Annals of Biomedical Engineering, Vol. 34, No. 1, January 2006 *(© 2006)* pp. 102–113
DOI: 10.1007/s10439-005-9008-1

Nanoscale Intracellular Organization and Functional Architecture Mediating Cellular Behavior

PHILIP R. LEDUC [1] and ROBERT M. BELLIN[2]

[1]Departments of Mechanical and Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA and [2] Department of Biology, College of the Holy Cross, Worcester, MA

(Received 23 February 2005; accepted 30 June 2005; published online: 3 February 2006)

Abstract— Cells function based on a complex set of interactions that control pathways resulting in ultimate cell fates including proliferation, differentiation, and apoptosis. The interworkings of this immensely dense network of intracellular molecules are influenced by more than random protein and nucleic acid distribution where their interactions culminate in distinct cellular function. By probing the design of these biological systems from an engineering perspective, researchers can gain great insight that will aid in building and utilizing systems that are on this size scale where traditional large-scale rules may fail to apply. The organized interaction and gradient distribution in intracellular space imply a structural architecture that modulates cellular processes by influencing biochemical interactions including transport and binding-reactions. One significant structure that plays a role in this modulation is the cell cytoskeleton. Here, we discuss the cytoskeleton as a central and integrating functional structure in influencing cell processes and we describe technology useful for probing this structure. We explain the nanometer scale science of cytoskeletal structure with respect to intracellular organization, mechanotransduction, cytoskeletal-associated proteins, and motor molecules, as well as nano- and microtechnologies that are applicable for experimental studies of the cytoskeleton. This biological architecture of the cytoskeleton influences molecular, cellular, and physiological processes through structured multimodular and hierarchical principles centered on these functional filaments. Through investigating these organic systems that have evolved over billions of years, understanding in biology, engineering, and nanometer-scaled science will be advanced.

Keywords—Cytoskeleton, Nanotechnology, Nanoscience, Intracellular organization, Structure.

INTRODUCTION

Cells are one of the essential units of life, yet their organization perplexes scientists and engineers on many levels. For many years, the analysis of natural systems has allowed designers to build better human-constructed systems. These include advances in optics based on the study of the eye and advances in flying based on the study of animal motion. Similarly, when working at the nanometer size scale, much can be learned by an analysis of nanometer-based biological systems. At this size-scale, the study of individual molecules and cells holds promise for many advances, especially when it is considered that this biological system has been optimized over billions of years. Because understanding the organization of the cell is critical to being able to emulate its complex functions at small scales, herein we will discuss the structure, components, and function of the cell.

The structural behavior in all mammalian cells, including cardiovascular, endothelial, smooth muscle, neural, and osteoclast cells, are known to be heavily influenced by the filament systems of the cytoskeleton.[5,33,47,57,93] The cytoskeleton is a complex and highly organized structure that has been shown to play a major role not only in controlling cell shape, but also in regulating motility,[8,60,84,94,95] division[7,16,46,86,98], and polarity.[21,23,32,45,72,74,99] The cytoskeleton itself has been shown to contribute directly to many processes in mechanical stimulation including its own remodeling under fluid shear stress, the strengthening of mechanical response by connecting the intermediate filaments to intercellular adhesion sites, and remodeling the actin cytoskeleton in the direction of minimal deformation under equibiaxial and uniaxial strain with elastomeric membranes.[12,43,113]. Moreover, forces distributed through the cytoskeleton have been shown to have long-range effects propagating into the nucleus, where single chromosomes can be displaced through mechanical manipulations of cells at their surface.[3,36] Mechanically stimulating cell transmembrane proteins such as integrins have resulted in a reorientation of cytoskeletal filaments and localized physical distortions of nuclei and nucleoli in the direction of the applied tension.[62,63] The cytoskeleton is interesting not only from a scientific standpoint, but also because many diseases and therapeutic methods are related to cell structure and the cytoskeleton, including wound healing, cancer metastasis, and potential treatments for vascular diseases including ischemic heart disease, stroke, and peripheral arterial disease.

Address correspondence to Philip R. LeDuc , Departments of Mechanical and Biomedical Engineering, Carnegie Mellon University 5000 Forbes Avenue, 415 Scaife Hall, Pittsburgh, PA 15213. Electronic mail: prleduc@cmu.edu, rbellin@holycross.edu

The cytoskeleton provides not only an interesting structure through which cells adapt and change mechanically, but also an organizational system that is similar to industrial configurations. For example, a cell has many organization elements that are suggestive of what one finds in an automobile factory. In a factory, the assembly line has allowed the production of complex systems (cars) through sequential time- and space-governed schemes. Based on ideas popularized by the assembly line methodology for the Ford Model T, automobiles are built by moving the frame along a line from one station to the next, where each station has a specific addition or modification of the frame to accomplish. Parallel to this structural organization, the ability for proteins to proceed through a set of modifying events (i.e., posttranslational modifications) is thought to be related to the cytoskeletal structures of the cell. This has been suggested due to the efficiency of the process, along with known association of protein complexes with the cytoskeleton. Complex cellular functions could be accomplished through using an organizational architecture in this and many diverse systems.

In most animal cell types, the cytoskeleton is composed of three major classes of cytoskeletal elements: actin filaments, microtubules, and intermediate filaments (Fig. 1). Actin filaments (F-actin) are double stranded polymers composed of monomer units of globular actin (G-actin). They are semiflexible with a high aspect ratio and a diameter of approximately 7 nm. F-actin is found dispersed throughout the cytoplasm, but most filaments are located just beneath the plasma membrane in the cortical region of the cell. Microtubules are approximately 25 nm in diameter. They are formed from 13 protofilaments, each made of polymerized α/β tubulin dimers that create a hollow cylinder. They are more rigid than F-actin in compression and are primarily located in the cell with one end attached to the microtubule organizing center just outside the nucleus. From this attachment point, microtubules extend toward the cell periphery. The third class of cytoskeletal elements, which is called the intermediate filaments, is a diverse class of protein filaments each of which is approximately 10 nm in diameter. Intermediate filaments are cell-type specific. For example, epithelial cells contain primarily intermediate filaments composed of the protein keratin, while muscle cells primarily have desmin-containing intermediate filaments. Intermediate filaments are positioned throughout the cytoplasm and exist as a specialized network. In the nucleus the network is called the nuclear lamina. Each of these three systems has also been shown to interact with a variety of associated proteins that influence both their structure and their biochemical signaling functions.

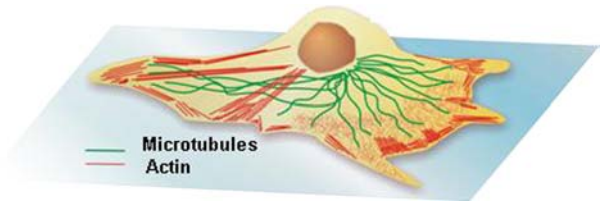

FIGURE 1. A nanometer-scaled engineering organization of a biological system (the cell) including structural components (the cytoskeleton) of actin filaments and microtubules. The actin cytoskeleton is just below the cell membrane and has connections across the entire length of a cell. The actin is anchored to the extracellular matrix through focal adhesion complexes and is involved in many processes including cell motility. Microtubules are distributed through the cytoplasm and mainly radiate from a central location in the microtubule organizing center to the cell membrane. The microtubules are involved in many cell functions including cell division and vesicle transport.

In this review, we will discuss the role of the cytoskeleton in cell function in terms of an essential nanoscale structural and organizational unit. We will focus on "nanobioengineering" in terms of understanding the engineering design of cellular systems at the nanometer size scale and also associated technology that has been interfaced with these biology systems. We will describe the cytoskeleton with respect to: (1) organizational structure and cell behavior; (2) mechanotransduction; (3) motor molecules; (4) additional cytoskeleton-associated proteins; and (5) nano- and microtechnology tools to probe it. It is our hope that the combination of these discussions will describe the intricate roles that the cytoskeleton plays in a variety of cell functions.

