Extracellular Compartments in Matrix Morphogenesis: Collagen Fibril, Bundle, and Lamellar Formation by Corneal Fibroblasts

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ABSTRACT The regulation of collagen fibril, bundle, and lamella formation by the corneal fibroblasts, as well as the organization of these elements into an orthogonal stroma, was studied by transmission electron microscopy and high voltage electron microscopy. Transmission and high voltage electron microscopy of chick embryo corneas each demonstrated a series of unique extracellular compartments. Collagen fibrillogenesis occurred within small surface recesses. These small recesses usually contained between 5 and 12 collagen fibrils with typically mature diameters and constant intrafibrillar spacing. The lateral fusion of the recesses resulted in larger recesses and consequent formation of prominent cell surface foldings. Within these surface foldings, bundles that contained 50–100 collagen fibrils were formed. The surface foldings continued to fuse and the cell surface retracted, forming large surface-associated compartments in which bundles coalesced to form lamellae. High voltage electron microscopy of 0.5 μm sections cut parallel to the corneal surface revealed that the corneal fibroblasts and their processes had two major axes at approximately right angles to one another. The surface compartments involved in the production of the corneal stroma were aligned along the fibroblast axes and the orthogonality of the cell was in register with that of the extracellular matrix. In this manner, corneal fibroblasts formed collagen fibrils, bundles, and lamellae within a controlled environment and thereby determined the architecture of the corneal stroma by the configuration of the cell and its associated compartments.

The rigid control of collagen fibril structure and the arrangement of fibrils into a specific three-dimensional architecture is necessary for the development of a transparent corneal stroma. In this paper we demonstrate how the corneal fibroblast exerts control over collagen fibrillogenesis and the positioning of newly formed fibrils into a highly ordered lamellar matrix.

The mature chick corneal stroma is composed of collagen fibrils arranged as lamellae parallel to the corneal surface. Collagen fibrils within a lamella have the same orientation; collagen fibrils in adjacent layers are oriented approximately at right angles, forming an orthogonal grid. The orthogonal corneal lamellae are composed of small diameter (25 nm) collagen fibrils equidistantly spaced (1, 2). The orthogonal lamellae describe a gradual clockwise shift from epithelium to endothelium of approximately 220°. This clockwise spiral pattern resembles a cholesteric liquid crystal and has the same handedness in both eyes (2, 3); in contrast, all other ocular features, such as the pattern of overlap of the scleral ossicles, demonstrate mirror symmetry (4).

Morphogenesis of the chick corneal stroma occurs in a sequence of relatively well-described stages (for review, see reference 5). Initially, the corneal epithelium deposits the primary corneal stroma beneath its basal surface. The pattern of this epithelially derived acellular stroma is identical to that found in the mature corneal stroma (2). Beginning late in the 5th day of development and continuing through the 12th day, the presumptive corneal fibroblasts migrate into the primary stroma using the primary stroma as a scaffold for their migration (1, 6). By the 14th day of development the fibroblasts have stopped migrating, reach the final adult number, and are producing and depositing the components of the mature or secondary corneal stroma (7, 8). The major macromolecular component of the secondary corneal stroma is type I

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collagen (9), while the minor components are type V collagen (10, 11), chondroitin sulfate proteoglycan (8), keratan sulfate proteoglycan (12, 13), and heparan sulfate proteoglycan (14).

It has been well-established that fibroblasts synthesize collagen and other matrix components. It is less acknowledged that fibroblasts play an active role in collagen deposition and in the assembly of the components producing the complex order found in the matrix. Fibroblasts regulate the stoichiometry and sequence of mixing of the extracellular matrix components within the cell and influence matrix formation through the vectoral discharge of these packaged matrix components into the extracellular space (15, 16). Postdepositional enzymatic modifications, such as procollagen processing and covalent cross-linking, are also important in the establishment of matrix order (17–20) and must be regulated by the fibroblasts (21). In embryonic tendons it has been demonstrated that fibril formation occurs in intimate association with the fibroblast cell surface (22). This association of collagen fibril assembly with the cell surface would provide a mechanism whereby the early events in fibrillogenesis could be regulated by the fibroblast.

In this study we used high voltage electron microscopy (HVEM)[1] to examine 0.5–1.0-μm-thick sections of developing chick corneas to evaluate the relationship between the fibroblast cell surface and the forming collagenous architecture.

MATERIALS AND METHODS

White Leghorn chick embryos were incubated at 37.5°C in a humidified atmosphere and staged according to Hamburger and Hamilton (23).

Corneas, at different stages in development, were dissected and fixed in 4% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4) with 8 mM $CaCl_2$ for 5 min at room temperature, followed by 30–60 min at 4°C. The tissues were washed in 0.1 M cacodylate buffer and postfixed in 1.3% osmium tetroxide in collidine buffer (pH 7.4) for 1 h at 4°C. After fixation, the tissues were dehydrated in a cold graded ethanol series followed by propylene oxide. Infiltration was with an equal mixture of propylene oxide and final embedding mixture for 4 h, propylene oxide and final embedding mixture at 1:5 for 12 h, and finally final embedding mixture for 8 h. The blocks were embedded in a fresh mixture of Polybed 812, nadic methyl anhydride and dodecenylsuccinic anhydride (Polysciences, Inc., Warrington, PA), polymerized at 68°C for 18 h, and sectioned.

Sections of the cornea were cut either perpendicular or parallel to the epithelial surface. For transmission electron microscopy (TEM), sections were cut with a silver to pale gold interference color, while sections for HVEM were cut at 0.5 to 1.0 μm. Sections for HVEM were picked up onto grids with a pale gold Formvar film stabilized by the evaporation of carbon.

TEM sections were stained with 2% aqueous uranyl acetate and 0.2% lead citrate in 0.1 M NaOH. HVEM sections were stained with 2% aqueous uranyl acetate for 1 h at 37°C followed by 30–45 min in 0.2% lead citrate at room temperature. In some cases, the tissues were stained en bloc with uranyl acetate. In these instances, the tissue, after postfixation, was rinsed in buffer and incubated for 1 h in 2% aqueous uranyl acetate at 4°C. The sections were then stained with lead citrate or a combination of uranyl acetate and lead citrate as described. Thin sections were examined and photographed using a Philips 300 transmission electron microscope. Thick sections were examined at an accelerating voltage of 1 million V using the AEI EM 7 high voltage electron microscope at the New York State Department of Health Laboratories in Albany, New York.

