Role of Collagenous Matrices in the Adhesion and Growth of Cells

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It has long been appreciated that, for many types of cells, attachment to a substrate is required for their replication. Collagen substrates enhance the growth (64, 70, 109, 238) as well as the differentiation (40, 69, 85, 86, 146, 161, 184), of many cells in culture above that observed with other substrates such as plastic and glass. As discussed in this report, many cultured cells including fibroblasts, myoblasts, hepatocytes, chondrocytes, and certain epithelial cells are thought not to bind directly to the collagen substrate or to the plastic surface of culture dishes (31, 73, 74, 76, 77, 89, 93, 113, 164, 220). Instead, extracellular glycoproteins bind the cells to the substrate. One of these attachment proteins, fibronectin, has been extensively studied (99, 151, 166, 227, 228, 244). Fibronectin is produced by fibroblasts (13, 197, 246) and endothelial cells (102, 140) as well as some other cell types. It is also present in high concentrations in blood and serum (152). In culture, serum fibronectin, as well as that produced by the cells, can bind to both the collagen substrates and the tissue culture plastic surface and mediate the attachment of cells (73, 113, 164, 242). Circulating fibronectin participates in a variety of reactions important to wound healing, including the adhesion and spreading of platelets on collagen (10, 14, 75, 100, 204), binding to the fibrin clot (74, 150, 198) and to collagen (37, 46, 94, 103, 119), promoting opsonization reactions by phagocytic cells (18, 94, 201), and promoting fibroblast migration (6, 61). Thus, fibronectin not only functions as an attachment protein, but also may play an important role in repair reactions.

In the case of certain differentiated cells, such as chondrocytes (89) and epithelial cells (159, 220), glycoproteins different from fibronectin are active in attachment. The components of these extracellular matrices of fibroblasts, chondrocytes, and epithelial cells differ with the cell type, and they require separate attachment proteins to provide additional specificity to the interaction of the cell with its matrix. Alterations in cell-substratum interactions are observed in differentiating cells and after spontaneous transformation of cells or exposure to oncogenic agents (99, 228). This review will briefly outline our current knowledge of the types of collagen and their distribution and biosynthesis. Then, the interaction of cells with collagen and other matrix components will be described, with emphasis on cell adhesion, differentiation, and growth.

COLLAGEN

Structure, Biosynthesis, and Degradation

Numerous criteria define a collagen and distinguish it from other proteins (22, 49, 50, 58, 141, 148, 184). Collagens contain a triple-helical segment composed of three chains that have a characteristic composition including 33% glycine, ~10% proline, 10% hydroxyproline, and variable amounts of hydroxylysine. Collagens, but not other proteins, are degraded by bacterial collagenases, which recognize and cleave the sequence gly-X↓-pro-gly. These, as well as other features such as their x-ray pattern, establish certain structural proteins as collagens and other proteins as containing collagenous domains. At present, two nonstructural proteins having collagenous segments have been identified: Clq, a component of the complement cascade (188), and acetylcholinesterase (192). Detailed molecular models for these molecules have been proposed.

At least five isotypes of collagen molecules have been characterized, and others probably exist (Table I) (22). Although the interstitial collagens, types I, II, and III, are the products of separate genes and have unique amino-acid sequences, in other respects they are quite similar. Each molecule contains a triple-helical domain, roughly 300 × 1.5 nm, composed of three chains, each ~94,500 daltons. Type I collagen, found in skin, bone, and tendon, has two α1(I)-chains and one α2(I)-chain. In addition, collagen molecules containing only α1(I)-chains with the composition $[\alpha 1(I)]_3$ have been identified and are usually referred to as type I trimer (142, 157). Type I trimer is found in tissues (105), as products of certain cultured cells (16, 33, 142, 144), and in tumors (149). Type II collagen is found mainly in cartilage and contains three α1(II)-chains (148). Type III collagen, at one time thought to be "fetal" collagen, is composed of three α1(III)-chains and is most prominent in blood vessels, skin, and the parenchyma of internal organs (63). Many investigators consider it to be the reticulin described by histochemical methods, although this has not been proved.

TABLE I

The Collagen Isotypes

Collagen type	Composition	Some distinguishing characteristics	Tissue location	Cell types that synthesize various collagen types
I	$[\alpha 1(I)]_2\alpha 2(I)$	Low carbohydrate, low hydroxylation of lysine	Skin, bone, tendon, cornea	Fibroblasts, osteoblasts, smooth muscle cells, epithelium
II	$[\alpha 1(II)]_3$	>10 hydroxylysines per chain	Cartilage, cornea, vitreous body	Chondrocytes, neural retinal cells, notochord cells
III	$[\alpha 1(III)]_3$	Contains cysteine, low hydroxylation of lysine	Fetal skin, blood vessels, organs	Fibroblasts, myoblasts
IV	$[\alpha 1(IV)]_3$ $[\alpha 2(IV)]_3$	Many lysines hydroxylated and glycosylated, sugars other than glucose and galactose, low alanine, high 3-hydroxyproline	Basement membrane	Endothelial and epithelial cells
V	$[\alpha 1(V)]_2\alpha 2(V)$ (formerly αA-and αB-chains)	Elevated hydroxylysine, low alanine, contains 3-hydroxyproline	Blood vessels, smooth muscle	Smooth muscle cells, chondrocytes under certain conditions

Types IV and V collagens have been described and partially characterized. Type IV collagen is found in basement membranes (22, 110) and probably contains two distinct chains that are larger than the chains of other collagens (222). In addition, type IV collagen has more carbohydrate, 3-hydroxyproline, and hydroxylysine than the other collagens. Type V collagen, which is present in skin, smooth muscle, placenta, and bone (24, 32, 143, 190), contains two types of chains designated αA and αB, or in the new terminology α2(V) and α1(V), respectively. Recent studies indicate that the two chains occur in the same molecule in type V collagen from placenta (15), and molecules containing only αB chains have been isolated from cartilage (190).

The amino-acid sequences of the α1(I)- and α1(II)-chains have been determined, and partial sequences of the α1(III)- and α2(I)-chains are known (51). For each chain there is considerable interspecies sequence homology (23).

The steps in the biosynthesis of collagen types I, II, and III have been described (for reviews, see references 50, 141, 183). These collagens are formed from procollagen, a larger, more soluble precursor containing pro-α-chains (Fig. 1). The pro-α1(I)-chain of type I collagen contains a central collagenous region plus additional peptide sequences of ~13,000 daltons at its amino-terminus and of 36,000 daltons at its carboxy-terminus. Pro-α2(I)-, pro-α1(II)-, and pro-α1(III)-chains contain analogous peptides, which are chemically distinct.

The chains are modified enzymatically after synthesis (Fig. 1) (183). These modifications include the introduction of hydroxyl groups into certain prolyl residues to form 3- and 4-hydroxyproline and the hydroxylation and glycosylation of some lysyl residues. Most prolyl residues that precede glycyl residues in the molecule are hydroxylated in the 4-position of the ring by an intracellular, probably membrane-bound enzyme, prolyl hydroxylase, which requires for activity ferrous ion, ascorbic acid, α-ketoglutarate, and oxygen (223). Prolyl residues hydroxylated in the 3-position have been found to occur in the sequence 3-hydroxyproline-4-hydroxyproline-glycine (1). Both proline and 4-hydroxyproline stabilize the collagen helix (23, 184), whereas the role of 3-hydroxyproline residues is unknown. Hydroxylation of lysyl residues is carried out by a separate enzyme that has the same cofactor requirements as prolyl hydroxylase (147). In addition, some hydroxylysyl residues are glycosylated by unique sugar transferases to form galactosyl-hydroxylysine and glucosyl-galactosyl-hydroxylysine (58, 183). The function of the sugar residues is not known.

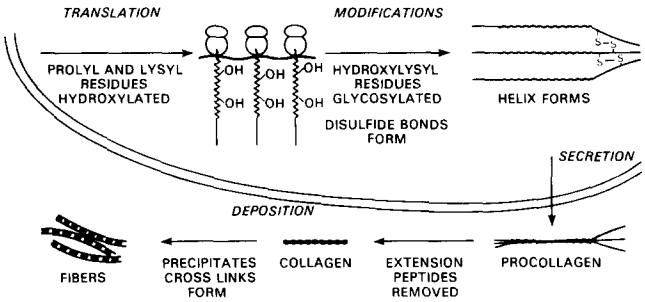

FIGURE 1 Synthesis and maturation of collagen. Certain prolyl and lysyl residues on the nascent chains are hydroxylated by specific enzymes. Although the hydroxylation begins on the nascent chains, it can continue after completion and release of the chains, until the triple helix forms. Helix formation also terminates glycosylation of hydroxylysyl residues. The procollagen molecule is then secreted from the cell where extracellular or membrane-bound enzymes remove the noncollagenous extension peptides. The collagen molecules are enzymatically cross-linked to form stable fibers.

Glycosylation and hydroxylation reactions occur in the pro-α-chains and terminate when the chains assume a helical configuration (183). To form type I procollagen, two pro-α1(I)-chains and one pro-α2(I)-chain associate, form disulfide bonds at the carboxy-terminus, and coil into a triple helical protein (50). Separate enzymes, procollagen N-protease and procollagen C-protease, remove the amino-terminal and carboxy-terminal peptides, respectively (50, 128, 183). Kinetic studies indicate that the amino-terminal peptides are removed *en bloc* either inside the cell or at the cell surface, whereas the carboxy-terminal peptides are removed later (50). The liberated amino-terminal peptides may regulate collagen synthesis by feedback inhibition of translation (163, 239). Because collagen molecules spontaneously aggregate into fibers, it is likely that the carboxy-terminal peptides confer solubility on procollagen and that procollagen C-protease may regulate fiber formation (21, 50). Collagen molecules in fibrils are cross-linked via the condensation of aldehydes formed from certain lysyl and hydroxylysyl residues in the molecule by the enzyme lysyl oxidase (49, 174, 175). Fibrillar type I collagen is readily identified in vivo at the electron microscope level by its characteristic banding pattern (80), whereas the appearance of the other collagens is either

less distinct (type II), nonfibrillar (type IV), or unknown (types III and V) (63).

Native collagen molecules and fibrous collagen are resistant to most proteases and the initial step in their breakdown in animals is carried out by a specific collagenase (80). Mammalian collagenase cleaves at a single site approximately one-fourth of the way from the carboxy-terminal end of the molecule (82). The resulting fragments are much less stable than the intact molecule and are presumed to denature and then be degraded by other proteases. There is evidence to suggest that there are collagenases specific for different collagen types. The fibroblast collagenase cleaves collagen types I, II, and III, whereas the neutrophil enzyme cleaves type I but not type III collagen (95). In addition, certain tumor cells produce an enzyme that cleaves type IV collagen (138) but not the others.

Synthesis of Collagen by Cultured Cells

With the exception of lymphocytes, reticulocytes, and other blood-borne cells, most cells in culture synthesize collagen (68, 72, 127). Further, it has been shown that cultured cells can accomplish all the reactions necessary to form and to cross-link collagen fibers (134, 219). However, this is not an efficient process and in culture up to 90% of the procollagen formed accumulates in the medium (131). Much of the procollagen in the medium lacks the amino-terminal peptides but maintains the carboxy-terminal extensions (50). Presumably, the protease that cleaves the carboxy-terminal portions is not sufficiently active in most cells under culture conditions to achieve complete conversion to collagen. Almost complete conversion, however, has been noted with certain mouse fibroblast lines (219).

The amount of collagen produced varies with the cell type. Most fibroblast strains produce collagen as 5–10% of their total protein, whereas other cell types synthesize from 0.001 to 10% of their total protein as collagen (68). Although cells freshly removed from embryonic tendon synthesize collagen as 20–50% of the total protein (36), once in culture, there is a decrease to ~3–5% of the total protein. Relatively minor modifications of the culture medium (Table II), such as the addition of sodium lactate and of ascorbic acid with lower serum levels, allow the cells to maintain a high rate of collagen synthesis (209). Ascorbic acid has long been recognized as essential for the formation of normal connective tissue (26). Although the translation of collagenous polypeptides is not affected in ascorbic acid deficiency, hydroxyproline formation is reduced (173), and the chains do not assume a helical configuration (185). Addition of ascorbic acid has been reported to stimulate proliferation of cells in culture (193), perhaps as a consequence of the enhanced deposition of a collagenous matrix. It should be noted that levels in most media are low and that ascorbic acid has a short half-life (<12 h) in tissue culture (173).

Synthesis of Collagen by Differentiated Cells

Most fibroblasts produce both types I and III collagen simultaneously (62). An even greater variety is observed with smooth muscle cells that synthesize types I, III, IV, and V collagens (143). Epithelial and endothelial cells synthesize type IV collagen, which is consistent with the role of these cells in the formation of the basement membrane (for examples, see references 8, 33, 96, 101, 202).

Freshly isolated chondrocytes synthesize type II collagen, a cartilage-specific protein (224). With time in culture, the cells become more fibroblastic and are found to synthesize type I collagen or both types I and II (16, 231). Late cultures of such cells synthesize types I, II, III, and V, and type I trimer (16, 142). Factors involved in the loss of characteristic chondrocyte activities during culture will be covered in the section Matrix Proteins and Cell Differentiation.

