Modulation of Collagen Synthesis by a Growth Factor and by the Extracellular Matrix: Comparison of Cellular Response to Two Different Stimuli

S. C. G. TSENG,* N. SAVION, D. GOSPODAROWICZ, and R. STERN*
Department of Pathology* and Cancer Research Institute and Departments of Medicine and Ophthalmology, University of California, Medical Center, San Francisco, California 94143

ABSTRACT
Cultured bovine corneal endothelial cells can be grown in three ways: on plastic, on plastic with fibroblast growth factor present in the media, and on their own preformed extracellular matrix. On plastic alone, cells grow in a disorderly fashion and secrete matrix on all cell surfaces. Cells grown on plastic with growth factor or on a matrix, at confluence, have matrix deposition only on the basal surface of the cells and an orderly contact-inhibited pattern of growth. This correlates with the polarity they demonstrate histologically. This cell-matrix pattern resembles the pattern observed in vivo. Both the soluble growth factor and the extracellular matrix are able to modulate the pattern of collagen synthesis and deposition by cells, but they do so in two entirely different ways. In cells grown on the extracellular matrix, total collagen synthesis is lower but more efficient. Collagen is deposited primarily into the cell layer even at the early sparse stage of culture. In cells grown on plastic with growth factor in the media, collagen is initially secreted into the media and does not become incorporated into the matrix. The deposition of collagen on the basal surface of cell occurs only late in the culture, and is achieved by increments in a stepwise manner. The in vivo-like pattern is not manifest until confluence has been reached. Thus, the extracellular matrix functions not only as a structural support, but is also instructional to the cells plated on it. In this case, the matrix regulates the level of collagen synthesis in the cells and modulates the pattern of collagen deposition. Soluble growth factors may act in part by enhancing a cell's ability to elaborate an appropriate matrix pattern necessary for the cell's own growth and accurate function.

The extracellular matrix (ECM) of cells is informational. It appears to be able to modulate the behavior and phenotypic expression of cells. In the present study, the manner by which the ECM modulates the level and pattern of collagen synthesis in cultured corneal endothelial cells is examined.

Corneal endothelial cells derived from adult bovine cornea can be grown in vitro in three different ways. Cells can be grown on plastic, or on plastic in the presence of fibroblast growth factor (FGF). Cells grown with FGF resemble corneal endothelium in vivo (7, 8, 22). If seeded at low density and grown continuously in the presence of FGF, at confluence they form a monolayer of closely apposed, highly flattened orderly cells that do not overgrow one another. A basement membrane-containing ECM is localized exclusively beneath cells, on the basal cell surface. In contrast, cells grown on plastic in the absence of FGF are large and pleomorphic, lose contact inhibition, and overgrow one another. They also lose their histologic polarity and now secrete ECM components over their entire cell surface.

A third way in which corneal endothelial cells can be grown is to plate them on their own preformed ECM. Cells are initially grown in the presence of growth factors. Cells are removed with detergent and new cells are then plated. When cells are grown in this manner, at confluence they resemble cells grown on plastic in the presence of FGF. They are highly orderly and secrete matrix components only on their basal surface. Thus, an ECM obviates the requirement of a growth factor for cells to achieve normal morphological phenotypic...
expression (4–6).

It appears that cultured corneal endothelial cells are able to achieve accurate phenotypic expression in two ways, by adding a peptide factor to the culture medium or by being plated on a preformed matrix. The state these cells reach at confluence is remarkably similar yet the mechanisms by which cells reach that state differ markedly. Cells grown on plastic only have an entirely different appearance, which at no stage of growth bears any resemblance to their in vivo counterparts.

