

# Type VII Collagen Forms an Extended Network of Anchoring Fibrils

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**Abstract.** Type VII collagen is one of the newly identified members of the collagen family. A variety of evidence, including ultrastructural immunolocalization, has previously shown that type VII collagen is a major structural component of anchoring fibrils, found immediately beneath the lamina densa of many epithelia. In the present study, ultrastructural immunolocalization with monoclonal and monospecific polyclonal antibodies to type VII collagen and with a monoclonal antibody to type IV collagen indicates that amorphous electron-dense structures which we term "anchoring plaques" are normal features of the basement membrane zone of skin and cornea. These plaques contain type IV collagen and the carboxyl-terminal domain of

type VII collagen. Banded anchoring fibrils extend from both the lamina densa and from these plaques, and can be seen bridging the plaques with the lamina densa and with other anchoring plaques. These observations lead to the postulation of a multilayered network of anchoring fibrils and anchoring plaques which underlies the basal lamina of several anchoring fibril-containing tissues. This extended network is capable of entrapping a large number of banded collagen fibers, microfibrils, and other stromal matrix components. These observations support the hypothesis that anchoring fibrils provide additional adhesion of the lamina densa to its underlying stroma.

**B**ASEMENT membranes are widely distributed in vertebrate tissues and serve a variety of functions including molecular ultrafiltration, tissue organization, and mediation of the interactions between specific cell layers or cells and their underlying stroma. Most basal laminae separate cells from adjacent matrix. The attachment of the epithelial cell layer to the lamina densa has been well studied and numerous anchoring devices have been postulated (Kanwar and Farquhar, 1980; Hay, 1982; Gipson et al., 1983), yet the mechanism of attachment of the lamina densa to the underlying stroma is unknown. Ruthenium red-staining filaments have been found at the lamina densa-stroma interface in all tissues. These have been suggested as one attachment device (Wasano and Yamamoto, 1985), although the molecular mechanisms of attachment are unknown. In addition, some tissues demonstrate structures known as anchoring fibrils (Palade and Farquhar, 1965; Bruns, 1969). Ultrastructural observations suggest that these specialized fibrous structures provide additional attachment between the basal lamina and the underlying matrix (Susi et al., 1969; Kawanami et al., 1979). Attachment has been suggested to occur by physical entrapment of banded collagen fibers and other microfibrillar matrix components between the anchoring fibril arches and the lamina densa. This role of anchoring fibrils in securing the lamina densa to the underlying dermis of skin has recently been questioned (Wasano and Yamamoto, 1985). An-

choring fibrils with arching morphology entrapping other matrix fibrils are a relatively rare occurrence. Far more often, anchoring fibrils project perpendicularly from the basement membrane, one end inserting into the lamina densa and the other often terminating in an amorphous electron-dense plaque. Since these anchoring fibrils do not have any apparent interaction with fibrous matrix components, their role in basal lamina attachment to matrix is unclear.

We have recently demonstrated that anchoring fibrils contain type VII collagen as a primary structural element (Bentz et al., 1983; Burgeson et al., 1986; Morris et al., 1986; Sakai et al., 1986b; Lunstrum et al., 1986). The fibrils are unstaggered parallel aggregates of antiparallel dimeric type VII collagen molecules. The distal ends of type VII procollagen molecules, and therefore anchoring fibrils, contain large and complex globular domains (Lunstrum et al., 1986). As illustrated in Fig. 1, type VII collagen molecules have been proposed to condense laterally to form anchoring fibrils. This condensation may involve proteolytic removal of the amino-terminal globular domains. Although illustrated as fully extended structures with the same length as type VII collagen molecules, most anchoring fibrils seen ultrastructurally appear arched, or otherwise contorted reflecting the flexibility of the collagen VII triple helix. Fully extended anchoring fibrils approach the lengths measured for type VII molecules (Bruns, 1969; Kawanami et al., 1979).

We have produced both monoclonal and monospecific polyclonal antibodies to the terminal globular domains. By ultrastructural immunolocalization studies, we have been able to define the relationship of the terminal ends to the architecture of the basement membrane zone (Sakai et al., 1986b). The studies detailed in this report strongly suggest that anchoring fibrils form an extended network within the subbasal lamina capable of physical entrapment of large numbers of matrix components. Since the postulated anchoring fibril network is far more extensive than previously appreciated, these studies lend credence to the hypothesis that these structures can serve as true anchoring devices.

## Materials and Methods

### Immunological Reagents

A monoclonal antibody to the carboxyl-terminal end of the type VII collagen triple-helical domain (mAb-VII) has been described (Sakai et al., 1986b). Polyclonal antiserum made to whole type VII procollagen in a New Zealand White Rabbit (pAb-VII) which recognizes the carboxyl-terminal globular domain has been recently reported (Lunstrum et al., 1986). Another monoclonal antibody (mAb-161) has been described (Hessle et al., 1984) and has recently been shown to be specific for an epitope within the carboxyl-terminal globular domain of type VII procollagen (Lunstrum et al., 1986). A monoclonal antibody to human type IV collagen (mAb-IV) has been described (Sakai et al., 1982), and this antibody recognizes an epitope ~60 nm from the "7S" domain (Dieringer et al., 1985).

### Immunoelectron Microscopy

En bloc labeling of tissue samples using monospecific antibodies has been described (Sakai et al., 1986b). Briefly, unfixed tissue pieces are incubated in first antibody, washed extensively, and then incubated with second antibody which is bound to colloidal gold of defined size. After further washing, the tissue is fixed, embedded, sectioned, stained, and examined using a Philips 410 LS electron microscope.

Double labeling was performed using two monospecific first antibodies raised in different species. Second antibodies, with differently sized colloidal gold particles attached, were species specific. Thus, in a single experiment, the binding of pAb-VII (a rabbit IgG) was detected by a goat anti-rabbit IgG second antibody bound to 15-nm gold, and the binding of mAb-IV (a mouse IgG) was detected by goat anti-mouse IgG second antibody bound to 5-nm gold.