For light microscopic autoradiography, the cornea was carefully removed with a ring containing the scleral ossicles, washed three times in Earle's balanced salt solution, and placed in organ culture. Labeling was done in Delbecco's modified Eagle's medium containing 0.5% fetal bovine serum, 50 μg/ml ascorbate, 4 mM glutamine, 50 U/ml penicillin G, and 30 μCi/ml [^{3}H]proline (specific activity 160 Ci/mmol) at 37°C in a humidified atmosphere of 5% CO_2, 95% air. The tissues were labeled continuously for 4 h. After the labeling period, the corneas were washed five times for >30 min with complete medium without labeled proline, followed by three washes with Earle's balanced salt solution over 15 min. The tissues were then fixed and embedded as previously described. 1-μm sections were cut for light microscopy, dip-coated with Kodak NTB-2 emulsion, exposed, developed, stained with toluidine blue, and examined.

Corneas were labeled as described above. The extent of procollagen processing was determined after SDS PAGE and fluorography. The central cornea was removed with a 2 mm trephine, frozen in liquid nitrogen, and pulverized with a mortar and pestle. The tissue was suspended in electrophoresis sample buffer and heated to 100°C for 5 min. The extracted collagen solution was clarified by centrifugation, dialyzed against buffer, and electrophoresed (24). After electrophoresis, the gel was fixed, impregnated with ENHANCE (New England Nuclear, Boston, MA), dried, and exposed to Kodak X OMAT film at −70°C. After exposure, the fluorogram was developed and the relative amounts of processed and unprocessed procollagen were determined.

RESULTS

A major finding in these studies was that the corneal fibroblast has a complex topography which serves to compartmentalize the extracellular space and that these extracellular compartments, defined by the fibroblast cell surface, are related to the deposition of matrix in an orthogonal pattern. The complex specializations of the corneal fibroblast cell surface are important for at least three different levels of stromal organization: collagen fibrils, fibril bundles, and lamellae.

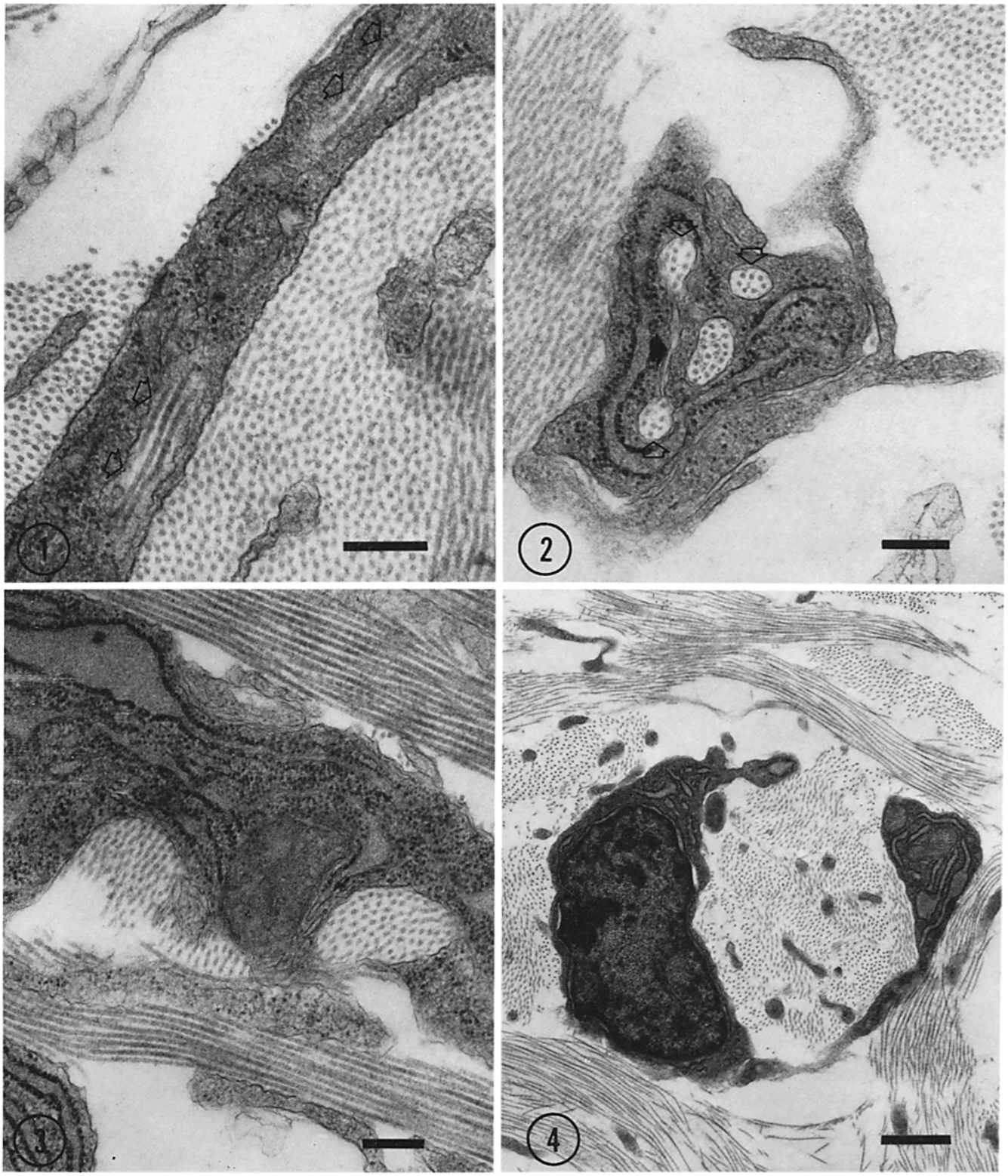
The 14-d-old (stage 40) chick embryo cornea was studied using conventional TEM and thin sections cut perpendicular to the corneal surface. The fibroblasts have small surface recesses that are delimited by the cell. Small surface recesses contain 5–12 collagen fibrils and are relativley uniform in size and content. When the corneal stroma was sectioned perpendicular to the corneal surface, these small surface recesses were seen in both longitudinal (Fig. 1) and cross section (Fig. 2). Fusion of two or three smaller recesses with consequent coalescence of the fibrils into small bundles was seen often. Occasionally, the actual process of fusion was observed as indicated by a membranous connection between two small recesses (Fig. 2). Small surface recesses continued to fuse laterally with a consequent convolution of the cell surface that resulted in a folding of the fibroblast surface (Fig. 3). Within these surface foldings, collagen fibrils coalesced to form fibril bundles that contained 50–100 collagen fibrils.