Tissue levels of one or more of the collagens are altered in a variety of diseases (21). In some cases, skin fibroblasts cultured from such patients retain the collagen defect. For example, tissue levels of type III collagen are reduced or absent in patients with the Ehlers-Danlos type IV syndrome (179), and the production of type III collagen by fibroblasts from the skin of these patients is markedly reduced. Fibroblasts from patients with some forms of osteogenesis imperfecta synthesize an unusually high ratio of type III to type I collagen (171). In addition, tumors often synthesize the collagen type characteristic of their tissue of origin. For example, osteosarcomas contain type I collagen (149), whereas chondrosarcomas contain type II collagen (212). In general, the type and amount of collagen synthesized by cells in culture reflect their origins.

RECONSTITUTED COLLAGEN MATRICES

Preparation of Collagen Matrices

Collagen is often employed as a substrate for cultured cells (40, 43–45, 64, 69, 85, 86, 109, 124, 125, 139, 146, 159, 205, 238). Such matrices can be prepared in a variety of forms differing in their properties and components. In many cases, an acid extract of rat tail tendon is dried on a culture dish.

TABLE II

Compounds Specifically Affecting Collagen Synthesis and Maturation

Compounds	Mechanism	Effect
Inhibitors		
cis-Hydroxyproline (50–200 μg/ml)*	Proline analogue	Inhibits helix formation (4, 183, 194, 225)‡
cis-4-Fluoro-L-proline		
L-Azetidine-2-carboxylic acid		
3,4-Dehydroproline		
β-Aminoproprionitrile (50 μg/ml)	Inhibits lysyl oxidase	Inhibits collagen cross-linking (175)
α,α′-Dipyridyl (0.3 mM)	Inhibits hydroxylases (iron chelator)	Inhibits prolyl and lysyl hydroxylation (183)
D-Penicillamine (1.0 mM)	Binds to aldehydes	Inhibits cross-linking (183)
Stimulators		
Ascorbic acid (50 μg/ml)	Enzyme cofactor	Stimulates prolyl and lysyl hydroxylase (173)
Sodium lactate (40 mM)	?	Activates prolyl hydroxylase (223)

* Amount in parentheses denotes effective concentration for tissue culture studies.
‡ Numbers in parentheses are references.

Such an extract would consist in large part of type I collagen, but would also have other constituents. Commercial preparations of type I collagen have been used that may contain substantial quantities of impurities including other proteins and even glycogen. Some of the commercial preparations are labeled types I, II, III, IV, and V (Sigma Chemical Co., St. Louis, Mo.). These designations refer to different purification procedures, not to the genetically distinct collagens.

With purified collagens, weighed amounts are dissolved in dilute acetic acid and this solution is placed on the surface of plastic or glass culture dishes. The solvent is evaporated either at room temperature or in a 37°C incubator. 10–50 μg of collagen are sufficient to cover the surface of a 35-mm-diameter dish (121). This substrate is probably not arranged as native-type fibers and may even be denatured (78). A firm and adherent gel can be generated from the acetic acid solution of collagen by exposure to NH_3 vapors (48). Again, the collagen may not be present as native-type fibers.

Heat-reconstituted collagen gels are also used as substrates. In this case, the collagen is dissolved in acetic acid and dialyzed in the cold against 0.15 M NaCl buffered to pH 7.4 with phosphate or Tris. The collagen remains in solution in the cold overnight but forms a firm gel of native fibrils when warmed (44, 81, 121). These gels can be used directly or can be liberated from the surface of the dish to serve as a floating support (45, 205). Higher concentrations of collagen are used to prepare heat-reconstituted substrates (1–4 mg/plate) than for the other coatings and, for this reason, heat-reconstituted gels require higher concentrations of attachment proteins or serum to support cell attachment (121). When nonphysiological levels of phosphate buffer (>0.01 M) are used, calcium and other divalent cations are precipitated from the medium used for cell attachment or culture. Cells attach to this precipitate but often do not spread or divide (121).

Biological Effects

Erhmann and Gey in 1956 (48) were the first to systematically compare growth of many cell strains and tissue explants on collagen substrates with growth on glass. They reported that collagen gels in many cases improved cell growth. Subsequently, it has been observed that collagen substrates alter the morphology (43, 235), migration (181), and adhesion (97, 113, 159) of cells and, in some cases, differentiation (124, 184). In fact, it appears that, for certain cells, growth factors may not be required if the cells are maintained on specific collagens (203) or on the extracellular matrix laid down by the cells (70).

Fibroblasts require a collagen substrate for migration (181) on the plastic filters used in the Boyden-chamber assay. Presumably, the fibroblasts must adhere to the filter in order to move. In addition, collagen α-chains and small collagenous peptides are reported to be chemotactic (180). Thus, collagen and degradation products of collagen may be attractants for fibroblasts in vivo during wound repair, fracture healing, and embryogenesis.

COLLAGEN AND CELL ADHESION

Recent studies suggest that specific glycoproteins bind cells to the substratum or to their collagenous matrix. There are a variety of collagen isotypes, and specific binding proteins have been described, while others probably exist. These proteins, of which fibronectin is the best described, are matrix and cell surface constituents. It has been demonstrated that certain cells utilizing these glycoproteins can adhere to specific collagens (Table III). For example, whereas fibroblasts adhere well to all collagens, chondrocytes bind preferentially to type II collagen and epithelial and endothelial cells to type IV collagen. The fibronectin-mediated attachment of fibroblasts to collagen substrates is the most studied. This adhesion is rapid (113), requires divalent cations (113, 115), is temperature dependent (114), and is inhibited by cytochalasin B (168). Fibronectin from one species functions in vitro with cells from other species.

Fibronectin

The "cold-insoluble globulin" (CIg) of blood, later identified as a form of fibronectin (198) (Table IV), was originally observed in 1948 in the precipitate that formed when blood was allowed to clot in the cold (150, 228). Although they were first thought to be part of the fibrinogen molecule (41), examination of the amino-acid sequences at the amino-termini of these proteins showed them to be distinct (178). Cold-insoluble globulin is a large molecule (~440,000 daltons) with two similar or identical chains linked by disulfide bonds (152). However, the chains separate slightly on electrophoresis (27) and could be modified forms of the same chain or different gene products. The protein contains about 6% carbohydrate linked through asparagine residues (25).

The presence of a protein closely related to CIg in connective tissues and on the surface of cells was not discovered until later. In 1973 and 1974, various studies indicated that one of the most abundant glycoproteins on the surface of cells with chains of ~200,000 daltons disappeared after transformation (59, 92, 98, 167, 191, 199, 229, 246). This glycoprotein has been known by a variety of synonyms including cell surface protein (CSP) (246) and "large, external, transformation-sensitive protein" (LETS) (99), but now is referred to as fibronectin (227).

The key role that a serum protein played in mediating the adhesion of trypsinized cells to collagen substrates was reported independently by Hauschka and White (86) and also by Klebe (113). Hauschka and White briefly reported that myoblasts require a serum protein to bind to collagen substrates, but those authors made no further studies. Klebe, while studying the attachment characteristics of established cells, found a serum requirement for fibroblast attachment to collagen substrates (113). Furthermore, he isolated the protein from serum, which he named "collagen-dependent cell attachment protein" (c-CAP). Subsequently, Pearlstein (164) demonstrated that CSP had the ability to promote cell attachment to collagen.

Opsonic proteins that facilitate the phagocytic activities of macrophages are known to be present in serum (3, 18, 94, 200, 241). These proteins, called anti-gelatin factor (241) and α2-SB-glycoprotein (18), were isolated from serum and subsequently shown by independent studies to be identical to serum fibronectin (38, 200). Fibronectin stimulates macrophages to engulf particles coated with gelatin. It also binds DNA (248) and actin (111), presumably also for opsonization.

The fibronectin in blood is not identical in all respects to that in extracellular matrices and on cell surfaces. The blood form is slightly smaller than the cell form (242). Furthermore, after reduction two bands are observed when serum fibronectin is electrophoresed in SDS, whereas a single diffuse band is observed under similar conditions with the cell-associated fibronectin. The serum form is fully soluble at neutral pH, whereas the material extracted from the cells remains in solution best at pH 11. The blood and cellular forms are equally active in mediating cell attachment at levels of 1–5 μg/ml. However, the cellular form is some 50 times more active in

restoring normal morphology to transformed cells (7, 242, 247). The molecular basis for these differences has not been defined but could arise from posttranslational modifications involving glycosylation reactions or proteolysis. Alternatively, the proteins could be genetically distinct molecules.

TABLE III

Adhesion of Cells to Collagen

Cell type	Preferred collagen substrate
Fibronectin-mediated	
Established cells lines (CHO, 3T3, etc.) (73, 122, 137, 168)*	I-IV
Primary fibroblasts (159)	I-IV
Rat embryonic periosteum (123)	I-IV
Rat hepatocytes (94)	I-IV
Osteosarcoma (123)	I-IV
Myoblasts (31, 112)	I-IV
PMT (highly metastatic pulmonary tumor cell) (158)	I-III
Embryonic sheep aortic smooth muscle cells‡ (67)	I-III
Chondronectin-mediated	
Chick sternal chondrocytes (89)	II
Laminin-mediated	
Breast epithelial cells (220)	IV
Guinea pig epidermal cells (220)	IV
EHS (basement membrane producing) tumor cells (220)	IV
TERA (transformed epidermal cells) (220)	IV
PAM 212 (transformed epidermal cells) (220)	IV
PMT (highly metastatic pulmonary tumor cell) (158, 220)	IV
Human choroid epithelial cells (220)	IV
PYS (parietal yolk sac cells) (220)	IV
Monkey pigmented epithelial cells (220)	IV
Sheep aorta vascular endothelial cells (220)	IV
Monkey lens epithelial cells (220)	IV
Bovine lens epithelial cells (220)	IV
Other adhesion mechanisms	
Platelets (14, 204)	Fibrillar collagen
Embryonic sheep smooth muscle cells‡	V

* Numbers in parentheses are references.
‡ Grotendorst, unpublished observations.

Current studies indicate that fibronectin is a highly asymmetric molecule in which the monomer has a Stokes radius of 110 Å (245). Physical data suggest that fibronectin contains several globular domains separated by more flexible segments (5, 233). Different fragments of the molecule have been isolated after protease treatment and found to have distinct binding sites as shown schematically in Fig. 2. A fragment of ~40,000 daltons near the amino-terminus contains the collagen-binding site, whereas another fragment of ~160,000 daltons binds to the cell surface (11, 12, 57, 66, 83, 145, 196, 210). The 160,000-dalton fragment does not bind to collagen but can promote cell adhesion to plastic, whereas the collagen-binding fragment (40,000 daltons) lacks cell-attachment activity (83, 84, 196). One report describes a 50,000-dalton peptide from fibronectin that can both bind to collagen and mediate cell adhesion (66). Thus, although these two regions on the fibronectin molecule are in distinct domains, they could be adjacent. Still another portion of the molecule including the amino-terminal region contains the site cross-linked by transglutaminase (156).

Fibronectin is quite antigenic and antibody to fibronectin has often been used to study its histological distribution (217, 234). Fibronectin is observed in the extracellular matrix around many cells (20, 30, 88, 136, 226, 234) both in regular association with collagen (20, 88, 226) and also as fibronectin filaments lacking any association with collagen (30). Fibronectin is observed on the surface of fibroblasts (197, 226, 246), astroglial cells (229), endothelial cells (102), certain epithelial cells (29), and myoblasts (55). The chondrocytes in cartilage lack the protein but begin to synthesize it when placed in culture (39).

Role of Circulating Fibronectin in Wound Repair

The multiple interactions and biological activities of plasma fibronectin are consistent with it having a role in wound repair. Fibronectin can bind and be cross-linked by transglutaminase (155, 156) to itself (153, 232), fibrin (74, 198, 216), and collagen (11, 12, 37, 46, 47, 66, 67, 78, 83, 84, 94, 103, 104, 106, 119, 120, 145, 164, 165, 196, 210, 242). It is likely that these interactions are essential for the formation of the fibrin clot. As discussed

TABLE IV

Synonyms for Fibronectin

Name	Property	References
Cold-insoluble globulin (CIg)	Coprecipitates with cryofibrinogen	150
Surface fibroblast antigen (SFA), renamed fibronectin	Present on the fibroblast cell surface and in serum and synthesized by fibroblasts	197, 199
Galactoprotein a (Gap a)	Glycoprotein labeled with galactose oxidase—[^{3}H]borohydride that is absent from transformed NIL cells	59
Large external transformation-sensitive protein (LETS)	Cell surface protein lost after in vitro transformation	98
Cell surface protein (CSP)	High molecular weight cell protein on the surface of chick embryo fibroblasts	246
L1 band	Absent from transformed cells	92
Zeta proteins	Absent from transformed chick embryo fibroblasts	191
Band 1	Absent from transformed BHK cells	167
Collagen-dependent cell attachment protein (c-CAP)	Serum glycoprotein that mediated cell adhesion to collagen	113
Anti-gelatin factor (AGF)	Serum protein required by macrophages to ingest gelatin-coated particles	94, 241
α_2-SB-opsonic glycoprotein	Serum protein that promoted phagocytic activity	18
Cell spreading factor (SF)	Serum fraction that promoted cell spreading	77

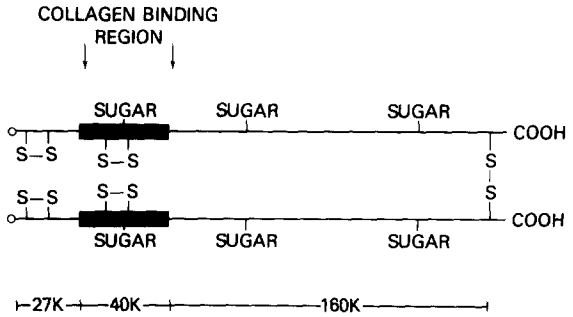

FIGURE 2 Schematic model of the fibronectin molecule including the location of specific domains. Peptides containing the collagen-binding site (*40K*), the transglutaminase cross-linking site (*27K*), and the cell-binding site (*160K*) are shown in their probable locations in the molecule (11, 57, 154, 210). Some or all of the sugars are in the 40,000-dalton region (154, 210). The position of the S-S bonds is from Wagner and Hynes (233).

below, fibronectin appears to be involved in some platelet reactions (10, 14, 204). As already mentioned, fibronectin has opsonic activity for macrophages (3, 18, 19, 94, 241), which could be important for debriding the wound. Fibronectin may also be one of the agents attracting fibroblasts into the wound, because it is highly chemotactic for fibroblasts (61). In addition, fibroblasts adhere to fibrin that has covalently bound fibronectin (74). Once in the wound area, fibronectin could help maintain the fibroblasts by promoting cell-matrix interactions and stimulating matrix production (53).