ORGANIZATIONAL STRUCTURE AND CELL BEHAVIOR

The necessary organizational capacity of a complex system such as a cell, which must integrate many processes including transport, intracellular and extracellular binding-reactions, and genetic transcription, suggests that an underlying architecture exists to compartmentalize these precise functions and allow the cell to function properly. In general, if a cell simply consisted of an aqueous environment surrounded by a lipid bilayer and governed by reactions mediated solely by diffusion, it would need little spatial organization. In actuality, cells exhibit densely packed molecules, sharp local concentration gradients, low numbers of individual subunits, and small or irregularly shaped compartments, all of which create deviations from the assumed idealistic environment. When examining a simplified solution-based system, diffusion of small molecules would immediately disperse cell components into an equilibration of homogenous distribution due to the micrometer width of individual cells and high diffusion constants of individual molecules. Models of pure aqueous environments are inadequate to describe findings on cellular organization. For example, it has been shown that particles greater than 20 nm exhibit limited diffusion within the

cytoplasm, likely due to the fact that intracellular distribution of protein molecules exist at densities ranging from 50 to 400 mg/ml causing macromolecular crowding (Fig. 2).[68,69,77,83] This high density of proteins in the cytoplasmic region reveals the difficulty with which diffusional transport would proceed and thus suggests that there must be an alternate plausible explanation for the execution of these intricate processes. The cytoskeleton is one of the most likely candidates.

One primary function mediated by the cytoskeleton in cells is molecular transport. The movement of materials within the cell and the overall organization of living cells, while relying on a number of factors, are both strongly influenced by the cytoskeleton. To influence these properties, any governing system must have access and a presence across the cell. The cytoskeleton spreads through the interior of the cell in its various forms and is known to be linked to additional networks in the nuclear domain via the intermediate filaments. This overall network of proteins provides the scaffolding for the complex functions to occur when coupled with the various cytoskeletal-associated proteins and motor molecules, which will be discussed later.

Structural organization exists at multiple scales in biological systems. At the tissue level, the extracellular matrix provides the organizational structure for developing complex tissues. The scaffolding in bones must be established for osteoblasts and osteoclasts to function in harmony and develop stable structures. Also, basement membranes, which are specialized mats of extracellular matrix, relay support and provide the necessary initial conditions for angiogenesis (i.e., the growth of vasculature). In general, self-assembly and organization are hallmarks of inherently smart materials. Units are joined together to form higher order stable systems, which have properties and functionality that are not obvious from the individual analyses of the solitary units. These traits exist at the cellular and molecular scales in the cytoskeleton.

For this structure to have more than just a mechanical influence, it must affect functions in living cells. Many cell tasks are known to be directly correlated to the cytoskeleton. Exocytosis allows the cell to move proteins from the intracellular compartments to the extracellular environment. This is accomplished through the encapsulation of specified proteins in a lipid-bilayer structured vesicle. The transport of the vesicles to the exterior of the cell is known to be heavily dependent upon the cytoskeleton, specifically, the microtubules. In studies using green fluorescent proteins, the transport efficiency of the fluorescent protein from the Golgi complex to the plasma membrane was observed to be significantly slowed when the microtubules were depolymerized using nocodazole—a tubulin binding agent that inhibits the polymerization of microtubules.[11] In cells with intact microtubules, the transport of the vesicles is observed to have a radial vector from the Golgi to the cell periphery (Fig. 3), yet when the microtubules are depolymerized, the transport that occurs is via random diffusion of the vesicles through the cytoplasm. Cytoskeletal-associated transport applies to other systems as well. The endoplasmic reticulum transports proteins to the Golgi apparatus for posttranslational processing. This transport is once again thought to be mediated by the microtubules because the depolymerization of these filaments inhibits this process.

The local organization and distribution of molecules in the cytoplasm leads to global cell response as well. The ability of a cell to move during chemotaxis is the integration of a complex set of molecular events occurring in a nonuniform environment. During cell motility, the

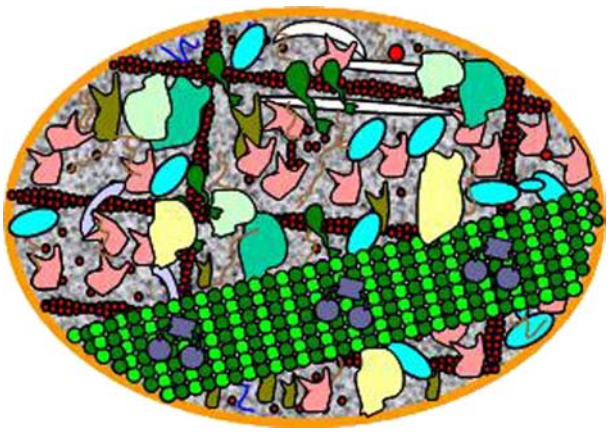

FIGURE 2. Schematic of the crowded distribution of molecules in living cells including microtubules and actin filaments. The distribution of molecules in the cytoplasmic space of a cell is a highly dense region. This closely packed arrangement can inhibit random diffusion and reaction processes due to particle interference and local binding-reaction events.

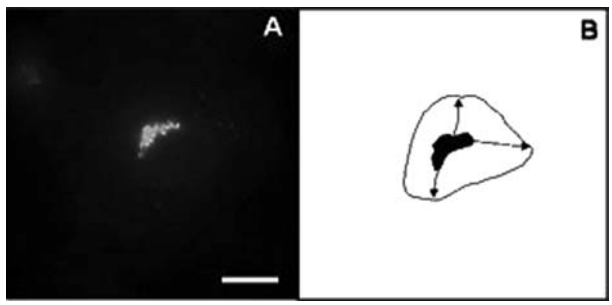

FIGURE 3. Green fluorescent protein chimeras visualized in transport from the Golgi apparatus to the plasma membrane during exocytosis. (A) Green fluorescent protein localized in the Golgi Apparatus and in small vesicles outside of the Golgi during transport. (B) Schematic of the path of the vesicles from the Golgi to the cell membrane. Tracking the vesicle mobility reveals the linear transport along the microtubules in various directions. After depolymerizing the microtubules, this process is inhibited. Scale *bar* = 10 μm.

leading edge of the cell experiences a significant increase in protein density. Specifically, actin monomers and filaments are found in highly amplified quantities in this region as the actin is actively moved toward the leading edge. This increase in the concentration of actin monomers and filaments is critical in the steps preceding motility where extension of lamella, lamellipodia, and filopodia requires high actin filament concentration for polymerization to impart mechanical force. To produce this force for extending podia, one hypothesis is that the actin filaments, which normally bear tensile loads, are bundled together and provide compressive strength for the extension of these cellular processes. If actin polymerization is inhibited through the introduction of agents, such as cytochalasin-D and latrunculin, podial extension and the associated motility is significantly restricted.

The ability of a cell to progress around the cell cycle and proliferate is also directly influenced by the cytoskeleton and its organization. In cell division, the ability of a cell to move from interphase to telophase is regulated by the microtubules and their reorganization to form the mitotic spindle. The microtubules assist in division as each chromosome lines up at a point that bisects the length of the aligned spindle. The chromosome is then pulled apart and each half moves along the microtubule path for the formation of two new and equal nuclei. Furthermore, in animal cells the actin cytoskeleton also plays a role during cell division in the formation of structures that define the plasma membrane. The two new cells are pinched off by actin filaments during cytokinesis at the end of M phase. Because the actin filaments are known to be located just beneath the plasma membrane in normal cells, this process for the separation and development of new plasma membrane structures seems ideal. Even prior to division, the progression into distinct stages of the cell cycle are mediated by the presence of intact cytoskeletal components.[41] Disruption of the cytoskeleton integrity inhibits the extracellular signal-regulated kinases and halts the cell cycle at the G_1 phase. The presence of the cytoskeleton in this time window allows for the progression of the cell machinery into the S phase and thus promotes cell growth. Through all of these different schemes, the cell exhibits an ability to organize itself through structures that influence numerous functions including transport and cell division. The cytoskeleton structure further affects the ability of a cell to respond to mechanical stimulation through chemical signaling.

MECHANOTRANSDUCTION

The cytoskeleton is directly related to the interconnected fields of cellular structure and mechanics, in which cells sense, generate, and respond to forces. The empirical effects of mechanotransduction (or mechanical stimulation inducing biochemical signaling) are known because cells sense and respond to mechanical stimulation. The mechanistic link though is not well understood. The mechanical stimulation of cells has produced a wide range of effects including responses in the proteome with the activation of the mitogen-activated protein kinase pathways such as p38 and jun-n-terminal kinase[22,56,90] and in the genome, as alterations in gene expression profiles.[31,82,105] The link between mechanical stimulation and biochemical response has recently focused more acutely on the cytoskeleton[2,6,34,44,88,121] and its associated extracellular matrix–cell connections.[24,85,116,118] Of particular interest is the manner in which forces are transmitted into cells from the extracellular environment. One of these links is through the focal adhesion complexes, which are a heterocomplex of proteins including paxillin, vinculin, talin, and the transmembrane integrins. These complexes are connected to the actin cytoskeleton as well as the extracellular matrix, which provides a direct link from the extracellular world (i.e., the extracellular matrix) to the intracellular architecture (i.e., the cytoskeleton).

