Modulation of Type III Collagen Synthesis in Bovine Corneal Endothelial Cells

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Bovine corneal endothelial cells in culture synthesize predominantly type III collagen, unlike rabbit corneal endothelial cultures which synthesize type IV collagen. In an attempt to document whether this type III collagen synthesis by bovine cells is a tissue culture-specific phenomenon, collagens synthesized by organ culture of bovine Descemet's membrane/corneal endothelium complex were compared with those of subsequent tissue culture cells, up to the eighth passage. The biosynthetically labeled collagens were analyzed on SDS electrophoresis. The soluble fractions of tissues extracted with neutral salt followed by pepsin digestion contained only type I collagen; no other radiolabeled collagens were detected in organ culture. When pepsin treatment was eliminated, type IV collagen was identified in the tissue extract by immunoblot analysis using monoclonal antibody; type III collagen failed to show a positive band by immunoblot analysis. The pepsin-treated medium fraction of the primary culture contained types I, III and V collagen; type IV collagen was identified by either the characteristic electrophoretic mobility or by immunoblot analysis only prior to the proteolysis step. The subsequent subcultures continued to synthesize types I, III and V collagen, but type IV collagen was no longer detectable from the third passage on. No substantial quantitative changes in the expression of individual collagens were observed during subculture. From the primary culture, type I collagen accounted for 30%, type III for 60% and type V for 10%. Enhanced expression of type III collagen was observed in the eighth passage and in primary cultures grown on type I collagen matrix. Type VIII collagen, appearing as a nonreducible 50K band after pepsin treatment, was not detectable at the biosynthetic level in either organ culture or tissue cultures. These data suggest that type III synthesis, and its major contribution to the collagen spectrum, may be a tissue culture-induced phenotypic modulation specific to bovine corneal endothelial cells. Invest Ophthalmol Vis Sci 29:200-207, 1988
Materials and Methods

Cell Cultures

Adult bovine eyes were purchased from a local abattoir, transported on ice, and processed within several hours of death. The following procedures were performed under sterile conditions. Following excision of the cornea, epithelial cells were removed. Corneas were then placed with the endothelial layer upward in concave glass wells placed in a petri dish. Trypsin solution (0.25%) containing 5 mM EDTA was added to the endothelial cell surfaces and incubated for 7–9 min at 37°C under humidified conditions. The trypsin solution was removed and the endothelial cell surfaces were washed three times with phosphate-buffered saline (PBS). The washing solutions were pooled into the original trypsin solutions, in which fetal calf serum (FCS) was added to a final concentration of 20% to inactivate the enzyme. The cell suspension was centrifuged and the cell pellet resuspended in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% FCS containing 50 µg/ml gentamicin. Cells were plated at 1.2 X 10^4 cells/cm^2. For subsequent passages, cells were treated with trypsin/EDTA solution as described previously. Cultures were maintained in a humidified atmosphere of 7.5% CO₂ in air.

Collagen Substratum

Type I collagen was prepared from fetal bovine skin by extraction with 0.5 M acetic acid, as previously described. Collagen-coated dishes were prepared by allowing a solution of 200 µg of collagen in 0.5 M acetic acid, placed in the bacteriologic plastic petri dish, to evaporate. Dishes were sterilized by ultraviolet irradiation before seeding. The freshly isolated primary cells were plated on the collagen-coated dishes and maintained for 7 days in culture before analysis. The primary cultures on the tissue culture plastic dishes served control.

Protein Synthesis

The confluent cells on day 7 from the primary to the subsequently passaged cultures were labeled for 20 hr with DMEM containing 200 µCi[5-3H]proline (27 Ci/mM) (Dupont, Boston, MA), 2% FCS, 50 µg/ml 2-aminopropionitrile (B-APN) and 50 µg/ml ascorbate. Medium was collected and the cell debris removed by centrifugation. Protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 mM N-ethylmaleimide, 4 mM EDTA) were added. The medium fraction was then concentrated with ammonium sulfate to 45% saturation. The precipitate was collected by centrifugation at 10,000 g for 15 min at 4°C and dissolved in buffer I (0.15 M NaCl, 0.05 M Tris-HCl, pH 7.4, 0.1% Triton-X-100) followed by dialysis in the same buffer. An aliquot of the precipitate was dialyzed against 0.5 M acetic acid and 0.1% Triton-X-100, followed by pepsin treatment (100 µg/ml) at 4°C for 24 hr. The enzyme reaction was stopped by raising pH to 8.0, and the reaction mixture was dialyzed against buffer I.

