

Regulation of Functional Cytodifferentiation and Histogenesis in Mammary Epithelial Cells: Role of the Extracellular Matrix

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Primary mammary epithelial cells provide a versatile system for the study of hormone and extracellular matrix (ECM) influences on tissue-specific gene expression. We have characterized the formation of aveolarlike morphogenesis and mammary-specific functional differentiation that occur when these cells are cultured on a reconstituted basement membrane (EHS). Cells cultured on EHS exhibit many ultrastructural and biochemical features indicative of polarized and functionally differentiated mammary epithelium *in vivo*. The increased expression and specific vectorial secretion of milk proteins into lumina formed in culture are accompanied by large increases in milk protein mRNA expression. However, when individual ECM components are tested, smaller increases in milk protein mRNA are measured on heparan sulfate proteoglycan (HSPG) and laminin, and these responses are not associated with full functional cytodifferentiation or histotypic configuration. This indicates that multiple levels of regulation are involved in mammary-specific gene expression, and that in addition to individual ligand requirements cooperative interactions between various ECM molecules and cells are necessary for functional differentiation in culture. We have also shown that endogenous production of ECM molecules and changes in cell geometry are correlated with changes in functional and histogenic gene expression. We have previously proposed a model of cell-ECM interactions that is consistent with these data.

"It is possible, then, that A should move B, and B move C . . . As, then, in these automatic machines the *external* force moves the parts in a certain sense . . . it is the innate motion that does this, as the act of building builds the house" Aristotle, from *De Generatione Animalium*, fourth century B.C.

Introduction: Mammary Gland as a Model for the Study of Hormone and Extracellular Matrix Regulation of Tissue-Specific Gene Expression

Previously, environmental regulation of cell growth and differentiation was implicitly confined to the study of humoral factors. Now insoluble macromolecules that comprise the extracellular matrix (ECM) are also known to exert profound influences on cells during differentiation (1-5). Grobstein (6) first speculated about possible ECM influences to explain inductive tissue interactions in development. The underlying mechanisms of such influences, however, have remained elusive. Now that older

descriptive embryological approaches can be combined with the tools of cell and molecular biology, it is possible to realistically address the question of how the ECM regulates the induction and/or stability of tissue-specific gene expression. The mammary gland is well suited for such studies for three basic reasons: a) systemic hormonal requirements for functional differentiation have been defined *in vivo* and in organ and cell cultures; b) functional and morphogenic differentiation can be regulated with exogenous substrata in culture; and c) milk-specific gene expression varies in a stage-specific manner during late pregnancy and lactation. Precise markers for functional differentiation of mammary epithelia have been defined. By measuring the expression of glucose metabolites and milk-specific products, stage-specific differences can be evaluated under various conditions in culture.

Our laboratory is specifically investigating the influence of ECM on functional differentiation and lactogenesis in culture. We are also interested in eventually studying and integrating our present research with that of earlier morphogenic events, including the role of ECM in mammary cell growth, migration, and tissue-specific histogenesis. It is our contention that cytodifferentiation and histogenesis are both regulated by recursive (see last section) progressions that exist between the cell and its ECM (as well as other cells' ECMs), and between adjoining cells,

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and that ECM in conjunction with the changing humoral milieu determine the ultimate form and function of the tissue *in vivo*.

Mammary Gland Histogenesis

Several cell types interact in the mammary gland to bring about full functional competence during lactation. The gland consists of at least four recognizable cell types: adipocytes, fibroblasts, myoepithelial, and epithelial. Regional specialization of epithelial types might also distinguish basal from ductal and secretory epithelium (7-10). While cytodifferentiation of mammary epithelial cells can occur without substantial amounts of stromal cells in culture, tissue-specific histogenic expression during various stages relies heavily on the active participation of the mesenchyme and its derivatives. Early work by Kratochwil (11) demonstrated differential tissue-specific response of embryonic mammary epithelium to embryonic mesenchyme in homeotypic and heterotypic recombinant organ cultures. When mammary epithelium is combined with mammary mesenchyme, the result is typical monopodial branching patterns indicative of normal mammary morphogenesis. In contrast, when mammary epithelium is combined with salivary mesenchyme, the outcome is dichotomous branching patterns typical of salivary gland. Thus, the mesenchyme is capable of determining differential tissue-specific morphology. Subsequently, it was shown by Sakakura (12) that while salivary mesenchyme induced salivary specific histogenic response in mammary epithelium, these cells still synthesized the milk protein α -lactalbumin. This indicates that tissue-specific histogenesis in this case is influenced by the mesenchyme, and not directly associated with the determination of tissue-specific cytodifferentiation (i.e., cytodifferentiation and histogenesis are not necessarily always coupled).

It has been shown that various mammary epithelia are capable of morphogenic expression in cell-free collagen gels, or when embedded in hydrated collagen gels and implanted into the gland (13). This morphogenic expression, however, is not as extensive and is usually characterized by spiked ends rather than end buds typical of normal mammary morphogenesis *in vivo* (Fig. 1). In fat pad transplants, epithelium that came into contact with adipocytes did form normal end bud structures. This indicates that collagen gels alone do not contain all the necessary information for full histogenic formation and emphasizes the importance of interactions with adipocytes for normal morphogenesis.

