Cell Biology International 2002, Vol. 26, No. 4, 313–317
doi:10.1006/cbir.2002.0857, available online at http://www.idealibrary.com on IDEAL®

MINI-REVIEW

MECHANICAL ASPECTS OF CELL SHAPE REGULATION AND SIGNALING

WOLFGANG H. GOLDMANN

Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, U.S.A.

Received 6 September 2001; accepted 7 January 2002

Physical forces play a critical role in cell integrity and development, but little is known how cells convert mechanical signals into biochemical responses. This mini-review examines potential molecular mediators like integrins, focal adhesion proteins, and the cytoskeleton in the context of a complex cell structure. These molecules—when activated by cell binding to the extracellular matrix—associate with the skeletal scaffold via the focal adhesion complex. Vinculin is presented as a mechanical coupling protein that contributes to the integrity of the cytoskeleton and cell shape control, and examples are given of how mechanical signals converge into biochemical responses through force-dependent changes in cell geometry and molecular mechanics.

KEYWORDS: integrin; vinculin; actin; mechano-transduction; extracellular matrix.

INTRODUCTION

To date, there are many known examples that suggest that mechanical tension between the various interconnected assemblies in the extracellular matrix and the cytoskeleton play a large role in determining cell shape and structure. Cell-generated forces have been shown to regulate biological functions ranging from growth and differentiation to gene expression, indicating that molecules are ultimate responsible for these functions (Chicurel *et al.*, 1998*a*). To understand how mechanical forces regulate cell behavior one has to assume that a subtle balance of force is maintained, which is brought about by many intracellular and extracellular components. Changes in tension influence the molecular structure and biochemical activity of the cell. This is in contrast with the typical, purely chemical signaling, which starts with an agonist-stimulated receptor that then propagates a cascade of biochemical events (Janmey, 1998).

Mathematical modeling of cells has shown that essentially pre-stress and architecture influence the mechanical stability of the cell. Pre-stress determines the initial stiffness of the structure and assures that the system will respond immediately when stressed externally, whereas architecture refers to the number of building elements as well as how they distribute forces (Stamenovic *et al.*, 1996). The pre-stressed system of molecular connections provides a discrete path for mechanical signal transfer through the cell as well as a mechanism for producing integrated changes in cellular and nuclear response to stress. Changes in local stresses may alter the cellular biochemistry by bringing different immobilized enzymes and substrates into position or by altering molecular mechanics and thereby changing local thermodynamic and kinetic parameters (Meyer *et al.*, 2000).

Stress-induced changes in the geometry of the cytoskeleton or mechanics may also influence signal transduction. Since signaling molecules lie in the path of mechanical force transfer, the focal adhesion complex (FAC) represents a potential site

To whom correspondence should be addressed: Wolfgang H. Goldmann M.B., Ch.B., Ph.D., Department of Medicine, Renal Unit, Massachusetts General Hospital, Harvard Medical School, Building 149, 13th Street, Room 8200, Charlestown, MA 02129 U.S.A. Fax: +1 (617) 726 5671. E-mail: wgoldmann@partners.org
This paper is dedicated to the late Elizabeth F. Finnigan.

1065–6995/02/$-see front matter

for translating mechanical stresses into biochemical responses. Molecules that are incorporated within insoluble macromolecular scaffolds that bear mechanical loads transmitted from, e.g. integrins could change their chemical potential as well as their shape and motion and determine their chemical behavior (Wang and Ingber, 1994).

Despite intensive investigation into understanding how the cytoskeleton responds to chemical stimuli, the mechanism by which external forces are transmitted across the cell surface and transduced into a cytoskeletal response is still poorly understood. This mini-review describes techniques that have been used to illustrate how the mechanics of the cell regulate cell shape, signaling, and function, and particularly how the mechanical coupling protein vinculin affects these parameters.

Control of cell shape and cytoskeleton

The extracellular matrix (ECM) is critical in determining whether cells will grow, differentiate, or undergo apoptosis (Galbraith and Sheetz, 1998). The ECM regulates cell morphogenesis through FAC by altering the structure of the intracellular cytoskeleton (CSK), which in turn orients much of the cell's metabolic machinery. So far much attention has been given to the molecular basis of CSK polymerization and assembly, but little is known about how cell geometrics control CSK mechanics and mechanical tension (Chen *et al.*, 1997).

