Expression of Extracellular Matrix Components Is Regulated by Substratum

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Abstract. Reconstituted basement membranes and extracellular matrices have been demonstrated to affect, positively and dramatically, the production of milk proteins in cultured mammary epithelial cells. Here we show that both the expression and the deposition of extracellular matrix components themselves are regulated by substratum. The steady-state levels of the laminin, type IV collagen, and fibronectin mRNAs in mammary epithelial cells cultured on plastic dishes and on type I collagen gels have been examined, as has the ability of these cells to synthesize, secrete, and deposit laminin and other extracellular matrix proteins. We demonstrate de novo synthesis of a basement membrane by cells cultured on type I collagen gels which have been floated into the medium. Expression of the mRNA and proteins of basement membranes, however, are quite low in these cultures. In contrast, the levels of laminin, type IV collagen, and fibronectin mRNAs are highest in cells cultured on plastic surfaces, where no basement membrane is deposited. It is suggested that the interaction between epithelial cells and both basement membrane and stromally derived matrices exerts a negative influence on the expression of mRNA for extracellular matrix components. In addition, we show that the capacity for lactational differentiation correlates with conditions that favor the deposition of a continuous basement membrane, and argue that the interaction between specialized epithelial cells and stroma enables them to create their own microenvironment for accurate signal transduction and phenotypic function.

THE synthesis and secretion of milk proteins in functional mammary epithelia are regulated by both hormones and extracellular matrix (ECM)[1] (3, 17), although the molecular mechanisms underlying the control of milk protein expression are not fully understood. A culture system is being used in our laboratory in which dissociated primary mouse mammary epithelial cells express high levels of milk products in response to prolactin, hydrocortisone, and insulin. Cells from pregnant mammary gland rapidly lose the ability to synthesize milk proteins if they are cultured on a plastic substratum, even when all lactogenic hormones are present (18, 37). However, when plated onto an exogenous stromal (type I collagen) matrix which is then floated, or onto a basement membrane matrix reconstituted from Engelbreth-Holm-Swarm tumor, the cells reacquire many of their differentiated properties, including the capacity to assume a polarized morphology, to accumulate casein and transferrin mRNAs, and to secrete most of the milk proteins (7, 12, 13, 36–38). Furthermore, cells cultured on the basement membrane matrix undergo striking multicellular architectural restructuring, resemble mammary alveoli in vivo, and secrete milk proteins vectorially (1, 13). The major conclusion from several years' study in this and other laboratories is that the ECM plays a fundamental role in controlling cellular phenotype in specialized tissues.

It is clear that an exogenous reconstituted basement membrane gel positively influences cell-specific differentiation not only in mammary cells (1, 13, 38), but also in other cell types, including Sertoli cells (24, 25), Schwann cells (11), hepatocytes (5, 47), and cells of alveolar lung type II (51), endothelial (33), and uterine epithelial origin (22). Although these systems provide direct evidence for the involvement of basement membranes in cytodifferentiation, they offer little insight into the molecular mechanism(s) by which ECM so profoundly alters tissue-specific gene expression. Nevertheless, studies with this type of substratum raise the possibility that induction of function in mammary cells cultured on a stromal matrix is due to the synthesis and deposition of an endogenous basement membrane rather than to a direct interaction between the cells and type I collagen. In this paper we have examined the expression of ECM components by mammary epithelial cells cultured on different substrata, and followed basement membrane formation by immunohistochemical techniques. A type I collagen gel system (18, 36), which itself contains no detectable laminin and type IV collagen, provides an ideal context for pursuing this goal.

Our data indicate that cells cultured on floating collagen gels organize an endogenously synthesized basement membrane. The expression and deposition of ECM components

1. *Abbreviation used in this paper*: ECM, extracellular matrix.

themselves are regulated by substratum. On tissue culture plastic, in the absence of a basement membrane, the cells continue to synthesize and secrete large quantities of laminin and other ECM proteins. The appearance of the basement membrane on floating collagen gel cultures, however, negatively correlates with the levels of ECM mRNAs, and positively correlates with the ability of mammary epithelial cells to undergo functional differentiation and to produce milk.

Materials and Methods

Cell Culture

Epithelial cells from the mammary glands of midpregnant (11–14 d gestation) CD-1 mice were prepared as described previously (1, 37), and plated at 3×10^5 cells/cm^2 onto the appropriate substrata in F12 medium with lactogenic hormones (5 μg/ml insulin, 1 μg/ml hydrocortisone, 3 μg/ml prolactin), 10% FCS, and 1 mg/ml fetuin. After 36 h this was replaced with serum-free medium containing only lactogenic hormones. These conditions encourage differentiation (37).

Stromal (type I collagen) substrata were prepared using established protocols (36). Thick collagen gels were made with 150 μl rat tail collagen per cm^2 of culture dish, and either left attached to the dish or released into the medium 48 h after plating. Thin collagen gels were made by covering dishes with 15 μl collagen per cm^2.

Northern Blotting

RNA was prepared by the guanidinium isothiocyanate method (14), separated in denaturing formaldehyde gels (40), and transferred to nylon membranes (Hybond-N; Amersham Corp., Arlington Heights, IL) for hybridization (21). Blots containing 2 μg total RNA per lane were probed for milk or fibronectin gene expression; those containing 10 μg total RNA were probed for laminin and type IV collagen sequences. High specific activity ($1–3 \times 10^9$ cpm/μg) random-primed probes (20) were prepared using gel-purified insert sequences from plasmids kindly provided by M. Kurkinen (Robert Wood Johnson Medical School) (mouse type IV [α1] collagen, pPE123 [34]), B. Hogan Vanderbilt University (mouse laminin B1, pPE47 [2], and R. Hynes (Massachusetts Institute of Technology) (human fibronectin, λrlf 1 [48]). Probes for milk gene sequences, mouse β-casein (pBCL-1, a homemade subclone derived from a plasmid from J. Rosen, Baylor College of Medicine), and mouse transferrin (pMTf-5, from this laboratory [12]), were prepared by nick translation (specific activity = 5×10^8 cpm/μg). After a 2-h prehybridization step, hybridization was carried out overnight at 42°C in the presence of 6 × SSC (SSC is 150 mM NaCl, 15 mM sodium citrate), 1 μg/ml BSA, 1 μg/ml Ficoll 400, 1 μg/ml polyvinylpyrollidine, 0.1% SDS, 25 mM NaH_2PO_4, 1.5 mM $Na_4P_2O_7$, 50% deionized formamide, 100 μg/ml sheared calf thymus DNA, and $3–8 \times 10^6$ cpm of ECM probe or $1–2 \times 10^6$ cpm of milk probe per ml of hybridization solution. Blots were washed (0.1% SDS, 0.1 × SSC, 68°C) and exposed to XAR-5 film (Eastman Kodak Co., Rochester, NY) for fluorography (8). The positions of RNA size standards (2 μg per lane; Life Technologies Inc., Gaithersburg, MD) were determined by staining the marker lane with methylene blue after transfer.

