Formation of Hemidesmosomes In Vitro by a Transformed Rat Bladder Cell Line

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Abstract. Two hemidesmosomal plaque components of 230 and 180 kD have recently been characterized using autoantibodies in the serum samples of bullous pemphigoid (BP) patients (Klatte, D. H., M. A. Kurpakus, K. A. Grelling, and J. C. R. Jones. 1989, *J. Cell Biol.* 109:3377–3390). These BP autoantibodies generate the type of staining patterns that one would predict for formed hemidesmosomes, i.e., a punctate staining pattern towards the substratum; in <50% of various primary epithelial and transformed epidermal cell lines even when such cells are maintained in culture for prolonged periods. In contrast, affinity-purified human autoantibodies against the 230-kD hemidesmosomal plaque component generate intense immunofluorescence staining along the region of cell–substratum interaction in the rat bladder tumor cell line 804G maintained on uncoated glass coverslips. This pattern is distinct from that observed in the 804G cells using an antibody preparation directed against vinculin, a component of adhesion plaques. Ultrastructural analyses of the 804G cells reveals that hemidesmosome-like structures occur along the basal surface of cells where they abut the substratum. These structures are present in 804G cells maintained in culture in reduced levels of Ca^{2+} and are recognized by

autoantibodies directed against the 230-kD hemidesmosomal plaque component as determined by immunogold ultrastructural localization.

To study hemidesmosome appearance in this cell line, 804G cells were trypsinized and then allowed to readhere to glass coverslips. In rounded, unattached 804G cells, hemidesmosome-like plaque structures occur along the cell surface. These structures are recognized by the 230-kD autoantibodies. At 1 h after plating, hemidesmosomes are observed along the substratum attached surface of cells. Protein synthesis is not required for the appearance of these hemidesmosomes. Within 4 h of plating, autoantibody staining and hemidesmosomes appear towards the cell periphery. Subsequently, the polypeptide recognized by the BP autoantibodies becomes concentrated in the perinuclear region, where there are numerous hemidesmosomes. We propose that the hemidesmosomes in 804G cells are involved in cell–substratum adhesion. We discuss possible mechanisms of assembly of hemidesmosomes in the 804G cells. Indeed, the 804G cells should prove an invaluable cell line for the biochemical and molecular dissection of hemidesmosome structure, function, and assembly.

HEMIDESMOSOMES are localized along the basal surface of epithelial cells where they abut the connective tissue (Kelly, 1966). They play a role in both the adherence of basal cells to the basement membrane zone and as attachment devices for bundles of intermediate filaments (IF)[1] (Staehelin, 1974). The hemidesmosome possesses an electron-dense tripartite cytoplasmic plaque, subjacent to the plasma membrane (Kelly, 1966; Jones et al., 1986). IF attach to the most cytoplasmic region of the hemidesmosome plaque (Shienvold and Kelly, 1976; Ellison and Garrod, 1984). Fine filaments, termed anchoring filaments, traverse from the plaque to the lamina densa region of the

basement membrane zone (BMZ). Anchoring fibrils, composed of collagen VII (Sakai et al., 1986), extend into the connective tissue from the lamina densa immediately subjacent to the hemidesmosome (Ellison and Garrod, 1984).

Autoantibodies in the serum samples of patients with the autoimmune blistering disease bullous pemphigoid (BP) recognize components of the hemidesmosomal plaque (Mutasim et al., 1985; Regnier et al., 1985; Westgate et al., 1985; Jones et al., 1986). Indeed, these autoantibodies have proved excellent probes for studying the morphogenesis of hemidesmosomes in various wound models and the fate of hemidesmosome plaque components in certain disease states (Gipson et al., 1989; Jones et al., 1989; Kurpakus et al., 1990a).

To determine the particular autoantibodies in BP serum samples which recognize hemidesmosomal components,

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1. *Abbreviations used in this paper*: BP, bullous pemphigoid; IF, intermediate filaments.

Klatte et al. (1989) prepared monospecific BP autoantibodies. These authors reported that autoantibodies directed against 180- and 240-kD polypeptides recognize elements of the hemidesmosomal plaque in the region where IF attach. Using 240-kD BP autoantibodies we have recently isolated clones encoding for this hemidesmosomal component from a human epithelial lambda gt11 cDNA library (Hopkinson, and J. C. R. Jones, unpublished observations). The sequences of certain of these clones show overlap, not surprisingly, with the published partial sequence of a 230-kD polypeptide termed the "bullous pemphigoid antigen" by Stanley et al. (1988). Because of this slightly different molecular weight designation for the same hemidesmosomal component, there is a possibility for confusion. Therefore, in the work we present here we will refer to the higher molecular weight polypeptide of the two hemidesmosomal plaque components described by Klatte et al. (1989) as a 230-kD protein.

In this paper we have used autoantibodies directed against the 230-kD hemidesmosomal plaque component to characterize hemidesmosome-like structures produced in vitro by an epithelial cell line termed 804G. Furthermore, we have monitored the appearance of the hemidesmosomes of 804G cells after trypsinization and replating of cells onto an uncoated glass substrate.

Materials and Methods

Cell Culture

The 804G cell line, derived from a rat bladder tumor was generously provided by Ryoichi Oyasu (Department of Pathology, Northwestern University Medical School, Chicago, IL) and is described in Izumi et al. (1981). 804G, the transformed mouse epidermal cell line, PAM, and the human pharyngeal squamous cell carcinoma epithelial cell line, FaDu, were maintained at 37°C in MEM with Earle's salts supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin and 10% FCS (Gibco Laboratories, Grand Island, NY). This medium contains ~1.9 mM Ca^{2+} (Jones and Grelling, 1989). 804G cells were also maintained in MEM without added Ca^{2+}, supplemented with 10% chelex-treated FCS, 50 U/ml penicillin, and 50 μg/ml streptomycin (Hennings et al., 1980). This medium contains ~0.04 mM Ca^{2+} (Jones and Grelling, 1989).

Bovine corneal epithelial cells were prepared as described in Kurpakus et al. (1990b) using a modification of a procedure by Ebato et al. (1987).