In the next stage, the surface foldings fused, then receded, and the surface retracted, forming large surface-related compartments that were surrounded by the cell (Fig. 4). Within these large surface compartments, fibril bundles coalesced to form lamellae. The forming lamellae within these spaces were completely enveloped by the fibroblast and were also intimately associated with the cell surface, with surface foldings and processes interdigitating between the fibril bundles.

Light microscopic autoradiography of the 14-d-old cornea after 4 h of continuous labeling with [^{3}H]proline revealed the site of fully processed and discharged collagen within the extracellular matrix. After 4 h of labeling, ~60% of the labeled collagen was fully processed (data not shown). This time point was chosen to specifically identify the site of labeled collagen in the extracellular matrix/cell perimeter. Label was not uniformly distributed throughout the stroma. Instead, label was localized in specific cell-surrounded sites (Fig. 5).

The corneal fibroblast and the extracellular matrix was studied further using HVEM and 0.5–1.0-μm sections. In most of these preparations, the 14-d-old chick embryo corneas were cut parallel to the corneal surface so that the corneal fibroblast could be studied in the same plane as the lamella. The use of the HVEM has contributed significantly to our characterization of the topography of the fibroblast and significantly clarified the structure and possible role of the dif-

[1] *Abbreviations used in this paper.* HVEM, high voltage electron microscopy; TEM, transmission electron microscopy.



FIGURES 1–4 Transmission electron micrographs of fibroblasts from 14 d (stage 40) chick embryo corneas cut perpendicular to the corneal surface. Figure 1: A fibroblast process contains a small recess in the cell surface (arrows) with several collagen fibrils in longitudinal section. The fibrils in the recess, which are presumably in the process of being deposited, are perpendicular to the orientation of the fibrils on either side of the cell process. × 50,000. Figure 2: A corneal fibroblast that contains four small surface recesses is pictured. Three of the recesses are similar in size and contain 7–10 collagen fibrils (open arrows) while the fourth recess contains about 19 fibrils. Two of the smaller recesses are undergoing coalescence as indicated by the membranous connection joining them (solid arrow). × 40,000. Figure 3: A corneal fibroblast with two large surface foldings in cross-section is shown. The continued lateral fusion of surface recesses results in the formation of larger recesses and surface foldings as seen in Fig. 2. The fusion causes a folding of the cell surface, and within these foldings collagen fibrils coalesce to form bundles which contain 50–100 collagen fibrils. × 36,500. Figure 4: A corneal fibroblast with a large surface-associated compartment is seen. The large surface-associated compartment forms as a result of continued fusion of surface folds and a retraction of the cell surface. The result is a large extracellular compartment completely enveloped by the corneal fibroblast with its fibrillar contents in intimate association with cell processes and surface infoldings. Within these compartments fibril bundles coalesce to form larger bundles and lamellae. × 12,000. (Bars: Figs. 1–3, 300 nm; Fig. 4, 1.0 μm.)

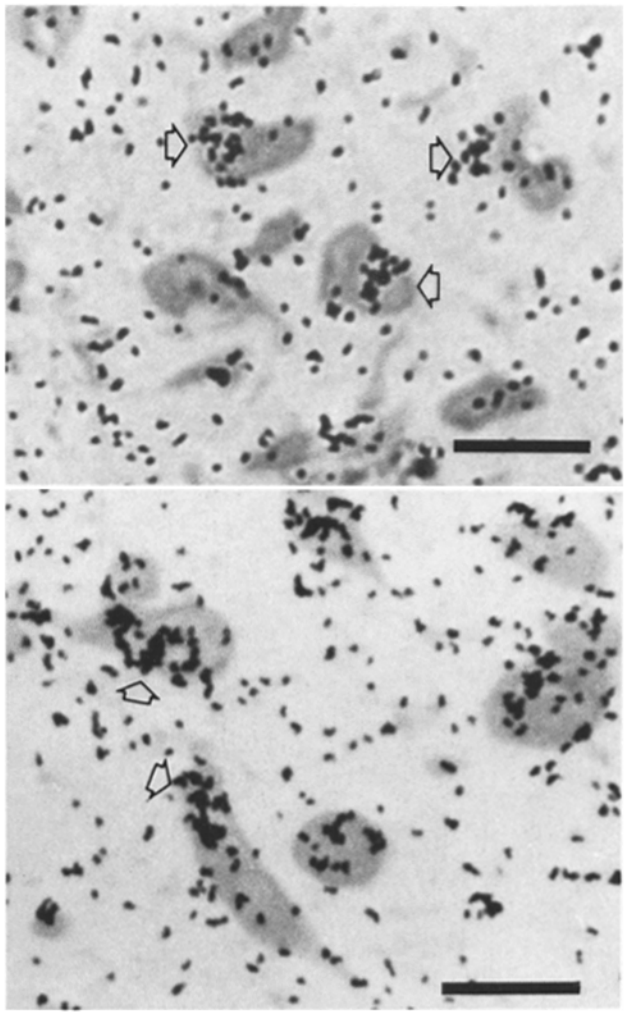


FIGURE 5 Light microscopic autoradiograms of 14-d-old chick embryo corneas labeled continuously with [^{3}H]proline for 4 h and cut perpendicular to the corneal surface. These micrographs show that collagen is not secreted randomly over the entire cell surface. Instead, release into the extracellular space and fibrillogenesis are localized at specific sites. These sites are often seen as lucent regions surrounded by the cell and cell processes (arrows). These regions are the large surface-associated compartments seen in Fig. 4. Bar, 10 μm. × 1,800.

ferent surface-defined spaces in collagen fibril formation and in the determination of tissue architecture. The use of thick sections and HVEM was similar to studying 5–15 perfectly aligned serial thin sections with the conventional transmission electron microscope. This substantially increased sample volume provided images in which the complexity of the fibroblast cell surface could be immediately appreciated. These thick sections also contained significant information on the three-dimensional relationship of the fibroblast to the extracellular matrix.

In thick sections, small surface recesses, that contained several collagen fibrils, were extensive in their length. The recesses coursed through a large portion of the corneal fibroblast (Fig. 6), often running from a perinuclear position to the more peripheral regions of the cell. The proximal ends of the small surface recesses, containing 5–12 collagen fibrils, were frequently observed in a perinuclear position in association with the Golgi apparatus and secretory vacuoles (Figs. 7 and 8). When these surface recesses were cut perpendicular to the corneal surface, as with the TEM data, they were seen to contain 5–12 collagen fibrils in cross section (Figs. 2, 6). In longitudinal section it was apparent that these recesses communicate directly with the extracellular space. The collagen fibrils seen within these recesses had diameters of ~25 nm and an interfibrillar spacing of 60 to 70 nm identical to that seen in the extracellular space.