Section surface labeling using colloidal gold has been previously described (Carlemalm et al., 1980). By this protocol, thin sections from weakly fixed tissue embedded at 4°C in Lowicryl K4M were incubated with first antibody, washed, incubated with gold-conjugated second antibody, washed, and examined.

For either protocol, control experiments included absence of first antibody and the substitution of another first antibody of the same immunological subtype but of known and unrelated specificity.

### Morphometric Measurements

The shortest distance from the edge of the lamina densa to an immunologically identified anchoring plaque was measured using a digitizing tablet from Bioquant (Nashville, TN).

## Results

### The Characterization of Immunological Reagents

Fig. 1 schematically illustrates the proposed relationship of type VII collagen to anchoring fibrils. In addition, the location of the epitopes recognized by mAb-VII, mAb-161, and pAb-VII are indicated. All antibodies have been previously well characterized with regard to specificity and epitope location (Sakai et al., 1986b; Lunstrum et al., 1986; Hessle et al., 1984).

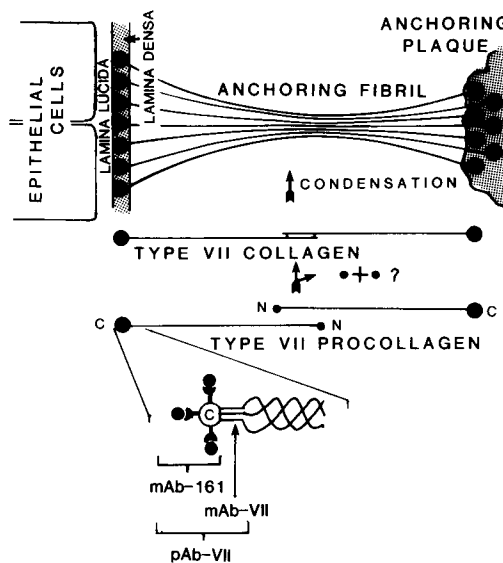
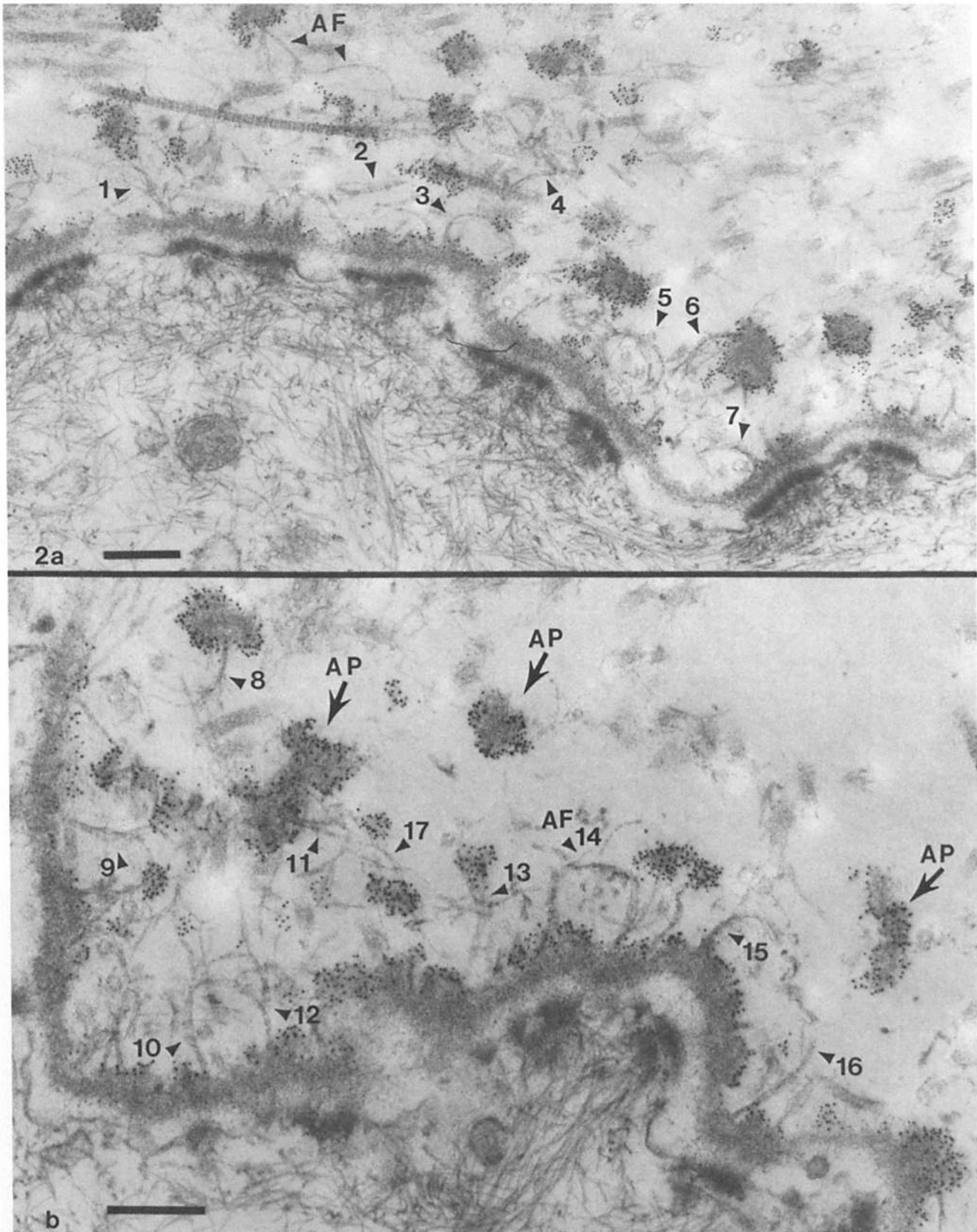


Figure 1. The cartoon illustrates the hypothetical relationship between type VII collagen and an anchoring fibril. An anchoring fibril is depicted between the lamina densa and an anchoring plaque. Type VII procollagen molecules dimerize, perhaps with the subsequent processing of the amino-terminal globular domain (● + ●). These dimers then condense to form anchoring fibrils. The anchoring fibrils interact with the lamina densa and anchoring plaques through the multidomained carboxyl terminus. An arrow indicates the epitope recognized by mAb-VII, and brackets indicate the regions recognized by mAb-161 and pAb-VII.

