

CELLS AS TENSEGRITY STRUCTURES: ARCHITECTURAL REGULATION OF HISTODIFFERENTIATION BY PHYSICAL FORCES TRANSDUCED OVER BASEMENT MEMBRANE

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INTRODUCTION

...the problem of organization is the central problem of biology, and the riddle of form is the fundamental riddle...

-Joseph Needham (Terry Lectures, Yale University, 1936)

The working hierarchy of symbiotic cellular arrangements that we call life is constructed through a combination of cell growth, differentiation, and progressive remodeling of increasingly complex tissue structures in a manner reminiscent of phylogeny. Yet embryogenesis in all of its incredible complexity involves the development of a living organism from a single cell (fertilized ovum) in only a matter of months.

All of the enzymatic and structural molecules that comprise the organism are the result of expression of specific gene products. However, our current knowledge of molecular genetics is not sufficient to explain embryonic development or even the generation of tissues. Similarly, specific molecular messengers and chemical gradients so long sought after as morphogenetic organizers have also proved unsatisfactory as a source of form organization. The central theme of the presentation which follows is that we may find some of these answers (i.e., how cells and tissues are organized) through study of the relationships between different cellular components rather than solely by analysis of their substance.

Sometimes when we work in simplified cell systems we forget that specialized tissues arise from distinct groupings of cells which exhibit characteristic patterns of organization. We tend to ignore that the cells and molecules which comprise normal tissues undergo continual turnover and that the function of a tissue is dictated by its structure. In this discussion, we attempt to reintroduce previously well established mechanical descriptions of cell form and functional organization and integrate them within a higher order architectural schema based upon more recent developments in the fields of art and architecture. Tissues, as specialized architectural assemblies, are characterized by an inherent distribution of structural forces (e.g., tension and compression). We would like to propose that these physical forces may be informative in nature serving as regulators of gene expression, cell growth, and histodifferentiation through their modulation of cell shape.

A theoretical model of architectural regulation of histodifferentiation is presented which stems from the observation that a cell is a tensegrity structure and thus its form (and related metabolism) is determined through a dynamic equilibrium of physical forces. In this system, intracellular structural components (e.g., DNA regulatory assemblies, nuclear protein matrix, cytoskeleton) intercommunicate with the architectural network of the external milieu as well as other cells via transduction of structural forces over specific cellular anchoring interconnections present in the extracellular matrix (ECM). These architectural concepts are placed in perspective of experimental data generated in various laboratories including our own which demonstrate that basement membrane (BM), as an *in vivo* foundation for cell anchorage, serves to spatially organize epithelial cells. In this manner, we propose that an entire society of individual epithelial cells may be coordinated as a single functional unit with morphogenetic changes in tissue form being guided through highly regulated alterations in architectural force distributions (e.g., via BM remodeling). We also suggest

that the disorganization of tissue morphology that underlies carcinogenesis may result from an inability to maintain normal systems of force transduction (e.g., gradual BM dissolution).

HISTORICAL BACKGROUND

Prior to the advent of a biology based on biochemistry and molecular genetics, the emphasis was on cellular mechanics. Cells and tissues were viewed as architectural structures and as such were expected to conform to the same physical and mathematical laws that rule manmade constructions. Biologists of the first quarter of this century did not have the plethora of data that we are blessed with today and thus leaned heavily upon morphology and visual concepts which were more wholistic in quality. They realized that Nature is at once engineer and architect and that organic form is constructed in direct response to physical forces without the convenience of blueprints, mathematics, or forethought. However, the beauty of their vision was not sufficient to explain the complex workings of living organisms or provide the basic scientific requirement: the ability to predict. Rather their movement, out of desperation, became one of vitalism claiming that all systems of organization remained secure from understanding attributing them to some overriding guiding force or *Entelechy* as first proposed by Aristotle and reintroduced by Driesch (1,2).

It was out of this vitalist conundrum that modern biology gained its incredible impetus towards dissection and reduction in order to explain what was previously viewed as beyond human understanding. While reduction was performed obviously out of necessity, it has been overwhelmingly successful in terms of generating detailed information about the components that comprise living organisms and they way in which they interact with one another. However, the power of structural thinking and the use of the *gestalt* so highly emphasized by earlier scientists was almost completely lost in programs of scientific training and practice. We are now at a time when massive amounts of data exist categorizing all levels of organic structure, but the search for unifying principles of organization continues.

The remainder of this discussion is designed to present the processes of cell and tissue organization from a more wholistic and structural perspective. We hope to convince the reader that: 1) biological architecture is based upon a coherent, tangible, and constructable system of structural engineering, 2) this form of architecture is special in that its structural integrity is predominantly dependent upon tensile forces, and 3) positional information and pattern-generating informative forces are inherent to this architectural scheme in the form of structural forces.

STRUCTURAL ANALYSIS

In architecture, stable form is generated through an equilibration between many interdependent structural elements, each of which is independently in a state of disequilibrium. Complex architecture can not be broken up into isolated pieces without losing qualities that are inherent to the structural whole. This is extremely important in biological systems in which every functional unit is literally more than the sum of its constituent parts. Thus, structural analysis must be applied at the appropriate system level at which architectural stability is recognized (e.g., cell, tissue). The significance of structural analysis lies in the fact that form and associated function are determined through arrangements of component parts rather than by their composition.

