Cell Shape

Determinants, Regulation, and Regulatory Role

Edited by
Wilfred D. Stein and Felix Bronner

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Preface

The relationship between form and function is one of the most intriguing themes in biology. It was D'Arcy Thompson's incisive treatment of the subject that forced biologists to think seriously about how the needs of organisms might have shaped their form. Since that seminal work was written, the flowering of biochemical and molecular analysis has led to the discovery and description of very many compounds that either by virtue of their location or their structure may play specific roles in cell shape or assembly. We felt, therefore, that this would be an opportune time for summarizing what we know and still need to know about cell shape and its regulation.

The book is divided into four sections. The first, Basic Determinants of Cell Shape, attempts to relate the physics and structural engineering of the cell to its molecular components. In Chapter 1, Ingber and Folkman try to answer the question of how cell shape provides regulatory information. They do this by analyzing the mechanism of shape determination, summarizing how the extracellular matrix, the cytoskeleton, and biochemical force transduction may affect and respond to cell shape control. They apply the creative ideas on tensegrity, pioneered by the architect Buckminster Fuller, to the determination of cell form, and show how tensile and compressive forces in the cell may be transduced into chemical changes.

Oster, in Chapter 2, further develops the mechanical modeling of cell shape and its changes. Oster's viewpoint is that the biochemical reactions underlying cell motility are complicated, but that the mechanical forces are few in number. In particular, he considers the gel-like properties of the cell cortex to be the determinants of cell shape. Changes in cell shape, in turn, lead to cell movement. Oster's cortical tractor model, presented in this chapter, provides a unifying theoretical framework for a large number of seemingly disparate experimental phenomenona.

In Chapter 3, Sachs builds on the exciting progress that has been made in the study of isolated ion channels in cell membranes. He shows that such channels may themselves be the transducers of changes in cell shape.

Stretching and compression of the cell membrane can be sensed mechanically by the cytoskeleton. A signal from the cytoskeleton then travels to a specific ion channel, leading to a change in transmembrane ion flow, thus activating (or inhibiting) the cell.

Section II describes the results of the many molecular studies that have, in recent years, provided detailed information on the building blocks that make up the cytoskeleton and that seem to direct tissue arrangement as cells grow, differentiate, migrate, and assemble.

Ben-Ze'ev, in Chapter 4, deals with the cytoskeletal proteins (vimentin, desmin, the cytokeratins, and talin) and the regulation of their expression in the cell. He analyzes how desmosomes and adherens junction proteins affect or are affected by cell–substrate and cell–cell contact and how these in turn alter gene expression.

Fibronectin is the theme of Peters and Mosher's Chapter 5. This high-molecular-weight glycoprotein, found in the extracellular space, is a determinant of cell adhesion. Peters and Mosher discuss the molecular biology of fibronectin, its primary structure, the structure of its gene, and the molecular biology of fibronectin variants. Cells display receptors for fibronectin. This chapter deals with these also and with the mutual interactions between fibronectin and its receptors as these regulate cell adhesion, shape, and motility.

Hay and Svoboda, in Chapter 6, discuss the particular role played by actin in the cytoskeleton and describe their studies in which cytochalasins were used to direct changes in the cytoskeleton. They analyze how interaction between the extracellular matrix and actin is mediated by receptors on the cell surface. The mutual interactions among cell shape, cytoskeleton, and gene expression are explored in mesenchymal cells (preadipocytes, cartilage cells, and primordial fibroblasts) and in epithelia (mammary gland, granulocytes, and cornea).

When a quiescent cell is stimulated to divide, it retracts from its substratum, rounds up, divides, and the two daughter cells then reassume the spread-out form of the mother cell. Clearly, cell growth is intimately related to changes in cell shape. This relationship is the subject of Chapter 7, by Farmer and Dike. Their theme is that growth is modulated by the extracellular environment, by cell–cell interactions, and by specific growth hormones; the authors describe how the cytoskeleton responds to these effectors in terms of the control of gene expression.