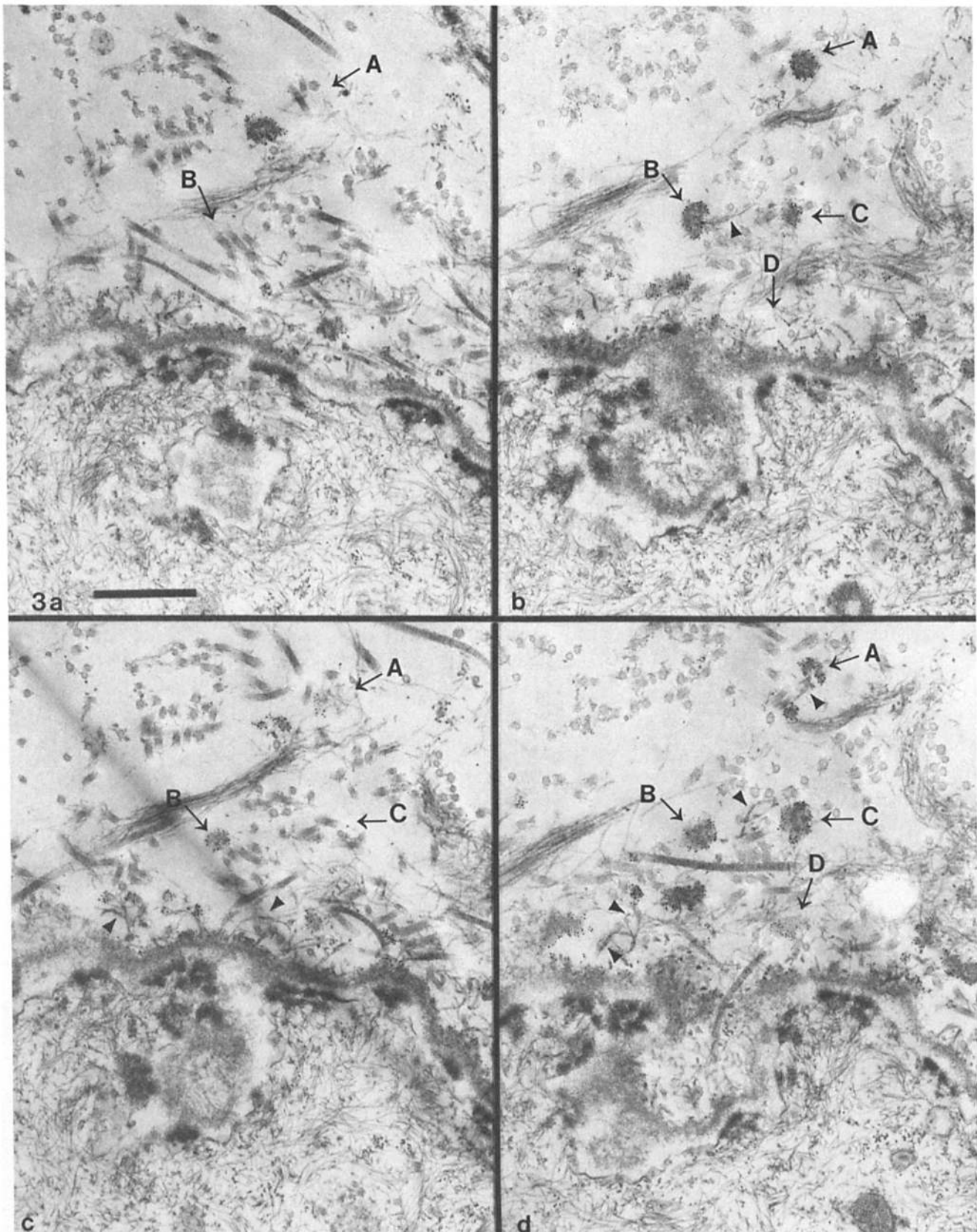
### Ultrastructural Immunolocalization of the Type VII Collagen Carboxyl-terminal Globular Domain to "Anchoring Plaques"

Ultrastructural immunolocalization of the carboxyl-terminal end of type VII collagen using en bloc procedures (see Materials and Methods) with pAb-VII (Fig. 2, a and b) give identical results to those obtained with mAb-VII (Sakai et al., 1986b). These antibodies directed intense colloidal gold deposition upon the lamina densa as well as upon amorphous electron-dense structures (marked AP in Fig. 2 b) in the dermal stroma beneath the basement membrane. Extensive gold deposition was not observed upon the lamina lucida. This result indicates that anchoring fibril carboxyl termini exist in both the lamina densa and these dermal plaques. As expected when using the en bloc technology, gold deposits are not seen within electron-dense structures such as the lamina densa as the metal has access to only the margins of these structures. The epitopes recognized by mAb-VII (and pAb-VII) are present throughout the lamina densa as previously shown (Sakai et al., 1986b).

The plaques have been previously assumed to be tangential sections of basement membrane undulations (Palade and Farquhar, 1965). To examine the relationship of the plaques to the basal lamina, 90-nm sequential serial sections were obtained from the subbasal lamina region of tissue stained en bloc with gold directed by mAb-VII. As shown in Fig. 3, serial sectioning demonstrates that the plaques are irregular amorphous electron-dense structures. Examination of multiple regions along the basement membrane conclusively established that these plaques represent independent islands of electron-dense material, whose only connections to the lam-