Role of Fibronectin in Cell Attachment to Collagen

Fibronectin promotes the attachment and subsequent spreading of many cells (31, 73, 75, 77, 84, 93, 100, 113, 123, 137, 158, 164). In these studies, freshly dissociated cells are incubated on a plastic or collagen substrate. Most cells will synthesize their own attachment protein with time but, in short incubations or when an inhibitor of protein synthesis is present, the attachment of many trypsinized fibroblasts is stimulated by serum and by fibronectin (76, 228). Under standard assay conditions, 50% attachment of Chinese hamster ovary cells is obtained with ~40 ng/cm^2 of fibronectin on a substrate containing 100 ng/cm^2 of collagen. The process is enhanced by higher levels of fibronectin but is saturable. Three steps in the process of fibronectin-mediated attachment of cells to collagen have been described (Fig. 3): (*a*) fibronectin binds to collagen, (*b*) cells bind to fibronectin-collagen complex, and (*c*) cell spreading requires fibronectin and cellular metabolism.

Fibronectin will bind to a variety of collagen types including types I–V (37, 46, 47, 103, 119, 123), but not to *Ascaris* collagen (123) or to various synthetic peptides (119). The affinity of fibronectin for denatured collagen is greater than for the native protein. For example, Jilek and Hörmann (103) found that native type I collagen bound 1/100 of the amount of fibronectin bound by denatured type I collagen at 4°C. Type III collagen was much more effective under these conditions, binding one-fourth as well as denatured type I collagen to fibronectin. It should be noted that the binding of fibronectin to native collagen occurred more slowly than to denatured collagen and that equilibrium was not reached at this temperature. Greater binding is found at physiological temperatures (106) and heparin has been shown to enhance this binding (104, 106). The fibrillar nature of the collagen is also an important factor in fibronectin binding, because the binding sites in the collagen molecules in the internal portions of fibers would be unavailable for reaction. Fibrillar type III collagen binds fibronectin more effectively than type I collagen does (47, 103), perhaps because type III fibers are thinner (129).

The fibronectin binding site on type I collagen comprises an unusual sequence of amino acids including the bond cleaved by mammalian collagenase (37, 120). Cleavage of the α1(I) chain by mammalian collagenase, between residues 775–776, is sufficient to destroy binding activity (120). A peptide of 35 amino-acid residues, which binds fibronectin and contains the mammalian collagenase cleavage site (120), has been isolated from the α1(I)-chain. A smaller synthetic peptide including a part of this region was also active (Fig. 4). The fibronectin-binding site, like the rest of the helical domain, contains glycine as every third residue, but lacks proline and hydroxyproline and is hydrophobic. This region is less stable as a triple helix than other segments of collagen (91). At physiological temperatures the helix may loosen, allowing fibronectin to bind. Homologous peptides with binding activity have been isolated from α2, α1(II), and α1(III) (37, 117) (Fig. 5). Each sequence contains the collagenase-sensitive bond, suggesting that the binding of fibronectin occurs in the same region of each collagen.

Various other components of the extracellular matrix may also participate in cell adhesion. Fibronectin binds to proteoglycans (172, 243), and heparan sulfate is present in the adhesion sites of cells (35). Fibronectin binds to both hyaluronic acid and heparan sulfate (104, 213, 215, 243), and the interaction of fibronectin with collagen appears to be stabilized by both hyaluronic acid and heparan sulfate (195, 243). It is possible that the relatively low binding of fibronectin to native collagen is attributable in part to the lack of an additional factor, such as a proteoglycan that promotes their interaction (104, 106).

Little is known about the nature of the cell surface component that interacts with fibronectin. Certain complex gangliosides with two or three sialic-acid residues have been shown to bind to fibronectin (117) and inhibit cell adhesion but not alter the binding of fibronectin to collagen (118). The activity resides in the carbohydrate portion of the glycolipid. Because gangliosides are cell surface molecules, it is possible that they or other glycoconjugates with a similar heteropolysaccharide are the cell surface receptors for fibronectin. A possible model for the interaction of fibronectin in an attachment site is depicted in Fig. 6.

The interaction between the cell surface and the fibronectin-collagen complex is being investigated by isolating and characterizing cell mutants that are defective in adhesion. Poorly adherent variants of Chinese hamster ovary cells have been

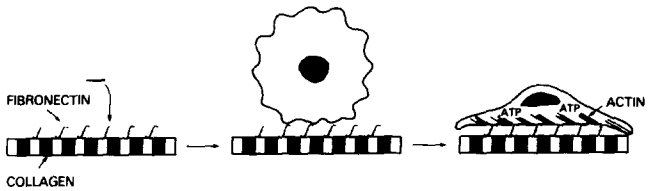

FIGURE 3 Schematic model of fibronectin-mediated cell adhesion to collagen in vitro. Fibronectin first binds to a specific sequence of amino acids on the collagen chain (113, 120, 165). Then the cells in the presence of Ca^{++} or Mg^{++} bind to the fibronectin-collagen complex (115) and spread.

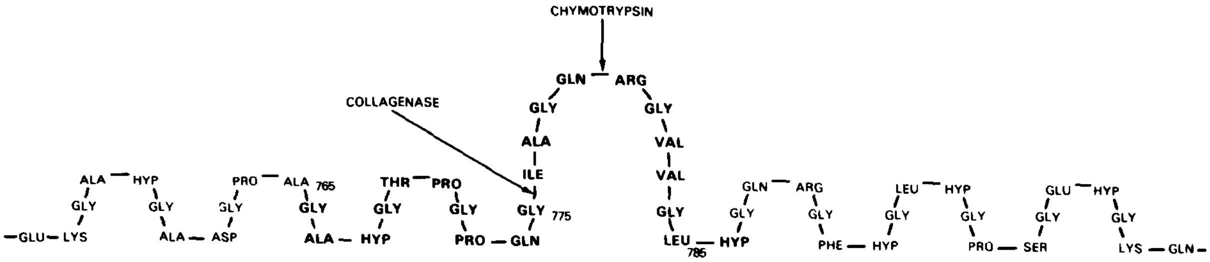

FIGURE 4 Amino acid sequence of the collagen $\alpha 1$(I)-chain known to bind fibronectin. The secondary structure of the active region (residues 774–785) is diagramatically depicted as being more open than the remainder of the molecule whose tight helical coil is depicted with regular curves. The fibronectin-binding site (residues 766–786) is shown in heavy letters.

isolated on the basis of their low adhesion to collagen (116). In addition, the mutant cells adhered poorly to glass and tissue culture plastic. Several adhesion-deficient cell strains have been isolated by other selection procedures (169, 182). One mutant has a defect in glycoprotein synthesis but reverts to a normal phenotype in the presence of added *N*-acetylglucosamine (182). A ricin-resistant fibroblast variant shows defective attachment but synthesizes biologically active fibronectin (169). Analysis of these mutants should help to clarify the interaction of the fibronectin-collagen complex with the cell surface.

Cell-Substrate Adhesion Not Involving Fibronectin

The attachment of baby hamster kidney (BHK) (78, 207) and BALB 3T3 (137) cells to denatured collagen is stimulated by added fibronectin, although some of these cells attach to native collagen without added fibronectin. This has led to the suggestion that different mechanisms are used to attach to the native and denatured protein (137). If this is so, it has not been defined and more likely explanations have not been excluded. For example, cells can utilize for attachment the fibronectin they themselves produce (76). In this case, it would appear that attachment to native collagen was more efficient with the newly synthesized fibronectin. More studies are needed in this area.

Clearly, fibronectin is not involved in the attachment of all cells (Table III). The matrix adjacent to epithelial cells and chondrocyte usually lacks fibronectin (39, 217). These cells abut on extracellular matrices that are quite distinct from fibrous connective tissues. As discussed below, additional glycoproteins, apparently cell specific, attach chondrocytes (89) and epithelial cells (159, 220) to their matrix.

Chondrocyte Attachment—Chondronectin

Cartilage does not contain fibronectin (39, 217), and purified fibronectin does not promote the adherence of freshly isolated chondrocytes, whereas fibronectin-free serum does (89). Chondronectin, the chondrocyte-attachment factor in serum, can be separated from fibronectin by DEAE-cellulose column chromatography. This protein is somewhat more heat-labile than fibronectin ($T_{1/2} = 52°C$ vs. $T_{1/2} = 57°C$) (Table V). As isolated from serum, chondronectin has a molecular weight of 180,000 daltons, and the molecule contains disulfide-linked chains (90). Chondronectin is secreted by cultured chondrocytes and can also be extracted from cartilage; it is presumed to be analogous in function to fibronectin, but specific for chondrocytes. Interestingly, once chondrocytes attach, fibronectin causes the cells to flatten and appear fibroblastic and, at the same time, the synthesis of cartilage-specific macromolecules is suppressed (170, 237).

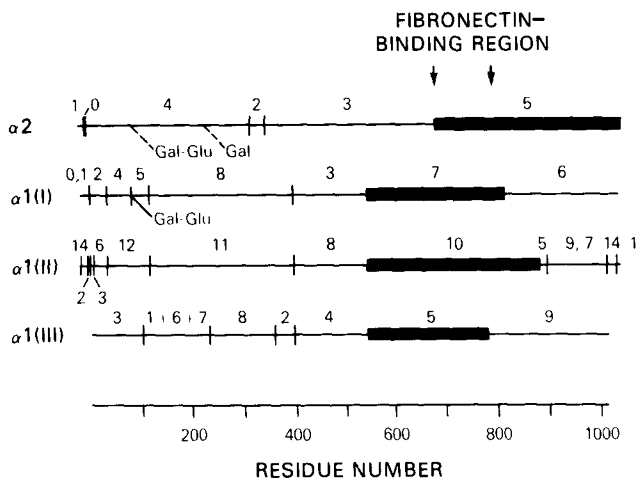

FIGURE 5 Activity of various cyanogen bromide-derived peptides of collagen α-chains in binding fibronectin. The numbers between the vertical lines indicate the cyanogen bromide peptide number. The major peptides active in binding fibronectin are shaded (■). Data on $\alpha 2$(I), $\alpha 1$(I), $\alpha 1$(II) are from Dessau et al. (37). Data on $\alpha 1$(I) and $\alpha 1$(II) are from Kleinman et al. (119). Data on $\alpha 1$(III) are from Kleinman and McGoodwin (unpublished observations).

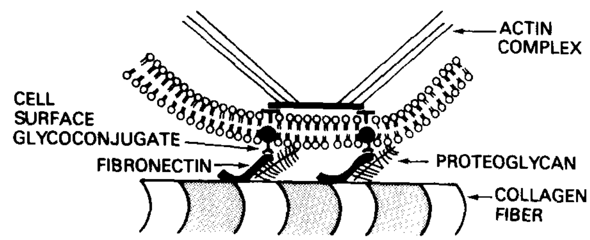

FIGURE 6 Schematic model of an adhesion site. Collagen fibers contain specific sites to which the fibronectin molecule binds. The fibronectin molecule contains a region that recognizes collagen and another region that recognizes the cell surface. The cell surface receptor is thought to be a glycoconjugate, possibly a glycolipid that interacts with cytoskeletal elements in some as yet unknown manner. Proteoglycans are also present and stabilize the interaction. The components of this model are not necessarily to scale.

Epithelial Cell Attachment—Laminin

Epithelial cells attach preferentially to type IV (basement membrane) collagen and do not attach well to plastic or to dishes coated with collagen types I, II, or III. The attachment of epidermal cells (159), lens epithelial cells (97), and breast epithelial cells (238) is slow and is not stimulated by serum or fibronectin. Laminin, an 800,000-dalton glycoprotein found in basement membranes (42, 52, 54, 132, 221), has been shown to be the attachment factor for these cells (220) (Tables III and

TABLE V

Characteristics of Adhesion Factors

	Fibronectin	Chondronectin	Laminin
Molecular weight	450,000 ↓ Reduction 220,000	180,000 ↓ Reduction 80,000	800,000 ↓ Reduction 200,000, 400,000
Activity in attachment	1–5 μg	5–50 ng	1–5 μg
Serum amount	200 μg/ml	Below 20 μg/ml	Below 1 μg/ml
Tissue location	Fibrous connective tissue, some basement membranes	Cartilage, vitreous body	Basement membrane
Heparin binding	Yes	Yes	Yes
Heat lability	57°C	52°C	49°C

V). In the presence of laminin (1–5 μg/ml), epithelial cells attach rapidly to type IV collagen. Laminin acts apparently in a manner analogous to that of fibronectin in that it first binds to the substrate, type IV collagen, and then the cells bind to the laminin–type IV collagen complex. Certain cells, such as regenerating liver cells[1] and metastatic cells from the T241 fibrosarcoma (158), attach via fibronectin to a type I collagen substrate and via laminin to a type IV collagen substrate. Because some cells are capable of synthesizing both proteins (53), it can be assumed that these cells utilize both mechanisms for adhesion. In the case of the metastatic cells, the ability to bind to different matrices may be important in their invasive behavior.

