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Physically based principles of cell adhesion mechanosensitivity in tissues

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Received 21 February 2012, in final form 12 July 2012
Published 19 October 2012
Online at stacks.iop.org/RoPP/75/116601

Abstract
The minimal structural unit that defines living organisms is a single cell. By proliferating and mechanically interacting with each other, cells can build complex organization such as tissues that ultimately organize into even more complex multicellular living organisms, such as mammals, composed of billions of single cells interacting with each other. As opposed to passive materials, living cells actively respond to the mechanical perturbations occurring in their environment. Tissue cell adhesion to its surrounding extracellular matrix or to neighbors is an example of a biological process that adapts to physical cues. The adhesion of tissue cells to their surrounding medium induces the generation of intracellular contraction forces whose amplitude adapts to the mechanical properties of the environment. In turn, solicitation of adhering cells with physical forces, such as blood flow shearing the layer of endothelial cells in the lumen of arteries, reinforces cell adhesion and impacts cell contractility. In biological terms, the sensing of physical signals is transduced into biochemical signaling events that guide cellular responses such as cell differentiation, cell growth and cell death. Regarding the biological and developmental consequences of cell adaptation to mechanical perturbations, understanding mechanotransduction in tissue cell adhesion appears as an important step in numerous fields of biology, such as cancer, regenerative medicine or tissue bioengineering for instance. Physicists were first tempted to view cell adhesion as the wetting transition of a soft bag having a complex, adhesive interaction with the surface. But surprising responses of tissue cell adhesion to mechanical cues challenged this view. This, however, did not exclude that cell adhesion could be understood in physical terms. It meant that new models and descriptions had to be created specifically for these biological issues, and could not straightforwardly be adapted from dead matter. In this review, we present physical concepts of tissue cell adhesion and the unexpected cellular responses to mechanical cues such as external forces and stiffness sensing. We show how biophysical approaches, both experimentally and theoretically, have contributed to our understanding of the regulation of cellular functions through physical force sensing mechanisms. Finally, we discuss the different physical models that could explain how tissue cell adhesion and force sensing can be coupled to internal mechanosensitive processes within the cell body.

(Some figures may appear in colour only in the online journal)

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Acknowledgments

References

1. Introduction

Cell adhesion has attracted the attention of physicists and engineers since it has become apparent that (i) managing it is a prerequisite to organize cells in a synthetic matrix for bioengineering applications, and (ii) that it could not be described with theories designed for dead matter, albeit being sophisticated.

1.1. Biological background

The interactions of tissue cells with the ECM and with their neighbors have a large influence on a wide variety of biological processes including cell adhesion, migration and differentiation (Lauffenburger and Horwitz 1996, Geiger et al 2001). Cell adhesion and migration are highly complex and multistep processes, which share many common features. They both involve several compartments of the cell, including surface receptors, signaling elements and the cytoskeleton, which is a cellular scale fiber structure at the origin of cell shape and tissue elasticity, as we describe later on (figure 1). Adhesion and migration then play an essential joint role in embryogenesis (Jacinto et al 2002), wound healing (Martin 1997) and inflammatory responses (Muller 2003). A dysregulation of cell attachment also has dramatic effects and can cause pathological states such as developmental defects, cancer invasion and metastasis (Thiery 2002).

Cell adhesion as well as the orientation and the velocity of cell migration are affected by extracellular cues such as biochemical gradients (chemokines or growth factors) (Swaney et al 2010, Phillipson and Kubes 2011) and surface chemistry (Maheshwari et al 2000, Peyton and Putnam 2005, Pompe et al 2009). Physical cues including external forces (Kamm 2002, Desprat et al 2008), topography (Curtis and Wilkinson 1997, Berry et al 2004, Dalby 2009, Ghibaudo et al 2009, le Digabel et al 2010) and the elastic properties of the ECM (Lo et al 2000, Discher et al 2005, Zaman et al 2006, Ghassemi et al 2012) are now considered as important regulators for various biological mechanisms involving cell adhesion and migration. Even for cells with the same genetic material, they could behave very differently depending on the mechanical properties of their microenvironments.