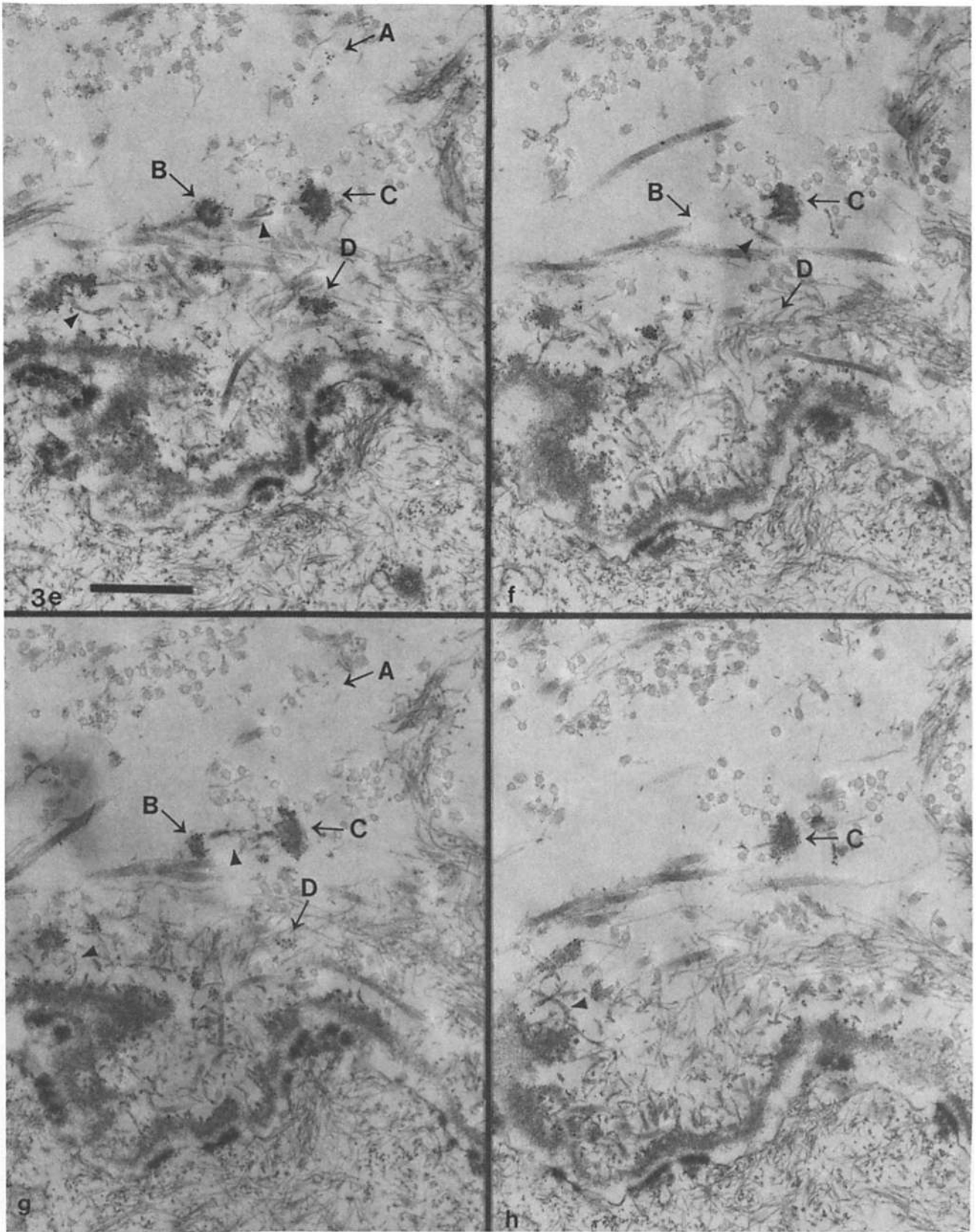


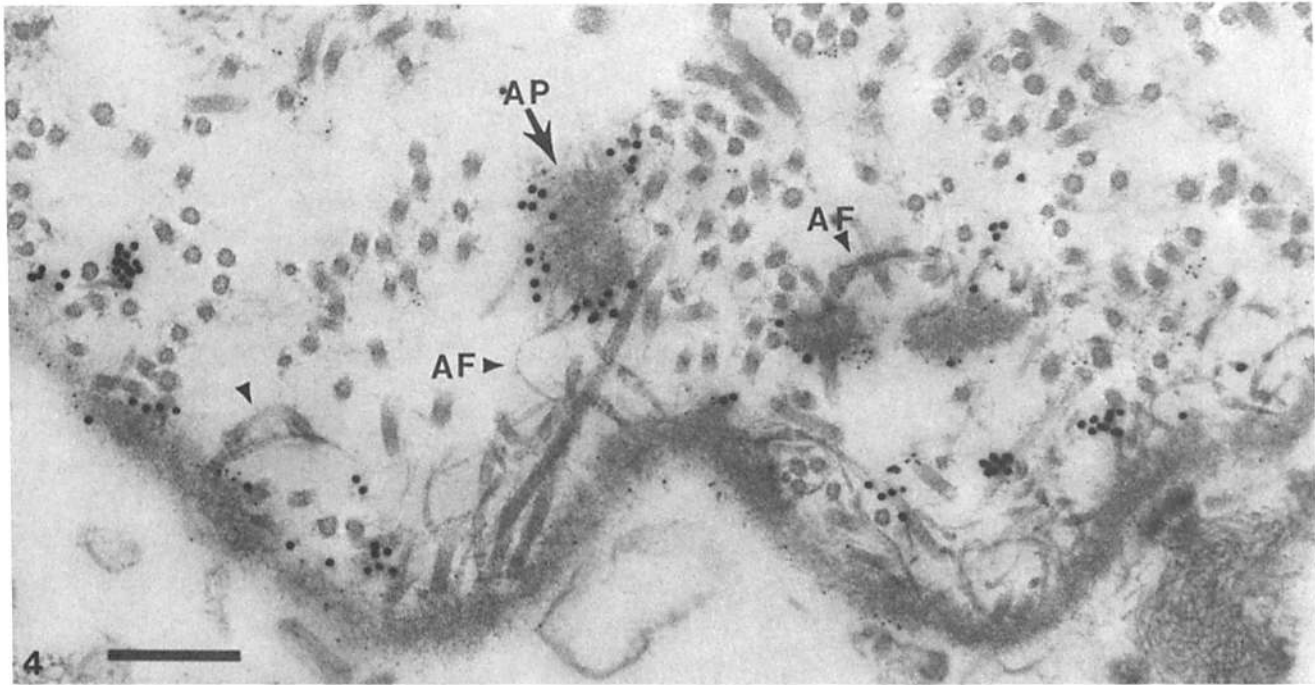
**Figure 2.** Ultrastructural immunolocalization of the carboxyl-terminal globular domain of type VII collagen within the dermal-epidermal junction of neonatal human foreskin. (*a* and *b*) Primary antibody = pAb-VII; secondary antibody = 5-nm colloidal gold-conjugated goat anti-rabbit IgG. *AF*, anchoring fibrils; *AP*, anchoring plaques. Numbered arrowheads indicate anchoring fibrils. Bars, 250 nm.



