



Lawrence Berkeley Laboratory

UNIVERSITY OF CALIFORNIA

Materials & Chemical Sciences Division

To be published as a chapter in 1992 McGraw-Hill Yearbook of
Science & Technology, S. Parker, Ed., McGraw-Hill, Inc.,
New York, NY, 1992

Extracellular Matrix (ECM) Guides Tissue-Specific Function & Developmental Processes

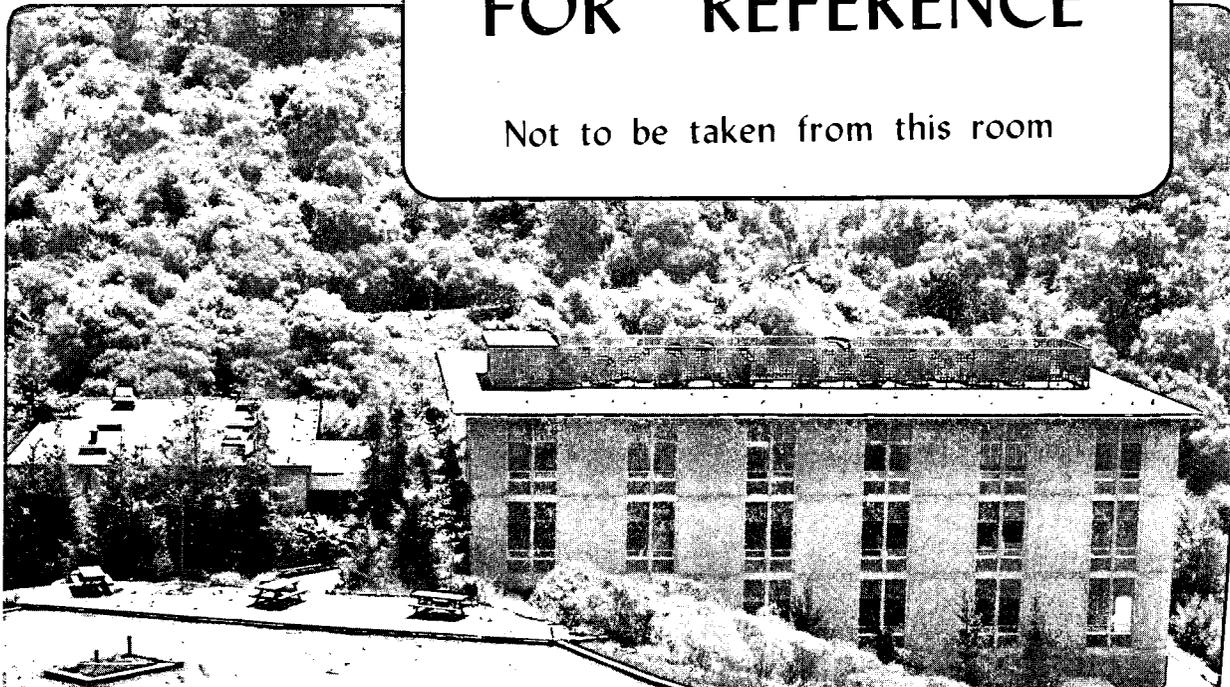
M.J. Bissell, A.R. Howlett, and C.H. Streuli

November 1990

U. C. Lawrence Berkeley Laboratory
Library, Berkeley

FOR REFERENCE

Not to be taken from this room



Prepared for the U.S. Department of Energy under Contract Number DE-AC03-76SF00098

Bldg. 50 Library.
Copy 1

LBL-30643

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

**Extracellular Matrix (ECM) Guides Tissue-Specific
Function & Developmental Processes**

Mina J. Bissell, Anthony R. Howlett, and Charles H. Streuli

Cell & Molecular Biology Division
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

November 1990

In: 1992 McGraw-Hill Yearbook of Science & Technology. In press. (1990)

**EXTRACELLULAR MATRIX (ECM) GUIDES TISSUE-SPECIFIC
FUNCTION & DEVELOPMENTAL PROCESSES**

MINA J. BISSELL, ANTHONY R. HOWLETT & CHARLES H. STREULI

**CELL & MOLECULAR BIOLOGY DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CA**

This report has been reproduced directly from the best available copy.