Single cell adhesion and migration have been extensively studied in 2D culture systems and described as a multistep event where the cell interacts with the matrix on a molecular level and where this interaction is amplified into conformational changes that lead to a cellular response called mechanosensitivity.
Figure 1. Cell adhesion on a 2D substrate and actin-based motility structures. (a) Schematic representation of a cell attached on a substrate. Actin filaments are the basic structural component found in a number of cellular structures used for cell motility and substrate sensing (insets: filopodium, lamellipodium and lamellum). Certain structures appear sheet-like (e.g. lamellipodia/lamellae, ruffles), whereas others are finger-like (e.g. filopodia). The cell migrates upwards and could be attached to a second cell on the right through cell–cell junctional proteins involving cadherins. Image courtesy of the Mechanobiology Institute, National University of Singapore. (b) Immunofluorescent image of a fibroblast showing the different adhesive structures (focal adhesions in red, actin filaments in green, nucleus in blue). Image courtesy of Leyla Kocgozlu.

process (Ridley 2011). All these steps form a cyclic process, based on the coordination of cell adhesion to the ECM and the cell’s ability to contract its body so that it can crawl like a slug (figure 2). Then it becomes obvious that cell adhesion and migration are tightly coupled.

However, the coupling is far from simple, and fast- or slow-moving regimes do not correlate in a simple way to the amount of adhesion proteins that are engaged (Peyton and Putnam 2005). The reason is that both cell adhesion and cell migration involve actin filaments. These are components of the cytoskeleton, a composite filamentous structure that influences cell shape and cell contractility on the cellular scale (Alberts et al 2002). Actin filaments are distributed throughout the cell and give the appearance of a gel network. Compared with ‘physical’ polymers, they present two striking abilities: (I) they continuously polymerize/depolymerize, their average length being regulated by different capping proteins (Nobes and Hall 1995, Borisy and Svitkina 2000, Pollard and Borisy 2003, Parsons et al 2010) (figure 3), (II) they self-assemble in the presence of cross-linker proteins. Some molecular motors, such as Myosin II, can contribute as ‘active’ cross-linkers. Energetically driven changes of conformation of the molecular motors make actin polymer chains slide respective to the others. The molecular motors then remodel the actin network, on the molecular or even on the cellular scale depending on the presence of other static cross-linkers. The phase diagram of the self-assembling actin filaments is quite complex (Backouche et al 2006, Lieleg et al 2010). On the one hand, it could be an extremely well organized and stably structured actin cytoskeleton in a striated muscle cell. On the other hand, it constitutes a very dynamic system that is under constant remodeling and reorganization to perform specific cellular functions such as cell motility and adhesion (Mogilner 2006, Vogel and Sheetz 2006, Sykes and Plastino 2010). Whatever the structure of the self-organized actin filaments, the collective contribution of the molecular motors leads to a global contraction of the network (Fletcher and Mullins 2010). Contracting bundles of actin play a dominant role in the cellular adhesion machinery, and are named stress fibers as a consequence of their morphology (Nobes and Hall 1999) (figures 4(a) and 5). Contraction of the non-polarized actin network plays a dominant role in cell migration, as it allows the retraction of the back edge of the cell (figure 2). The resulting cellular scale contraction of any of these structures is adaptive and resembles muscle contraction (Hill 1938, Mitrossilis et al 2009): the contractile stress they impose increases as a resistance is faced. As such, actin cytoskeleton is intrinsically mechanosensitive, in the sense that it adapts to mechanical forces. Connection of the actin cytoskeleton to clusters of proteins that are anchored to the ECM makes it suitable to probe the mechanical properties of the extracellular environment, as a response to the resistance the adhesion-mediated anchorage makes to its contraction. However, cell/matrix adhesions do not limit as stress transducers between the actin cytoskeleton and the extracellular environment.