In 0.5-μm sections, the corneal fibroblast had a complex surface topography. The cell surface was intimately associated with the collagenous extracellular matrix (Fig. 9). When thick sections were cut parallel to the corneal surface, fibril bundles were found within prominent surface foldings and within these foldings the fibril bundles were intimately associated with the cell surface and foldings from the cell surface (Fig. 9*a*, curved arrows). The surface foldings fused more peripherally, giving rise to large surface compartments in which bundles coalesced to form larger bundles and fused to form lamella (Fig. 9*a*, arrows). In thick sections cut perpendicular to the corneal surface through a region similar to that designated by the arrows in Fig. 9*a*, the large surface compartments were seen in cross section (Fig. 9*b*). These surface compartments contained large bundles which coalesced to form lamella. The bundles and forming lamellae were in intimate association with the cell surface through foldings and processes from the cell surface that interdigitate among fibril bundles.

When the corneal fibroblasts and their surrounding extracellular matrix were studied, using 0.5–1.0-μm sections cut parallel to the corneal surface, both the collagenous extracellular matrix and the fibroblasts were arranged with two major axes at approximately right angles to one another. The orthogonality of the stroma is in register with that of the cells (Figs. 9*a*, 10).

Within the corneal stroma there were very few single collagen fibrils in the extracellular space. The collagen fibrils were organized as fibril bundles composed of 50–100 collagen fibrils. The collagen fibril bundle was a distinct structural element within the chick secondary corneal stroma. The fibril bundle, not the collagen fibril, was the principal structure produced by the fibroblast and used in the construction of the corneal stroma. The fibril bundles were positioned by the fibroblast within large surface-associated compartments forming orthogonal lamellae in the corneal stroma (Figs. 9 and 10).

In sections cut parallel to the corneal surface, often there are 90° changes in the direction of fibril bundles within the corneal stroma. These changes in direction are along the established orthogonal axes. In sections cut perpendicular to the corneal surface, the collagenous lamellae are not always parallel to the corneal surface, but are "sinusoidal" or "undulating." Accordingly, in some sections cut parallel to the corneal surface, fibrils were observed in oblique planes of section.

The corneal fibroblasts and their cell processes were also observed to have two major axes at approximately right angles to one another (Figs. 9*a* and 10). The positioning of surface recesses, foldings, and surface-associated compartments with their related collagen fibrils, fibril bundles, and forming lamellar structures defined the functional axes of the corneal fibroblast. These surface specializations were positioned so that the physical and functional axes of the corneal fibroblast were superimposed during the morphogenesis of the second-