To demonstrate the importance of cell shape a technique was used, which was originally developed for the microchip industry, that allows spontaneous assembly of monolayers of alkanethiols to create micropatterned surfaces, producing chemically identical adhesive islands of arbitrary size and geometry at a micrometer level (Singhvi *et al.*, 1994). Goldmann *et al.* (2000) describe in detail the various stages involved in generating patterned substrates to create islands of ECM surrounded by nonadhesive regions for single cells to attach and spread only on adhesive regions. Using this technique has shown that ECM appears to be the dominant regulator that dictates whether cells proliferate, differentiate, or die. Studies with living and membrane-permeabilized cells confirm that changes in cell shape result from the action of mechanical tension, which is generated within microfilaments and balanced by resistance sites within the underlying ECM. Analysis of the molecular basis of these effects reveals that ECM molecules alter cell growth via both biochemical and biomechanical signaling mechanisms (Sheetz *et al.*, 1998).

Application of mechanical methods

Wang *et al.* (1993) developed a magnetic twisting device in which controlled mechanical stresses are applied directly to cell surface receptors and hence to the cytoskeleton, using ferromagnetic microbeads pre-coated with specific ligands. In this technique cellular responses to applied stress are measured simultaneously by quantifying changes in the rotation (i.e. the angular strain) of the surface-bound magnetic beads. These researchers found that the stiffness (i.e. the ratio of stress to strain) of the cytoskeleton increased in direct proportion to the applied stress and that the cytoskeleton functions as a tensionally integrated structure.

Visualizing cellular effects of force application

In addition to measuring physical parameters of single cell populations via magnetometry as outlined above, the magnetic twisting device can be used to apply a controlled force to specific receptors within a population of spread cells. For this application the magnetometer is used only to verify the application of the vertical 'twisting' magnetic field rather than to record experimental data. Following mechanical force application by magnetic bead twisting, biologic outputs can be measured by conventional laboratory techniques. For example, using *in situ* hybridization to quantify mRNA and ribosome recruitment to the FAC, Chicurel *et al.* (1998*b*) used the magnetic twisting device to apply mechanical stress to ECM-coated magnetic microbeads and found that these elements of the protein-translation machinery are preferentially recruited to the FAC in a stress-dependent manner. Their data suggest that altering the balance of mechanical forces, specifically across integrins, induces formation of a micro-compartment at the FAC specialized for protein translation.

Stiffening response of cells

In studies using different cells and beads, the stiffening response was found to be linear although of varying intensity. The response to mechanical stresses of cultured pulmonary smooth muscle and capillary endothelial cells using microbeads coated with either RGB peptides or specific antibodies against integrins were in the order $\beta1>\alpha V\beta3>\alpha5>\alpha2>\alpha V$ (Lee *et al.*, 1998). In general, integrin receptors mediate mechanical force transfer across the cell surface and to the cytoskeleton, whereas other transmembrane receptors (e.g. scavenger receptors, i.e. AcLDL and platelet

endothelial cell adhesion molecule, i.e. PECAM) do not. In contrast, experiments where beads were coated with the receptor (uPAR) of the urokinase plasminogen activator (uPA) found at the leading edge of migrating monocytes suggested a mechanical link between uPAR and the cytoskeleton (Planus *et al.*, 1997). In another set of experiments these authors demonstrated that beads coated with H18/7 and bound to the surface of IL-1β-activated human umbilical vein endothelial cells (HUVEC) induce transmembrane cytoskeletal linkage of E-selectin through its cytoplasmic tail. Recently, Potard *et al.* (1997) compared RGD-coated with HECD-1 (anti-E-cadherin antibody) in confluent epithelial cell lines (MCF7) and found that the stiffness of the cytoskeleton through integrins was significantly higher than through E-cadherins. Comparing results with those of non-confluent cells suggests that the degree of confluency may be associated with different mechanisms and functions of the cytoskeletal network. More recently, using a vimentin-deficient and vinculin-deficient cell line and RGD-coated beads in twisting experiments showed reduced mechanical stability, motility, and other cellular functions depending on mechanical stability (Ezzell *et al.*, 1997; Eckes *et al.*, 1998).

Role of tension of the cytoskeletal network in intracellular signaling

There are many examples that suggest that the various interconnected assemblies in the extracellular matrix and the cytoskeleton play an important role in signal transduction events accompanying cell differentiation, gene expression, and even the induction of chronic myelogeneous leukemia. Mechanical force in form of stress or shear has been shown to regulate the expression of a number of genes. However, the mechanism by which the macromolecular networks or mechanical forces may participate in signaling remains elusive.