Human Sera and Antibody Preparations

Bullous pemphigoid (BP) serum samples were generously donated by Ruth Frienkel (Department of Dermatology, Northwestern University Medical School).

A monoclonal antibody preparation against vinculin was obtained from Sigma Chemical Co. (St. Louis, MO).

Immunofluorescence

Cultured cells grown on glass coverslips were rinsed briefly in PBS (6 mM Na^+/K^+ phosphate, pH 7.4, 171 mM NaCl, 3 mM KCl) and then fixed in −20°C acetone for 2 min and air dried. In the case of cells processed for localization of antivinculin, cells were prefixed for 5 min in 3.7% formaldehyde in PBS before extraction in −20°C acetone. Coverslips were processed for single and double labels as detailed in Jones et al. (1985). Immunofluorescence controls included incubation of cells on coverslips in secondary conjugated antibodies alone and the use of antibodies that do not recognize epithelial cells to determine nonspecific binding sites of primary and secondary antibodies. Stained cells were viewed using a Photomicroscope III equipped with epifluorescence and phase contrast optics. Fluorescence and phase contrast micrographs were taken using Kodak Plus-X film. Films were developed in Diafine two-stage developer (Acufine, Inc., Chicago, IL).

Conventional and Immunoelectron Microscopy

804G cells grown on glass coverslips or rat bladder tissue were fixed in 1% glutaraldehyde in sodium cacodylate buffer pH 7.4 for 1 h at room temperature. The preparations were then postfixed with 1% OsO_4 in sodium cacodylate pH 7.4 containing 0.8% potassium ferricyanide ($K_3Fe(CN)_6$). They were dehydrated and embedded in BEEM capsules in Epon/Araldite as previously described in Starger et al. (1978). The BEEM capsules containing cells were removed from the glass coverslip after immersion in liquid nitrogen. Sections were prepared perpendicular to the growth substrate. Thin sections were stained in uranyl acetate and lead citrate and viewed in a JEOL 100CX electron microscope at an accelerating voltage of 60 kV.

For immunogold localization, cells grown on glass coverslips were processed as detailed in Jones and Grelling (1989). Controls were similar to those detailed above for immunofluorescence with substitution of gold conjugated secondary antibodies. Thin sections were prepared and viewed in the electron microscope as described above.

SDS-PAGE and Western Blotting

SDS-PAGE using 6.0% polyacrylamide gels with 3.0% stacking gels was performed on a basal cell extract of bovine tongue enriched in hemidesmosome components (Klatte et al., 1989) and 804G whole cell extracts according to Laemmli (1970). Approximately 10 μg of protein per lane was used. After SDS-PAGE, separated polypeptides were transferred to sheets of nitrocellulose (Towbin et al., 1979). Immunoblotting was carried out using the described antibodies as detailed in Zackroff et al., (1984).

Affinity Purification of Human Autoantibodies

Affinity purification of 180- and 230-kD autoantibodies from BP serum samples was carried out following the procedure of Olmsted (1981) as detailed in Klatte et al., (1989).

Protein Preparations

Bovine tongue mucosa was incubated overnight in 20 mM EDTA in PBS containing 1 mM PMSF. The pieces of tissue material were then treated in the same buffer containing 1M KCl. The epithelium was peeled from the underlying connective tissue. The basal cells of the epithelium were solubilized in a sample buffer consisting of 8 M urea, 1% SDS, 5% 2-mercaptoethanol, and 64 mM Tris-HCl (pH 6.8) (Klatte et al., 1989).

Confluent 100-mm petri dishes of 804G cells were rinsed briefly in PBS and then 1 ml of the sample buffer described above was added to the cells. Cells were scraped from the dish in the sample buffer and disrupted by sonication. Protein solutions were stored at −70°C for later use.

Cycloheximide Treatment of 804G Cells

804G cells were seeded onto a glass substrate in medium containing 2 μCi/ml of [^{35}S]trans-methionine (ICN Radiochemicals, Costa Mesa, CA) in the presence or absence of 10 μg/ml cycloheximide. After various time points cells were lysed in buffer containing 0.5 M NaCl, 5 mM EDTA, and 0.5% SDS at pH 7.4. BSA to a concentration of 0.5 mg/ml was added to the resulting solubilized cell material that was then precipitated by addition of 20% TCA. After incubation for 1 h on ice, the precipitated material was collected on Whatman glass microfiber GF/C filters (Whatman, Maidstone, England). The filters were air dried and then counted on a Beckman LS6800 scintillation counter (Beckman Instruments, Fullerton, CA).

Results

Expression of Hemidesmosomal Components in Cultured Epithelial Cells

Bullous pemphigoid (BP) autoantibodies generate punctate staining along the substratum attached surface of certain cultured primary epithelial cells (Klatte et al., 1989). This is the type of pattern that one would expect if the cells were forming hemidesmosomal connections with the substratum. However, <50% of these corneal cells appear to express hemidesmosomal antigens in this form, even 2 wk after plating on glass substrata (Klatte et al., 1989). Approximately
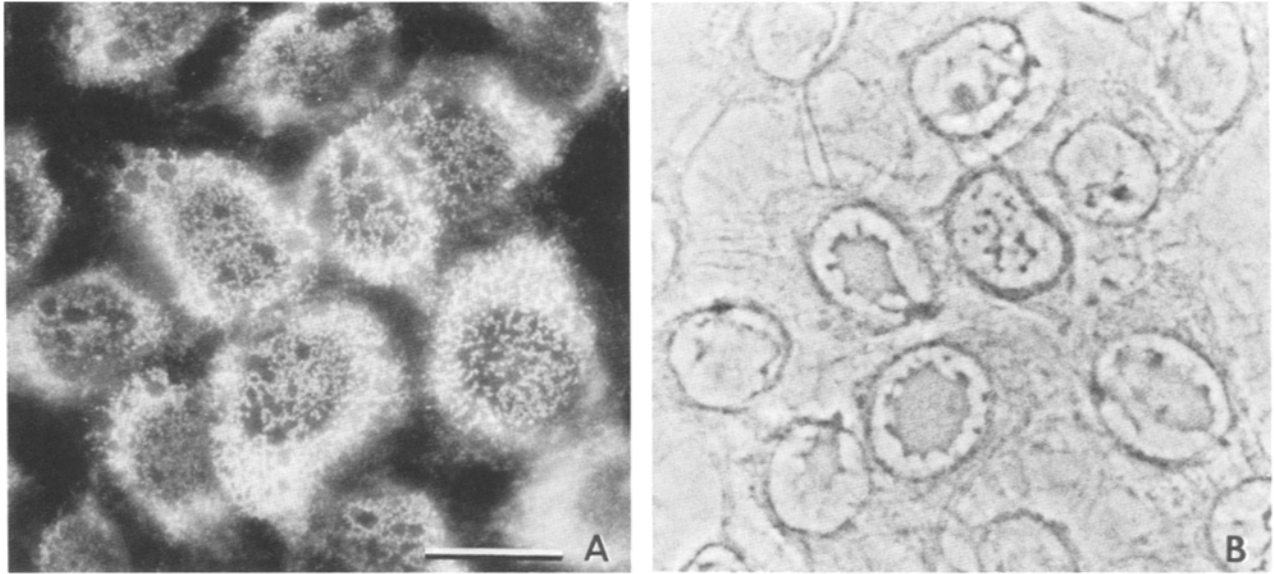
Figure 1. 804G cells processed for indirect immunofluorescence using affinity-purified 230-kD autoantibodies prepared from a BP serum sample. Note that the autoantibodies generate an intense stain towards the perinuclear region of each cell (*A*). *B* shows a phase-contrast micrograph of the cells shown in *A*. Bar, 4 μm.