Platelet Adhesion

The cells in the blood are largely nonadherent. However, in culture, monocytes and neutrophils adhere to all collagen types and to bacteriological plastic (122). It has been suggested that fibronectin is the collagen receptor on the platelet cell surface (14). Platelets contain fibronectin (14, 152, 176), which is released during their aggregation by collagen or by thrombin (249); however, only small amounts are found on the cell surface (65). Antibody against fibronectin, however, only blocks adhesion by 20% and has no effect on aggregation (204). Furthermore, whereas fibronectin binds best to denatured collagen (37, 46, 47, 103, 119), platelets are aggregated by native and not by denatured collagen (60, 240). These observations suggest that fibronectin does not participate in the initial step in the platelet-collagen interaction. Once platelets adhere to collagen, exogenous fibronectin enhances their flattening (75, 100). Recent evidence of a role for fibronectin in platelet aggregation is suggested by studies on a patient with the Ehlers-Danlos syndrome with poor wound healing and abnormal platelet aggregation (10). This patient was found to have normal levels of fibronectin, using an immunological assay. However, only a slight aggregation of the patient's platelets occurred in plasma while the addition of fibronectin from a control subject corrected the defect. These data suggested that the fibronectin from the patient was not functional in platelet aggregation. Clearly, fibronectin is present in platelets and involved in platelet function, but its role as the collagen receptor is unlikely.

COLLAGEN AND CELL GROWTH

It is well established that normal cells must attach to a substrate in culture to survive and divide (211). Such cells deposit material on the surface of the dish as they adhere (34, 35, 133, 208). Under appropriate conditions, collagenous protein is identified in the substrate attachment matrix (34, 208). As expected, little collagenous protein is present when ascorbic acid levels in the medium are low (30), because ascorbic acid is necessary for maximal synthesis. Similarly, little collagen is present when transformed cells are used, because they make less collagen than normal cells (2, 71, 107, 135) or fail to convert procollagen to collagen (9). It is likely that the plastic or glass surface is adequate without collagen. Although it is not clear how glass and plastic surfaces interact with the cell membrane, more recent studies suggest that tissue culture plastic adsorbs fibronectin and other glycoproteins that the cells use for attachment (31, 73, 76, 77, 93). In this case, the plastic and glass would serve as a surrogate for the natural collagenous matrix.

Collagenous matrices, however, do alter the growth of normal and transformed cells in culture. Cells such as hepatocytes (205), corneal endothelial cells (69), breast epithelial cells (238), and epidermal cells (159) are maintained in a viable state longer on a collagenous substrate than on plastic. Similarly, a collagen substrate was found to prolong the lifespan of cultured chick fibroblasts (64). Growth-promoting effects have been noted in culture with ascorbic acid (193) and attributed to the ability of ascorbic acid to increase the production of a collagenous matrix (173). Dexamethasone (56) as well as butyrate (87) induce normalization of the morphology in transformed cells along with increasing the deposition of collagen (56) and fibronectin (56, 87) by the cell. Possibly the reduced adhesiveness of transformed cells and their failure to assume a normal parallel alignment could be attributed to the reduced production of matrix proteins.

Proline analogues that prevent the deposition of collagen have been used to determine the necessity for collagen synthesis during cell culture. Fibroblasts cultured in the presence of *cis*-hydroxyproline begin to round up within a few hours and eventually detach (108). However, *cis*-hydroxyproline has no effect on growth (139) when cells are plated on a collagen substratum (as low as 0.1 μg/cm^2). This indicates that the *cis*-hydroxyproline is not toxic to the cells but prevents the cells from producing a collagenous matrix required for growth. Using this analogue, it has been possible to determine the requirement that certain cells have for specific collagens. Breast epithelial cells will not grow on type I collagen in the presence of *cis*-hydroxyproline but will grow on type IV collagen (238). Thus, these cells specifically require a type IV collagen substrate for growth.

Cis-hydroxyproline inhibits the growth of chick embryo tendon fibroblasts in primary culture (108) and a variety of other cells, but does not affect the growth of several trans-

[1] Carlsson, R., E. Engvall, Y. Yoshida, A. Freeman, and E. Ruoslahti. Laminin and fibronectin in cell adhesion. Enhanced adhesion of cells from regenerating liver. Manuscript in preparation.

formed cell lines including KB cells, neuroblastoma cells, and other cells listed in Fig. 7. These data (Fig. 7) suggest that the tumorigenic cells that make only small amounts of collagen are not dependent on collagen for growth.

MATRIX PROTEINS AND CELL DIFFERENTIATION

Possible roles for collagen in differentiation have been assessed by growing cells on collagen substrates, by treating cells or tissues with bacterial collagenase, and by treating cells with drugs that interfere with collagen synthesis (Table VI).

Purified collagen substrates and collagenous matrices have been shown to maintain differentiated functions as well as to induce differentiation in cultured cells. One of the best-documented roles for collagen in differentiation is in the fusion of myoblasts into multinucleated myotubes (85, 124). Myoblasts are unable to form myotubes when plated in the absence of fibroblasts (125). However, in the presence of conditioned medium (124) or on collagen-coated dishes (85), they form myotubes. Both native and denatured collagen support differentiation, but only when the collagen is used as a substrate and not when it is added to the medium (86). All mammalian collagens tested are equally active in promoting muscle differentiation (112), and it was found that the α1(I)-CB7 peptide, the fibronectin-binding peptide (37, 119), contains the active region (86). A serum factor was shown to be necessary for the attachment of myoblasts to collagen (86), and this was subsequently shown to be fibronectin (31). It is interesting to note that as myoblasts fuse they lose their cell surface fibronectin (28, 55), and the addition of exogenous fibronectin delays fusion (177). Thus, myoblasts appear to require fibronectin for attachment, whereas loss of fibronectin from the cell surface is necessary for differentiation.

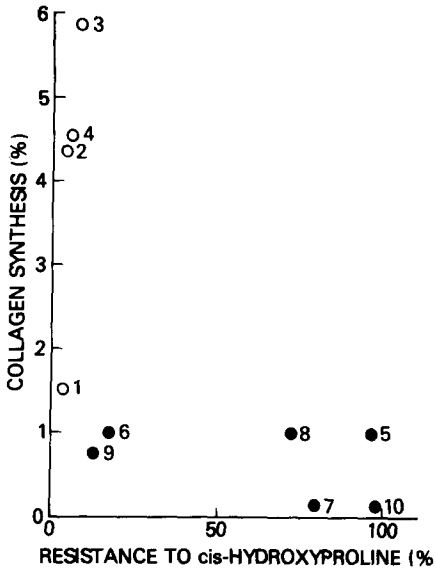

FIGURE 7 Effect of *cis*-hydroxyproline on the growth of normal and tumorigenic cells. Several normal (*1*, 3T3; *2*, WI38; and *3*, early and *4*, late passage adult connective tissue cells), and tumorigenic cells (*5*, spontaneously transformed mouse connective tissue cells; *6*, cells derived from the tumor produced by injection of the transformed mouse connective tissue cells; *7*, cells from tumor derived from skin of mice painted with methylcholanthrene; *8*, cells derived from the tumor arising as a result of subcutaneous injection of methylcholanthrene; *9*, SV-3T3 cells; and *10*, cells from a metastatic fibrosarcoma of the T241 fibrosarcoma) were tested for their ability to grow in the presence of 25 μg/ml *cis*-hydroxyproline for 5 d. ●, Tumorigenic; O, nontumorigenic. Values are expressed as the percentage of cells remaining after treatment. Data are from reference 230, with permission.

The differentiation of a variety of other cell types is enhanced by collagen, including somite chondrogenesis (126, 130), the induction of matrix formation by corneal epithelium (146), and the adhesion and development of some epidermal cells (159). Somite chondrogenesis is improved by type I procollagen and collagen, and type II collagen is even more effective (126, 130). It is possible that the type II collagen synthesized by the notochord induces chondrogenesis in somites in vivo. The production of the epithelial stroma of the cornea is enhanced by all collagens (40). Epidermal cells in culture often require feeder layers of mesenchymal cells (189) or the presence of collagen gels for growth (109). Type IV (basement membrane) collagen has been found to promote the attachment and the subsequent differentiation of epidermal cells into squamous epithelium (159).

One of the most impressive activities of a collagenous matrix is the induction of bone by devitalized and decalcified bone powder implanted in syngeneic hosts (186). This material induces an outgrowth of mesenchyme cells that differentiate into chondrocytes. Subsequently, blood vessels enter the area, and the cartilage is replaced by osteoblasts. Later, osteoclasts and marrow spaces containing hematopoietic cells form (186). The cells at each stage synthesize unique matrix proteins (187), and it is the matrix produced by the osteoblasts rather than the implanted bone powder that mineralizes. In culture, the bone powder also induces chondrocytes to develop from cells in minced muscle tissue (160). It is not clear how the bone powder, which is mostly collagen, acts. However, it has been suggested that the collagenous matrix contains a firmly attached "inducer" molecule (160).

Further evidence that collagen participates in differentiation has been obtained by use of bacterial collagenase or drugs that block collagen biosynthesis. Treatment with collagenase prevents the development of feathers (218), lung (236), and salivary epithelium (79) as well as the induction of notochord cartilage by spinal cord (162). It is possible that noncollagenous macromolecules are also involved, because the collagenases used may have contained other enzymes including proteases. Further, proteoglycans have been implicated as necessary for salivary epithelial morphogenesis (17). Several drugs that inhibit collagen formation, including proline analogues, have been shown to prevent lung morphogenesis (4, 214), salivary gland formation (214), and ameloblast differentiation (194).

Collagen is not the only matrix protein known to influence cell differentiation. For example, the phenotype of chondrocytes is altered by fibronectin. Although cartilage does not contain fibronectin, freshly isolated chondrocytes, when cultured at low cell density in the presence of serum, become flattened, synthesize fibronectin, and retain it on their cell surface (39). These effects are observed in cells grown on plastic substrates but, as expected, occur more rapidly in cells grown on collagen substrates (170). As cell density increases, chondrocytes become less flattened, synthesize increased amounts of proteoglycan, and no longer retain fibronectin on their cell surfaces. Chondrocytes grown in medium with serum depleted of fibronectin are less flattened, produce more proteoglycan, and synthesize and retain less fibronectin than cells grown with fibronectin (170, 237). The "dedifferentiation" of chondrocytes observed in long-term culture (206) could be attributed in part to the alterations in synthetic activities resulting from fibronectin-dependent changes in cell shape.

TABLE VI

Role of Matrix Proteins in Cell Differentiation

System	Differentiated product	Evidence for matrix protein requirement
Effect of pure collagen substrates		
Chick embryonic myoblast (124)*	Multinucleated myotube cells	Requires (*a*) conditioned medium or (*b*) collagen substrate and a serum component
Corneal epithelium (40)	Production of stroma	Requires (*a*) lens as a substratum or (*b*) collagen substrate
Chick embryonic somites (126, 130)	Cartilage formation	Requires collagen substrate, type II collagen most effective
Epidermal cells (159)	Multilayered squamous epithelium	Requires (*a*) feeder layer of cells, (*b*) crude collagen substrate, or (*c*) type IV collagen substrate
Effect of collagenous matrices		
Subcutaneous implant of decalcified bone (186)	Bone formation	Requires demineralized bone powders
Muscle mesenchymal cells (160)	Cartilage and bone	Requires (*a*) Demineralized bone powders or (*b*) freeze-thawed bone
Neural crest mesenchymal cells (161)	Cartilage formation	Requires extracellular matrix of retinal pigmented epithelium
Effect of enzymes or drugs		
Chick embryonic skin tracts (218)	Feather morphogenesis	Blocked by collagenase but not trypsin
Embryonic salivary rudiment epithelium (79, 214)	Branched salivary glands	Blocked by collagenase and by L-azetidine-2-carboxylic acid and α,α'-dipyridyl
Chick embryonic lung epithelium, chick or mouse embryonic ureteric bud (236)	Branched epithelium and maintenance of shape of ureteric bud	Blocked by exposure to collagenase but can be restored by adding back lung or kidney mesoderm
Embryonic lung or tooth mesenchyme (4, 214)	Lung morphogenesis or ameloblast differentiation	Blocked by proline analogue, L-azetidine-2-carboxylic acid
Effect of fibronectin		
Chick sternal chondrocytes (170, 237)	Cartilage formation	Inhibited by fibronectin, restored by its removal

* Numbers in parentheses are references.

CONCLUSIONS

At present there are five well-identified isotypes of collagen that vary in composition, fiber structure, and tissue distribution, suggesting that they play more than just a structural role. The collagen isotypes are known to have somewhat different compositions depending on the type or age of the tissue. Because collagens can interact with various attachment proteins and proteoglycans, it is likely that unique and complex matrices are formed that maintain the cells within the tissue and direct their growth and differentiation. Some examples of the affinity of cells for the different collagen isotypes are known, including chondrocytes that prefer type II collagen, and epidermal cells and breast epithelial cells that prefer type IV collagen.