One of the mechanisms for this mechanotransduction response is that these structural alterations are triggered through molecular interactions with specific cell surface receptors that bind to insoluble adhesion molecules. Thus, receptor signaling pathways can be utilized to control cell structure and function.[1,76,112,114] Studies have revealed the importance of focal adhesion complexes in the activation of mechanochemical cascades. Within the focal adhesion complexes, integrins have been shown to be critical. When localized forces are applied to a cell through integrin-specific antibodies, the cytoskeletal stiffness response increases in proportion to the applied forces. Through these links, the role of the cytoskeleton has been shown to regulate many processes in mechanical stimulation including the remodeling of the cytoskeleton under fluid shear stress, the strengthening of mechanical response through connecting intermediate filaments to intercellular adhesion sites, and remodeling the actin cytoskeleton

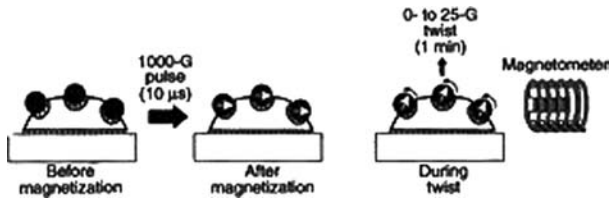

FIGURE 4. Mechanotransduction through the cytoskeleton. Schematic of magnetic twisting cytometry. Magnetic beads coated with integrin binding molecules including arginyl-glycyl-aspartic acid are compared to nonbinding molecules including bovine serum albumin. The magnetic beads are brought into contact for attachment to individual cells and then a magnetic field is introduced onto the system causing an angular rotation of the beads. The connection of the beads to the actin through the focal adhesion complex reveals a direct link of the force–strain response of the cells when linked to the cytoskeleton[112].

in the direction of deformation.[12,43,113] Furthermore, studies of stress and strain on cells have increased the understanding of mechanics with respect to intracellular behavior involving the cytoskeleton.[35,39]

From a biochemical perspective, local modulation of integrin receptors affects cell behavior because applying mechanical stresses alters the expression of cyclic AMP as well as induces local recruitment of mRNA and ribosomes to focal adhesions.[10,65] Membrane receptor signaling and gene transcription are activated in a force-dependent manner by applying magnetic twisting forces to these receptors via ferromagnetic beads coated with specific receptor ligands (Fig. 4).[65,112] Magnetically twisting cell surface adhesion receptors that are clustered (oligomerized) within focal adhesion complexes result in a rapid increase in the production of the second messenger, cyclic AMP. This increase in cyclic AMP leads to downstream signaling events resulting in the activation of gene transcription, as confirmed by demonstrating that magnetic activation of a reporter gene that encodes the enzyme, β-galactosidase, is driven by cyclic AMP-response elements. In addition to the proteins described earlier, associated structural candidates are likely to be involved in mechanotransduction. The membrane-associated proteins of guanine nucleotide binding proteins (G proteins) are associated with this cascade. They are heterotrimers composed of Gα, Gβ, and Gγ subunits. These subunits activate second messengers which have direct effects on the regulation of adenylyl cyclase, cyclic AMP, and inositol triphosphate. It is also quite likely that a family of proteins called syndecans may play a role in mechanotransduction similar to integrins. Syndecans are transmembrane heparan sulfate proteoglycans located at the cell surface with known binding affinities for the extracellular matrix and the actin cytoskeleton. Recent studies indicate that heparan–sulfate linked proteins likely play a role in mechanical signaling, and syndecans are the most likely candidates of this class of proteins due to their membrane spanning nature.[70]

MOTOR MOLECULES

Molecular motors are an active control mechanism that can move and organize entities from molecules to organelles often associated with the cytoskeleton through mechanochemical interfaces. Motor molecules, including kinesin, dynein, and myosin, have the ability to travel individually, or in combination with complexed proteins. These motors are able to convert the energy from adenosine triphosphate (ATP) hydrolysis into mechanical force, thereby creating a chemical-to-mechanical actuator for intracellular transport (Fig. 5). Even though these motors are nanometers in size, they move rapidly with velocities reaching up to 60 μm per s for myosin XI (considering the nanometer scale of these motors, this is analogous scale-wise to an automobile traveling km per s).

The ATP is the critical "fuel" and generates the power stroke necessary for motility of these motors. This fuel is thought to arise from the complex and tight coupling in the transition of ATP to adenosine diphosphate (ADP); thus with the addition of ATP, mobility of these proteins can be induced. The motion of these particular molecules is directly linked to the organization structure of the cytoskeleton. Myosin relies on the binding and actuation along the actin filaments, while kinesin and dynein likewise bind to microtubules. The discrete steps of these motors (e.g., 8 nm for kinesin) are based upon the ability to attach and detach successively while moving along monomers and dimers of the filaments. Through this motion, the motors are also able to apply forces which are used for intracellular transport as the motors bind and pull molecules and vesicles to reposition them to alternative areas in the cell; kinesin produces around 5 pN of force. Recent studies have analyzed the motor-molecule directed motion of organelles down to a nanometer size scale, thereby providing even more insight into these motor molecules and their movement.[53] These motors are also being harnessed by researchers who are creating biological hybrid devices for mechanical actuation as they move distinct distances while attaching

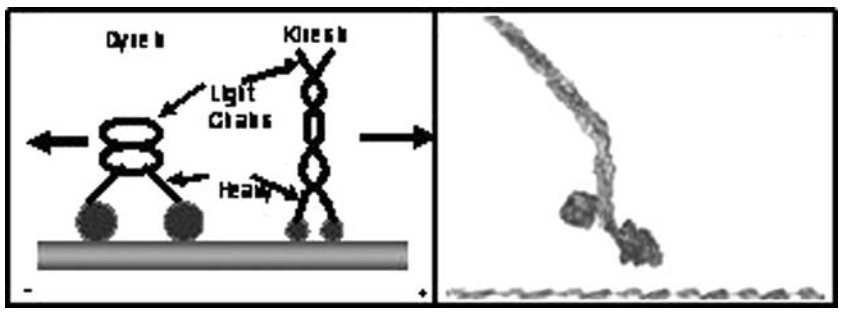

FIGURE 5. Molecular motors for transport and organization in living cells. Model of kinesin capturing the resulting mechanical motion of the motor from the chemical energy conversion.[109] They attach to the cytoskeleton scaffolding and to systems including vesicles in living cells through their light chains. The two heavy chains of these motors "walk" along the cytoskeleton tracks to actively transport and organize systems within cells.

to organic and inorganic systems. Thus, the motion of the motor molecules that is used for active transport inside living cells also provides the scaffolding for the directional movement of these nanometer-sized mechanochemical power engines. This capability is involved in cell functions including exocytosis, as the vesicles are transported along microtubule tracks. This also is critical in cell motility with respect to the force generation at the leading edge of cells, where actin filaments push the leading edge of the cell forward for attaching the cell to the substrate often through the focal adhesion complexes, which are also linked to the actin cytoskeleton.

ADDITIONAL CYTOSKELETAL-ASSOCIATED PROTEINS

A major unresolved issue in the study of signaling pathways relates to the intracellular signaling proteins. The ability for proteins to organize in cells involves parameters of both time and space. Thus a mechanism must be in place within the cellular environment for this to proceed. As discussed earlier, the cytoskeleton is integrally related to trafficking of molecules in the cell. In this, the ability to form scaffolding systems where proteins are assembled for complex spatiotemporal signaling may be mediated by cytoskeletal-associated proteins beyond motor molecules; these provide a variety of functions. In the case of F-actin networks, there are specific proteins which control structural organization in terms of the formation of the stress fibers in the cytoplasmic region. For example, filamin is an actin-associated protein that is responsible for binding the actin filaments into a gel-like network. The binding of filamin inhibits the binding of the competing associated proteins, tropomyosin and α-actinin. Actin filaments in the absence of filamin can bind to tropomyosin, which attaches along the length of the filaments increasing its strength. In turn, this tropomyosin binding allows the α-actinin to bind to the plus end for bundling of the actin filaments, creating stress fibers. The result of this competitive system is two distinct organizations: 1) a loose network of actin filaments, and 2) closely bundled actin filaments. These bundles are involved in contractility response along with myosin-II. In addition to the filamin/tropomyosin system, the ARP 2/3 complex is involved with actin branching for controlling the structural organization in cells.