the same percentage of cultured primary human and mouse epidermal cells and the continuous epithelial cell lines PAM and FaDu also show similar staining patterns with bullous pemphigoid autoantibodies as determined by immunofluorescence microscopy (results not shown).

In contrast, affinity- purified BP autoantibodies that recognize a 230-kD hemidesmosomal plaque component in bovine tongue mucosa (Klatte et al., 1989) generate a dramatic substratum associated particulate fluorescence pattern in cells of the rat bladder tumor cell line termed 804G (Fig. 1). The 804G cells shown in Fig. 1 were maintained in culture for 24 h on uncoated glass coverslips. The staining in cells

is primarily in the perinuclear region and is excluded from the periphery of cells or regions where cells are in contact (Fig. 1). Confocal microscopical analysis of the 804G cells reveals that the human autoantibodies generate staining within 0.5 μm of the substratum attached surface of the 804G cells (results not shown).

There is no correlation between hemidesmosomal antibody localization and staining generated by antibodies directed against vinculin, a component of the microfilament-associated adhesion plaque whose molecular composition has been extensively studied (see, for example Burridge et al., 1987). For these studies we used a monoclonal vinculin
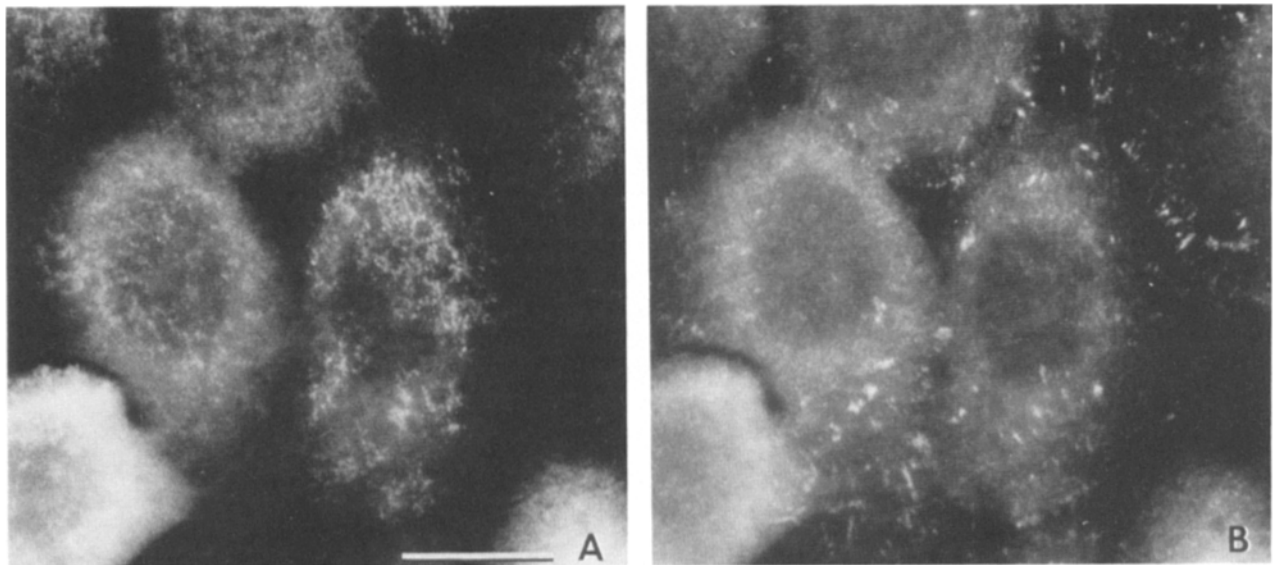

Figure 2. 804G cells were processed for double label immunofluorescence using BP autoantibodies (*A*) and an mAb directed against vinculin (*B*). At the substratum attached surface of the cells the vinculin antibody stains a series of spots and streaks, a very different pattern than that observed using BP autoantibodies. Bar, 4 μm.
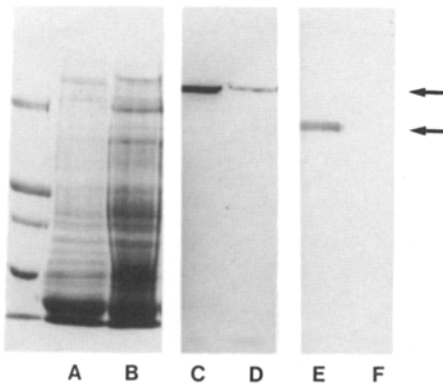
Figure 3. A bovine tongue basal epithelial cell extract enriched in hemidesmosomal components (Klatte et al., 1989) (*A, C,* and *E*) and a whole cell extract of 804G cells (*B, D,* and *F*) were processed for SDS-PAGE using a 6% resolving gel and then transferred to nitrocellulose. Molecular mass standards of 200, 116, 97, and 66 kD are shown in the first lane. Lanes *A* and *B* are amido black stains of the two transferred protein preparations. Affinity-purified anti-230-kD autoantibodies prepared from BP serum samples recognize 230-kD polypeptides in both lanes *C* and *D* (*top arrow,* lane *F*). Although 180-kD autoantibodies prepared from BP serum recognize an 180-kD polypeptide in the bovine tongue basal epithelial cell extract, they fail to recognize a polypeptide in the 804G cell protein extract (compare lanes *E* and *F, bottom arrow*).