Protein Analysis

Cultured cells were metabolically labeled to steady state with 0.1–0.25 mCi/ml ^{35}S-methionine for 24 h. Pulse-labeled cells were starved of methionine for 1 h before labeling and pulsed in methionine-free medium. For the chase, cultures were washed with and incubated in normal medium containing 6 mM *l*-methionine. Medium fractions were cleared of cellular debris and adjusted to radioimmune precipitation (RIPA) salt conditions (150 mM NaCl, 50 mM Tris-HCl, 5 mM EDTA, 0.1% SDS, 1% Nonidet-P40, 1% sodium deoxycholate, 0.5% aprotinin, 0.5 mM PMSF, pH 7.4). Cells were scraped into RIPA buffer, extensively vortexed, and sonicated (3 × 5-s bursts) on ice, and the insoluble fractions cleared (16,000 *g*, 5 min, 4°C). This insoluble fraction could be solubilized in RIPA salts containing 2 M urea. Fractions were stored at −70°C until use. The total incorporation of radioactivity was calculated from the TCA-precipitable counts contained in the medium, cell, and urea fractions; and aliquots representing equal numbers of total TCA-precipitable cpm were used for immune precipitations. These samples were adjusted to 500 μl with fresh RIPA, incubated (2 h, 4°C) with 10 μl rabbit anti-mouse laminin (E.-Y. Laboratories, Inc., San Mateo, CA), and subsequently (40 min, 4°C, shaking) with 50 μl 10% wt/vol protein A Sepharose (Sigma Chemical Co., St. Louis, MO). The protein A-Sepharose beads were collected, washed (3 × RIPA buffer; 1 × 50 mM Tris-HCl, pH 7.4), and the precipitated proteins eluted (3 min, 100°C) in sample buffer (10% glycerol, 50 mM Tris-HCl, 2% SDS, 0.02% bromophenol blue, 5% β-mercaptoethanol, pH 6.8) before separating by PAGE (3–6% linear gradient gels, standard discontinuous Tris/glycine system [27]). For fluorography, gels were treated with Amplify (Amersham Corp.), dried and exposed at −70°C to preflashed XAR-5 film.

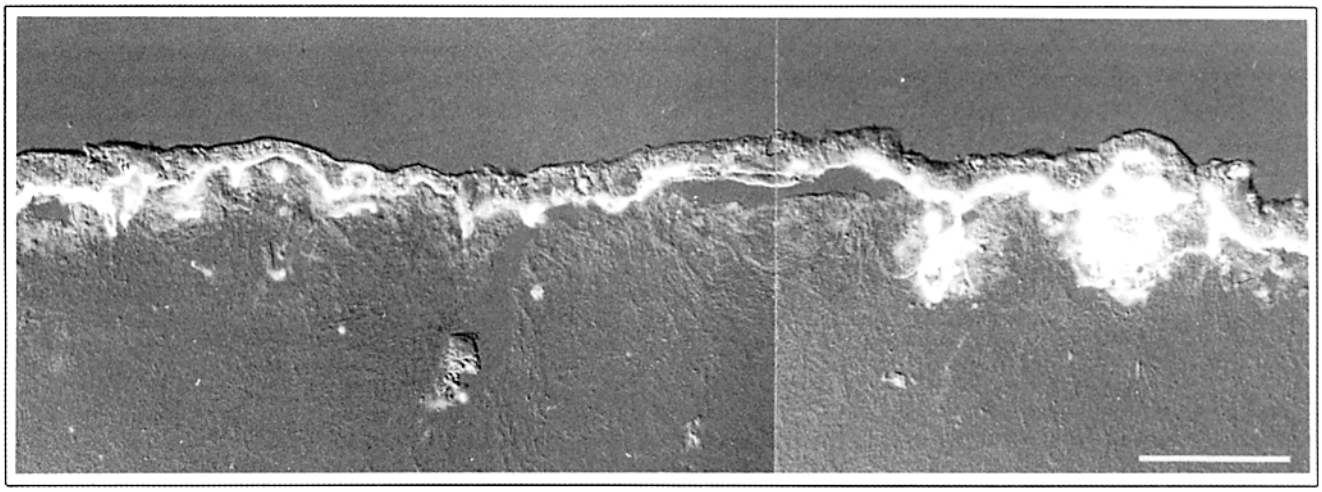

Figure 1. Mammary epithelial cells cultured on floating collagen gels organize type IV collagen into an intact basement membrane. Thick, transverse sections of cells cultured on collagen gels were stained for the presence of type IV collagen. This exposure illustrates the spatial relationship between type IV collagen and the cell layer, photographed simultaneously using epifluorescence and Nomarski optics. The staining profile of this 19-d culture is typical of that in cultures from day three onwards (see also Fig. 5). The observed thickness of the basement membrane components is not an artifact of tissue culture techniques and is also seen in sections of mammary gland tissue stained by immunofluorescence for laminin or type IV collagen. Control slides incubated without primary antibody showed no staining reaction (data not shown). Bar, 50 μm.

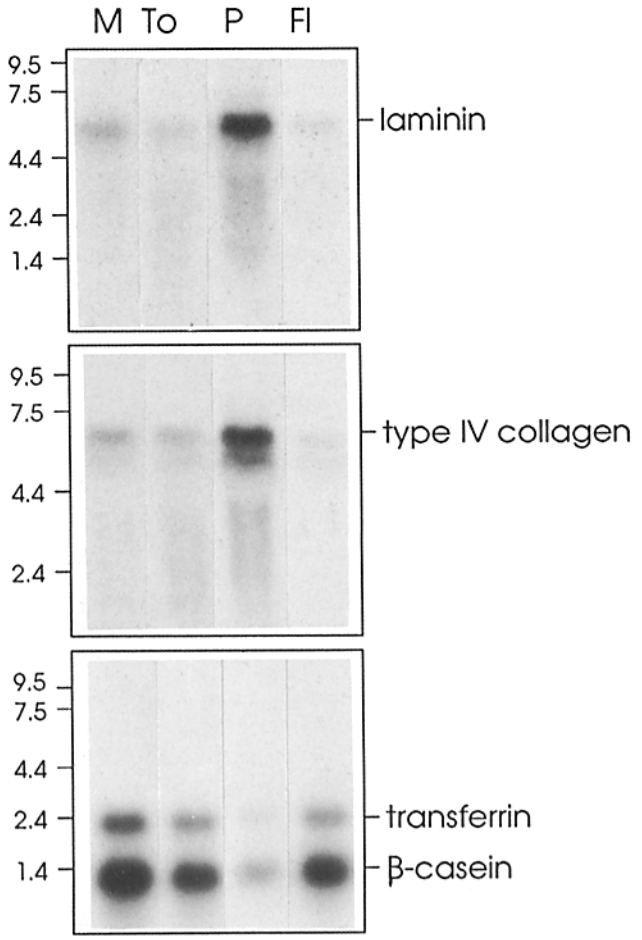

Figure 2. Influence of matrix on the expression of ECM and milk mRNAs. Northern blots illustrate the steady state levels of mRNA for laminin (B1 chain), type IV collagen, or transferrin and β-casein in mammary epithelial cells cultured on (*P*) plastic or on (*Fl*) floating collagen gels. The mRNA levels expressed in the gland of a late pregnant mouse (*M*), and in dissociated epithelial cells just before plating (T_o) are also shown. The positions of RNA-size markers (in kb) are noted in the margin.

