggerated by the detergent treatment. From [20]. Bar = 200 nm.

labeled molecules are able to flatten the basal surface. Albumen-coated or plain Covaspheres do not bind to the cell surface. Moreover, the basal epithelia binding sites for laminin and collagen are removed by treatment of the isolated tissue with trypsin [1,38]. Additional evidence that specific binding sites are present in the cell surface was obtained by competing the labeled molecules off the basal cell surface with unlabeled collagen or laminin [38].

We are currently exploring the possibility that the cell surface binding sites are membrane-intercalated HSPG and/or glycoproteins. In order to determine whether or not isolated corneal epithelial cells contain membrane-intercalated HSPG, the cells were cultured in ^{3}H-leucine or $^{35}SO_4$ and the newly synthesized proteoglycans analysed by Sepharose CL-2B chromatography [31,38]. A 4 M guanidine-HCl extract applied to Cl-2B resolves as two populations: one eluting at $K_{av} = 0.626$ with GAG composed of 70% heparan sulfate and 20% chondroitin sulfate (molecular weight approximately 500 Kd) and one eluting in the void volume with GAG composed of 85% heparan sulfate. Detergent treatment depletes the V_O HSPG, suggesting lipid association. In addition, the V_O HSPG exhibits hydrophobic properties when applied to octyl-separose [38] and can be inserted into liposomes. Moreover, this HSPG is not released from the cell surface by NaCl or excess heparin, as a proteoglycan associated with the cell via its sugar groups would be. Our preliminary experiments, however, indicate that this putative plasmalemmal HSPG may not be the cytoskeleton-connected receptor that organizes the basal epithelial surface, at least not by itself [38].

In addition to HSPG [38,48], specific glycoprotein binding sites [33–37] appear to be present in the corneal plasmalemma for laminin and collagen. Sugrue [38] has sonicated corneal epithelia and isolated the membranes by sucrose gradient centrifugation. The membrane proteins solubilized in detergent were run over a laminin-sepharose column and those binding to laminin were eluted with a linear salt gradient [34] and subjected to SDS-PAGE. A 65 Kd protein with laminin affinity and lipophilic qualities was recovered from corneal epithelial membranes in this way. It is planned to assay the effect of antibodies to this protein in the biological assay alluded to above in order to determine that it is a cytoskeleton-organizing receptor. Additional studies suggest that a separate collagen receptor with a 47–48 Kd and 70 Kd component also exists [38]. Our working hypothesis at the present time (Fig. 2A) is that corneal epithelial cells have separate receptors for collagen and laminin and that this interaction is stabilized by additional HSPG bonds, perhaps to collagen [38]. We have not as yet searched for a fibronectin receptor because fibronectin is not a consistent component of the corneal basal lamina [43] and needs endogenous collagen to affect the corneal epithelium [46].

It is tempting to think that these putative glycoprotein receptors interact with the cytoskeleton directly or via actin binding proteins (Fig. 2A) to bring about the stimulatory effect of molecules like laminin and collagen on production of ECM by the corneal epithelial cells. How could organization of the cytoskeleton affect protein synthesis? Penman and coworkers [49] hypothesize that translating mRNA is bound with polyribosomes to the cytoskeleton. mRNA has been localized to the cytoskeletal framework by several methods [49,50]. It is likely that residual RER is included in detergent-extracted frameworks [51], but the detergent-resistant protein substructure of the reticulum might be considered part of the cytoskeletal framework [49]. Other ways in which the cytoskeleton could influence protein synthesis that have been

hypothesized include an effect on organization of the intermediate filaments and nuclear matrix [52,53]. We are presently exploring these alternatives using cytoskeletal disrupting drugs to interfere with ECM-stimulated collagen production by corneal epithelium [54].