Subcellular trafficking along the cytoskeleton reveals a rich, dynamic, and spatial process when activated with extracellular stimuli.[37,73] Signaling from the cytoplasmic to the nuclear regions has been proposed to be linked to the cytoskeleton-associated protein plectin and to nuclear pore structures. Plectin provides a molecular organization for mediating association with an array of cytoskeletal elements. The interactions of this large protein (over 500 kDa) include association with actin and intermediate filaments as well as binding to the transmembrane integrins, which are involved in the mechanotransduction cascade. Through these associations, the regulation of signaling including p34(cdc2) kinase is induced, which has further effects on cell functions including mitosis through the intermediate filaments.[81,97]

The cytoskeleton of individual cells can influence trafficking through a mechanism whose association with the cytoskeleton is debated.[13] Specifically, glucocorticoid receptors move from the cytoplasm to the nucleus under external stimulation of a steroid such as dexamethasone. This transport is linked to the cytoskeleton, but the exact mechanism is not known.[38] One postulate is that chaperones, proteins that are associated with both the glucocorticoid receptors and the cytoskeleton, alter the transport properties of the cell. The glucocorticoid receptor is a gene regulatory protein that is bound in the cytoplasm to the chaperone heat shock protein 90 (Hsp90) as well as interacting with cochaperones such as hsp40, and other peptides including cyp40; Hsp90 also binds to both actin and tubulin. When the receptor is complexed to the chaperone proteins, it can bind to the associated steroid hormone, which induces a conformational alteration in the complex. This binding in turn releases the chaperones, which allows the receptor to traverse the nuclear membrane and initiate transcription of steroid-responsive genes.[4,107,108] This biochemical activation of nuclear redistribution can be temporarily suppressed with geldanamycin, which disrupts the molecular binding of Hsp90 to glucocorticoid receptor, yet when the cytoskeleton is depolymerized with cytochalasin-D treatment, the process resumes normal transport speeds. This indicates the necessity of the cytoskeleton in this process,[29,78,115] although the specific mechanism of this nucleocytoplasmic transport is still unclear.

From a mechanistic standpoint, studies postulate that glucocorticoid receptor diffuses through the cytoplasm with protein interactions that bind the mobile proteins at specific sites. Alternately, it is proposed that the association and active movement through machinery associated with the cytoskeleton plays a role in this nucleocytoplasmic transport. This mechanism is thought to work through cytoskeleton-linker proteins and in the case of glucocorticoid receptor transport, a potential protein is Hsp-90, aforementioned. Hsp-90 has the presence and binding affinities to implicate it since it is: (1) a ubiquitous phosphorylated dimer; and (2) a prevalent eukaryotic chaperone that can compose 1–5% of the total cellular proteins.[14] Further, Hsp-90 is known to associate with cytoskeleton proteins such as tubulin and actin, so it could function to affect this transport through the cell structure. Although the mechanism needs to be elucidated, the cytoskeleton is definitely involved and likely through cytoskeleton-associated proteins that help regulate spatiotemporal signaling in cells.

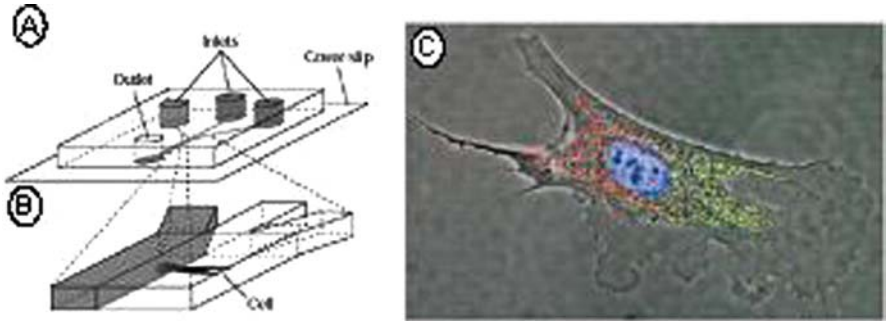

FIGURE 6. Fluidic technology for subcellular stimulation to control cytoskeletal integrity. This technology is able to overcome a significant limitation in chemical stimulation where normally due to the size scale of single cells local stimulation with aqueous solutions will diffuse across the length of a cell within seconds. (A, B) The lithographically constructed fluidics system with a close-up of the region where the inlet channels converge into the main channel. (C) Mitotracker Green FM labeled the mitochondria using the fluidics device only in the local cytoplasmic domains on the right region of the cell with simultaneous labeling of the mitochondria on the left region of the cell with Mitotracker Red.[100] Scale *bar* = 5 μm.

NANO- AND MICROTECHNOLOGY TO ADDRESS THE CYTOSKELETON

To understand and dissect the influence of the cytoskeleton on the function of the cell, novel techniques must be developed and interfaced with this complex biological system from a bioengineering perspective. Here, we describe a sampling of technologies that have been applied to biological systems such as cells and individual biological molecules. Technology has provided insights into the response of single cells and cell populations in relation to the structural cytoskeleton on numerous fronts. Over the past several decades, microtechnology such as micropipette aspiration devices has improved the understanding of membrane mechanics including: the interconnection between the membrane of the outer hair cell with its cortical lattice; the cell-cycle-regulated viscoelastic properties of hepatocellular carcinoma cells; and the volume differential behavior of red blood cells under an anisotonic environment, which has direct and indirect links to the cytoskeleton.[19,71,113] Fluid shearing devices have helped uncover protein-specific attachment sites and the contributions of the cytoskeleton to them.[17,49,61,87,106,110] Alternately, other methods can examine local domains.[20,42,51,54,79,80,102,103,111] Lithographic fabrication techniques have been used to develop tools to study how cell shape is regulated by the attachment and spreading of individual cells.[9,25,55,64,66,67,73,75,92]. Technology has been applied to cell biology questions using techniques such as soft lithography[28,40,48,58,104] at a cell monolayer or whole cell size-scale.[15,18,27,28,50,52,59,96,119].

Nanotechnology has become a popularly discussed subject over the past several years. Nanotechnology though is challenging to define depending on the interests and backgrounds of individual researchers. For example, a chemist may define nanotechnology as chemistry, the material scientist may define it as fabrication on the nanometer scale, and the biologist may define it as actively manipulating molecules to name a few perspectives. This description includes nanotechnology such as: the fabrication of thin films; the synthesis of self-assembled monolayers (SAMs); and genetic constructs include developing biological molecules. These are all on the nanoscale and could be debatably described as nanotechnology. Irregardless of the varying perspective, the development of smaller and smaller technology, which is requisite to the interface with individual molecules on the nanometer size scale generally has focused around two approaches, the top-down and bottom-up. In one facet of the top-down approach, lithography has been utilized and improved as the resolution of these techniques increases to the nanometer size scale through utilizing techniques such as electron beam lithography. One facet of the bottom-up approach uses the assembly and utilization of molecular complexes on the single nanometer size scale including chemical synthesis or leveraging biological building blocks of proteins or nucleic acids.

Many cellular processes are spatially and temporally responsive including cell structure, motility, and apoptosis. Thus, if internal cell responses are to be elucidated in cellular biology, methods for working at the resolution of the cytoskeletal components that have control over these gradient behaviors must be developed. Nano- and microtechnology are ideally suited for this purpose. A number of novel techniques to probe the localized domains of the cytoskeleton have been reported recently. For example, a fluidic device, which was built using soft lithographic methods, is able to probe subcellular domains in a single cell using laminar fluid flow separation. This procedure has allowed the delivery of pharmacological drugs locally including cytoskeletal targeting agents (Fig. 6).[100,101] This technique utilizes micrometer-sized streams of fluid flowing over spread mammalian cells. Due to the low velocity of the fluid flow and the small dimensions of the channel, low Reynolds number flow is established and the separated streams intersect in the main channel with minimal mixing. Through

this, chemical step gradients are delivered to subdomains of single cells (Fig. 6). This has been used to locally depolymerize the nanometer scale cytoskeleton inside a subcellular domain using chemicals including cytochalasin-D.