Living organisms emerge out of a hierarchy of cellular arrangements which are at once plastic and dynamic. However, we speak of stable tissue structure as our definition of stability is limited by technological methods and limits of resolution. In fact, change is the only constant. Normal tissues undergo continual orderly renewal of their constituent cells and molecules and so it is the complexity of pattern that is maintained. Thus, we must analyze the physical forces that are generated within tissues and determine the architectural patterns in which they are distributed in order to begin to visualize organic systems of structural organization.

ARCHITECTURAL SYSTEMS

The structural integrity of most man-made structures is dependent upon compressive forces (2-4). A simple example would be an arch built out of bricks that is inherently unstable until the last key stone is set in place compressing the other structural elements upon which it sits. This sort of structure is obviously dependent upon gravity to resist the compressive forces that result from the weight of each individual stone. Man-made structures are usually inefficient in terms of the quantity of materials required to enclose a space or support a given load. Compression-dependent structures are inherently rigid and poorly adapted for a rapidly

changing environment. They are of course useless at a cell level where gravitational forces are of minimal significance.

On the other hand, most natural structures depend upon tensile forces for their integrity (2). The malleability and adaptability of most organic forms require this type of a system of structural organization which is independent of gravity. Artists and architects commonly describe the human body as a tensile structure in which tensional integrity is maintained by muscles suspended across compression-resistant bones (3). Tensile systems of architecture have only recently come to be utilized for man-made structures.

TENSEGRITY

Many of the understandings necessary for use of tensile systems of construction have come out of the labors of R. Buckminster Fuller and his students. Fuller spoke for many years of a universal system of structural organization of the highest efficiency based upon a continuum of tensional integrity or "tensegrity" (5,6). However, it was a sculptor studying under Fuller, Kenneth Snelson, who was first able to physically construct a tangible model based upon this system of tensile organization (7). The theory of tensegrity developed out of the discovery of the geodesic dome, the most efficient of architectural forms, and through study of the distribution of stress forces over its structural elements. It is important to note that some years after Fuller's discovery, the structural organization of viral capsids was discovered to be built according to these basic rules of architectural organization (6).

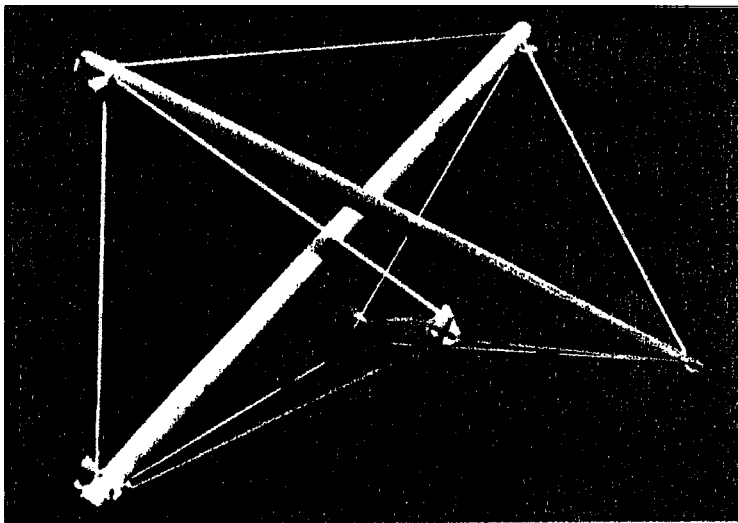


Fig. 1. Basic tensegrity structure constructed out of wood dowels and monofilament line. The rigid compression-resistant dowels do not touch but rather are held up by a continuity of tension transmitted over the monofilament line.

A tensegrity system is defined as an architectural construction that is comprised of an array of compression-resistant struts (e.g., bones, wood dowels) that do not physically touch one another but are interconnected by a continuous series of tension elements (e.g., muscles, monofilament line)(4,5). Thus, tensegrity structures are literally pulled up and open rather than compressed in place and so are by definition independent of gravity. The simplest tensegrity form is visualized using a model in Fig. 1. Disruption of the continuity of tension transmission within this sort of structure results in destabilization and breakdown of the entire structure (e.g., severing the achilles tendon results in upward contraction of the foot and loss of controlled movement).

The beauty of this sort of structural organization lies in its incredible efficiency. A minimum of materials are required to cover a given space. Furthermore, as physical forces are distributed equally over all elements of a tensegrity system, its load bearing capability per weight of construction material is the highest known to man and its potential size is unlimited. Perhaps it is for this reason that Nature so often utilizes this form of construction. The solar system has been described as a natural tensegrity system in which gravitational forces serve as tensile elements interconnecting a discontinuous array of compression-resistant planetary masses (5). Similarly, an analogous tensegrity model for the architectural organization of

the atom has been proposed (7). That nature always seeks out the most economical of possibilities has been understood since the time of the Greeks (2).