In signaling, where neighboring cells communicate via locally secreted chemical mediators either directly or through the extracellular matrix, the first step in the pathway is the binding to cell surface receptors (i.e. integrins). In physical terms, this corresponds to conformational energy. Conformational changes may be drastic, and the accompanying energy changes are quite substantial. The energy may manifest itself in the form of stress, and therefore, through the intracellular domain of the receptor and its connection to the cytoskeleton, may cause or at least contribute to the deformation of the microfilament and possibly via intermediate and microtubule networks. If the networks are interconnected assemblies, the deformation can propagate through the cell and eventually be sensed in the nucleus (Chicurel *et al.*, 1998*b*).

There are a number of examples that suggest that the purely mechanical and purely biochemical mechanisms of signal transduction are complementary rather than mutually exclusive. The activated receptor through direct or indirect connections modifies the mechanical state of the cytoskeletal network. A signaling molecule (i.e. PKC) bound to the network is activated (or deactivated) by this modification, for example by the increase or decrease of local stress and/or tension. It may remain attached to the network or it may dissociate from it. In both cases, the assembly may change its architecture locally. The actin network may also reorganize or completely disassemble. A kinase detached from the network, may diffuse for a short time and then bind again either to the network or to another kinase. Such a combination of mechanical and chemical signals may provide considerable variability and redundancy, and with this, an additional level of regulation.

The suggested mechanism for biological signal transduction, which relies on and combines the biochemical and mechanical views of signaling, provides the means to transmit signals in a fast and reliable way and to accommodate the various needs of the organism by shifting to a more mechanical or more biochemical signaling pathway. This can be accomplished simply by dynamic restructuring of the cytoskeletal network (Pourati *et al.*, 1998).

Vinculin: a mechanical coupling protein

Vinculin is required for the efficient coupling of integrins with the cytoskeleton and provides a starting point at which to define the intermolecular interactions that mediate mechanical signal transfer in living cells (Ezzell *et al.*, 1997). As described in work by Johnson and Craig (1994), vinculin can dimerize with itself and bind to actin; however, it does not appear to bind directly to integrins. Rather it interacts indirectly, binding with other focal adhesion proteins such as talin, alpha-actinin, or paxillin. This illustrates that there are several interactions that could mediate the connection of actin to the plasma membrane.

In one study, the stiffness of mouse F9 embryonic carcinoma cells (treated with the mutagen ethanemethylsulfonate to produce an adhesion-defective cell line), called 5.51, was reduced to 50% of that measured in the wild-type cells. Transfection of vinculin into 5.51 cells then