MESENCHYME-MATRIX INTERACTION

General Rules

The cell surface and cytoskeleton of mesenchymal cells are quite different from those of epithelial cells. In contrast to substrate-attached epithelial cells, fibroblasts do not show polarized budding of viruses [55]. The structural organization [1,24], as well as the biochemical composition [56], of the cytoskeleton of mesenchymal cells can be distinguished from that of epithelial cells. The general rule mesenchymal cells follow is to invade rather than reside on collagen lattices [22,23]. Within collagen lattices, they become highly elongate in shape and resemble their counterparts in situ [21,24]. We do not yet know whether or not elongate fibroblasts in collagen gels are metabolically more active than their abnormally flat counterparts on plastic, but cell shape does affect chondrocyte metabolism [14,15,57].

The nature of the binding site(s) of fibroblasts to ECM is not well understood at present (Fig. 2B,C). One of the problems is that cells that are studied by attachment assays are growing on planar substrata that distort their morphology. There is evidence that fibroblasts [58] and chondrocytes [59] possess direct binding sites for collagen, perhaps in addition to those that seem to bind to collagen via fibronectin [60], and fibroblasts may even develop binding sites for laminin [for further review see 1,37].

The Role of the Cytoskeleton in Corneal Fibroblast Cell Shape Change

Fibroblasts freshly isolated from the corneal stroma with trypsin and collagenase are round in shape. When suspended in collagen gels, they extend filopodia that within a few hours become localized to two opposite poles on the spherical cell (Fig. 6). The cell extends pseudopodia from these same two poles to become bipolar and over the next 15–18 hr the pseudopodia lengthen, causing the cell to become highly elongate [61]. The filopodia contain an actin meshwork but little or no myosin; the mechanism of their formation and movement is not understood [24,61]. The entire cell is rimmed by an actin-rich cortex (Fig. 2B) and it is possible that the pseudopodia extend by sliding of cortical microfilaments past myosin in the cytosol, a mechanism that has also been postulated for migration of the cells through the collagenous matrix [24].

On plastic, the stress fibers composed of actin and myosin that form in the highly flattened cells are strongly attached to the substratum and probably interfere with cell movement [62]. The extreme spreading that occurs on plastic has been equated to an attempt on the part of the fibroblasts to phagocytose the dish [63]. Further distortion of fibroblast morphology on planar substrata includes development of ruffling membranes on leading edges and focal junctions where stress fibers approach the substratum (Fig. 2C). Focal junctions or fibronexi [64] do not occur on fibroblasts in situ [24].

Treatment of corneal fibroblasts suspended in collagen gels with cytoskeletal-disrupting drugs, such as cytochalasin and nocodazole, shows that the first three steps

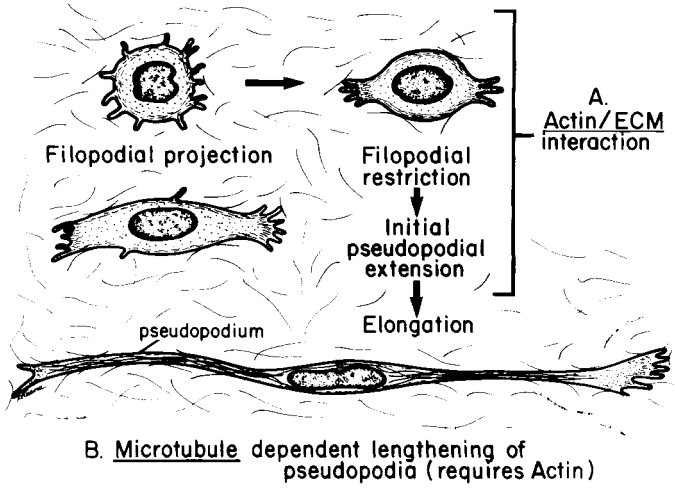

Fig. 6. Diagram summarizing the steps in elongation of corneal fibroblast cultured in collagen gel. A) the first three steps require an intact actin microfilamentous cortex, as they are sensitive to cytochalasin D treatment. B) the final lengthening of pseudopodia requires both intact actin and microtubules. Either cytocholasin or nocodazole prevent elongation. From Tomasek and Hay [61].

in assumption of the bipolar shape (Fig. 6) require actin microfilaments but not microtubules [61]. The subsequent slow elongation of the cells in the collagen gel requires both microtubules and an intact actin cortex. Microtubules run the length of the elongated fibroblast [61] and are probably formed from newly synthesized tubulin. There is evidence in the literature that microtubules interact with actin [65–67] and it is tempting to conclude that interaction of the actin-rich cell cortex with external ECM may be a controlling factor in the organization of microtubules as well as the actin cytoskeleton [61]. It is generally believed that only mesenchymal cells elongate in this fashion when suspended in ECM, but, as we shall now see, epithelial cells retain an inherent capacity to behave like mesenchymal cells when exposed to particular ECM environments.