TENSEGRITY CELL MODELS

Tensegrity structures may be built which utilize elastic elements in place of non-extensible tension cables (Fig. 2a). We would like to propose that this sort of elastic tensegrity unit may serve as a conceptual model for the cell as well as other biological systems above and below the cell in the organic systems hierarchy (e.g., tissue, nucleus). Cells may be described as tensegrity structures as they generate their own tensional forces and exhibit an architectural integrity independent of gravity. At a cell level, compression-resistant struts may be thought to represent stressfibers or other cytoskeletal filamentous structures (e.g., intermediate filaments) which appear as non-elastic elements isolated within the elastic cytoplasm as seen, for instance, by acoustic microscopy (8). The elastic elements that hold the model together may represent contractile microfilaments, which are known to generate tensile forces (9,10), as well as viscoelastic cytoplasm and plasmalemmal components. Actual cell morphology is of course also dependent upon pneumatic considerations (e.g., osmotic regulation) but these will not be discussed here.

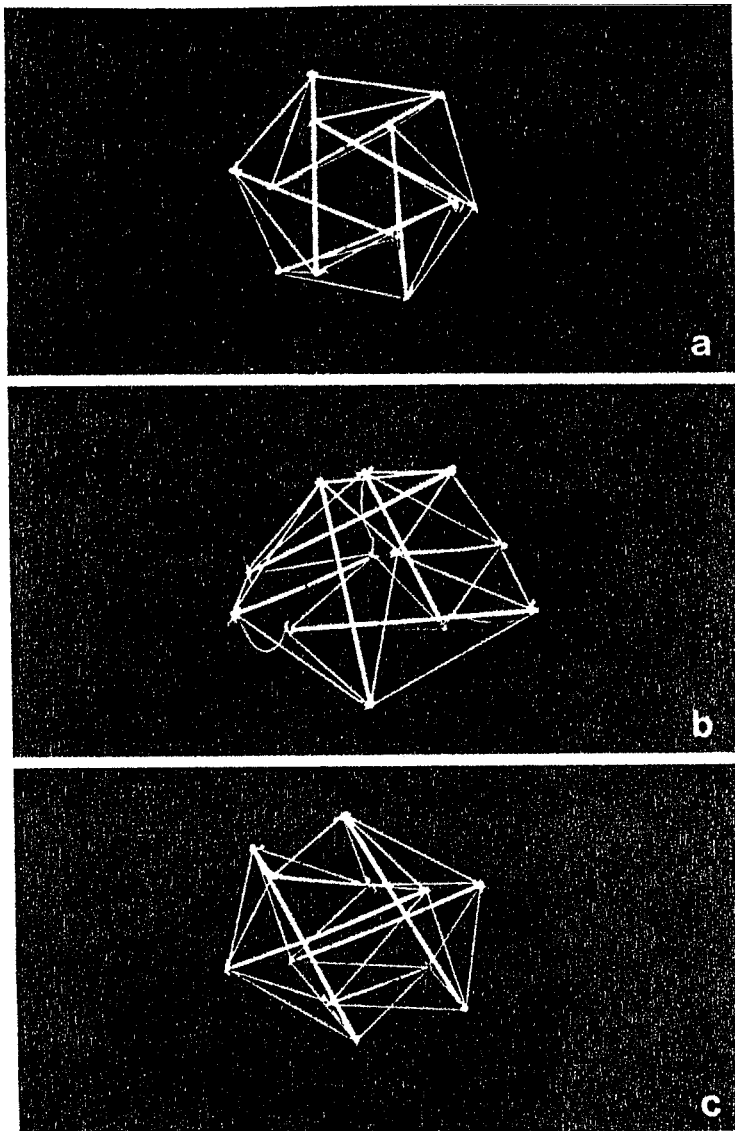


Fig. Z. Tensegrity cell models constructed with wood applicator sticks and elastic thread. a) An elastic tensegrity cell structure takes on a round form when free of anchorage as this is its minimum energy state.

b) If the structure is attached to a rigid substratum that can resist "cell"-mediated tensions, its form will depend upon its distribution of anchors. c) if the structure is anchored in a similar manner as in (b) but to a malleable substratum, the "cell" will pull in on the foundations and remain round.

Cell form alterations can be shown to be inherent to this structural system of organization using the model described above. As dynamic tensile structures, cell models alter their shape until an equilibrium configuration is attained which most efficiently and evenly distributes the load given the characteristic architectural distribution of anchors within the substratum. The model spreads out when attached to a rigid substratum (Fig. 2b) but pulls in on a malleable foundation and remains round (Fig. 2c). Cell form is thus dependent upon the tension that the structure (cell) generates and the structural rigidity of its anchoring foundation.

Construction of tensegrity cell model containing a smaller "nuclear" tensegrity assembly built by additional elastic elements allows further analysis of the structural coordination between cell and nuclear form. When the cell/nuclear tensegrity structure is maintained free of anchorage both cell and nuclear forms are round (Fig. 3a). However, when the "cell" attaches and spreads over a rigid substratum (Fig. 3b), its nucleus moves towards its base and spreads out horizontally in a coordinated fashion. Thus, if cells are indeed constructed based upon the rules of tensegrity, then the structural alterations demonstrated by these models should predict the behavior of living-cells under similar conditions.