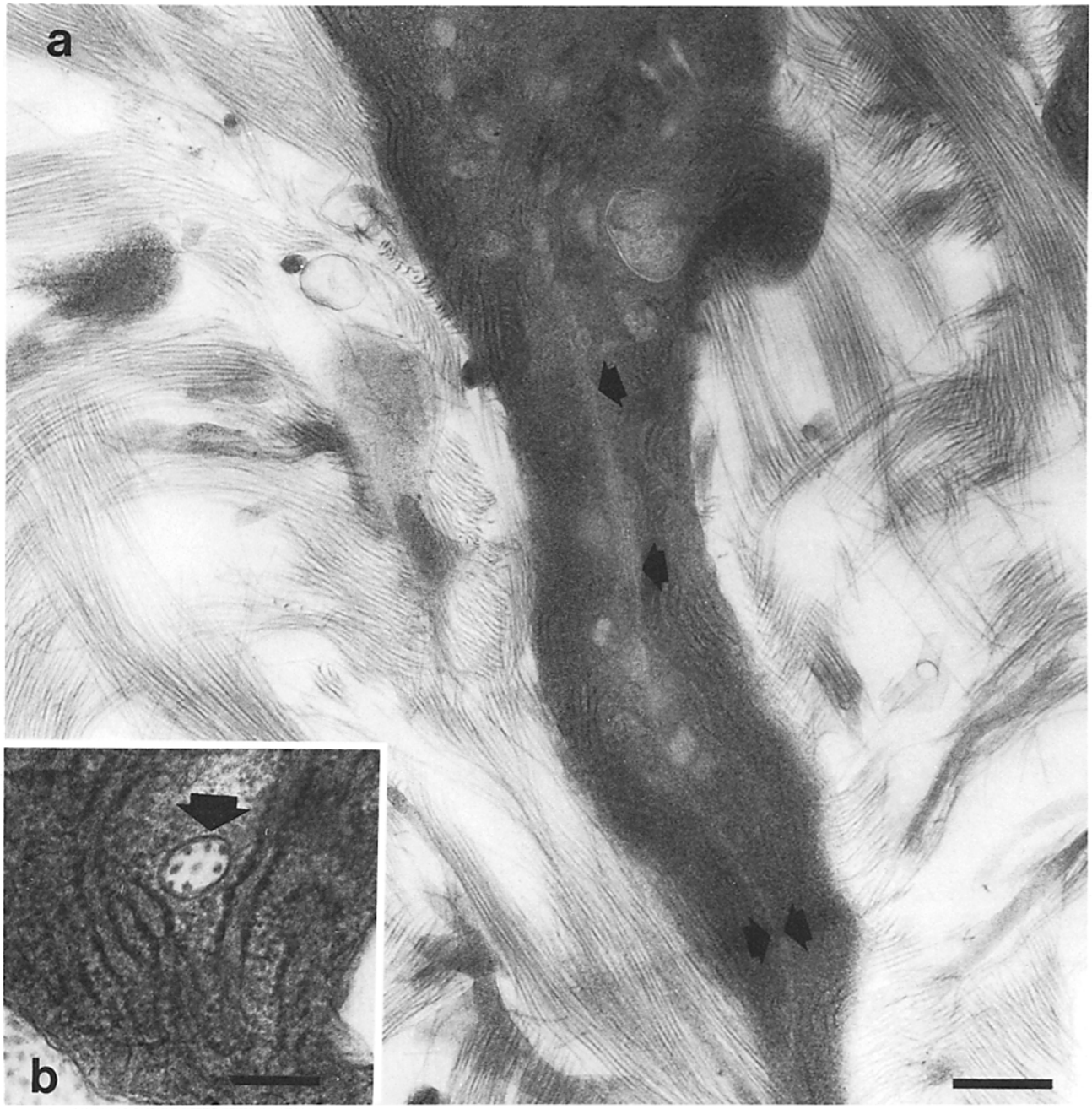


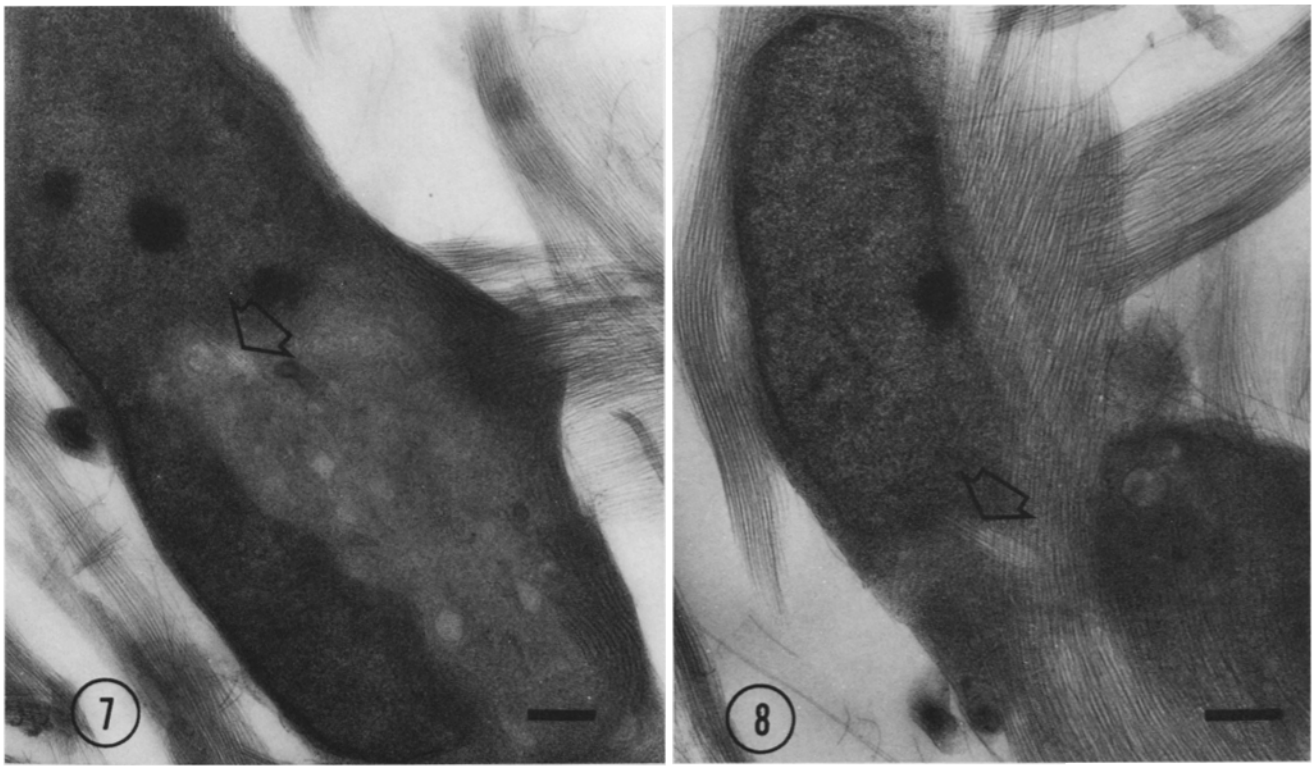
FIGURE 6 (a) High voltage electron micrograph of a corneal fibroblast cut parallel to the corneal surface. A small surface recess that contains collagen fibrils (arrows) is seen to course through most of this section. This micrograph illustrates how extensive the surface recesses can be in their extension from within the cell to the cell surface. Bar, 1.0 μm. × 15,500. (*b*) This micrograph shows a small surface recess that contains eight collagen fibrils, similar to that seen in *a*, cut in cross section (arrow). Compare with those seen in Fig. 2. The fibril diameters are small and the intrafibrillar spacing in the small recesses is relatively regular. Bar, 300 nm. × 50,000.

ary corneal stroma. The orthogonality of the corneal fibroblasts and their processes was in register with that of the collagen fibril bundles and forming lamellae in the extracellular matrix.

DISCUSSION

The corneal fibroblast cell surface defined at least three major extracellular compartments that were under cellular regulation and involved in the control of collagen fibrillogenesis, fibril bundle formation, and the morphogenesis of the corneal stroma.

The first compartment was a small recess initimately associated with the fibroblast cell surface. These surface recesses normally contained from 5 to 12 collagen fibrils, although occasionally recesses that contained 1 or 2 fibrils were observed. The formation of the small recesses probably occurred by the tandem fusion of secretory vacuoles with the cell surface and consequently with one another. A similar process of compound exocytosis has been described in the chromaffin and mast cell (25, 26). The small surface recesses were the initial extracellular sites of collagen fibrillogenesis. This is in agreement with data from embryonic tendon fibroblasts



FIGURES 7 and 8 High voltage electron micrographs of fibroblasts from 0.5-μm sections of 14-d-old chick embryo corneas cut parallel to the corneal surface. The micrographs show small surface recesses cut in longitudinal section (arrows) that contain collagen fibrils. The proximal ends of small recesses are often found in a perinuclear position and associated with the secretory components of the corneal fibroblast. Bars, 1.0 μm. Fig. 7, × 9,200; Fig. 8, × 10,000.

which has demonstrated that small surface recesses are the initial sites of fibril formation (22).

In the corneal fibroblast, the next compartment was a larger surface folding in which collagen fibrils were collected into small fibril bundles that contained 50–100 collagen fibrils. These spaces appeared to result from the lateral fusion of several of the small recesses.

The third cell-defined space which we distinguished was the large surface compartment in which bundles coalesced into larger bundles and lamellae. These spaces were surrounded by the cell and its processes, and the contents of this compartment remained associated with the cell surface through surface foldings and the interdigition of cell processes. These spaces appeared to form after the fusion of the bundle-forming surface foldings, and after a breakdown and retraction of the fibroblast surface.

The corneal fibroblast thus partitioned the extracellular space into at least three distinct types of compartments and it was within these compartments that collagen fibril formation, bundle formation, and the initial organization into lamellae occurred in a sequential manner.