Developmentally Regulated Changes in ECM Expression in the Mammary Gland *In Vivo*

While some basement membrane (BM) components are consistently present throughout mammary gland development (e.g., laminin and type IV collagen), substantial quantitative variation still does occur in a stage-specific manner. Other BM molecules such as glycosaminoglycans (GAGs) not only exhibit quantitative variation through de-

velopment, but also show dramatic qualitative changes, shifting from one type to another and back again during involution of the gland. Such changes might indicate which molecules modulate or stabilize the functional cytodifferentiation of mammary epithelia. Specific variations in BM organization and metabolism might also correlate with certain aspects of histogenesis, including directed morphogenic movement. If we are to develop model systems in culture that emulate stage-specific responses operating *in vivo*, it is crucial to always keep in mind trends of ECM composition and turnover seen *in vivo*.

Using immunohistochemistry, it has been shown in the mouse that laminin is present during various stages of mammary gland development (7). Its distribution is continuous throughout the BM, except in endbuds associated with branching and proliferation during morphogenesis. Laminin, type IV collagen, and fibronectin have been demonstrated using immunohistochemistry in rat lactating gland BM (14). While laminin and type IV collagen are continuous throughout the BM, fibronectin exhibits punctate distribution. Other immunohistochemical studies show that the sulfated glycoprotein entactin is also present in the BM of lactating rat mammary gland. In the virgin gland, however, entactin is associated only with interstitial ECM (15). Our preliminary data indicate that the quantitative expression of laminin, fibronectin, and type IV collagen mRNA are developmentally regulated in the mouse mammary gland (16). This indicates that changes in expression of BM components correlate with the onset and stabilization of functional differentiation *in vivo*.

Silberstein and Daniel (17) have shown that hyaluronic acid (HA) is distributed predominantly in the BM of growing endbuds of virgin mouse mammary gland. While ducts appear to be lined by chondroitin sulfate (CS), heparan sulfate (HS) is conspicuously absent at this undifferentiated stage of development. However, it has been shown that HA, CS, and HS are all synthesized in midpregnant mouse mammary gland rudiments in substantial amounts (18). This may be indicative of similar changes in GAG composition that have been correlated with differentiation *in vivo* in several embryonic and regenerative systems (19). In these systems, HA appears to be exclusively associated with earlier morphogenic stages when cell migration and proliferation are predominant, while the sulfated GAGs are expressed during later stages associated with functional differentiation.

Appropriate ECM is Necessary for the Functional Differentiation of Mammary Epithelia in Culture

Early attempts to culture isolated mammary epithelia usually resulted in stromal overgrowth and/or complete loss of tissue-specific gene expression (20). It was shown subsequently, however, that if cultured mammary cells were injected back into gland-free fat pads, they underwent ductal morphogenesis, alveolar formation, and were still capable of milk production once the impregnated animal gave birth.