Most cells in higher organisms are in contact with each other and with an extensive network of glycoproteins and proteoglycans referred to as extracellular matrix (ECM) [Fig. 1]. In connective tissues the ECM is composed mainly of type I and III collagen, proteoglycans and accessory glycoproteins. The collagens are a family of large proteins, rich in glycine and proline, and characterized by a triple helical structure. We currently know of thirteen distinct collagens. In organs such as exocrine and endocrine glands or skin, epithelial and mesenchymal components are separated by a form of ECM referred to as basement membrane (bm). While it is clear that there are some differences between the bm in different tissues, the overall composition is similar. All bm's contain laminin, type IV collagen, heparan sulphate proteoglycan (now referred to as perlecan), entactin and a number of other glycoproteins. Extracellular matrices are instrumental in guiding cell migration, adhesion, differentiation, morphogenesis and tissue-specific gene expression. The biochemistry of ECM molecules has been well studied; most of the genes have been cloned and the proteins are well characterized [Fig. 2].

Historically, ECM molecules were thought to provide only structural and physical support. There was, however, a rich literature identifying the stroma as a key regulator of morphogenesis and function, and with that in mind, we suggested a number of years ago that ECM molecules may be involved in directing tissue-specific function. We proposed that the ECM is linked to the nucleus via transmembrane receptors and the cytoskeleton and hence had a direct effect on gene expression. This model implies that the unit of cellular function is the cell plus its ECM, and that the functional unit *in vivo* is the tissue itself (epithelium, mesenchyme and the ECM). Since then, much evidence has accumulated to support this concept. ECM proteins, as well as hormones and growth factors, have emerged as important regulatory elements in determining tissue-specific form and function. Here we briefly review some of the evidence for the involvement of ECM molecules in development and in expression of tissue-specific functions.

1] Three recent experimental approaches have been useful in unravelling the role of ECM in development: Natural mutants, where the phenotype is manifested but the genes responsible have to be tracked down; Transgenic animals where a gene is introduced and the resultant phenotype is dissected; Disruption of developmental processes by specific inhibitors or antibodies to ECM molecules.

Three developmental mutants have aberrations in ECM structure and function: First the lethal spotted (*ls/ls*) mutant mouse has been shown to be deficient in ganglion cells [derived from neural crest {nc}] in the terminal segment of the colon. The animals develop a congenital megacolon in which functional motility is absent in the aganglionic zone. The nc cells, however, appear to be normal in co-culture experiments, suggesting that non-nc-derived cells of the *ls/ls* terminal bowel are responsible for the failure of nc cells to migrate. Recent studies have shown that bm components in the terminal bowel are abnormal, which argues that the defect in *ls/ls* mutant is related to faulty ECM accumulation. Second, in white axolotl mutants, trunk pigmentation is restricted because the epidermis does not support the subepidermal migration of pigment cells from the nc. Using membrane microcarriers to absorb ECM molecules *in vivo* and transplanting them into embryos, recent studies indicate that ECM from mutant skin fails to promote migration at the correct developmental juncture. Finally, in *Steel* mice, where a non-cell autonomous mutation adversely affects the differentiation of three migratory stem cell populations (including nc-derived melanoblasts), it has been shown that melanogenesis is enhanced on normal ECM but not on mutant-derived ECM.

Studies with transgenic animals are not yet refined. However, the importance of intact collagen I can be seen in transgenic animals where introduction of a mutant gene leads to an embryonic lethal phenotype. In addition, there are many human diseases that are known to be related to mutations in collagen genes.

Finally, studies with antagonists indicate that an intact ECM is required for many developmental processes including neural tube morphogenesis. Thus ECM is becoming firmly established as an important component of inductive processes in development.

2] It has become abundantly clear that ECM is also necessary for maintenance of tissue-specific function once the cells have differentiated. This discovery is based mainly on the failure of cells cultured on tissue culture plastic to retain differentiated functions. To study the regulation of gene expression under strictly defined conditions has necessitated the use of cell culture. However, no sooner are the cells removed and placed under conventional culture conditions (plastic dishes, presence of serum) than the characteristic traits to be studied are lost. This is because conditions that were defined for growing cells in culture are not necessarily conducive for keeping them differentiated. The importance of cell-cell and cell-ECM interactions in functional differentiation was realized when epithelial cells (notably hepatocytes and mammary epithelial cells) were cultured on exogenous physiological matrices. In many cases the cells, which on tissue culture plastic had lost both their epithelial morphology and tissue-specific traits, could now resume their "correct" form and some specialized functions. With the recognition that a functional bm can provide an even more physiologically relevant substratum for epithelial cells, and with the availability of a preparation of bm from a tumor known as Engelberth-Holm-Swarm tumor (EHS-matrix or "Matrigel"), high levels of tissue-specific gene expression can now be achieved in a wide variety of cell types. Hepatocytes in culture, for example, require such a matrix for sustained expression of liver function. Albumin synthesis and secretion, hermopexin, α -1-acid glycoprotein and cytochrome P-450 expression are dramatically elevated. Individual components of the ECM do not appear to substitute for the complex bm. Biological effects of the ECM apply to mesenchymal as well as epithelial cells. Liver-derived lipocytes for example, express one kind of ECM on tissue culture plastic (type I