Further, a specific stimulation at a spatially localized subcellular region could lead to insight for directional sensing or polarized sensitivity with respect to the cytoskeleton. Nucleocytoplasmic transport has been visualized in this directional transport through green fluorescent protein labeled glucocorticoid receptor, which is related to cytoskeleton organization (Fig. 7). Manipulating the cytoskeleton is also possible through the introduction of laser energy. Through focusing femto-second pulses of lasers through an objective with a high numerical aperture, large quantities of discrete energy have been delivered to nanometer-sized volumes inside a single cell with no apparent damage to adjacent domains (Fig. 8). This technique has been combined with fluorescent labeling of the cytoskeleton to target actin filaments in single cells [89]. Individual actin fibers have been severed while the surrounding cytoplasm and membrane remain intact. Also, SAMs have been used to control biological responses including cytoskeletal and ECM behavior.[9,92] SAMs can control not only *in vitro* but *in vivo* components as well as being used for encapsulated *in vivo* environments with liposomal systems. Nanoelectromechanical systems (NEMS) can also be applied to the investigation of molecular behavior. NEMS are often based on the scaling down of conventional microelectronics and have allowed a greater ability to detect the presence of small amounts of molecules; this is accomplished through monitoring the deformation of long cantilevered beams due to loading of the molecules. Furthermore, semiconductor nanocrystals (quantum dots) can be used for *in vivo* sensing. A recent example demonstrated the ability to identify prostate-specific membrane antigen with simultaneous visualization of cancerous regions *in vivo* via near-infrared imaging.[30] These are a few examples of how technology has been implemented to

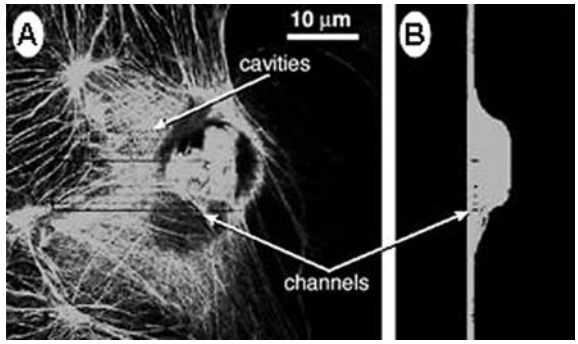

FIGURE 8. Manipulation of individual cytoskeleton filaments in living cells with a femtosecond pulsed laser. Through the introduction of high energy packets of laser focused into the interior of a single cell, the individual actin filaments have been severed. (A) A top and (B) side view of a three-dimensional reconstruction using confocal microscopy of a single cell.[89] The channels and cavities are formed within the cytoplasm through continuous and pulsed laser excitation, respectively, while actuating the laser across the cell. Localized disruption and manipulation of the cytoskeleton filaments is possible with minimal damage to adjacent zones in the cell.

probe the cellular functions of the cytoskeleton. In the future, the use of nano- and microtechnology to study intracellular structural responses can lead to many insights into disease diagnostics and potential treatments. This will require multidisciplinary approaches including engineering, biology, physics, mathematics, and chemistry to develop novel combinations of cellular, molecular, and technological research.[26,91,117,120]

CONCLUSIONS

The cytoskeleton is a central and integrating functional structure in influencing cell processes. This structure though has evolved with the development of the cell to provide an ultimate optimization in design and organi-

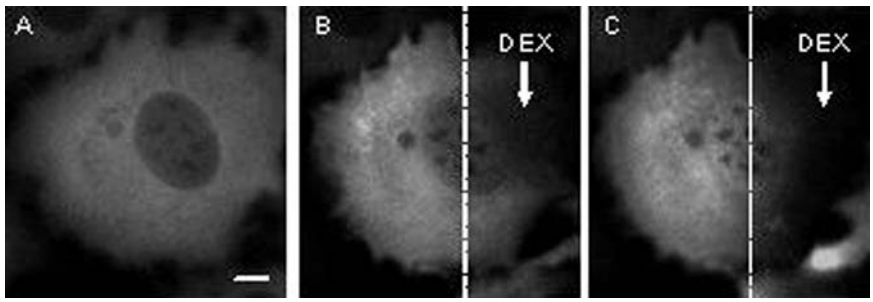

FIGURE 7. Localized cytoplasmic receptor response through a possible cytoskeleton association. (A) The glucocorticoid receptor is labeled with green fluorescent protein and is initially distributed throughout the cytoplasm in its unstimulated state. When steroids including dexamethasone (DEX) were added to local domains (the right half) of single living cells, the transport of the glucocorticoid receptor, which is thought to be associated with the cytoskeleton through heat shock protein-90, reveals a gradient response. This gradient shows (B) a biased distribution into the unstimulated cytoplasmic region after 5 min and (C) a reverse gradient behavior in the nucleus with the green fluorescent protein distribution also in the unstimulated portion of the cell in the nuclear compartment after 10 min. Scale *bar* = 7 μm.

zation in this system. Through understanding the cell at the nanoscale from a design perspective, many advances in nanoscience, engineering, and biology can be made in fields such as nanobioengineering. Over the course of evolution that has resulted in the development of more complex eukaryotic cells, billions of genetic changes likely have occurred. In these changes, many characteristics that are beneficial to cells have been passed along to the following generations. Through this progression, the necessity for an organized hierarchical system for many cellular processes that cannot be accomplished through traditional diffusion reaction schemes has developed. Diffusion definitely occurs and governs processes, but each generation of advanced research is finding that the cell exhibits multiple processes to ensure that cell functions are accomplished even if there is an inhibition of the primary mechanism.

The presence of these complementary schemes for transport and organization systems involving the cytoskeleton suggest that cell function is optimized for these superfluous functions in cytoskeleton-mediated solid-state biochemistry. The organization in cells further exhibits redundancy, which ensures the completion of cellular functions; this is also found as a duplicate method that exists in both chemically governed and structurally governed scenarios giving rise to highly ordered systems established by millions of years of evolution. This has indicated that as cells evolved over the course of history, the ability for them to form more complex systems initially relied on the establishment of a system that could regulate cell processes in highly organized schemes. The cytoskeleton would be one potential system for this type of evolution scenario. Regardless, the cytoskeleton definitely plays important roles in complex signaling pathways by providing a scaffolding for them as well as alternate schemes for their completion. This field of structure and organization through internal structures in a cell has a wide range of implications in fields from cell biology and medical therapeutics, to computational biology.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Science Foundation (R.B.), National Science Foundation-CAREER (P.L.), Pennsylvania Infrastructure Technology Alliance, and the Department of Energy-Genome to Life Program. The authors would also like to thank M. L. Ledbetter, S. LeDuc, S. Lawrence, and W. Messner for their helpful discussions and input on the manuscript.

REFERENCES

[1]Alenghat, F. J., B. Fabry, K. Y. Tsai, W. H. Goldmann, and D. E. Ingber. Analysis of cell mechanics in single vinculin-deficient cells using a magnetic tweezer. *Biochem. Biophys. Res. Commun.* 277:93–99, 2000.

[2]Alenghat, F. J., and D. E. Ingber. Mechanotransduction: All signals point to cytoskeleton, matrix, and integrins. *Sci. STKE* 119:PE6, 2002.

[3]Baluska, F., and P. W. Barlow. The role of the microtubular cytoskeleton in determining nuclear chromatin structure and passage of maize root cells through the cell cycle. *Eur. J. Cell Biol.* 61:160–167, 1993.

[4]Beato, M., M. Truss, and S. Chavez. Control of transcription by steroid hormones. *Ann. N. Y. Acad. Sci.* 784:93–123, 1996.

[5]Bellin, R. M., T. W. Huiatt, D. R. Critchley, and R. M. Robson. Synemin may function to directly link muscle cell intermediate filaments to both myofibrillar z-lines and costameres. *J. Biol. Chem.* 276:32330–32337, 2001.

[6]Benjamin, M., C. W. Archer, and J. R. Ralphs. Cytoskeleton of cartilage cells. *Microsc. Res. Tech.* 28:372–377, 1994.

[7]Bogre, L., O. Calderini, I. Merskiene, and P. Binarova. Regulation of cell division and the cytoskeleton by mitogen-activated protein kinases in higher plants. *Results Probl. Cell Differ.* 27:95–117, 2000.

[8]Casanova, J. E. Epithelial cell cytoskeleton and intracellular trafficking v. Confluence of membrane trafficking and motility in epithelial cell models. *Am. J. Physiol. Gastrointest. Liver Physiol.* 283:G1015–G1019, 2002.

[9]Chen, C. S., M. Mrksich, S. Huang, G. M. Whitesides, and D. E. Ingber. Geometric control of cell life and death. *Science* 276:1425–1428, 1997.

[10]Chicurel, M. E., R. H. Singer, C. J. Meyer, and D. E. Ingber. Integrin binding and mechanical tension induce movement of mRNA and ribosomes to focal adhesions. *Nature* 392:730–733, 1998.

[11]Cole, N. B., C. L. Smith, N. Sciaky, M. Terasaki, M. Edidin, and J. Lippincott-Schwartz. Diffusional mobility of golgi proteins in membranes of living cells. *Science* 273:797–801, 1996.