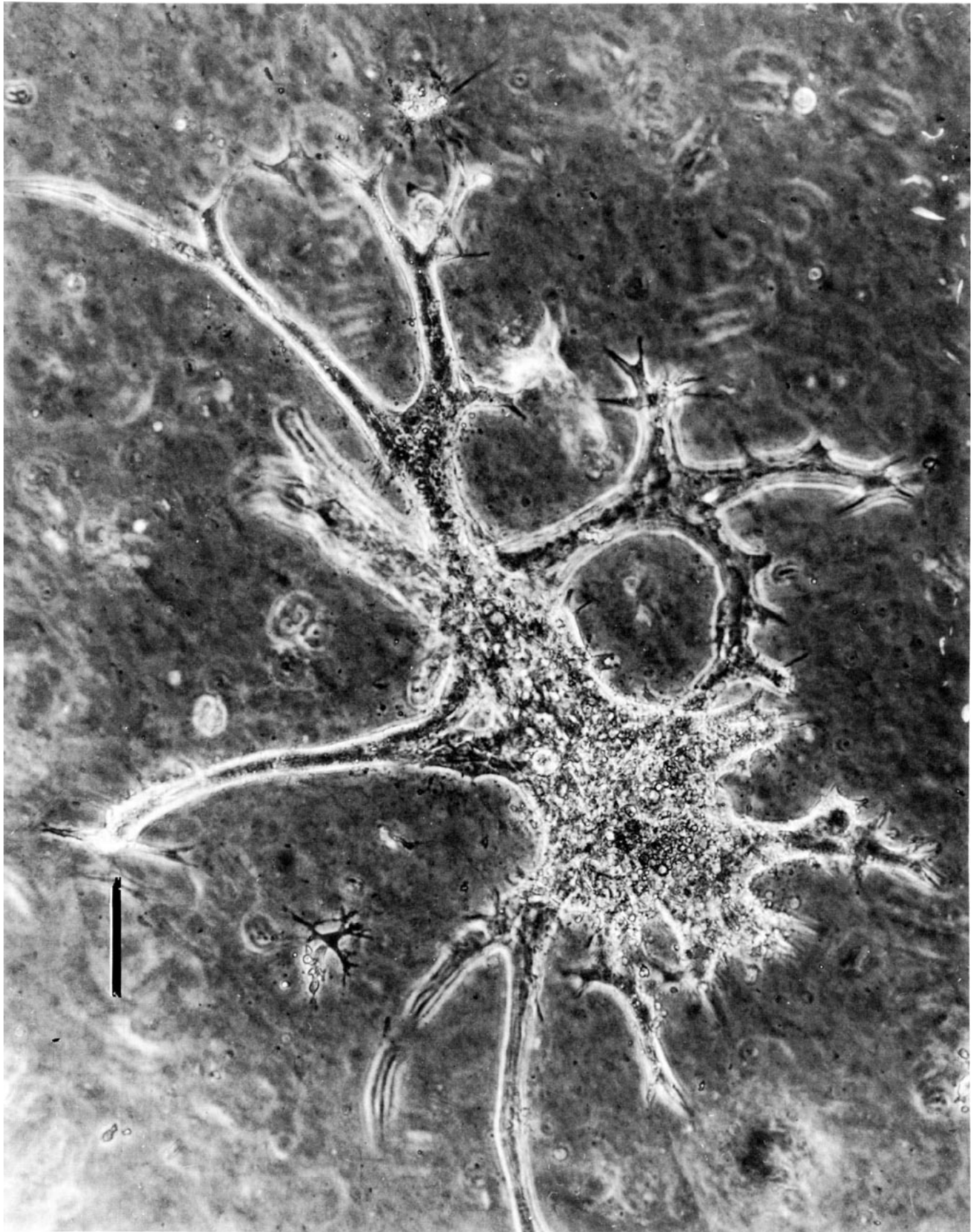


FIGURE 1. Morphology of mammary epithelial cells embedded in type I collagen gels. Phase contrast microscopy of PMME embedded in type I collagen gels and cultured for 10 days in F-12 with lactogenic hormones, bars = 100 μ m (unpublished photo).

This indicated that the loss of differentiated expression seen in culture was reversible. Organ culture model systems were instrumental in defining the basic hormonal requirements for milk-specific gene expression in culture (21-24). Isolated epithelia plated onto plastic substrata and cultured in the presence of lactogenic hormones (i.e., prolactin, insulin, hydrocortisone), however, fail to respond substantially when compared to explant cultures. This emphasizes the importance of cellular environment in addition to lactogenic hormones for mammary epithelial differentiation.

Since its introduction as a substratum for cultured cells (25), collagen gels have been shown to induce or maintain tissue-specific gene expression in numerous systems. Following the examples of Elsdale and Bard (26), and Michaelopoulos and Pitot (27), Emerman and Pitelka (28) placed primary mouse mammary epithelial cells (PMME) on type I collagen gels. While cells on attached gels increased secretory expression slightly, it was found that releasing the gel to float resulted in ultrastructure typical of secretory activity *in vivo* and was associated with elevated levels of casein (29) later shown to be synthesized *de novo* (30). It was further shown by our laboratory in the case of PMME (31), and by others with rabbit mammary cells (32), that on released collagen gels, mammary cells exhibit dramatic increases in milk-specific mRNA levels over cells on attached collagen gels or plastic substrata. If cells were plated onto glutaraldehyde prefixed gels that were subsequently floated, the gels did not contract, and cells exhibited cytostructure typical of those cultured on plastic, such as flattened morphology, and lacked polarized organelle distribution associated with secretory activity (33). We have shown that these cells do not support functional differentiation (30). These experiments emphasize a possible requirement for malleability of substrata, leading to cell shape changes that result in tissue-specific gene expression.

Model Systems for the Study of Hormone and ECM Interactions in Culture

Through the use of a BM extract derived from the Engelbreth-Holm-Swarm (EHS) mouse tumor (34), our laboratory has established a model system for the study of the reestablishment, induction, and maintenance of PMME functional differentiation under defined culture conditions. EHS extract contains large amounts of laminin, and lesser amounts of type IV collagen, heparan sulfate proteoglycan (HSPG), and entactin (35). EHS is used commonly now to attain differentiation of a number of cell types in culture. In our system, a thin coating of culture plates with EHS allow isolated PMME cultured in the presence of lactogenic hormones to develop into hollow spheres highly reminiscent of secretory alveoli *in vivo* (Fig. 2b). In contrast, cells cultured on plastic form confluent monolayers with occasional domelike structures (Fig. 2a). While there is approximately 30% greater plating efficiency on EHS, there is negligible growth on either substrata when cells are plated at high density ($\sim 2.5 \times 10^5$ cells/cm²) in serum-free (from day 2 on) culture conditions (36). SEM micrographs reveal striking

topographic differences between cells on different substrata. Cells on plastic appear flattened and exhibit minimal surface activity (Fig. 2c); cells on EHS are rounded and show prominent microvilli formation (Fig. 2d). TEM micrographs clearly show that cells on EHS are clustered in structures with hollow centers (lumina), (Fig. 2f). The cells are columnar with tight apical junctions, prominent luminal microvilli, apical golgi, and secretory granules, extensive endoplasmic reticulum, and basally located nuclei, all morphological features associated with the establishment of polarity and functional gene expression *in vivo* (37) and in culture (28). In contrast, cells on plastic lack all these features (Fig. 2e). Distinct zones of electron-dense material underlie cells on EHS (Fig. 2g), representative of organized BM formation and possibly endogenous ECM deposition. Within the first 24 hr of plating, cells on EHS actively pull up SEM visible matrix and surround the developing spheres with it (36). In the absence of lactogenic hormones, cells on EHS do not exhibit full secretory phenotype and develop slightly rounded SEM morphology intermediate between those described above (38). These cells do not form distinct BM or hollow spheres, but rather remain in aggregate or limited monolayer form throughout culture (unpublished data). Thus, the synergistic action of ECM and lactogenic hormones is necessary for functional cytodifferentiation and sphere formation in culture.

