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To be published as a chapter in **Fundamentals of Medical Cell Biology**, E.E. Bittar, Ed., JAI Press, Inc., Fall 1990

The Extracellular Matrix

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January 1990

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TALHOUK et al. (1990)
In: "Fundamentals of Medical Cell Biology", E. E. Bittar, ed., JAI Press Inc.

SECTION III

THE STRUCTURAL BASIS AND ORGANIZATION OF LIVING MATTER

CHAPTER ___

THE EXTRACELLULAR MATRIX

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Introduction

Cells in multicellular organisms are in contact with an intricate network of extracellular macromolecules composed largely of glycoproteins and proteoglycans. This network is collectively known as the extracellular matrix (ECM) and includes the interstitial matrix and basement membranes. It has long been recognized that cells interact with their ECM, and indeed, studies implicating a role for an extracellular environment in histogenesis have been carried out since the early 1950s. Numerous subsequent studies have established the importance of mesenchymal/epithelial and epithelial/epithelial interactions in organogenesis and the differentiation of tissues. Much of the early work focussed on the effect of the ECM/cell or mesenchymal/epithelial cell interactions on tissue morphology and growth, whereas those studies that described changes in cell function and differentiation were few. In the last decade considerable information has accumulated documenting the effect of ECM on cell attachment, cell polarity, cell morphology, and gene expression (For reviews see reading list). Whereas considerable information is now available that points to ECM as an important agent regulating tissue morphogenesis and cell function, little is known about the underlying mechanism(s) by which an insoluble matrix regulates these processes.

Almost a decade ago a model was proposed suggesting an interaction between the ECM on the one hand and the cellular cytoskeleton and nuclear matrix on the other (Bissell, Hall and Parry 1982). The model postulates that the ECM affects the cytoskeleton via transmembrane receptors. The cytoskeleton in turn is associated with messenger RNA and has connections to the nuclear matrix, which itself is associated with DNA. Hence the unit of function in normal cells of higher organisms is seen as the cell plus its extracellular matrix.

Although this model provided a general framework for ECM/cell interactions, the experimental evidence that supports the model is only now beginning to emerge. Recent data show that cells possess receptors for various components of the ECM, such as laminin, collagen, and fibronectin. These receptors appear to be transmembrane proteins that, in addition to interacting with ECM components on the outside of the cell, bind cytoskeletal proteins on the cytoplasmic side. The discovery of these transmembrane linkages between the ECM and the cytoskeleton is helping provide answers as to how the ECM could affect cellular structure and function.

As we learn more about cell/cell and cell/matrix interactions, it is becoming clear that both cytoskeleton and ECM are required for normal cell function. The aim of the present chapter is to describe the different components of ECM, to provide some insights into the diversity of cell/matrix interactions, and to discuss some of the current literature on how the ECM influences gene expression and function in normal cells.

The discussion presented here is based on the following premises:

1- The ECM and the cell are to be viewed as one functional unit; that is, there exists a "dynamic reciprocity" between the cell and its ECM. A change in one will affect the other. A disruption in proper interactions could lead to abnormal function and hence disease and possibly cancer.

2- Transmembrane proteins are involved in signal transmission between extracellular molecules and elements that regulate gene expression.

3- Cell/ECM interactions not only affect the cytoskeleton and cellular organization, but also influence the way that cells interact with one another as well as the overall three dimensional integrity of the tissues as a whole.

Extracellular Matrix Molecules

Before attempting to discuss the effects of ECM on cell morphology and function, it is important to define the components of the ECM and how they interact amongst themselves to create an intricate network that profoundly affects numerous aspects of cell behavior. Irrespective of the tissue, all matrices are composed of collagens, glycoproteins, and proteoglycans, although the type and the ratio of these molecules is tissue-specific. A specialized form of ECM is the basement membrane, a layer of macromolecules that closely underlies or surrounds epithelia, nerve, fat, muscle, and endothelial cells (Fig. 1).

Close to fifty different components comprise the ECM. However, in this review we will briefly discuss only its major components and how they interact with each other and with the cell. We will describe two of the major stromal components, type I collagen and fibronectin, and then discuss biochemical aspects of some basement membrane components, laminin, type IV collagen and proteoglycan, before briefly mentioning entactin, hyaluronic acid, tenascin and sparc. It should be made clear that these proteins do not act in isolation *in vivo*, and that the function of the extracellular environment requires cooperation between more than one type of component. For more comprehensive reviews on the extracellular matrix the reader is invited to consult the reading list at the end of this chapter.

Type I Collagen: Collagens, which are the most prevalent proteins in the body, form the main architectural elements in connective tissue such as tendon and cartilage, and are crucial for bone formation. They are also abundant in stromal tissues that underlie the dermis of the skin and form the supporting structure for

epithelia of many organs (Fig. 1). Of the numerous collagen types (Fig. 2), type I collagen is the most abundant and has been studied in greatest detail. The basic molecular unit of the collagen molecule is a right-handed triple helix about 295 nm long, formed by three polypeptide chains, referred to as procollagen molecules, winding round each other. Most of the collagens are comprised of two or more different subunits, with the type I triple helix most commonly containing two $\alpha 1$ and one $\alpha 2$ subunits (Fig. 2).

The ability to form a tightly wound helix depends on a basic repeating unit of three amino acids, gly-X-Y. Glycine is the only amino acid that is small enough to fit into the center of the helix. A proline often occurs in position Y; about half these residues become hydroxylated in the endoplasmic reticulum by the membrane-bound enzyme, prolyl hydroxylase. The presence of a hydroxylated proline is required for maximal inter-chain hydrogen bonding, which increases triple helix stability.

The initial assembly of procollagen type I chains (shown schematically in Fig. 3) takes place in the endoplasmic reticulum. After the three procollagen chains have been aligned in the correct direction, they become chemically linked at the C' (carboxy) end by inter-chain disulfide bridges (Fig. 3, stage 1). Hydroxylation of proline residues also occurs at this stage and continues until the helix is actually formed. Helix-winding then begins at the C' terminus of the molecule and proceeds towards the N' (amino) terminus of the triplet region (stage 2). Following exocytosis (stage 3), the globular regions that flank the rigid helical domain of the fiber-forming collagens (types I, II, III, V) are removed (stage 4) in order not to interfere with the integrity of the completed fiber. The fully processed trimeric molecule is now referred to as collagen, rather than procollagen. Subsequently (stage 5), triple-helical molecules are assembled with a defined periodicity to form fibrils of varying diameters, from 10 - 300 nm depending on the tissue. These then aggregate with specific geometry into high tensile-

strength fibers; extensive intermolecular cross-linking between collagen molecules greatly increases the strength of completed fibers. This process resembles the production of cotton thread, with disulfide and hydrogen bonding taking place of the fibril twisting that maintains integrity in cotton.

The globular regions of other collagens are often retained. These non-collagenous, or NC, domains are the molecular units that reflect tissue specificity and confer the unique collagen-specific function to different members of the group (Fig. 2).

Improper winding of the triple helical domain has devastating consequences for the integrity of connective tissue; disruption in the collagen assembly process for two severe types of such defect is shown in Fig. 4. Although scurvy is less common nowadays, scorbutic people have serious problems with their teeth, and wounds fail to heal. Osteogenesis imperfecta affects unborn and newborn children, who have abnormally fragile bones.

Fibronectin: As with many ECM molecules, fibronectin (FN) is a glycoprotein that has diverse roles. Its function depends on the precise structure of the molecule and the cell type with which it interacts. Perhaps FN is best characterized as an adhesion molecule, linking certain cells to substrata such as collagen and proteoglycan. During development mesenchymal cells such as fibroblasts, which synthesize components of connective tissue, adhere to FN and depend on it for their guidance through stroma. FN also plays an important role in wound healing. At sites of tissue damage, one of the many newly synthesized proteins is a form of FN. In conjunction with chemotactic molecules for fibroblasts, it provides a molecular attachment zone so that the newly-arrived fibroblasts can adhere and begin to synthesize connective tissue for repairing the wound. It is also involved in the migration of epidermal cells of the skin layer, endothelial cells of blood vessels, and macrophages to damaged sites in

tissues. A soluble form of FN, plasma FN, is secreted by hepatocytes into the bloodstream. This type of FN plays a role in the blood clotting cascade helping many cell types, including fibroblasts, to adhere to the fibrin clot (see below). During vertebrate development, FN appears in growth areas where morphogenetic events are taking place; it acts as a molecular "highway" for the motile cells that prefer to adhere to FN than to anything else (Fig. 5).

Fibronectin is composed of two similar subunits, 60-70 nm in length, joined together by 2 disulfide linkages near their C' termini. It is extensively glycoslyated and the dimer has a MW of about 550 kD. There are more than 20 forms of the protein, although FN is encoded by a single gene. Such considerable heterogeneity is achieved by alternative splicing of the FN mRNA, which produces a spectrum of molecules, each with a distinct function (Fig. 6). These multiple FN species may be involved in the different processes outlined above. Despite its heterogeneity, the polypeptide contains three types of homologous repeat unit (types I, II and III) that are arranged in such a way as to correspond to the different functional units. These repeat units often reappear as the building blocks for other extracellular molecules: Types I and II repeats, for example, are shared by proteins that are involved in blood clotting mechanisms, such as factor XII and plasminogen. Some regions of other ECM molecules, such as tenascin (see below), have sections containing type III repeats.

FN has binding domains for several proteins, such as fibrin and other ECM molecules, and contains two cell binding domains (Fig. 6). The glycine - arginine - glycine - aspartic acid - serine (GRGDS) pentapeptide sequence is involved in binding FN to cell surfaces. This peptide contains the arginine - glycine - aspartic acid, or RGD sequence, which is a common motif present in many ECM molecules and recognized by specific cell-surface receptors (see below for more details). Understanding the way in which cells interact with their environment may help to devise unique and

effective therapies for cells that lose their normal communication with the ECM (Fig. 7).

Laminin: The basement membranes that contact numerous cell types contain several ECM glycoproteins, the most abundant of which is laminin. Among its many functions, laminin is a potent regulator of cell behavior. It can stimulate cell attachment and migration, most notably influencing neuronal outgrowth (Fig. 8). It can also influence myoblast motility (Fig. 15), and some of its functional domains have growth factor activity. In conjunction with other basement membrane components, it plays a significant role in the induction and maintenance of epithelial cell differentiation; this crucial function of basement membranes is discussed in the last section of the chapter.

Laminin is a multidomain glycoprotein. The 850 kD molecule is a trimer, composed of A, B1, and B2 subunits. The three polypeptide chains are joined together in a cross, with the C' termini of each at the end of the long arm; the large globular region is formed by the A chain (Fig. 9). Laminin has several cell binding domains. A pentapeptide with the sequence tyrosine - isoleucine - glycine - serine - arginine (YIGSR) located on the short arm of the B1 chain mediates attachment to certain cell surface receptors for laminin. There is also an RGD cell-binding motif on the short arm of the A chain. At the bottom of the long arm is a region that binds to other receptors. This region is responsible for promoting neurite outgrowth. The various receptors that interact with the different sequences on the laminin molecule are discussed in more detail below. In addition, laminin has separate domains that bind to collagen, entactin and heparin. This type of binding activity enables different ECM molecules to interact and form macroscopic multi-component polymers, such as basement membranes.

The multiple functions of laminin suggest that additional laminin species may direct some of its specific properties that were outlined above. The finding of a novel laminin, s-laminin, encoded by its own unique gene as a component of the synaptic basement membrane at the neuromuscular junction supports the existence of a family of laminin genes.

Type IV Collagen: Type IV collagen, which has a structure and composition distinct from other collagens, is found only in basement membranes. Instead of aggregating into the striated fibers typical of interstitial collagens such as types I or II, a network structure is the usual type IV configuration. This is built up from standard triple-helical collagen molecules, although these are somewhat longer (350 nm) than those of interstitial collagens (295 nm). The two types of α-chain range in size from 135-180 kD, although most commonly they are 185 kD and 170 kD for the α1 and α2 chains, respectively. A non-collagenous domain, which is present in the final secreted form and plays a role in assembly of type IV collagen matrices (Fig. 10), forms a globular structure at the C' terminus of each monomer (Fig. 2). In contrast to other collagen types, whose subunit genes are encoded on different chromosomes, the α1(IV) and α2(IV) subunit genes are located adjacent to each other.

Two models have been proposed for the macromolecular organization of type IV collagen and its assembly into the matrix (Fig. 10). The "network model" proposes that tetramers, joined together via their N' termini, create an irregular polygonal network resembling chicken wire. In the "hexagonal model", dimers linked at their globular domains interact by lateral association to form a hexagonal or irregular polygonal array. Networks of these type may exist *in vivo* and form the basic molecular lattice of basement membranes, which can be added to by the incorporation of other basement membrane components such as laminin and proteoglycan.

Proteoglycans: Proteoglycans consist of a core protein to which are attached sugar macromolecules known as glycosaminoglycans. These are composed of linear polymers of repeating disaccharides, covalently linked through a serine residue to the core (Fig. 11). Proteoglycans constitute a vastly heterogeneous family of ECM molecules. They can differ in the type of core protein, in the glycosaminoglycan attached to the core protein, and in the length of disaccharide repeat (Fig. 11). Proteoglycans exist both in connective tissue and in basement membranes, although the most abundant forms in the latter are heparan sulfate proteoglycan (HSPG) and chondroitin sulfate proteoglycan. Since the sugars in HSPG and chondroitin sulfate proteoglycan contain sulfate residues, they are negatively charged molecules. Glomerular basement membranes in the kidney contain these type of sulfated proteoglycans that constitute polyanionic sites and function as a selective charge barrier in the process of macromolecular filtering in this tissue. HSPGs interact with other components of the basement membrane contributing to its assembly into a functional unit (see below).

Entactin: Entactin, also referred to as nidogen, is a widely distributed basement membrane glycoprotein (MW 150 kD). Although little is known about its function, entactin may modulate the way that laminin interacts with cell surfaces. It is often found in association with laminin and contains a region that binds tightly to one of the globular domains in laminin. Entactin also has its own RGD sequence - the peptide motif recognized by some ECM receptors - and is able to promote adhesion of cells to various surfaces. Therefore entactin appears to contribute to the variety of mechanisms by which laminin binds to cells. Additionally, it may modulate the effects that laminin and other basement membrane components have on cell functions, such as

movement or differentiation.

Hyaluronic acid: Hyaluronic acid is a gigantic, space-filling glycosaminoglycan of the ECM, found mainly in connective tissue, cartilage, synovial fluid and vitreous body. The molecule is composed of up to 50,000 repeats of a non-sulfated disaccharide (glucuronic acid - N-acetyl glucosamine) joined together by β-linkages and extensive hydrogen bonding. The hydrophilic nature of hyaluronic acid allows it to bind water to such an extent that it can swell to over a thousand times its original size, giving the molecule a space filling property. The resulting viscosity permits it to act as a lubricant at joints, and as a shock absorber in connective tissue. Hyaluronic acid is one of the ECM molecules involved with certain cell migrations that occur during development. Its viscosity makes it interfere with cell - cell and cell - matrix interactions, separating cells from one another and contributing to an environment that favors migration of motile cells. This is illustrated in elegant studies that have demonstrated a role for hyaluronic acid in corneal development (Fig. 12). It is also involved with aspects of cell differentiation; for example, hyaluronic acid contributes to regulating the fusion of myoblasts into multinucleated muscle fibers.

Tenascin: Tenascin was first described as a component of tendon-muscular junction, being originally known as myotendinous antigen. It appears to be involved in two distinct situations, both in adult tissues that have low turnover rates, such as skin or myotendinous junction, and in regions of active cell growth and tissue remodelling. In regard to the latter, tenascin is a significant mesenchymal component during embryonic development, found in association with actively growing epithelial tissues such as mammary gland, kidney, lung, and tooth. It is also expressed in developing bone and cartilage. In addition, it is present in tissues undergoing extensive

remodelling, such as at sites of wound repair and in the stroma surrounding certain epithelial neoplasias. The tenascin molecule is a hexamer with its subunits, like laminin and FN, being joined together by disulfide linkages. It forms a "hexabrachion" structure of six arms radiating from a central point. The arms are composed of one of three different forms of the molecule, each derived by alternative splicing from a single tenascin gene. In this regard, tenascin is similar to other ECM molecules that have multiple forms, such as FN. The core region of the tenascin hexamer contains numerous EGF-like repeats, similar to those in laminin (see Fig. 9). Towards the ends of the arms, at their C' termini, are a series of FN-like type III repeats (see Fig. 6), which contain sites for cell binding and interaction with other ECM molecules.

SPARC: SPARC (secreted protein, acidic and rich in cysteine) is a calcium-binding glycoprotein of 43 kD originally isolated from parietal endoderm of mouse embryo. These endodermal cells synthesize a thick basement membrane (Reichert's membrane) that surrounds the embryo. SPARC is identical to two other proteins called BM40 and osteonectin. As with a number of ECM proteins, it is likely to have diverse roles, although currently these have not been studied very extensively. SPARC appears to be a fairly common basement membrane component and may be involved with some aspects of ECM assembly and/or remodelling. It is also present as a component of interstitial matrix in a variety of mesenchymal tissues, and may play a role in mineralization of bone.

Plasma Membrane And ECM Association

ECM molecules interact with a variety of types of specific cell-surface receptor in ways that can be influenced by other extracellular proteins. Laminin, for example, has many different receptors and its binding to the plasma membrane can be affected by other proteins such as entactin. Cell surface proteins or lipids also modulate this interaction; for example, gangliosides, the negatively charged lipids on the external leaflet of the plasma membrane influence the way that FN interacts with cells. In addition, other charged molecules play a role; divalent cations such as Ca^{++} or Mn^{++} are crucial in stabilizing the activity of many ECM receptors. The complexity of the cell - ECM interaction is only beginning to be unravelled, but the very diversity of mechanisms has significant implications for regulation of the tissue-specific activity of each ECM molecule. For example, their different roles may be controlled by the particular set of receptors that is expressed by the responding cell. In addition, it should be remembered that proteins such as FN and tenascin have multiple forms that are likely to represent an additional order of control for their tissue-specific function.