Extraction of Collagen Synthesized by DM/Endothelium Organ Culture

Descemet’s membrane/corneal endothelium complex was scraped from the overlying corneal stroma under a dissecting microscope as described previously. The membranes were rinsed in sterile HEPES-Spinner solution followed by labeling with DMEM containing 500 µCi[5-3H]proline, 2% FCS, 50 µg/ml B-APN and 50 µg/ml ascorbate for 20 hr. The medium layer was collected and processed as described above, while tissues were homogenized in buffer I in the presence of protease inhibitors. Collagen was extracted in buffer I at 4°C for 24 hr and the insoluble pellet removed by centrifugation. The supernatant was dialyzed against buffer I. An aliquot was dialyzed against 0.5 M acetic acid followed by pepsin treatment (100 µg/ml) at 4°C for 24 hr. The reaction mixture was dialyzed against buffer I.

SDS-Polyacrylamide Gel Electrophoresis

Polypeptides were electrophoresed under the conditions described by Laemmli. Gels were fixed in 20% trichloroacetic acid for 30 min and processed for fluorography as described. For the quantitation of individual collagen types the density of each monomeric band separated by electrophoresis and localized by fluorography was measured by densitometric scanning (LKB, Cambridge, England). To measure the ratio of α1/α2 of type I collagen, each monomeric band separated by electrophoresis and localized by fluorography was hydrolyzed with 0.4 N NaOH for 16 hr at 55°C, neutralized with 1 M acetic acid and counted.

Immunoblotting

Monoclonal antibody against type III procollagen was a gift from Dr. SundarRaj (University of Pittsburgh, Pittsburgh, PA), and monoclonal antibody against human type IV collagen was purchased from Dr. SundarRaj.

Electrophoretic transfer of proteins to a nitrocellulose filter and immunoblot analysis followed published procedures, with some modifications. All subsequent incubations were carried out at room...
Type III

\( \alpha_1(IV) \)

\( \alpha_2(IV) \)

- 95K

Fig. 1. Fluorogram on 4.5% SDS-PAGE and immunoblot of collagen synthesized by bovine corneal endothelial cells on Descemet's membrane. 1, proline-labeled medium concentrated with ammonium sulfate; 2, collagen marker; 3, proline-labeled medium treated with pepsin; 4, sample in lane 1 stained with anti-type IV collagen antibody. The arrowheads indicate the position of bacterial collagenase-sensitive bands.

Temperature in PBS containing 0.05% Triton-X-100. The nitrocellulose sheet was first washed and remaining accessible sites blocked with 4% bovine serum albumin. Primary antibody incubations, at 1:100 dilution, were carried out for 3 hr. Sheets were washed and incubated with horseradish peroxidase-conjugated secondary antibody (1:500 dilution) for 2 hr. Following wash, sheets were incubated with diaminobenzoic acid (DAB) (1 mg/ml PBS) containing hydrogen peroxide (10 \( \mu \)l/10 ml DAB).

Enzyme Digestion

Bacterial collagenase digestion was performed as described by Peterkofsky and Diegelman.18

Results

Collagen Synthesis by Organ Cultures of DM/Corneal Endothelium Complex

There appear to be several factors contributing to the controversial findings on the presence and synthesis of type IV collagen in bovine corneal endothelial cells. The general pattern of the findings is that cultured bovine CEC appear to synthesize very little type IV collagen, while its presence was demonstrated in the extracted tissue samples or by immunofluorescent staining of Descemet’s membrane in vivo.6-12