The corneal fibroblast replicates a template provided by the corneal epithelium and thereby determines the orthogonal architecture of the secondary corneal stroma. How matrix order is interpreted by cells and consequently replicated is poorly understood. We found that the corneal fibroblast had an orthogonal configuration when studied in thick sections cut in the plane of the corneal lamellae. Both the fibroblast and its cell processes were aligned along two distinct axes at ~90° to one another. The orthogonal shape of the fibroblast was thus in register with the epithelially derived template it invaded and the collagen fibril bundles in the extracellular space which it deposited. Our interpretation of these data is that the corneal fibroblasts migrated into the primary corneal stroma, using the initial orthogonal collagen lattice as a scaffold, and positioned their axes in register with it. The fibroblasts were then aligned with the same orientation as the primary corneal stroma, and the collagen fibril bundles and lamellae that the fibroblasts produced were ordered because the cell that deposited them was ordered. We tentatively conclude that the transfer of the epithelial template to the stroma requires the unique spatial organizing capacity of the intermediary fibroblast and does not simply represent a situation in which the fibroblast-derived matrix "crystallizes" onto the epithelial template.

It is apparent that newly formed collagen fibril bundles were growing away from the fibroblast or that the fibroblast migrated away from the fibrils, or both. During the period in which the secondary stroma forms, the eye is rapidly growing (27). Growth in size could provide the forces necessary for the fibroblast to "spin" fibril bundles from its surface, and could partially account for the necessary translocation of cells and fibrils. In addition, as the fibroblast forms bundles there may be a migration of the fibroblast away from the bundle. One explanation for the large number of 90° bends seen in fibril bundles is that as the bundles form, the fibroblast migrates along one of the orthogonal axes of the cornea. Since the corneal fibroblast simultaneously forms bundles in two directions perpendicular to one another, movement along one of the axes would result in one straight bundle and one bundle with a 90° bend. This process would provide the necessary translocation and explain the 90° changes in bundle direction.

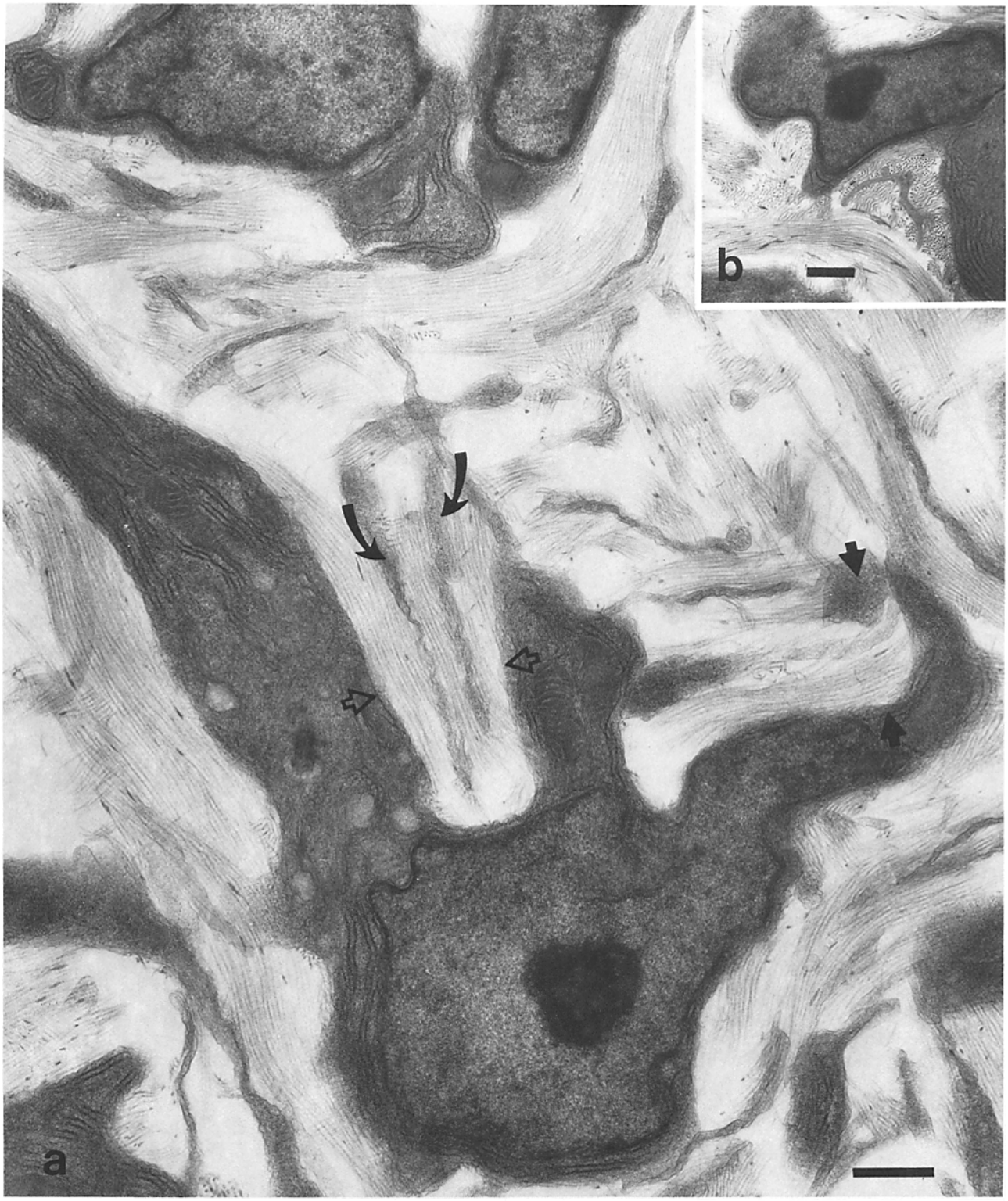


FIGURE 9 (*a*) A high voltage electron micrograph of a 0.5-μm–thick section from a 14-d-old chick embryo cornea cut parallel to the corneal surface. The orthogonality of the corneal fibroblast and its processes is illustrated. Bundles of collagen fibrils are seen within cell surface foldings. Cell processes (curved arrows) or ridges in the cell surface are seen separating fibril bundles. A fusion of these compartments and a retraction of the cell surface forms large surface-associated compartments as seen in longitudinal section at the arrows. The formation of these compartments is seen along two major axes at approximately right angles to one another (open vs. closed arrows). It is within these cell-associated compartments that collagen fibril formation, fibril bundle formation, and lamellar formation occurs. These processes presumably are occurring in two orthogonal directions simultaneously. Bar, 1.0 μm. × 13,500. (*b*) A high voltage electron micrograph of a 14-d-old chick embryo corneal fibroblast. This micrograph is of a 0.5-μm section cut perpendicular to a region similar to that designated by the arrows in *a*. A large surface compartment with fibril bundles coalescing to form larger bundles and lamellae is illustrated. This occurs in association with the fibroblast surface with cell processes and surface foldings seen interdigitating between bundles within this compartment. Bar, 1.0 μm. × 8,000.