We have previously determined the percentage of β -casein-producing cells on plastic and EHS using indirect immunofluorescence (39). While 40% of freshly isolated cells from midpregnant gland expressed β -casein, 95% expressed β -casein after 6 days of culture on EHS, and only 7% expressed it on plastic. Thus, induction of tissue-specific gene expression occurs in cells on EHS (as well as increased amounts per cell), whereas a substantial number of cells on plastic lose the ability to express detectable amounts of β -casein.

By treating [³⁵S] methionine-labeled cells with low concentrations of EGTA in calcium-free media intraluminal contents of cultures can be isolated and assayed separately from media and intracellular fractions (36,40). Immunoprecipitation of milk proteins with a broad spectrum polyclonal rabbit-anti-mouse milk antibody yields secretion profiles of media, lumina, and intracellular pools, respectively. The levels of immunoprecipitable milk proteins released from PMME cultures on plastic and EHS after 6 days show striking differences in the relative amounts and distribution of secretory products (Fig. 3). Cells on plastic secrete relatively small amounts of milk proteins into either media, or EGTA released fractions. Cells on EHS, however, secrete substantial amounts of milk proteins into both media and luminal compartments. It is also apparent that substantially greater secretion of milk-specific proteins occurs into lumina on EHS than into the media. Time course experiments indicate that this is a result of increased lumina formation as cultures progress. Lumina formation on EHS can also be followed morphologically, both with TEM and optically with the introduction of dye into the developing lumina (36).

Separation of immunoprecipitated samples from PMME cultured on plastic and EHS (Fig. 4) show that on EHS the