**Figure 3.** Serial sections of immunolocalized type VII collagen in human neonatal foreskin. Type VII collagen was localized en bloc using mAb-VII and 5-nm gold-conjugated goat anti-mouse IgG. Consecutive serial 90-nm-thick sections were taken and the appearance and disappearance of "anchoring plaques" was examined (lettered A-D). Bar, 500 nm.







**Figure 4.** Ultrastructural immunolocalization of the carboxyl-terminal globular domain of type VII collagen and the triple-helical domain of type IV collagen within the dermal-epidermal junction of human foreskin. Double immunolabel: primary antibody = pAbVII; secondary antibody = 15-nm colloidal gold-conjugated goat anti-rabbit IgG; and, second primary antibody murine mAb-IV; secondary antibody, 5-nm colloidal gold-conjugated goat anti-mouse IgG. AP, anchoring plaque; AF, anchoring fibril. Bar, 250 nm.

ina densa are through anchoring fibrils. The sizes of these plaques vary considerably. For example, plaque A appears in Fig. 3b, is clearly evident in Fig. 3c, and is not seen in Fig. 3e. As these are  $\sim 90$ -nm sections, plaque A is an ellipsoid, measuring  $\sim 200 \times 170$  nm when calibrated using collagen fibril interband distances in the same sections. In contrast, plaque B is more irregular with dimensions of  $\sim 270 \times >500$  nm. Plaque D is  $\sim 200 \times 100$  nm. We have termed these elements "anchoring plaques."

#### **Anchoring Plaques Contain Both Collagen Types VII and IV**

Since these plaques ultrastructurally resemble basement membrane, the possible localization of type IV collagen within these anchoring plaques was examined using a monoclonal type IV-specific antibody. The anti-type IV antibody produced gold distribution identical to that directed by antibodies to the carboxyl-terminal globular domain of type VII procollagen. Monoclonal antibodies of the same immunological subtype but of irrelevant specificity demonstrate no labeling of the anchoring plaques (Sakai et al., 1986a).

To verify the coincidence of the type IV labeling with the carboxyl terminus of type VII collagen, double-labeling experiments were performed using 5-nm colloidal gold-conjugated anti-mouse IgG, directed specifically to type IV collagen monoclonal antibodies, and 15-nm gold-conjugated anti-rabbit IgG, specific to the pAb-VII antibodies. The results (Fig. 4) clearly demonstrate that type IV collagen co-

distributes with the carboxyl-terminal domain of type VII collagen and is present in both the lamina densa and in anchoring plaques.

#### **The Relationship of Anchoring Fibrils to Anchoring Plaques**

Since all of the antibodies used recognize the carboxyl end of type VII collagen and not the triple-helical domain, in this study it is impossible to unambiguously determine the location of the type VII collagen triple helix immunologically. However, partially banded anchoring fibrils can be recognized extending from both the lamina densa and the anchoring plaques (Figs. 2-4; arrowheads). The banding of the anchoring fibril has previously been shown to correspond to the triple-helical domain of type VII collagen (Burgeson et al., 1985; Sakai et al., 1986b). These observations indicate that anchoring fibrils can originate and/or terminate in both the lamina densa and the anchoring plaques. The anchoring fibrils seen in skin appear quite random in orientation. Fibrils with arching morphology are seen which originate and terminate in the lamina densa (Fig. 2, arrowheads 3, 5, and 14) while other anchoring fibrils originate in the lamina densa and extend into the dermis to insert into anchoring plaques (Fig. 2, arrowheads 1, 2, 8, 9, and 13). Additional anchoring fibrils originate in an anchoring plaque and terminate in another (Fig. 2, arrowheads 4, 11, and 17). Still others appear to branch (Fig. 2, arrowheads 10 and 12). In the serial sections of Fig. 3, anchoring fibrils can be seen ex-

**Figure 5.** Anchoring plaques are present in human skin regardless of site of biopsy. Type VII collagen was immunolocalized en bloc using pAb-VII and 5-nm gold-conjugated goat anti-rabbit IgG in (A) aged human foreskin (84 years); (B) human thigh skin; (C) human hand skin (palmar surface); and (D) human heel skin. Bar, 500 nm.



tending from all the anchoring plaques visualized in at least one of the sections containing that plaque. The fibrils between plaques B and C are especially well visualized in Fig. 3, *c-f*. The serial sections also demonstrate that few if any of the gold particles are present in the space below the lamina densa that are not associated with anchoring plaques, suggesting that anchoring fibrils do not remain free in this space, but more usually associate with other anchoring fibrils through their carboxyl termini. These sections also indicate that plaques are seen, though infrequently, at considerable distance from the lamina densa. Plaque A is  $\sim 1700$  nm from the nearest lamina densa.

### The Distribution of Anchoring Plaques in Human Skin

Human skin from a variety of locations, and at several ages was examined ultrastructurally after optimal fixation and by immunolocalization using either the en bloc or section surface labeling techniques. The tissues examined included neonatal foreskin; adult foreskin (84 years) (Fig. 5 *A*); young forearm skin (individuals less than 10 years); adult thigh (31, 74, and 84 years) (Fig. 5 *B*); and adult (84 years) heel skin (Fig. 5 *D*), forearm skin, hand palmar (Fig. 5 *C*), and back surface skin, and back skin. Anchoring fibrils and anchoring plaques could be identified in all cases. Regardless of site of excision, both anchoring fibrils and anchoring plaques were consistently present along the basement membrane and appeared to penetrate the stroma to the same extent. All sites showed the bridging of anchoring plaques by anchoring fibrils independently from the lamina densa (for example, Fig. 2, *a* and *b*, in neonatal foreskin). In all human skin locations, and at all ages, the distribution of gold directed by the antibodies specific to the carboxyl-terminus of type VII collagen was equivalent (Figs. 2 and 5 are representative). The results indicate that in skin, anchoring plaques are an ultrastructural feature of this organ regardless of site of biopsy, and the numbers of anchoring plaques do not significantly vary with age.