[12]Costa, K. D., W. J. Hucker, and F. C. Yin. Buckling of actin stress fibers: A new wrinkle in the cytoskeletal tapestry. *Cell Motil. Cytoskeleton* 52:266–274, 2002.

[13]Csermely, P. A nonconventional role of molecular chaperones: Involvement in the cytoarchitecture. *News Physiol. Sci.* 16:123–126, 2001.

[14]Csermely, P., T. Schnaider, C. Soti, Z. Prohaszka, and G. Nardai. The 90-kda molecular chaperone family: Structure, function, and clinical applications. A comprehensive review. *Pharmacol. Ther.* 79:129–168, 1998.

[15]Dalby, M. J., S. J. Yarwood, M. O. Riehle, H. J. Johnstone, S. Affrossman, and A. S. Curtis. Increasing fibroblast response to materials using nanotopography: Morphological and genetic measurements of cell response to 13-nm-high polymer demixed islands. *Exp. Cell Res.* 276:1–9, 2002.

[16]de Castro, R. D., A. A. van Lammeren, S. P. Groot, R. J. Bino, and H. W. Hilhorst. Cell division and subsequent radicle protrusion in tomato seeds are inhibited by osmotic stress but DNA synthesis and formation of microtubular cytoskeleton are not. *Plant Physiol.* 122:327–336, 2000.

[17]Dekker, R. J., S. van Soest, R. D. Fontijn, S. Salamanca, P. G. de Groot, E. VanBavel, H. Pannekoek, and A. J. Horrevoet. Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung kruppel-like factor (klf2). *Blood* 100:1689–1698, 2002.

[18]Desai, T. A. Microfabrication technology for pancreatic cell encapsulation. *Expert Opin. Biol. Ther.* 2:633–646, 2002.

[19]Engstrom, K. G., and H. J. Meiselman. Combined use of micropipette aspiration and perifusion for studying red blood cell volume regulation. *Cytometry* 27:345–352, 1997.

[20]Farinas, J., A. W. Chow, and H. G. Wada. A microfluidic device for measuring cellular membrane potential. *Anal. Biochem.* 295:138–142, 2001.

[21]Fath, K. R., S. N. Mamajiwalla, and D. R. Burgess. The cytoskeleton in development of epithelial cell polarity. *J. Cell Sci.* 17(Suppl.):65–73, 1993.

[22]Ferrer, I., R. Blanco, M. Carmona, B. Puig, M. Barrachina, C. Gomez, and S. Ambrosio. Active, phosphorylation-dependent mitogen-activated protein kinase (mapk/erk), stress-activated protein kinase/c-jun n-terminal kinase (sapk/jnk), and p38 kinase expression in parkinson's disease and dementia with lewy bodies. *J. Neural Transm.* 108:1383–1396, 2001.

[23]Fleming, T. P., P. M. Cannon, and S. J. Pickering. The cytoskeleton, endocytosis and cell polarity in the mouse preimplantation embryo. *Dev. Biol.* 113:406–419, 1986.

[24]Fluck, M., J. A. Carson, S. E. Gordon, A. Ziemiecki, and F. W. Booth. Focal adhesion proteins fak and paxillin increase in hypertrophied skeletal muscle. *Am. J. Physiol.* 277:C152–C162, 1999.

[25]Folch, A., and M. Toner. Cellular micropatterns on biocompatible materials. *Biotechnol. Prog.* 14:388–392, 1998.

[26]Fox, J. A., T. E. Hotaling, C. Struble, J. Ruppel, D. J. Bates, and M. B. Schoenhoff. Tissue distribution and complex formation with ige of an anti-ige antibody after intravenous administration in cynomolgus monkeys. *J. Pharmacol. Exp. Ther.* 279:1000–1008, 1996.

[27]Freitas, R. A. Jr. Exploratory design in medical nanotechnology: A mechanical artificial red cell. *Artif. Cells Blood Substit. Immobil. Biotechnol.* 26:411–430, 1998.

[28]Fu, A. Y., H. P. Chou, C. Spence, F. H. Arnold, and S. R. Quake. An integrated microfabricated cell sorter. *Anal. Chem.* 74:2451–2457, 2002.

[29]Galigniana, M. D., J. L. Scruggs, J. Herrington, M. J. Welsh, C. Carter-Su, P. R. Housley, and W. B. Pratt. Heat shock protein 90-dependent (geldanamycin-inhibited) movement of the glucocorticoid receptor through the cytoplasm to the nucleus requires intact cytoskeleton. *Mol. Endocrinol.* 12:1903–1913, 1998.

[30]Gao, X., Y. Cui, R. M. Levenson, L. W. Chung, and S. Nie. *In vivo* cancer targeting and imaging with semiconductor quantum dots. *Nat. Biotechnol.* 22:969–976, 2004.

[31]Garcia-Cardena, G., J. I. Comander, B. R. Blackman, K. R. Anderson, and M. A. Gimbrone. Mechanosensitive endothelial gene expression profiles: Scripts for the role of hemodynamics in atherogenesis? *Ann. N. Y. Acad. Sci.* 947:1–6, 2001.

[32]Hable, W. E., and D. L. Kropf. Roles of secretion and the cytoskeleton in cell adhesion and polarity establishment in pelvetia compressa zygotes. *Dev. Biol.* 198:45–56, 1998.

[33]Hasezawa, S., and F. Kumagai. Dynamic changes and the role of the cytoskeleton during the cell cycle in higher plant cells. *Int. Rev. Cytol.* 214:161–191, 2002.

[34]Helmke, B. P., and P. F. Davies. The cytoskeleton under external fluid mechanical forces: Hemodynamic forces acting on the endothelium. *Ann. Biomed. Eng.* 30:284–296, 2002.

[35]Helmke, B. P., A. B. Rosen, and P. F. Davies. Mapping mechanical strain of an endogenous cytoskeletal network in living endothelial cells. *Biophys. J.* 84:2691–2699, 2003.

[36]Holth, L. T., D. N. Chadee, V. A. Spencer, S. K. Samuel, J. R. Safneck, and J. R. Davie. Chromatin, nuclear matrix and the cytoskeleton: Role of cell structure in neoplastic transformation (review). *Int. J. Oncol.* 13:827–837, 1998.

[37]Htun, H., J. Barsony, I. Renyi, D. L. Gould, and G. L. Hager. Visualization of glucocorticoid receptor translocation and intranuclear organization in living cells with a green fluorescent protein chimera. *Proc. Natl. Acad. Sci. U.S.A.* 93:4845–4850, 1996.

[38]Htun, H., L. T. Holth, D. Walker, J. R. Davie, and G. L. Hager. Direct visualization of the human estrogen receptor alpha reveals a role for ligand in the nuclear distribution of the receptor. *Mol. Biol. Cell* 10:471–486, 1999.

[39]Hu, S., J. Chen, B. Fabry, Y. Numaguchi, A. Gouldstone, D. E. Ingber, J. J. Fredberg, J. P. Butler, and N. Wang. Intracellular stress tomography reveals stress focusing and structural anisotropy in cytoskeleton of living cells. *Am. J. Physiol. Cell Physiol.* 285:C1082–C1090, 2003

[40]Hu, J., Z. H. Wang, and Z. L. Tao. Micropatterning of biotin-avidin layers and cell location. *Sheng Wu Gong Cheng Xue Bao* 18:619–621, 2002.

[41]Huang, S., and D. E. Ingber. A discrete cell cycle checkpoint in late g(1) that is cytoskeleton-dependent and map kinase (erk)-independent. *Exp. Cell Res.* 275:255–264, 2002.

[42]Huang, Y., E. L. Mather, J. L. Bell, and M. Madou. Mems-based sample preparation for molecular diagnostics. *Anal. Bioanal. Chem.* 372:49–65, 2002.

[43]Huen, A. C., J. K. Park, L. M. Godsel, X. Chen, L. J. Bannon, E. V. Amarg, T. Y. Hudson, A. K. Mongiu, I. M. Leigh, D. . Kelsell, B. M. Gumbiner, and K. J. Green Intermediate filament-membrane attachments function synergistically with actin-dependent contacts to regulate intercellular adhesive strength. *J. Cell Biol.* 159:1005–1017, 2002.

[44]Hutcheson, I. R., and T. M. Griffith. Mechanotransduction through the endothelial cytoskeleton: Mediation of flow- but not agonist-induced edrf release. *Br. J. Pharmacol.* 118:720–726, 1996.

[45]Ishikawa, H. Cell polarity and cytoskeleton. *Tanpakushitsu Kakusan Koso* 34:1742–1748, 1989.