collagen) but on bm matrix they switch exclusively to type III collagen synthesis, a phenotype resembling the *in vivo* situation.

Vascular endothelial cells form tubular networks on EHS in a laminin-dependent process; peptides that inhibit interaction of these cells with laminin, inhibit tube formation. Angiogenesis is regulated by the extent of interaction of endothelial cells with ECM molecules. It should be pointed out that in addition to directly influencing cellular function, such matrices also sequester growth factors and thus additionally affect functional differentiation and growth. The subendothelial matrix, for example, binds basic fibroblast growth factor (bFGF), which directly stimulates endothelial cell growth. Kidney tubulogenesis depends on both ECM and on sequestered transforming growth factor alpha (TGF- α).

Sertoli cells, when cultured on a bm matrix form seminiferous cord-like structures resembling testicular cords *in vivo*. Analogous to endothelial tube formation, the morphogenesis of these seminiferous cords is prevented by inhibitors of cell-laminin interactions.

Mammary epithelial cells from midpregnant mice present one of the more dramatic examples of cell-ECM interactions. Epithelial cells from the glands of midpregnant animals, plated on type I collagen gels which are then floated, contract the gel and establish intimate cell-cell interactions (a 3-dimensional culture model closer to the actual structure of the gland). Under these conditions the cells change shape, become polar, develop extensive microvilli, and secrete a number of milk proteins. All mRNAs for milk proteins are upregulated (some as much as 50-70 fold) and the degree of response to lactogenic hormones is considerably elevated; these processes are regulated at both transcriptional and post-transcriptional levels. On an exogenously supplied bm (EHS matrix), the cells undergo complex morphological reorganization leading to formation of functional "alveoli" (Fig. 3). Here, most milk proteins are secreted vectorially from the apical surface of the polarized epithelial cells into an enclosed luminal space, whereas other proteins such as

ECM-degrading enzymes are secreted basally. In addition, cells on this type of bm matrix are capable of producing whey acidic protein (WAP), a major mouse milk protein that is absent in cells on tissue culture plastic, and greatly reduced on other substrata. The inability to synthesize WAP under the latter conditions appears to be related to the production of a secreted inhibitor which, by autocrine action, selectively turns off WAP. Thus the formation of the inhibitor and hence the ability of cells to make WAP is dependent on the nature of the ECM on which the cells are cultured.

In conclusion, ECM is a key regulator of differentiation and tissue-specific gene expression. Recent advances in cell culture and genetic engineering techniques promise to make it possible to unravel the detailed molecular mechanisms of signal transduction by ECM in the near future.

FIGURE LEGEND

- FIGURE #1:** Defining the microenvironment. [Stoker *et al.*, (1990) *Current Opinion in Cell Biology*. In press, used with permission.]
- FIGURE #2:** Major extracellular matrix components diagramatized approximately to scale, and key features commented on. [The molecular diagrams are reproduced with permission from Trelstad *Ann. N.Y. Acad. Sci.* (1990) 580:391-420.]
- FIGURE #3:** Model of alveolar-like morphogenesis by mammary epithelial cells cultured on EHS matrix. [Barcellos-Hoff & Bissell (1989). In: Autocrine and Paracrine Mechanisms in Reproductive Endocrinology (L. C. Krey, B. J. Gulyas and J. A. McCracken, eds.) Plenum Press, pp. 137-155. Reproduced with permission.]

REFERENCES

1. Bissell, MJ, Hall, HG and Parry, G (1982) How does the extracellular matrix direct gene expression? *J. Theoret. Biol.* **99**:31-68.
2. Morrison-Graham, K and Weston, JA (1989) Mouse mutants provide new insights into the role of extracellular matrix in cell migration and differentiation. *TIG* **5**:116-121.
3. Sanders, EJ (1988) The roles of epithelial-mesenchymal cell interactions in developmental processes. *Biochem. Cell Biol.* **66**:530-530.
4. Stoker, AW, Streuli, CH, Martins-Green, M and Bissell, MJ (1990) Designer microenvironments for the analysis of cell and tissue function. *Current Opinion in Cell Biol.* (In press.)