MATERIALS AND METHODS

Materials: FGF was purified from bovine brains as previously described (3). Brain FGF yielded a single band on PAGE at pH 4.5 on an isoelectric focusing column (total volume 100 ml, pH range 3.4 to 11.0). All the activity focused within an isoelectric range of 9.2 to 9.6. Bovine calf serum, Dulbecco’s minimal essential medium (DME), type H-16, and glutamine were purchased from Gibco Laboratories (Santa Clara, CA). Tissue culture dishes were obtained from Falcon Plastics (Oxnard, CA). L-2,3,4,5-[3H]proline (80–100 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL), and l-ascorbic acid from Calbiochem-Behring, Corp. (La Jolla, CA). The β-aminopropionitrile (βAPN), phenylmethylsulfonylfluoride (PMSF), and β-hydroxymercuribenzoate (PCMB) were the products of the Sigma Chemical Co. (St. Louis, MO) and benzamidine of the Eastman Kodak Co. (Rochester, NY). DEAE-cellulose (DE-52) and CM-cellulose (CM-S2) were obtained from Whatman (Clifton, NJ). Pepsin A was obtained from Worthington Biochemical Corp. (Freehold, NJ) and Aquasol from New England Nuclear (Boston, MA).

Preparation of Plastic Plates Coated with an ECM: The ECM were prepared as previously described (4). In brief, bovine corneal endothelial (BCE) cells were plated at an initial density of 10^4 cells per 35-mm dish and were maintained in DME (H-16) supplemented with 10% (vol/vol) fetal calf serum (FCS), 5% (vol/vol) calf serum, 5% (wt/vol) dextran T-40 (Sigma Chemical Co.), Gentiomycin (50 μg/ml), and Fungizone (2.5 μg/ml). FGF (100 ng/ml) was added every other day. Once the cultures became confluent, ordinarily within 6, the media were renewed and the cultures were incubated for an additional 6 d. The cultures were then washed with PBS and exposed for 30 min to 0.5% (vol/vol) Triton X-100 in the same buffer. Once the nuclei and the extracellular matrix (ECM) became visible by phase-contrast microscopy, the cultures were washed three times with PBS. After three washings, only a few cytoskeletal structures and nuclei were associated with the intact ECM.

Tissue Culture of BCE Cells: BCE cells were established from steer corneas. Stock cultures were maintained in DME (H-16) supplemented with 10% (vol/vol) fetal calf serum (FCS). 5% (vol/vol) calf serum, 5% (wt/vol) dextran T-40 (Sigma Chemical Co.), Gentamycin (50 μg/ml), and Fungizone (2.5 μg/ml). FGF (100 ng/ml) was added every other day until cultures were subconfluent. Three different culture conditions were used in these experiments. In Group A, BCE cells were grown on plastic plates without addition. In Group B, cells were grown on plastic plates but FGF was added in the same fashion as described above for stock cultures. In Group C, cells were grown on their natural ECM without other additions. On day 0, three tissue cultures were initiated at a density of 1–2 × 10^4 cells per 35-mm dish or at ~10 cells/mm^2. The remainder of the cell culture conditions were identical for the three groups, as described. Cell cultures in Groups B and C reached confluence on day 6. The cell density in cultures of Group A was less than that in Groups B and C on each designated day (8) and correlated with the observation that cells were larger in size.

Metabolic Labeling: Both sparse and subconfluent cultures of BCE cells were preincubated for 24 h in glutamine-free DME (H-16) supplemented with 10% bovine calf serum and ascorbic acid (25 μg/ml) and then with L-(2,3,4,5-[3H]proline (40 μCi/ml) on the following day, (day 1) or on days 3 or 5 for 24 h at 37°C.

In one group of early experiments, βAPN was added (80 μg/ml) to determine its effect on the synthesis and distribution of collagen molecules, since βAPN is known to prevent collagen cross-linking. It was discovered that the effect of βAPN was negligible. βAPN was therefore withdrawn from all experiments measuring radiolabeled hydroxyproline. The exceptions were the experiments quantitating procollagen types by DEAE-cellulose chromatography.

After 24 h, the medium containing the secreted procollagen was removed and the cultures were washed three times with cold (4°C) PBS. The plates were then stored at ~70°C. Culture medium and the first wash were combined. To inhibit further proteolysis, Tri-HCl (pH 8.0), EDTA, PMSF, PCMB, and benzamidine were added at final concentrations of 50, 20, 0.1, 1, and 0.1 mM respectively. The media were then centrifuged (800 g, 10 min) to remove cell debris and the supernatants were stored at ~70°C. In Group B, additional measurements were made when cultures were 3 wk old, i.e., 2 wk after confluence.