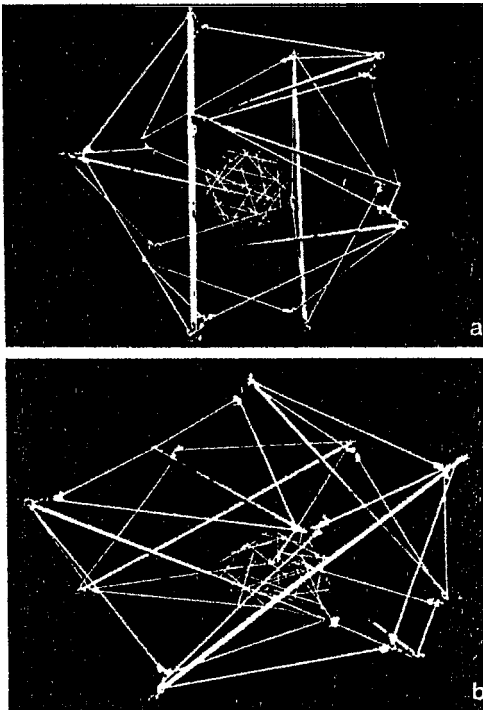


Fig. 3. Elastic tensegrity cell/nucleus model. The "cell" is constructed with aluminum struts and nylon shock cord. The "nucleus" is made of applicator sticks and elastic thread and is interconnected with the "cell" surface using elastic thread.

EXPERIMENTAL SYSTEMS

Studies were carried out in our laboratory to test the hypothesis that cellular structures are tension-dependent architectural assemblies and thus that BM, as a physiologic anchoring foundation, may regulate epithelial cell form and histodifferentiation (e.g., polarity). We have previously described a transplantable carcinoma of the rat exocrine pancreas that is comprised of cells which retain the ability to reorganize and display normal epithelial orientation only when associated with intact BM in vivo (11). Cells that are mechanically dispersed from this epithelial tumor do not spontaneously adhere to standard culture substrata

such as plastic, glass, Millipore filters, or type I collagen gels. This inability for spontaneous attachment in vitro makes the pancreatic acinar cell tumor a rare system for studies on epithelial cell-substratum interactions. We have recently used this system to demonstrate that exogenous intact BM (prepared from human amnion) and dishes coated with purified laminin are sufficient to support epithelial tumor cell attachment and spreading in vitro even in the absence of new protein synthesis (12).

In order to demonstrate that cell form is determined by the structural integrity of the anchoring substratum (as predicted by the tensegrity models described above in Fig. 2a-c), we coated purified laminin over culture substrata with different abilities to resist cell-mediated tensions. Purified laminin that was free of any major contaminant as detected by electrophoresis was kindly provided by Dr. J.A. Madri (Dept. of Pathology, Yale University School of Medicine). This laminin was air-dried (300ug/35mm dish) on rigid plastic dishes or on pre-dried malleable type I collagen gels which alone will not support tumor cell attachment.

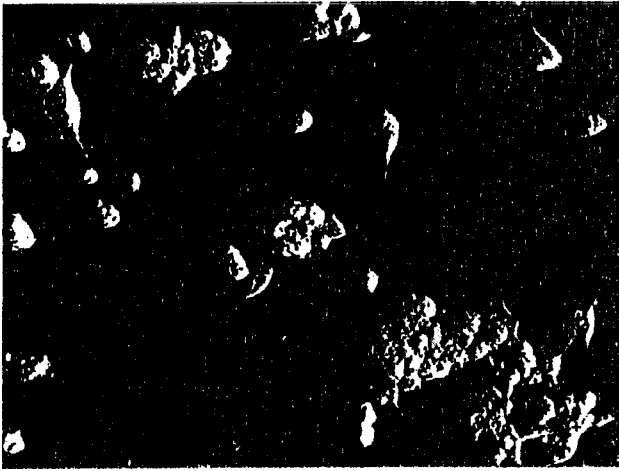


Fig. 4. Light micrograph of tumor cells grown on a laminin-coated plastic dish as viewed by Hoffman optics at 6 hrs (x 480).

Tumor cells were mechanically dispersed to single cells from subcutaneous tumor implants using a series of siliconized pipets and nylon meshes. These epithelial tumor cells attach and spread over laminin-coated plastic dishes within 6 hours of plating (Fig. 4). Acinar tumor cells will also attach to laminin that has been dried on floating collagen gels, however, these cells do not spread. Electron microscopy demonstrates that individual tumor cells apparently pull the denatured amorphous laminin matrix up and around their surface and remain round after 6 hours of culture (Fig. 5). Similar results were observed at 24 hours and no cells were observed to spread when viewed from above by inverted phase contrast microscopy of living cultures (not shown). These findings confirm previous observations from other laboratories which demonstrate that all types of cells generate tensile forces (9,10,13) and that cell shape is substratum-dependent (10,13,14).