The signaling pathways that coordinate the formation of new adhesions as well as their maturation are intimately linked to the dynamical reorganization of the actin cytoskeleton (figure 5) (Bershadsky et al 2003). On the one hand, the engagement of tissue cell receptors with their ligands in the ECM triggers a local intracellular response that governs the assembly of adhesion complexes and associated cytoskeleton
Figure 2. Schematic representation of the different steps of cell migration on 2D substrates. 1. Polymerization of actin filaments at the leading edge is translated into protrusive force. 2. Membrane protrusion facilitates the binding of transmembrane cell surface receptors to the substratum components. New adhesions are rapidly linked to the network of actin filaments. 3. The combined activity of retrograde actin movement and contractile forces produced by stress fibers generates tension to pull the cell body forward. 4. The forces produced by the contractile network combined with actin filament and FA disassembly help to retract the trailing cell edge. Image courtesy of the Mechanobiology Institute, National University of Singapore.

Some more information is required to understand why tissue cell adhesion differs from the adhesion of a partially wetting droplet to a surface, and how it contributes to the cell’s ability to probe its physical environment (Bruinsma and Sackmann 2001, Discher et al 2005). As previously mentioned, tissue cell adhesion organizes as micrometer-sized patches, with non-uniform distribution (figure 1(b)). Specific interactions between the adhesion proteins in the cell membrane and the ECM are required to observe such dot-like organization of the adhesion regions. In vivo ECMs indeed contain proteins such as fibronectin, laminin, elastin or various forms of collagen, which strongly interact with transmembrane adhesion proteins from the integrin family (Zaidel-Bar et al 2007, Parsons et al 2010). Formation of ‘key–lock’ bonds between the extracellular and transmembrane proteins triggers the activation of multiple signaling cascades (Alberts et al 2002). In the absence of such an engagement of the adhesion proteins, as would occur on a non-adhesive surface or in the case of red blood cells that do not have any of these adhesion proteins, cell adhesion on the surface relies on the non-specific interactions with the surface of the extracellular coat that wraps the cell membrane. Then the cell maintains a regular, round shape and adhesion resembles the wetting of a partially wetting droplet (Cuvelier et al 2007). However, tissue cells soon degrade and die in the absence of engagement of the adhesion proteins. The linkage of specific transmembrane proteins to proteins in the ECM is crucial for the adhesion of tissue cells. Signaling cascades that follow their engagements are extensively being studied, since dysfunction of one of them usually impacts other cellular mechanisms, such as division, differentiation or even cell survival. They result in the aggregation of numerous proteins, which are finely connected to the actin cytoskeleton (figure 5). At present, this complex architecture of proteins, called adhesome (Zaidel-Bar and Geiger 2010), is shown to share about 200 potential interactions.
As detailed in section 2, these overall adhesive structures are crucial for tissue cells to probe their microenvironment, exert traction forces to promote adhesion and migration, and control cell shape and function. As such, they are subjected to a constant remodeling to adapt themselves to external cues. Hence, not only is the biochemical composition of the ECM important, but also its geometry, topography and mechanical properties (Discher et al 2005, Ingber 2006). In this context, the conversion of physical signals, such as external forces, matrix stiffness and topography, into chemical signaling processes has become crucial to understand various fundamental cellular mechanisms. This emerging field has been called mechanotransduction, since it addresses the conversion of mechanical cues into biochemical, signaling events (Khan and Scott 2009).

1.2. Historical perspective

It has been postulated for a very long time by physical biologists that mechanics was crucial for the shape, organization and functioning of tissues. In his treatise On Growth and Form, originally published in 1917, D’Arcy Thompson involved a problem of mechanics, physical forces and cellular motions to explain morphogenesis (Thompson 1992). This highly original concept did not reach the mainstream and took a back seat in favor of the notions of genetic control and chemical communication. However, recent studies have shed new lights on the role of mechanical forces in shaping and organizing cells and tissues, as presented in different reviews (Discher et al 2005, Lecuit and Le Goff 2007, Montell 2008, Farge 2011). From single cell studies up to the integration of multicellular functions to describe living organisms, these studies reveal the importance of a number of mechanical entities that could coordinate to lead to complex shaping of cells, tissues and organisms. Among them, we can cite cell protrusion and cytoskeleton dynamics (Borisy and Svitkina 2000), cell–ECM interactions (Vogel and Sheetz 2006) and intercellular adhesions (Leckband et al 2011). It is thus tempting to reinterpret cell biochemistry within the framework of physical interactions in order to combine these different aspects—molecular biology, genetics and mechanics—and thus explain the wide diversity in the shapes of biological systems.