One class of ECM receptors are the integrins, which form a large family of heterodimeric proteins. The archetype of the family is the FN receptor. It contains an α and a β chain, both of which are required for binding to ECM molecules (Fig. 13). Numerous different integrin molecules have now been identified, each with a specificity for one or more ligands (Fig. 14). In general, β chains interact with a spectrum of α chains that provide the ligand specificity. Although some of the integrin families such as the β_1 family are widespread, others are only present on certain types of cell. The β_2 integrins, for example, appear to be specific for members of the hemopoietic lineage.

Integrins "integrate" the ECM with the cytoskeleton of the cell. Many integrins recognize the afore-mentioned RGD motif present in certain ECM molecules. Some, such as the vitronectin receptor (composed of $\alpha_v\beta_3$), are fairly promiscuous and interact with many proteins containing an RGD sequence. Others, such as VLA-5 ($\alpha_5\beta_1$), are much more monospecific and require neighboring amino acid sequences, as well as the correct tertiary structure of the ECM molecule; for their specificity.

The short cytoplasmic domains of the integrins bind to cytoskeletal elements. Talin and α-actinin are two proteins that interact directly with these regions and together they are believed to form multi-component complexes. At focal contacts, for example, the cell's internal scaffold is anchored (Fig. 15, part 1). This type of contact is crucial for influencing not only the shape of various cultured cell types (Fig. 16), but also the way in which they move (Fig. 15, part 2). Formation and dissolution of these complexes are highly regulated inside the cell. We know little about this process, but phosphorylation and dephosphorylation at tyrosine residues on the integrin molecule by specific kinases and phosphatases may be of great importance. The integration of molecules outside the cell with regulatory structures inside also plays a major role in controlling cell function at the levels of both growth and differentiation (Fig. 15, part 3). As we learn more about the biochemistry of integrins and the way they interact with intra and extracellular molecules, we are likely to understand more clearly the mechanisms by which different ECM molecules influence tissue-specific processes.

Many other types of ECM receptors and binding proteins exist. This is most clearly illustrated by the example of numerous proteins that interact with laminin. In addition to the various ECM proteins already mentioned that bind to laminin, i.e. entactin, type IV collagen and HSPG, laminin binds to other proteins some of which are specific cell-surface receptors. Several integrins, $\alpha_1\beta_1$, $\alpha_3\beta_1$, and $\alpha_6\beta_1$ (each on a different cell type), have laminin as a ligand. A 67/69 kD protein also interacts with

laminin. This transmembrane protein, which interacts with the YIGSR pentapeptide sequence in the laminin B1 chain, is present on endothelial and neuronal cells and is likely to be important in mediating neurite outgrowth (Fig. 8). It may also be involved with the binding of a number of tumor cell types to basement membranes. In addition, a newly characterized muscle-specific secreted protein binds to laminin and might regulate the interaction between muscle cells and their basement membranes. This peripheral membrane protein, called aspartactin, may react with cell surfaces via its unusual C' terminal poly-aspartic acid sequence. Finally, a cell-surface galactosyl transferase can interact with some of the sugar residues on glycosylated regions of laminin. In this way, membrane-bound enzymes can modulate the behavior of cells when they are already bound to ECM molecules such as laminin.

The Architecture Of Basement Membranes

The basement membrane underlies all epithelial cells in the body and is also found surrounding fat cells, Schwann cells, and muscle cells (Fig. 17; see also Fig. 25). The exact composition and distribution of basement membrane components varies between different tissues, but generally consists of type IV collagen, laminin, entactin, and proteoglycans. Currently there are three different models for the assembly of basement membranes from their major components: First, the "layer" model postulates the self-assembly of laminin and type IV collagen into sheets that overlay each other; type IV collagen networks (Fig. 10) may provide such a framework. The proteoglycan sulfates attach to collagen and laminin but are not incorporated between the layers. Second, the "matrisome" model suggests that laminin, type IV collagen, entactin and proteoglycan form individual units that subsequently become assembled together.

Although either of these models might form the structural basis for basement membrane formation it is likely that in addition, adjacent cells play a crucial role in its construction. They have after all, multiple surface receptors for basement membrane components and the morphological structure and the differentiated function of many cell types is dependent upon this interaction. Thus, the third model - the "assembly polymorphism" model - incorporates this aspect of cell function into the creation and maintenance of basement membrane structures. At present, however, the kinetics and stoichiometry of the interaction between various components and different cell types is unclear. The latter model has the additional advantage of predicting that the same molecules of the basement membrane, alternatively organized, could affect different cell functions.

Basement membranes are more than just structural entities to which cells attach. Produced by the epithelial cells and/or the neighboring stromal cells (Fig 18), they also act as selective barriers separating epithelial cells from direct contact with connective tissue and fibroblasts. In the kidney, for example, one function of the basement membrane is to act as a semi-permeable barrier allowing the passage of macromolecules that are to be excreted from the blood. Additionally, basement membranes are involved in wound repair; upon injury of epithelial or muscle cells, numerous cell types migrate to the site of tissue damage (see the section on FN). The uninjured basement membrane serves as a foundation for the arriving cells to attach to and reestablish tissue integrity. A major function of basement membranes is their role in regulating cell function. For example, they are involved with the regulation of tissue specific differentiation in numerous cell types including hepatocytes, Schwann cells, kidney cells, endothelial cells, Sertoli cells, and mammary cells. For the past several years our laboratory has utilized the mammary system for studying the effect of the ECM on tissue specific gene regulation. This model will be described in detail below.

The Influence of ECM on Cell Function: Mammary Gland as a Model

The mammary gland is a versatile model for studies of cell/ECM interactions because it undergoes cycles of growth, differentiation and involution that are accompanied by alterations in the ECM. The development and differentiation of the mammary gland into an intricate network of ducts and alveoli embedded within a stroma is a complex multistep process that starts early in fetal life and undergoes structural, compositional and functional changes through pre-puberty, puberty, and pregnancy, culminating in lactation (Fig. 19). In mid-pregnant and fetal stages the basement membrane surrounding the penetrating ducts contains non-sulfated glycosaminoglycans and laminin, whereas the fully differentiated mammary epithelium in lactating mammary gland rests on a basement membrane that contains sulfated glycosaminoglycans, laminin, entactin, type IV collagen, and fibronectin. Studies in vivo have shown that normal mammary growth and development are interrupted when ECM deposition is disrupted. Involution, a stage characterized by loss of mammary epithelial cell differentiation is preceded by enzymatic hydrolysis and degeneration of the basement membrane. Thus it is reasonable to assume that, in addition to hormones and other soluble mammary effectors, cell/ECM interactions play a critical role in maintaining the functional status of the mammary cell in vivo.

Studies in the last decades have defined and characterized a variety of mammary specific factors such as hormones, growth factors, and enzymes required for development, lactation, and involution of the gland. Furthermore, the protein products and the genes of many of the milk proteins secreted by the mammary gland have been purified or cloned, providing useful probes for determining the differentiated status of

mammary cells in culture. Such knowledge, initially accumulated in vivo and in organ cultures, has been used to develop defined mammary cell culture systems. The latter will allow us to address mechanistic questions relating to the effects of cell/ECM interactions on mammary cell function and differentiation, questions that are otherwise difficult to address in vivo.

Effect of ECM on Mammary Cells in Culture: In order to illustrate the role of the ECM in regulation of cell function, we will draw on examples from research conducted primarily in our laboratory using cultures of mammary epithelial cells derived from midpregnant mice. The progress made in cell culture techniques over the past decade has allowed us and several others to establish primary cultures of mammary cells that regulate their milk protein production in response to hormonal modulation when placed on an appropriate substratum.

The classic tissue culture substratum, plastic, used by many investigators, does not support mammary epithelial cell differentiation, in that epithelial cells do not maintain their cuboidal morphology, produce only some of the milk proteins and secrete even less. By providing a stromal type matrix, type I collagen, as the substratum for cultured cells, it was first observed by Emerman and Pitelka (1977) that mammary epithelial cells were able to establish intracellular and intercellular organization that was reminiscent of that found in secretory epithelial cells in vivo. Cells were grown on a thick layer of type I collagen that was then floated in the culture medium. The cells contract the collagen gel forming a cuboidal morphology concomitantly with the synthesis and secretion of milk proteins. A basement membrane was observed at the epithelial cell/collagen interface only when the gel was floated. This observation led several investigators to utilize different extracellular matrices to optimize further conditions that favor differentiation of mammary epithelial cells in culture. Given that

a basement membrane underlies epithelia in vivo, we cultured mammary epithelia on a reconstituted-basement membrane matrix.

Thus, we will briefly address the pertinent features that the various substrata (plastic, type I collagen, and basement membrane) elicit from mammary epithelial cells. There are two levels at which one can observe the functional state of a particular cell type. The most obvious is how the cellular components are organized, i.e. how do the cells look ? This can be investigated using light microscopy, or electron microscopy. The cellular ultrastructure and morphology indicate how closely the cells resemble their counterparts in vivo. The other level is to assess the functional status of the cells, which can be assayed using biochemical and molecular techniques. One can ask whether the genes for differentiated products (in this case milk proteins) are being transcribed and translated and whether the protein products are secreted. In the case of a secretory epithelium such as the mammary gland, this would include questions related to composition, concentration and the direction of secretion.

Ultrastructure: Mammary epithelial cells cultured on a plastic substratum in the presence of lactogenic hormones assume a flat, elongated morphology, with a high nuclear to cytoplasmic ratio. They are deficient in organized rough endoplasmic reticulum and golgi apparatus and have short "stubby" microvilli. Cells cultured on attached collagen gels develop a cuboidal morphology with some evidence of secretory capacity. In contrast, mammary epithelial cells cultured on a floating type I collagen or reconstituted basement membrane assume the morphological characteristics of a simple epithelium (see Fig. 1), as is demonstrated by basally located nuclei, tight junctional complexes, well developed apical microvilli, highly organized rough endoplasmic reticulum and golgi apparatus, and secretory vesicles with casein micelles and fat droplets (Fig. 20). In addition basal lamina is observed at the interface of the

cells and substratum.

Despite the presence of lactogenic hormones, mammary epithelial cells on plastic are unable to develop or maintain cuboidal morphology or a differentiated cytostructure. In contrast, providing a permissive substratum allows the mammary epithelial cells to re-express their epithelial morphology and assemble an organized and differentiated ultrastructure typical of lactating mammary cells.

Morphology: The effect of ECM extends beyond cytostructural organization of mammary cells in culture. Multicellular organization and growth of mammary cells in culture is dramatically altered by the type of substratum provided. Whereas cells on plastic and attached type I collagen gels spread and form confluent sheets, cells cultured on reconstituted basement membrane aggregate into clumps during the first 2 days after plating and then organize into three-dimensional structures (Fig. 21). A cross section view of these structures shows that the cells are polarized with their apical side oriented toward a lumen and are highly reminiscent of secretory alveoli (Fig. 22).

The mechanism by which the ECM exerts its effect on multicellular organization is not yet clear. Individual components of the basement membrane are not as effective in eliciting the cellular reorganization brought about by the complex matrix. Interestingly, cells cultured on air dried or prefixed matrix (a procedure that provides a non-malleable organization of the matrix components), do not aggregate into alveolar-like structures and instead spread into sheets of densely packed cells. In the first approximation, the composition of the EHS matrix is not changed by fixing or drying. However, the matrix becomes cross linked and is thus not malleable. Whereas cells on an unfixed floating collagen gel become reorganized and functional, there is little function on fixed floated gel. Thus malleability is important for function. On the other

hand, a basement membrane matrix (EHS) is required for alveoli formation and optimal functional differentiation of mouse mammary cells.

Gene Expression And Protein Synthesis: Concomitant with the organizational changes that are induced by the ECM, gene expression and synthesis and secretion of milk and ECM proteins are also modulated. There are several reasons why it is interesting to compare the regulation of milk and ECM proteins. 1) Milk proteins (caseins, whey acidic protein, and transferrin) are highly regulated differentiated products of the mammary gland, and as such, provide markers for functional differentiation of these cells. ECM proteins, on the other hand, are not necessarily tissue specific and are implicated in the maintenance of functional epithelium. 2) Milk proteins are secreted apically whereas ECM proteins are secreted basally, indicating two secretory pathways and the existence of polarity. 3) The establishment of a basement membrane corresponds to high production of mRNA for milk proteins but a turn off of mRNA for ECM proteins.

Regulation of Milk Proteins: Mammary cells cultured on plastic transcribe the genes and translate the mRNA for milk-specific caseins but fail to secrete much of the proteins. The cells do not express whey acidic protein (WAP) and produce little or no fat. However, on plastic the cells do secrete low levels of the iron-binding proteins, transferrin and lactoferrin. In contrast, cells cultured on floating type I collagen express more milk protein mRNA than cells on plastic and secrete relatively large amounts of the proteins into the medium. The induction of high levels of caseins in cells on type I floating collagen is thought to be influenced by two factors. Cells on floated type I collagen are able to contract the gel so that they are at high local densities and have a cuboidal morphology. The latter in turn may influence the ability

to organize an intact basement membrane and to express and secrete caseins. In addition their basolateral membranes have better access to nutrients than cells on attached type I collagen or plastic.

Is an organized cytostructure, cuboidal morphology, and a basement membrane sufficient to elicit differentiation and expression of all milk proteins by mammary cells in culture? This appears not to be the case since some milk proteins such as WAP and α-lactalbumin are not detected in mouse mammary cell cultures on floating type I collagen. Whereas mammary cells on EHS matrix exhibit similar ultrastructural organization to those on floating type I collagen and express and secrete similar levels of caseins and transferrin, only cells on EHS matrix express (Fig. 23) and secrete WAP. Our studies have indicated that WAP mRNA is suppressed by a secreted inhibitor when cells are cultured under most conditions tested. It appears that culturing cells on native basement membrane matrix either suppresses the inhibitor or sequesters it within the apical lumen thereby making it inaccessible to its receptor. This receptor must be present on the basolateral surface since addition of conditioned medium from cells on plastic, and even floating gel inhibits WAP production by the cells cultured on native basement membrane. These results further demonstrate that the ECM directly, or indirectly via the three dimensional organization of cells (Fig. 21A and 21B), can affect gene expression and protein synthesis and secretion.

Regulation of ECM Proteins: Does an exogenous ECM regulate the de novo production and deposition of basement membrane components by mammary cells in culture? And if so, does the deposition of the basement membrane or the lack of it correlate with cell differentiation events? Mammary cells on plastic express high levels of laminin and type IV collagen mRNAs and secrete these proteins. In this case the cells are not able to deposit and assemble a basement membrane and do not

differentiate. In contrast, mammary cells on floating type I collagen express low levels of mRNA for ECM components (Fig. 24) but, by comparison with cells on plastic, are able to deposit their own basement membrane. The formation of the latter corresponds to the onset of differentiation (Fig. 25).

Conclusion: Most of the studies outlined for the mammary cells emphasize the concept that the more organized and complete a basement membrane, the closer the cells are to a functional differentiated state, in this case lactation. It is important to note that this concept does not imply that an undifferentiated cell lacking the correct composition of ECM for differentiation is a non-functional cell. As the mammary gland goes through puberty, pregnancy, lactation, and involution the ECM is continuously remodelling and, along with soluble mammary cell effectors, influences cell functions. The cell in turn is responding to its environment and modulating its production of ECM components and ECM degrading proteinases. This interaction of the cell with its stroma could act both as an inhibitor or inducer of differentiation.

The latter part of this chapter was intended to demonstrate in a single system how ECM regulates cellular form and function. A model that originally postulated that linkage between ECM, the cytoskeleton, and the nuclear matrix could modulate gene expression, has provided the framework for the above studies. During the last decade, evidence for the association of active genes with cellular skeletal elements has emerged. Thus it is tempting to suggest that the stability of milk protein mRNAs is governed, at least in part, through transmembrane links between cytoskeleton and ECM. Thus the unit of function in higher organisms is not the cell itself but the cell plus its ECM, and that ECM and soluble cell effectors are both needed for a fully functional secretory gland.

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Fig 1. Schematic diagrams of simple and complex epithelium

The basement membrane forms a thin sheet that separates stroma from epithelium, shown below in the schematic diagram of simple epithelium. The underlying supportive stroma, otherwise known as "connective tissue" contains ECM copmonents in the form of "interstitial matrix" as well as capillaries and various cell types, such as fibroblasts. The epithelial cells directly interact with components of the basement membrane and the stroma. Cells such as fibroblasts migrate through the stroma, and turn over its components to maintain functional integrity.

More components are involved with the architecture of complex epithelium, shown on the right. This cross-section of skin (cutis) shows multiple layers of keratinizing epithelium (epidermis). Beneath this layer, and separated from it by a basement membrane, is the connective tissue, comprised of both dermis and below this (but not shown here), the subcutaneous fatty stroma. Hair follicles and sweat glands originate in the dermis, which also contains blood and lymph capillaries and has abundant nerve endings.

Fig 2. The collagen family

Currently 12 vertebrate collagens have been identified, each with varying numbers (subscripts) and types of chains. In the standard notation, chain types are shown in arabic numerals, followed in parentheses by the collagen type (roman numerals). The fibrils formed by types I, II, III, and V are able to assemble into fibers. The overall structure of the collagen fibrils is drawn approximately to scale, with their triple helical and globular domains; some of these are removed after formation of the collagen trimer (open circles and rods). The filled regions show the extent of the molecule that remains when embedded in the ECM.

(modified from K. Kuhn (1987) Structure and function of collagen types. Acad. Press, Inc., New York; and R.E. Burgeson (1988) Ann. Rev. Cell Biol., 4:551-557)

Fig 3. Assembly of the collagen molecule

Fig 4. Improper assembly of collagen leads to severe connective tissue defects

Prolyl hydroxylase relies on ascorbic acid (vitamin C) for its activity. Scurvy is a disease caused by a severe lack of vitamin C. This deficiency results in low activity of prolyl hydroxylase, which ultimately leads to the inability of collagen to form a triple helix.