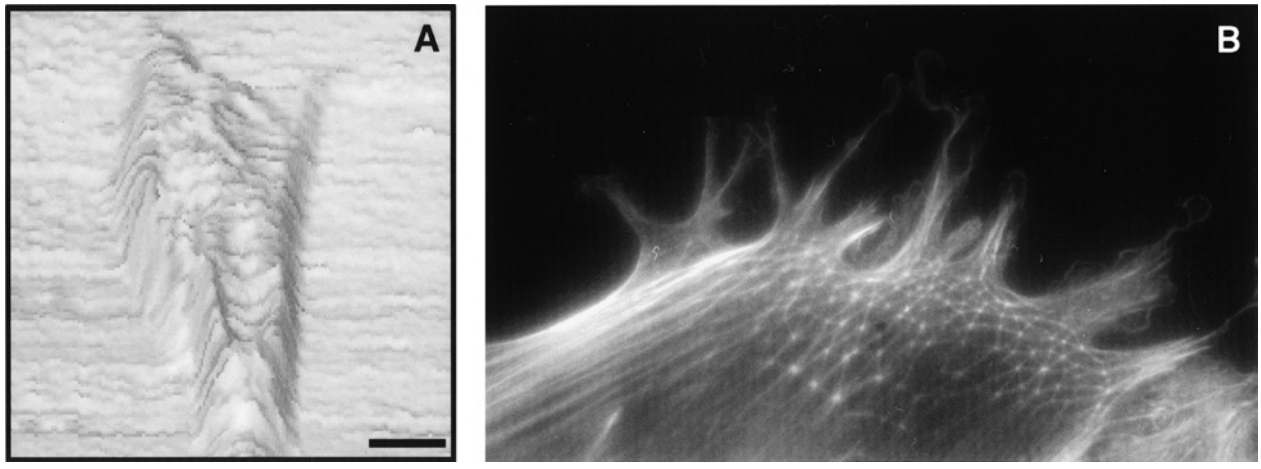

Fig. 1. (A) The 3D *elastic* image of a F9 wild-type cell using atomic force microscopy and NIH imaging (shading) technique, where 'softer' regions are shown by their height relative to 'harder' regions. (B) The leading edge of a F9Wt cell using confocal microscopy, where focal adhesion complexes (FACs) are clearly visible connecting to the cytoskeleton. Conditions: 35 mm NUNC dishes are coated with 2 μg/ml poly-D-lysine and WtF9 cells are incubated for 4 hours prior to imaging. Bar in (A) equals 10 μm.

restored the mechanical linkage. These results indicate that the vinculin-deficient cells are less able to resist mechanical deformation and less effective at transmitting mechanical stress to the internal cytoskeleton (Ezzell *et al.*, 1997).

Given the findings concerning the F9 wild-type and vinculin-deficient cells, however, it is expected that the vinculin constructs that restore normal adhesion, spreading, and actin organization also restore the mechanical linkage between the integrins and the cytoskeleton, and thus restore the mechanical properties of the cell. The ability of the magnetic twisting device to detect and quantify differences in the mechanical properties of focal adhesions has been indispensable for examining the transfected cells. An increased mobility of bound RGD-coated beads and a decreased resistance to deformation by twisting reflected a weak mechanical link to the cytoskeleton. If a stronger link between the extracellular matrix and the cytoskeleton is necessary for normal cell motility, this would help to explain why the vinculin-deficient cells adhere to fibronectin but spread less and have fewer lamellipodia than do the wild-type parental cells (Goldmann *et al.*, 1998).

Recently several techniques have been used to examine another vinculin-deficient F9 cell line (which was generated using homologous recombination to selectively target and disrupt the expression of both copies of the vinculin gene, Coll *et al.*, 1995), F9Vin(-/-), where significant differences in viscoelasticity between them and wild-type cells were measured. The viscosity of F9Vin(-/-) cells was restored to wild-type levels after transfection with increasing amounts of vinculin. Transfection of vinculin missing either the head or tail domain only partially restored the viscosity of F9Vin(-/-) cells (Goldmann and Ingber, 2002). Only when intact vinculin was added did the assembly of focal adhesion complexes and transmembrane mechanical coupling to integrins during cell adhesion and spreading return to wild-type level. Atomic force microscopy (AFM) was used on F9 wild-type and vinculin-deficient F9Vin(-/-) cells to examine the relation between vinculin protein structure and function in the context of control of cell shape, cell mechanics, and lamellipodia formation and it was shown that F9Vin(-/-) cells failed to spread, extend lamellipodia, or maintain effective cell stiffness relative to WT cells. Transfection of F9Vin(-/-) cells with the head or tail domain alone was unable to reverse these effects. Simultaneous expression of the head and tail domains was slightly more effective, but only replacement with intact vinculin completely restored normal cell shape or mechanics. These results demonstrate that vinculin's ability to mechanically couple integrins to the cytoskeleton, to modulate cell mechanics, and to promote changes in cell shape all require more than individual protein–protein binding interactions and depend on its intact three-dimensional structure. However, mechanical coupling between vinculin, integrins, and the cytoskeleton is not essential for biochemical signaling in F9 cells, as demonstrated by Goldmann (2002). Fig. 1 shows in (A) a 3D-image of a wild-type F9 cell generated by atomic force microscopy and in (B) the leading edge of a wild-type F9 cell using confocal microscopy.

ACKNOWLEDGEMENTS

The author thanks Judith Feldman, Ph.D. for copyediting and proofreading this manuscript. W. H. Goldmann is a recipient of a *grant* from the German Government and from NATO.

REFERENCES

Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE, 1997. Geometric control of cell life and death. *Science* **276:** 1425–1428.

Chicurel ME, Chen CS, Ingber DE, 1998*a*. Cellular control lies in the balance of forces. *Curr Opin Cell Biol* **2:** 232–239.

Chicurel ME, Singer RH, Meyer CJ, Ingber DE, 1998*b*. Integrin binding and mechanical tension induce movement of mRNA and ribosomes to focal adhesion. *Nature* **392:** 730–733.