Assay of Nondialyzable Radiolabeled Proline and Hydroxyproline: To measure the amount of newly synthesized collagen under each of the culture conditions, a paper electrophoresis method of quantitating radiolabeled proline and hydroxyproline was used (23). The ratio of [3H]-hydroxyproline/[3H]proline + [3H]hydroxyproline was presented as such without correction for the loss of radioactivity that occurs when proline residues become hydroxylated. Total hydroxyproline calculated from values of the total radioactive incorporation and the above ratios were regarded as newly synthesized collagen. A correction factor for cell number was made for each experiment. Triplicate samples were measured and the mean ± SD derived from these three measurements are presented.

DEAE-Cellulose Chromatography: To examine the procollagens that were secreted into the media of the subconfluent cultures under the three culture conditions, DEAE-cellulose chromatography was performed (19). After labeling for 24 h with [3H]proline in the presence of ascorbic acid and βAPN, media were dialyzed at 4°C against 0.15 M NaCl, 50 mM Tris-HCl, pH 7.5 containing 20 mM EDTA, 1 mM PMSF, 1 mM PCMB, and 1 mM benzamidine, to remove the traces of free radiolabeled amino acid and to prevent conversion of procollagen to collagen. Saturated ammonium sulfate (100% vol/vol) was added to make the final concentration 30% (vol/vol). The resulting suspension was then stirred gently overnight at 4°C in the presence of 3 mg of unlabeled carrier lathyritic rat skin collagen. Precipitates were collected by centrifugation (7,000 g, 30 min, 4°C), dissolved in the initial buffer, 2 M urea, 50 mM Tris-HCl, pH 7.5, and dialyzed overnight (4°C) against the same solution. Before chromatography, any insoluble material was removed by filtering through cotton. Samples were applied to a column of DEAE-cellulose (1.5 cm × 4 cm) and washed with 25 ml of the initial buffer. Bound proteins were eluted using a linear gradient of 0–0.2 M of NaCl in a total volume of 160 ml, and a flow-rate of 6.2 ml/h. Fractions of 2.45 ml were collected, of which a 0.3-mL aliquot was removed for counting. 3 ml Aquasol was added and samples were counted in a Beckman Counter CS 8000 (Beckman Instruments, Palo Alto, CA), with a counting efficiency of 17.6% for the tritiated material.

Assay of Lysyl Oxidase Activity: To determine whether the extent of deposition of collagen was affected by the activity of lysyl oxidase, an extracellular enzyme responsible for the cross-linking of collagen, the enzymatic activity was measured in both the media and cell layers of the three different cultures on day 6, at confluence. The procedure for the preparation of the radiolabeled substrate is described in detail by Siegel et al. (18) and Siegel (16). The assay of lysyl oxidase activity was performed by a procedure described by Siegel and Fu (17).

Cell Counting: The cell number was obtained by trypsinizing parallel cultures and counting the cells in triplicate with a Coulter counter (Coulter Electronics Inc., Hialeah, FL).

RESULTS

In the present experiments, we examined the mechanisms by which cells can respond to two entirely different environmental situations and yet achieve the same morphology by the time confluence is reached. Examining cell behavior at intervals in the sequential stages of growth yields a dynamic picture of how that occurs and sheds light on the mechanism involved.

Tissue Culture of BCE Cells

BCE cells were established from steer corneas. They were passed weekly at a 1:64 split ratio and FGF was added until cultures were confluent. BCE cells were initially plated at a density of 1–2 × 10^4 cells per 35-mm dish or at ~10 cells/mm^2. Three different culture conditions were used in these experiments: in Group A, BCE cells were grown on plastic plates; in Group B, cells were grown on plastic plates but FGF was added in the same fashion as described under Materials and Methods; in Group C, cells were grown on their preformed matrix, as described under Materials and Methods.
Comparison of Growth and Morphology of BCE Cells Grown under Different Conditions

As observed previously by Gospodarowicz et al. (8), under the three culture conditions, BCE cells exhibit major differences in growth rate, and in their final cell density (Fig. 1). Cells grown on plastic (Group A) were larger, haphazard in orientation, overlapping and irregular in shape (Fig. 1a and b). They had the slowest growth rate. By the time other cells had reached confluence, cells in Group A had only half the cell density. Cells in Group A never reached the density of the other cells even when cultured for longer periods of time, for 3 wk.