In the inherited disesase, osteogenesis imperfecta, single mutations or gross deletions in the collagen chains prevent formation of the triple helix. Osteogenesis imperfecta is a spectrum of disease, with some mutations having more severe consequences than others. In general, mutations towards the C' end of the procollagen molecule correlate with a more serious prognosis as they interfere more with the winding process.

In both cases, the improperly formed helices are either rapidly degraded inside the cell -- by "procollagen suicide" -- or, if they get out, collagen fibrils are incorrectly assembled into fibrils resulting in considerably weakened fibers with less tensile strength.

Fig 5. FN as a molecular highway in embryonic development

During early embryonic development, massive migrations of primitive cells take place. These processes ultimately lead to the correct spatial positioning of various tissues and organs which make up the mature body form. One example of embryonic migration is that of the neural crest (see picture, right), whose cells give rise to numerous cell types such as sensory and sympathetic ganglia, connective tissue forming cells and pigment cells. These cells migrate along the pathways shown (A, B, C), on sheets of ECM proteins which are deposited before movement takes place. FN is a crucial component of these pathways, as can be demonstrated by injecting embryos with antibodies directed against the cell surface receptors for FN. In such experiments, neural crest migration is completely (but reversibly) blocked. (By courtesy of R. Perris, and M. Bronner-Fraser).

The potent role of FN in providing these cells with a molecular highway for migration can be illustrated (below) by coating dishes with strips of FN. Populations of neural crest cells (seeded at left) migrate only on the FN strips (top picture), in a process that can be documented by time lapse videomicroscopy (bottom picture).

(from J.P. Thiery; J-L. Duband, S. Dufour, P. Savanger, and B.A. Imhof (1988). Fibronectin. D.F. Mosher, Acad. Press Inc. New York)

Fig 6. Features of the FN molecule

Alternative splicing at three distinct sites (E III B, E III A and V) of the rat FN gene allow 12 possible FN polypeptides to be synthesized. Since the final FN molecule is a dimer, at least 100 dimer variants are conceivable, although many fewer than this have been currently identified.
(modified from R. Hynes (1985). Ann. Rev. Cell Biol. 1:67-90)

FN is an adhesive molecule which links cells to other extracellular macromolecules, such as collagen and proteoglycans. The cell binding domains, "a" and "b", are indicated, as are the regions which bind to other ECM components such as collagen. FN binds to proteoglycans at the heparin site. The fibrin sites are crucial in the binding of FN to blood clots. The main region in FN which binds to cells is domain "a", which contains the sequence GRGDS. Peptides containing only these amino acids mediate cell attachment to plastic dishes via the cell surface FN receptor. Numerous proteins contain this sequence and its interaction with the FN receptor is discussed in more detail below. An additional cell binding domain (site "b") occurs in the region V. Since this cell binding site can be modified by alternative splicing, shown as the three alternate splice patterns in the figure, it may regulate the tissue specificity of different types of FN molecule.

Fig 7. Peptides which interfere with binding of cells to their ECM can inhibit experimental metastasis

The B16-F10 cell line derived from a mouse malignant melanoma is an example of an aggressive experimental tumor that has a strong tendency to metastasize to lung and other tissue. As with many cell types, the binding of these melanoma cells to substrata is mediated by ECM components such as FN. This interaction is one feature of these cells that allows them to invade neighboring tissues and to form metastatic tumors, shown below (left) as lung metastases. Peptides, derived from the cell binding region, which contains the sequence GRGDS, interfere with the binding of these cells to their natural substrata. When mice are co-injected with B16-F10 melanoma cells and high doses of the peptide (3 mg/mouse, figure on right) fewer tumors develop. Control peptides with the sequence GRGES or GRDGS fail to inhibit metastasis.

Although this is a simplistic model and the B16 melanoma cell line is uniquely sensitive to such treatment, the principle of the approach may provide a rational basis for the therapy of certain types of aggressively malignant human tumors.
(courtesy of M.J. Humphries, and K.M. Yamada)

Fig 8. Laminin guides the outgrowth of neurites

When central nervous system (CNS) neurons are plated onto culture dishes, neurites grow out if the dishes have been previously coated with laminin. Although some other ECM components such as FN and certain collagens have some "neurite outgrowth" promoting effect, laminin is by far the most potent. Here, sensory axons are being guided onto stripes of laminin, avoiding the other areas which have been coated with type IV collagen.

A fragment of laminin, its large globular region (hatched domain, Fig 9) mimics this type of activity, indicating that this is the region in the laminin molecule which promotes neurite outgrowth.

This type of experiment, coupled with the observation that laminin is transiently expressed by astrocytes after brain injury, suggests that laminin may be involved in regeneration of damaged CNS tissue. It may also be a key regulator in the development of the CNS.

(Courtesy of R.W. Gundersen)

Fig 9. A structural model for laminin

Arrangement of the 3 chains that make up the laminin "crucifix" are the A chain (M.Wt. 400 kDa), the B1 chain (M.Wt. 215 kDa) and the B2 chain (M.Wt. 205 kDa). The globular domains (○) on the short arms of the molecule are seperated by cysteine rich domains (═), which share homology to similar regions on other extracellular molecules such as growth factors. Because of their similarity to sequences on epidermal growth factor (EGF), they are called EGF-like repeats. These domains appear to play a role in binding many types of extracellular molecules to their cell-surface receptors. The position of the YIGSR and RGD cell binding domains are shown. Note that these sequences occur within EGF-like repeats. The squiggly lines represent an α -helical region where the three chains are linked together.

Fig 10. Assembly of type IV collagen into two-dimensional networks

Monomers of type IV collagen contain a triple-helical region (rod) with a non- collagenous domain (circle) at the C' terminus, shown on the right. The network model of type IV collagen assembly proposes that monomers aggregate via their N' and C' ends to form tetramers and oligomers, which subsequently form networks. Type IV collagen monomers are additionally able to assemble into hexagonal arrays. In this model, lateral aggregation beween monomers also occurs via the triple-helical domains.

(figure from R.M. Glanville (1987). Structure and function of collagen types. R. Mayne, and R.E. Burgeson. Acad. Press, Inc., New York).

The network model is based on solution studies (below) , in which adjusting the salt concentration of dilute solutions of type IV collagen leads to the initial production of oligomers (left picture) which finally associate to form networks (right picture). These images are of type IV collagen molecules, coated by rotary shadowing with carbon-platinum to make them show up easily in the electron microscope.

(micrographs from H. Furthmayr (1988). Self assembling architecture. J.E. Varner. Alan R. Liss, Inc., New york)

Fig 11. Proteoglycans

A schematic drawing of a proteoglycan (right) consisting of linear molecules of repeating disaccharide units that are covalently linked to a core protein. Proteoglycans are 90-95% carbohydrate by weight. Different types and sizes of the glycosaminoglycans can be found attached to the same core protein. The extent and location of the sulfate groups on the glycosaminoglycan (table below) vary greatly, contributing to the complexity and heterogeneity of this family of macromolecules.

(Modified from Molecular Biology Of The Cell (1983). B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J.D. Watson. Garland Pub. Inc. New York)

Figure 12. Schematic diagram showing development of the avian cornea

Inductive interaction between the lens and corneal epithelium leads to :

secretion of a primary stroma by corneal epithelium and migration, beneath the stroma, of mesenchymal cells destined to become corneal endothelium.

Secretion of hyaluronic acid by endothelial cells causes the primary stroma to swell. This allows a second wave of migration of mesenchymal cells which give rise to fibroblasts (keratocytes).

The mesenchymal cells secrete type I collagen and proteoglycans. At this stage hyaluronidase appears which may degrade hyaluronic acid and the stroma shrinks. Thyroxine from the developing thyroid gland causes loss of water and the stroma condenses, eventually becoming the transparent cornea.

(Modified from Developmental Biology (1988). S.F. Gilbert. Sinauer Associates Inc., MA)

Fig 13. The integrin heterodimer

A general structural model of the integrin heterodimer composed of an α and a ß chain, is shown here. Both integrin monomers are classical transmembrane proteins, containing a hydrophobic membrane-spanning domain and a short C' terminal cytoplasmic region, which links the molecule to elements of the cytoskeleton. Both chains of the large extracellular domain are involved with binding to ECM molecules, with important Ca^{++} binding sites at the N' terminus of the α chain. The cysteine-rich region of the ß chain plays a role in generating the correct tertiary structure of the complex.

(Modified from E. Ruoslahti (1988). Ann Rev. Biochem. 57:375-413).

Fig 14. The integrin superfamily

Distinct classes of integrins exist, classified by the type of ß chain that is present in the heterodimer. At least 3 ß families exist as shown above, although as this manuscript goes to press three more ß chains have been identified. Within each ß family are a number of chains. Each combined heterodimer has a specificity for a different ligand.

LM, laminin; VN, vitronectin; VWF, von Willebrand factor; TSP, thrombospondin. The latter three are serum proteins, involved with platelet aggregation, wound healing and the blood clotting cascade. C3bi is a complement receptor on monocytes.

(Modified from R. Hynes (1987). Cell 48:549-554).

Fig 15. One type of intracellular-extracellular linking structure: the focal contact

(1) Focal contacts, otherwise known as focal adhesions, are the cell's "feet". These are areas of the cell surface that interact with the ECM. Molecules such as FN, shown in this diagram (right) bind to their cell surface receptors, integrins, coupling cells to other ECα components or, in the case of cultured cells, to the surface of the petri dish. This structure is linked to architectural elements of the cell - the actin containing cytoskeleton - via a complex containing talin, vinculin, -actinin and other proteins. Stationary cells that adhere tightly to a FN substratum (below) show co- distribution of some of these components particularly well when they are stained with fluorescent antibodies. (From W. Chen, E. Hasegawa, T. Hasegawa, C. Weinstock and K. Yamada (1985). J. Cell Biol. 100: 1103-1114).

(2) Focal contacts illustrate one type of physical interaction that can occur between cells and their environment, although ECM components other than FN may interact with cells in different ways: Myoblasts, for example, adhere strongly to FN and as a response form focal contacts and organize their cytoskeleton to form the actin fibres characteristic of stationary cells: they probably form structures similar to those shown in the diagram above. Laminin interacts with mouse myoblasts in a completely different way. If mouse myoblasts are plated onto dishes coated with laminin, they become highly motile. They don't form focal contacts and don't redistribute proteins such as vinculin.

Myoblasts on laminin or on FN substrata, stained with antibodies against vinculin. Only when the cells spread out on FN do they form focal contacts, visible here as the vinculin stipples. (From S.L. Goodman, G. Risse, and K. von der Mark (1989). J. Cell Biol. 109:799-809).

3

Response to an ECM component through a specific ECM receptor may be necessary for the differentiation that occurs when myoblasts fuse together to form myotubes: Antibodies which block the interaction between FN and its receptor on myoblasts block their fusion into myotubes. (From A.S. Menko, and D. Boettiger (1987). Cell 51:51-57).

myoblasts fuse to form myotubes in culture

this type of differentiation is blocked by antibodies which bind to the FN-receptor

Fig 16. Tensegrity, or "how cells stand up"

The architectural elements of eukaryotic cells - tubulin, actin, keratins and their various associated proteins - are involved with many aspects of cell function, including the maintenance of cell shape. Exactly how these proteins scaffold cells into their various shapes, such as flat, round or columnar, is unclear. An attractive model proposes that they form two main types of structural element; compression-resistant and elastic. With just these two types, which might be represented by microtubules and actin fibers (respectively), numerous dynamic shapes can be built. Rather than relying on classic architectural systems, these structures are maintained by tensional integrity- or "tensegrity".

The diagram (upper figure) is drawn from a model in which compression-resistant elements (rods) are held in space by elastic fibers (thin double lines). A similar, but more complex model built on the same principle might more accurately describe the type of structure which "holds up" a cell in vivo. Consider now that the ends of the compression-resistant elements interact with clusters of molecules (such as vinculin, talin, integrin), some of which are embedded in the plasma membrane. These are represented by the knobs in the model. The three hatched ones are already in contact with a surface. By merely attaching the stippled knobs to the same surface (this requires some force and the elastic elements stretch), the shape of the whole structure is dramatically altered (lower figure). In cell terms, this change represents the formation of new focal contacts which occurs when a cell flattens out and adheres to a suitable surface - one coated with fibronectin, for example. By including more elements, and looking down onto the cell, rather than obliquely as in the diagram, one can extrapolate to the type of cytoskeletal patterns actually seen inside the flattened, stationary cells shown in photographs of Fig 15.

Fig 17. The basement membrane

Scanning electron micrograph of the cornea in a chick embryo (left). The sheet-like basement membrane (BM) separates epithelial cells (E) from the stroma, visible as an intricate network of collagen fibrils (C). The close association of basement membrane with mammary epithlial cells is also evident in the fluorescent micrograph stained by immunofluorescence for the presence of laminin (see fig. 25).

(By courtesy of R. Trelstad)

Fig 18. Origin of basement membrane components

The basement membranes that separate simple epithelia from their underlying stroma are produced by either epithelia or mesenchyme or both, depending on the tissue and the specific ECM component itself. As will become clear at the end of this chapter (Fig. 25), the milk-producing epithelial cells of mammmary glands synthesize and deposit their own basement membranes when they are cultured on artificial stroma. In a different tissue, the intestine, different ECM proteins are made by the epithelium and the mesenchyme.

A schematic diagram of intestine, on the right, shows the juxtaposition of epithelium to underlying stroma, which is composed of lamina propria and muscle layers. The basement membrane separates these two compartments.

During development of this tissue, intestinal endoderm becomes the mature epithelial layer, while mesenchymal tissue develops into the stromal component. Correct development of intestinal tissue can also occur if endoderm from one animal is mixed with mesenchyme from another, and then grown as a graft in a host animal. Species-specific antibodies can be used to identify which tissue different components come from. In the example below, rat endoderm was mixed with chicken mesenchyme and implanted into a developing embryonic host. A new intestine developed, composed of rat epithelium and stromal tissue derived from the chicken mesenchyme. Cross-sections of the newly formed tissue were stained by immunofluorescence with species-specific antibodies that recognize either chick or rodent proteoglygcan (HSPG). The basement membrane is stained only with the anti-rodent antibodies (left picture) and not detected with anti-chick antibodies (right picture), indicating that its origin is the rodent epithelium. On the other hand, the (chick) stromal tissue contains some HSPG, demonstrating that although the mesenchyme is able to make this ECM protein, it is not incorporated into basement membrane.

Similar experiments conducted with antibodies specific for chick or rodent type IV collagen show that this protein is deposited into the basement membrane by mesenchyme. These types of experiments elegantly show that two different tissue compartments, separated by a basement membrane, contribute to its formation. In the case of intestine, HSPG is supplied by epithelium and type IV collagen by mesenchyme. Correct intestinal development thus requires interaction and cooperation between these two types of tissue. (Picture from P. Simon-Assman, F. Bouziges, M. Vigny, and M. Kedinger (1989). J. Biol. Chem. 263:16536-16544).

Fig 19. Growth of mammary epithelia into the surrounding stroma

A schematic diagram (top panel) illustrating the morphogenesis of the mouse mammary gland in a virgin (V), pregnant (P), and lactating (L) animal. From a mostly ductular structure embedded in a prominent fat pad in the virgin mammary gland, lobuloalveolar development replaces the fat pad during pregnancy, to occupy the whole gland in lactation. This is illustrated in the transverse sections (lower panel) of a paraffin embedded mammary gland from each of the respective stages. In the virgin gland, ductular (D) structures composed of epithelia and stromal fibroblasts (SF) are surrounded by fat cells (F). The latter are replaced during pregnancy by ducts and budding alveoli (arrow head). Upon parturition and the onset of lactation, the alveolar development is complete. The mammary epithelia grouped in an alveolus (A) are polarized and rest on a basement membrane (BM), as shown in figure 25.

Fig 20. Ultrastructure of secretory mammary epithelial cells on a reconstituted basement membrane.

Transmission electron micrograph of mammary epithelial cells from mid-pregnant mice cultured on on a reconstituted basement membrane matrix derived from Engelbreth-Holm-Swarm (EHS) tumor for 6 days. A view of a section through a secretory epithelial cell (panel a) shows many characteristics of a differentiated secretory cell; a basally located nuclues (Nu), abundant rough endoplasmic reticulum (RER), an organized golgi apparatus (G), lipid droplets (L), apically located secretory vesicles. Neighbouring cells are joined together by tight junctions at their apical ends (panels a and b, arrows), and are surrounded on their basal side by a distinct basement membrane (panels a and c, small arrows). Bar 1 μm (panel a), bar, 0.5 μm (panel b and c). (From M. H. Barcellos-Hoff, J. Aggeler, T.G. Ram, and M.J. Bissell (1989). Development 105:223-235)

Fig 21. Effect of ECM on mammary cell morphology

Phase contrast micrograph of mammary cells from mid-pregnant mice on day 6 of culture. Cells on plastic (A) or on floating collagen (B) spread and form confluent sheets of cells. On a reconstituted basement membrane matrix (C), clusters of cells reorganize into alveolar-like structures. Bar 100 μm. From L-H. Chen and M. J. Bissell (1989). Cell Regulation 1:45-54.

Fig 22. Traverse section of mammary cell aggregates cultured on a reconstituted basement membrane matrix.

(A) Traverse section of an alveolus from a lactating mouse mammary gland (Paraffin embedded, stained with hematoxylin and eosin). (B) Transmission electron micrograph of a section through a spherical cell structure formed by aggregation of dissociated mammary cells from mid-pregnant mice grown on a reconstituted basement membrane matrix. Bar 10 μm. (From C. H. Streuli and M.J. Bissell (1990a). In press).