To better characterize this distribution in skin, the distance of anchoring plaques from the lamina densa was measured. Anchoring plaques were scored which demonstrated gold deposition in response to pAb-VII. As the lamina densa of skin undulates, in cases where two lengths were possible, the shorter was taken. The results from human neonatal foreskin are shown in Fig. 6, and indicate that anchoring plaques were an average of 341 nm from the lamina densa. Only 2.4% were farther away from the lamina densa than the maximum length of an anchoring fibril ( $\sim 800$  nm).

### The Tissue Distribution of Anchoring Plaques

To evaluate the relevance of these observations in skin to other anchoring fibril-containing tissues, human amniotic membranes and bovine cornea were similarly examined by ultrastructural immunolocalization using pAb-VII by section surface labeling. The results are shown in Fig. 7. Anchoring plaques were readily observed in bovine cornea as shown by

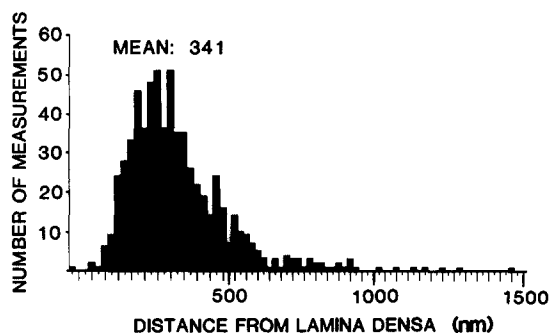


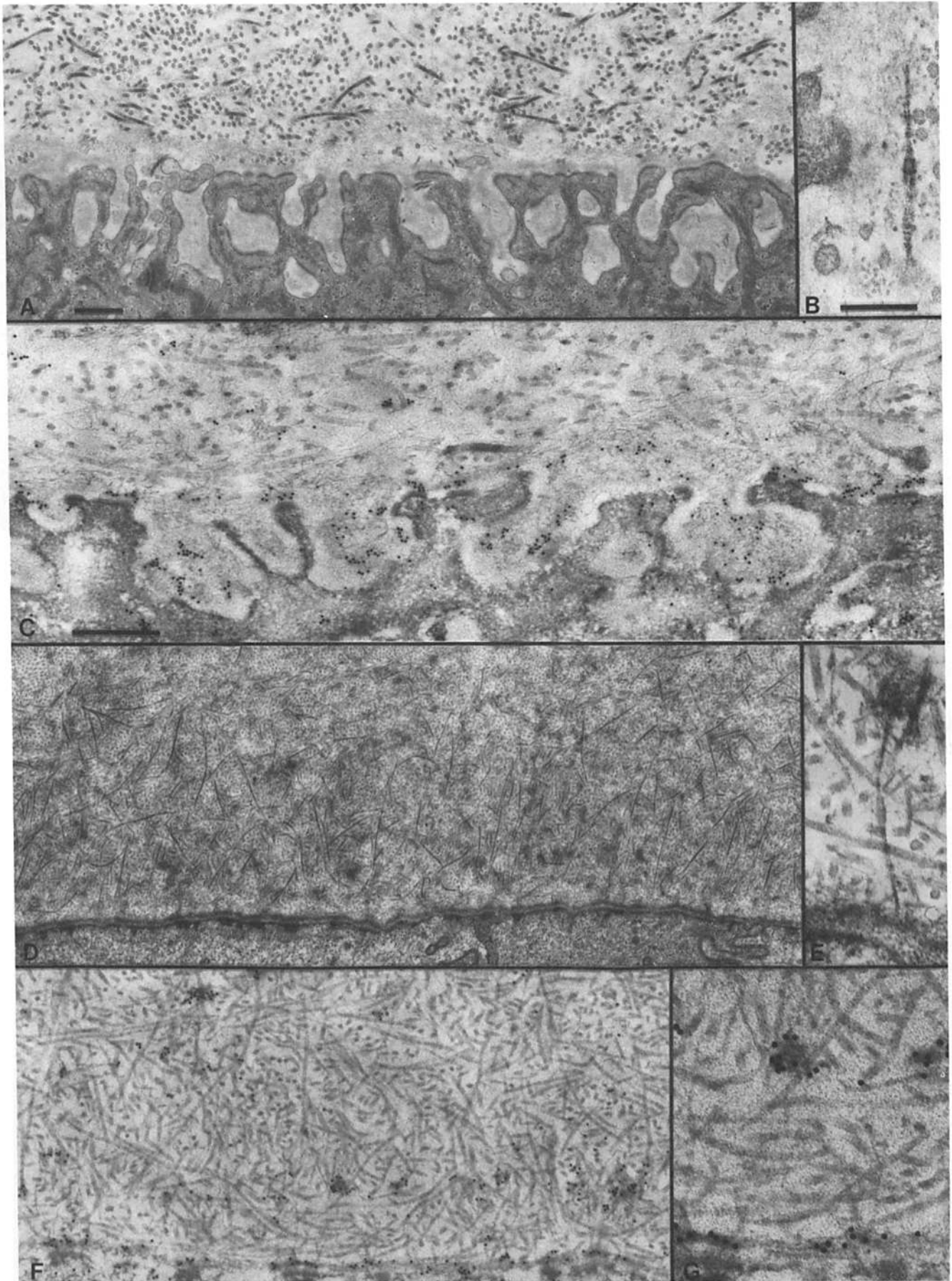
Figure 6. In human neonatal foreskin, anchoring plaques average 341 nm distant from the lamina densa. Type VII collagen was immunolocalized en bloc using pAb-VII and 15-nm gold-conjugated goat anti-rabbit IgG to human neonatal foreskin. The distance from the lamina densa to the center of the anchoring plaque was measured. The average distance measured was 341 nm. 2.4% (16 out of 658 measurements) of the plaques are  $>800$  nm from the lamina densa.