Highly specialized cells of the body often have a shape that seems to reflect their function. Some examples of these are discussed in Section III. Steck, in Chapter 8, describes design requirements and the underlying molecular structure which permit red blood cells to travel through the widely varying diameters of the body's vasculature, undergoing compres-

sion and deformation as they pass through the capillaries. In particular, Steck considers the dominant role of spectrin as a determinant of membrane elasticity and describes disease models in which spectrin is defective. The role of actin and of protein bands 2.1 and 4.1 and their interactions in building the erythrocyte skeleton are discussed.

Of all the cells in the body, neurons have the most unusual shape. Their long, thin axons can extend as much as 10 meters in a great blue whale, distances along which the electrical signals travel efficiently and rapidly. Chapter 9 by Letourneau describes the functional polarization of a neuron into its three regions: the central soma, the multiple dendrites, and the single axon. Microtubules are a key element in neuron function, for the tracks along which membrane organelles are transported for great distances, and also constitute the major supportive elements of the axon. Interactions of the microtubules with the actin network regulate the progress of neuron elongation. The migration of the growth cone neuron and its guidance by chemotaxis and electrical fields are areas of intensive research which are discussed at length. Letourneau also points out what still needs to be established to give a firm molecular basis to our understanding of neuron structure.

The fact that cartilage cells are embedded in the extracellular matrix has given rise to many studies that have characterized matrix molecules and explored their interaction with these cartilage cells, the chondrocytes. Cartilage cells, grown in culture, are also a favorite object for investigating the interrelationships between cell shape and cell activity. Zanetti and Solursh, in Chapter 10, discuss the effect of cell shape on cartilage differentiation as mediated through the cytoskeleton and components of the extracellular matrix (such as fibronectin and the ectodermal antichondrogenic factor). In addition, they describe the effects of vitamin A, of phorbol esters, and of cAMP. They conclude that a round cell shape reflects the expression of chondrogenesis, while a spread cell is one arrested in a state that precedes chondrogenesis or is undergoing dedifferentiation.

Sato and Rodan, in Chapter 11, discuss the two cell types of bone, the osteoblasts, concerned with bone formation, and the osteoclasts, bone-resorbing cells. Bone, an extracellular structure, undergoes continuous remodeling, mediated by these two types of cells. To act at specific sites in bone, osteoblasts and osteoclasts not only need to reach those sites (and hence need to be motile), but they need to replace one another in turn if formation of bone is to be succeeded by resorption or vice versa. These movements require shape changes, the molecular determinants of which are discussed in this chapter. Systemic signals, such as parathyroid hormone which induces bone resorption, cause osteoblasts to signal to osteoclasts, these latter cells possessing no receptors for the hormone. Bone

adapts to mechanical forces, modifying its structure so as to support the load to which it is exposed. A major emphasis of this chapter is a discussion of how bone cells respond to and mediate mechanical perturbations.

The last section (IV) discusses how the shape of whole organisms is determined. In Chapter 12, Goodwin considers those whole organisms which are themselves single cells, protozoa and unicellular algae. The complicated process of morphogenesis in *Paramecium* involves a templatelike reproduction of the details of cell shape when the cell divides, essentially utilizing cytoplasmic inheritance. In *Tetrahymena,* as another example, the reproduction of the oral apparatus seems to involve the expression of a morphogenetic field. The roles of such fields are discussed in detail. In *Acetabularia,* mechanochemical fields, mediated by cytoskeleton–calcium interactions, seem to be the basis of morphogenesis.

Finally, in Chapter 13, Watt and Smith review the shape changes that occur during embryogenesis. They discuss how the polarity of the embryo is determined and the processes involved in gastrulation and notochord formation. The role of the unusually shaped "bottle" cells and of cell migration are stressed. The main driving force in gastrulation is "convergent extension," a process involving intercalation of cells to form a long and narrow array, the extension of which drives gastrulation. In neurulation, the first visible sign is a change in shape of the prospective neural cells that become tightly packed and columnized. Watt and Smith discuss how these shape changes bring about that bending of the cell sheet which forms the neural tube.

We trust the book will give the reader insight into how the shape of a cell fits it for its specialized function, how this shape is determined at a molecular level, and how the shape of the cell is itself a signal, controlling gene expression in the cell and modulating intercellular interactions. For most cells, we have not yet arrived at that detailed understanding of cell shape that we have for the red blood cell. We hope, however, that this book will lead investigators to generate, for other types of cells, the knowledge that we will need if we are to pass with confidence from understanding shape at the molecular level to understanding it at higher levels of biological organization.