Fig. 5. Electron micrograph of an acinar tumor cell grown for 6 hours on a laminin-coated native collagen gel (x 6,000). The tumor cell does not spread but instead pulls the malleable amorphous laminin matrix in and around its surface. A thick layer of laminin physically separates the cell from underlying collagen fibrils.

In additional studies, we have utilized computerized morphometric analysis to show that pancreatic epithelial tumor cells consistently reorient when in contact with intact amniotic BM in vitro as they exhibit a normal polarized distribution of lipid droplets, nuclei, Golgi complexes, and zymogen granules (from base to apex) even in the absence of new protein synthesis (12). This is in contrast to tumor cells that are cultured on the stromal side of the amnion which remain round and randomly oriented. As the elastic cell/nucleus tensegrity model predicted that cell nuclei should spread and move basally in a coordinated fashion, the repolarization of intracellular organelles within acinar tumor cells attached to amniotic BM may be explained in purely structural terms. Of course, more specialized movements of intracellular components and cell processes may be fine-tuned by additional dynamic systems of intracellular organization and construction as it is clear that cell form may be stabilized at both intracellular and extracellular levels (9,15). However, these results in addition to those of many other laboratories (16,17), suggest that anchorage (e.g., to BM) may serve as an initial point of structural stabilization upon which subsequent steps of the epithelial organization cascade may build.

TENSION AND COMPRESSION AS INFORMATIVE FORCES

I Perhaps more important than the description of cell architecture as a system based upon tensegrity is the information potential that is intrinsic to this system of structural organization. Every position in a complex architectural structure may be defined by the tensions, compressions, moments, and shear forces characteristic of that point in the structural assembly. Thus, the positional information that is central to embryological development (18) and, has of yet not been satisfactorily explained in terms of chemical information, may be physical in nature.

It is now clear that cell shape alterations may serve to regulate cell growth as well as many other aspects of cellular metabolism such as gene expression, translation, and the production of specific differentiated products (13,14,19-21). For instance, while protein synthesis is made possible through attachment of normal anchorage-dependent cells, recovery of DNA synthesis requires extensive cell spreading (19). Similarly, Folkman and Moscona were able to regulate the proliferative rates of cells by altering their shape using substrata of varying adhesiveness (20). The morphological differentiation of mammary epithelia (including their production and secretion of specialized milk proteins) also appears to be modulated by changes in cell form (13, 21).

Cells may normally alter their shape through deposition of specialized attachment molecules which link surface constituents to neighboring anchoring substrata. For instance, BM constituents are central to the process of epithelial cell anchorage in vitro. The attachment and spreading of various epithelia and endothelia require de novo BM (e.g., type IV) collagen deposition (22,23). Laminin may mediate this process as epithelia can use laminin to bind to type IV collagen (24) and, as mentioned above, purified laminin supports adhesion and spreading of pancreatic acinar tumor cells. Fibronectin and proteoglycans are also involved in cell attachment (25,26).

Alterations of cell geometry which are mediated by deposition of BM components may then be translated into biochemical process through associated cytoskeletal changes. For example, laminin promotes changes in cell geometry as well as assembly of actin-containing filaments inside the cell (12,27). This may be mediated through specific cell surface molecules which bind to both laminin and intracellular actin (28). Similarly, addition of fibronectin promotes intracellular assembly of actin-containing filaments, associated cell shape alterations, and so affects the differentiated state of cells (29,30). Conversely, trypsinization of cell surfaces removes attachment molecules, depolymerizes intracellular filaments, and results in cell rounding (31). Cell shape changes also serve to modulate vimentin biosynthesis and phosphorylation (32).

However, while interaction of a specific anchoring molecule such as laminin with its receptor is necessary for cell attachment, it is not sufficient for cell shape alterations (as shown above in Fig. 5). Rather cell form results from a physical interaction between a cell and its anchoring substratum. Cell geometry represents an equilibration between an array of architectural forces which are transmitted over specialized molecular anchoring assemblies (e.g., BM) that interconnect subcellular elements with the extracellular milieu. Thus, if cell shape and related metabolism are structurally determined, then the growth and differentiation of entire societies of cells may be regulated through a directed redistribution of structural forces with the common BM anchoring foundation.

Bissell et al. (33) have recently reviewed the role of extracellular matrix (ECM) in the regulation of gene expression. They present an excellent review of data from many laboratories which clearly demonstrate a structural and functional continuity between ECM, cytoskeleton, and organelle (e.g., nuclear) assemblies. As described above, certain ECM (e.g., BM) molecules interact directly with cell surface components (28) and effect changes in the distribution of cytoskeletal elements (12,27,29). Cytoskeletal filaments in turn appear to associate directly with the nucleus (34) as well as with polysomes that are actively involved in the translation of mRNA coding for a variety of proteins (35). The nuclear protein matrix (an organic geodesic sphere) may itself serve as an organizing site for DNA-regulatory assemblies (e.g., DNA polymerases) (36).