Although we regard most cells as permanent residents of the tissues to which they belong because of their attachments to other cells and to the extracellular matrix, a variety of evidence suggests that tumorigenic and particularly metastatic cells behave differently. Many of the properties of these cells relate to their reduced adhesion to a matrix and reduced synthesis of fibronectin and collagen. Many tumorigenic cells synthesize less collagen and probably less fibronectin, but synthesize larger amounts of proteases including collagenases. As a result, the cells are less flattened in culture and less dependent on an extracellular matrix for growth. Such factors would also increase their invasiveness.

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REFERENCES

1. Adams, E., and L. Frank. 1980. Metabolism of proline and the hydroxyprolines. *Annu. Rev. Biochem.* 49:1005–1061.
2. Adams, S. L., M. E. Sobel, B. H. Howard, K. Olden, K. M. Yamada, B. DeCrombrugghe, and I. Pastan. 1977. Levels of translatable mRNAs for cell surface protein, collagen precursors, and two membrane proteins are altered in Rous sarcoma virus-transformed chick embryo fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* 74:3399–3403.
3. Adelmann, B. C. 1973. The structural basis of cell-mediated immunological reactions of collagen. Recognition by the cutaneous delayed hypersensitivity reaction in guinea-pigs of conformational alterations of rat and calf skin collagen. *Immunology.* 24:871–880.
4. Alescio, T. 1973. Effect of a proline analogue, azetidine-2-carboxylic acid, on the morphogenesis *in vitro* of mouse embryonic lung. *J. Embryol. Exp. Morphol.* 29:439–451.
5. Alexander, S. S., G. Colonna, K. M. Yamada, I. Pastan, and H. Edelhock. 1978. Molecular properties of a major cell surface protein from chick embryo fibroblasts. *J. Biol. Chem.* 253:5820–5824.
6. Ali, I. U., and R. O. Hynes. 1978. Effect of LETS glycoprotein on cell motility. *Cell.* 14: 439–446.
7. Ali, I. U., V. M. Mautner, R. Lanza, and R. O. Hynes. 1977. Restoration of normal morphology, adhesion and cytoskeleton in transformed cells by addition of a transformation-sensitive surface protein. *Cell.* 11:115–126.
8. Alitalo, K., M. Kurkinen, A. Vaheri, T. Krieg, and R. Timpl. 1980. Extracellular matrix components synthesized by human amniotic epithelial cells in culture. *Cell.* 19:1053–1062.
9. Arbogast, B. W., M. Yoshimura, N. Kefalides, H. Holtzer, and A. Koji. 1977. Failure of cultured chick embryo fibroblasts to incorporate collagen into their extracellular matrix when transformed by Rous sarcoma virus. *J. Biol. Chem.* 252:8863–8868.
10. Arneson, M. A., D. E. Hammerschmidt, L. T. Furcht, and R. A. King. 1980. A new form of Ehlers-Danlos Syndrome. Fibronectin corrects defective platelet function. *JAMA (J. Am. Med. Assoc.).* 244:144–147.
11. Balian, G., E. M. Glick, and P. Bornstein. 1980. Location of a collagen binding domain in fibronectin. *J. Biol. Chem.* 255:3234–3236.
12. Balian, G., E. M. Glick, E. Crouch, J. M. Davidson, and P. Bornstein. 1979. Isolation of a collagen-binding fragment from fibronectin and cold-insoluble globulin. *J. Biol. Chem.* 254:1429–1432.
13. Baum, B., J. A. McDonald, and R. G. Crystal. 1977. Metabolic fate of the major cell surface protein of normal human fibroblasts. *Biochem. Biophys. Res. Commun.* 79:8–15.
14. Bensusan, H. B., T. L. Kah, K. G. Henry, B. A. Murray, and L. A. Culp. 1978. Evidence that fibronectin is the collagen receptor on platelet membranes. *Proc. Natl. Acad. Sci. U. S. A.* 75:5864–5868.

15. Bentz, H., H. P. Bächinger, R. Glanville, and K. Kühn. 1978. Physical evidence for the assembly of A and B chains of human placental collagen in a single triple helix. *Eur. J. Biochem.* 92:563–567.
16. Benya, P. D., S. R. Padilla, and M. E. Nimni. 1978. Independent regulation of collagen types by chondrocytes during loss of differentiated function in culture. *Cell.* 15:1313–1321.
17. Bernfield, M. R., S. D. Banerjee, and R. H. Cohn. 1972. Dependence of salivary epithelial morphology and branching morphogenesis upon acid mucopolysaccharide (proteoglycan) at the epithelial surface. *J. Cell Biol.* 52:674–689.
18. Blumenstock, F. A., J. M. Saba, P. Weber, and E. Cho. 1976. Purification and biochemical characterization of a macrophage stimulating alpha-2-globulin opsonic protein. *Res. J. Reticuloendothel. Soc.* 19:157–172.
19. Blumenstock, F. A., J. M. Saba, P. Weber, and R. Laffin. 1978. Biochemical and immunological characterization of human opsonic α_2SB glycoprotein: its identity with cold-insoluble globulin. *J. Biol. Chem.* 253:4287–4291.
20. Bornstein, P., and J. F. Ash. 1977. Cell surface-associated structural proteins in connective tissue cells. *Proc. Natl. Acad. Sci. U. S. A.* 74:2480–2484.
21. Bornstein, P., and P. H. Byers. 1980. Disorders of collagen metabolism. *In* Metabolic Control and Diseases. 8th ed. P. K. Bondy and L. E. Rosenberg, editors. W. B. Saunders Company, Philadelphia. 1089–1153.
22. Bornstein, P., and H. Sage. 1980. Structurally distinct collagen types. Annu. Rev. Biochem. 49:957–1003.
23. Bornstein, P., and W. Traub. 1979. The chemistry and biology of collagen. *In* The Proteins. Vol. 4. 3rd ed. H. Neurath and R. Hill, editors. Academic Press, Inc., New York. 411–632.
24. Burgeson, R. E., F. A. El Adli, I. I. Kaitela, and D. W. Hollister. 1976. Fetal membrane collagens: identification of two new collagen alpha chains. *Proc. Natl. Acad. Sci. U. S. A.* 73:2579–2583.
25. Carter, W. G., and S. Hakomori. 1979. Carbohydrate structure of galactoprotein a, a major transformation sensitive glycoprotein released from hamster embryo fibroblasts. *Biochemistry.* 18:730–735.
26. Chatterjee, G. C. 1969. Effects of ascorbic acid deficiency in animals. *In* The Vitamins. Vol. 1. W. H. Sebrell and R. S. Harris, editors. Academic Press, Inc., New York. 407–457.
27. Chen, A. B., D. L. Armani, and M. W. Mosesson. 1977. Heterogeneity of the cold-insoluble globulin of human plasma (CIg), a circulating cell surface protein. *Biochim. Biophys. Acta.* 493:310–322.
28. Chen, L. B. 1977. Alterations in cell surface LETS protein during myogenesis. *Cell.* 10: 393–400.
29. Chen, L. B., N. Maitland, P. H. Gallimore, and J. K. McDougall. 1977. Detection of large external transformation-sensitive protein on some epithelial cells. *Exp. Cell Res.* 106:39–46.
30. Chen, L. B., A. Murray, R. A. Figal, A. Bushnell, and M. L. Walsh. 1978. Studies on intercellular LETS glycoprotein matrices. *Cell.* 14:377–391.
31. Chicquet, M., E. C. Puri, and D. C. Turner. 1979. Fibronectin mediates attachment of chicken myoblasts to a gelatin-coated substratum. *J. Biol. Chem.* 254:5475–5482.
32. Chung, E., R. K. Rhodes, and E. J. Miller. 1976. Isolation of three collagenous components of probable basement membrane origin from several tissues. *Biochem. Biophys. Res. Commun.* 71:1167–1174.
33. Crouch, E., and P. Bornstein. 1978. Collagen synthesis by human amniotic fluid cells in culture: characterization of a procollagen with three identical pro α1(I) chains. *Biochemistry.* 17:5499–5509.
34. Culp, L. A., and H. Bensusan. 1978. Search for collagen in substrate adhesion site of two murine cell lines. *Nature (Lond.).* 273:680–682.
35. Culp, L. A., B. J. Rollins, J. Buniel, and S. Hitri. 1978. Two functionally distinct pools of glycosaminoglycans in the substrate adhesion site of murine cells. *J. Cell Biol.* 79:788–801.
36. Dehm, P., and D. J. Prockop. 1971. Synthesis and extraction of collagen by freshly-isolated cells from chick embryo tendon. *Biochim. Biophys. Acta.* 264:375–382.
37. Dessau, W., B. C. Adelmann, R. Timpl, and G. R. Martin. 1978. Identification of the sites in collagen α-chains that bind serum anti-gelatin factor (cold insoluble globulin). *Biochem. J.* 169:55–59.
38. Dessau, W., F. Jilek, B. C. Adelmann, and H. Hörmann. 1978. Similarity of antigelatin factor and cold insoluble globulin. *Biochim. Biophys. Acta.* 533:227–237.
39. Dessau, W., J. Sasse, R. Timpl, F. Jilek, and K. von der Mark. 1978. Synthesis and extracellular deposition of fibronectin in chondrocyte cultures. *J. Cell Biol.* 79:342–355.
40. Dodson, J. W., and E. D. Hay. 1974. Secretion of collagen by corneal epithelium. Effect of the underlying substratum on secretion and polymerization of epithelial products. *J. Exp. Zool.* 189:51–72.
41. Edsall, J. T., G. A. Gilbert, and H. A. Scheraga. 1955. The nonclotting component of the human plasma fraction 1-1 ("cold insoluble globulin"). *J. Am. Chem. Soc.* 77:157–161.
42. Ekblom, P., K. Alitalo, A. Vaheri, R. Timpl, and L. Saxén. 1980. Induction of a basement membrane glycoprotein in embryonic kidney: possible role of laminin in morphogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 77:485–489.