Immunofluorescence

Cells cultured on collagen gels were fixed in 2% paraformaldehyde in PBS, quenched with 0.1 M glycine, and equilibrated first with sucrose and subsequently with Tissue-Tek OCT compound (Miles Scientific Div., Elkhart, IN) before freezing on a dry ice/ethanol bath. 5-μm sections were cut with a Leitz cryotome and stained by conventional immunofluorescence methodology (27). Monolayer cultures, grown on acid-washed glass coverslips, were stained after a 5-min methanol:acetone (1:1) fixation at −20°C. Primary antibodies to detect laminin, type IV collagen (J. Roll, University of California at San Francisco), entactin (A. Chung, University of Pittsburgh), and keratin markers (LE61 and LP34; B. Lane, Imperial Cancer Research Fund, London) were kindly supplied. To obtain a qualitative estimate of the staining intensity at different days in the time course experiments, negatives were exposed for equal times, as were the photographic prints.

Results

Formation of a Basement Membrane by Mammary Cells Cultured on a Stromal Matrix

Primary mouse mammary cells cultured for several days in the presence of lactogenic hormones respond according to the exogenous substratum on which they are plated. Multicellular rearrangements take place in cultures seeded onto a type I collagen gel made flexible by floatation into the medium; the cells maintain a cuboidal–columnar morphology, but do not proliferate. On gels that remain attached to the dish, the cells remain flat in most areas, although eventually the gels release spontaneously. On plastic, the cells become squamous and are nonpolarized (18, 55). To confirm that the basal lamina-like structure in electron micrographs of mammary cells cultured on floating collagen gels (18, 26) actually contains ECM components, thick sections of cells on collagen gels were stained by immunofluorescence. A continuous basement membrane, containing type IV collagen (Fig. 1), laminin (see below, Fig. 5), and entactin (data not shown) was laid down between the cell layer and collagen gel.

Expression of ECM Components Is Regulated by Substratum

We examined the capacity of cells cultured on different substrata to synthesize and deposit ECM components. The steady state levels of ECM and milk protein mRNAs were compared by Northern blot analysis of total RNA extracted from cells cultured for 6 d on plastic or on floating type I collagen gels. On the latter, the levels of laminin B1 and type IV collagen mRNA were low and compared with those in the mammary gland of pregnant mice and in freshly dissociated epithelial cells before plating (Fig. 2). They also contained abundant mRNAs for the milk proteins, transferrin, and β-casein. By contrast, cells cultured on a plastic substratum (Fig. 2) produced low levels of β-casein and transferrin mRNA, and milk protein secretion was barely detectable (37). However, in these same cells laminin B1 and type IV collagen mRNA were induced, with substantially higher steady state levels than in midpregnant mammary gland or in cells cultured on floating gels.

The levels of ECM protein synthesis and secretion mirrored this RNA profile. Many high molecular weight proteins were secreted by cells cultured on plastic, including major species of apparent molecular weight 290, 250, 230, 190, and 130 kD, at least one of which (290 kD) was sensitive to bacterial collagenase (data not shown). Although the 130-kD protein appeared at similar levels in both culture systems, and significantly more of a differentiation antigen (transferrin; 80 kD) was present in the medium from collagen gels, the higher molecular weight species were secreted principally by cells on a plastic substratum (data not shown). In addition, both pulse and steady-state labeling demonstrated substantially more secreted and cell-associated laminin in the plastic cultures than in those on floating collagen gels (Fig. 3 *a*). The laminin antibody appeared to recognize three laminin B chains (B_1, B_2, $_pB$), of which two were secreted in a slightly higher molecular weight form (B_1-S, B_2-S), presumably due to glycosylation (Fig. 3 *a*, steady state). $_pB$ represents the laminin B subunit precursor forms (44). Three high molecular weight proteins were apparent, of which one (A) was secreted into the medium (A-S) at least by cells cultured on plastic. The identities of the two additional higher molecular weight bands (A_x and A_y) are unclear at this stage, but may represent novel laminin A chains. In a pulse–chase experiment, the only laminin species to accumulate extracellularly were the A-S, B_1-S, and B_2-S