The information potential of ECM may lie in its ability to locally affect the supramolecular organization of neighboring molecules. For instance, Bissell et al. (33) suggest that ECM, as a multivalent ligand with a distinct periodicity, may cluster surface growth factor receptors or transmembrane molecules which in turn alter intracellular filament nucleation sites and polymerization. ECM generated changes may subsequently affect nuclear pore structures and mRNA processing as well as associated translation rates. In this manner, the concept of an "extended cytoskeleton" is presented, however, the specific form and source of information transduced by this system remain unidentified.

The centriole has been proposed to serve as a mechano-transducer which aligns with intended direction of cell movement and when organized in orthogonal pairs are perfectly designed to locate hypothetical signals within the cell (37). Again the form and quality of these signals are not specified. Every tension has by definition an associated compression oriented perpendicular to its lines of force. Furthermore, every complex structure exhibits simple harmonic oscillations (e.g. Tacoma Bridge disaster) which may correspond to the cytoskeleton-related oscillatory signals hypothesized by past authors (37,38).

If there is a direct structural linkage between ECM and DNA (i.e., mediated by cytoskeletal and nucleoskeletal assemblies), then by definition a continuity of information transmission exists in the form of structural forces. Cells appear to be dependent upon tensile forces and, as shown above with models, are most likely constructed by the rules of tensegrity. This means that even though each cell constituent is not in direct contact with every other (e.g., nucleus and plasmalemma), they would be able to communicate via a continuous system of tension transmission. In this manner, physical force alterations resulting from a localized perturbation would be distributed over all structural elements and so an intrinsic harmony would exist between part and whole.

RELATION OF STRUCTURE To PROCESS: A MATHEMATICAL BASIS

If physical forces are informative, then the central question is that of how structural alterations are transduced into biochemical process. This question will most likely remain unanswered for many years. However, we would like to present a basic thermodynamic consideration which may provide an initial mathematical orientation to work from. In 1924, A.J. Lotka wrote of the limitations of physical chemistry being a structureless chemistry based upon in vitro studies of isolated biochemical components and suggested that geometrical and mechanical forces may play a dominant role in the regulation of biological structures (39).

To this day, thermodynamics remains based upon a structureless chemistry and thus pressure and volume are generally considered constant. For example, Gibbs Free Energy is one of the most commonly

used thermodynamic parameters in biochemistry as it predicts the spontaneity of chemical reactions and so relates directly to the equilibrium constant for a given reaction. Gibbs Free Energy (ΔG) is commonly defined in basic biochemistry texts as:

$$\Delta G = \Delta E - T\Delta S$$

where ΔE is change in the total energy of the system, T is temperature, and ΔS is entropic change. However, the full definition for Gibbs Free Energy is actually:

$$\Delta G = \Delta E + P\Delta V - T\Delta S$$

where P is pressure and ΔV represents the change in volume. Pressure and volume are commonly viewed as constant for biological systems. Yet, it is clear that a cell can significantly alter its volume while maintaining structural and functional integrity. The cell may be an open system when viewed from a cybernetic perspective in that molecules and nutrients continually pass in and out of the cell. However, the cell is a closed architectural system as it is structurally and functionally autonomous.

As architectural stresses can be translated into pressure and volume changes through the use of stress tensors, cell volumes and pressures may vary greatly depending upon different systems of anchorage. This may be central to the mechanism by which the structural integrity of an anchoring substratum (e.g., BM) regulate cell shape, cytoskeletal organization, and associated metabolic activity. In support of this hypothesis, moderate levels of hydrostatic pressure reversibly depolymerize actin filament bundles in whole sea urchin eggs although F-actin is not depolymerized under similar conditions when isolated *in vitro* (40). The growth or extension phase during self-assembly of thick myosin-containing filaments can also be modulated by pressure changes (41).

Stimulation of cell growth by cell spreading may also be directed by physical force alterations as mechanical tension stimulates cell division in vertebrate cells (42). Furthermore, macroscopic growth of tissues is clearly dependent upon physical stresses including those created by the expansion of its own mass (43). For example, Koch demonstrated that the pattern of trabecular bone deposition within the human femur corresponds directly to engineering lines of tension and compression characteristic for a structure of that form with similar weight-bearing characteristics (44).

Cells may grow into the lines of force as a result of transduction of stress forces into cell and nuclear shape changes. Tensegrity cell/nucleus models predict coordinated alterations in cell and nuclear form. Nuclear shape changes may increase access of stimulatory molecules into the nucleus by spreading open nuclear pores. On the other hand, alterations of nuclear structure may have direct effects on gene expression and replication as chemically-induced swelling of isolated nuclear protein matrices results in release of DNA template restrictions *in vitro* (36). In this manner, cell growth and organization may be generally regulated by spatial constraints in a most economical and efficient manner with chemicals serving as, fine-tuning.

ARCHITECTURAL REGULATION OF HISTODIFFERENTIATION

As tensegrity structures, cells exhibit a structural continuity of tension transmission and thus information processing throughout ontogeny. During early stages of embryogenesis, these informative forces are limited to transmission over the intracellular structural components of a single cell (e.g., ovum). Individual cells living free in a fluid environment are effectively insulated from physical forces that are distributed in distinct patterns in nearby organic architectural assemblies unless they are physically interconnected (exhibit a continuity of tension) with these structures at selective anchoring sites as hydrostatic pressure is distributed equally to every point on the cell surface.