43. Elsdale, T., and J. Bard. 1972. Cellular interaction in mass cultures of human diploid fibroblasts. *Nature (Lond.).* 236:152–155.
44. Elsdale, T., and J. Bard. 1972. Collagen substrate for studies on cell behavior. *J. Cell Biol.* 54:626–637.
45. Emerman, J. T., S. J. Burwen, and D. R. Pitelka. 1979. Substrate properties influencing ultrastructural differentiation of mammary epithelial cells in tissue culture. *Cell.* 11:109–119.
46. Engvall, E., and E. Ruoslahti. 1977. Binding of soluble form of fibroblast surface protein, fibronectin, to collagen. *Int. J. Cancer.* 20:1–5.
47. Engvall, E., E. Ruoslahti, and E. J. Miller. 1978. Affinity of fibronectin to collagen of different genetic types and to fibrinogen. *J. Exp. Med.* 147:1584–1595.
48. Erhmann, R. L., and G. O. Gey. 1956. The growth of cells on a transparent gel of reconstituted rat tail collagen. *J. Natl. Cancer Inst.* 16:1375–1403.
49. Eyre, D. R. 1980. Collagen: molecular diversity in the body's protein. *Science (Wash. D. C.).* 207:1315–1322.
50. Fessler, J. H., and L. I. Fessler. 1978. Biosynthesis of procollagen. *Annu. Rev. Biochem.* 47:129–142.
51. Fietzek, P. P., and K. Kühn. 1976. The primary structure of collagen. *Int. Rev. Connect. Tissue Res.* 7:1–60.
52. Foidart, J. M., E. W. Bere, M. Yaar, S. I. Rennard, M. Gullino, G. R. Martin, and S. I. Katz. 1980. Distribution and immunoelectron microscopic localization of laminin, a noncollagenous basement membrane glycoprotein. *Lab. Invest.* 42:336–342.
53. Foidart, J. M., J. J. Berman, L. Paglia, S. I. Rennard, S. Abe, A. Perantoni, and G. R. Martin. 1980. Synthesis of fibronectin, laminin, and several collagens by a liver-derived epithelial cell line. *Lab. Invest.* 42:525–532.
54. Foidart, J. M., and A. H. Reddi. 1980. Immunofluorescent localization of type IV collagen and laminin during endochondral bone differentiation and regulation by pituitary growth hormone. *Dev. Biol.* 75:130–136.
55. Furcht, L. T., D. F. Mosher, and G. Wendelschafer-Craab. 1978. Immunocytochemical localization of fibronectin (LETS proteins) on the surface of L_6 myoblasts: light and electron-microscopic studies. *Cell.* 13:263–271.
56. Furcht, L. T., D. F. Mosher, G. Wendelschafer-Craab, and J. M. Foidart. 1979. Reversal by glucocorticoid hormones by the loss of a fibronectin and procollagen matrix around transformed human cells. *Cancer Res.* 39:2077–2083.
57. Furie, M. B., and D. B. Rifkin. 1980. Proteolytically derived fragments of human plasma fibronectin and their location within the intact molecule. *J. Biol. Chem.* 255:3134–3140.
58. Gallop, P. M., O. O. Blumenfield, and S. Seifter. 1972. Structure and metabolism of connective tissue proteins. *Annu. Rev. Biochem.* 41:617–672.
59. Gahmberg, C. G., and S. Hakomori. 1973. Altered behavior of malignant cells associated with changes in externally labeled glycoprotein and glycolipid. *Proc. Natl. Acad. Sci. U. S. A.* 70:3329–3333.
60. Gastpar, H., K. Kühn, and R. Marx. 1976. Collagen-platelet interaction. In Proceedings of the First Munich Symposium on Biology of Connective Tissue. F. K. Schattauer Verlag, Stuttgart.
61. Gauss-Müller, V., H. K. Kleinman, G. R. Martin, and E. Schiffmann. 1980. Role of attachment and attractants in fibroblast chemotaxis. *J. Lab. Clin. Med.* In press.
62. Gay, S., G. R. Martin, P. K. Müller, R. Timpl, and K. Kühn. 1976. Simultaneous synthesis of types I and III collagen by fibroblasts in culture. *Proc. Natl. Acad. Sci. U. S. A.* 73:4037–4040.
63. Gay, S., and E. J. Miller. 1978. Collagen in the Physiology and Pathology of Connective Tissue. Gustav-Fischer Verlag, Stuttgart.
64. Gey, G. O., M. Svotelis, M. Foard, and F. B. Bang. 1974. Long-term growth of chicken fibroblasts on a collagen substrate. *Exp. Cell Res.* 84:63–71.
65. Ginsberg, M. H., R. G. Painter, J. Forsyth, C. Birdwell, and E. F. Plow. 1980. Thrombin increases expression of fibronectin on the platelet surface. *Proc. Natl. Acad. Sci. U. S. A.* 77:1049–1053.
66. Gold, L. I., A. Garcia-Pardo, B. Frangione, E. C. Franklin, and E. Pearlstein. 1979. Subtilisin and cyanogen bromide cleavage products that retain gelatin-binding activity. *Proc. Natl. Acad. Sci. U. S. A.* 76:4803–4807.
67. Gold, L. I., and E. Pearlstein. 1980. Fibronectin-collagen binding and requirement during cellular adhesion. *Biochem. J.* 186:551–554.
68. Goldberg, B., and H. Green. 1969. Relationship between collagen synthesis and collagen proline hydroxylase activity in mammalian cells. *Nature (Lond.).* 221:267–268.
69. Gospodarowicz, D., G. Greenburg, and C. R. Birdwell. 1978. Determination of cellular shape by the extracellular matrix and its correlation with the control of cellular growth. *Cancer Res.* 38:4155–4171.
70. Gospodarowicz, D., and C. R. Ill. 1980. Do plasma and serum have different abilities to promote cell growth? *Proc. Natl. Acad. Sci. U. S. A.* 77:2726–2730.
71. Green, H., G. Todaro, and B. Goldberg. 1966. Collagen synthesis in fibroblasts transformed by oncogenic viruses. *Nature (Lond.).* 209:916–917.
72. Green, H., B. Goldberg, and G. J. Todaro. 1966. Differentiated cell types and the regulation of collagen synthesis. *Nature (Lond.).* 212:631–633.
73. Grinnell, F. 1978. Cellular adhesiveness and extracellular substrata. *Int. Rev. Cytol.* 53: 65–144.
74. Grinnell, F., M. Feld, and D. Minter. 1980. Fibroblast adhesion to fibrinogen and fibrin substrates: requirement for cold-insoluble globulin (plasma fibronectin). *Cell.* 19:517–525.
75. Grinnell, F., M. Feld, and W. Snell. 1979. The influence of cold insoluble globulin on platelet morphological response to substrata. *Cell Biology International Reports.* 3:585–591.
76. Grinnell, F., and M. K. Field. 1979. Initial adhesion of human fibroblasts in serum-free medium: possible role of secreted fibronectin. *Cell.* 17:117–128.
77. Grinnell, F., D. G. Harp, and D. Minter. 1977. Cell adhesion and spreading factor. *Exp. Cell Res.* 110:175–190.
78. Grinnell, F., and D. Minter. 1978. Attachment and spreading of baby hamster kidney cells to collagen substrata: effects of cold-insoluble globulin. *Proc. Natl. Acad. Sci. U. S. A.* 75:4408–4412.
79. Grobstein, C., and J. Cohen. 1965. Collagenase: effect on the morphogenesis of embryonic salivary epithelium *in vitro*. *Science (Wash. D. C.).* 150:626–628.
80. Gross, J. 1974. Collagen biology: structure, degradation and disease. *Harvey Lect.* 68: 351–432.
81. Gross, J., and D. Kirk. 1958. The heat precipitation of collagen from neutral salt solutions: some rate regulating factors. *J. Biol. Chem.* 233:355–360.
82. Gross, J., and Y. Nagai. 1965. Specific degradation of the collagen molecule by tadpole collagenolytic enzymes. *Proc. Natl. Acad. Sci. U. S. A.* 54:1197–1201.
83. Hahn, L. E., and K. M. Yamada. 1979. Identification and isolation of a collagen-binding fragment of the adhesion glycoprotein fibronectin. *Proc. Natl. Acad. Sci. U. S. A.* 76: 1160–1163.
84. Hahn, L. E., and K. M. Yamada. 1979. Isolation and biological characterization of active fragments of the adhesive glycoprotein fibronectin. *Cell.* 18:1043–1051.
85. Hauschka, S. D., and I. R. Konigsberg. 1966. The influence of collagen on the development of muscle colonies. *Proc. Natl. Acad. Sci. U. S. A.* 55:119–126.
86. Hauschka, S. D., and N. K. White. 1972. Studies of myogenesis *in vitro*. *In* Research in Muscle Development and the Muscle Spindle. B. Q. Banker, R. J. Przybylski, J. P. van der Meulen, and M. Victor, editors. Excerpta Medica, Amsterdam. 53–71.
87. Hayman, E. G., E. Engvall, and E. Ruoslahti. 1980. Butyrate restores fibronectin at cell surface of transformed cells. *Exp. Cell Res.* 127:478–481.
88. Hedman, K., H. Kurkinen, K. Alitalo, A. Vaheri, S. Johansson, and M. Höök. 1979. Isolation of the pericellular matrix of human fibroblast cultures. *J. Cell Biol.* 81:83–91.
89. Hewitt, A. T., H. K. Kleinman, J. P. Pennypacker, and G. R. Martin. 1980. Identification of an adhesion factor for chondrocytes. *Proc. Natl. Acad. Sci. U. S. A.* 77:385–388.
90. Hewitt, A. T., H. H. Varner, and G. R. Martin. Isolation of chondonectin. *In* Immunochemistry of Collagen. Vol. I: Methods. H. Furthmayr, editor. CRC Press, Boca Raton, Florida. In press.
91. Highberger, J. H., C. Corbett, and J. Gross. 1979. Isolation and characterization of a peptide containing the site of cleavage of the chick skin collagen α1(I) chain by animal collagenases. *Biochem. Biophys. Res. Commun.* 89:202–208.
92. Hogg, N. M. 1974. A comparison of membrane proteins of normal and transformed cells by lactoperoxidase labeling. *Proc. Natl. Acad. Sci. U. S. A.* 71:489–492.
93. Höök, M., K. Rubin, A. Oldberg, B. Öbrink, and A. Vaheri. 1977. Cold-insoluble globulin mediates the adhesion of rat liver cells to plastic petri dishes. *Biochem. Biophys. Res. Commun.* 79:726–731.