EPITHELIAL-MESENCHYMAL TRANSFORMATION IN COLLAGEN GELS

There are a number of reports in the literature that epithelia covered on their apical surface with collagen gel form closed structures with basal surface facing the gel, either by migration of cells onto the overlying gel or by an actual reversal of cell polarity [68–71]. We expected, therefore, that when epithelia from embryonic and adult eye tissues were suspended within collagen gels the epithelia would form central lumens and basal surfaces facing the gel. On the contrary, corneal epithelia and endothelium, anterior lens epithelium, notochord, limb epidermis, and even adult thyroid follicles placed in gelling solutions of collagen give off individual, elongated cells that migrate through the collagen lattice and seem to acquire the phenotype of mesenchymal cells [72–74]. Anterior lens epithelium can be dissected readily from the embryo or adult with the basal lamina (lens capsule) intact; because there is no mesenchyme in the vicinity, enzyme treatment is unnecessary to obtain pure epithe-

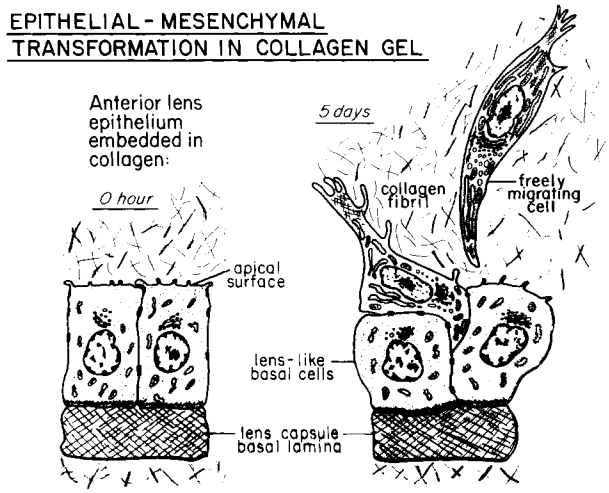

Fig. 7. Diagram showing the transformation of cells derived from lens epithelium to mesenchymelike cells in collagen gel. The epithelium was isolated with its basal lamina intact and the cells next to this lamina (lens capsule) remain epithelial. The elongate cells leaving the former apical surface acquire RER and begin to secrete type I collagen. Based on data of Greenburg and Hay [72].

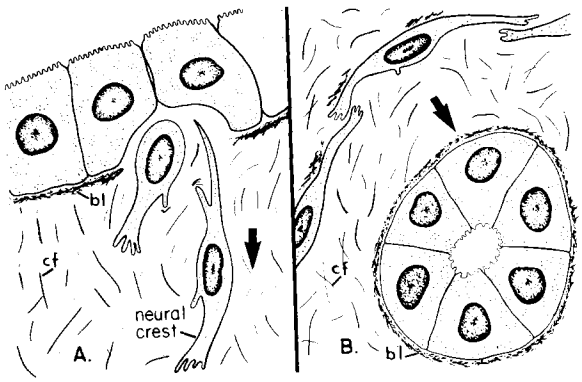

Fig. 8. Diagram showing epithelial-mesenchymal transformation in the embryo. A) mesenchymal cells may arise from simple epithelia by a transformation of the cell surface that permits the cells to extend filopodia and pseudopodia into the ECM [80]. B) some mesenchymal cells redifferentiate into epithelia, as in the case of the kidney. The cells line up and start to produce laminin [82]. bl, basal lamina; cf, collagen fibril. From Hay [1].

lium. The cells attached to the lens capsule remain epithelial, but after a few days the epithelium multilayers and then mesenchymelike cells begin to migrate away from the former apical surface (Fig. 7). The migrating cells acquire RER and new cytoskeletal elements, stop making crystallins, and start making type I collagen [73].