Subsequent embryonic (as well as phylogenetic) development involves interconnection between developing cells via specific cell products (e.g., surface agglutinins). In this manner, tiers of cellular systems begin to be constructed in which the structural organization of constituent cells is the result of translation of spatial information (i.e., physical forces). For instance, the primordial cell polarization occurs at the S cell stage of development with the axis of polarity being determined by cell-cell contacts, yet no polarity develops in cells which are totally surrounded by other cells (45). Higher order tissue architecture effectively results from an extension of this system of multicellular organization as individual cells interact with macromolecular complexes comprised of specialized cell products (e.g., BM) rather than directly with other cells. However, spatial constraints may again serve as a regulatory influence since these extracellular anchoring foundations have their own patterns of structural forces inherent.

The model that follows describes the role of physical forces in the regulation of more advanced epithelial structures and concentrates on BM as a central component in a higher order tensegrity system (i.e., tissue structure) which acts to transmit these forces between adjacent cellular societies. While it will not be discussed here, physical forces most likely also play a significant role in the regulation of other anchorage-dependent cells (e.g., bone, muscle) although forces are transmitted over different ECM anchoring molecules (e.g., type I collagen) in these tissues. In other systems in which cells exist free of anchorage (e.g., immune system), the growth and coordinated action of cellular populations may be greatly dependent upon highly specialized systems of chemical communication and coordination. However, cells that are normally free would most likely be sensitive to similar types of force transmission and shape modulation if anchored to an exogenous substratum (e.g., diapycnosis during inflammation).

Basement Membrane as an Architectural Anchoring Foundation

BM molecules most likely function in an anchoring capacity *in vivo* as they do *in vitro* (22-26). In fact, higher order tissue architecture may be constructed as a result of specific binding affinities between BM molecules and other ECM components that are produced by neighboring epithelial and mesenchymal societies. For example, laminin binds to specific cell surface receptors (28) and to both type IV collagen (24) and heparin-like molecules (46). Heparan-sulfate proteoglycan may appear as an integral membrane protein (47) and sulfated proteoglycans interact directly with type I collagen (48) and fibronectin (49) in addition to laminin. Fibronectin in turn binds to interstitial collagens and attaches to the fibroblast cell surface (50).

This BM anchoring foundation is probably maintained under tension through the actions of adjacent epithelia and mesenchyme *in vivo* as each tissue is comprised of cells capable of generating their own tensile forces. Thus, BM suspended between compression-resistant collagen fibrils may itself be viewed as a tension element of a higher order tensegrity system. In this manner, BM serves to equally distribute the physical forces characteristic for a given three dimensional orientation and to provide a continuity (tensional integrity) between all epithelial and mesenchymal cells. The form, orientation, and growth of cells within each component tissue may then be orchestrated with that of the cells of its neighbor through a dynamic equilibration of architectural forces.

If we view the epithelial cell as an elastic tensegrity structure then BM should regulate cell shape (as well as growth and differentiation) through its transmission of the physical forces of tension and compression for a given spatial configuration. In this manner, the structural coordination, homogeneity of cell form, and effective systems of intercellular communication required for successful tissue function are sustained by normally constant architectural relationships. Alteration of the pattern of physical forces within the structural framework of the epithelium (e.g., cell death or removal) results in cell spreading and division until the original state of compression is regained. Thus, maintenance of BM of constant dimension may assure for the stability of normal epithelial morphology. (i.e., low turnover rate with equal rates of BM synthesis and degradation; Fig. 6a)

EPITHELIAL ORGANIZATION AND DISORGANIZATION: Role of Basal Lamina

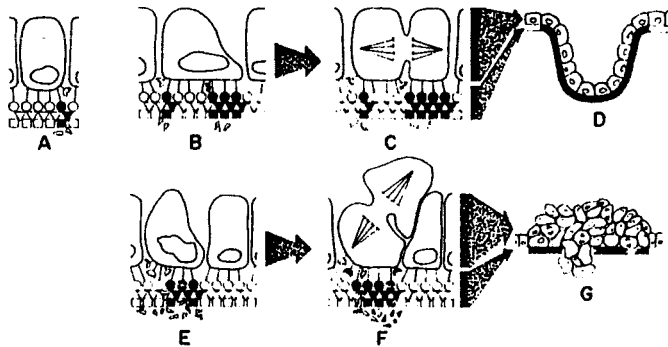


Fig. 6. Schematic diagram of a model for architectural regulation of epithelial tissue morphology. Circles, triangles, and squares indicate different integral BM components undergoing continual turnover. (open figures- preexisting BM components; closed figures- components added to preexisting BM by *de novo* synthesis and deposition; broken figures- BM components removed by ongoing degradation).