In the present study, a nonenzymatic technique using homogenization in neutral salt buffer was applied to extract intact soluble collagens newly synthesized by the corneal endothelium on Descemet’s membrane. The neutral salt extract contains several collagenase-sensitive bands when analyzed on SDS electrophoresis, the identities of which are rather ambiguous because of the existence of procollagens (Fig. 1, lane 1); therefore, the soluble fraction of tissue extract was examined by immunoblot analysis using specific antibodies. Anti-type IV collagen antibody demonstrates several bands: a predominant band migrating between \( \alpha_1(IV) \) and \( \alpha_2(IV) \) and two closely migrating bands just below \( \beta_1(II) \), the molecular sizes of which appear to be identical to \( \alpha_1(IV) \) and \( \alpha_2(IV) \) (Fig. 1, lane 4). The size of the former band (approximately 95K) shares its identity with the major pepsin-fragment of type IV collagen which was used for the production of monoclonal antibody (personal communication with Dr. SundarRaj). It has been known that frequent imperfection of the triplet sequence Gly-X-Y makes type IV collagen susceptible to protease digestion.19 The predominance of this band confirms current understanding of the protease-sensitivity of native type IV collagen. Immunoblot analysis on type III procollagen demonstrates no positive band identified by the specific antibody (data not shown), suggesting that bovine corneal endothelial cells on basement membrane may not synthesize type III collagen. When the soluble fraction was further analyzed following pepsin digestion, it is of interest that type I collagen is the only biosynthetically labeled collagen (Fig. 1, lane 3). The absence of the newly-synthesized type IV collagen may be due to the further fragmentation induced by pepsin treatment. Since the native type IV collagen in organ culture...
Type III collagen was synthesized by bovine corneal endothelial cells in culture. Cells on Day 7 were labeled with \[^3H\]proline for 20 hr. The medium fraction was treated with pepsin prior to electrophoresis on 4.5% SDS-PAGE under nonreduced (1~6) and reduced (7~12) conditions. 1 and 7, primary culture; 2 and 8, first passage; 3 and 9, second passage; 4 and 10, third passage; 5 and 11, fourth passage; 6 and 12, eighth passage.

Fig. 2. Collagen phenotypes synthesized by bovine corneal endothelial cells in culture. Cells on Day 7 were labeled with \[^3H\]proline for 20 hr. The medium fraction was treated with pepsin prior to electrophoresis on 4.5% SDS-PAGE under nonreduced (1~6) and reduced (7~12) conditions. 1 and 7, primary culture; 2 and 8, first passage; 3 and 9, second passage; 4 and 10, third passage; 5 and 11, fourth passage; 6 and 12, eighth passage.

demonstrates the susceptibility to protease, which yields a major 95K band, further fragmentation by pepsin occurs producing smaller peptides, which cannot be detected on a 4.5% gel electrophoresis.

The recently described type VIII collagen has been identified in bovine Descemet's membrane. One of the unique features of the molecule is that the native molecule yields a nonreducible fragment of M.W. 50,000 following pepsin treatment. Biosynthesis of type VIII collagen has been reported in both rabbit and bovine corneal endothelial cells in culture. Unlike these findings, the 50K nonreducible pepsin-resistant fragment was not detectable in the organ culture extract (Fig. 1, lane 3).

Collagen Synthesized by Tissue Cultured Bovine Corneal Endothelial Cells

It appears that adult bovine CEC, when cultured, synthesize predominantly type III collagen under a variety of culture conditions, including early passage, postconfluent cultures and the presence of fibroblast growth factor. Furthermore, a previous study found some variation in relative quantitation of each collagen type. In order to accurately assess the expression of collagen phenotypes of bovine CEC in culture, identical culture conditions were maintained throughout the experiment (eg, same batch of fetal calf serum and media, same inoculation cell density). Cell growth of each subculture was monitored by cell numbers, while cell morphology was evaluated by phase-contrast light microscopy. Growth potential of each culture (from primary to eighth passage cultures) was similar, with cell numbers at confluency (day 7) ranging from 3 \times 10^6 cells to 4 \times 10^6 cells (not shown). In addition, the biosynthetic capacity of these cultures was similar in overall protein and collagen synthesis (data not shown).

Collagenous peptides were characterized primarily from the soluble fraction of medium, and the collagen phenotypes were evaluated by several criteria. First, the medium fraction was analyzed on SDS-PAGE, following pepsin treatment. Biosynthetically labeled collagens obtained from the primary culture through the eighth passage culture were fractionated into three collagen types (Fig. 2): type III collagen as disulfide-bonded molecules, which yielded \( \alpha \)-size chain upon reduction; type V collagen as \( \alpha_1(V) \) and \( \alpha_2(V) \); and type I collagen as \( \alpha_1(I) \) and \( \alpha_2(I) \). No qualitative changes were observed in these collagens from the primary culture to the eighth passage culture. The slow migrating band above the trimeric type III collagen appears to be type III collagen, which is reducible to \( \alpha_1(III) \); reduction does not generate any other smaller molecular weight bands that would be candidates for pepsin fragments of type IV collagen.