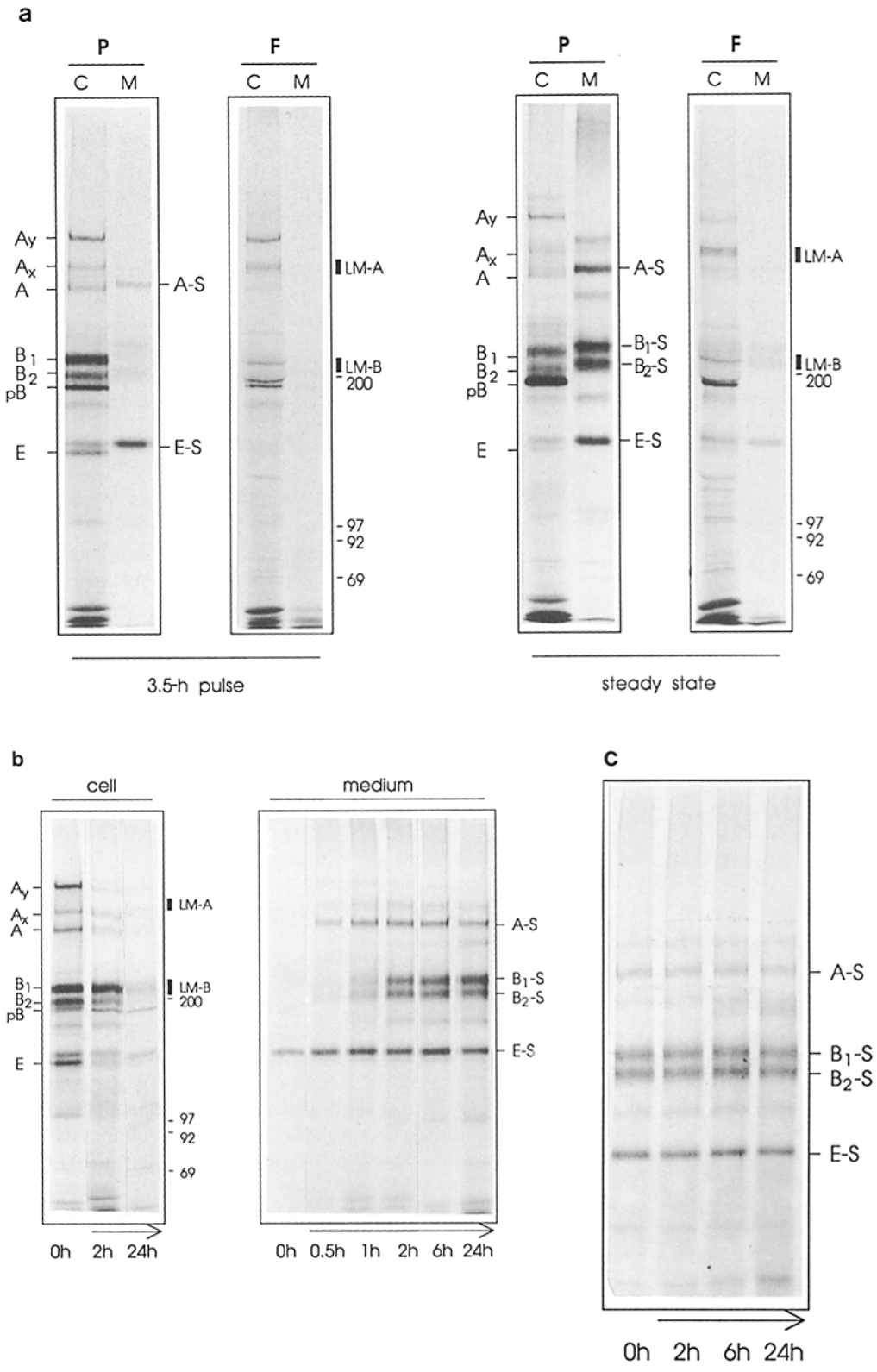

Figure 3. Influence of matrix on synthesis and secretion of ECM components. (*a*) Immune precipitates of cell (*C*) and media (*M*) fractions collected after metabolic labeling of plastic (*P*) and floating gel (*F*) cultures. The 3.5-h pulse and 24-h steady-state samples were from identical dishes harvested simultaneously at day 5 of culture, and aliquots (1.5×10^6 cpm) representing equal total TCA precipitable counts were precipitated with an antilaminin antibody. The three cell-associated B chains (B_1, B_2, $_pB$) were also detected in Western blots using the same antibody (data not shown). The antibody preparation also contains a species that independently reacts with entactin on Western blots (data not shown), so the entactin (*E*, *E-S*) in this figure may be present by virtue of this reactivity rather than by being laminin associated. The positions of radiolabeled size markers (in kD) are noted in the margin, as are the positions of laminin A and B chains isolated from Engelbreth-Holm-Swarm tumor (*LM-A*, *LM-B*). (*b*) Cells cultured on plastic dishes were pulse labeled for 1.5 h, and chased for up to 24 h. Media samples were collected at various times during this chase. Aliquots corresponding to 1.5×10^6 total TCA-precipitable cpm from both cell and media fractions were precipitated with an antilaminin antibody as in Fig. 3 *a*. (*c*) Media from the steady state–labeled culture (see Fig. 3 *a*) was cleared of cell debris and immediately incubated at 37°C for the indicated times. Subsequently, equal aliquots were precipitated with the antilaminin antibody to look for the presence of any cell-secreted laminin degrading activity.

forms (Fig. 3 *b*). The continued presence of these species at 24 h pulse indicates a lack of degradation by cell-secreted proteases. This stability was confirmed by incubating medium from the steady state–labeled cultures at 37°C. In the absence of any inhibitors, no laminin was degraded over a 24-h period (Fig. 3 *c*). Entactin (E) was also recognized by this antibody preparation. A higher molecular weight form (E-S) appeared to be secreted rapidly after synthesis and accumulated in the medium as rapidly as 30 min postchase (Fig. 3 *c*). It was also present to a greater extent in the medium of plastic cultures than in that of the collagen gels (Fig. 3 *a*).