Morphogenesis

The coordinated alterations of tissue form that underly morphogenesis represent reciprocal interactions acting in a spatially organized manner. This sort of regulatory scheme is inherent to a tensegrity system of architectural organization. If BM is under tension, localized sites of increased BM turnover may destabilize this anchoring foundation resulting in a subtle release of tensile constraints reminiscent to the way in which a weak spot in a balloon expands more readily than the rest of its surface. Associated epithelial cells, in turn, may respond to this local decrease in cell compression in a manner analogous to the way in which epithelial cells act during wound healing: by depositing new BM components, spreading, and proliferating until an equilibrium of physical forces is restored (20,23). A directed loss of structural integrity maintained over time within a limited region of BM could then theoretically lead morphogenesis and result in both tissue growth and lateral BM extension (Fig. 6a-d).

The work of Bernfield and collaborators elegantly demonstrates a direct correlation of this kind between an increased rate of localized BM turnover, associated cell proliferation, and subsequent budding out of epithelial forms during salivary morphogenesis (51). Studies in our laboratory suggest a similar role for BM turnover in pattern formation during the development of the pancreas. This system of morphogenetic regulation might function dynamically in a manner analogous to the way in which the cellular society inhabiting bone is able to recognize and respond to physical loads and in turn grow and lay down their bone matrix directly along lines of tension and compression (44). Similarly, D'Arcy Thompson relates the structural forms and growth patterns of a great variety of organisms to physical forces and spatial constraints in his classic book on growth and form (2). As BM is continually extending laterally during morphogenesis, net BM synthesis must be greater than net BM *degradation*. However, inherent to this system of architectural regulation would be a fine coordination between epithelial mass expansion and BM extension and thus the ability to generate new tissue forms in the absence of significant alterations in cell shape or BM morphology that are detectable by electron microscopy.

Neoplastic Disorganization

If normal adult epithelium experiences a local net increase in BM degradation (decrease in tensile constraints) then adjacent epithelial cells may respond by increasing BM deposition and proliferating in a manner reminiscent of embryogenesis. This process must be gradual since rapid BM dissolution results in a complete loss of cell viability as normal epithelial cells are anchorage-dependent (e.g., mammary gland involution; 52). However, if BM synthetic and degradative capacity are increased to similar levels (i.e., maintenance of a high turnover rate) then this release of spatial constraints could be maintained within a normal adult epithelium over an extended period of time. In this manner, a loss of organized epithelial cell arrangements may result from stimulation of cell proliferation in the absence of neighboring epithelial boundary extension as there is no net BM accumulation.

Early stages of this process would remain reversible (e.g., dysplasia) as long as cell growth remained dependent upon continued anchorage, BM deposition, and shape regulation. Continued release of tensile constraints with sustained cell growth over an extended time could then lead to spontaneous transformation *in vivo* just as continued culturing may do *in vitro* (i.e., selection of an anchorage-independent subpopulation, expression of oncogenes). As this neoplasm grew in size, autonomous epithelial cells would become separated from neighboring connective tissue by many cell diameters and so would be less susceptible to the regulatory influences of associated connective tissue that may normally assure for maintenance of integral BM. For instance, mixture of certain epithelial tumors with embryonic mesenchyme results in normal differentiation and production of linear BM (53; for a review see ref. 54).

Thus, a positive feedback system could develop which results in malignant invasion through either loss of BM synthetic capacity, increased degradative potential, or through the acquisition of some new transformed cell product which in some way further compromises BM integrity. The development of metastatic potential may in fact be dependent upon increased production of specific BM degrading enzymes. Liotta and coworkers have clearly demonstrated a direct correlation between production of BM (e.g., type IV) collagenase activity and metastatic potential in a variety of tumor systems (55). However, malignant invasion may require maintenance of some BM synthetic capacity in association with relatively higher levels of degradative activity since cell movement appears to depend upon continued deposition of BM components (22,23).

CONCLUSION

The central objective of this presentation is to propose that physical forces may be informative in nature and that it is as a transducer of these forces that BM is involved in the regulation of epithelial cell shape, differentiation, and growth. We propose that this system may be based upon an organic scheme of hierarchical organization as conceptualized by the cell/nuclear tenegrity model (Fig. 3). This model clearly visualizes the concept of a structural "systems jump" with each individual tenegrity system (e.g., cell) being integrated with larger and smaller tenegrity structures (i.e., tissue and nucleus, respectively) through the provision of tensional continuity. In this manner, many individually complex schemes of structural organization may be integrated and coordinated in a hierarchical fashion. However, successful transmission of forces throughout these different sized architectural systems would then be dependent upon continued maintenance of tensional integrity between proper attachment sites. Thus, malignancy may result from a breakdown of this architectural scheme. Any physical or functional interruption in the continuity of tension transmission between a cell's nuclear regulatory assemblies and its external environment (e.g., gradual BM dissolution, Src gene effects on vinculin) might produce a cell which is effectively blind to the informative forces that normally limits its growth and orientation. Similarly, a break at any other point in the transduction of these forces into a biochemical response could result in a similar state of autonomy. While accelerated BM turnover may underly early stages of oncogenesis in certain systems, other tumors may only fail to maintain BM integrity after having gained the ability to proliferate autonomously by some other pathologic mechanism (e.g., viral transformation).

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