antibody preparation as a probe for the adhesion plaque (Geiger, 1981) and BP autoantibodies as probes for hemidesmosomal antigens (Fig. 2). Double-label immunofluorescence analysis reveals that vinculin antibodies stain mainly towards the periphery of cells in contrast to the staining generated by the BP autoantibodies (Fig. 2).

To further characterize the antigens recognized in the 804G cells by the human hemidesmosomal autoantibodies, we undertook immunoblotting analyses.

Immunoblotting Characterization of Hemidesmosomal Antigens in 804G Cells

The reactivity of affinity-purified BP autoantibodies on whole cell extracts of 804G cells was compared with that seen with these antibody preparations on the basal cell extract of bovine tongue which contains components of the hemidesmosome (Klatte et al., 1989).

Immunoblotting analyses using affinity-purified 180- and 230-kD human autoantibody reveals that the 230-kD autoantibodies show reactivity with both the 804G whole cell extracts and the tongue preparation (Fig. 3). In contrast, the 180-kD antibody preparation shows reactivity with the bovine tongue preparation but does not recognize a polypeptide in the 804G whole cell extracts (Fig. 3). The above immunochemical finding was confirmed by immunofluores-
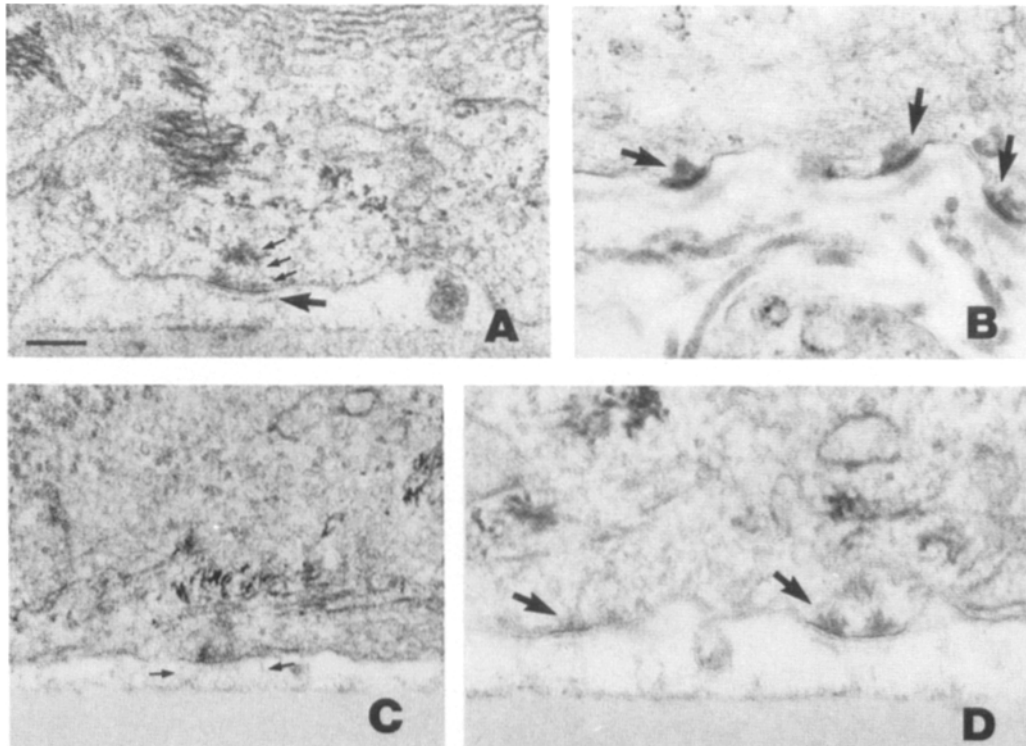