Coll JL, Ben-Ze'ev A, Ezzell RM, Rodriguez Fernandez JL, Baribault H, Oshima RG, Adamson ED, 1995. Targeted disruption of the vinculin gene in F9 and ES cells changes cell morphology, adhesion and locomotion. *Proc Nat Acad Sci USA* **92:** 9161–9165.

Eckes B, Dogic D, *et al.* 1998. Impaired mechanical stability, migration and contractile capacity in vimentin-deficient fibroblasts. *J Cell Sci* **111:** 1897–1907.

Ezzell RM, Goldmann WH, Wang N, Parasharama N, Ingber DE, 1997. Vinculin promotes cell spreading by mechanically coupling integrins to the cytoskeleton. *Exp Cell Res* **231:** 14–26.

Galbraith CG, Sheetz MP, 1998. Forces on adhesive contacts affect cell function. *Curr Opin Cell Biol* **10:** 566–571.

Goldmann WH, Galneder R, Ludwig M, Xu W, Adamson ED, Wang N, Ezzell RM, 1998. Differences in elasticity of vinculin-deficient F9 cells measured by magnetometry and atomic force microscopy. *Exp Cell Res* **239:** 235–242.

Goldmann WH, Alonso JL, *et al.* 2000. In: *Cytoskeleton: Signaling and Cell Regulation*. Carraway KL, Carraway CAC, eds., Oxford University Press. pp. 245–276.

Goldmann WH, Ingber DE, 2002. Intact vinculin protein is required for control of cell shape, cell mechanics, and rac-dependent lamellipodia formation. *Biochem Biophys Res Comm* **290:** 749–755.

Goldmann WH, 2002. Coupling of vinculin to the cytoskeleton is not essential for mechano-chemical signaling in F9 cells. *Cell Biol Int* **26:** in press.

Janmey PA, 1998. The cytoskeleton and cell signaling: Component localization and Mechanical coupling. *Physiol Reviews* **78:** 763–781.

Johnson RP, Craig SW, 1994. F-actin binding site masked by intramolecular association of vinculin head and tail domains. *Nature* **373:** 261–264.

Lee KM, Tsai KY, Wang N, Ingber DE, 1998. Extracellular matrix and pulmonary hypertension: Control of vascular smooth muscle cell contractility. *Amer J Physiol* H76–82.

Meyer CJ, Alenghat FJ, Rim P, Fong JH, Fabry B, Ingber DE, 2000. Mechanical control of cyclic AMP signalling and gene transcription through integrins. *Nat Cell Biol* **2:** 666–668.

Planus E, Barlovatz-Meimon G, Rogers RA, Bonavaud S, Ingber DE, Wang N, 1997. Binding of urokinase to plasminogen activator inhibitor type-1 mediates cell adhesion and spreading. *J Cell Sci* **110:** 1091–1098.

Potard US, Butler JP, Wang N, 1997. Cytoskeletal mechanics in confluent epithelial cells probed through integrins and E-cadherins. *Amer J Physiol* **272:** C1654–1663.

Pourati J, Maniotis A, Spiegel D, Schaffer JL, Butler JP, Fredberg JJ, Ingber DE, Stamenovic D, Wang N, 1998. Is cytoskeletal tension a major determinant of cell deformability in adherent endothelial cells? *Amer J Physiol* **274:** C1283–1289.

Sheetz MP, Felsenfeld DP, Galbraith CG, 1998. Cell migration: regulation of force on extracellular-matrix-integrin complexes. *Trends Cell Biol* **8:** 51–54.

Singhvi R, Kumar A, Lopez GP, Stephanopoulos GN, Wang DI, Whitesides GM, Ingber DE, 1994. Engineering cell shape and function. *Science* **264:** 696–698.

Stamenovic D, Fredberg JJ, Wang N, Butler JP, Ingber DE, 1996. A microstructural approach to cytoskeletal mechanics based on tensegrity. *J Theor Biol* **181:** 125–136.

Wang N, Butler JP, Ingber DE, 1993. Mechanotransduction across the cell surface and through the cytoskeleton. *Science* **260:** 1124–1127.

Wang N, Ingber DE, 1994. Control of cytoskeletal mechanics by extracellular matrix, cell shape, and mechanical tension. *Biophys J* **66:** 2181–2189.