Despite the considerable increase in mRNA levels, and

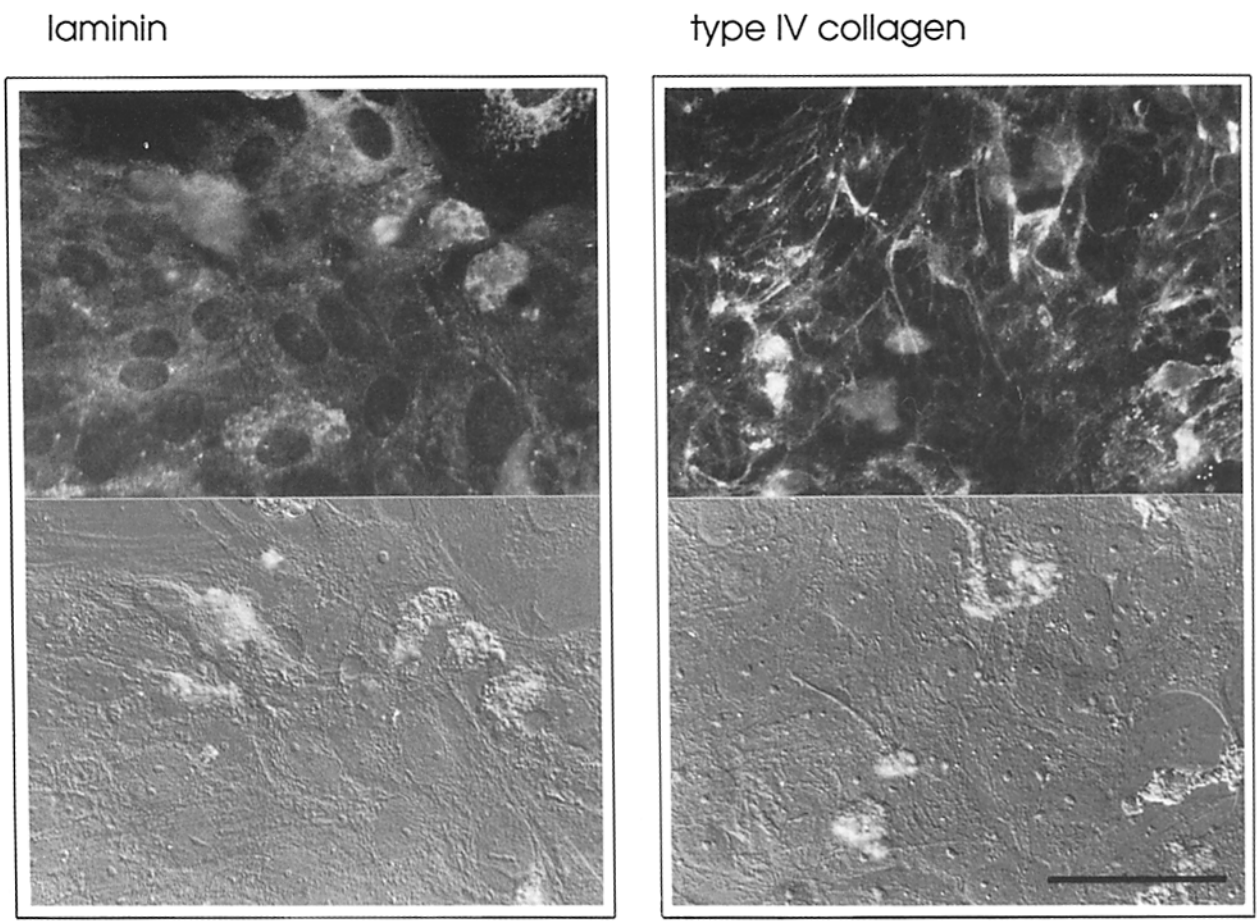

Figure 4. Immunofluorescence of basement membrane components in monolayer cultures. Monolayers of cells cultured on coverslips were stained for the presence of laminin and type IV collagen. Note that the laminin staining appears to be intracellular, while type IV collagen is present in an extracellular lattice. Below each fluorescence micrograph are Nomarski images of the same area. Bar, 50 μm.

higher levels of secreted ECM proteins, cells cultured on plastic showed little deposition of laminin or type IV collagen by immunofluorescence analysis (Fig. 4). In epithelial areas (i.e., in cells with a packed cuboidal morphology that stain with monoclonal antibody LE61, a marker for epithelial cells [35], data not shown) there was intracellular staining with laminin antibodies which, in occasional cells, appeared to be reticular. This was suggestive of newly synthesized material located in the ER and may reflect the marked accumulation of laminin mRNA and newly synthesized protein in these cultures. Although there was no obvious laminin-containing extracellular mesh, which would be expected if a basement membrane had been formed, there was a type IV collagen lattice that appeared to be located on the upper surface of the cell layer rather than below it. This confirmed electron microscope studies in which no basal lamina was detected beneath cells cultured on plastic substratum (18, 26, 55). Thus, although these cells have high levels of ECM mRNAs capable of being translated into secreted proteins, additional factors are required for the assembly of the ECM components into a continuous basement membrane.

An advantage of using floating gels to study mammary cell gene expression is that a variety of events can be synchronized to the time of floatation and correlated with one another. Time course experiments demonstrated that the lamina beneath cells cultured on floating gels appeared de novo. A basement membrane containing laminin (Fig. 5 *a*) and type IV collagen (Fig. 5 *b*) accumulated after gel release on day 2, most clearly demonstrated in monolayer, but polarized, regions (Fig. 5 *a*). In areas of cell clustering, the staining was still situated between the outermost cell layer and the collagen gel, although it was more diffuse, with ECM deposits occurring among cells within the aggregates (Fig. 5 *b*). The formation of the basement membrane, however, was not solely dependent on the presence of a collagen matrix, since the cells that remained spread on nonreleased (attached) gels had little laminin or type IV collagen, except in areas of clustering (Fig. 5 *b*). Furthermore, cells cultured on a thin layer of type I collagen that remained squamous had a staining profile similar to that shown in Fig. 4 for cells cultured on plastic (data not shown). This indicates that changes in cell shape and density may also be required for the stromal matrix to be able to induce polarized deposition of an endogenous basement membrane.

The induction of laminin expression was an early event in the establishment of these cultures (Fig. 6 *a*). This occurred both on a plastic substratum (P_2), and on collagen gels until the second day of culture (A_2). Its expression was stimulated further on plastic cultures, but the trend was reversed on a collagen substratum. This was particularly apparent in