Cells in Groups B and C grew more rapidly reaching confluence at days 6 and 5, respectively. Their morphology was similar to each other (Fig. 1c-f). They form a monolayer of highly orderly, flat, cobblestoned cells that do not overgrow one another. The basement membrane-containing ECM is localized exclusively beneath cells.

Levels of Protein Synthesis

The patterns of protein synthesis were examined next. Incorporation of radiolabeled proline into protein was used as an index of protein synthesis and was measured at three stages of growth, at sparse, confluent, and postconfluent stages under the three cell culture conditions. Cells were labeled for 24 h with $[^3]$H]proline at each of the various stages. As shown in Table I incorporation differed widely. Incorporation was highest in Group A. On day 1, the value for Group A, which were also the largest cells, was about twice that of Groups B and C. On day 5 of culture, Group A was about two and three times that of B and C respectively (Table 1). A pattern of decreasing incorporation of radiolabel was observed as cells in Groups A and C reached confluence. The decrease was greatest for Group C, whose value fell 55% as cells went from sparse to confluent. For Group A, the decrease was only 20%.

The media and the cell layers were then analyzed separately to examine the distribution of incorporated proteins into these two compartments. The level of total $[^3]$H]proline incorporated into the proteins of the media and the cell layers from each group gave a similar pattern. More proteins were consistently incorporated into the cell layer than secreted into the media, regardless of the stage of culture (Fig. 2). When the three groups were compared, the highest proportion was found in Group C, in which cells were grown on their own natural ECM.

The highest value for Group C, 80%, appeared at the earliest stage of growth, on day 1. This indicated that a pre-existing ECM instructed cells to incorporate newly synthesized proteins primarily into the cell layer at the earliest stage of culture.

| Table 1 | $[^3]$H]Proline Incorporation into Bovine Corneal Endothelial Cells at Different Stages and under Various Culture Conditions |
|---------|---------------------------------------------------------------|
| Culture condition | Day 1 | Day 3 | Day 5 | Week 3 |
|-------------------|-------|-------|-------|--------|
| Plastic           | 17.7 ± 0.5 | 16.0 ± 0.6 | 14.6 ± 0.4 | —      |
| Plastic and FGF   | 7.5 ± 0.5 | 7.7 ± 0.4 | 8.1 ± 0.4 | 7.5 ± 0.3 |
| ECM               | 9.8 ± 0.3 | 7.3 ± 0.3 | 4.5 ± 0.2 | —      |

Mean value ± SD are reported here; each value represents cpm x 10^6/10^6 cells.

Figure 1. Morphological appearance of low density and confluent cultures of bovine corneal endothelial cells maintained on plastic or ECM-coated dishes and grown in the absence or presence of serum. Bovine corneal endothelial cells ($2 \times 10^4$ cells/35-mm dish) were seeded on either plastic (A–D) or ECM-coated dishes (E and F) and exposed to DME supplemented with 10% fetal calf serum, 5% calf serum, either with (C and D) or without (A, B, E, and F) FGF. Phase-contrast micrographs were taken after 2 (A, C, and E) and 7 d (B, D, and F) in culture.
HI PROLINE INCORPORATION

Figure 2. Level of [3H]proline incorporation into nondialyzable material in media and cell layers of bovine corneal endothelial cells under three different culture conditions, as described under Materials and Methods. Different stages, i.e., days 1, 3, and 5 were analyzed. The mean values of triplicates are presented here, and denoted as D1, D3, and D5 from each Group. 3-wk-old cultures (of Group B only) are denoted as 3W.

Table II

| Culture condition | Day 1          | Day 3          | Day 5          | Week 3          |
|-------------------|----------------|----------------|----------------|-----------------|
| Plastic           | 10.5 ± 0.6     | 6.5 ± 0.5      | 6.0 ± 0.5      |                 |
| Plastic and FGF   | 4.5 ± 0.4      | 2.4 ± 0.3      | 2.4 ± 0.4      | 1.9 ± 0.3       |
| ECM               | 1.8 ± 0.3      | 1.5 ± 0.2      | 1.2 ± 0.2      |                 |

Mean value ± SD are reported here; each value represents cpm x 10^4/10^6 cells.