The editing of this book was completed while one of us (F. B.) held the Varon Visiting Professorship at the Weizmann Institute of Science, Rehovot, Israel. Our thanks go to the Weizmann Institute for its hospitality and to the Weizmann Renal Research Fund for its continuing support of the research efforts of W.D.S.

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I

Basic Determinants of Cell Shape

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Tension and Compression as Basic Determinants of Cell Form and Function: Utilization of a Cellular Tensegrity Mechanism

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I. Introduction

Can the form of a cell or tissue dictate its function? If so, how can cell shape provide regulatory information to the cell? In this chapter, we will attempt to answer these questions by analyzing the mechanism of cell shape determination.

Cell shape may be viewed as a visual manifestation of underlying pat-

terns of structural forces, since it results from the action of tensile forces generated by intracellular contractile microfilaments and resisted by extracellular matrix attachment points. We have constructed three-dimensional cell models in order to analyze the role of mechanical forces during cell form modulation. Our cell models are comprised of a discontinuous array of compression-resistant struts that do not physically touch one another, rather they are pulled open through interconnection with a continuous series of tension elements. The stability of this type of architectural system, like that of the cell, therefore depends on maintenance of tensional integrity, or what has come to be termed "tensegrity" (Buckminster Fuller, 1975; Edmondson, 1987).

We now extend our previous studies (Ingber and Jamieson, 1985) and demonstrate that inorganic tensegrity cell models can be used to predict a variety of dynamic, substratum-dependent alterations of cell form. Based on these findings, we propose that cell shape changes may convey regulatory information through modulation of physical force distributions. A possible mechanochemical mechanism for transduction of tensile and compressive forces into changes of cellular biochemistry is also presented.

II. Cell Shape and Regulation of Cell Function

Hormones and other soluble mediators clearly play a central role in the regulation of mammalian cell physiology. However, cell growth and differentiation can vary greatly across small distances, suggesting that the action of humoral factors is subject to local control at the level of the cell. For example, normal cells are "anchorage dependent" in that they proliferate in response to stimulation by soluble mitogens when attached to a substratum, but fail to grow if suspended in the same medium.

We have previously suggested that anchorage-dependent cell growth may be the result of cell "shape dependence" (Folkman and Moscona, 1978). This hypothesis was based on the finding that cell proliferation requires more than contact with a planar surface; cells must be able to spread and change their shape in order for them to enter the synthetic phase of the cell cycle (Maroudas, 1973b; Folkman and Moscona, 1978; Iwig *et al.*, 1981; Ingber *et al.*, 1987). In general, DNA synthesis increases exponentially in response to linear increases in projected cell and nuclear area (Ingber *et al.*, 1987). Other aspects of nuclear metabolism, such as RNA synthesis and DNA virus replication, are similarly regulated by cell configuration, although cytoplasmic functions (e.g., protein synthesis and cytoplasmic RNA virus replication) are controlled independently (Benecke

et al., 1978; Ben-Ze'ev *et al.*, 1980; Ben-Ze'ev, 1983). In addition, cell geometry is centrally involved in the regulation of cell differentiation, gene expression, secretion, and in the generation of tissue form (Emerman and Pitelka, 1977; Spiegelman and Ginty, 1983; Lee *et al.*, 1984; Aggeler *et al.*, 1984; Ingber *et al.*, 1986a).

Cell shape may also serve as a physiological control element *in vivo*. For example, modulation of cell form appears to be central to the process by which angiogenesis (i.e., capillary development) is regulated. While angiogenesis can be triggered by soluble growth factors that act over large distances, we found that alterations in the integrity and composition of the extracellular matrix (ECM) can regulate growth factor responsiveness locally (Folkman, 1982; Ingber *et al.*, 1986b, 1987; Folkman and Ingber, 1987). The growth-modulating effects of different ECM molecules, in turn, correlate directly with their ability to support capillary endothelial spreading *in vitro* (Ingber *et al.*, 1987). Furthermore, antiangiogenic drugs that induce basement membrane breakdown within growing capillaries result in loss of anchorage, endothelial cell rounding, and capillary involution (Ingber *et al.*, 1986b; Folkman and Ingber, 1987). Basement membrane dissolution has been observed during involution of other epithelial tissues (Wicha *et al.*, 1980; Ikawa *et al.*, 1984), although associated changes of cell form have not been characterized.