94. Hopper, K. E., B. C. Adelmann, G. Gentner, and S. Gay. 1976. Recognition by guinea-pig peritoneal exudate cells of conformationally different states of the collagen molecule. *Immunology.* 30:249–259.
95. Horwitz, A. L., A. J. Hance, and R. G. Crystal. 1977. Granulocyte collagenase: Selective degradation of type I relative to type III collagen. *Proc. Natl. Acad. Sci. U. S. A.* 74:897–901.
96. Howard, B. V., E. J. Macarak, D. Gunson, and N. A. Kefalides. 1976. Characterization of the collagen synthesized by endothelial cells in culture. *Proc. Natl. Acad. Sci. U. S. A.* 73:2361–2364.
97. Hughes, R. C., G. Mills, Y. Courtois, and J. Tassin. 1979. Role of fibronectin in the adhesiveness of bovine lens epithelial cells. *Biol. Cell.* 36:321–330.
98. Hynes, R. O. 1973. Alteration of cell-surface proteins by viral transformation and by proteolysis. *Proc. Natl. Acad. Sci. U. S. A.* 70:3170–3173.
99. Hynes, R. O. 1976. Cell surface protein and malignant transformation. *Biochim. Biophys. Acta.* 458:73–107.
100. Hynes, R. O., I. U. Ali, A. T. Destree, V. M. Mautner, M. E. Perkins, D. R. Senger, D. D. Wagner, and K. K. Smith. 1978. A large glycoprotein lost from the surfaces of transformed cells. *Ann. N. Y. Acad. Sci.* 312:317–342.
101. Jaffee, E. A., C. R. Minick, B. Adelmann, C. G. Becker, and R. Nachman. 1976. Synthesis of basement membrane collagen by cultured human endothelial cells. *J. Exp. Med.* 144: 209–225.
102. Jaffee, E. A., and D. F. Mosher. 1978. Synthesis of fibronectin by cultured human endothelial cells. *J. Exp. Med.* 147:1779–1791.
103. Jilek, F., and H. Hörmann. 1978. Cold-insoluble globulin (fibronectin). IV. Affinity to soluble collagen of various types. *Hoppe-Seyler's Z. Physiol. Chem.* 359:247–250.
104. Jilek, F., and H. Hörmann. 1979. Fibronectin (cold-insoluble globulin). VI. Influence of heparin and hyaluronic acid on the binding of native collagen. *Hoppe-Seyler's Z. Physiol. Chem.* 360:597–603.
105. Jimenez, S. A., R. I. Bashey, M. Binditt, and R. Yankowski. 1977. Identification of collagen α1(I) trimer in embryonic chick tendons and calvaria. *Biochem. Biophys. Res. Commun.* 78:1354–1361.
106. Johansson, S., and M. Höök. 1980. Heparin enhances the binding of fibronectin to collagen. *Biochem. J.* 187:521–524.
107. Kamine, J., and H. Rubin. 1977. Coordinate control of collagen synthesis and cell growth in chick embryo fibroblasts and the effect of viral transformation on collagen synthesis. *J. Cell. Physiol.* 92:1–12.
108. Kao, W.-Y., and D. J. Prockop. 1977. Proline analogue removes fibroblasts from cultures with mixed cell populations. *Nature (Lond.).* 266:63–64.
109. Karasek, M. A., and M. E. Charlton. 1971. Growth of post-embryonic skin epithelial cells on collagen gels. *J. Invest. Dermatol.* 51:247–252.
110. Kefalides, N. A. 1978. Biology and Chemistry of Basement Membranes. Academic Press, Inc., New York.
111. Keski-Oja, J., A. Sen, and G. J. Todaro. 1980. Direct association of fibronectin and actin molecules in vitro. *J. Cell Biol.* 85:527–533.
112. Ketley, J. N., R. W. Orkin, and G. R. Martin. 1976. Collagen in developing chick muscle *in vivo* and *in vitro*. *Exp. Cell Res.* 99:261–268.
113. Klebe, R. J. 1974. Isolation of a collagen-dependent cell attachment factor. *Nature (Lond.).* 250:248–251.
114. Klebe, R. J. 1975. Cell attachment to collagen: the requirement for energy. *J. Cell. Physiol.* 86:231–237.
115. Klebe, R. J., J. R. Hall, P. Rosenberger, and W. D. Dickey. 1977. Cell attachment to collagen: the ionic requirements. *Exp. Cell. Res.* 110:419–425.
116. Klebe, R. J., P. G. Rosenberger, S. L. Naylor, R. L. Burns, R. Novak, and H. K. Kleinman. 1977. Cell attachment to collagen. Isolation of a cell attachment mutant. *Exp. Cell Res.* 104:119–125.
117. Kleinman, H. K., A. T. Hewitt, J. C. Murray, L. A. Liotta, S. I. Rennard, J. P. Pennypacker, E. B. McGoodwin, G. R. Martin, and P. H. Fishman. 1979. Cellular and metabolic specificity in the interaction of adhesion proteins with collagen and with cells. *J. Supramol. Struct.* 11:69–78.
118. Kleinman, H. K., G. R. Martin, and P. H. Fishman. 1979. Ganglioside inhibition of fibronectin-mediated cell adhesion to collagen. *Proc. Natl. Acad. Sci. U. S. A.* 76:3367–3371.
119. Kleinman, H. K., E. B. McGoodwin, and R. J. Klebe. 1976. Localization of the cell attachment region in types I and II collagen. *Biochem. Biophys. Res. Commun.* 72:426–432.
120. Kleinman, H. K., E. B. McGoodwin, G. R. Martin, R. J. Klebe, P. P. Fietzek, and D. E. Woolley. 1978. Localization of the binding site for cell attachment in the α1(I) chain of collagen. *J. Biol. Chem.* 253:5642–5646.
121. Kleinman, H. K., E. B. McGoodwin, S. I. Rennard, and G. R. Martin. 1979. Preparation of collagen substrate for cell attachment. Effect of collagen concentration and phosphate buffer. *Anal. Biochem.* 94:308–312.
122. Kleinman, H. K., J. C. Murray, E. B. McGoodwin, and G. R. Martin. 1978. Connective tissue structure: cell binding to collagen. *J. Invest. Dermatol.* 71:9–11.
123. Kleinman, H. K., J. C. Murray, E. B. McGoodwin, G. R. Martin, and I. Binderman. 1978. Attachment of bone cells to collagen. *Calcif. Tissue Abstracts.* (special suppl) 61–70.
124. Konigsberg, I. R. 1970. The relationship of collagen to the clonal development of embryonic skeletal muscle. *In* Chemistry and Molecular Biology of the Intracellular Matrix. E. A. Balazs, editor. Academic Press, Inc., New York. 3:1799–1809.
125. Konigsberg, I. 1971. Diffusion-mediated control of myoblast fusion. *Dev. Biol.* 26:133–152.
126. Kosher, R. A., and R. L. Church. 1975. Stimulation of *in vitro* chondrogenesis by procollagen and collagen. *Nature (Lond.).* 258:327–330.
127. Langness, U., and S. Udenfriend. 1974. Collagen biosynthesis in non-fibroblastic cell lines. *Proc. Natl. Acad. Sci. U. S. A.* 71:50–51.
128. Lapière, C. M., A. Lenaers, and L. D. Kohn. 1971. Procollagen peptidase: an enzyme excising the coordination peptides of procollagen. *Proc. Natl. Acad. Sci. U. S. A.* 68: 3054–3058.
129. Lapière, C. M., B. Nusgens, and G. E. Pierard. 1977. Interaction between collagen type I and type III in conditioning bundle organization. *Conn. Tiss. Res.* 5:21–29.
130. Lash, J. W., and N. S. Vasan. 1978. Somite chondrogenesis *in vitro*. Stimulation by exogenous extracellular matrix components. *Dev. Biol.* 66:151–171.
131. Layman, D. L., E. B. McGoodwin, and G. R. Martin. 1971. The nature of the collagen synthesized by cultured human fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* 68:454–458.
132. Leivo, I., A. Vaheri, R. Timpl, and J. Wartiovaara. 1980. Appearance and distribution of collagens and laminin in the early mouse embryo. *Dev. Biol.* 79:100–114.
133. Lembach, K. J., R. E. Branson, P. B. Hengley, and L. W. Cunningham. 1977. The synthesis of macromolecular 3-hydroxyproline by attaching and confluent cultures of human fibroblasts. *Eur. J. Biochem.* 72:379–383.
134. Levene, C. I., C. J. Bates, and A. J. Bailey. 1972. Biosynthesis of collagen cross-links in cultured 3T6 fibroblasts; effect of lathrogens and ascorbic acid. *Biochim. Biophys. Acta.* 263:574–578.
135. Levinson, W., R. S. Bhatnagar, and T.-Z. Liu. 1975. Loss of ability to synthesize collagen in fibroblasts transformed by Rous sarcoma virus. *J. Natl. Cancer Inst.* 55:807–810.
136. Linder, E., A. Vaheri, E. Ruoslahti, and J. Wartiovaara. 1975. Distribution of fibroblast surface antigen in the developing chick embryo. *J. Exp. Med.* 142:41–49.
137. Linsenmayer, T.F., E. Gibney, B. Toole, and J. Gross. 1978. Cellular adhesion to collagen. *Exp. Cell Res.* 116:470–474.
138. Liotta, L. A., S. Abe, P. G. Robey, and G. R. Martin. 1979. Preferential digestion of basement membrane collagen by an enzyme derived from a metastatic murine tumor. *Proc. Natl. Acad. Sci. U. S. A.* 76:2268–2272.
139. Liotta, L. A., D. Vembu, H. K. Kleinman, G. R. Martin, and C. Boone. 1978. Collagen required for proliferation of cultured connective tissue cells but not their transformed counterparts. *Nature (Lond.).* 272:622–624.
140. Macarak, E. J., E. Kirby, T. Kirk, and N. A. Kefalides. 1978. Synthesis of cold-insoluble globulin by cultured calf endothelial cells. *Proc. Natl. Acad. U. S. A.* 75:2621–2625.
141. Martin, G. R., P. H. Byers, and K. A. Piez. 1975. Procollagen. *Adv. Enzymol.* 42:167–191.
142. Mayne, R., M. S. Vail, and E. J. Miller. 1975. Analysis of changes in collagen biosynthesis that occur when chick chondrocytes are grown in 5-bromo-2′-deoxyuridine. *Proc. Natl. Acad. Sci. U. S. A.* 72:4511–4515.
143. Mayne, R., M. S. Vail, and E. J. Miller. 1978. Characterization of the collagen chains synthesized by cultured smooth muscle cells derived from rhesus monkey thoracic aorta. *Biochemistry.* 17:446–452.
144. Mayne, R., M. S. Vail, P. M. Mayne, and E. J. Miller. 1976. Changes in type of collagen synthesized as clones of chick chondrocytes grow and eventually lose division capacity. *Proc. Natl. Acad. Sci. U. S. A.* 73:1674–1678.
145. McDonald, J. A., and D. G. Kelley. 1980. Degradation of fibronectin by human leukocyte elastase: release of biologically active fragments. *J. Biol. Chem.* 255:8848–8858.
146. Meier, S., and E. D. Hay. 1974. Control of corneal differentiation by extracellular materials. Collagen as a promoter and stabilizer of epithelial stroma production. *Dev. Biol.* 38:249–270.
147. Miller, R. L. 1971. Chromatographic separation of the enzymes required for hydroxylation of lysine and proline residues of procollagen. *Arch. Biochem. Biophys.* 147:339–342.
148. Miller, E. J., and V. J. Matukas. 1974. Biosynthesis of collagen: the biochemist's view. *Fed. Proc.* 33:1197–1201.
149. Moro, L., and B. D. Smith. 1977. Identification of collagen α1(I) trimer and normal type I collagen in a polyoma virus-induced mouse tumor. *Arch. Biochem. Biophys.* 182:33–41.
150. Morrison, P. R., J. T. Edsall, and S. G. Miller. 1948. Preparation and properties of serum and plasma proteins. XIII. The separation of purified fibrinogen from fraction I of human plasma. *J. Am. Chem. Soc.* 70:3103–3108.
151. Mosesson, M. W., and D. L. Armani. 1980. The structure and biological activities of plasma fibronectin. *Blood.* 56:145–158.
152. Mosesson, M. W., and R. A. Umfleet. 1970. The cold-insoluble globulin of human plasma. I. Purification, primary characterization, and relationship to fibrinogen and other cold-insoluble fraction components. *J. Biol. Chem.* 245:5728–5732.
153. Mosher, D. F. 1975. Cross-linking of cold-insoluble globulin by fibrin-stabilizing factor. *J. Biol. Chem.* 250:6614–6621.
154. Mosher, D. F. 1980. Fibronectin. *Prog. Hemostasis Thromb.* 5:111–151.
155. Mosher, D. F., P. E. Schad, and H. K. Kleinman. 1979. Cross-linking of fibronectin to collagen by blood coagulation factor $XIII_a$. *J. Clin. Invest.* 64:781–787.
156. Mosher, D. F., P. E. Schad, and J. W. Vann. 1980. Cross-linking of collagen and fibronectin by factor XII_a: Localization of participating glutaminyl residues to a tryptic fragment of fibronectin. *J. Biol. Chem.* 255:1181–1188.
157. Müller, P. K., W. N. Meigel, B. G. Pontz, and K. Raisch. 1974. Influence of a,a′-dipyridyl on the biosynthesis of collagen in organ cultures. *Hoppe-Seyler's Z. Physiol. Chem.* 355: 985–996.
158. Murray, J. C., L. A. Liotta, S. I. Rennard, and G. R. Martin. 1980. Collagen adhesion characterization of murine metastatic and nonmetastatic tumor cells *in vitro*. *Cancer Res.* 40:347–351.
159. Murray, J. C., G. Stingl, H. K. Kleinman, G. R. Martin, and S. I. Katz. 1978. Epidermal cells adhere preferentially to type IV (basement membrane) collagen. *J. Cell Biol.* 80: 197–201.
160. Nakagawa, M., and M. R. Urist. 1977. Chondrogenesis in tissue culture under the influence of a diffusible component of bone matrix. *Proc. Soc. Exp. Biol. Med.* 154:568–572.
161. Newsome, D. A. 1976. In vitro stimulation of cartilage in embryonic chick neural crest cells by products of retinal pigmented epithelium. *Dev. Biol.* 49:496–507.
162. O'Hare, M. J. 1972. Aspects of spinal cord induction of chondrogenesis in chick embryo somites. *J. Embryol. Exp. Morphol.* 27:235–243.
163. Paglia, L., J. Wilczek, L. Diaz de Leon, G. R. Martin, D. Hörlein, and P. Müller. 1979. Inhibition of procollagen cell-free synthesis by amino acid terminal extension peptides. *Biochemistry.* 18:5030–5034.
164. Pearlstein, E. 1976. Plasma membrane glycoprotein which mediates adhesion of fibroblasts to collagen. *Nature (Lond.).* 262:497–500.
165. Pearlstein, E. 1977. Substrate activation of cell adhesion factor as a prerequisite for cell attachment. *Int. J. Cancer* 22:32–35.
166. Pearlstein, E., L. I. Gold, and A. Garcia-Pardo. 1980. Fibronectin: a review of its structure and biological activity. *Mol. Cell. Biochem.* 29:103–128.
167. Pearlstein, E., and M. D. Waterfield. 1974. Metabolic studies on ^{125}I-labeled baby hamster kidney cell plasma membranes. *Biochim. Biophys. Acta.* 362:1–12.
168. Pena, S. D. J., and R. C. Hughes. 1978. Fibronectin-plasma membrane interactions in the adhesion and spreading of hamster fibroblasts. *Nature (Lond.).* 276:80–83.
169. Pena, S. D. J., J. Mills, and R. C. Hughes. 1979. Two-dimensional electrophoresis of surface glycoproteins of normal BHK and ricin resistant mutants. *Biochim. Biophys. Acta.* 550:100–109.
170. Pennypacker, J. P., J. R. Hassell, K. M. Yamada, and R. M. Pratt. 1979. The influence of an adhesion cell surface protein on chondrogenic expression *in vitro*. *Exp. Cell Res.* 121:411–415.
171. Penttinen, R. P., J. R. Lichtenstein, G. R. Martin, and V. A. McKusick. 1975. Abnormal collagen metabolism in cultured cells in osteogenesis imperfecta. *Proc. Natl. Acad. Sci. U. S. A.* 72:586–589.
172. Perkins, M. E., T. H. Ji, and R. O. Hynes. 1979. Cross-linking of fibronectin to sulfated proteoglycans at the cell surface. *Cell.* 16:941–952.
173. Peterkofsky, B. 1972. The effect of ascorbic acid on collagen polypeptide-synthesis and proline hydroxylation during the growth of cultured fibroblasts. *Arch. Biochem. Biophys.* 152:318–328.