The most unique pattern was that of Group B. Proportionately more [3H]proline was incorporated into cell layers as cultures approached confluence (Group B, Fig. 2). But a ratio similar to that of Group C cultures, with incorporation predominantly into the cell layer, was not observed in Group B until cells had reached confluence, on day 5.

Levels of Collagen Synthesis

Hydroxyproline is an amino acid found in high concentrations in collagen. The collagen content of the three culture groups was analyzed in both media and cell layers by measuring levels of [3H]hydroxyproline in acid hydrolysates. The levels in the three cell culture conditions differed markedly, which indicated cells varied in their capacity to make collagen. Cells grown on plastic plates synthesized the most collagen. [3H]hydroxyproline recovered in Group A was 2.3 times that of Group B and six times that of Group C on day 1 (Table II). This was also observed at subsequent stages of culture. These results demonstrated that cells were most actively involved in collagen synthesis when they were first plated and this decreased as cells reached confluence. The difference in collagen synthesis between Groups A and C (Table II and Fig. 3) was noteworthy. The decrease in collagen synthesis per cell was greater than that for total protein synthesis (Table I and Fig. 2). This demonstrated that the effect of a pre-existing ECM was greater on collagen synthesis than on general protein synthesis.

The media and cell layers were then analyzed separately to examine the pattern of distribution of newly synthesized collagen. In Group A, a higher proportion of newly synthesized collagen was found in the media than in the cell layers (Fig. 3), an observation that was made at all stages of growth. Examining the percentage of total [3H]hydroxyproline that was incorporated into the cell layers as a function of time in culture reveals the percentage rose from 21% to 34%, and finally to 40% on days 1, 3, and 5, respectively (Table III). Thus, proportionally more collagen was deposited into the cell layers as the cells reached confluence, despite the fact that the total amount of collagen synthesis decreased.

In Group B a similar pattern in distribution of collagen was...
observed with most newly synthesized collagen being secreted into the media. However, a gradual increase occurred in the level of collagen deposited into the cell layers (Fig. 3). The percentage of total [3H]hydroxyproline present in cell layers increased from 13% on day 1 to 28% on day 3 and 34% on day 5. A percentage of 56% was eventually reached when culture were 2-wk postconfluent.

Group C was then analyzed. The distribution of newly synthesized collagen was entirely different from that of Groups A and B. Most newly synthesized collagen was present in the cell layer from from the very earliest stages of cell culture. On day 1, a percentage of 52% was observed, which increased to even higher levels, to 62% and 74% on days 3 and 5. This increase was primarily due to the decrease in the amount of collagen elaborated into the media, since the actual level of new collagen in the cell layers remained almost constant (Fig. 3). Thus a pre-existing ECM caused preferential deposition of newly synthesized collagen into the cell layers, even at the sparse stage of growth.

Distribution of Radiolabel between Cells and the Extracellular Matrix

The values presented here for cell layers include both cells and their surrounding ECM. A question regarding the distribution of newly made proteins between cells and their ECM was addressed. Cells were removed from plates by the same procedure described for preparing the extracellular matrix in Materials and Methods. Less than 30% of total [3H]proline remained in the matrix. However, >85% of total [3H]hydroxyproline was found in the ECM, indicating that newly synthesized collagen was present in the ECM and did not represent retained intracellular collagen.

Ratios of Collagen and Noncollagenous Protein Synthesis

The proportion of collagen to noncollagenous proteins was examined to determine whether the modulation of collagen synthesis by FGF or ECM was specific for collagen, or involved total protein synthesis. The ratio of [3H]hydroxyproline and [3H]proline incorporation was used and is summarized in Table IV. Groups A and B had levels of 6% on day 1, while Group C had a level of only 1.8%. This indicated that on plastic, in the presence and absence of FGF, the proportion of collagen synthesis was much higher than when cells were only an ECM. Thus, a pre-existing ECM specifically suppressed collagen synthesis. As cultures progressed, the pattern of these ratios changed, with values decreasing steadily. The decrease in B was more dramatic than in A. In Group C, the ratio increased from 1.8% on day 1 to 2.7% on day 5. The difference in patterns in the three groups suggested different mechanisms were operative for the modulation of collagen synthesis by FGF and ECM.