It has been known that several pepsin-resistant fragments of \( \alpha_1(IV) \) and \( \alpha_2(IV) \), ranging from 27K to 140K, were generated during extraction with pepsin.
in various tissues. Since some pepsin-resistant fragments of type IV collagen may comigrate with other interstitial collagen chains, the individual collagen chains resolved in Figure 2 were identified by their characteristic CNBr-peptide profiles by two-dimensional SDS electrophoresis. The resulting peptide profiles of the primary culture showed characteristic CNBr-peptides of $\alpha_1$ (I), $\alpha_2$ (I), $\alpha_1$ (III), and $\alpha_1$ (V) chains, indicating that these pepsin-resistant fragments are not derived from type IV collagen. Identical profiles were obtained from the fourth passage cultures (data not shown). In order to determine whether the absence of type IV collagen synthesized by bovine CEC was due to the nonspecific degradation by pepsin, the culture media obtained from the primary cultures and the fourth passage cultures were analyzed prior to pepsin treatment. The profiles of the biosynthetically labeled molecules demonstrate bands corresponding to 180K, $\alpha_1$(IV), and 160K, $\alpha_2$(IV) in the primary culture medium; however, the intensities of the bands were very faint when compared with other procollagen chains (Fig. 3, lane 2). Furthermore, the two bands were not detectable in the fourth passage culture medium (Fig. 3, lane 3), although they were present in the medium up to the second passage cultures, albeit at a low level. These findings suggest that the level of newly synthesized type IV collagen is very low in the primary culture, and expression of the molecule appeared to be lost as the cultures were passaged. The presence of type IV collagen in the primary culture was further evidenced by immunoblot analysis (Fig. 3, lane 5). Bands corresponding to both $\alpha_1$(IV) and $\alpha_2$(IV) were recognized by the monoclonal antibody against type IV collagen. The partially degraded 95K band is quite insignificant in the primary culture medium when compared to the organ culture product, suggesting that nonspecific protease activity is much less in the tissue culture medium than in tissues.

Biosynthesis of type III collagen was not demonstrated by organ cultures; in contrast, the molecule was synthesized from the primary culture. Immunoblot analysis using the specific antibody further demonstrates type III procollagen synthesis in the primary culture medium, while the same antibody does not react with the salt-soluble tissue extract in organ culture (data not shown).

In order to study the relationship between the phenotypic expression of collagen and culture age, the proportion of the individual collagen types was analyzed; individual chains separated by SDS-electrophoresis were then treated mathematically according to the known stoichiometry of chains of each collagen type (Table 1). In the first passage culture, type I collagen accounted for 30% of the total collagen, type III for 60%, with the remainder being type V collagen. No substantial quantitative changes in the expression of individual collagens occurred up to the fifth passage culture, at which time type III collagen increased until it was the major collagen constituent in the eighth passage culture.

The stoichiometry of type I collagen does not appear to be altered significantly throughout the subcultures; the higher values (approximately 2.8) ob-
Table 1. Quantitative analysis of the collagen phenotype synthesized by the sequentially passaged bovine corneal endothelial (CEC) cells in culture

<table>
<thead>
<tr>
<th>Passage number</th>
<th>Type I collagen (%)</th>
<th>Type III collagen (%)</th>
<th>Type V collagen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1°</td>
<td>29.5 ± 1.8</td>
<td>60.3 ± 3.0</td>
<td>10.1 ± 2.0</td>
</tr>
<tr>
<td>2°</td>
<td>31.5 ± 1.7</td>
<td>58.6 ± 2.9</td>
<td>10.0 ± 1.1</td>
</tr>
<tr>
<td>3°</td>
<td>32.1 ± 3.7</td>
<td>61.3 ± 4.5</td>
<td>6.4 ± 0.8</td>
</tr>
<tr>
<td>4°</td>
<td>36.1 ± 1.8</td>
<td>55.6 ± 2.1</td>
<td>8.3 ± 0.3</td>
</tr>
<tr>
<td>5°</td>
<td>41.5 ± 0.6</td>
<td>52.0 ± 1.3</td>
<td>6.5 ± 0.7</td>
</tr>
<tr>
<td>8°</td>
<td>23.6 ± 4.7</td>
<td>72.7 ± 5.5</td>
<td>3.7 ± 1.1</td>
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</tbody>
</table>

The density of each monomeric band separated by electrophoresis and localized by fluorograph was measured by densitometer as described in the text. Values are the means of four samples ± SD.