In this context, considerable attention has been paid to the mechanical interactions of tissue cells with the ECM. The development of various tools based on microscopy techniques, micromanipulation or microfabrication allowed one to examine how these cells apply mechanical forces as well as how they respond to changes in their mechanical environment. Early experiments exposed fibroblastic or endothelial cells to spatially uniform mechanical environments such as confined environments (Chen et al 1997), flexible substrates (Harris et al 1980, Dembo et al 1996) or shear stresses (Davies and Tripathi 1993). Using these techniques, it has been possible to measure the averaged response of tissue cells to physical signals.
Figure 4. Organization of the various structures of actin cytoskeleton within an adherent cell and role of adhesive structures in actin organization. (a) The distribution of adhesions (nascent and mature FAs) and actin structures (actin cortex, stress fibers, actin bundles) inside the cell, indicating the role of the different Rho family GTPases (Cdc42, Rho, Rac1) at specific regions in a migrating cell. (b) Adhesion acts like a ‘molecular grip’ and influences the protrusion of filopodia (2a). Adhesion between membrane-anchored integrins and the extracellular substrate (e.g. fibronectin) causes clustering of FA components (represented by a hand, 2b). Connections between the adhesions and the actin cytoskeleton convert the force generated by actin polymerization into protrusion (2c). Image courtesy of the Mechanobiology Institute, National University of Singapore.

However, the discovery of the importance of sub-cellular adhesion sites as major sites of force transduction for cell adhesion and migration led to the development of techniques that were first able to probe cell mechanical properties on the micrometer scale, the size of a single adhesive patch. Together with the improvement of microscopy techniques, advanced micromanipulation and microfabrication techniques allowed one to study the cell response at sub-cellular resolution either to locally applied external forces (Choquet et al 1997, Riveline et al 2001, Sniadecki et al 2007, Icard-Arcizet et al 2008, Paul et al 2008) or to map the distribution of nano-Newton forces exerted by various tissue cell types (including fibroblasts, neurons, endothelial and epithelial cells, cardiomyocytes, myoblasts and stem cells) on their underlying substrate (Galbraith and Sheetz 1997, Dembo and Wang 1999, Balaban et al 2001, Tan et al 2003, du Roure et al 2005, Ganz et al 2006, Chan and Odde 2008, Kajzar et al 2008, Sabass et al 2008, Zemel et al 2010).

1.3. Principles of mechanotransduction

The development of these techniques has demonstrated that tissue cells sense mechanical cues that guide their growth and movements by probing their microenvironment. Cell interactions with the ECM as well as with their neighboring cells (Mege et al 2006, Borghi et al 2010) through their respective adhesive structures play a key role in such processes. Even though cell–cell interactions might have an essential role for a cell probing its physical environment, its contribution is a very emerging field of research. Its investigation requires innovative strategies to limit perturbations of the adhesion complex by the probe. Therefore, we focus our review to the role of cell–ECM interactions in tissue cell sensitivity to physical cues.