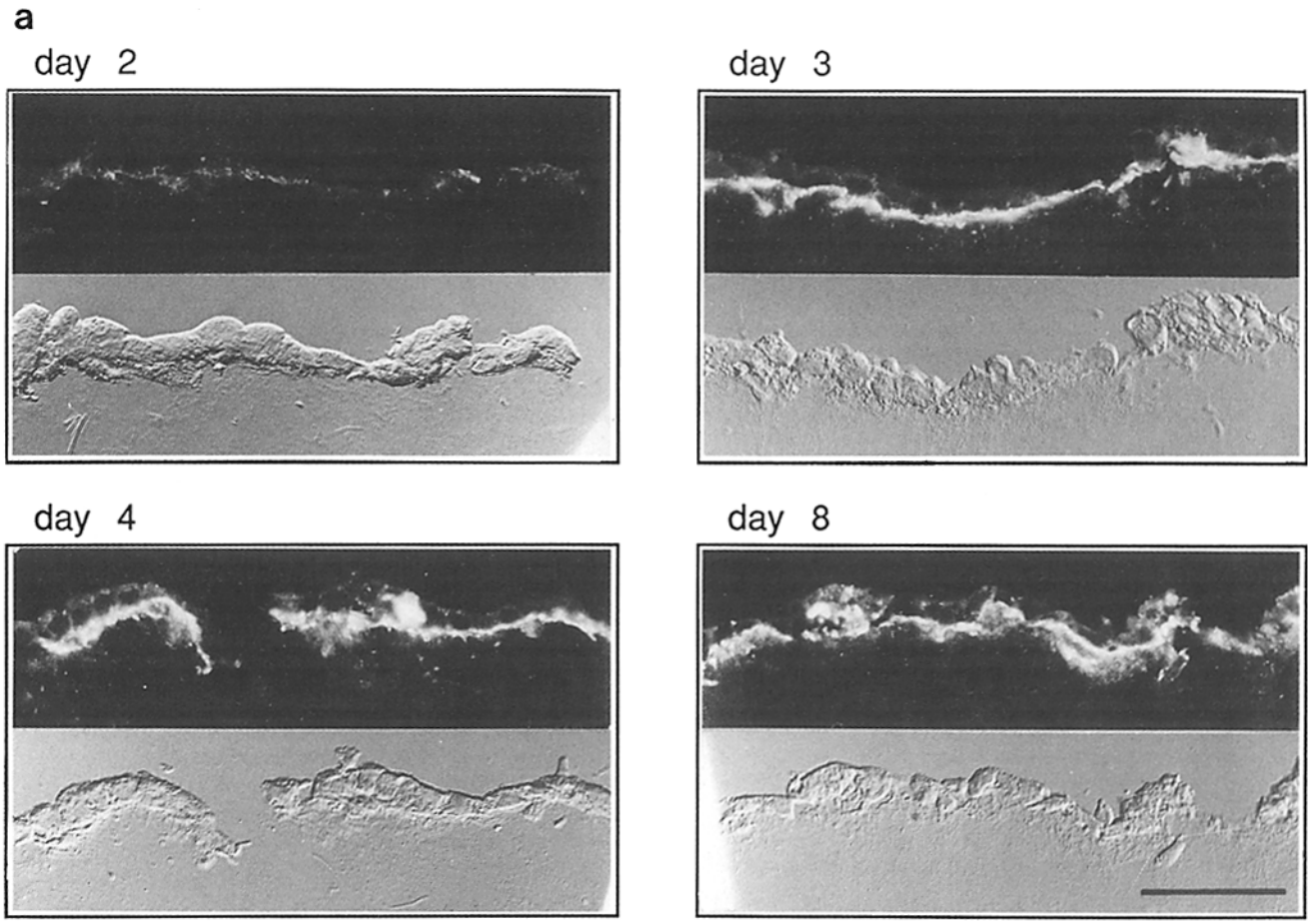

Figure 5. Time course of basement membrane deposition by cells cultured on collagen gels. (*a*) Thick sections of cells cultured on collagen gels for 2, 3, 4, or 8 d were stained for the presence of laminin. The day 3, 4, and 8 cultures were floated after 2 d in culture. In this figure, monolayer regions were photographed. Below each fluorescent micrograph are Nomarski images of the same area. (*b*) Thick sections of cells cultured on collagen gels for 2, 3, or 6 d were stained for the presence of type IV collagen. The floating gels were released on day 2. Compare the low level of staining on an attached gel at day 3 of culture to that on a gel which had been released the previous day. Bars, 50 μm.

the case of the floating gel cultures, suggesting that either the interaction between the epithelial cells and their basement membrane, or the cell shape changes that occurred after floatation, exerted a negative influence on the expression of the mRNA for the laminin B1 chain.

Other ECM proteins were regulated differently, as illustrated by the example of fibronectin (Fig. 6 *b*) which was downregulated in a stromal environment. On a plastic substratum the expression of its mRNA was rapidly and sustainably induced, whereas only low levels of this component accumulated on type I collagen gels.

The Appearance of the Basement Membrane Correlates with the Onset of Functional Differentiation

Whereas the steady-state levels of transferrin and β-casein mRNAs (Fig. 6 *c*), as well as their protein products (37), remained low in cells cultured on a plastic substratum, they became substantial on floating collagen gels at the time (day 3) when a basement membrane was laid down, and were maintained for at least a further three days (Fig. 6 *c*, lanes F_3, F_4, F_6). Transferrin and β-casein mRNA were present before floatation (A_2) and may reflect residual function in groups of cells that remained as clusters (small organoids) after gland dissociation. This activity subsequently disappeared on attached gels (A_3, A_4) when the cells had had greater opportunity to spread onto the available areas of collagen. In contrast, the action of floating the gel (F_3–F_6) allowed for considerable induction of functional differentiation.

Discussion

Formation of a Basement Membrane

Basement membrane formation is a complex process requiring dynamic interaction between epithelial cells and their environment. Our results demonstrate that it is not sufficient for cells to synthesize and secrete large quantities of laminin, glycosaminoglycans (42), and other high molecular weight proteins, nor is simple contact with a stromal-type matrix of type I collagen enough to initiate basement membrane formation. A suitable context for this process, however, is provided by the flexibility achieved when the collagen gel cul-

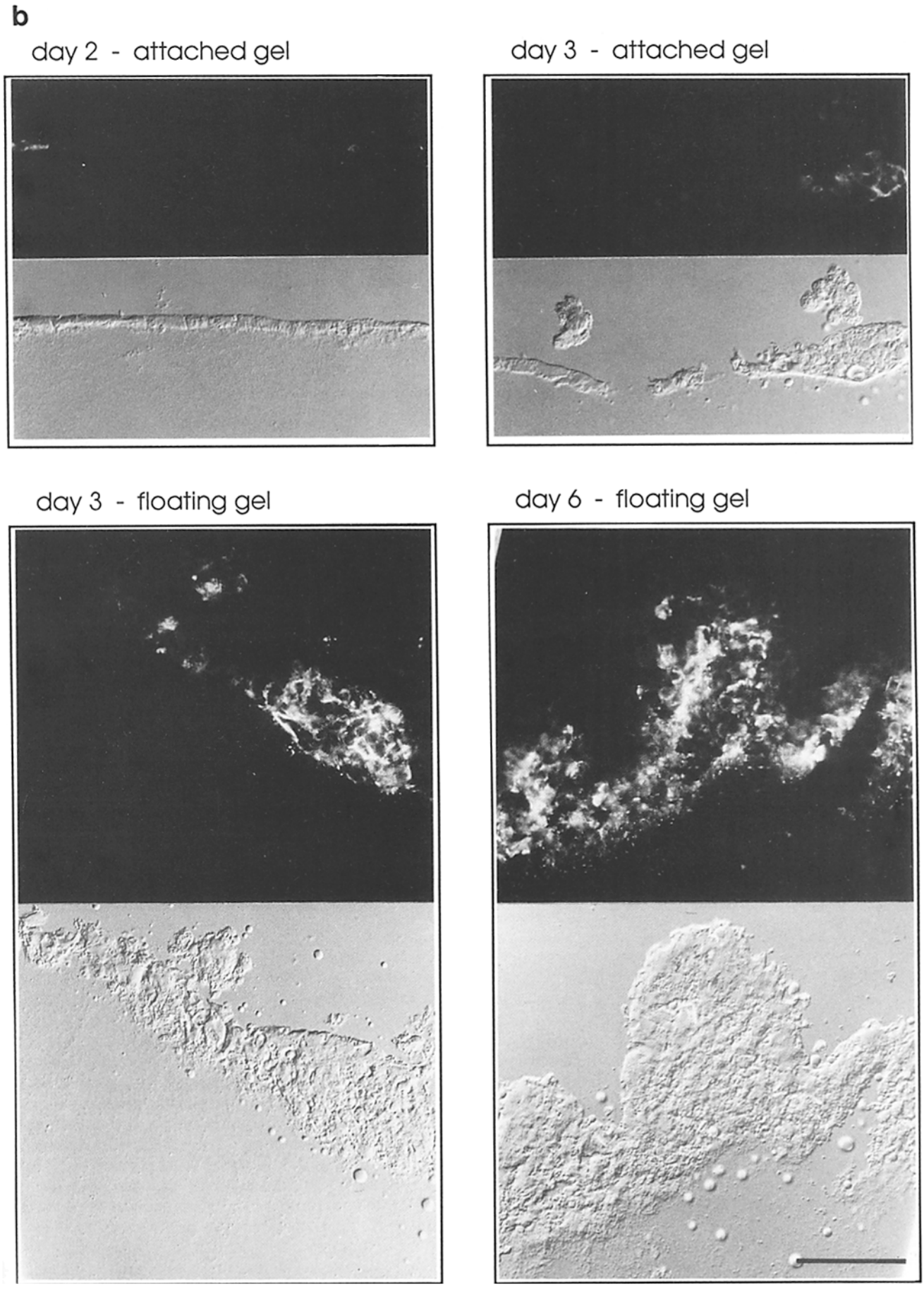

Figure 5 (Continued).