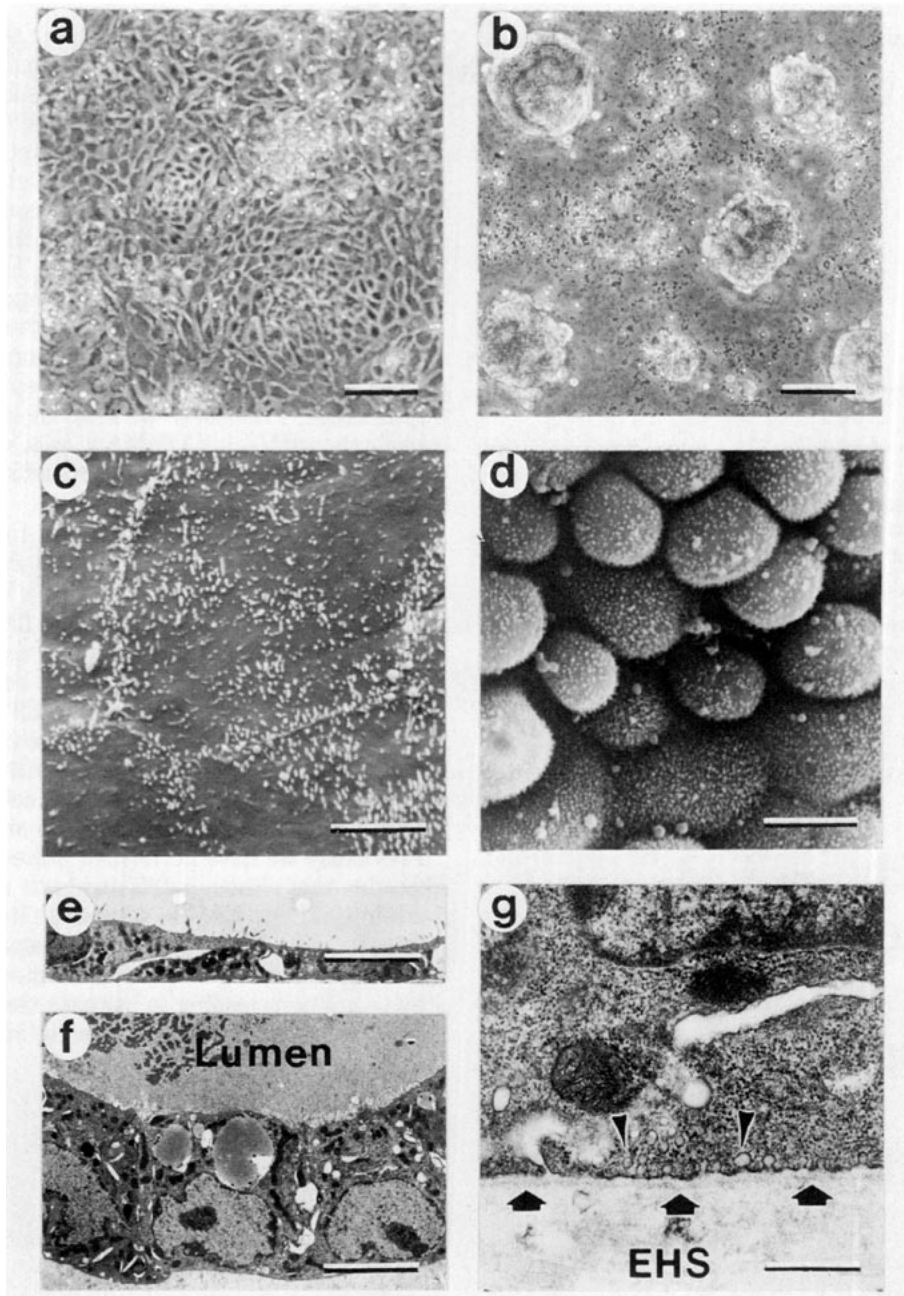


FIGURE 2. Morphology and ultrastructure of mammary epithelial cells cultured on different substrata. Phase contrast of PMME on plastic (a), and EHS (b), bars = 100 μM . SEM of PMME on plastic (c), and EHS (d), bars = 5 μM . Notice the prominent rounded shape and microvilli in this view of the apical region of cells on EHS. TEM of PMME on plastic (e), and EHS (f), bars = 5 μM . Cells on EHS form lumina and exhibit many ultrastructural features typical of secretory alveoli *in vivo*. Higher magnification of PMME on EHS (g), bar = 1 μM . Notice the distinct electron-dense BM formed in culture (arrows). (Photos a-c, unpublished, photos d-g; from Bissell and Aggeler (38,44), reproduced with permission.)

relative proportion of β -casein to other proteins secreted into the lumina is significantly greater than that in the media. In contrast, transferrin and lactoferrin are secreted equally well into both compartments (36). Recently it has been shown that COMMA-D cells (a mouse mammary cell strain) cultured in minicell chamber cultures also secrete β -casein apically; transferrin is secreted both apically and basally

(41). The specific vectorial secretion of β -casein relative to that of transferrin is consistent with our previous speculations regarding a possible dual role for transferrin in the mammary gland: In addition to being an important constituent of milk for the infant, transferrin might also be necessary for the development of the gland itself (42). Such differences in vectorial secretion of various proteins, in ad-

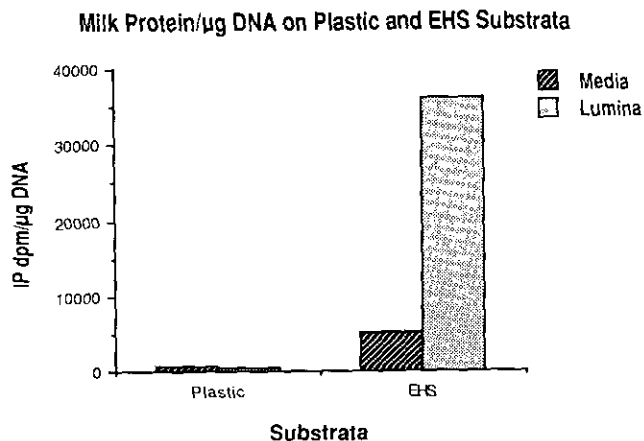


FIGURE 3. Immunoprecipitation of milk proteins secreted by mammary epithelial cells on different substrata. Cells were labeled for 4 hr with 100 μ Ci/mL 35 S-methionine in methionine-free medium; after removal of media, the cells were treated with 2.5 mM EGTA in calcium-free media for 10 min at 37°C. Both media and EGTA releasable (luminal) fractions were immunoprecipitated with rabbit-anti-mouse polyclonal milk antisera (unpublished data). IP, immunoprecipitable.

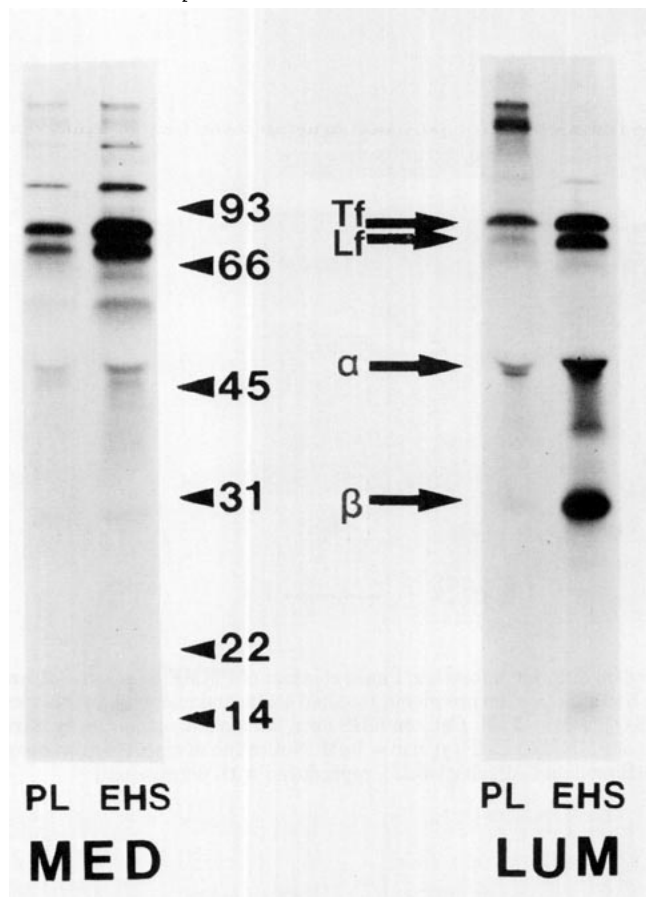


FIGURE 4. Vectorial secretion of milk proteins by mammary epithelial cells cultured on EHS-matrix. SDS-PAGE electrophoresis of immunoprecipitated samples taken from the media and luminal fractions of PMME on plastic and EHS show the relative amount of milk proteins in the separate fractions. Notice the specific vectorial secretion of β -casein into lumina (EGTA fraction) on EHS, while transferrin and lactoferrin are secreted both apically and basally (unpublished data). Tf, transferrin; Lf, lactoferrin, α , α -casein, β , β -casein.

dition to ultrastructural and histotypic properties of cells on EHS, reinforce our supposition that mammary spheres on EHS are analogous to secretory alveoli *in vivo*.