Fig 23. Regulation of milk protein gene expression by ECM

Milk proteins gene expression is regulated by the type of substratum the cells are grown on. The expression of the whey acidic protein (WAP) mRNA is most notable in tissue from mid-pregnant mice (lane 0), or when the cells are cultured on a reconstituted basement membrane matrix and organize into alveolar-like structures (lane 1). Although transferrin (Tf) mRNA is expressed by cells on all substrata (EHS, lane 1; Fixed EHS, lane 2; floating type I collagen, lane 3; and plastic, lane 4), ß-casein is not expressed by cells on plastic. (From L-H. Chen and M.J. Bissell (1989). Cell Regulation1:45-54).

Fig 24. Reciprocal expression of basement membrane components and milk protein mRNA

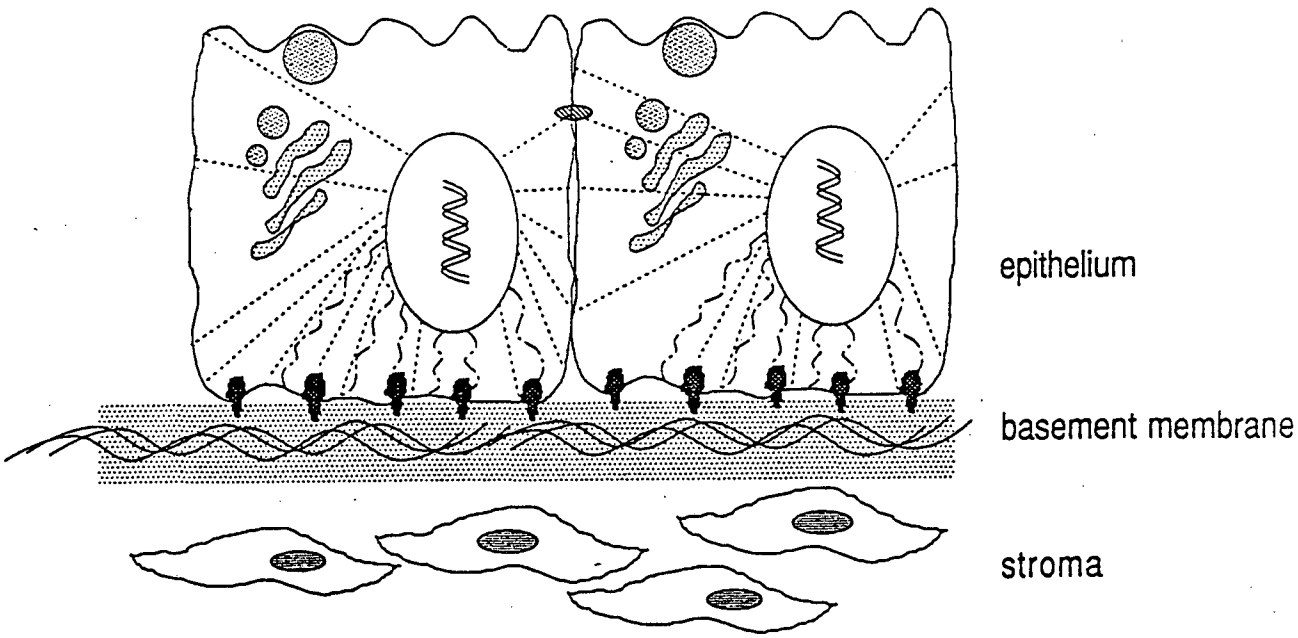
Mammary epithelial cells of mid pregnant mice from an intact gland (M), freshly dissociated (To), and cells on day 6 of culture on floating type I collagen (FI) express abundant levels of milk protein mRNA, whereas their mRNA levels for laminin and type IV collagen are very low. Although expressing low levels of basement membrane component mRNA, mammary cells on floating type I collagen and in the gland deposit their own basement membrane (see Fig. 25). The reverse pattern is true for undifferentiated cells cultured on plastic (P). (From C. H. Streuli and M. J. Bissell (1990b). J. Cell Biol., In press).

Fig 25. Deposition of basement membrane by mammary cells in culture and in vivo.

Thick transverse sections of cells on type I collagen stained for the presence of laminin on days 2 and 3 of culture (top panel). Lower panel is a Nomarski image of the same section. Mouse mammary cells cultured on type I collagen deposit their own basement membrane only after floatation of the collagen gel on day 2 of culture. The deposition of the basement membrane corresponds to differentiation events and expression of milk proteins. (From C. H. Streuli and M. J. Bissell (1990b). J. Cell Biol., In press). Below a thick section of a lactating mouse mammary gland shows epithelial cells grouped together as circular acini (see also Fig. 19), with the laminin-containing basement membrane delineating the basal side of the cells.

Fig 1. Schematic diagrams of simple and complex epithelium

The basement membrane forms a thin sheet that separates stroma from epithelium, shown below in the schematic diagram of simple epithelium. The underlying supportive stroma, otherwise known as " connective tissue", contains ECM components in the form of "interstitial matrix" as well as capillaries and various cell types, such as fibroblasts. The epithelial cells directly interact with components of the basement membrane and the stroma. Cells such as fibroblasts migrate through the stroma, and turn over its components to maintain functional integrity.



More components are involved with the architecture of complex epithelium, shown on the right. This cross-section of skin (cutis) shows multiple layers of keratinizing epithelium (epidermis). Beneath this layer, and separated from it by a basement membrane, is the connective tissue, comprised of both dermis and below this (but not shown here), the subcutaneous fatty stroma. Hair follicles and sweat glands originate in the dermis, which also contains blood and lymph capillaries and has abundant nerve endings.

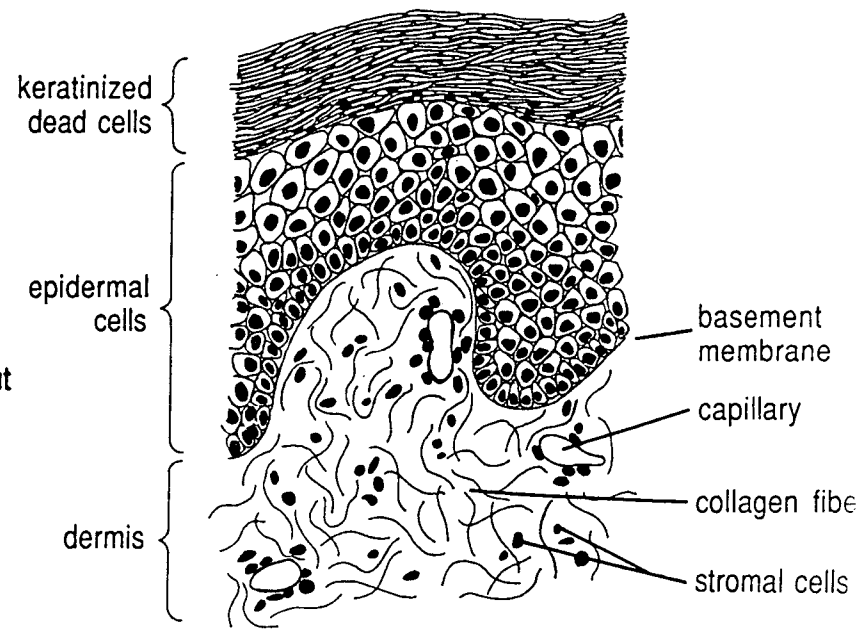
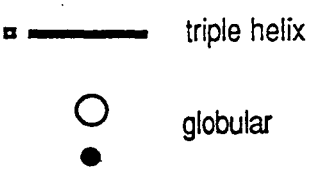


Fig 2. The collagen family

type	composition	structure	location
I	$\alpha 1(I)_2$ $\alpha 2(I)$		skin, bone, tendon
II	$\alpha 1(II)_3$		cartilage, vitreous body
III	$\alpha 1(III)_3$		skin, blood vessels, organs
IV	$\alpha 1(IV)_2 \alpha 2(IV)$		basement membranes
V	$\alpha 1(V)$ $\alpha 2(V)$ $\alpha 3(V)$		interstitial tissue, basement membranes
VI	$\alpha 1(VI)$ $\alpha 2(VI)$ $\alpha 3(VI)$		blood vessels, kidney, skin, placenta, muscle, liver
VII	$\alpha 1(VII)_3$		skin, chorioamniotic membranes
VIII	$\alpha 1(VIII)_3$		endothelial cells
IX	$\alpha 1(IX)$ $\alpha 2(IX)$ $\alpha 3(IX)$		cartilage
X	$\alpha 1(X)_3$		cartilage
XI	$\alpha 1(XI)$ $\alpha 2(XI)$ $\alpha 3(XI)$		cartilage
XII	$\alpha 1(XII)$		



Currently 12 vertebrate collagens have been identified, each with varying numbers (subscripts) and types of chains. In the standard notation, chain types are shown in arabic numerals, followed in parentheses by the collagen type (roman numerals). The fibrils formed by types I, II, III, and V are able to assemble into fibers. The overall structure of the collagen fibrils is drawn approximately to scale, with their triple helical and globular domains; some of these are removed after formation of the collagen trimer (open circles and rods). The filled regions show the extent of the molecule that remains when embedded in the ECM.

(modified from K. Kuhn (1987) Structure and function of collagen types. Acad. Press, Inc., New York; and R.E. Burgeson (1988) Ann. Rev. Cell Biol. 4:551-557)

Fig 3. Assembly of the collagen molecule

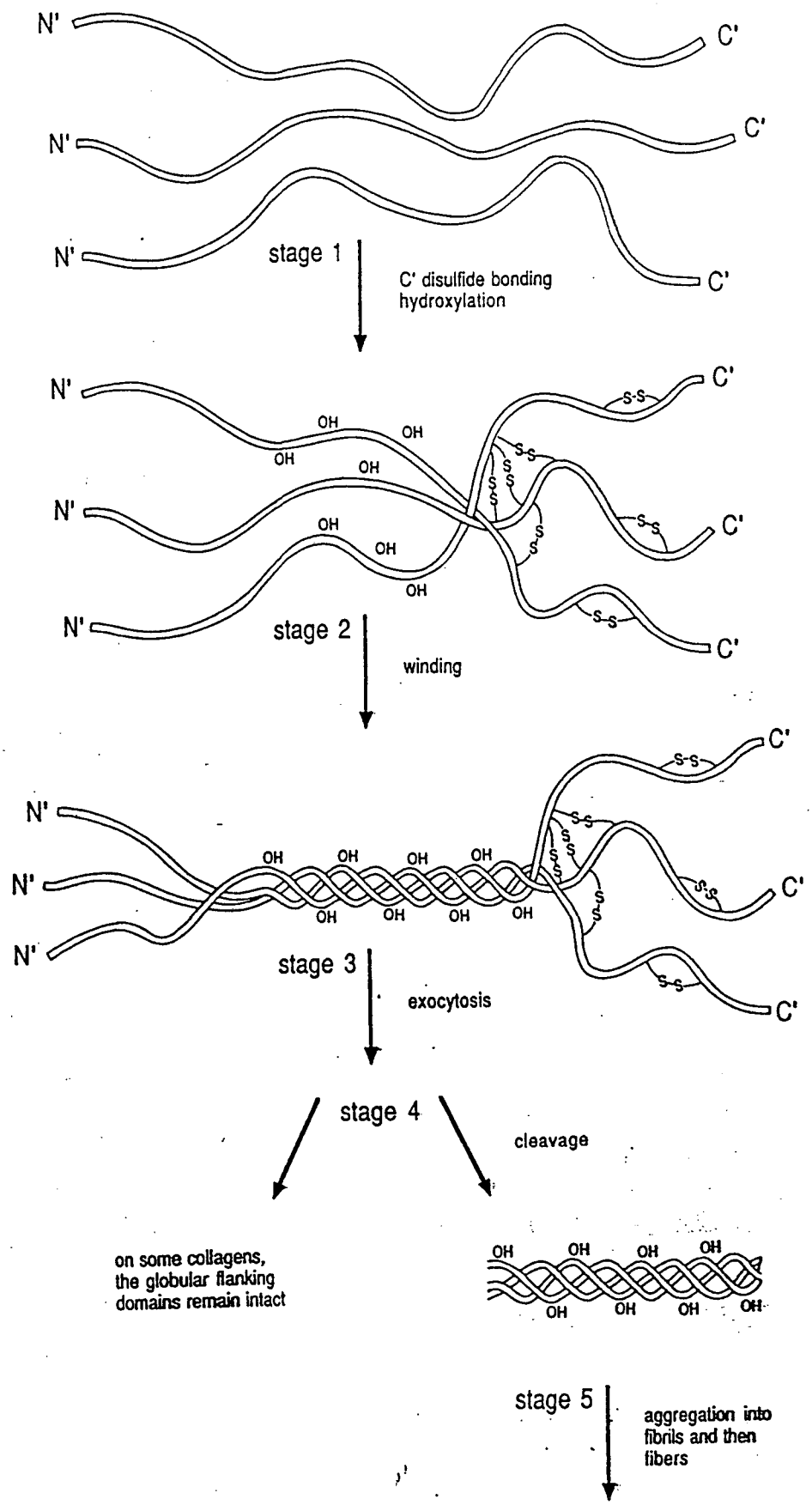
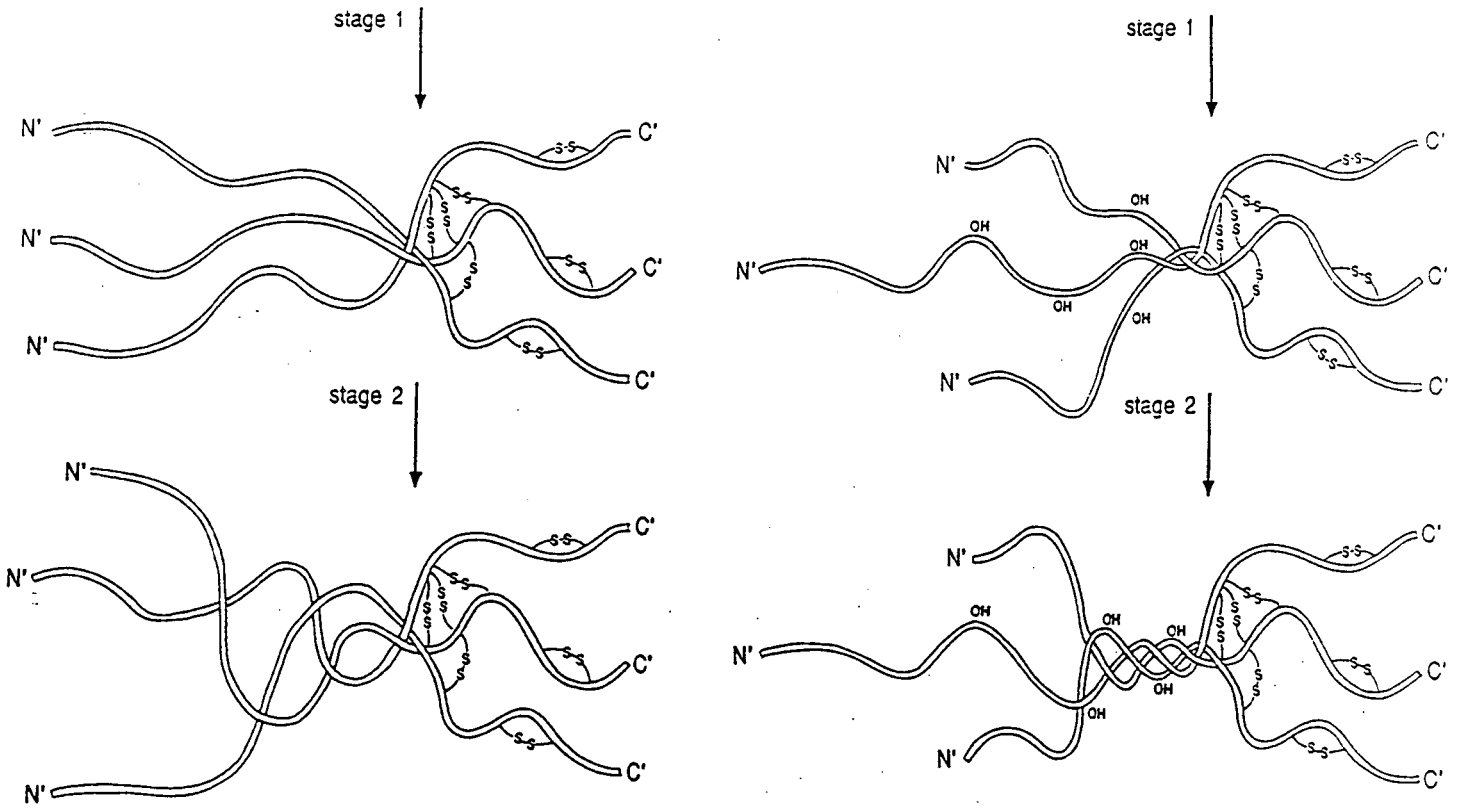


Fig 4. Improper assembly of collagen leads to severe connective tissue defects



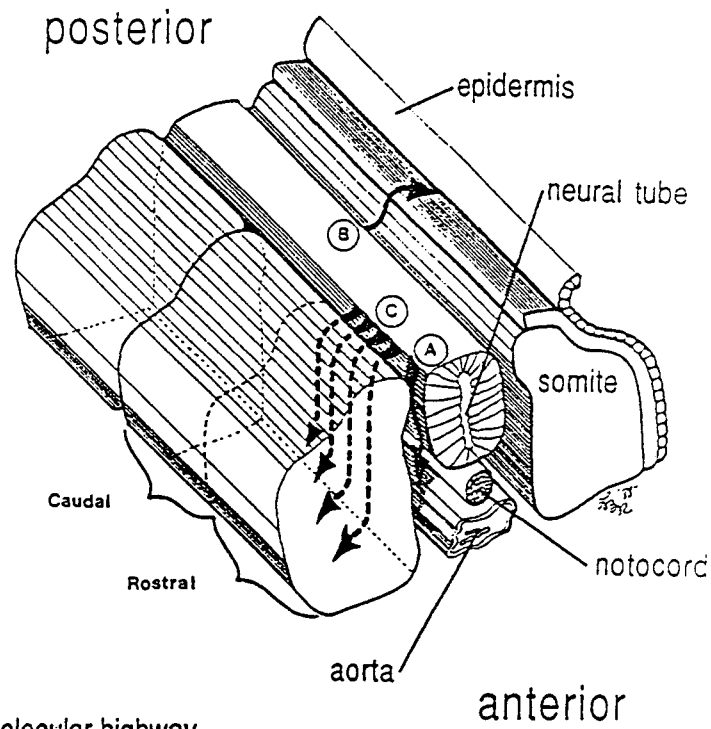
Prolyl hydroxylase relies on ascorbic acid (vitamin C) for its activity. Scurvy is a disease caused by a severe lack of vitamin C. This deficiency results in low activity of prolyl hydroxylase, which ultimately leads to the inability of collagen to form a triple helix.