174. Piez, K. A. 1968. Crosslinks of collagen and elastin. *Annu. Rev. Biochem.* 37:547–570.
175. Pinnell, S., and G. R. Martin. 1968. Cross-linking of collagen and elastin: the enzymatic conversion of lysine in peptide linkage to α-aminoadipic-semialdehyde by an extract from bone. *Proc. Natl. Acad. Sci. U. S. A.* 61:708–714.
176. Plow, E. F., C. Birdwell, M. H. Ginsberg, V. Byers, L. Taylor, and A. Braisier. 1979. Identification and quantitation of platelet-associated fibronectin antigen. *J. Clin. Invest.* 63:540–543.
177. Podleski, T. R., I. Greenberg, J. Schlessinger, and K. M. Yamada. 1979. Fibronectin delays the fusion of L_6 myoblasts. *Exp. Cell Res.* 122:317–326.
178. Pollara, B. 1963. Studies on the nature of cryofibrinogens. PhD Thesis. University of Minnesota.
179. Pope, F. M., G. R. Martin, J. R. Lichtenstein, R. Penttinen, B. Gerson, D. W. Rowe, and V. A. McKusick. 1975. Patients with Ehlers-Danlos syndrome type IV lack type III collagen. *Proc. Natl. Acad. Sci. U. S. A.* 72:1314–1316.
180. Postlethwaite, A. E., J. M. Seyer, and A. H. Kang. 1978. Chemotactic attraction of human fibroblasts to type I, II and III collagens and collagen-derived peptides. *Proc. Natl. Acad. Sci. U. S. A.* 75:871–875.
181. Postlethwaite, A. E., R. Snyderman, and A. H. Kang. 1976. Chemotactic attraction of human fibroblasts to a lymphocyte derived factor. *J. Exp. Med.* 144:1188–1203.
182. Pouysségur, J., M. Willingham, and I. Pastan. 1977. Role of surface carbohydrate and proteins in cell behavior: Studies on the biochemical reversion of an *N*-acetylglucosamine-deficient fibroblast mutant. *Proc. Natl. Acad. Sci. U. S. A.* 74:243–247.
183. Prockop, D. J., K. I. Kivirikko, L. Tuderman, and N. A. Guzman. 1979. The biosynthesis of collagen and its disorders. *N. Engl. J. Med.* 301:13–23, 77–85.
184. Ramachandran, G. N., and A. H. Reddi. 1976. *Biochemistry of Collagen.* Plenum Press, New York.
185. Ramaley, P. B., S. Jimenez, and J. Rosenbloom. 1973. Conformation of underhydroxylated collagen synthesized by 3T3 cells in culture. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 33:187–191.
186. Reddi, A. H., and W. A. Anderson. 1976. Collagenous bone matrix-induced endochondral ossification and hemopoiesis. *J. Cell Biol.* 69:557–572.
187. Reddi, A. H., R. Gay, S. Gay, and E. J. Miller. 1977. Transition in collagen types during matrix-induced cartilage, bone, and bone marrow formation. *Proc. Natl. Acad. Sci. U. S. A.* 74:5589–5592.
188. Reid, K. B. M., and R. R. Porter. 1976. Subunit composition and structure of subcomponent C1q of the first component of human complement. *Biochem. J.* 155:19–23.
189. Rheinwald, J. G., and H. Green. 1975. Serial cultivation of strains of human epidermal keratinocytes the formation of keratinizing colonies from single cells. *Cell.* 6:331–344.
190. Rhodes, R. K., and E. J. Miller. 1978. Physiochemical characterization and molecular organization of the collagen A and B chains. *Biochemistry.* 17:3442–3448.
191. Robbins, P. W., G. G. Wickus, P. E. Branton, B. J. Gaffrey, C. B. Hirschberg, P. Fuchs, and P. M. Blumberg. 1974. The chick fibroblast cell surface following transformation by Rous sarcoma virus. *Cold Spring Harbor Symp. Quant. Biol.* 39:1173–1180.
192. Rosenberry, T., and J. Richardson. 1977. Structure of 18S and 14S acetylcholinesterase. Identification of collagen-like subunits that are linked by disulfide bonds to catalytic subunits. *Biochemistry.* 16:3550–3558.
193. Rowe, D. W., B. J. Starman, W. Y. Fujimoto, and R. H. Williams. 1977. Differences in growth response to hydrocortisone and ascrobic acid by human diploid fibroblasts. *In Vitro* (Rockville). 13:824–830.
194. Ruch, J. V., M. Fabre, V. Karcher-Djercie, and A. Staublei. 1974. The effects of L-azetidine-2-carboxylic acid (analogue of proline) on dental cytodifferentiation *in vitro*. *Differentiation.* 2:211–220.
195. Ruoslahti, E., and E. Engvall. 1980. Complexing of fibronectin, glycosaminoglycans and collagen. *Biochim. Biophys. Acta.* 631:350–358.
196. Ruoslahti, E., and E. G. Hayman. 1979. Two active sites with different characteristics in fibronectin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 97:221–224.
197. Ruoslahti, E., and A. Vaheri. 1974. Novel human serum protein from fibroblast plasma membranes. *Nature (Lond.).* 248:789–791.
198. Ruoslahti, E., and A. Vaheri. 1975. Interaction of soluble fibroblast surface antigen with fibrinogen and fibrin. Identity with cold insoluble globulin. *J. Exp. Med.* 141:497–501.
199. Ruoslahti, E., A. Vaheri, P. Kuusela, and E. Linder. 1973. Fibroblast surface antigen: a new serum protein. *Biochim. Biophys. Acta.* 322:352–358.
200. Saba, T. M., F. A. Blumenstock, P. Weber, and E. Kaplan. 1978. Physiological role of cold-insoluble globulin in host defense: implications of its characterization as the opsonic α_2 surface binding glycoprotein. *Ann. N. Y. Acad. Sci.* 312:43–55.
201. Saba, T. M., and E. Jaffee. 1980. Plasma fibronectin (opsonic glycoprotein). Its synthesis by vascular endothelial cells and role in cardiopulmonary integrity after trauma as related to reticuloendothelial function. *Am. J. Med.* 68:577–594.
202. Sage, H., E. Crouch, and P. Bornstein. 1979. Collagen synthesis by bovine aortic endothelial cells in culture. *Biochemistry.* 18:5433–5442.
203. Salomon, D. S., L. A. Liotta, and W. R. Kidwell. Differential growth factor responsiveness of rat mammary epithelium plated in different collagen substrata in serum-free medium. *Proc. Natl. Acad. Sci. U. S. A.* In press.
204. Santoro, S. A., and L. W. Cunningham. 1979. Fibronectin and the multiple interaction model for platelet-collagen adhesion. *Proc. Natl. Acad. Sci. U. S. A.* 76:2644–2648.
205. Sattler, C. A., G. Michalopoulos, G. A. Sattler, and H. C. Pitot. 1978. Ultrastructure of adult rat hepatocytes cultured on floating collagen membranes. *Cancer Res.* 38:1539–1549.
206. Schiltz, R. R., R. Mayne, and H. Holtzer. 1973. The synthesis of collagen and glycosaminoglycans by dedifferentiated chondroblasts in culture. *Differentiation.* 1:97–108.
207. Schor, S. L., and J. Court. 1979. Different mechanisms involved in the attachment of cells to native and denatured collagen. *J. Cell. Sci.* 38:267–281.
208. Schwartz, C. E., C. G. Hellerquist, and L. W. Cunningham. 1978. A collagenous component of the microexudate carpet secreted by attaching human skin fibroblasts. *Ann. N. Y. Acad. Sci.* 312:450–452.
209. Schwartz, R. I., and M. J. Bissell. 1979. Dependence of differentiated state on cellular environment. Modulation of collagen synthesis in tendon cells. *Proc. Natl. Acad. Sci. U. S. A.* 74:4453–4457.
210. Sekiguchi, K., and S. Hakomori. 1980. Functional domain structure of fibronectin. *Proc. Natl. Acad. Sci. U. S. A.* 77:2661–2665.
211. Shin, S., V. H. Freedman, R. Risser, and R. Pollack. 1975. Tumorigenicity of virus transformed cells in nude mice is correlated specifically with anchorage independent growth *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* 72:4435–4439.
212. Smith, B. D., G. R. Martin, E. J. Miller, A. Dorfman, and R. Swarm. 1975. Nature of the collagen synthesized by a transplanted chondrosarcoma. *Arch. Biochem. Biophys.* 166: 181–186.
213. Smith, R. T., and R. W. von Korff. 1957. A heparin-precipitable fraction of human plasma. I. Isolation and characterization of the fraction. *J. Clin. Invest.* 36:596–604.
214. Spooner, B. S., and J. M. Faubion. 1980. Collagen involvement in branching morphogenesis of embryonic lung and salivary gland. *Dev. Biol.* 77:84–102.
215. Stathakis, N. E., and M. W. Mossesson. 1977. Interaction among heparin, cold-insoluble globulin, and fibrinogen in formation of the heparin-precipitable portion of plasma. *J. Clin. Invest.* 60:855–865.
216. Stemberger, A., and H. Hörmann. 1976. Affinity chromatography on immobilized fibrinogen and fibrin monomer. II. The behavior of cold-insoluble globulin. *Hoppe-Seyler's Z. Physiol. Chem.* 357:1003–1005.
217. Stenman, S., and A. Vaheri. 1978. Distribution of a major connective tissue protein, fibronectin, in normal human tissues. *J. Exp. Med.* 147:1054–1064.
218. Stuart, E. S., B. Garber, and A. A. Moscona. 1972. An analysis of feather germ formation in the embryo and *in vitro*, in normal development and in skin treated with hydrocortisone. *J. Exp. Zool.* 179:94–118.
219. Taubman, M. B., and B. Goldberg. 1976. The processing of procollagen in cultures of human and mouse fibroblasts. *Arch. Biochem. Biophys.* 173:490–494.
220. Terranova, V. P., D. H. Rohrbach, and G. R. Martin. Role of laminin in the attachment of PAM 212 (epithelial) cells to basement membrane collagen. *Cell.* In press.
221. Timpl, R., H. Rohde, P. Gehron Robey, S. I. Rennard, J. M. Foidart, and G. R. Martin. 1979. Laminin—a glycoprotein from basement membrane. *J. Biol. Chem.* 254:9933–9937.
222. Tryggvason, K., P. G. Robey, and G. R. Martin. 1980. Biosynthesis of type IV procollagens. *Biochemistry.* 19:1284–1289.
223. Udenfriend, S. 1966. Formation of hydroxyproline in collagen. *Science (Wash. D. C.).* 152:1335–1340.
224. Uitto, J. 1977. Biosynthesis of type II collagen. Removal of amino- and carboxy-terminal extension from procollagen synthesized by chick embryo cartilage cells. *Biochemistry.* 15: 3421–3429.
225. Uitto, V., J. Uitto, W.-Y. Kao, and D. J. Prockop. 1978. Procollagen polypeptides containing *cis*-hydroxylproline are overglycosylated and secreted as nonhelical pro-γ-chains. *Arch. Biochem. Biophys.* 185:214–221.
226. Vaheri, A., M. Kurkinen, V.-P. Lehto, E. Linder, and R. Timpl. 1978. Codistribution of pericellular matrix proteins in cultured fibroblasts and loss in transformation: fibronectin and procollagen. *Proc. Natl. Acad. Sci. U. S. A.* 75:4944–4948.
227. Vaheri, A., and D. F. Mosher. 1978. High molecular weight cell surface glycoprotein (fibronectin) lost in malignant transformation. *Biochim. Biophys. Acta.* 516:1–25.
228. Vaheri, A., E. Ruoslahti, and D. F. Mosher. 1978. Fibroblast surface protein. *Ann. N. Y. Acad. Sci.* Vol. 312.
229. Vaheri, A., E. Ruoslahti, B. Westermark, and J. Pontén. 1976. A common cell-type specific surface antigen in cultured human glial cells and fibroblasts: loss in malignant cells. *J. Exp. Med.* 143:64–72.
230. Vembu, D., L. A. Liotta, M. Paranjpe, and C. W. Boone. 1979. Correlation of tumorigenicity with resistance to growth inhibition by *cis*-hydroxyproline. *Exp. Cell. Res.* 124: 247–252.
231. von der Mark, K., V. Gauss, H. von der Mark, and P. Müller. 1977. Relationship between cell shape and type of collagen synthesized as chondrocytes lose their cartilage phenotype in culture. *Nature (Lond.).* 267:531–532.
232. Vuento, M., T. Vartio, M. Saraste, C. Von Bonsdorf, and A. Vaheri. 1980. Spontaneous and polyamine-induced formation of filamentous polymers from soluble fibronectin. *Eur. J. Biochem.* 105:33–42.
233. Wagner, D. D., and R. O. Hynes. 1980. Topical arrangement of the major structural features of fibronectin. *J. Biol. Chem.* 255:4304–4312.
234. Wartiovaara, J., E. Linder, E. Ruoslahti, and A. Vaheri. 1974. Distribution of fibroblast surface antigen. Association with fibrillar structures of normal cells and loss upon viral transformation. *J. Exp. Med.* 140:1522–1533.
35. Weiss, P. 1945. Experiments on cell and axon orientation in vitro: the role of colloidal exudates in tissue organization. *J. Exp. Zool.* 100:353–386.
236. Wessels, N. K., and J. H. Cohen. 1968. Effects of collagenase on developing epithelial *in vitro*: lung, ureteric bud and pancreas. *Dev. Biol.* 18:294–309.
237. West, C. W., R. Lanza, J. Rosenbloom, M. Lowe, H. Holtzer, and N. Avdalovic. 1979. Fibronectin alters the phenotypic properties of cultured chick embryo chondroblasts. *Cell.* 17:491–501.
238. Wicha, M., L. A. Liotta, S. Garbisa, and W. R. Kidwell. 1979. Basement membrane collagen requirements for attachment and growth of mammary epithelium. *Exp. Cell Res.* 124:181–190.
239. Wiestner, M., T. Krieg, D. Hörlein, R. W. Glanville, P. P. Fietzek, and P. K. Müller. 1979. Inhibiting effect of procollagen peptides on collagen synthesis in fibroblast culture. *J. Biol. Chem.* 254:7016–7023.
240. Wilner, G. D., H. L. Nossel, and E. C. LeRoy. 1968. Aggregation of platelets by collagen. *J. Clin. Invest.* 47:2616–2621.
241. Wolff, I., R. Timpl, I. Pecker, and C. Steffen. 1967. A two-component system of human serum agglutinating gelatine-coated erythrocytes. *Vox Sang.* 12:443–456.
242. Yamada, K. M., and D. W. Kennedy. 1979. Fibroblast cellular and plasma fibronectins are similar but not identical. *J. Cell Biol.* 80:492–497.
243. Yamada, K. M., D. W. Kennedy, K. Kimata, and R. M. Pratt. 1980. Characterization of fibronectin interactions with glycosaminoglycans and identification of active proteolytic fragments. *J. Biol. Chem.* 255:6055–6063.
244. Yamada, K. M., and K. Olden. 1978. Fibronectins—adhesive glycoproteins of cell surface and blood. *Nature (Lond.).* 275:179–184.
245. Yamada, K. M., D. H. Schlesinger, D. W. Kennedy, and I. Pastan. 1977. Characterization of a major fibroblast cell-surface glycoprotein. *Biochemistry.* 16:5552–5556.
246. Yamada, K. M., and J. A. Weston. 1974. Isolation of a major cell surface glycoprotein from fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* 71:3492–3496.
247. Yamada, K. M., S. S. Yamada, and I. Pastan. 1976. Cell surface protein partially restores morphology, adhesiveness and contact inhibition of movement to transformed fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* 73:1211–1221.
248. Zardi, L., A. Siri, B. Carnemolla, L. Ganti, W. D. Gardner, and S. O. Hoch. 1979. Fibronectin: a chromatin-associated protein. *Cell.* 18:649–657.
249. Zucker, M. B., M. W. Mossesson, M. J. Brockman, and K. L. Kaplan. 1979. Release of platelet fibronectin (cold-insoluble globulin) from alpha granules induced by thrombin or collagen: lack of requirement for plasma fibronectin in ADP-induced platelet aggregation. *Blood.* 54:8–12.