Table 2. Ratio of α1 to α2 of type I collagen synthesized by bovine CEC

<table>
<thead>
<tr>
<th>Passage number</th>
<th>α1/α2</th>
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<tbody>
<tr>
<td>1°</td>
<td>2.09 ± 0.64</td>
</tr>
<tr>
<td>2°</td>
<td>2.26 ± 0.08</td>
</tr>
<tr>
<td>3°</td>
<td>2.57 ± 0.01</td>
</tr>
<tr>
<td>4°</td>
<td>2.81 ± 0.01</td>
</tr>
<tr>
<td>5°</td>
<td>2.74 ± 0.16</td>
</tr>
<tr>
<td>8°</td>
<td>2.86 ± 0.26</td>
</tr>
</tbody>
</table>

Each monomeric band separated by electrophoresis and localized by fluorograph was treated as described in the text. Values are the means of four samples ± SD.

Table 3. Quantitative analysis of the collagen phenotype synthesized by the primary bovine CEC

<table>
<thead>
<tr>
<th></th>
<th>Type I collagen (%)</th>
<th>Type III collagen (%)</th>
<th>μt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control cultures</td>
<td>39.2 ± 7.7</td>
<td>60.8 ± 7.7</td>
<td>1.55</td>
</tr>
<tr>
<td>On type I collagen matrix</td>
<td>11.1 ± 0.3</td>
<td>88.9 ± 0.3</td>
<td>8.01</td>
</tr>
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</table>

Each monomeric band separated by electrophoresis and localized by fluorograph was treated as described in the text. Values are the means of three samples ± SD.

Collagen Synthesis by Primary Corneal Endothelial Cells on Collagen Matrix

While organ cultures produce types I and IV collagen, the common collagen spectrum in the cultured cells consists of types I, III and V; type IV collagen is demonstrated only in the early passage cultures. Since type I collagen is readily synthesized by bovine corneal endothelium on Descemet’s membrane, collagen synthesis by the primary cultures on type I collagen matrix was studied to determine if such artificial substratum induced the phenotypic expression of collagen observed in vivo, reflecting the physiologic constituents in Descemet’s membrane. Cell morphology and growth potential of the cells plated on collagen matrix were compared with those of the control cultures plated on tissue culture dishes. Plating efficiency and growth rate appear to be identical (data not shown). Collagen phenotypes on Day 7 were analyzed to determine if there were qualitative changes in phenotypic expression of collagen. When intact chains were fractionated using SDS electrophoresis following pepsin treatment, there were no changes in the expression of types I, III and V collagen by the cells on type I collagen matrix; however, major changes were observed in the proportion of types I and III collagen. Type I collagen accounted for 39% in control cultures, while its synthesis was significantly decreased by the cells on collagen matrix. The decrease of the molecule was compensated for by an increase in type III collagen (Table 3). Therefore, the ratio of type III/I collagen is dramatically altered in the cultures on collagen matrix, suggesting that type I collagen matrix enhances the expression of type III collagen. Of interest is that cells on the artificial matrix do not enhance expression of type IV collagen, which is one of the physiologic components of the basement membrane in vivo. The cellular fraction and extracellular matrices produced by both cultures were also examined; type III collagen was relatively more associated with cell layer recovered from the cells on collagen matrix. However, type I collagen synthesized by the cells either on plastic dish or on collagen gel substratum is predominantly present in the medium fraction (data not shown).

Discussion

Over the years, studies on corneal endothelial cells in culture have provided conflicting reports on collagen synthesis. Some of these conflicts can be easily explained, while other phenomena defy explanation. Some of the conflicts are apparently inherent to the animal species studied; rabbit CEC have yielded less conflicting results than observed in bovine CEC. It appears that there is a common finding on the synthesis of type IV collagen by rabbit corneal endothelial cells in culture, in which either primary cultures or early passaged cultures were used. Furthermore, these cells, regardless of passage numbers, retain biosynthetic capacity of type IV collagen, with no evidence of a switch of collagen phenotypes. Type III collagen has also been reported to be synthesized by rabbit CEC, while others do not find its synthesis. Nevertheless, the contribution of type III to the total collagen spectrum is very negligible. Whether synthesis of the molecule is related to tissue culture-specific phenomenon rather than reflecting...
its physiologic presence in the rabbit Descemet's membrane is currently not known. Type VIII collagen has been reported to be the major collagenous peptide synthesized by rabbit CEC; however, similar sized pepsin fragments of type IV collagen could not be distinguished from each other, thus leading to uncertainty regarding the contribution of the molecule to the total collagen. There is no apparent conflict regarding the synthesis of types IV and VIII collagen or as to the minor contribution of type III collagen to the entire collagen spectrum in rabbit CEC in culture. However, compatible occurrence of these molecules in rabbit Descemet's membrane should be evidenced to understand the entire physiologic collagen phenotype.