Based on these findings, we suggested that capillary development may be regulated by a series of "solid-state" controls in which matrix-dependent alterations of cell form act locally to prohibit proliferation in response to a general angiogenic stimulus (Ingber *et al.*, 1987). In this manner, pattern formation during mammalian development may be similar to establishment of leaf position in plants; local growth differentials and complex tissue forms result from interplay between a global positive growth stimulus and multiple localized inhibitory signals (Steeves and Sussex, 1972).

III. Determinants of Cell Shape

Many scientists have a difficult time accepting the concept of cell shape as a biological regulator, because they view cell "form" as a phenomenological entity that is inaccessible to testing and measurement. Use of vague terms such as cell "shape," "form," "geometry," and "configuration" can also be the source of confusion. For example, migrating cells can change their shape without growing; however, cell spreading is coupled to growth when the cells become nonmotile and form focal adhesions (Couchman *et al.*, 1982). Blockage of cell cycle progression after the S

phase (e.g., secondary to irradiation damage or treatment with chemicals such as mitomycin C or ICRF-159) can also induce extensive cell spreading while suppressing both DNA synthesis and cell proliferation (Mitchison, 1973; Lazo *et al.*, 1978). Inhibition of cell growth could be due to interference with dynamic alterations of cell structure, since cells appear to almost become physically fixed in greatly extended forms during blockage in the G_2 phase. Furthermore, we have shown that growth is not linked to cell shape per se, but rather to cell spreading or extension; bipolar and polygonal cells of the same size (i.e., projected cell areas) display similar DNA synthetic rates even though they exhibit different shapes (Ingber *et al.*, 1987). The significance of cell extension may be due to associated changes of nuclear structure, since cell spreading results in nuclear matrix rearrangement and expansion. Thus, one of the major goals of this chapter is to redefine cell shape at the molecular level in terms of chemical and physical determinants that can be measured and quantitated.

Every organism is comprised of cells of various types, shapes, and sizes. However, all cells display common properties which suggest some universal rules of organization. Mammalian cells that are capable of undergoing division contain a nucleus and specialized plasma membrane, interconnected by a continuous series of cytoskeletal filaments. Cytoskeletal elements also interconnect with ECM molecules via specific cell surface transmembrane receptors. We believe that one of the major principles of cell shape-dependent regulation relates to the mechanism by which these different systems are structurally and functionally interconnected.

A. The Cytoskeleton

The mammalian cytoskeleton is a highly integrated structural network that is comprised of three major classes of filamentous polymers: microfilaments, microtubules, and intermediate filaments. Changes of cell shape and motility are the result of directed alterations of cytoskeletal dynamics: filaments polymerize, depolymerize, contract, elongate, and change their position. The cytoskeleton is integrally involved in a variety of cell functions, including force production and transduction, cell surface modulation, phagocytosis, secretion, intracellular transport, organelle translocation, cell migration, and mitogenesis.

1. Microfilaments

In cells, actin may exist in a nonfilamentous form (G-actin) or in a polymerized state (F-actin) within microfilaments (4–6 nm in diameter) and higher-order "stress fibers." Most actin-containing microfilaments also

contain myosin as well as various other "actin-associated proteins" including α-actinin, troponin, tropomyosin, calmodulin, and filamin. Actin fibril length is maintained by a steady-state polymerization of G-actin. However, some actin-binding proteins (e.g., gelsolin, profilin, villin, and the actinins) are also involved in the local regulation of microfilament assembly (Stossel *et al.*, 1985).

Actin-containing microfilaments are centrally involved in cell shape determination since they are responsible for force production by cells (Korn, 1978; Clarke and Spudich, 1977; see also Section II,D). Actin–myosin interactions within contractile microfilaments apparently act in a manner analogous to that observed in muscle to generate tension (Murray and Weber, 1974). Force production is required for changes of intracellular consistency as well as cell extension and movement (Taylor *et al.*, 1973). Contractile microfilaments are also responsible for movements during cytokinesis (Schroeder, 1975). Microfilaments appear to be functionally interlinked with intermediate filaments and microtubules (Pollard *et al.*, 1984), although the structural basis of these interconnections is not known.