Figure 1. Defining the Microenvironment.

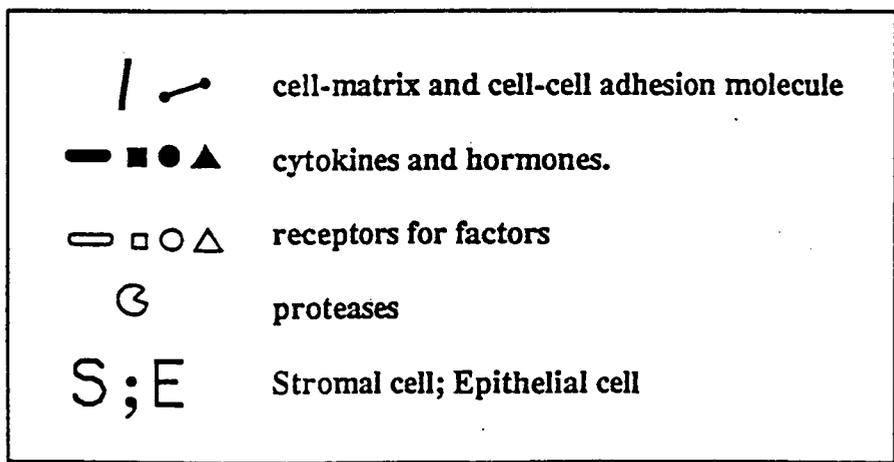
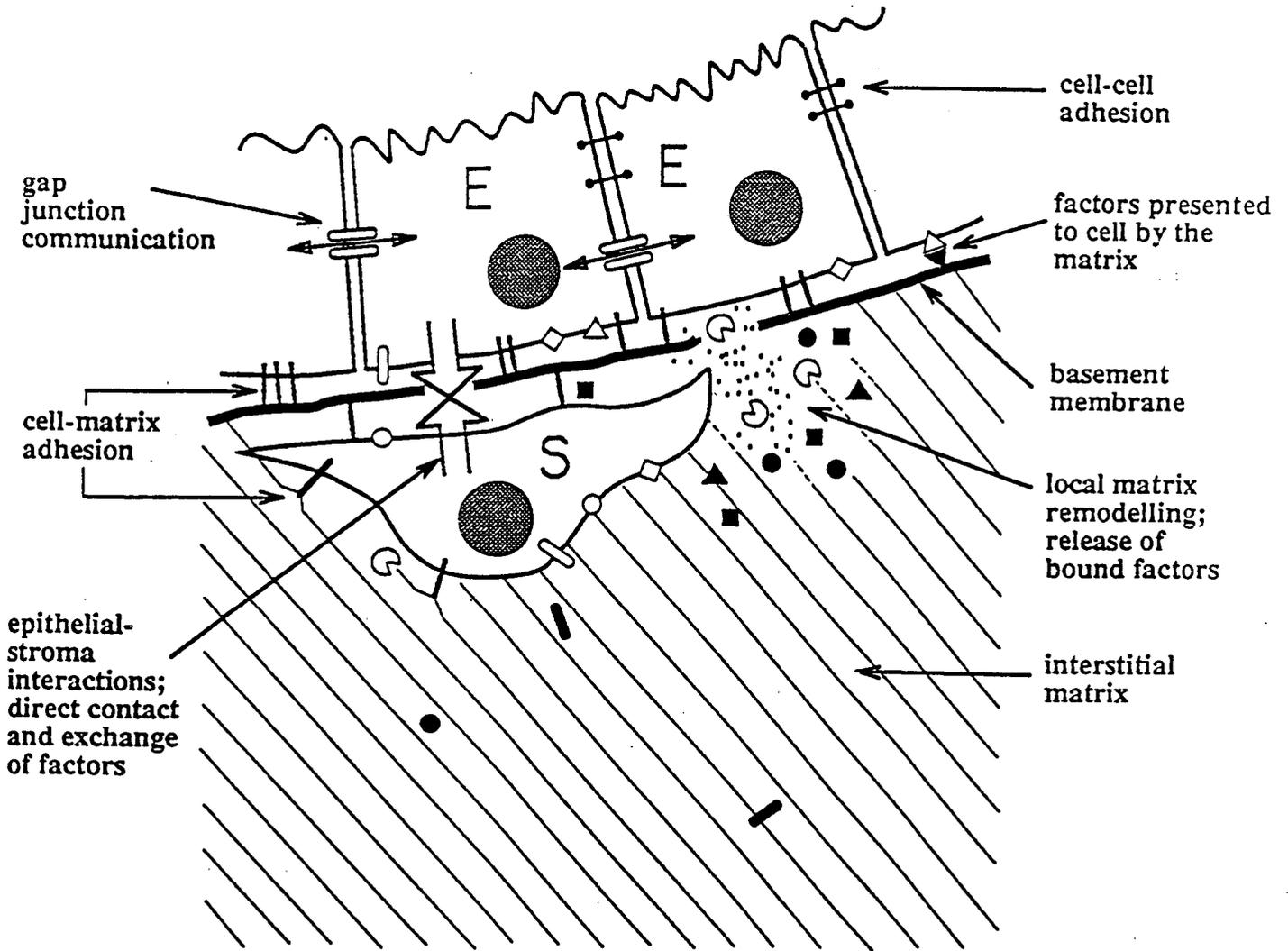