In the inherited disesase, osteogenesis imperfecta, single mutations or gross deletions in the collagen chains prevent formation of the triple helix. Osteogenesis imperfecta is a spectrum of disease, with some mutations having more severe consequences than others. In general, mutations towards the C' end of the procollagen molecule correlate with a more serious prognosis as they interfere more with the winding process.

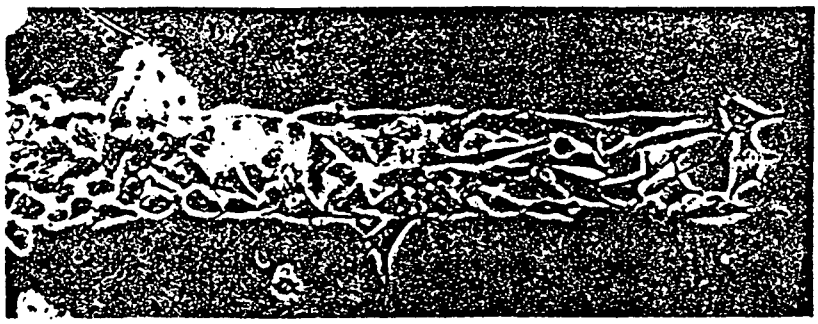
In both cases, the improperly formed helices are either rapidly degraded inside the cell -- by "procollagen suicide" -- or, if they get out, collagen fibrils are incorrectly assembled into fibrils resulting in considerably weakened fibers with less tensile strength.

Fig 5. FN as a molecular highway in embryonic development

During early embryonic development, massive migrations of primitive cells take place. These processes ultimately lead to the correct spatial positioning of various tissues and organs which make up the mature body form. One example of embryonic migration is that of the neural crest (see picture, right), whose cells give rise to numerous cell types such as sensory and sympathetic ganglia, connective tissue forming cells and pigment cells. These cells migrate along the pathways shown (A, B, C), on sheets of ECM proteins which are deposited before movement takes place. FN is a crucial component of these pathways, as can be demonstrated by injecting embryos with antibodies directed against the cell surface receptors for FN. In such experiments, neural crest migration is completely (but reversibly) blocked. (By courtesy of R. Perris, and M. Bronner-Fraser).

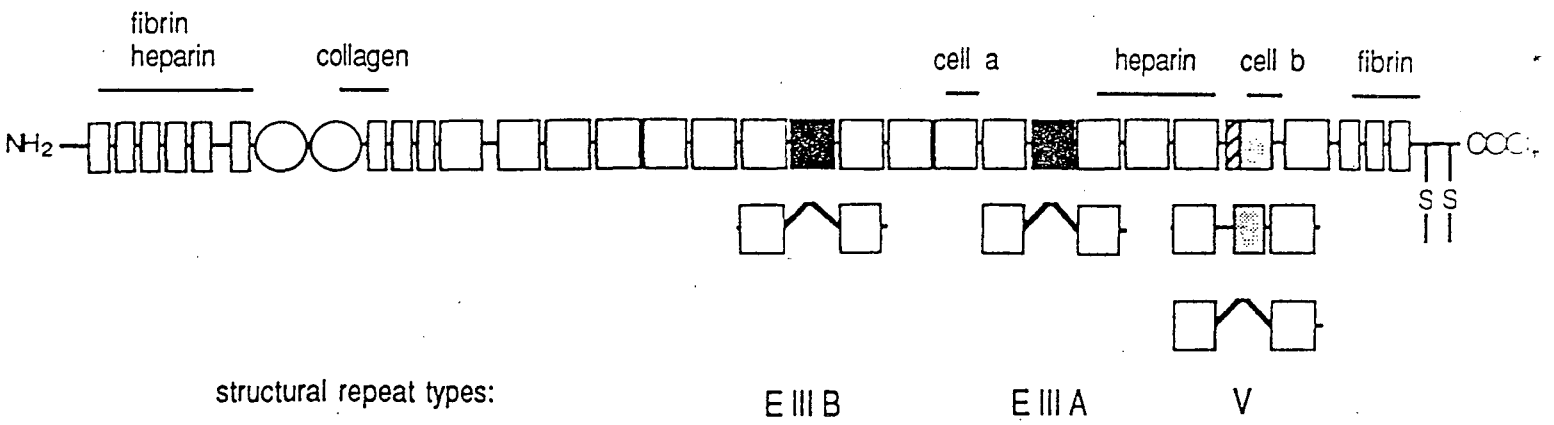


The potent role of FN in providing these cells with a molecular highway for migration can be illustrated (below) by coating dishes with strips of FN. Populations of neural crest cells (seeded at left) migrate only on the FN strips (top picture), in a process that can be documented by time lapse videomicroscopy (bottom picture).



(from J.P. Thiery; J-L. Duband, S. Dufour, P. Savanger, and B.A. Imhof (1988). Fibronectin. D.F. Mosher, Acad. Press Inc. New York)

Fig 6. Features of the FN molecule



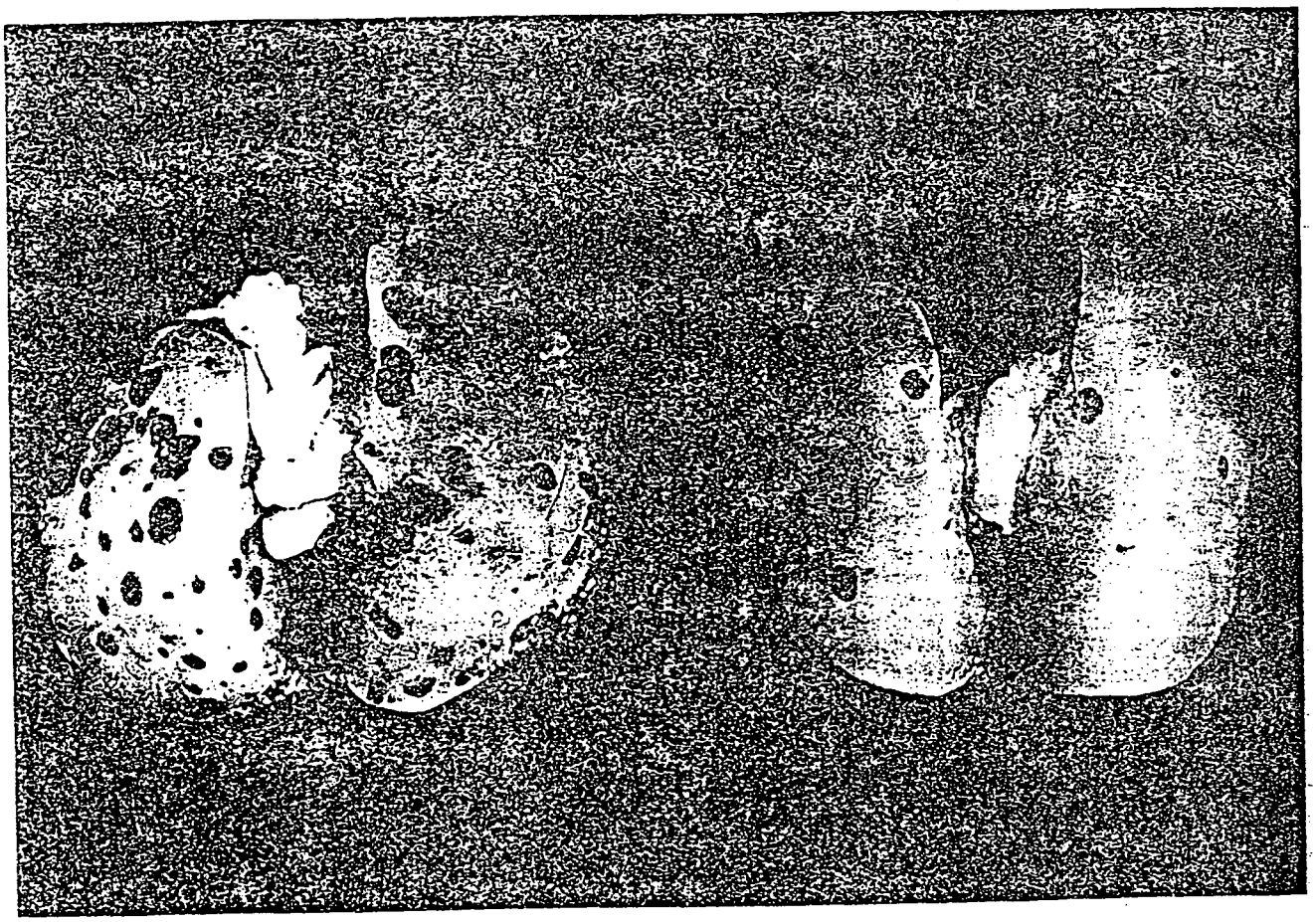
Alternative splicing at three distinct sites (E III B, E III A and V) of the rat FN gene allow 12 possible FN polypeptides to be synthesized. Since the final FN molecule is a dimer, at least 100 dimer variants are conceivable, although many fewer than this have been currently identified.

(modified from R. Hynes (1985). Ann. Rev. Cell Biol. 1:67-90)

FN is an adhesive molecule which links cells to other extracellular macromolecules, such as collagen and proteoglycans. The cell binding domains, "a" and "b", are indicated, as are the regions which bind to other ECM components such as collagen. FN binds to proteoglycans at the heparin site. The fibrin sites are crucial in the binding of FN to blood clots. The main region in FN which binds to cells is domain "a", which contains the sequence GRGDS. Peptides containing only these amino acids mediate cell attachment to plastic dishes via the cell surface FN receptor. Numerous proteins contain this sequence and its interaction with the FN receptor is discussed in more detail below. An additional cell binding domain (site "b") occurs in the region V. Since this cell binding site can be modified by alternative splicing, shown as the three alternate splice patterns in the figure, it may regulate the tissue specificity of different types of FN molecule.

Fig 7. Peptides which interfere with binding of cells to their ECM can inhibit experimental metastasis

The B16-F10 cell line derived from a mouse malignant melanoma is an example of an aggressive experimental tumor that has a strong tendency to metastasize to lung and other tissue. As with many cell types, the binding of these melanoma cells to substrata is mediated by ECM components such as FN. This interaction is one feature of these cells that allows them to invade neighboring tissues and to form metastatic tumors, shown below (left) as lung metastases. Peptides derived from the cell binding region, which contains the sequence GRGDS, interfere with the binding of these cells to their natural substrata. When mice are co-injected with B16-F10 melanoma cells and high doses of the peptide (3 mg/mouse, figure on right) fewer tumors develop. Control peptides with the sequence GRGES or GRDGS fail to inhibit metastasis.

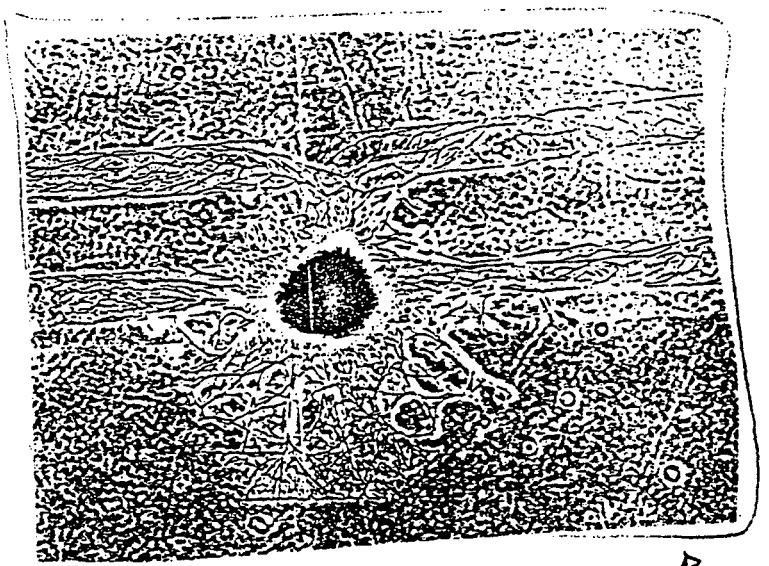


Although this is a simplistic model and the B16 melanoma cell line is uniquely sensitive to such treatment, the principle of the approach may provide a rational basis for the therapy of certain types of aggressively malignant human tumors.

(courtesy of M.J. Humphries, and K.M. Yamada)

Fig 8. Laminin guides the outgrowth of neurites

When central nervous system (CNS) neurons are plated onto culture dishes, neurites grow out if the dishes have been previously coated with laminin. Although some other ECM components such as FN and certain collagens have some "neurite outgrowth" promoting effect, laminin is by far the most potent. Here, sensory axons are being guided onto stripes of laminin, avoiding the other areas which have been coated with type IV collagen.



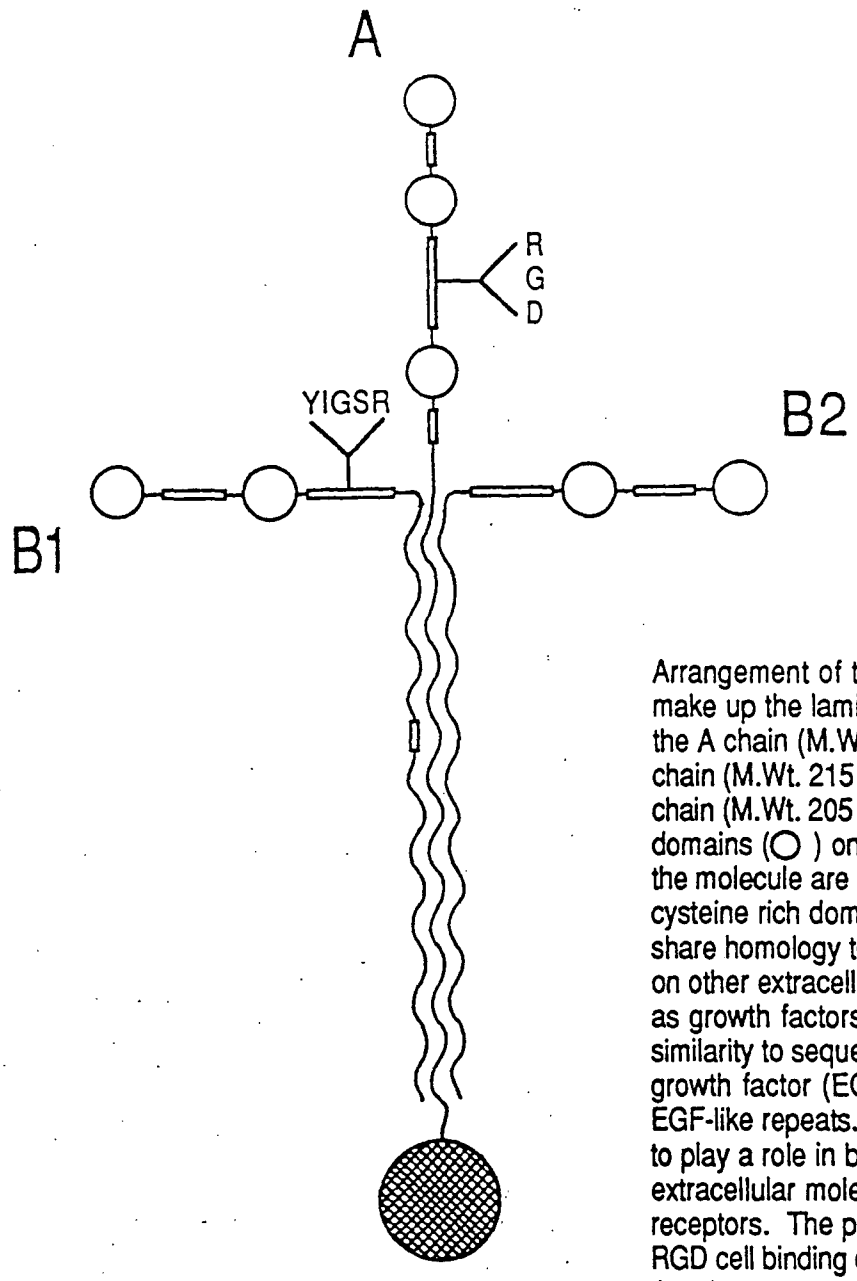
A fragment of laminin, its large globular region (hatched domain, Fig 9) mimics this type of activity, indicating that this is the region in the laminin molecule which promotes neurite outgrowth.

similar fig to this one

This type of experiment, coupled with the observation that laminin is transiently expressed by astrocytes after brain injury, suggests that laminin may be involved in regeneration of damaged CNS tissue. It may also be a key regulator in the development of the CNS.