Northern blot analysis of accumulated mRNA in cells cultured on plastic and EHS show substantial increases in both transferrin and β -casein mRNA levels in cells on EHS (Fig. 5). Whereas β -casein message expression on both substrata is almost totally dependent on the presence of lactogenic hormones, transferrin mRNA is still expressed in measurable amounts, even in the absence of hormones (43). Therefore, while the expression of both mRNAs are dependent on the ECM and lactogenic hormones, transferrin mRNA expression is especially susceptible to modulation by the ECM. Previously we have shown that the level of transferrin mRNA in COMMA-D cells, while completely independent of the presence of prolactin in culture, is also modulated by the ECM (44).

We have conducted experiments to determine which ECM components of EHS are most crucial for full functional expression in culture (39,45,46). We found that coating plates with type IV collagen and fibronectin had no effect on β -casein levels, whereas HSPG and laminin increased β -casein mRNA levels 8- and 3-fold, respectively (Fig. 6). These levels, though enhanced, are still substantially lower than those seen for cells on EHS extract. Secretion of β -casein does not increase on HSPG. Cells cultured on HSPG are extremely flat and do not exhibit cell shape changes (or sphere formation) observed for cells on EHS.

Previously we have performed pulse-chase experiments indicating that although cells on plastic are capable of some β -casein synthesis, they do not secrete it as it gets degraded intracellularly (Fig. 7) (31). Similar aspects of posttranslational regulation might help explain mechanisms that operate in conditions leading to increase tissue-specific mRNA levels, without a concomitant increase in protein levels. This

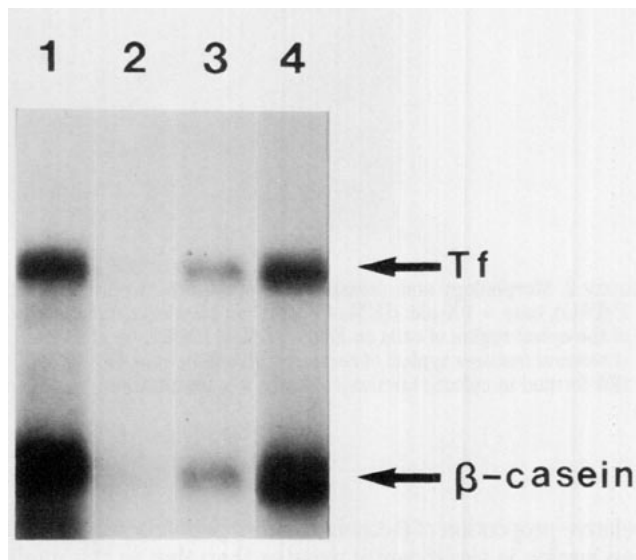


FIGURE 5. mRNA levels of milk proteins in mammary epithelial cells on different substrata. Northern blot containing 10 μ g RNA/lane was hybridized with nick-translated transferrin and β -casein probes. RNA isolated from lactating mammary gland (lane 1); PMME on plastic (lane 2); PMME on EHS (lane 3); PMME on EHS-released gel (lane 4). From Chen and Bissell (43). Reproduced with permission.

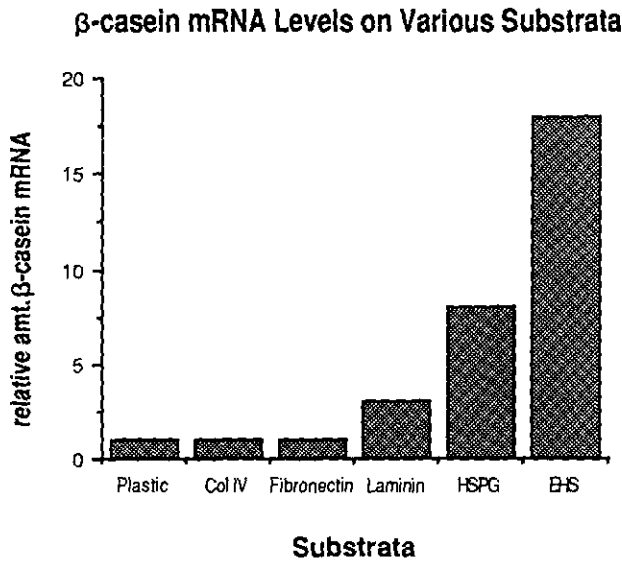


FIGURE 6. β -casein mRNA levels in mammary epithelial cells on various ECM components. Dot blot loaded with equal amounts of RNA was hybridized with β -casein cDNA probe. X-ray film exposures of blot was scanned using densitometry. The fold increase in relation to levels for PMME on plastic substratum = 1 are shown [plotted from Li et al. (39)].

indicates that ECM regulates gene expression at multiple levels and emphasizes the necessity of cooperative macromolecular assemblies in addition to individual specific ligand requirements for full functional differentiation.