Qualitative Study of Collagen Synthesis in the Media Proteins under Three Different Culture Conditions

The types of procollagen synthesized were analyzed using DEAE-cellulose chromatography. The three elution profiles did not differ profoundly. Procollagen type III was the major collagen species elaborated into the media under all culture conditions (Fig. 4a–c). The identity of the type III procollagen was established by its co-elution with type III procollagen from human skin fibroblasts, as shown previously (22). The major differences in the chromatographic patterns of the three cell culture conditions were observed in the fractions in which...
proteoglycans would be eluted, in the fractions of acidic proteins eluted by concentrations higher than 0.2 M NaCl (14). These results suggested the possibility of enhanced synthesis of proteoglycans under the influence of the ECM. A 10-fold increase in levels of such material was observed in cells cultured on an ECM compared to cells grown in the presence of FGF (Fig. 4 b and c). The action of FGF may not be restricted to the modulation of collagen synthesis. A broader range of action, involving the deposition of other macromolecules is implied, particularly those which eluted with the highly acidic fractions of the DEAE-cellulose chromatogram.

Lysyl Oxidase Activity

In the preceding sections, different patterns of collagen synthesis and deposition were observed. This suggested that complex control mechanisms were operative under the three culture conditions, particularly in the compartmentalization between media and extracellular deposition. The ECM itself may participate in cross-linking and fibrillogenesis. The effect of FGF and ECM on collagen deposition into the cell layers might involve the cross-linking of collagen. Therefore, the pattern of newly synthesized collagen was determined in the presence of βAPN (80 μg/ml). This lathyrogen effectively prevents the cross-linking of collagen fibrils by inhibiting lysyl oxidase activity. In the presence of βAPN, a pattern was achieved that was similar to that in Fig. 3 without βAPN (data not shown). Thus the pattern of collagen deposition was not affected by cross-linking and the enzyme lysyl oxidase did not play a major role in organizing the collagenous matrix of cultured endothelial cells. This was confirmed by the low values of lysyl oxidase activity in these cultures compared to that of fibroblasts (Table V). Some changes of activity did occur when the three culture conditions were compared.

DISCUSSION

Cells are intimately associated with their surrounding ECM to which they attach, and on which they migrate, proliferate, and differentiate. Such cellular activities are in part determined by the ECM. However, the mechanism by which cells detect and respond to that matrix is unknown. In the endothelial culture system described here, cells grown at low density lose normal phenotypic properties unless FGF is present or unless cells are grown on their preformed natural ECM. Collagen is implicated as an important molecule in cell-matrix interactions. Synthesis of collagen may itself be modulated by the extracellular substrata, so that the ECM thus formed exerts effects on subsequent cell functions. Evidence is provided herein to support this hypothesis. We also attempted to establish that the mechanism of action of FGF on cell function may be through the changes in the ECM that it induces. Corneal endothelial cells synthesize an abundant ECM. This system provides an excellent model for determining whether collagen synthesis is modulated by the ECM. We have observed that collagen synthesis and deposition were indeed modulated significantly. A sixfold difference in collagen synthesis was observed between cells plated on plastic and on their own matrix. In addition, collagen incorporation into the cell layer was much lower when grown on plastic plates. This study is the first to demonstrate that collagen synthesis and deposition can be modulated by the matrix in this manner.

Endothelial cells grown on plastic alone failed to elaborate collagen primarily into the cell layer at any stage of culture. The greatest proportion of newly synthesized collagen was secreted into the media. This seemed to correlate with the histologic appearance of these cells in which matrix material is present on the total cell surface.

Cells grown on plastic plates in the presence of FGF developed the ability to secrete collagen primarily into the cell layer in progressive step-like increments. Only late in the cell culture did the pattern of collagen distribution between media and cell layer resemble that of the cells grown on their preformed ECM. By the final stage of culture, the cells grown in FGF and on an ECM resembled each other. In both cases a prominent basal lamina was found exclusively on the undersurface of the cell. It is tempting to assume that the asymmetry of the ECM seen histologically in these cells correlated with the preponderance of newly synthesized collagen being incorporated into the cell layer.

