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Mini-Review

Mechanotransduction in cells[1]

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Abstract

Cell–matrix and cell–cell adhesions critically influence cell metabolism, protein synthesis, cell survival, cytoskeletal architecture and consequently cell mechanical properties such as migration, spreading and contraction. An important group of adhesive transmembrane receptors that mechanically link the ECM (extracellular matrix) with the internal cytoskeleton are integrins which are intimately connected with the FAs (focal adhesions) which consists of many proteins. The transient formation of FAs is greatly augmented either through externally applied tension to the cell or internally through myosin II-driven cell contractility. Exactly which protein(s) within FAs sense, transmit and respond to mechanical stress is currently debated and numerous candidates have been proposed.

Keywords: cell–cell adhesion; cell–matrix adhesion; cytoskeleton; focal adhesion protein; integrin; mechanotransduction; strain; stress

1. Introduction

Nearly all cell species react sensitive to external mechanical stress and changes to their mechanical milieus. This is described as mechanotransduction, a process that brings about important cellular changes in shape, motility, cytoskeletal remodelling, FA (focal adhesion) reorganization and gene expression, which has been the focus of much research over the years (Sheetz et al., 1998; Meyer et al., 2000; Chen et al., 2001; Goldmann, 2002; Ingber, 2003; Goldmann and Isenberg, 2004; Discher et al., 2009; Hoffman et al., 2011). However, the mechanism by which cells transmit mechanical stress throughout the cytoplasm and the cytoskeleton and by which signals are sensed and converted into biochemical signals, is still not understood. So far there is little direct experimental evidence on how intracellular cytoskeletal structural elements in living cells are able to deform and to signal stress in response to internal and external mechanical forces. To date many biophysical and biochemical models have been proposed which include the genetic model (Syntichaki and Tavernarakis, 2004), protein polymerization and depolymerization model (Drew et al., 2005), viscoelastic continuum model (Lim et al., 2006), thermal fluctuation model (Brannigan and Brown, 2006), and protein conformation 'switch-like' model (Hoffman et al., 2011) to explain how cells reorganize and change shape in response to mechanical input, however, rigorous tests of these models on cells are missing. The lack of understanding how changes in protein composition, dynamics and mechanics are processed is a major obstacle in identifying molecular mechanisms and sites of force transmission in cells.

2. Elucidating cellular force transmission

Many laboratories have used novel methods and approaches to elucidate the force transmission pathway in the cell body. For instance, Fabry et al. (2001a, b) used magnetic microbeads coated with ECM (extracellular matrix) proteins or antibodies and applied arbitrary force patterns on to specific cell surface receptors such as integrins. They tagged FA proteins to quantify intracellular displacement distribution in response to local mechanical input. By plating cells on to a deformable matrix of known elasticity, they also measured where and how intracellular forces are exerted on the matrix. Results showed that stresses and strains within the cell body did not decay smoothly over a short distance as predicted by the continuum model, but rather exhibited substantial long-range heterogeneity (Hu et al. 2003). Stress applied at the apical surface could be transmitted over distances of more than 30 μm and was concentrated at isolated FA sites at the basal cell surface (Hu et al., 2003). Other observations demonstrate the existence of long-range force transfer that could greatly exceed the cell boundaries (Ingber, 1997; Hoffman et al., 2011).

Force transmission over considerable distances made FA sites prime candidates for mechanosensing. Experiments by our group and others showed that the FA proteins vinculin, p130Cas, FAK (focal adhesion kinase), etc. are crucial for the ability of cells to transmit external forces and to generate cytoskeletal tension (Grashoff et al., 2010; Mierke et al., 2010; Dey et al., 2011; Fabry et al., 2011; Margadant et al., 2011). We studied the 'mechanism of action' of these proteins for cellular mechanotransduction and mechanosensing in MEFs (mouse embryonic fibroblasts). In particular, we examined the conformational changes that are mechanically induced in MEFs using the magnetic tweezers and cell stretcher method. Modelling studies based on vinculin's structure had shown that vinculin is clamped by direct dipole interactions of the D1 and D4 region (Bakolitsa et al., 2004; Cohen et al., 2005). Introducing a point mutation into the D1 domain (A50→I) strengthened the head–tail interaction and its regulatory function in FAs (Diez et al. 2011). Structural analyses of FAK indicated that point mutations at V954A and L961A of the FAT (focal adhesion targeting) region regulates paxillin binding (Dixon et al., 2004).