Conditions that Modulate ECM Expression in Culture

The composition and distribution of *de novo*-synthesized ECM deposited in culture could be markedly altered on various culture substrata. This in turn could result in the observed changes in tissue-specific expression. We have conducted experiments to test this possibility. Our preliminary results indicate that mRNA levels for laminin, fibronectin and type IV collagen are dramatically altered in PMME on various substrata in culture (16). Cells on released gels syn-

thesize an organized BM containing laminin, fibronectin, entactin, and type IV collagen, while cells on plastic do not (16,47). Thus, increased BM production appears to correlate with increased functional differentiation in culture.

GAG synthesis and deposition by PMME is also differentially responsive to various substrata in culture (48). On plastic and attached collagen gels, the relative amount of *de novo*-synthesized HA to sulfated GAGs (CS and HS) incorporated into ECM is quite high in comparison to floating gels (Fig. 8). Thus, there is an inverse correlation between HA expression and functional differentiation in culture. Increased synthesis and incorporation of HA into ECM in less functional culture conditions is possibly indicative of an antagonistic role for HA in mammary epithelial cytodifferentiation during morphogenesis, as has been previously proposed for in other systems (19,49). On the other hand, there appears to be a positive correlation between increased incorporation of HS into the matrix and the increased expression of differentiated functions. This is consistent with the increase in β -casein mRNA expression seen in response to exogenous HSPG in the previously discussed experiments (39). More recently we have shown that PMME cultured on various substrata in the presence of β -xyloside exhibit increased synthesis and secretion of milk proteins (50). The addition of β -xyloside causes a relative enrichment of HS into endogenous ECM, while the major proportion of CS is released as free polysaccharide into the media. Thus, perturbation of endogenous ECM expression increases functional differentiation, which in this case is also associated with a relative increase in HS deposition into the ECM in culture.

In addition to the modulation of ECM expression seen on various substrata, it is likely that ECM expression is also regulated by hormones in culture. While EGF stimulates the synthesis of type IV collagen, hydrocortisone suppresses its degradation in rat mammary epithelial cells cultured on type I collagen gels (51). As mentioned earlier, cells cultured on EHS in the absence of lactogenic hormones do not form a recognizable BM (38). Little is known about how the lactogenic hormones each specifically affect *de novo* ECM synthesis and deposition in culture, an area of further investigation in our laboratory.

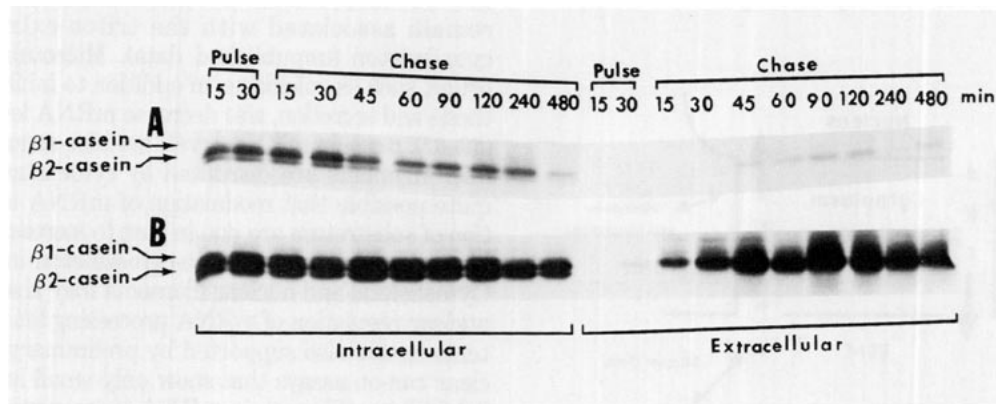


FIGURE 7. Intracellular degradation of β -casein by mammary epithelial cells on plastic. PMME on plastic (A) or on released gels (B) were incubated in methionine-free medium for 1 hr and then pulsed with ^{35}S -methionine at $150 \mu\text{Ci}/\text{mL}$. One dish was harvested after a 15-min pulse; the rest were pulsed for 30 min, then washed once with medium containing excess unlabeled methionine and chased for various periods. Some extracellular degradation of caseins occurs in the media of cells on both plastic dishes and collagen gels (32).

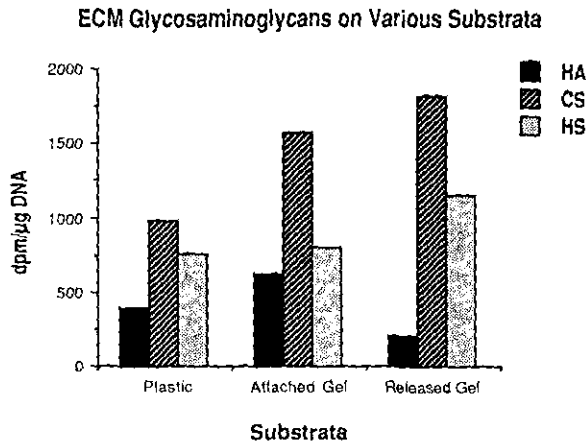


FIGURE 8. Incorporation of glycosaminoglycans into ECM by mammary epithelial cells on various substrata. Cells were labeled with ^3H -glucosamine for 24 hr, and GAGs were extracted from the different culture fractions (media, ECM, and intracellular). Individual GAGs were characterized using ion exchange chromatography, and selective enzymatic and nitrous acid degradation. The relative proportion of HA to sulfated GAGs (CS and HS) incorporated into ECM is higher on plastic and attached gels (less functional culture conditions) than it is on released gels [replotted from Parry et al. (50), reproduced with permission]. HA, hyaluronic acid, CS, chondroitin sulfate, HS, heparan sulfate.