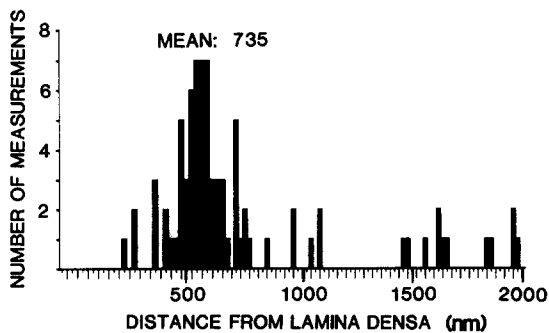
extensive gold deposits over the epithelial subbasal lamina, often as far as 1–2  $\mu\text{m}$  from the lamina densa. This anchoring plaque distribution was documented by measurements of the distances of immunologically identified anchoring plaques from the lamina densa, as was done with skin. The results, shown in Fig. 8, indicate that these plaques averaged a distance of 735 nm from the lamina densa, with 21% occurring at distances greater than the maximum length of a single anchoring fibril. This is in good agreement with recent studies of Gipson and co-workers (Gipson, I. K., S. J. Spurr-Michaud, and A. S. Tisdale, manuscript submitted for publication), who have recently reported the average maximum depth of penetration of anchoring fibrils in human cornea to be 0.6  $\mu\text{m}$ , and in rabbit cornea to be 0.54  $\mu\text{m}$ . As these authors have indicated, there appears to be no significant difference between anchoring fibrils in the rabbit and human cornea, even though there is no obvious Bowman's layer in the rabbit. Our data likewise indicate that the bovine cornea is not different from that of the human in this regard.

In contrast, amnion demonstrates infrequent labeling outside the folds between the amniotic epithelial foot processes, although some label is consistently seen between 0.5 and 1  $\mu\text{m}$  of the lamina densa. Recognizable anchoring fibrils and anchoring plaques are difficult to observe even in well-fixed cross sections of amnion, but anchoring fibrils are more readily seen when amnion is examined after sectioning parallel to the epithelial cell-matrix interface (Fig. 7 *B*). In amnion, much of the labeling appears to result from anchoring fibrils between the walls of the epithelial cell folds. Only occasional labeled plaques can be identified which appear to be clearly independent of the lamina densa. This anchoring plaque distribution appears quite distinct from that seen in either skin or cornea.

Figure 7. The anchoring plaque distribution in human amniotic membranes and bovine cornea appear different from that of human skin. Type VII collagen was immunolocalized by section surface labeling using pAb-VII and 15-nm gold-conjugated goat anti-rabbit IgG to the amniotic epithelial subbasal lamina (*C*) and bovine corneal epithelial subbasal lamina (*F* and *G*). Optimally fixed tissue sections of both amnion (*A*) and cornea (*D*) are also shown for comparison. Recognizable centrosymmetrically banded anchoring fibrils can be seen in well-fixed sections of both amnion (*B*) and cornea (*E*), but are not easily visualized by section surface staining. Bars: (*A*, *C*, *D*, and *F*) 500 nm; (*B*, *E*, and *G*) 200 nm.







**Figure 8.** The average distance of anchoring plaques from the corneal epithelial basement membrane is greater than the distance measured in skin. Type VII collagen was immunolocalized en bloc using pAb-VII and 15-nm gold-conjugated goat anti-rabbit IgG. Measurements were made from the lamina densa to the center of a labeled anchoring plaque. The average distance measured was 735 nm. 21% (17 out of 81 measurements) of the plaques measured >800 nm from the lamina densa.

## Discussion

The data clearly show the existence of irregularly shaped electron-dense bodies below the lamina densa of skin and cornea, which we have called "anchoring plaques." These plaques resemble fragments of lamina densa and contain type IV collagen and the carboxyl termini of type VII collagen. Anchoring plaques appear to be true features of skin and cornea and do not arise artifactually from trauma or age-related phenomena since the occurrence and distribution of these entities are unchanged with age or site of observation.

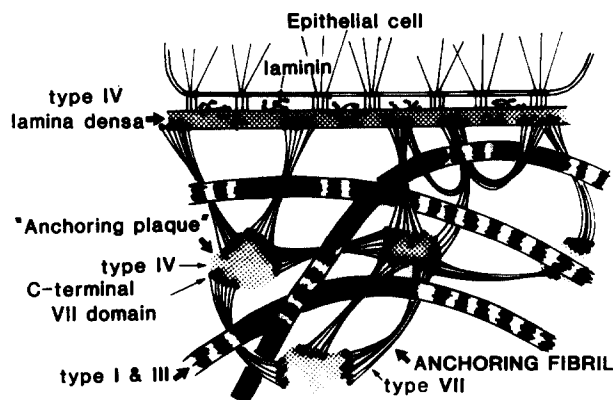
These data also document that anchoring fibrils are associated with the anchoring plaques in addition to being conjoint with the lamina densa. Ultrastructurally identifiable anchoring fibrils are observed associated with the vast majority of labeled anchoring plaques. From the density of gold deposition upon both the lamina densa and the anchoring plaques, more visible anchoring fibrils might be expected. From this study, the possibility cannot be excluded that the helical domain of type VII collagen is contained within either the lamina densa or the anchoring plaque, or that the carboxyl termini of type VII collagen is present in both locations without the helical domain. However, it is more likely that not all anchoring fibrils are sufficiently thick to be visualized either because there is a great deal of variance in the number of molecules within a fibril, or because the observed lateral aggregation of type VII molecules seen ultrastructurally as anchoring fibrils is an artifact of dehydration and tissue processing and may not occur uniformly for all type VII molecules.