[1] This work is dedicated to Hugh Finnigan who passed away in 2011.
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Abbreviations: ECM, extracellular matrix; FAC, focal adhesion complex; FAK, focal adhesion kinase; MEF, mouse embryonic fibroblast.

Temporal and spatial changes in the cytoskeletal protein configuration due to mechanical stimulation have been detected and characterized by a wide range of biophysical techniques, including magnetic twisting, magnetic tweezers, traction microscopy, intracellular stress tomography, nanoscale bead tracking, single cell rheology, cell stretching, FRAP (fluorescence recovery after photobleaching), FRET (fluorescence resonance energy transfer) and FLIM (fluorescence-lifetime imaging microscopy). A combination of these techniques enabled us and others to study force transmission and structural remodelling in cells under loading conditions that closely mimic the physiologic situation.

3. Future directions

Our research has advanced the understanding how the cytoskeleton of cells deforms and transmits strains and stresses to the FA sites and that force transmission through the cytoskeleton is mechanically highly heterogeneous, and that the FA proteins, e.g. vinculin, p130Cas and FAK are not only important mechanoregulators but also serve as mechanosensors. Since force transmission, sensing and response represent basic biological processes, which are crucial for a variety of higher fundamental cell functions, the following aspects should be investigated in more detail.

(*i*) *The magnitude and distribution of force in cell–matrix and cell–cell adhesions in response to altered internal and external mechanical stress*. As fluid shear stress and cell deformation as well as internal (contractile) forces are transmitted through the cell *via* preferential pathways formed by the cytoskeleton, the experimental focus should be on mechanical stresses and strains at FA sites (Hoffman et al., 2011) (Figure 1). When external forces are applied to the (apical) cell surface and transmitted and distributed throughout the cytoskeleton, they must be counter-balanced by equal and opposite forces at the attachment sites to the ECM and neighbouring cells. This holds also true for internally generated (contractile) forces of the cell. The aim should be to elucidate details of stress propagation and distribution through the cytoskeleton to focal and cell–cell adhesion sites, i.e. to determine the changes in protein composition, dynamics and mechanics.

(*ii*) *The FA and cytoskeletal remodelling (e.g. cellular reorganization and reinforcement) in response to mechanical stress*. Since mechanical stress within the FA is a regulated entity, acute changes in stress are reversed or counteracted by structural remodelling and motor protein regulation. When cells are mechanically stimulated, they respond in a multitude of ways, e.g. they modulate their spread area or change their shape and orientation, activate actomyosin interactions and recruit integrins and FA proteins (Schmidt et al., 1993; Choquet et al., 1997; Balaban et al., 2001; Riveline et al., 2001; Galbraith et al., 2002; Geiger and Bershadsky, 2002; Deng et al., 2004; Goldmann et al., 2005; Lehoux et al., 2006). The transient formation and regulation of FA proteins in a complex, and the physiologic function of these processes are still elusive, but are thought to be directly regulated by the stress acting on FA proteins due to externally applied tension or myosin II-driven cell contractility (Hoffman et al., 2011). For instance, mechanical stress at the FA sites can trigger reinforcement processes that are characterized by integrin clustering, recruitment of FA proteins as well as actin polymerization. The reinforcement process allows the cell to generate higher traction forces and to withstand greater external forces. It is expected that cellular responses to local mechanical perturbation change force propagation through cytoskeletal structures and ultimately result in changes of mechanical stress within the FAC (focal adhesion complex) (Hoffman et al. 2011). In particular, these complex cellular responses are orchestrated such that mechanical stresses within the FAC remain within a narrow range. This idea has been proposed, among others, by Balaban et al. (2001) who have shown that FA stress remains at a constant level during