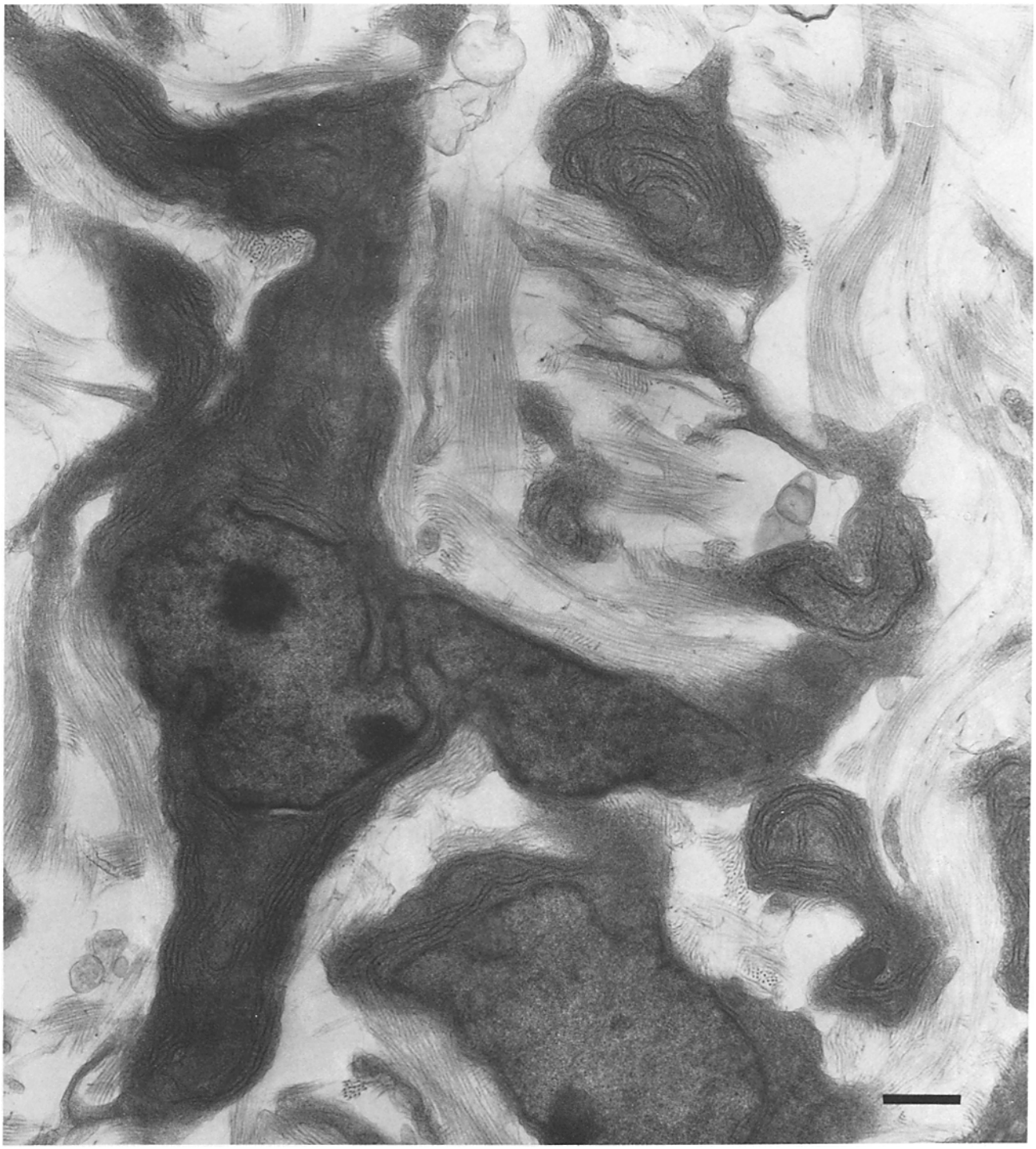


FIGURE 10 A high voltage electron micrograph of a 14-d-old chick embryo fibroblast cut parallel to the corneal surface. In this 0.5-μm section the orthogonality of the corneal fibroblast and its processes is apparent. The positioning of the cell mirrors the orthogonality of the collagenous bundles within the developing stroma and the primary stroma it invades. We conclude that the corneal fibroblast migrates into the ordered primary stroma and aligns itself using this initial orthogonal lattice. This results in the fibroblasts being aligned with their axes at ~90° to one another. These aligned fibroblasts then deposit fibril bundles and lamellae with an orthogonal orientation. Bar, 1.0 μm. × 13,000.

The compartmentalization of the extracellular space by the fibroblast as seen in the developing secondary corneal stroma would permit the cell to control all of the important events in collagen fibril, bundle, and lamellae formation. Table I and Fig. 11 (a diagramatic representation of a corneal fibroblast) summarize our current model.

The compartmentalization of the intracellular space for the initial events necessary for collagen fibrillogenesis, synthesis, triple helix formation, and posttranslational modifications is well-established (28, 29). It is within the Golgi apparatus and secretory vacuoles that packaging and initial aggregate formation occurs between collagens, collagenous and noncolla-