Dynamic Reciprocity: General Model for Epigenetic Determination and Progressive Stabilization of the Functional State

We have emphasized two points in particular: a) appropriate ECM and lactogenic hormones are essential for functional differentiation of PMME in culture; b) endogenous ECM expression by PMME is modulated in culture by exogenous ECM, cell geometry, and possibly by lactogenic hormones. A number of years ago we proposed a model of cell-ECM interactions, postulating a dynamic reciprocity (52) between the cell and its ECM (4). An essential element of this model is that the cell, while continually responding to its environment, is also shaping it as well. This process continues in periodic fashion until specific signals for change oc-

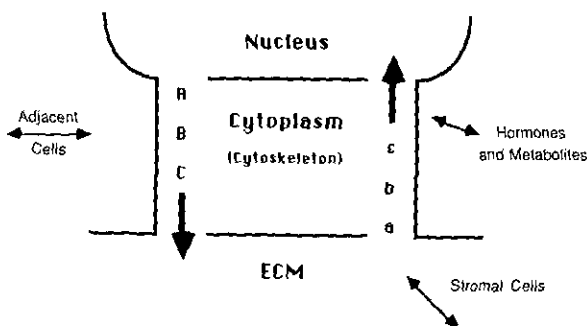


FIGURE 9. Recursive progression illustrating the principle of dynamic reciprocity. Letters represent progressive stages of gene expression (upper case), and environmental stimuli (lower case) that elicit responses in the opposite direction.

cur, or a specific condition is met (e.g., hormone concentration, density). These are recursive progressions in biological form. Here we are emphasizing that dynamic reciprocity could encompass both biochemical cytodifferentiation and histogenic configuration, both regulated by ECM and hormones.

The model is presented in a highly simplified diagrammatic form in Figure 9. The total integrated unit of these molecular events define the cell at a given point in time (i.e., for that particular developmental stage). The fundamental aspect of this model is that phenotype acts on genotype, which in turn acts on phenotype in reciprocal fashion. The expression of tissue-specific histogenic properties can also be envisioned as a dynamic reciprocity between mesenchyme (or stroma), and epithelial parenchyma. The BM that forms the foundation of developing parenchyma is a combination of epithelial basal lamina and reticular ECM derived from surrounding stroma: the two acting as a functional unit that receives, integrates, and imparts signals that could feasibly endow regional specificity in a developing system. While not emphasized in this chapter, it is clear that intercellular interactions (via cell adhesion molecules, gap junctions, etc.) are involved in tissue-specific gene expression in epithelia, where cooperative aggregate form is usually essential for normal function that responds to extrinsic stimuli.

Studies with EHS matrix may shed further light on the mechanisms by which ECM regulates tissue-specific gene expression. EHS matrix induces or restores mammary function only to mammary cells (our data), liver function only to hepatocytes (53), and Sertoli cell function only to Sertoli cells (54). Thus, it appears that this particular ECM acts to stabilize or allow predetermined tissue-specific functions to be manifest, possibly through similar mechanisms. Our data also indicate that this process in PMME is reciprocal in nature, involving *de novo* ECM expression in culture.

Studies involving rare and moderately abundant mRNA in mouse hepatocytes indicate that posttranscriptional regulation may be the prevalent control in eukaryotic cells to determine the level of individual mRNAs (55). In many cell types, 70 to 80% of the poly(A)⁺ mRNA is found in the cytoskeletal fraction (56-58). Our preliminary results indicate that more than 90% of β -casein and transferrin mRNA remain associated with the triton-extracted PMME cytoskeleton (unpublished data). Microtubule disrupting drugs, such as colchicine, in addition to inhibiting milk synthesis and secretion, also decrease mRNA levels for β -casein (59-61). β -casein mRNA levels are also reduced when actin microfilaments are disrupted by cytochalasin B (62). It is quite possible that modulation of mRNA levels as a function of substratum are due in part to increased mRNA half-life as a result of polysome-cytoskeleton interactions (63). Cytoskeletal and nuclear filaments may also be involved in nuclear regulation of mRNA processing (64-68). These contentions are also supported by preliminary data using nuclear run-on assays that show only small increases (1.5- to 3.0-fold) in milk protein mRNA transcription on EHS (unpublished data). In general, the cytoskeleton emerges as the structure most likely to integrate intrinsic and extrinsic reciprocity; defining the cytoplasm not simply as a bag of

enzymes, but rather as the decisive element in the continuum that regulates gene expression (69).

We have briefly touched on some mesenchymal-dependent properties involving tissue-specific morphogenic expression in the mammary gland. One essential aspect of defining etiological relationships within a developmental system is to separate the processes involved on larger levels of cell and tissue organization. While much progress has been made recently with regard to understanding some of the mechanisms involved in mammary-specific cytodifferentiation (e.g., importance of specific hormones, appropriate ECM, polarity, etc.), histogenesis is still rather poorly understood. While the literature on ECM molecules (70,71) and their receptors (72) is expanding quite rapidly, the profound influence of ECM on tissue-specific gene expression is also being explored in many systems as described. It is thus hoped that the exact mechanisms by which these molecules regulate form and function in higher multicellular organisms could be unraveled in the not-to-distant future.

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