[46]Izutsu, K. Cell division and the microtubular cytoskeleton. *Hum. Cell* 4:100–108, 1991.

[47]Kaibuchi, K., S. Kuroda, and M. Amano. Regulation of the cytoskeleton and cell adhesion by the rho family gtpases in mammalian cells. *Annu. Rev. Biochem.* 68:459–486, 1999.

[48]Kane, R. S., S. Takayama, E. Ostuni, D. E. Ingber, and G. M. Whitesides. Patterning proteins and cells using soft lithography. *Biomaterials* 20:2363–2376, 1999.

[49]Kano, Y., K. Katoh, and K. Fujiwara. Lateral zone of cell–cell adhesion as the major fluid shear stress-related signal transduction site. *Circ. Res.* 86:425–433, 2000.

[50]Kataoka, N., K. Iwaki, K. Hashimoto, S. Mochizuki, Y. Ogasawara, M. Sato, K. Tsujioka, and F. Kajiya. Measurements of endothelial cell-to-cell and cell-to-substrate gaps and micromechanical properties of endothelial cells during monocyte adhesion. *Proc. Natl. Acad. Sci. U.S.A.* 99:15638–15643, 2002.

[51]Kenis, P. J., R. F. Ismagilov, and G. M. Whitesides. Microfabrication inside capillaries using multiphase laminar flow patterning. *Science* 285:83–85, 1999.

[52]Kubicek, J. D., S. Brelsford, P. Ahluwalia, and P. R. Leduc. Integrated lithographic membranes and surface adhesion chemistry for three-dimensional cellular stimulation. *Langmuir* 20:11552–11556, 2004.

[53]Kural, C., H. Kim, S. Syed, G. Goshima, V. I. Gelfand, and P. R. Selvin. Kinesin and dynein move a peroxisome *in vivo*: A tug-of-war or coordinated movement? *Science* 308:1469–1472, 2005.

[54]LeDuc, P., C. Haber, G. Bao, and D. Wirtz. Dynamics of individual flexible polymers in a shear flow. *Nature* 399:564–566, 1999.

[55]LeDuc, P., E. Ostuni, G. Whitesides, and D. Ingber. Use of micropatterned adhesive surfaces for control of cell behavior. *Methods Cell Biol.* 69:385–401, 2002.

[56]Li, C., Y. Hu, M. Mayr, and Q. Xu. Cyclic strain stress-induced mitogen-activated protein kinase (mapk) phosphatase 1 expres-

sion in vascular smooth muscle cells is regulated by ras/rac-mapk pathways. *J. Biol. Chem.* 274:25273–25280, 1999.
[57]Li, Y. Q., A. Moscatelli, G. Cai, and M. Cresti. Functional interactions among cytoskeleton, membranes, and cell wall in the pollen tube of flowering plants. *Int. Rev. Cytol.* 176:133–199, 1997.
[58]Li Jeon, N., H. Baskaran, S. K. Dertinger, G. M. Whitesides, L. van de Water, and M. Toner. Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device. *Nat. Biotechnol.* 20:826–830, 2002.
[59]Liu, J., Q. Zhang, E. E. Remsen, and K. L. Wooley. Nanostructured materials designed for cell binding and transduction. *Biomacromolecules* 2:362–368, 2001.
[60]Machesky, L. M., and J. A. Cooper. Cell motility. Bare bones of the cytoskeleton. *Nature* 401:542–543, 1999.
[61]Malek, A. M., and S. Izumo. Mechanism of endothelial cell shape change and cytoskeletal remodeling in response to fluid shear stress. *J. Cell Sci.* 109(Pt 4):713–726, 1996.
[62]Maniotis, A. J., K. Bojanowski, and D. E. Ingber. Mechanical continuity and reversible chromosome disassembly within intact genomes removed from living cells. *J. Cell. Biochem.* 65:114–130, 1997.
[63]Maniotis, A. J., C. S. Chen, and D. E. Ingber. Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc. Natl. Acad. Sci. U.S.A.* 94:849–854, 1997.
[64]Matsuda, T., K. Inoue, and T. Sugawara. Development of micropatterning technology for cultured cells. *ASAIO Trans.* 36:M559–M562, 1990.
[65]Meyer, C. J., F. J. Alenghat, P. Rim, J. H. Fong, B. Fabry, and D. E. Ingber. Mechanical control of cyclic amp signalling and gene transcription through integrins. *Nat. Cell Biol.* 2:666–668, 2000.
[66]Miller, C., S. Jeftinija, and S. Mallapragada. Micropatterned schwann cell-seeded biodegradable polymer substrates significantly enhance neurite alignment and outgrowth. *Tissue Eng.* 7:705–715, 2001.
[67]Miller, C., H. Shanks, A. Witt, G. Rutkowski, and S. Mallapragada. Oriented schwann cell growth on micropatterned biodegradable polymer substrates. *Biomaterials* 22:1263–1269, 2001.
[68]Minton, A. P. Implications of macromolecular crowding for protein assembly. *Curr. Opin. Struct. Biol.* 10:34–39, 2000.
[69]Minton, A. P. The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media. *J. Biol. Chem.* 276:10577–10580, 2001.
[70]Moon, J. J., M. Matsumoto, S. Patel, L. Lee, J. L. Guan, and S. Li. Role of cell surface heparan sulfate proteoglycans in endothelial cell migration and mechanotransduction. *J. Cell. Physiol.* 203:166–176, 2004.
[71]Morimoto, N., R. M. Raphael, A. Nygren, and W. E. Brownell. Excess plasma membrane and effects of ionic amphipaths on mechanics of outer hair cell lateral wall. *Am. J. Physiol. Cell Physiol.* 282:C1076–C1086, 2002.
[72]Nelson, W. J., R. W. Hammerton, A. Z. Wang, and E. M. Shore. Involvement of the membrane-cytoskeleton in development of epithelial cell polarity. *Semin. Cell Biol.* 1:359–371, 1990.
[73]Ogawa, H., S. Inouye, F. I. Tsuji, K. Yasuda, and K. Umesono. Localization, trafficking, and temperature-dependence of the aequorea green fluorescent protein in cultured vertebrate cells. *Proc. Natl. Acad. Sci. U.S.A.* 92:11899–11903, 1995.
[74]Parry, G., J. C. Beck, L. Moss, J. Bartley, and G. K. Ojakian. Determination of apical membrane polarity in mammary epithelial cell cultures: The role of cell–cell, cell–substratum, and membrane–cytoskeleton interactions. *Exp. Cell Res.* 188:302–311, 1990.
[75]Patel, N., R. Bhandari, K. M. Shakesheff, S. M. Cannizzaro, M. C. Davies, R. Langer, C. J. Roberts, S. J. Tendler, ad P. M. Williams. Printing patterns of biospecifically-adsorbed protein. *J. Biomater. Sci. Polym. Ed.* 11:319–331, 2000.
[76]Plopper, G., and D. E. Ingber. Rapid induction and isolation of focal adhesion complexes. *Biochem. Biophys. Res. Commun.* 193:571–578, 1993.
[77]Pollack, G. Cells, Gels, and the Engines of Life. Seattle, WA: Ebner, 2001,
[78]Prima, V., C. Depoix, B. Masselot, P. Formstecher, and P. Lefebvre. Alteration of the glucocorticoid receptor subcellular localization by non steroidal compounds. *J. Steroid Biochem. Mol. Biol.* 72:1–12, 2000.
[79]Puskar, K., L. Apelstein, S. Ta'asan, R. Schwartz, and P. LeDuc. Understanding actin organization in cell structure through lattice based monte carlo simulations. *MCB* 1:123–132, 2004.
[80]Quake, S. R., and A. Scherer. From micro- to nanofabrication with soft materials. *Science* 290:1536–1540, 2000.
[81]Reipert, S., F. Steinbock, I. Fischer, R. E. Bittner, A. Zeold, and G. Wiche. Association of mitochondria with plectin and desmin intermediate filaments in striated muscle. *Exp. Cell Res.* 252:479–491, 1999.
[82]Resnick, N., H. Yahav, L. M. Khachigian, T. Collins, K. R. Anderson, F. C. Dewey, and M. A. Gimbrone. Endothelial gene regulation by laminar shear stress. *Adv. Exp. Med. Biol.* 430:155–164, 1997.
[83]Rivas, G., J. A. Fernandez, and A. P. Minton. Direct observation of the enhancement of noncooperative protein self-assembly by macromolecular crowding: Indefinite linear self-association of bacterial cell division protein ftsz. *Proc. Natl. Acad. Sci. U.S.A.* 98:3150–3155, 2001.
[84]Rivero, F., B. Koppel, B. Peracino, S. Bozzaro, F. Siegert, C. J. Weijer, M. Schleicher, R. Albrecht, and A. A. Noegel. The role of the cortical cytoskeleton: F-actin crosslinking proteins protect against osmotic stress, ensure cell size, cell shape and motility, and contribute to phagocytosis and development. *J. Cell Sci.* 109(Pt 11):2679–2691, 1996.
[85]Salter, D. M., S. J. Millward-Sadler, G. Nuki, and M. O. Wright. Integrin-interleukin-4 mechanotransduction pathways in human chondrocytes. *Clin. Orthop.* S49–S60, 2001.
[86]Sanger, J. W., and J. M. Sanger. The cytoskeleton and cell division. *Methods Achiev. Exp. Pathol.* 8:110–142, 1979.
[87]Schnittler, H. J., S. W. Schneider, H. Raifer, F. Luo, P. Dieterich, I. Just, and K. Aktories. Role of actin filaments in endothelial cell–cell adhesion and membrane stability under fluid shear stress. *Pflugers Arch.* 442:675–687, 2001.
[88]Shafrir, Y., and G. Forgacs. Mechanotransduction through the cytoskeleton. *Am. J. Physiol. Cell Physiol.* 282:C479–C486, 2002.
[89]Shen, N., D. Datta, C. B. Schaffer, P. LeDuc, D. E. Ingber, and E. Mazur. Ablation of cytoskeletal filaments and mitochondria in live cells using a femtosecond laser nanoscissor. *Mech. Chem. Biosyst.* 2:17–25, 2005.
[90]Shrode, L. D., E. A. Rubie, J. R. Woodgett, and S. Grinstein. Cytosolic alkalinization increases stress-activated protein kinase/c-jun nh2-terminal kinase (sapk/jnk) activity and p38 mitogen-activated protein kinase activity by a calcium-independent mechanism. *J. Biol. Chem.* 272:13653–13659, 1997.
[91]Silverstein, R. L., L. L. Leung, P. C. Harpel, and R. L. Nachman. Complex formation of platelet thrombospondin with plasminogen. Modulation of activation by tissue activator. *J. Clin. Invest.* 74:1625–1633, 1984.
[92]Singhvi, R., A. Kumar, G. P. Lopez, G. N. Stephanopoulos, D. I. Wang, G. M. Whitesides, and D. E. Ingber En-