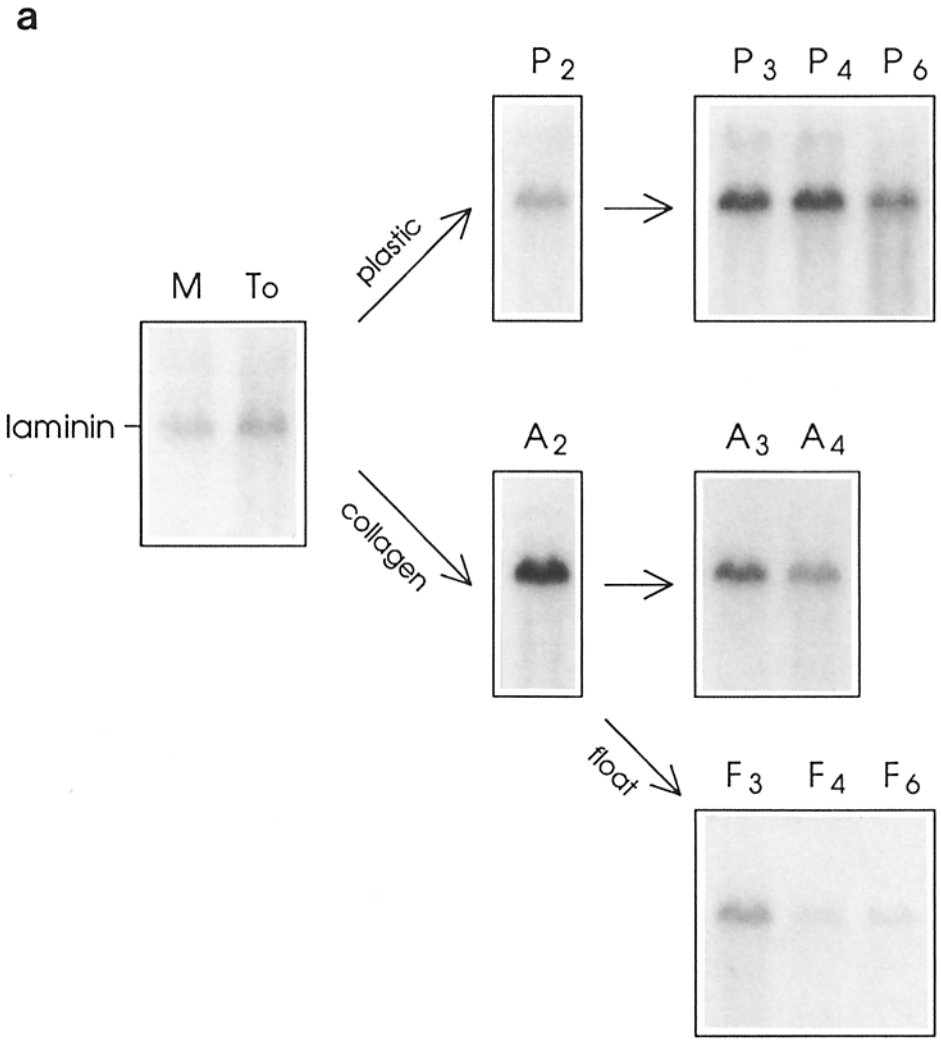

Figure 6. Time course of ECM and milk protein mRNA production by cells cultured on different substrata. Total RNA was prepared from midpregnant mammary gland (*M*), from epithelial cells dissociated from the same series of midpregnant mice (T_o), and from these cells cultured for 2, 3, 4, and 6 d after plating onto plastic (*P*) and attached (*A*) or floating (*F*) collagen gels. Northern analysis of equal quantities of these total RNAs illustrate the steady state levels of the mRNAs for (*a*) laminin (B1 chain), (*b*) fibronectin, and (*c*) transferrin and β-casein.

tures are floated. We suggest that in an environment where cells are not able to undergo the necessary changes for generating functional polarity, ECM components are not deposited into a basement membrane. Increased ECM synthesis and secretion into the medium may represent an attempted rescue mechanism for overcoming this inability.

Although there is some evidence that fat pad precursor cells are responsible for laminin and heparan sulphate proteoglycan synthesis in the embryonic mammary gland (31), our data show that during pregnancy epithelial cells themselves are able to carry out this function, at least in culture. These results extend previous observations that both sulfated glycosaminoglycans (42) and collagenous material (16, 54) are preferentially assembled into the ECM of mammary epithelial cells cultured on floating gels. In this type of flexible environment, multicellular restructuring is possible (9, 18, 26, 36, 37), with the cell shape changes and cell–cell interactions being accompanied by cytoplasmic reorganization that allows epithelial cells to maneuver themselves into a polarized orientation (46). Since mammary function is characterized by the presence of apical secretory vesicles for milk proteins such as β-casein (1, 41, 43), the correct organization of intracellular organelles similarly could be a necessity for directional secretion and subepithelial deposition of an intact basement membrane (3). Indeed, heparan sulphate proteoglycan and laminin are secreted preferentially in a basal direction by MDCK cells (10), and fibronectin and types I/III collagen are secreted basally by endothelial cells (53). Thus, basement membranes may form in conjunction with the establishment of polarity, and only when the local concentrations of their constituents at the basal side are sufficiently high.

The profile of protein synthesis is presumably also important for this process since basement membranes are complex structures containing defined amounts of several different components. It is likely that these need to be present in the correct ratios for deposition of a functional structure (23, 32). We have demonstrated that sulphated glycosaminoglycans (42), laminin, type IV collagen, and entactin are synthesized by cells cultured on plastic; nevertheless, a key ECM component may be absent or modified. Alternatively, cell-secreted proteases may change the profile of extracellular ECM accumulation. Proteoglycans secreted by NMuMG cells, a mammary cell line, were degraded when the cells were grown on plastic (15), although under the conditions used in our experiments primary cells secreted more glycosaminoglycans than when cultured on collagen gels (42). Our present data suggest that laminin is also stable in the medium