Figure 4. Electron micrographs of thin sections of 804G cells maintained for 24 h in culture on glass coverslips (*A, C,* and *D*) or the basal surface of a rat bladder epithelial cell in situ (*B*). The sections of 804G cells were prepared perpendicularly to the substrate. In *D*, 804G cells were maintained in medium containing 0.04 mM Ca²⁺. Note the electron densities with associated filament bundles that occur along the substratum attached surface of the 804G cells (*large arrows* in *A* and *C*). These densities are morphologically similar to hemidesmosomes observed in B (*arrows*). Each of these structures possesses a tripartite plaque structure (*small arrows* in *A*). In many instances an electron dense line lies immediately subjacent to the plasma membrane (*tip of large arrow* in *A*). Note in *C* fine filaments that span the region between the plasma membrane and the substratum (between the arrows). Bar, 200 nm.
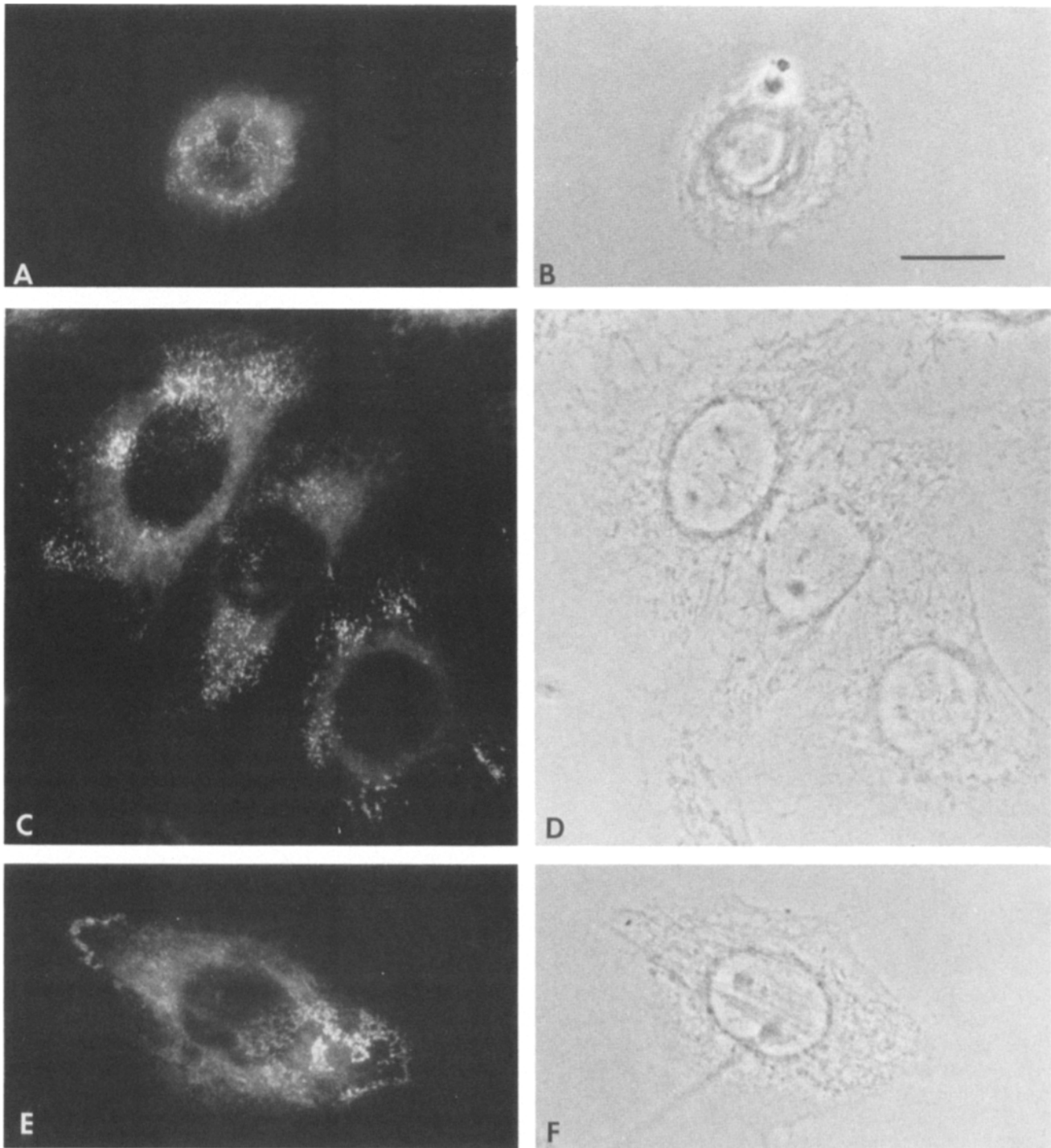
Figure 5. 804G cells were seeded onto glass coverslips and processed for immunofluorescence using the 230-kD autoantibodies at 1 (*A* and *B*), 4 (*C* and *D*), and 24 h (*E* and *F*) after plating. *B, D,* and *F* show phase-contrast micrographs of cells for orientation. At 1 h, the 804G cells are still relatively rounded and the autoantibodies stain a series of spots that appear to underlie the nucleus (*A* and *B*). At 4 h the staining pattern is mainly peripherally located (*C* and *D*). At 24 h after plating, a cell that possesses no contacts with its neighbors shows staining with the 230-kD autoantibodies towards the cell periphery (*E* and *F*). This staining is quite distinct from that observed in the contacting cells shown in Fig. 2. Bar, 5 μm.

cence. Whereas the 230-kD autoantibodies localize to the substratum attached surface of the 804G cells as shown above in Fig. 1, the 180-kD autoantibodies do not generate any obvious staining patterns in the 804G cells (results not shown).

Electron Microscopical Analyses

804G cells were processed for EM to determine the ultra-structural equivalent of the immunofluorescence patterns observed with the hemidesmosomal antibody preparations de-
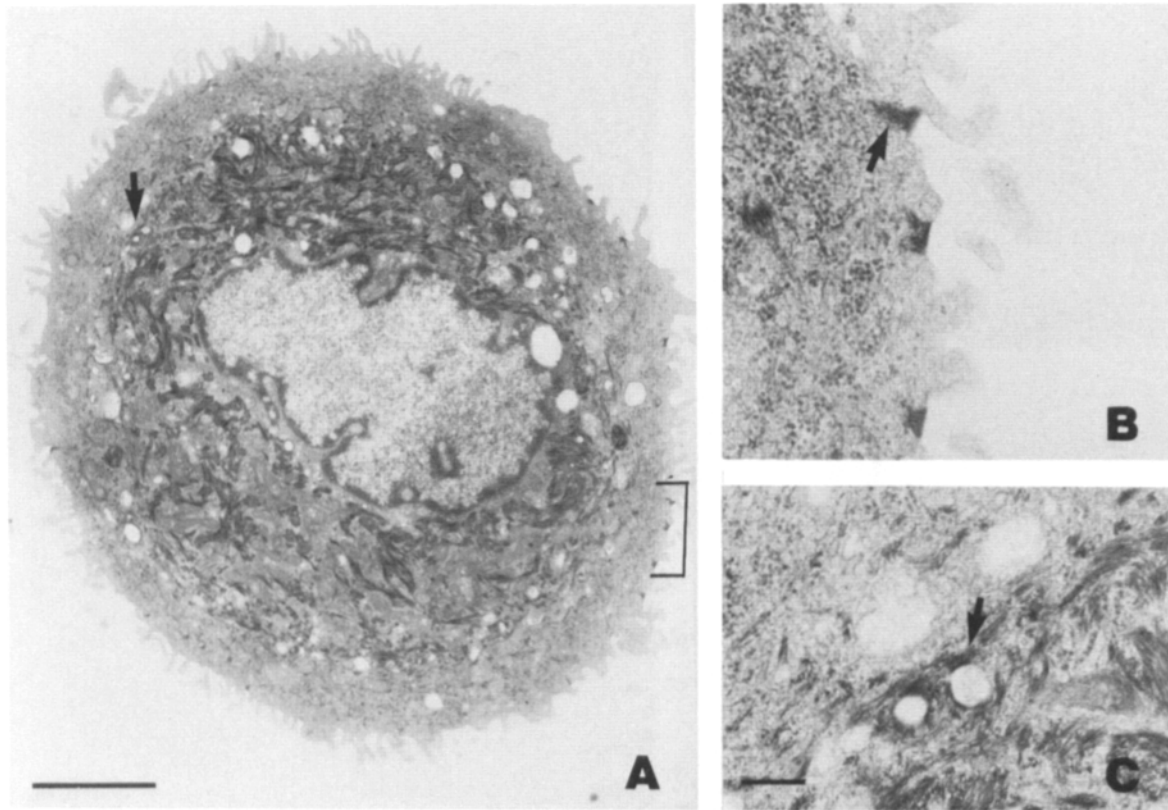
Figure 6. 804G cells were trypsinized and rounded cells were processed for electron microscopy. Many electron densities are observed along the cell surface (*A*). The region between the lines is enlarged in *B*. Note the filamentous tails attached to these electron densities (*arrow*) and their similarity to hemidesmosomes. The arrow in A indicates two vesicles which are enlarged in *C*. These vesicles possess electron dense structures on their cytoplasmic surface to which bundles of IF are attached (*arrow*). Bars: (*A*) 2 μm; (*C*) 200 nm.