(Courtesy of R.W. Gundersen)

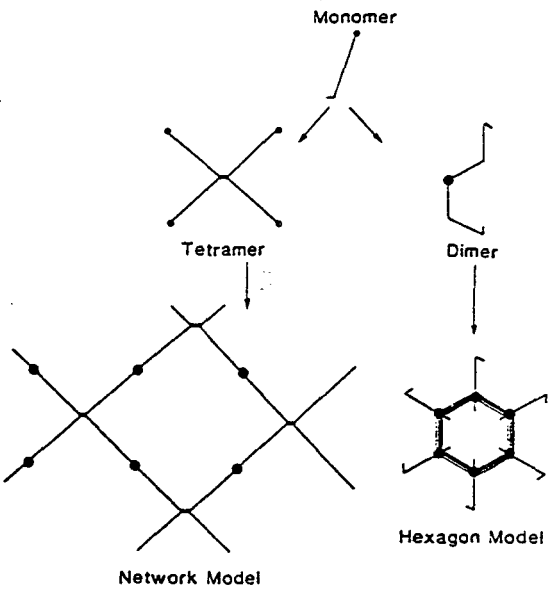
Fig 9. A structural model for laminin



Arrangement of the 3 chains that make up the laminin "crucifix" are the A chain (M.Wt. 400 kDa), the B1 chain (M.Wt. 215 kDa) and the B2 chain (M.Wt. 205 kDa). The globular domains (○) on the short arms of the molecule are seperated by cysteine rich domains (═), which share homology to similar regions on other extracellular molecules such as growth factors. Because of their similarity to sequences on epidermal growth factor (EGF), they are called EGF-like repeats. These domains appear to play a role in binding many types of extracellular molecules to their cell-surface receptors. The position of the YIGSR and RGD cell binding domains are shown. Note that these sequences occur within EGF-like repeats. The squiggly lines represent an α-helical region where the three chains are linked together.

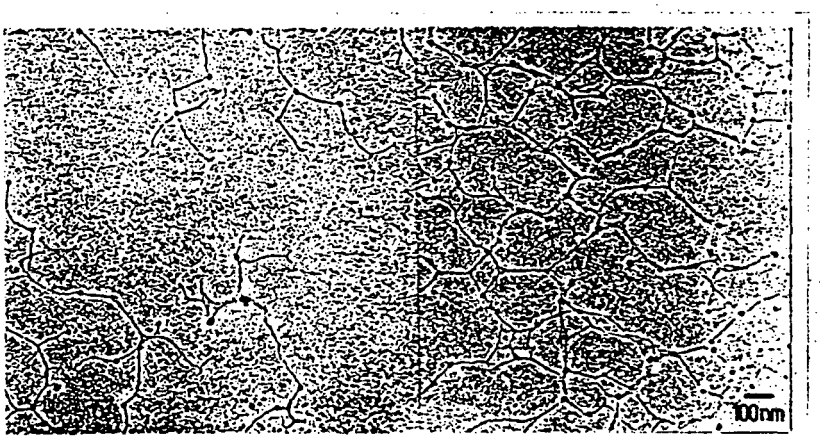
Fig 10. Assembly of type IV collagen into two-dimensional networks

Monomers of type IV collagen contain a triple-helical region (rod) with a non-collagenous domain (circle) at the C' terminus, shown on the right. The network model of type IV collagen assembly proposes that monomers aggregate via their N' and C' ends to form tetramers and oligomers, which subsequently form networks. Type IV collagen monomers are additionally able to assemble into hexagonal arrays. In this model, lateral aggregation beween monomers also occurs via the triple-helical domains.



(figure from R.M. Glanville (1987). Structure and function of collagen types. R. Mayne, and R.E. Burgeson. Acad. Press, Inc., New York).

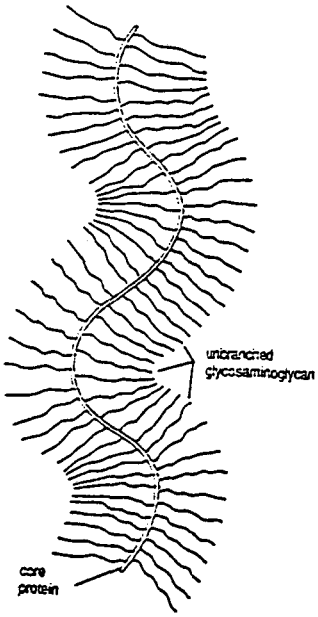
The network model is based on solution studies (below) , in which adjusting the salt concentration of dilute solutions of type IV collagen leads to the initial production of oligomers (left picture) which finally associate to form networks (right picture). These images are of type IV collagen molecules, coated by rotary shadowing with carbon-platinum to make them show up easily in the electron microscope.



(micrographs from H. Furthmayr (1988). Self assembling architecture. J.E. Varner. Alan R. Liss, Inc., New york)

Fig 11. Proteoglycans

Schematic drawing of a proteoglycan (right) consisting of a core protein that is covalently linked to linear molecules of repeating disaccharide units. Proteoglycans are 90-95% carbohydrate by weight. Different types and sizes of the glycosaminoglycans can be found attached to the same core protein. The extent and location of the sulfate groups on the glycosaminoglycan (table below) vary greatly, contributing to the complexity and heterogeneity of this family of macromolecules.

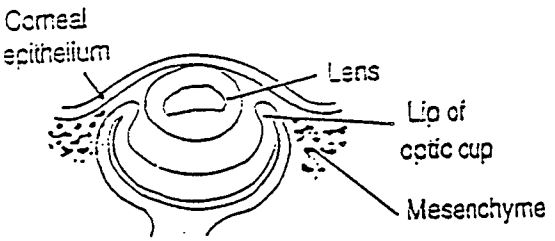


The major proteoglycan groups

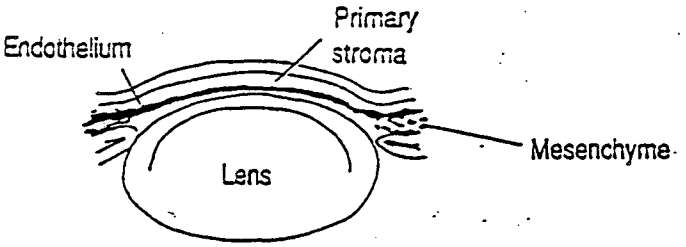
Proteoglycan	Molecular Weight	Repeating Disaccharide $(A\text{-}B)_n$		Sulfates per Disaccharide	Other Sugars	Tissue Distribution
		Monosaccharide A	Monosaccharide B			
Heparan sulfate	5,000 - 12,000	D-glucuronic acid or L-iduronic acid	N-acetyl-D-glucosamine	0.2 - 0.3	D-galactose D-xylose	lung, arteries, cell surfaces mammary gland
Heparin	6,000 - 25,000	D-glucuronic acid or L-iduronic acid	N-acetyl-D-glucosamine	2.0 - 3.0	D-galactose D-xylose	lung, liver, skin, mast cells
Chondroitin sulfate	5,000 - 50,000	D-glucuronic acid	N-acetyl-D-galactosamine	0.2 - 0.3	D-galactose D-xylose	cartilage, cornea, bone, skin, arteries, mammary gland
Dermatan sulfate	15,000 - 40,000	D-glucuronic acid or L-iduronic acid	N-acetyl-D-galactosamine	1.0 - 2.0	D-galactose D-xylose	skin, heart blood vessels, mammary gland
Keratan sulfate	4,000 - 19,000	D-galactose	N-acetyl-D-glucosamine	0.9 - 1.8	D-galactos-amine, D-mannose, L-fucose, sialic acid	cartilage, cornea, inter-vertebral disc

(Modified from Molecular Biology Of The Cell (1983). B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J.D. Watson. Garland Pub. Inc. New York)

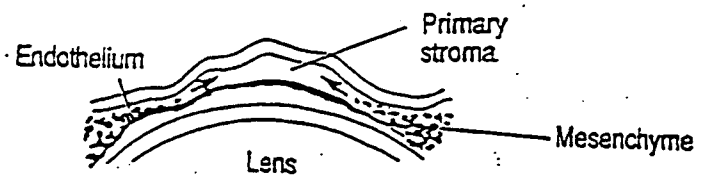
Figure 12. Schematic diagram showing development of the avian cornea



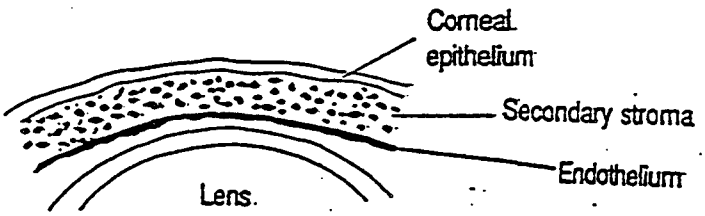
Inductive interaction between the lens and corneal epithelium leads to :



secretion of a primary stroma by corneal epithelium and migration, beneath the stroma, of mesenchymal cells destined to become corneal endothelium.



Secretion of hyaluronic acid by endothelial cells causes the primary stroma to swell. This allows a second wave of migration of mesenchymal cells which give rise to fibroblasts (keratocytes).

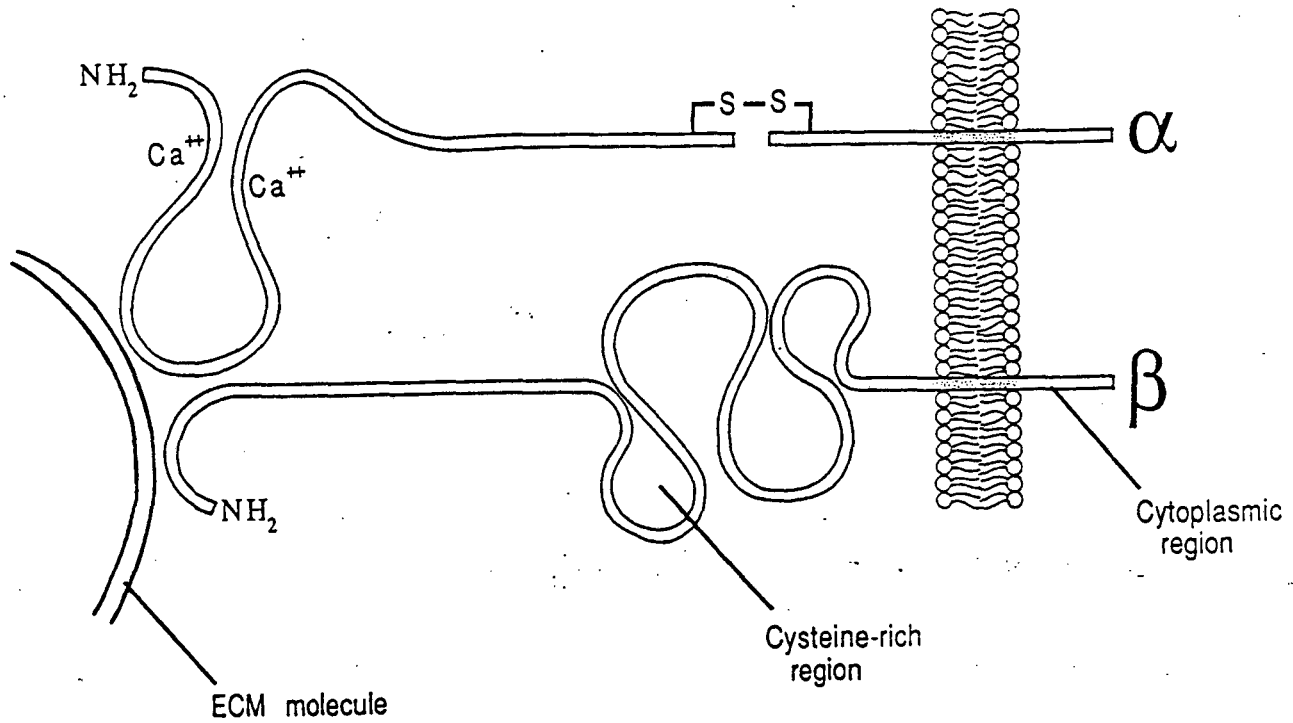


The mesenchymal cells secrete type I collagen and proteoglycans. At this stage hyaluronidase appears which may degrade hyaluronic acid and the stroma shrinks. Thyroxine from the developing thyroid gland causes loss of water and the stroma condenses, eventually becoming the transparent cornea.

(Modified from Developmental Biology (1988). S.F. Gilbert. Sinauer Associates Inc., MA)

Fig 13. The integrin heterodimer

A general structural model of the integrin heterodimer composed of an α and a ß chain, is shown here. Both integrin monomers are classical transmembrane proteins, containing a hydrophobic membrane-spanning domain and a short C' terminal cytoplasmic region, which links the molecule to elements of the cytoskeleton. Both chains of the large extracellular domain are involved with binding to ECM molecules, with important Ca++ binding sites at the N' terminus of the α chain. The cysteine-rich region of the ß chain plays a role in generating the correct tertiary structure of the complex.



(Modified from E. Ruoslahti (1988). Ann Rev. Biochem. 57:375-413.

Fig 14. The integrin superfamily

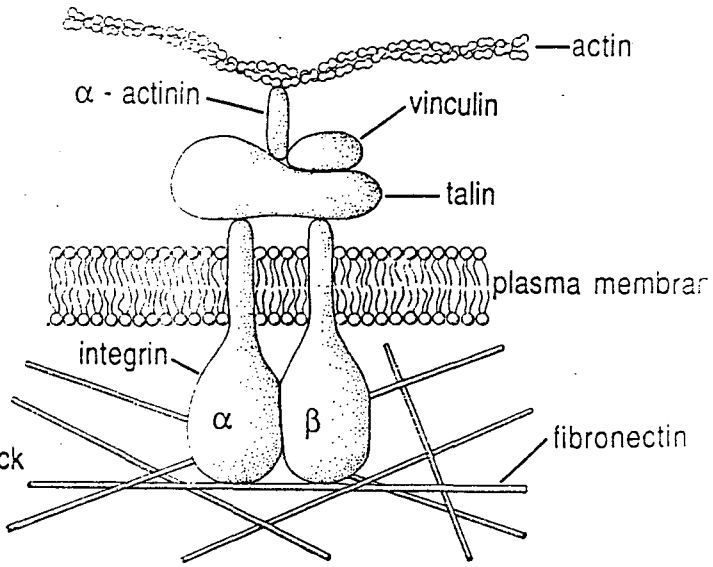
family	associated α chain	name of the complex	known ligands
β_1	α_1	VLA-1	LM
	α_2	VLA-2	collagens
	α_3	VLA-3	FN, LM, collagens
	α_4	VLA-4	FN
	α_5	VLA-5	FN
	α_6	VLA-6	LM
β_2	α_L	LFA-1	?
	α_M	Mac-1	C3bi
β_3	α_{IIb}	glycoprotein IIb IIIa	VN, FN, VWF, TSP
	α_V	vitronectin receptor	

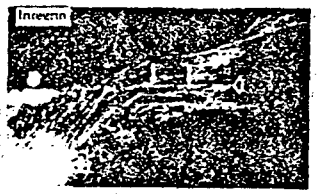
Distinct classes of integrins exist, classified by the type of ß chain that is present in the heterodimer. At least 3 ß families exist as shown above, although as this manuscript goes to press three more ß chains have been identified. Within each ß family are a number of α chains. Each combined heterodimer has a specificity for a different ligand.
LM, laminin; VN, vitronectin; VWF, von Willebrand factor; TSP, thrombospondin. The latter three are serum proteins, involved with platelet aggregation, wound healing and the blood clotting cascade. C3bi is a complement receptor on monocytes.

(Modified from R. Hynes (1987). Cell 48:549-554.

Fig 15. One type of intracellular-extracellular linking structure: the focal contact

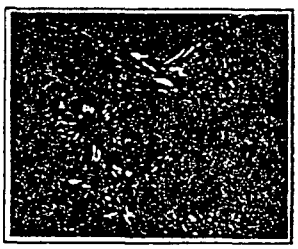
(1) Focal contacts, otherwise known as focal adhesions, are the cell's "feet". These are areas of the cell surface that interact with the ECM. Molecules such as FN, shown in this diagram (right) bind to their cell surface receptors, integrins, coupling cells to other ECM components or, in the case of cultured cells, to the surface of the petri dish. This structure is linked to architectural elements of the cell - the actin containing cytoskeleton - via a complex containing talin, vinculin, α -actinin and other proteins. Stationary cells that adhere tightly to a FN substratum (below) show co-distribution of some of these components particularly well when they are stained with fluorescent antibodies. (From W. Chen, E. Hasegawa, T. Hasegawa, C. Weinstock and K. Yamada (1985). J. Cell Biol. 100: 1103-1114).





a similar fig to this one

(2) Focal contacts illustrate one type of physical interaction that can occur between cells and their environment, although ECM components other than FN may interact with cells in different ways: Myoblasts, for example, adhere strongly to FN and as a response form focal contacts and organize their cytoskeleton to form the actin fibres characteristic of stationary cells: they probably form structures similar to those shown in the diagram above. Laminin interacts with mouse myoblasts in a completely different way. If mouse myoblasts are plated onto dishes coated with laminin, they become highly motile. They don't form focal contacts and don't redistribute proteins such as vinculin.



Myoblasts on laminin ▲ or on FN ▲ substrata, stained with antibodies against vinculin. Only when the cells spread out on FN do they form focal contacts, visible here as the vinculin stipples. (From S.L. Goodman, G. Risse, and K. von der Mark (1989). J. Cell Biol. 109:799-809).

(3) Response to an ECM component through a specific ECM receptor may be necessary for the differentiation that occurs when myoblasts fuse together to form myotubes: Antibodies which block the interaction between FN and its receptor on myoblasts block their fusion into myotubes. (From A.S. Menko, and D. Boettiger (1987). Cell 51:51-57).