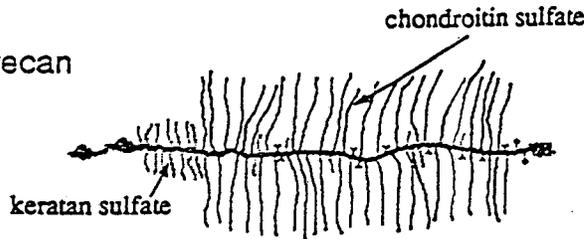
FIGURE #1: BISSELL ET AL., in: 1992 McGraw-Hill Yearbook of Science & Technology.

Interstitial ECM

collagen I, II, III



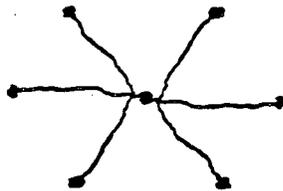
aggrecan



fibronectin



tenascin



The fibrillar collagens are triple-helical molecules composed of $\alpha 1$ and $\alpha 2$ chains; these aggregate with defined periodicity to produce fibrils of exceptional tensile strength. Collagen I is the major species in tendon forming highly organized, striated fibers. Less ordered arrays of collagens I and III constitute the basic framework of loose connective tissue, such as dermis. Collagen II is predominant in cartilage.

This example of a "space-filling" proteoglycan has a protein core of 207 kD, but the whole molecule is 2500 kD when coupled to more than 100 sulfated glycosaminoglycan (GAG) chains, such as chondroitin sulfate and keratan sulfate. It forms highly acidic, hydrophilic aggregates within the collagenous matrix of cartilage.

Dimers of fibronectin (MWt 550 kD) are important cell adhesion glycoproteins, linking many cell types to collagen and proteoglycan matrices. Fibronectin is of key significance for cell migration during embryogenesis, and in wound healing.

Tenascin is present in tissues undergoing extensive remodelling, such as at sites of wound repair and in regions of active cell growth. Two trimers of the glycoprotein are disulfide-linked to produce a hexamer (MWt >1000 kD) with a characteristic "hexabrachion" structure.

Basement membrane components

laminin



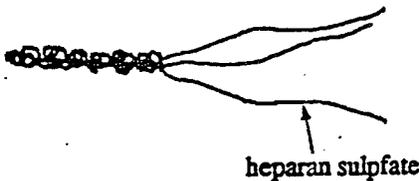
collagen IV



entactin



perlecan



50 nm

The A, B1 and B2 chains of the glycoprotein laminin assemble into a cruciform structure with a MWt of ~ 800 kD. Laminin is a major component of basement membranes, aggregating to form a highly malleable network. It is a potent regulator of cell behavior.