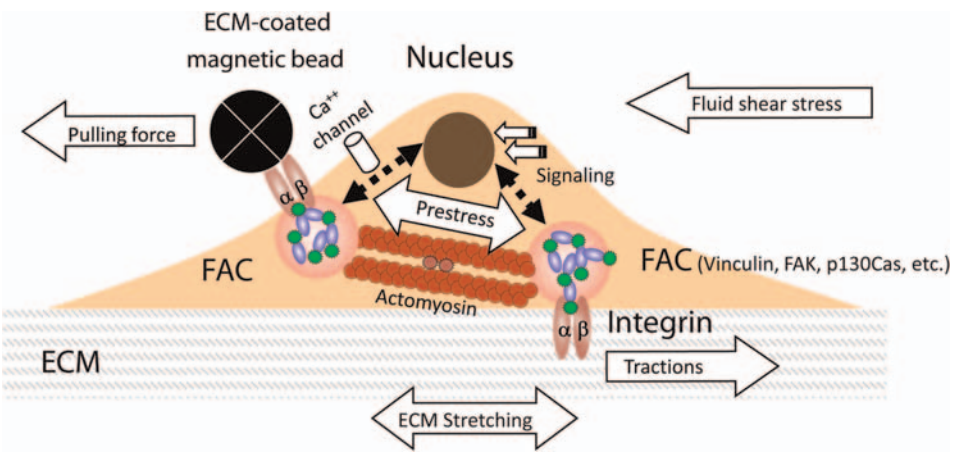

Figure 1 **Schematic diagram of how forces influence cellular mechanotransduction**
Fluid shear stress affects the cell surface directly and *via* cell receptors' intracellular signalling, and integrin-anchored FAs impact through matrix attachments cytoskeletal filaments. Internally generated tension and forces transmitted through cell–cell contact similarly reach FAs through the cytoskeleton. Forces located within FAC can stimulate integrin clustering, induce recruitment of additional cytoskeletal linker proteins, and activate integrin-associated signal cascades. Vinculin, p130Cas and FAK are involved in intracellular signalling which is regulated by calcium influx. Integrin attachment to the ECM produces tractions; ECM stretching influences outside-in signalling and cell morphology; shear stress affects calcium influx and mechanochemical signalling; and ECM-coated magnetic bead pulling determines cell stiffness, cytoskeletal dynamics, adhesion strength and prestress. In all, mechanical stimulation results in chemical signalling to and from the nucleus.

drug-induced actomyosin relaxation. The significance and novelty would be to test whether this holds true under external force perturbations.

(*iii*) *The molecular mechanism of mechanosensation involved in cellular (mechano)chemical signal transduction*. Numerous proteins [e.g. PKA (protein kinase A), PKC (protein kinase C), *src, rho*] that are activated by integrin-mediated mechanochemical signalling cascades can bind to calcium-gated channels on the cell surface. Increased Ca^{2+} influx, in turn, reinforces cytoskeletal structures and triggers actomyosin contraction that can lead to a cycle of further integrin signalling and calcium channel sensitization. Previous studies have identified both integrin- and Ca^{2+} influx-mediated signalling as a prerequisite for mechanical responses in cells (Ghosh and Greenberg, 1995; Chen et al., 2001). Mechanical force stimulation *via* integrins activates signalling cascades leading to *src*, *rho* and ERK (extracellular-signal-regulated kinase)/PAK (p21-activated kinase)/MAPK (mitogen-activated protein kinase) activation versus mechanical stretch and shear stress that lead to Ca^{2+} influx *via* mechanosensitive Ca^{2+} channels in cells. The possibility of mutual interaction and amplification between both signalling pathways has so far not been investigated. In particular, it is suggested that the interaction and coupling between both signalling pathways occurs through the modulation of contractile stress within the cytoskeleton. The modulation of contractile stress in cells through channel-mediated Ca^{2+} influx has thus far not been characterized. Apart from a direct activation of actomyosin interactions after Ca^{2+} influx, actin is rapidly polymerized and both can lead to an increase of contractile stresses. The aim here should be to find out how contractile responses are modulated by integrin-dependent signalling mechanisms.

The objective of further investigations should be to study molecular remodelling responses and mechanotransduction pathways that have previously been regarded as being independent, i.e. integrins and associated FA and cytoskeletal proteins as well as mechanosensitive Ca^{2+} channels. A long-term goal should be (i) to illuminate key roles of integrin-Ca^{++}-channel cross-talk for mechanotransduction which may lead to dynamic changes in cell function and (ii) to establish a platform for future studies aimed at developing new strategies of intervention.

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