scribed above. Numerous hemidesmosome-like structures can be seen along the substratum-attached surface of the 804G cells that had been maintained for 24 h on glass coverslips (Fig. 4). These structures are concentrated beneath the nucleus and are rarely found towards the cell periphery. This finding is consistent with the work of Riddle (1986) who showed that hemidesmosome distribution is nonuniform in basal cells in developing fetal skin with the majority of hemidesmosomes being located towards the cell center.

The hemidesmosome-like structures of 804G cells are morphologically similar to hemidesmosomes in rat bladder tissue in that they possess a tripartite electron-dense cytoplasmic plaque with which IFs associate (compare Fig. 4, *A* and *B*). The tripartite plaque appears triangular in cross-section (Fig. 4 *A*). In certain of the hemidesmosomes of 804G cells an electron-dense line occurs immediately subjacent to the plasma membrane (Fig. 4 *A*). This appears to be similar to the sub-basal dense plate of a hemidesmosome in intact tissue (Tidman and Eady, 1984). Furthermore, a set of fine filaments appears to anchor the hemidesmosomes of 804G cells to the substratum (Fig. 4 *C*).

Changes in Localization of Hemidesmosomal Antigens in 804G Cells after Trypsinization and Replating

It has been reported that hemidesmosomes do not form in an in vitro model system in the presence of low levels of extracellular Ca^{2+} (Trinkaus-Randall and Gispon, 1984). We determined whether the same was true for the hemidesmosomes of 804G cells. It was our hope that we could use a

switch from low to normal Ca^{2+} to induce and thereby monitor hemidesmosome formation in the 804G cells.

After trypsinization, 804G cells were plated into medium containing 0.04 mM Ca^{2+}, then maintained in culture for 24 h and subsequently processed for either immunofluorescence or EM. Under these conditions hemidesmosome-like structures form along the region of cell-substratum interaction (Fig. 4 *D*). These structures appear identical to those observed in 804G cells maintained in medium containing normal cell culture levels of Ca^{2+} (compare Fig. 4, *A* and *D*). Furthermore, the staining patterns generated by BP autoantibodies in cells maintained for 24 h in reduced levels of extracellular Ca^{2+} are similar to those observed in cells maintained in normal culture medium levels of Ca^{2+} for the same period as determined by immunofluorescence (results not shown).

Thus, our studies on hemidesmosomal assembly in the 804G cells, have involved analyses of the location of the 230-kD hemidesmosomal plaque component during cell spreading and reestablishment of cell–cell contact after trypsinization and replating. 804G cells were seeded onto glass coverslips and processed for indirect immunofluorescence and both conventional and immunogold electron microscopy using affinity purified 230-kD autoantibodies at various times after plating (Figs. 5–8).

Light microscopical observations of 804G cells at 1 h after plating, reveals that cells are still rounded but have begun to spread (Fig. 5, *A* and *B*). The human 230-kD autoantibodies generate punctate staining along the substratum attached
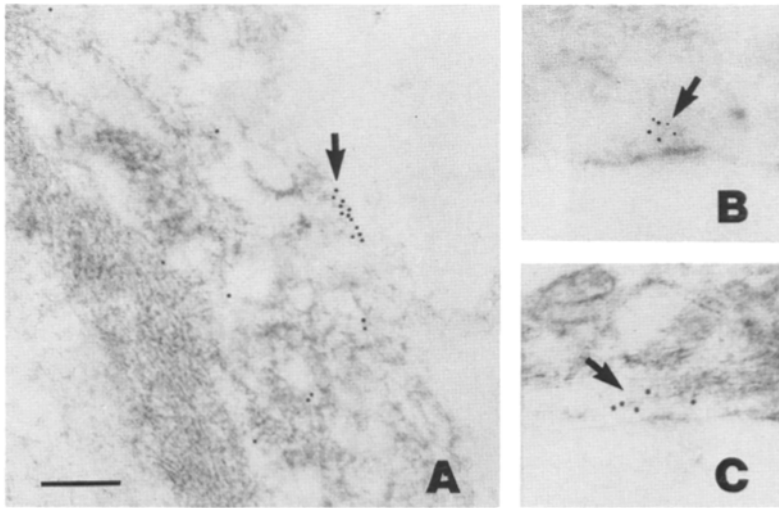
Figure 7. Rounded 804G cells (*A*) and cells at 1 (*B*) and 24 h (*C*) after seeding onto coverslips were processed for indirect immunogold localization using 230-kD autoantibodies. In *B* and *C*, thin sections were prepared perpendicular to the substrate. Note that in *A*, gold particles are associated with an electron dense structure at the cell surface. In *B* and *C* gold particles occur at electron-dense structures along the region of cell-substratum interaction (*arrows*). In *C*, gold particles are observed at the site of interaction of filaments with the basal cell surface. Bar, 200 nm.

surface in the perinuclear region of the cells (Fig. 5 *A*). In contrast, at 4 h after plating, the 230-kD autoantibodies produce staining towards the periphery of the cells (Fig. 5, *C* and *D*). This pattern is also observed in cells 24 h after trypsinization, provided the cell is not in contact with other cells (Fig. 5, *E* and *F*). The latter is distinct from the more perinuclear staining that is observed in contacting cells in the same preparation (Fig. 1).