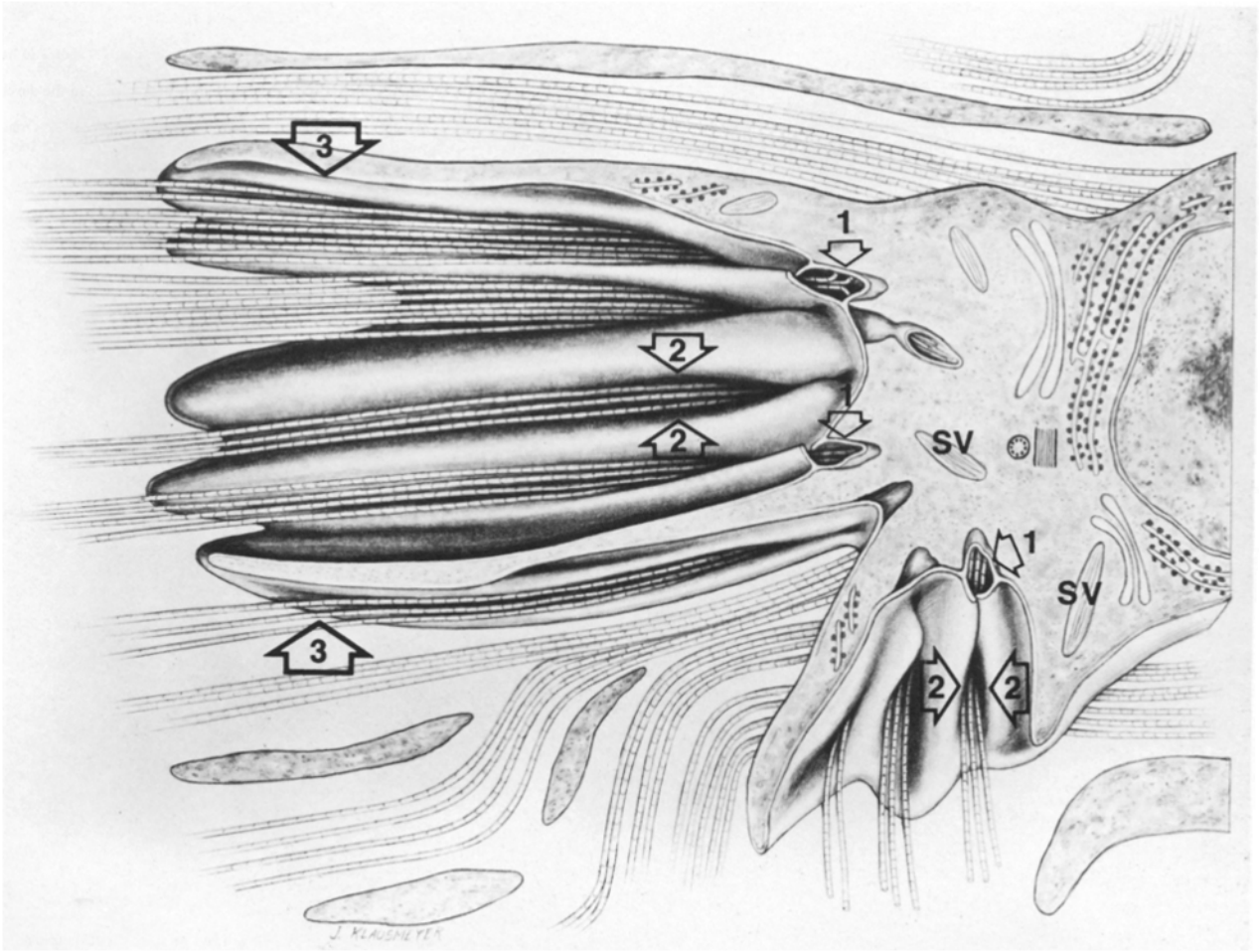


FIGURE 11 This diagram and Table I (at right) summarize our proposed model of the compartmentalization of the extracellular space by the corneal fibroblast and the role of these different compartments in the regulation of collagen fibril, bundle, and lamellar formation, and the consequent determination of tissue architecture. The synthesis, posttranslational modification, and packaging of procollagen occurs within a series of intracellular compartments. The intracellular pathway involves the rough endoplasmic reticulum, the Golgi apparatus, and secretory vacuoles (*SV*). The secretory vacuoles release their contents by fusion with the cell surface. This fusion forms the first extracellular compartment and if vacuoles fuse in a compound manner, the small surface recess (*1*) is formed. With this event the process of collagen fibrillogenesis becomes an extracellular process. it is within these small surface recesses that the initial extracellular events in collagen fibril formation occur. These recesses then fuse laterally forming larger recesses and surface folds (*2*). Within this compartment, collagen fibrils are collected into fibril bundles which contain 50–100 collagen fibrils. These surface foldings continue to fuse with a breakdown and retraction of the cell surface forming large surface-associated compartments (*3*). Within this compartment fibril bundles coalesce to form larger bundles and lamellae. In this manner the corneal fibroblast forms collagen fibrils, bundles, and lamellae within a sequence of regulated compartments in which matrix-macromolecular interactions as well as postdepositional enzymatic modifications such as procollagen processing and covalent cross-linking can occur. The corneal fibroblast positions its axes orthogonally, presumably using spatial cues provided by the primary stroma, and in this way determines the orthogonal arrangement of the newly formed fibril bundles and lamellae in the secondary corneal stroma.

genous matrix components (15, 16, 30, 31). Finally, the secretory vacuoles discharge their contents into the extracellular compartments via exocytosis.

An additional intracellular compartment, the phagocytic vacuole, has been described in a number of systems in which there is a rapid turnover or remodeling of connective tissue. Intracellular collagen fibrils have been described within these phagocytic vacuoles (32–35) that differ considerably in detail from the extracellular compartments described in the cornea. Moreover, the corneal fibroblast during this stage in development is a very active secretory cell with a large net positive synthesis. For example, we have calculated from the data of Coleman et al. (7) and Conrad (8) that each corneal fibroblast secretes ~1–3 million collagen molecules per hour during days 11 to 16 of development. The recesses and folds contain native collagen fibrils identical in morphology and in direct continuity with those seen in the extracellular space and these recesses do not contain amorphous material indicative of a secondary lysosome.

The distinct extracellular compartments are important not only in the physical positioning of collagen fibrils, fibril bundles, and lamellae, but also because this extracellular compartmentalization effected by the fibroblast provides a series of unique spaces, intimately associated with the cell surface, in which the extracellular events in collagen fibrillogenesis can occur sequentially. Through the control of these distinct microenvironments the fibroblast is able to manipulate extracellular processes in the same way intracellular processes are

TABLE I

Intracellular and Extracellular Compartments Involved in Collagen Fibrillogenesis

Compartment	Event
Rough endoplasmic reticulum	Collagen polypeptide synthesis. Triple helix formation.
Golgi apparatus	Packaging.
Secretory vacuoles (*SV*)*	Homo/Heteropolymeric mixing. Initial steps in aggregation.
Secretory vacuoles (*SV*)*	Discharge from cell.
Small surface recesses (*1*)*	Collagen fibril formation. Procollagen processing?
Surface foldings (*2*)*	Fibril bundle formation. Cross-linking?
Surface-associated compartments (*3*)*	Bundle alignment and formation of lamellae.

*Refers to cellular compartments in Fig. 11.

controlled. A modification of the milieu within small surface recesses through a change in the ionic conditions, and/or addition of other matrix components such as proteoglycans, fibronectin, or other collagen types could be important in the control of collagen fibril structure. The environments within recesses and surface foldings might be controlled to favor a particular postdepositional enzymatic event, such as the processing of the amino and/or carboxy propeptides of procollagen and covalent cross-linking.

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