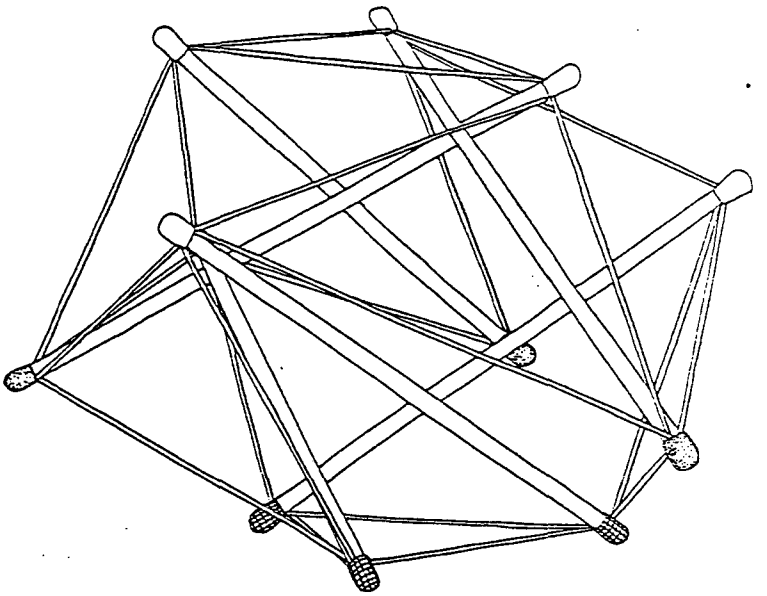
myoblasts fuse to form myotubes in culture

this type of differentiation is blocked by antibodies which bind to the FN-recept

Fig 16. Tensegrity, or "how cells stand up"

The architectural elements of eukaryotic cells - tubulin, actin, keratins and their various associated proteins - are involved with many aspects of cell function, including the maintenance of cell shape. Exactly how these proteins scaffold cells into their various shapes, such as flat, round or columnar, is unclear. An attractive model proposes that they form two main types of structural element; compression-resistant and elastic. With just these two types, which might be represented by microtubules and actin fibers (respectively), numerous dynamic shapes can be built. Rather than relying on classic architectural systems, these structures are maintained by tensional integrity- or "tensegrity".

The diagram (upper figure) is drawn from a model in which compression-resistant elements (rods) are held in space by elastic fibers (thin double lines). A similar, but more complex model built on the same principle might more accurately describe the type of structure which "holds up" a cell in vivo. Consider now that the ends of the compression-resistant elements interact with clusters of molecules (such as vinculin, talin, integrin), some of which are embedded in the plasma membrane. These are represented by the knobs in the model. The three hatched ones are already in contact with a surface. By merely attaching the stippled knobs to the same surface (this requires some force and the elastic elements stretch), the shape of the whole structure is dramatically altered (lower figure). In cell terms, this change represents the formation of new focal contacts which occurs when a cell flattens out and adheres to a suitable surface - one coated with fibronectin, for example. By including more elements, and looking down onto the cell, rather than obliquely as in the diagram, one can extrapolate to the type of cytoskeletal patterns actually seen inside the flattened, stationary cells shown in photographs of Fig 15.



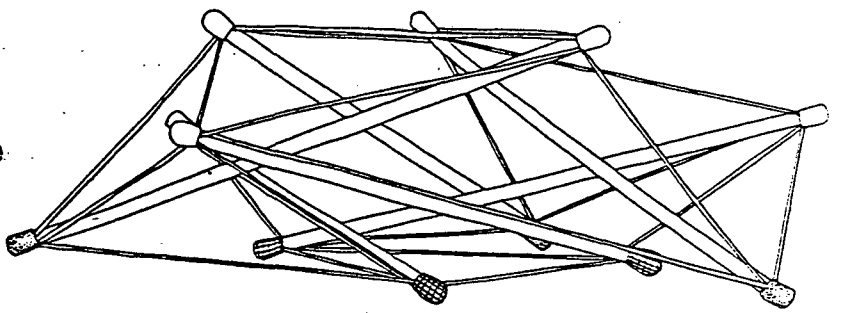
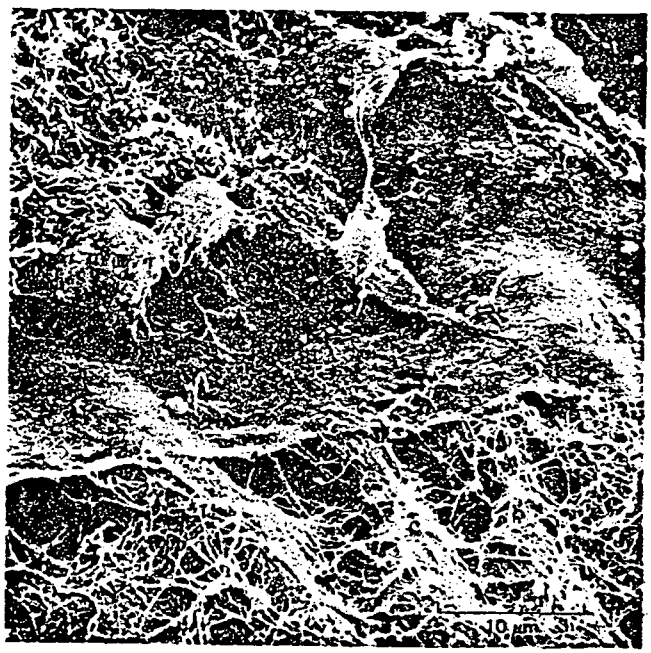


Fig 17. The basement membrane



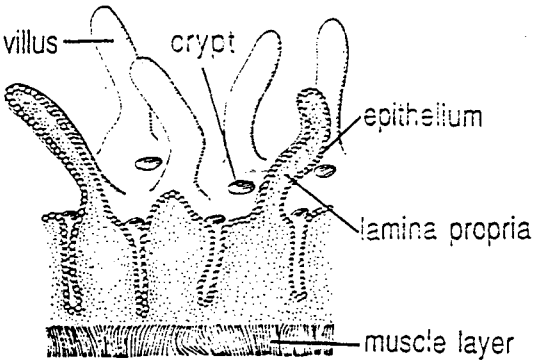
Scanning electron micrograph of the cornea in a chick embryo (left). The sheet-like basement membrane (BM) separates epithelial cells (E) from the stroma, visible as an intricate network of collagen fibrils (C). The close association of basement membrane with mammary epithlial cells is also evident in the fluorescent micrograph stained by immunofluorescence for the presence of laminin (see fig. 25).

(By courtesy of R. Trelstad)

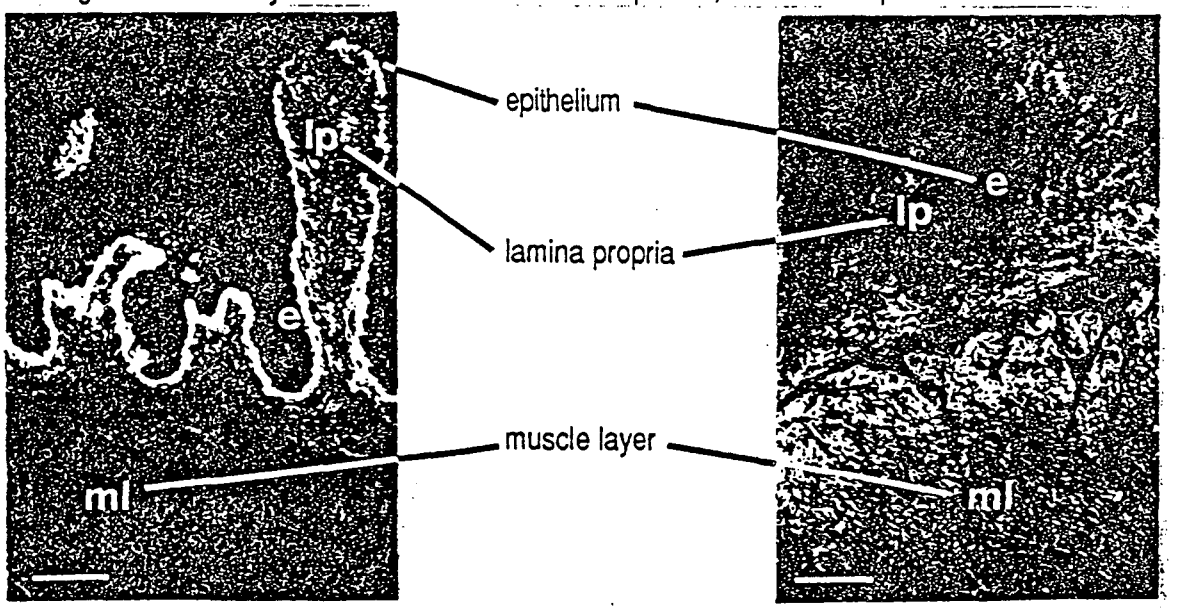
Fig 18. Origin of basement membrane components

The basement membranes that separate simple epithelia from their underlying stroma are produced by either epithelia or mesenchyme or both, depending on the tissue and the specific ECM component itself. As will become clear at the end of this chapter (Fig. 25), the milk-producing epithelial cells of mammmary glands synthesize and deposit their own basement membranes when they are cultured on artificial stroma. In a different tissue, the intestine, different ECM proteins are made by the epithelium and the mesenchyme.

A schematic diagram of intestine, on the right, shows the juxtaposition of epithelium to underlying stroma, which is composed of lamina propria and muscle layers. The basement membrane separates these two compartments.

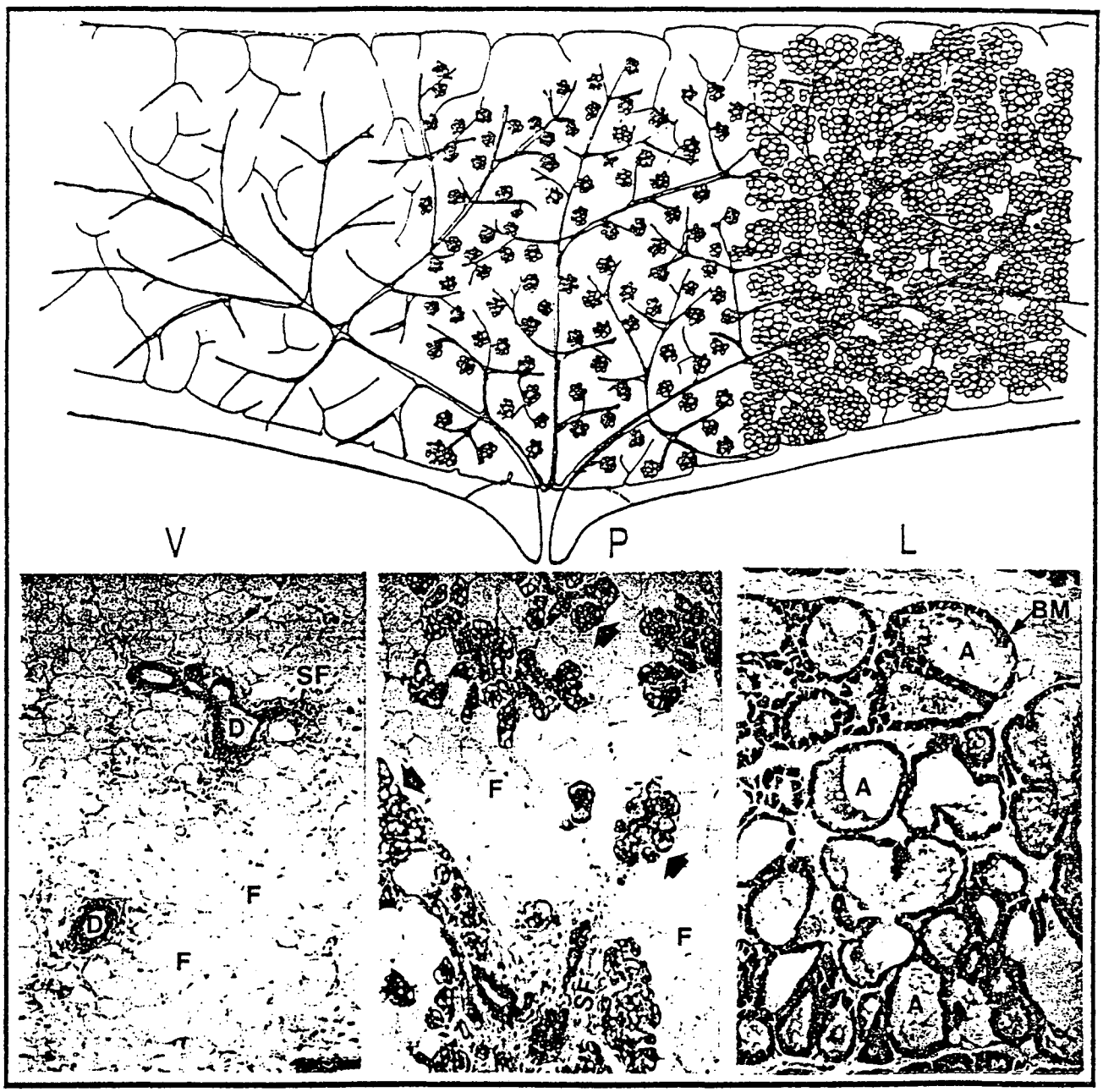


During development of this tissue, intestinal endoderm becomes the mature epithelial layer, while mesenchymal tissue develops into the stromal component. Correct development of intestinal tissue can also occur if endoderm from one animal is mixed with mesenchyme from another, and then grown as a graft in a host animal. Species-specific antibodies can be used to identify which tissue different components come from. In the example below, rat endoderm was mixed with chicken mesenchyme and implanted into a developing embryonic host. A new intestine developed, composed of rat epithelium and stromal tissue derived from the chicken mesenchyme. Cross-sections of the newly formed tissue were stained by immunofluorescence with species-specific antibodies that recognize either chick or rodent proteoglygcan (HSPG). The basement membrane is stained only with the anti-rodent antibodies (left picture) and not detected with anti-chick antibodies (right picture), indicating that its origin is the rodent epithelium. On the other hand, the (chick) stromal tissue contains some HSPG, demonstrating that although the mesenchyme is able to make this ECM protein, it is not incorporated into basement membrane.



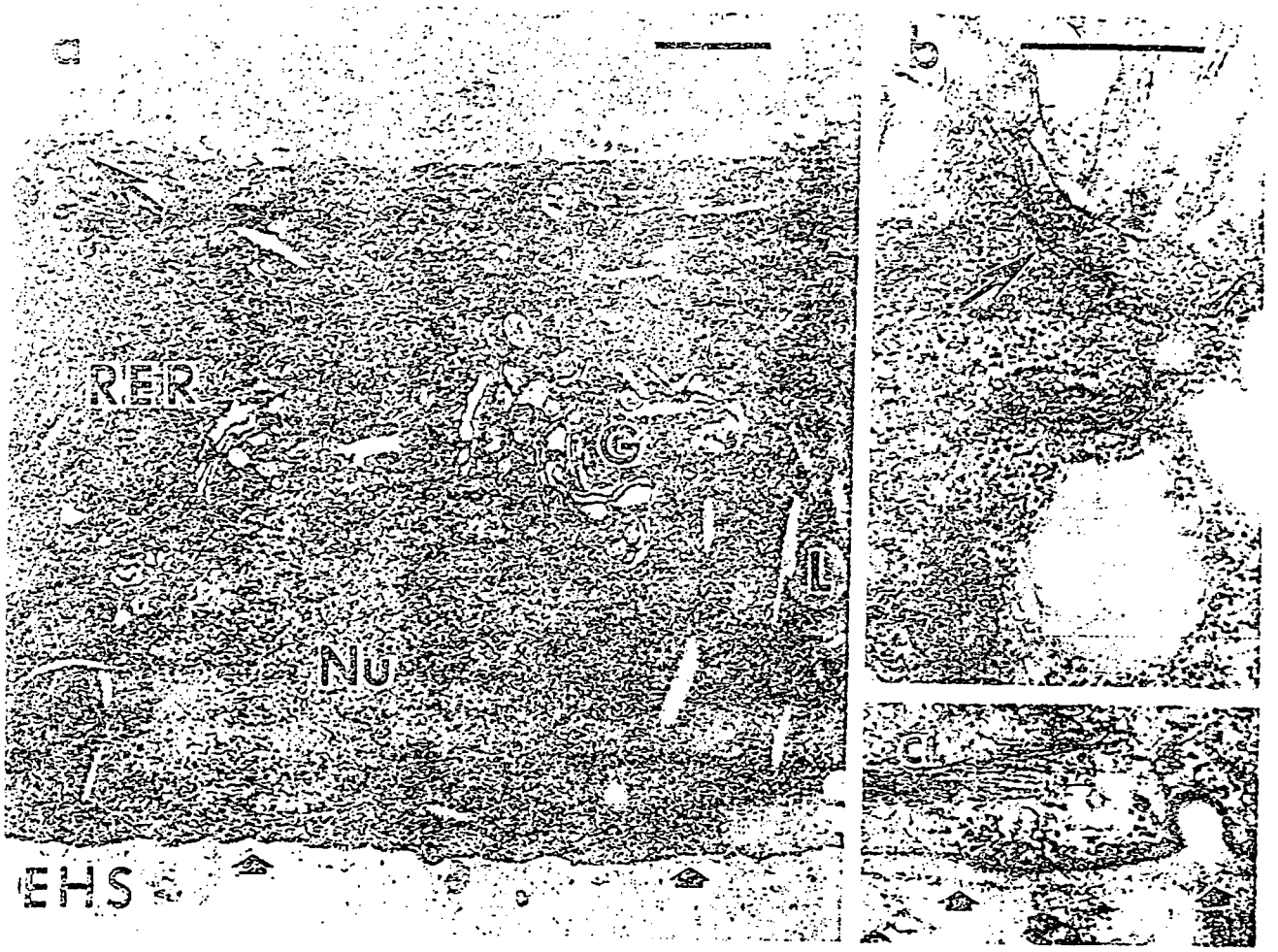
Similar experiments conducted with antibodies specific for chick or rodent type IV collagen show that this protein is deposited into the basement membrane by mesenchyme. These types of experiments elegantly show that two different tissue compartments, separated by a basement membrane, contribute to its formation. In the case of intestine, HSPG is supplied by epithelium and type IV collagen by mesenchyme. Correct intestinal development thus requires interaction and cooperation between these two types of tissue. (Picture from P. Simon-Assman, F. Bouziges, M. Vigny, and M. Kedinger (1989). J. Biol. Chem. 263:16536-16544).