If similar lens explants are placed on top of collagen gels, the cells that migrate away from it give rise to cuboidal epithelia that remain on top of the gel. New basal lamina rich in type IV collagen forms on their basal surface. The epithelial cells do

not produce type I collagen and they continue to produce crystallins [73]. Their morphology is that of typical anterior lens epithelium.

Greenburg and Hay [74] have recently focused attention on the thyroid epithelial-mesenchymal transformation we observed, because of the earlier reports that thyroid epithelium forms follicles when suspended in collagen lattices [68,69]. As in the case of lens, the epithelium multilayers before the transformation occurs, and this takes a few days, which is presumably enough time for the cells facing the gel to proliferate and to change their cell surface and/or cytoskeleton in order to extend filopodia and pseudopodia into the collagen lattice. The apical surface in the center of the follicle remains intact and the cells facing the lumen continue to contain thyroglobulin granules. The cells migrating away from the follicles develop mesenchymelike RER and lose thyroglobulin. We cannot explain the difference between our result [74] and that of Mauchamp's group [68,69]. At least one other laboratory has now observed the formation of individually migrating, elongate cells from thyroid follicles in collagen gels, and they report that concentration of follicles in the gel may affect the result [75].

In attempting to understand this phenomenon, it is important to keep in mind the fact that the transforming cells are leaving a newly multilayered epithelium (Fig. 7). By sending out filopodia and pseudopodia, the surface and cytoskeleton of cells apposed to ECM now behave as those of mesenchymal cells do. The basal surface attached to the lens capsule, however, continues to obey the rules for the basal epithelial surface, remaining flat and well organized, and the apical surface of the thyroid follicle not exposed to collagen retains its expected organization. We conclude that, following a period of proliferation, the cells contacting the collagen gel lose their ability to maintain epithelial polarity and somehow switch on a genetic program that leads to the development of mesenchymal characteristics.

In the embryo, only specific, presumably preprogrammed epithelia give rise to mesenchyme and these epithelia are not multilayered. They are simple or pseudostratified (Fig. 8A). Matrix-filled spaces appear between the lateral surfaces of epithelial cells as they prepare to leave the embryonic epithelium and this change in the cell surface occurs before basal lamina breaks appear [76–80]. Well known examples of epithelial-mesenchymal transformation include primitive streak [76], somites [77], neural crest cells [78], and cardiac cushion cells [79]. An unexpected epithelial-mesenchymal transformation in the embryo has recently been reported by Trelstad et al [81]. During Mullerian duct regression, lateral spaces appear around the epithelial cells, the basal lamina disappears, and the epithelial cells apparently transform into mesenchyme [81]. In all cases, however, it is likely that a change in the epithelial cell surface occurs before it can break the rules of epithelial polarity to extend pseudopodia into the ECM.

Certain mesenchymal cells in the embryo transform into epithelia [82], presumably by developing a new cell surface that is capable of polarizing to form a basal epithelial cortex (Fig. 8B). Based on earlier work [76], it seems reasonable to speculate that only primary mesenchymal cells derived from the primitive streak are capable of undergoing this transformation to epithelium. Mesenchyme that forms later from neural tube, somites, and other mesodermal epithelia rarely [83] if ever gives rise to epithelia and, interestingly enough, our lens-derived mesenchymal cells will not redifferentiate into epithelia either [73]. It also follows from the rules governing the normal basal epithelial surface that malignant epithelial cells must do

more than secrete enzymes to digest underlying basal lamina [84] in order to be able to extend pseudopodia into connective tissue stromas [85]. They must also lose the ability to maintain the organized basal cytoskeleton and flat basal cell surface that normal epithelial cells present to all underlying collagen lattices [18–22].

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