Unlike these findings, the equivalent bovine cells in culture synthesize predominantly type III collagen, which is deposited in the extracellular matrix. Interestingly there is very little if any synthesis of type IV collagen in the same cultures. On the other hand, the extracellular matrix deposited by postconfluent bovine CEC in culture contained type IV collagen as well, as determined by immunoblot analysis. Such controversy was manifested when collagen phenotypes of bovine Descemet's membrane were analyzed from the tissue extract, either with or without pepsin, and revealed the presence of types IV and VIII collagen. Type III collagen is found only in fetal bovine Descemet's membrane. In addition to types III, IV and VIII collagens, synthesis of types I and V was detected, compatible with their presence in Descemet's membrane.

In view of these reported differences in the synthetic activity of bovine CEC, and to minimize variations that may affect biosynthetic expression of collagen, the present study compared the biosynthetic pattern of organ cultures with that of bovine CEC in culture. Our results demonstrate qualitative changes in collagen phenotype between organ culture and cell culture, and between the primary culture and the serially passaged subcultures. Organ culture of DM/corneal endothelium appears to synthesize two collagenous components; type I collagen was detected by gel electrophoresis while type IV collagen was identified by immunoblot analysis. The other collagen types were not detectable; type III was not detected by either technique (characteristic electrophoretic mobility, with or without reduction, or immunoblot), and the absence of type VIII collagen was supported by the absence of the characteristic 50K nonreducible pepsin-resistant fragment. This may suggest three possibilities: type VIII collagen is not synthesized under the conditions applied in the present studies (the molecule is not readily synthesized by the confluent cells; personal communication with Dr. Rajesh Kapoor, University of Washington, Seattle); the initial recovery of the molecule was hindered by the absorptive nature of the molecule; or the level of type VIII collagen synthesized by the corneal endothelium on Descemet's membrane is negligible, resulting in negative findings. On the other hand, freshly isolated primary endothelial cells appear to maintain synthesis of types I and IV collagen and to acquire biosynthetic expression of types III and V collagen.

The most striking effect of tissue culture was the expression of type III collagen, which is apparently the major collagen type in both the primary culture and in the serially passaged cultures. These results essentially corroborate the previous findings on the predominant synthesis of type III collagen in culture. Significant enhancement of type III collagen synthesis was observed in the primary cultures grown on collagen matrix. A similar finding was reported by using types I or IV collagen for collagen substratum. Thus, the major contribution of type III collagen to the entire collagen spectrum in bovine CEC appears to be tissue culture-specific phenotypic modulation, and the expression of this molecule is highly preserved during subcultures. In contrast, the biosynthetic capacity of type IV collagen is lost; only the primary cultures and the very early passaged cells maintain synthesis of the molecule, albeit at a low level. It is not clear whether induction of a modulated phenotype (type III) suppresses the expression of one of the physiologic phenotypes (type IV), subsequently leading to a major maintenance phenotype in culture conditions. Unlike induction and suppression of these two molecules, the expression of type I collagen is not changed by the altered expression of types III and IV collagen, suggestive of independent regulation of the expression of type I collagen.

Type I collagen appears to be the ubiquitous modulated phenotype in most of the de-differentiated cells: subcultured chondrocytes and postconfluent sprouting vascular aortic endothelial cells. Therefore, it remains unclear why type III collagen becomes the major modulated phenotype in bovine CEC. As indicated by the findings from many laboratories, regulation of collagen gene expression occurs at both transcriptional and post-transcriptional levels. There are several possible explanations for the phenomenon observed in bovine CEC: (1) type III collagen gene has a more permissive conformation, which is readily transcribed; (2) the steady-state mRNA levels of type III are elevated; or (3) translatability of type III mRNA is enhanced.

The present findings reflect the current controversial results on the collagen synthetic activities of bovine CEC in vitro; the general pattern includes syn-