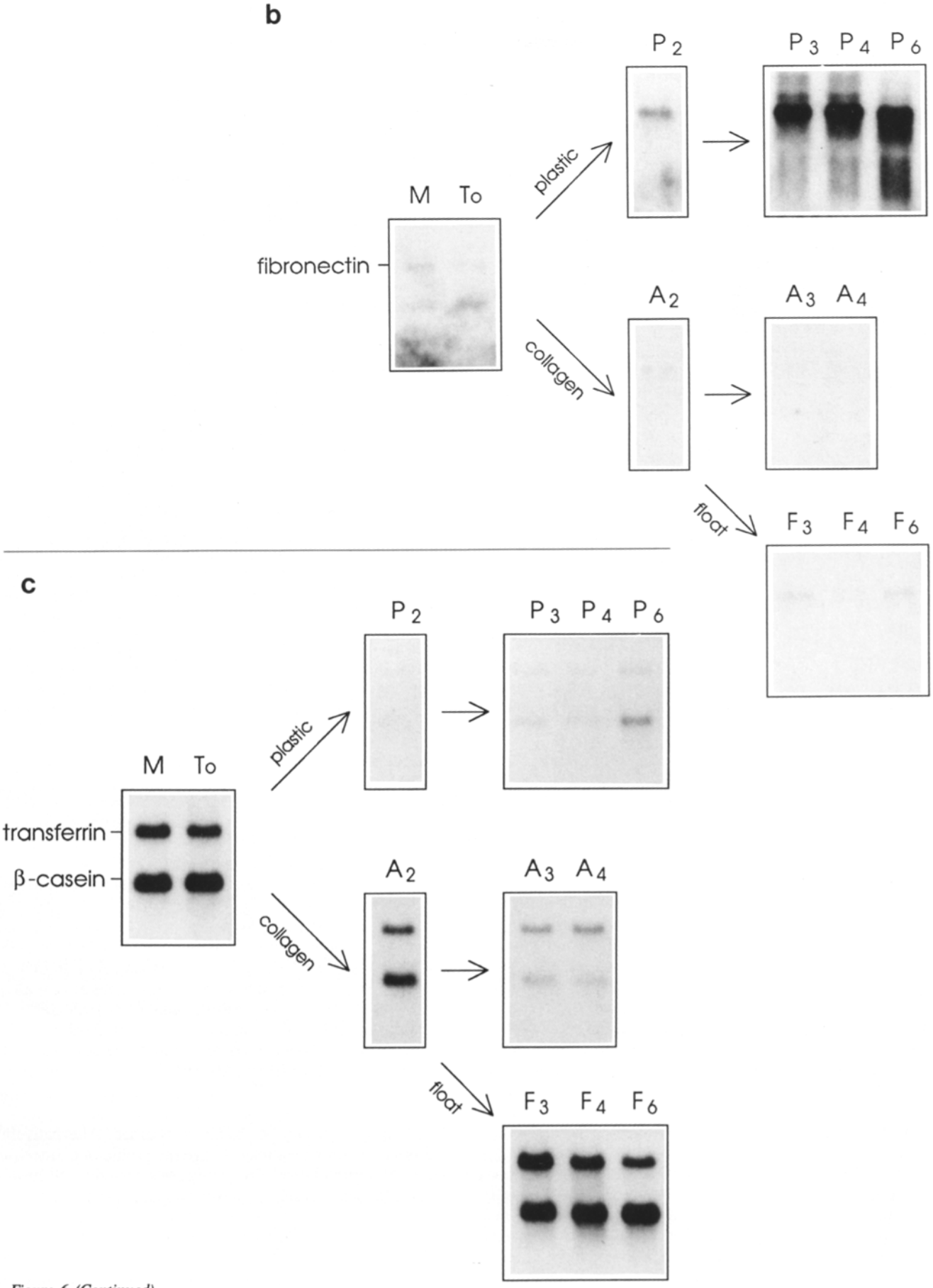

Figure 6 (Continued).

of cells grown on plastic dishes. Thus, the inability of these cells to deposit a basement membrane is unlikely to be related to the elaboration of ECM-degrading proteases.

Regulation of ECM Expression

A major finding of our studies is that the expression of ECM components themselves is regulated by substratum. In comparison to the situation in cells cultured on plastic dishes, the levels of laminin mRNA and protein are down regulated by type I collagen matrices. This is especially apparent on floating gels, when the cells are in contact with a newly made basement membrane. The data suggest that direct interaction of ECM components with the basal cell surface negatively regulates their own expression at the mRNA level. Furthermore, the mRNAs for other ECM proteins, such as those primarily present in the mesenchyme, are also regulated by substratum. The induction of fibronectin by plastic, and its corresponding inhibition by type I collagen, is dramatic. Together, these results show that components of both stroma and basement membrane influence the expression of various ECM proteins.

In support of our conclusion that the extracellular environment modulates expression of ECM molecules is the observation that fibronectin biosynthesis by smooth muscle cells is much lower in cells grown on a fibronectin substratum than in those cultured on laminin (28). In addition, endothelial cells respond to a type I collagen gel by increasing type V collagen and decreasing fibronectin incorporation into their matrix (39), and soluble ECM molecules modulate collagen biosynthesis in embryonic corneal epithelium (52).

It is possible that soluble factors such as TGF-β, which profoundly influence the expression of ECM components in some cell types (29, 39, 56), are increased and/or activated in mammary cells cultured on plastic and mediate the observed increase in ECM expression. Such effects could be in response to the "culture shock" that cells receive when they are plated onto a plastic substratum.

Control of Mammary Differentiation through Basement Membrane

The problem of finding culture conditions that enable mammary epithelial cells to express their differentiated functions in the presence of lactogenic hormones was initially addressed by the use of a collagen gel system (18, 19, 36). Increased basal access to nutrients is insufficient for the acquisition of function, since cultures plated onto glutaraldehyde-fixed collagen gels that are subsequently floated, remain flat, and are unable to express their differentiated phenotype (36, 50). The change of cell shape from squamous to cuboidal–columnar, and the establishment of polarity, both of which accompany lactational differentiation, influence the processing and stability of milk proteins and their mRNAs, possibly via an intact and accurately organized cytoskeleton (3, 4, 6, 49). Such alterations in cellular organization require structural and mechanical integrity between the cell and its molecular environment (30). On floating collagen gels, this is provided both by flexible stroma and by specific ECM components contained within the basement membrane. Our data indicate that the basement membrane may indeed significantly influence differentiation, since its appearance correlates with the onset of milk protein expression and secretion. Conversely, under conditions that permit neither basement membrane formation nor cell shape changes, the cells are unable to differentiate. Whether the basement membrane appears concomitantly with other required cellular changes such as the establishment of polarity, or whether it is a prima facie determinant in epithelial cell differentiation is unclear at this time. It is likely that the events leading to polarity and basement membrane formation are intimately related, and that both are required for optimal differentiation to occur.

Lactational differentiation has been achieved also by mammary cells cocultured with stromally-derived cells. Adipocytes (from the 3T3-L1 stock) provide a suitable substratum for morphological and functional differentiation of primary midpregnant mammary cells, but the lactational phenotype occurs only in conjunction with the appearance of a continuous basal lamina (55). More recently, epithelial and fibroblastic subpopulations have been established from an immortal mammary cell line (45). When cocultured, the epithelial cells developed competence to synthesize casein, but only in areas of laminin deposition. These data support our conclusion that stromal components provided by the collagen gel matrix, or derived from mesenchymal cells themselves, participate in the controlled production of a basal lamina, which in turn plays a key role in the establishment and maintenance of the differentiated phenotype.

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