Regardless of the difficulty in readily visualizing anchoring fibrils, multiple incidences have been observed and here documented of anchoring fibrils bridging the lamina densa and anchoring plaques, and of anchoring fibrils bridging neighboring anchoring plaques. From these observations and the logical extension of them, we postulate that the gold deposition observed upon anchoring plaques in these studies represents the termini of anchoring fibrils which interconnect either adjacent anchoring plaques or anchoring plaques and the lamina densa. Thus fibrils may form an extensive network of connections which might be conceptualized as a

scaffolding immediately adjacent to the lamina densa within the subbasal laminal space. We further postulate that this network extends into the subbasal lamina greater than the length of one anchoring fibril. This conclusion is based on the observation that in cornea, 21% of the anchoring plaques are more distant from the basement membrane than the length of one anchoring fibril ( $\sim 800$  nm). The number observed in skin is far less (2.4%), but it is our impression from examining numerous micrographs of skin that many of the plaques at the dermal limit of the basement membrane zone are bridged to other plaques and not directly to the basal lamina despite the fact that they are <800 nm from the lamina densa. This is difficult to convincingly demonstrate since the structures involved are considerably larger than the thin section thickness, and therefore the observation of anchoring fibrils of full length is a relatively rare event. This postulated network is illustrated in Fig. 9.

The significance of the apparent ultrastructural differences between the anchoring fibril network in skin, cornea, and amnion is unclear. A certain degree of tissue distortion occurs in skin when the biopsy is excised, and skin is not nearly as rigid as cornea. Even greater change in dimension occurs upon excision of a small piece of chorioamnion. It is possible that some of the differences in the morphology or dimensions of the fibril networks could be related to these changes. Therefore the absolute value of the dimensions of anchoring plaque placement should not be overemphasized. However, the relationships of the anchoring plaques to each other is believed to be correct. It is our impression that the amniotic anchoring fibril network is actually quite different from that of skin or cornea. The fibrils appear to bridge the basement membranes between the convolutions of the epithelial cell basal surfaces. It is unclear if the amorphous electron-dense materials seen within these folds represents tangential sections of basement membrane, or if they may indicate the presence of anchoring plaques. It would appear that in amnion the anchoring fibrils function to maintain the extensive cell basal surface convolutions.

While the distance of dermal penetration by the anchoring



**Figure 9.** A model of the postulated anchoring fibril network. Anchoring fibrils composed of type VII collagen are depicted between anchoring plaques and between the basal lamina and anchoring plaques. The anchoring plaques resemble basement membrane ultrastructurally, and contain type IV collagen in addition to the complex carboxyl-terminal domain of type VII collagen. This extended network entraps large banded collagen fibers, effectively anchoring the basal lamina to the underlying stroma.

fibril network is independent of the site of biopsy, the number of anchoring fibrils contributing to that network varies with the site. It is our impression that adult samples can be ranked in order of increasing anchoring fibril density as follows: foreskin < arm, back and back of hand skin < hand palm skin < heel skin. In general, the anchoring fibril density appears to correlate with the frictional exposure of the tissue, again suggesting that the network is involved in epidermal-dermal stabilization.

The role of type IV collagen in this complex may be that of a junctional element linking the carboxyl-terminal domains of type VII collagen. How this occurs is unclear. It is not presently known if the carboxyl-terminal globular domains of type VII collagen interact directly with type IV collagen, or with some additional component of the anchoring plaques. Attempts to localize laminin to the anchoring plaques have thus far been equivocal, and the presence of other basement membrane components has not been tested. The ultrastructural similarity of anchoring plaques to lamina densa suggests that additional proteins or proteoglycans may be present.

It has been frequently suggested that a portion of the anchoring fibril extends from the dermis, through the lamina densa, the lamina lucida, and directly intersects the hemidesmosomes (Susi et al., 1969). Our results suggest that type VII collagen is not directly involved in the proposed interaction with hemidesmosomes. The pAb-VII antiserum recognizes the entire carboxyl-terminal domain of type VII collagen (Lunstrum et al., 1986; and Lunstrum, G. P., unpublished data). Since gold deposition directed by pAb-VII is not seen by surface labeling techniques over the lamina lucida, the simplest interpretation of the observation is that the entire carboxyl-terminal domain is contained within the lamina densa. Further, this conclusion indicates that the ultrastructural elements referred to as anchoring filaments are most likely to be distinct from, although perhaps ultrastructurally continuous with type VII collagen. The apparent continuity of anchoring fibril with hemidesmosomes probably involve multiple gene products.

While entrapment of large collagen fibers by anchoring fibrils with arching morphology has been documented, it is rare (Wasano and Yamamoto, 1985). The function of anchoring fibrils extending perpendicularly into the matrix has not been understood. The postulation of a multilayered scaffold intertwined with and encompassing large banded collagen fibers and fine reticular fibers lends credence to the hypothesis that anchoring fibrils may function to secure the lamina densa to the underlying stromal matrix by physical entrapment of connective tissue elements.

If one function of the anchoring fibril network were to fortify the attachment of the lamina densa to the underlying dermis, then disruption of this network would be expected to produce dermal-epidermal separation. A correlation of anchoring fibril absence or diminution and recessive-dystrophic Epidermolysis Bullosa has been made (Goldsmith and Briggaman, 1983). However, in other forms of Epidermolysis Bullosa subbasal laminal separation can also occur even though anchoring fibrils extending from the lamina densa are present in normal amounts. If our hypothesis regarding the function of the anchoring fibril network is correct, these individuals which demonstrate subepidermal blisters but nor-

mal or nearly normal anchoring fibril numbers need to be reappraised. It is possible that the anchoring fibril network could be nonfunctional while the numbers of anchoring fibrils counted along the basement membrane might be little changed. Alternatively, previous studies document the presence of another anchoring device for lamina densa-matrix stabilization in tissues lacking anchoring fibrils. This Ruthenium red-positive structure exists in skin in addition to anchoring fibrils (Wasano and Yamamoto, 1985). If dermal-epidermal stability is an additive phenomenon of these two factors, then it is possible that the latter could fail, resulting in dermal-epidermal separation and an apparently intact anchoring fibril system.

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