Fig 19. Growth of mammary epithelia into the surrounding stroma



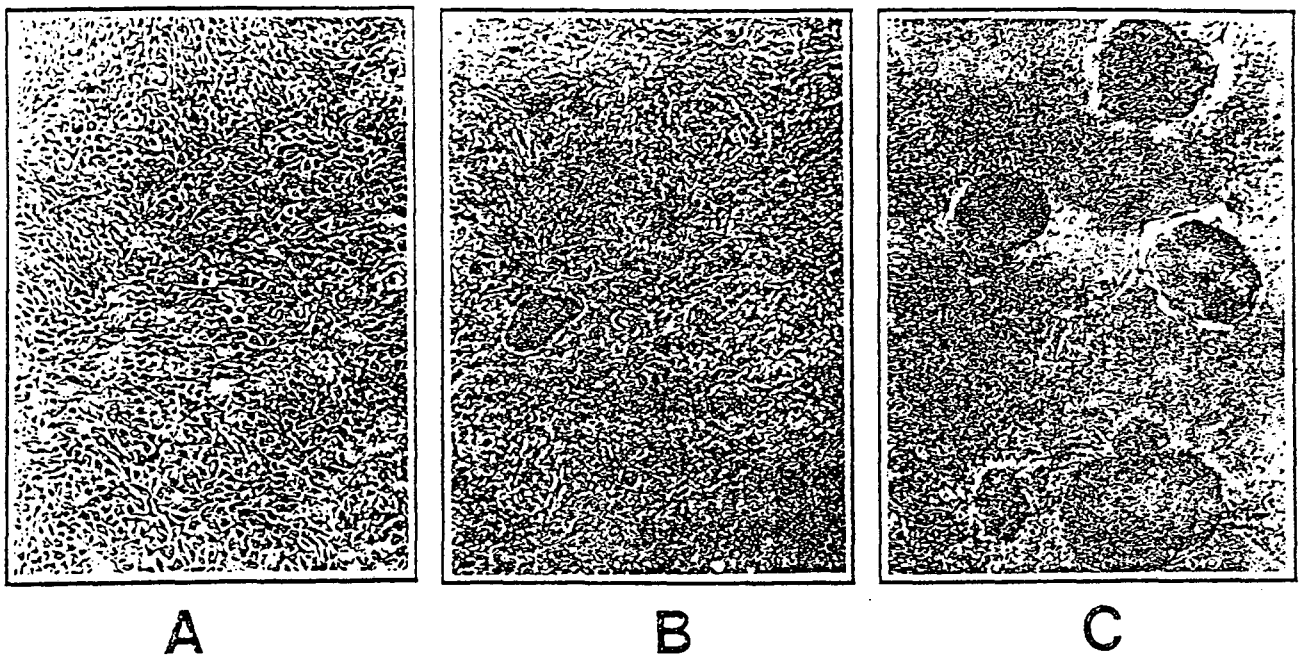
A schematic diagram (top panel) illustrating the morphogenesis of the mouse mammary gland in a virgin (V), pregnant (P), and lactating (L) animal. From a mostly ductular structure embedded in a prominent fat pad in the virgin mammary gland, lobuloalveolar development replaces the fat pad during pregnancy, to occupy the whole gland in lactation. This is illustrated in the transverse sections (lower panel) of a paraffin embedded mammary gland from each of the respective stages. In the virgin gland, ductular (D) structures composed of epithelia and stromal fibroblasts (SF) are surrounded by fat cells (F). The latter are replaced during pregnancy by ducts and budding alveoli (arrow head). Upon parturition and the onset of lactation, the alveolar development is complete. The mammary epithelia grouped in an alveolus (A) are polarized and rest on a basement membrane (BM), as shown in figure 25.

Fig 20. Ultrastructure of secretory mammary epithelial cells on a reconstituted basement membrane.



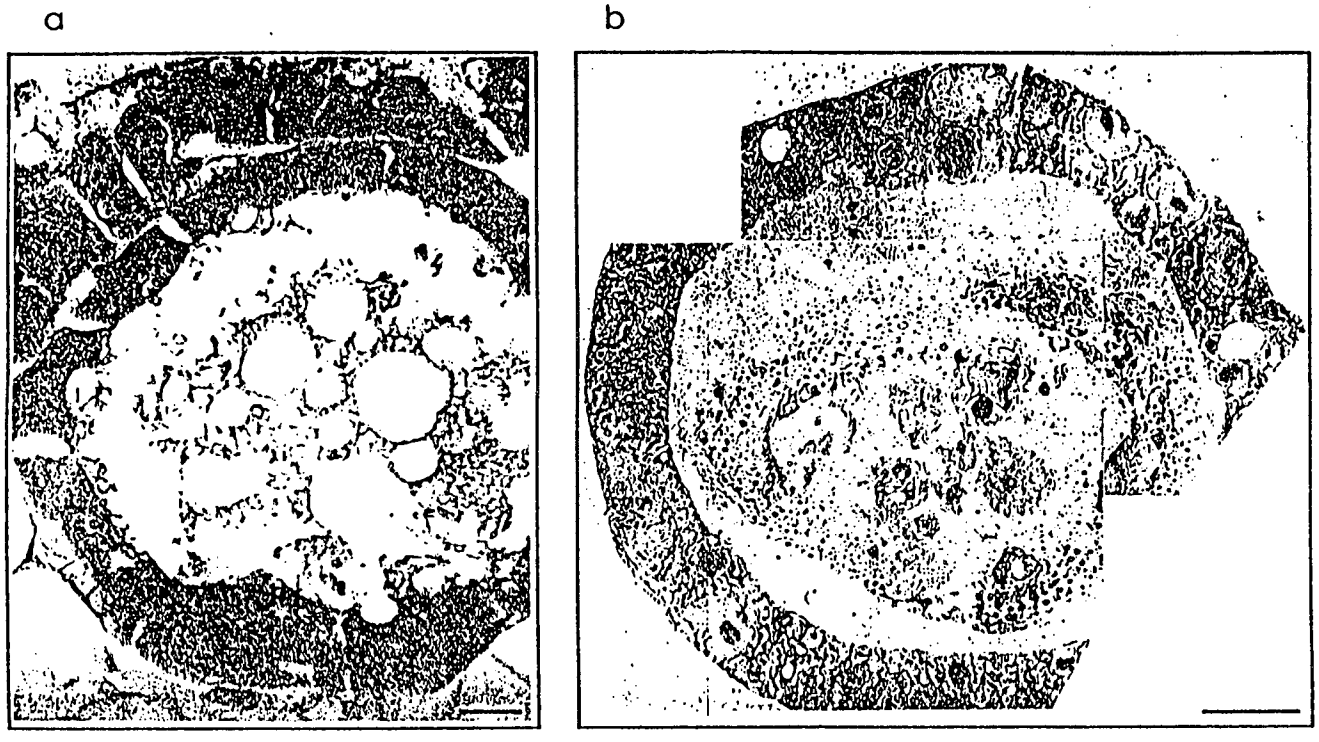
Transmission electron micrograph of mammary epithelial cells from mid-pregnant mice cultured on on a reconstituted basement membrane matrix derived from Engelbreth-Holm-Swarm (EHS) tumor for 6 days. A view of a section through a secretory epithelial cell (panel a) shows many characteristics of a differentiated secretory cell; a basally located nuclues (Nu), abundant rough endoplasmic reticulum (RER), an organized golgi apparatus (G), lipid droplets (L), apically located secretory vesicles. Neighbouring cells are joined together by tight junctions at their apical ends (panels a and b, arrows), and are surrounded on their basal side by a distinct basement membrane (panels a and c, small arrows). Bar 1 µm (panel a), bar, 0.5 µm (panel b and c). (From M. H. Barcellos-Hoff, J. Aggeler, T.G. Ram, and M.J. Bissell (1989). Development 105:223-235)

Fig 21. Effect of ECM on mammary cell morphology



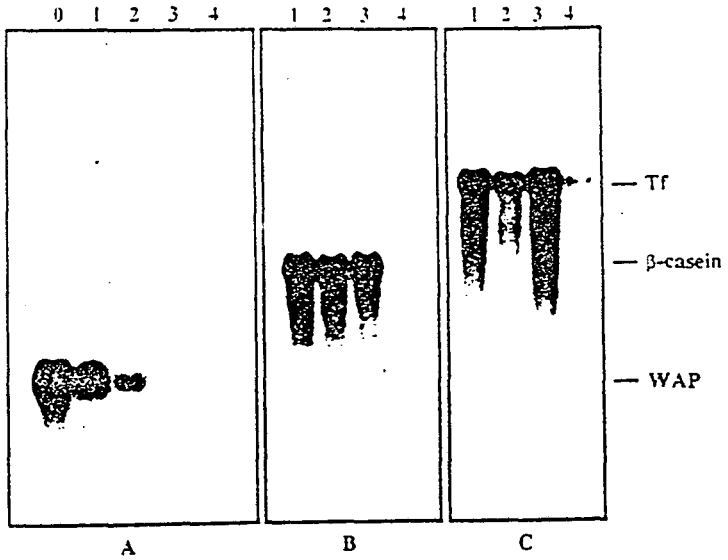
Phase contrast micrograph of mammary cells from mid-pregnant mice on day 6 of culture. Cells on plastic (A) or on floating collagen (B) spread and form confluent sheets of cells. On a reconstituted basement membrane matrix (C), clusters of cells reorganize into alveolar-like structures. Bar 100 μm. From L-H. Chen and M. J. Bissell (1989). Cell Regulation 1:45-54.

Fig 22. Traverse section of mammary cell aggregates cultured on a reconstituted basement membrane matrix.



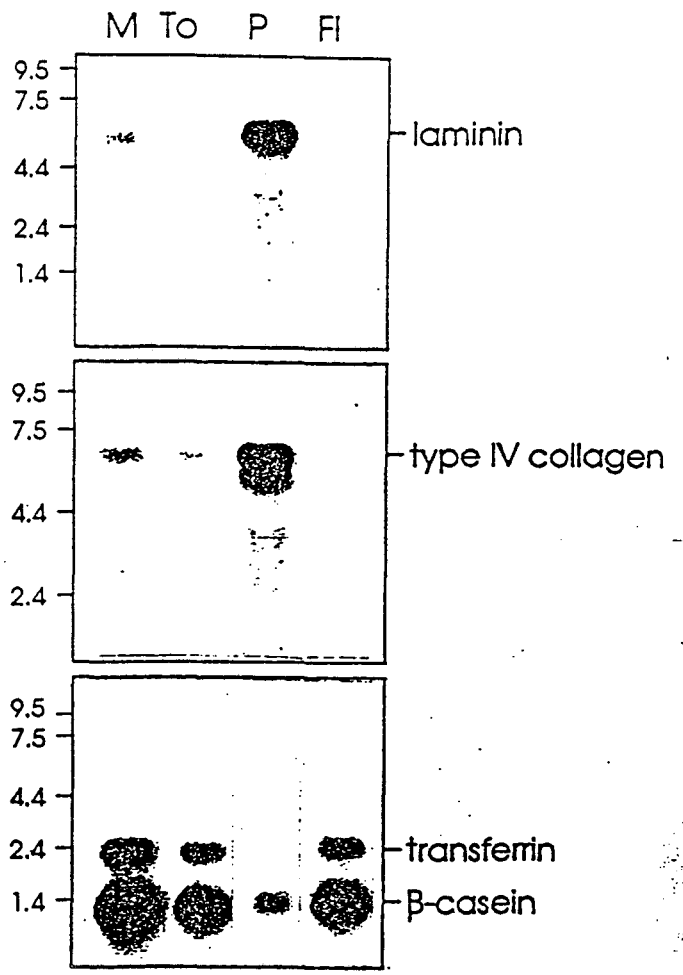
(A) Traverse section of an alveolus from a lactating mouse mammary gland (Paraffin embedded, stained with hematoxylin and eosin). (B) Transmission electron micrograph of a section through a spherical cell structure formed by aggregation of dissociated mammary cells from mid-pregnant mice grown on a reconstituted basement membrane matrix. Bar 10 μm.
(From C. H. Streuli and M.J. Bissell (1990a). In press).

Fig 23. Regulation of milk protein gene expression by ECM



Milk proteins gene expression is regulated by the type of substratum the cells are grown on. The expression of the whey acidic protein (WAP) mRNA is most notable in tissue from mid-pregnant mice (lane 0), or when the cells are cultured on a reconstituted basement membrane matrix and organize into alveolar-like structures (lane 1). Although transferrin (Tf) mRNA is expressed by cells on all substrata (EHS, lane 1; Fixed EHS, lane 2; floating type I collagen, lane 3; and plastic, lane 4), ß-casein is not expressed by cells on plastic. (From L-H. Chen and M.J. Bissell (1989). Cell Regulation1:45-54).

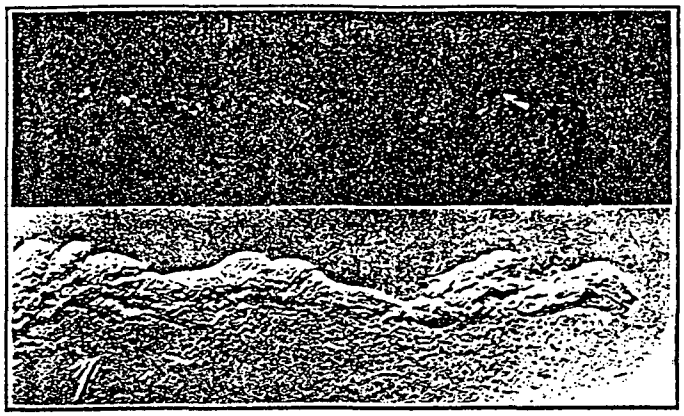
Fig 24. Reciprocal expression of basement membrane components and milk protein mRNA



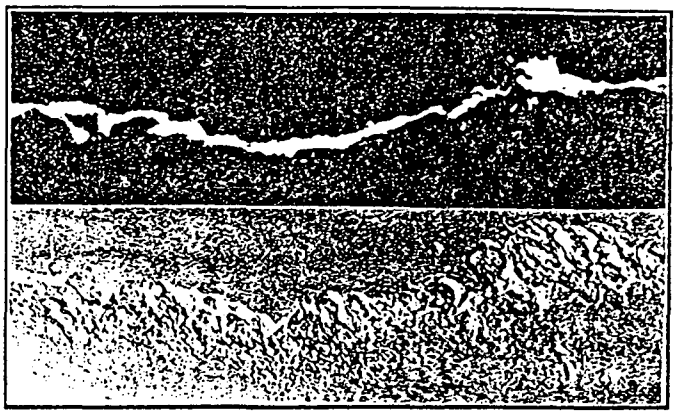
Mammary epithelial cells of mid-pregnant mice from an intact gland (M), freshly dissociated (To), and cells on day 6 of culture on floating type I collagen (Fl) express abundant levels of milk protein mRNA, whereas their mRNA levels for laminin and type IV collagen are very low. Although expressing low levels of basement membrane components mRNA, mammary cells on floating type I collagen and in the gland deposit their own basement membrane (see Fig. 25). The reverse pattern is true for undifferentiated cells cultured on plastic (P). (From C. H. Streuli and M. J. Bissell (1990b). J. Cell Biol., In press).

Fig 25. Deposition of basement membrane by mammary cells in culture and in vivo.

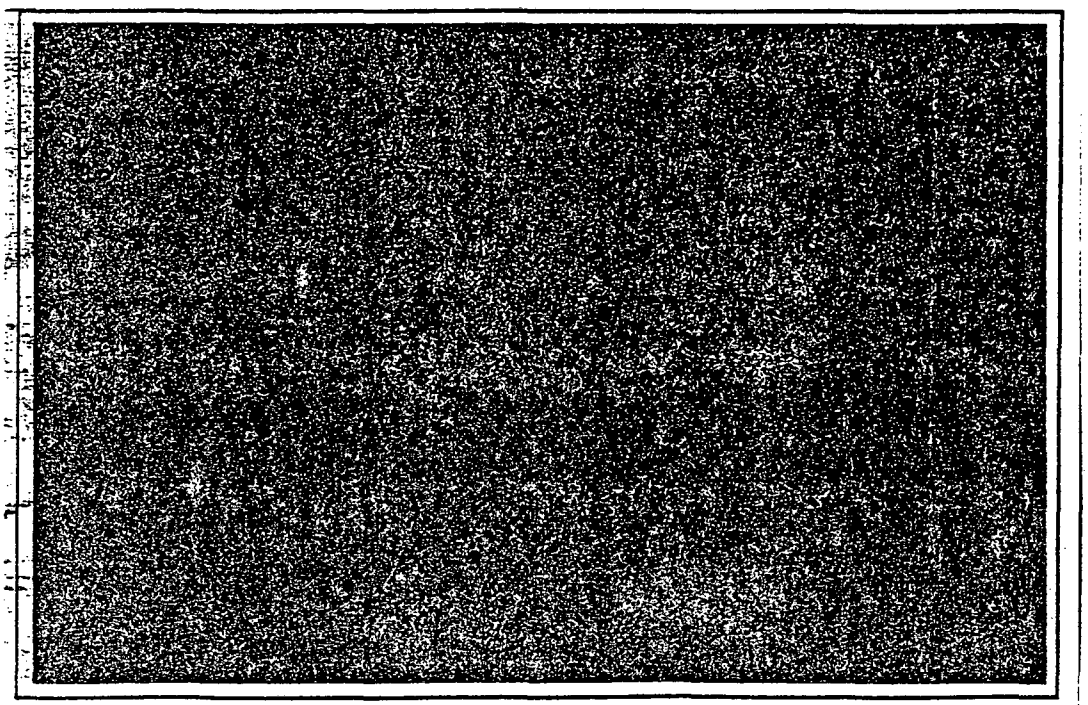
day 2



day 3



Thick transverse sections of cells on type I collagen stained for the presence of laminin on days 2 and 3 of culture (top panel). Lower panel is a Nomarski image of the same section. Mouse mammary cells cultured on type I collagen deposit their own basement membrane only after floatation of the collagen gel on day 2 of culture. The deposition of the basement membrane corresponds to differentiation events and expression of milk proteins. (From C. H. Streuli and M. J. Bissell (1990b). J. Cell Biol., In press). Below a thick section of a lactating mouse mammary gland shows epithelial cells grouped together as circular acini (see also Fig. 19), with the laminin-containing basement membrane delineating the basal side of the cells.



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