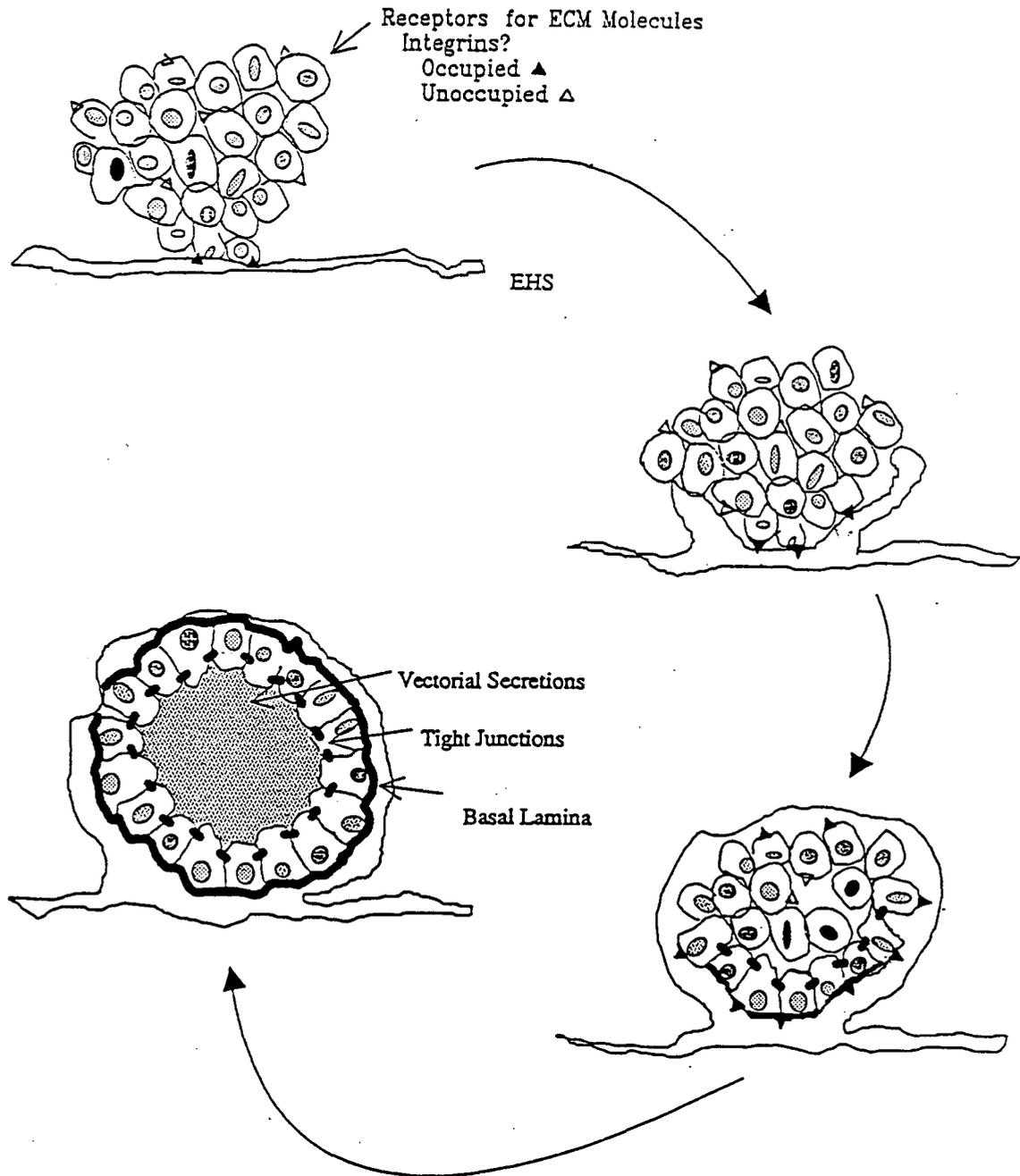
Two $\alpha 1$ and one $\alpha 2$ chains of collagen IV twist around each other, resulting in a triple-helical molecule (MWt ~540 kD). The globular C' terminal domain forms intermolecular bonds with other collagen IV molecules, producing a rigid network that is a key building block of basement membranes.

The basement membrane protein, entactin (MWt 150 kD) interacts with laminin and may modulate its interaction with cells.

The major heparan sulphate proteoglycan (HSPG) of basement membranes has a complex protein core (MWt ~400 kD) with some domains similar to laminin and others that resemble cell adhesion molecules. With several covalently-attached GAG chains, it is highly anionic.

FIGURE #2: BISSELL ET AL., in: 1992 McGraw-Hill Yearbook of Science & Technology.

Model of Alveolar-Like Morphogenesis on EHS Matrix



XBL 892-650

FIGURE #3: BISSELL ET AL., in: 1992 McGraw-Hill Yearbook of Science & Technology.

LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
INFORMATION RESOURCES DEPARTMENT
BERKELEY, CALIFORNIA 94720