At the ultrastructural level in rounded cells after trypsinization, hemidesmosome-like structures are distributed along the cell surface (Fig. 6, *A* and *B*). Some of these structures, which are recognized by the 230-kD autoantibodies, appear to have a filamentous tail (Figs. 6 *B* and 7 *A*). In the perinuclear region of rounded cells, large numbers of vesicles are apparent (Fig. 6 *A*). Electron-dense material to which bundles of IF attach occur on the cytoplasmic surface of certain of these vesicles but this material is not recognized by 230-kD autoantibodies (Fig. 6 *C*).

At 1 h after plating hemidesmosome-like structures are observed along the substratum attached surface of cells in the perinuclear region (Fig. 8). The plaques of these structures are morphologically similar to the hemidesmosomes of 804G cells in cells at 24 h after plating (compare Figs. 6 *A* and 8 *B*). Moreover, filamentous material on the cytoplasmic side of the hemidesmosomal plaques in cells at 1 h and 24 h after plating is recognized by the 230-kD autoantibodies (Fig. 7, *B* and *C*). These localization data are consistent with that presented by Klatte et al. (1989) who showed that antibodies directed against the 230-kD polypeptide recognize the most cytoplasmic region of the hemidesmosomal plaque in bovine tongue mucosal hemidesmosomes. At 4 h after plating, hemidesmosomes are observed primarily towards the cell periphery (results not shown).

Is Protein Synthesis Necessary for Appearance of Hemidesmosomes in Freshly Plated 804G Cells?

The rapidity of appearance of hemidesmosomes in the 804G cells suggests that protein synthesis is not required, at least in the short term. To examine this possibility 804G cells were incubated in medium containing [^{35}S]methionine and 10 μg/ml cycloheximide. At 1 h after plating cells were either processed for electron microscopy or incorporation of label into cellular protein was assessed as detailed in Materials

and Methods. Hemidesmosomes can be observed in the cycloheximide treated cells, where they abut the substratum even though protein synthesis is inhibited by >93% over control levels.

Discussion

Several reports in the literature suggest that epithelial cells will only form hemidesmosomes if they are grown on substrata coated with components of the extracellular matrix (Mann and Constable, 1977; Chapman and Eady, 1985; Eady, 1988). For example, hemidesmosomes are formed by epithelial cells within 3–5 d when such cells are cultured on collagen gels but hemidesmosome assembly is not observed in cells maintained on bare plastic (Mann and Constable, 1977; Chapman and Eady, 1985; Eady, 1988). In contrast, Shellswell et al. (1984) have shown that embryonic corneal cells form electron densities along their basal surface when cultured on tissue culture plastic. They term these densities rudimentary hemidesmosomes although these "hemidesmosomes" do not appear to be associated with the cytoskeleton and were not characterized immunologically. Christophers and Wolff (1975) also reported that hemidesmosomes form in epithelial cells maintained on plastic provided cells are treated with retinoic acid.

In agreement with Klatte et al. (1989), we have observed that hemidesmosomal antigens are expressed in a particulate form in a subpopulation of epithelial cells maintained on glass substrata. This is true for both primary cells and cloned continuous cell lines, with the exception of 804G cells, that we have so far studied. It is tempting to speculate that this staining represents formed hemidesmosomes, although we have yet to detect hemidesmosomes in such cells at the ultrastructural level to confirm this. Moreover, it is not clear why only certain cells stain in this manner even in cloned cell lines. Indeed, cells that do not show the punctate substratum staining using the hemidesmosomal antibodies may very well express hemidesmosomal antigens in another form which we have been unable to observe by conventional immunofluorescence microscopy. Nevertheless, the absence of detectable staining leads us to suppose that the majority of these cells do not possess formed hemidesmosomes.

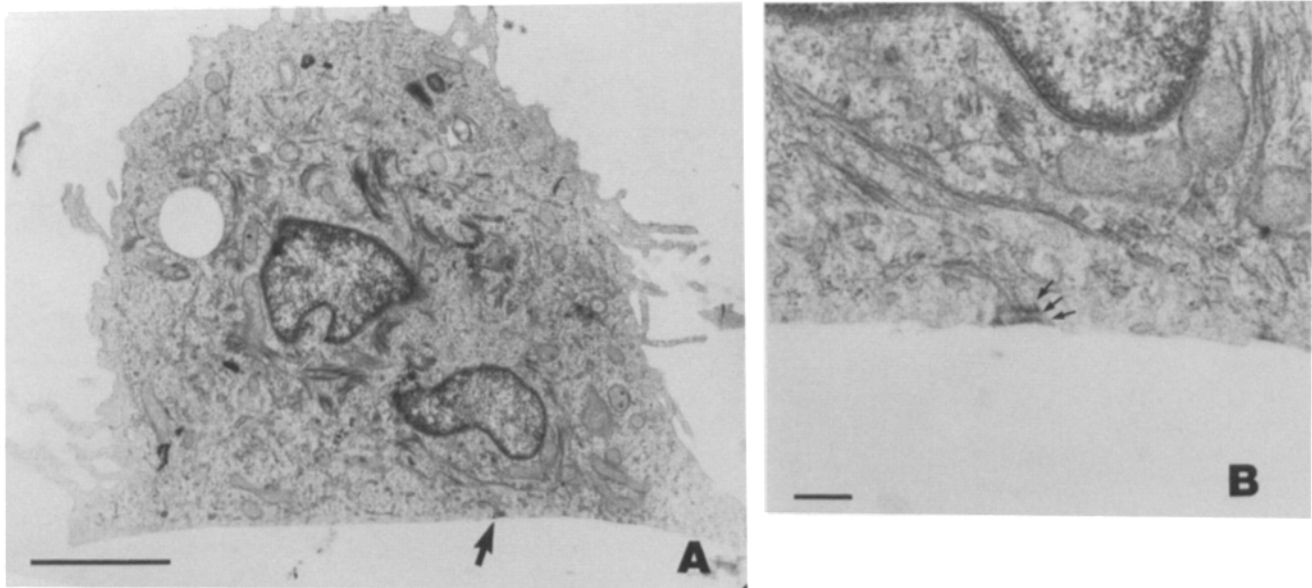The above immunofluorescence analyses taken together