Microfilaments interconnect with extracellular molecular assemblies at distinct sites on the cell surface. Disruption of tight junctions that normally link membranes of neighboring epithelial cells results in rapid microfilament disorganization (Meza *et al.*, 1980). However, actin may not interact directly with junctional elements. Rather, different actin-associated molecules (e.g., vinculin and α-actinin) appear to join microfilaments with the cell surface in these regions (Geiger *et al.*, 1981; Chen and Singer, 1982). Actin also appears in a reticular pattern within the cell cortex, directly beneath the plasma membrane. Interestingly, the mobility and function of a variety of other cell surface proteins, including receptors involved in growth regulation, also appear to be functionally dependent upon the state of actin filament assembly (Yahara and Edelman, 1975; Gall and Edelman, 1981; Weatherbee, 1982).

ECM components that mediate cell attachment to external sites can modulate intracellular patterns of microfilament organization (for an excellent review of this, see Burridge, 1986). For example, contact of the cell surface with exogenous ECM molecules triggers polymerization of actin into fibrous bundles (Sugrue and Hay, 1981; Ingber *et al.*, 1986a) and results in consistent orientation of intracellular organelles within polarized epithelial cells (Ingber *et al.*, 1986a). Morphological studies show that ECM molecules, such as fibronectin, colocalize with actin within cell adhesion sites (Hynes and Destree, 1978; Chen and Singer, 1982). Talin, α-actinin, vinculin, and nonerythroid spectrin may interlink microfilaments with the cell surface in these regions (Singer and Paradiso, 1981; Geiger, 1979; Pratt *et al.*, 1984; Horwitz *et al.*, 1986). Enzymatic removal of ECM

proteins results in loss of anchorage, cell rounding, and dissociation of cytoskeletal filaments (Pollack and Rifkin, 1975). Other cell adhesion molecules (i.e., non-ECM) may also be involved in structural linkages between neighboring cells (Edelman, 1984; Edelman *et al.*, 1987). Thus, cell surface receptors involved in both cell–cell and cell–substratum adhesion appear to be physically linked to actin-containing microfilaments.

2. *Microtubules*

Microtubules are tubular polymers (24 nm in diameter) comprised of tubulin and its associated binding proteins. Centrosomes within the cell's microtubule organizing center serve as nucleation sites for most microtubules, although free tubulin polymers may also form (Mitchison and Kirschner, 1984). The microtubular lattice is extremely dynamic; interphase microtubules exchange with soluble subunits and alter in length within minutes.

In vitro studies suggest that microtubule assembly is best characterized by the term "dynamic instability"; microtubules coexist in growing and shrinking populations that only rarely interconvert (Mitchison and Kirschner, 1984). The ends of growing microtubules appear to have guanosine triphosphate (GTP)-liganded caps, whereas shrinking microtubules do not. Microtubules therefore have a built-in instability based upon GTP hydrolysis by tubulin. Interestingly, tubulin polymer concentration usually remains relatively constant. The reason for this is that, as microtubules decrease in number, remaining tubulin polymers increase their length to a commensurate degree; the total amount of polymer is stable although that of individual microtubules is not.

In living cells, microtubule-associated proteins may promote nucleation as well as stabilize the ends of tubulin polymers. The mechanism by which specific spatial arrangements of microtubules are produced remains unclear. However, it is possible that alterations of microtubule assembly may be regulated by selective stabilization within a cell (e.g., along sites of cell–substratum attachment) in a manner analogous to that observed in studies with isolated centrosomes (Kirschner and Mitchison, 1986). Relative stabilization of one end of a microtubule results in much more rapid addition of tubulin monomers at the opposite end; polarized microtubular assembly results (Dentler *et al.*, 1974). Selective stabilization may play a central role in the establishment of cell orientation (Kirschner and Mitchison, 1986), since a cell's axis of polarity can be defined by determining the location of the microtubule organizing center relative to its nucleus (Gotlieb *et al.*, 1981).