gineering cell shape and function. *Science* 264:696–698, 1994.
[93]Small, J. V., and M. Gimona. The cytoskeleton of the vertebrate smooth muscle cell. *Acta Physiol. Scand.* 164:341–348, 1998.
[94]Small, J. V., I. Kaverina, O. Krylyshkina, and K. Rottner. Cytoskeleton cross-talk during cell motility. *FEBS Lett.* 452:96–99, 1999.
[95]Soll, D. R. Researchers in cell motility and the cytoskeleton can play major roles in understanding aids. *Cell Motil. Cytoskeleton* 37:91–97, 1997.
[96]Spector, A. A., M. Ameen, P. G. Charalambides, and A. S. Popel. Nanostructure, effective properties, and deformation pattern of the cochlear outer hair cell cytoskeleton. *J. Biomech. Eng.* 124:180–187, 2002.
[97]Steinbock, F. A., and G. Wiche. Plectin: A cytolinker by design. *Biol. Chem.* 380:151–158, 1999.
[98]Streiblova, E., and J. Hasek. Role of the cytoskeleton and homologs of retrovirus genes in yeast cell division. *Izv. Akad. Nauk SSSR Biol.* 353–360, 1987.
[99]Svoboda, A., I. Slaninova, and A. Holubarova. Cytoskeleton in regenerating protoplasts and restoration of cell polarity in the yeast saccharomyces cerevisiae. *Acta Biol. Hung.* 52:325–333, 2001.
[100]Takayama, S., E. Ostuni, P. LeDuc, K. Naruse, D. E. Ingber, and G. M. Whitesides. Subcellular positioning of small molecules. *Nature* 411:1016, 2001.
[101]Takayama, S., E. Ostuni, P. LeDuc, K. Naruse, D. E. Ingber, and G. M. Whitesides. Selective chemical treatment of cellular microdomains using multiple laminar streams. *Chem. Biol.* 10:123–130, 2003.
[102]Terray, A., J. Oakey, and D. W. Marr. Microfluidic control using colloidal devices. *Science* 296:1841–1844, 2002.
[103]Thiebaud, P., L. Lauer, W. Knoll, and A. Offenhausser. Pdms device for patterned application of microfluids to neuronal cells arranged by microcontact printing. *Biosens. Bioelectron.* 17:87–93, 2002.
[104]Tien, J., C. M. Nelson, and C. S. Chen. Fabrication of aligned microstructures with a single elastomeric stamp. *Proc. Natl. Acad. Sci. U.S.A.* 99:1758–1762, 2002.
[105]Topper, J. N., and M. A. Gimbrone Jr. Blood flow and vascular gene expression: Fluid shear stress as a modulator of endothelial phenotype. *Mol. Med. Today* 5:40–46, 1999.
[106]Truskey, G. A., and J. S. Pirone. The effect of fluid shear stress upon cell adhesion to fibronectin-treated surfaces. *J. Biomed. Mater. Res.* 24:1333–1353, 1990.
[107]Tsai, M. J., and B. W. O'Malley. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu. Rev. Biochem.* 63:451–486, 1994.
[108]Tumlin, J. A., J. P. Lea, C. E. Swanson, C. L. Smith, S. S. Edge, and J. S. Someren. Aldosterone and dexamethasone stimulate calcineurin activity through a transcription-independent mechanism involving steroid receptor-associated heat shock proteins. *J. Clin. Invest.* 99:1217–1223, 1997.
[109]Vale, R. D., and R. A. Milligan. The way things move: Looking under the hood of molecular motor proteins. *Science* 288:88–95, 2000.
[110]van Kooten, T. G., J. M. Schakenraad, H. C. van der Mei, A. Dekker, C. J. Kirkpatrick, and H. J. Busscher. Fluid shear induced endothelial cell detachment from glass—influence of adhesion time and shear stress. *Med. Eng. Phys.* 16:506–512, 1994.
[111]Wallrabe, U., P. Ruther, T. Schaller, and W. K. Schomburg. Microsystems in medicine. *Int. J. Artif. Organs* 21:137–146, 1998.
[112]Wang, N., J. P. Butler, and D. E. Ingber. Mechanotransduction across the cell surface and through the cytoskeleton. *Science* 260:1124–1127, 1993.
[113]Wang, J. H., P. Goldschmidt-Clermont, J. Wille, and F. C. Yin. Specificity of endothelial cell reorientation in response to cyclic mechanical stretching. *J. Biomech.* 34:1563–1572, 2001.
[114]Wang, N., and D. E. Ingber. Control of cytoskeletal mechanics by extracellular matrix, cell shape, and mechanical tension. *Biophys. J.* 66:2181–2189, 1994.
[115]Whitesell, L., E. G. Mimnaugh, B. de Costa, C. E. Myers, and L. M. Neckers. Inhibition of heat shock protein hsp90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: Essential role for stress proteins in oncogenic transformation. *Proc. Natl. Acad. Sci. U.S.A.* 91:8324–8328, 1994.
[116]Williams, B. Mechanical influences on vascular smooth muscle cell function. *J. Hypertens.* 16:1921–1929, 1998.
[117]Williams, S. E., I. Valenzuela, A. S. Kadish, and K. M. Das. Glomerular immune complex formation and induction of lymphoma in athymic nude mice by tissue filtrates of crohn's disease patients. *J. Lab. Clin. Med.* 99:827–837, 1982.
[118]Wozniak, M., A. Fausto, C. P. Carron, D. M. Meyer, and K. A. Hruska. Mechanically strained cells of the osteoblast lineage organize their extracellular matrix through unique sites of alphavbeta3-integrin expression. *J. Bone Miner. Res.* 15:1731–1745, 2000.
[119]Xu, C. W. High-density cell microarrays for parallel functional determinations. *Genome Res.* 12:482–486, 2002.
[120]Yanagi, S., N. Shimbara, and T. Tamura. Tissue and cell distribution of a mammalian proteasomal atpase, mss1, and its complex formation with the basal transcription factors. *Biochem. Biophys. Res. Commun.* 279:568–573, 2000.
[121]Yang, F., and Y. H. Li. Roles of integrins and cytoskeleton in cellular mechanotransduction. *Space Med. Med. Eng. (Beijing)* 15:309–312, 2002.