Analysis of collagen production on substrates other than plastic or collagen-coated plates has been demonstrated in only one other instance. Meier and Hay (12, 13) demonstrate that synthesis and deposition of collagen and glycosaminoglycans by corneal epithelial cells are stimulated when the cells are grown on killed lens capsule. Here the killed lens capsule represents a matrix provided by an adjacent tissue in situ rather than by the cells themselves.

In the present studies, when exogenous ECM was provided, the total amount of collagen synthesis was dramatically decreased, presumably because the pre-existing collagenous material provided a signal that the matrix was already present. Cells grown on plastic plates produced much more collagen. Thus, a regulatory mechanism must exist whereby cells sense and respond to the collagen in their immediate environment. Such a mechanism is important in understanding the regulation of collagen biosynthesis. Evidence for a receptor for soluble collagen on the cell surface has been documented (1, 2) as well as for other macromolecules of the ECM (15, 21). How a cell senses its total environment and what role these receptors play remains to be elucidated.

It is noteworthy that in cells grown on a preformed ECM, the ability to incorporate newly synthesized collagen primarily into the cell layer was present at the early sparse stage of growth. The matrix signals were present from the beginning when cells were first plated. In marked contrast, when FGF was added to the culture media, the synthesis and deposition of collagen was modulated in a stepwise manner so that elaboration of collagen primarily into the cell layer was achieved, but only after confluence had been reached. This demonstrated an active role for FGF throughout the course of cell growth. The amount of collagen synthesized decreased progressively, but the proportion deposited into the cell layer

| TABLE V |
| Lysyl Oxidase Activity under Different Culture Conditions |
| Plastic | Plastic and FGF | ECM |
| Media | plast | plast | plast |
| Cell Layers | 0 | 26 | 18 |

Activity is expressed as cpm of 3H release per 10^6 cells. In each assay tube, 1 × 10^6 cpm of (6-3H)lysine-labeled chick calvarial collagen was used as substrate. The assay was performed according to the procedure described by Siegel and Fu (13). Cultures were used at day 6. The results represent the mean of duplicates. Newborn human foreskin fibroblasts grown on plastic at confluence under the same conditions contained 5,200 cpm total activity per 10^6 cells.
gradually increased. The matrix gradually became organized. Finally, the profile of synthesis resembled that of cells plated initially on a natural preformed matrix. It appears that modulation of collagen differs from that of a pre-existing ECM (Fig. 3).

The ECM functions not only as a structural support, but also contributes to the control of cellular function (24) particularly during embryonic development (9–11). Further support for this concept is provided by the evidence in this study. The present study also contributes to the concept that soluble growth factors may operate by modulating ECM production.

One proposed role for the ECM in vivo is to sequester biologically activated factors including growth factors such as FGF. This would provide a persistent localized stimulation for cells (20). Such a phenomenon might provide an explanation for the phenomena described in the present communication. The BCE matrix might have sequestered growth factors that accumulated during the initial production of the ECM. However, the pattern of collagen modulation is very different when cells, grown in the presence of FGF and the preformed ECM, are compared. Therefore this was deemed an unlikely explanation for our observations.

Less collagen is synthesized in cells grown in presence of FGF or on the ECM, and in each case proportionately more collagen is retained in the cell layer. We drew the conclusion here that the matrix instructs the cell to do this. However an alternative explanation is that when less collagen is synthesized, more tends to remain with the cell layer, preventing it from being released into the media. It would be difficult to test this hypothesis without availability of an additional independent mechanism to modulate collagen synthesis.

In culture, cells are grown after being dissociated from tissue or passed from a previous culture by proteolytic treatment. Invariably the surrounding ECM is lost. The ability to grow and differentiate depends upon the cells’ capacity to manufacture rapidly an appropriate ECM. This capacity varies among different cell types. It is rather limited in epithelial cells, but preserved in mesenchymal cells. Cell behavior in culture which resembles that in situ is best achieved when an appropriate ECM is provided. This can be achieved either by providing a preformed ECM, or by enhancing the cells’ capacity to make an ECM, such as by adding various soluble growth factors. This may be the mechanism by which growth factors are necessary for some cultured cells.

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