Figure 8. 804G cells at 1 h after seeding onto a glass coverslip were processed for electron microscopy and thin sections of fixed cells were prepared perpendicular to the substrate. The arrow in *A* marks an electron density at the region of cell-substratum interaction. This structure is enlarged in *B*. Note that it is similar to a hemidesmosome in that it possesses a tripartite plaque to which IF are attached. Bars: (*A*) 2 μm; (*B*) 200 nm.

with a lack of hemidesmosome-like structures in a variety of epithelial cells in vitro at the ultrastructural level, emphasize the problems inherent in the use of cultured cells for the study of hemidesmosome assembly or even the expression of hemidesmosomal polypeptides. The 804G cells are therefore remarkable in that they readily form structures along their basal surface which are morphologically related to the hemidesmosomes of rat bladder in situ. Furthermore, the rosette-like or linear distribution of hemidesmosome components along the substratum attached surface of the 804G cells is consistent with the arrangement of hemidesmosomes in tissues (Buck, 1982, 1983). We have confirmed that these structures in 804G cells are immunologically related to hemidesmosomes by immunoelectron microscopy using human autoantibodies directed against a 230-kD plaque component of the hemidesmosome affinity purified from BP serum (Klatte et al., 1989). Indeed, we speculate that the hemidesmosomes of 804G cells, in concert with adhesion plaques, play a role in cell–substratum attachment.

The hemidesmosomes of 804G cells do not show reactivity with human autoantibodies which recognize the 180-kD polypeptide component of bovine tongue epithelial hemidesmosomes recently described by Klatte et al. (1989). Thus it is possible that the 180-kD polypeptide is not present in 804G cells. However, a protein related to the 180-kD component of bovine tongue epithelial hemidesmosome may be present in the 804G cells but this polypeptide may lack the epitope(s) recognized by the human autoantibodies.

The hemidesmosomes of 804G cells form even when cells are plated onto glass substrata in medium containing low levels of Ca^{2+}. This contrasts with data presented by Trinkaus-Randall and Gipson (1984) who showed that hemidesmosome assembly is Ca^{2+} sensitive in an in vitro model where epithelial cell sheets are added back to denuded stromal tissue. We cannot easily explain these differences in results particularly since desmosome assembly in 804G cells is sen-

sitive to modulation in extracellular Ca^{2+} levels (Green et al., 1990).

Immunogold localization of the 230-kD hemidesmosomal component in 804G cells in rounded cells reveals that the 230-kD polypeptide is associated with electron densities at the surface of cells. These structures may represent old hemidesmosomes which remain on the cell surface even after trypsinization. In addition, we also observed electron-dense material in association with perinuclear vesicles in rounded cells. Takahashi et al. (1987) have observed similar bodies in trypsin treated keratinocytes and have suggested that they are a result of internalization of hemidesmosomes. In rounded 804G cells we did not observe association of 230-kD antigen with these perinuclear vesicles. Thus these vesicles may not be a result of hemidesmosomal internalization. Rather, they may represent desmosomal remnants which have been shown to be internalized in cultured epithelial cells as a result of trypsinization or after a reduction in the level of Ca^{2+} in the cell culture medium (Kartenbeck et al., 1982; Mattey and Garrod, 1986; Duden and Franke, 1988). However, we cannot rule out the possibility that antigenic sites recognized by 230-kD autoantibodies on any internalized hemidesmosomal components may be masked, for example, by the collapse of filament bundles onto the surface of the vesicles.

As the 804G cells flatten after trypsinization, hemidesmosomes rapidly appear along the cell–substratum interface. The speed of appearance of hemidesmosomes in the 804G cells is comparable to that seen when dispase separated epithelial sheets are allowed to reassociate with denuded connective tissue in vitro and may proceed by a similar mechanism (Gipson et al., 1983). Furthermore, protein synthesis does not appear to be required for hemidesmosomes to occur along the substratum attached surface of freshly plated 804G cells. We are tempted to explain this phenomenon by proposing that hemidesmosomal remnants that occur on the cell

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surface of rounded cells are reused by cells as they establish substratum interactions. This is certainly an attractive hypothesis in light of our results. However, it is possible that hemidesmosomal polypeptides in a soluble and/or cytoskeleton associated pool may be the source of components for rapid de novo assembly of hemidesmosomes in 804G cells. Indeed, both these mechanisms may play a role in hemidesmosome formation as 804G cells spread onto a substrate.

Hemidesmosomes or, at least their 230-kD plaque component, "move" from the cell periphery in the 4 hour spread cells to the perinuclear zone by 24 h after plating provided cells possess intercellular contacts. We speculate that membranous elements of the hemidesmosome, which connect plaque components such as the 230-kD polypeptide to the cell surface, are free to move laterally in the plasma membrane. Lateral mobility of such elements could then result in relocation of the 230-kD polypeptide and possibly the entire hemidesmosomal plaque complex to the perinuclear region. This has a precedent since Klymkowsky et al. (1983) suggested that desmosomal plaque components are able to move within the plane of the plasma membrane.

In summary, we have identified a novel cell line that forms hemidesmosomes when grown on uncoated glass coverslips. Compared with the in vitro models so far developed for the study of hemidesmosome morphogenesis, the 804G cell line should prove invaluable for the biochemical and molecular analysis of hemidesmosome structure and hemidesmosomal plaque assembly.

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