At the cell–ECM interface, tissue cells sense the physical properties of ECM by translating mechanical forces and deformations into biochemical signals. In turn, these signals can adjust cellular and extracellular structures. This mechanosensitive feedback modulates various cellular functions such as adhesion (Pelham and Wang 1997, Riveline et al 2001, Giannone et al 2004), migration (Lo et al 2000), and even differentiation (Engler et al 2006). In this context, mechanotransduction refers to the conversion of these mechanical forces into biochemical signals. For instance, most tissue cells probe the elasticity of their environment as they anchor and pull on their surroundings. These mechanical steps are related to the acto-myosin contractility and the activation of the transcellular adhesion sites to transmit forces to substrates. Consequently, as they apply forces, the internal architecture of the cell actively changes through cytoskeleton reorganization, focal adhesion (FA) dynamics as well as other cellular processes that depend on the external load encountered by the cell (Discher et al 2005). Along the same lines, a similar active response has been observed as cells are subjected to external mechanical
time scales that control cell–substrate interactions. The integration of different processes over various length and contractility. Finally, we will discuss different approaches and as well as large-scale mechanisms that involve acto-myosin different scales including the role of FAs in the force response will present theoretical aspects of tissue cell adhesion on (local) or cellular (global) scale, thereby identifying possible candidates directing mechanosensitive responses. Then we will consider the various cellular responses on the molecular of tissue cell adhesion and cellular responses to physical stimuli, namely external forces and substrate stiffness. We will consider the various cellular responses on the molecular (local) or cellular (global) scale, thereby identifying possible candidates directing mechanosensitive responses. Then we will present theoretical aspects of tissue cell adhesion on different scales including the role of FAs in the force response as well as large-scale mechanisms that involve acto-myosin contractility. Finally, we will discuss different approaches and the integration of different processes over various length and time scales that control cell–substrate interactions.

2. Cellular responses to mechanical cues

Cells are exposed to a large variety of external mechanical stimulations such as forces exerted by neighboring cells or blood flow. These external forces induce mechanical stresses that are transmitted through the plasma membrane to the cytoplasm. Hence tissue cells could respond to the stresses through various mechanisms including assembly or disassembly of adhesion patches, actin remodeling and ion channel activation (Vogel and Sheetz 2006, Kobayashi and Sokabe 2010) over various time scales from seconds to hours.

1.4. Outline

In this review, we will first introduce biophysical aspects of tissue cell adhesion and cellular responses to physical stimuli, namely external forces and substrate stiffness. We will consider the various cellular responses on the molecular (local) or cellular (global) scale, thereby identifying possible candidates directing mechanosensitive responses. Then we will present theoretical aspects of tissue cell adhesion on different scales including the role of FAs in the force response as well as large-scale mechanisms that involve acto-myosin contractility. Finally, we will discuss different approaches and the integration of different processes over various length and time scales that control cell–substrate interactions.

2. Cellular responses to mechanical cues

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Figure 6. Schematic representation of the different mechanosensitive elements. Tissue cells respond to the external stresses and/or applied stresses that modify their internal organization through various mechanisms including assembly or disassembly of adhesion patches, actin remodelling (stress fiber formation) and ion channel activation over various time scales from seconds to hours. (Deng et al 2006, Wang et al 2009) (figure 6). We should consider different types of mechanical stimulations that can be categorized as those that come from the outside environment of the cells and those that are directly produced by the cells themselves. Cells are exposed to various external forces such as stretching (muscle cells), compression (bones) or shear flows (blood cells) and also respond to mechanical and biochemical changes of their surrounding environments. Here we focus on the impact of mechanical parameters that include external forces and substrate elasticity on the adhesion and migration of tissue cells. However, other exogenous cues such as substrate topography (Yim et al 2010, McNamara et al 2012, Nikkhah et al 2012), 3D versus 2D environments (Friedl et al 1998, Zaman et al 2006, Doyle et al 2009) or substrate chemistry (Maheshwari et al 2000, Peyton and Putnam 2005, Pompe et al 2009) could trigger various cellular responses. In particular, the interplay between ECM ligand density and matrix stiffness remains a puzzling issue to understand their effect on cell functions. We chose to describe and interpret mechanotransduction events on 2D substrates where surface chemistry and topography were kept as constant parameters. In such a context, cells interact with their mechanical environments and by doing so, adapt their own mechanical tension (figure 7). As a consequence, external and internal forces might be linked to each other (Chen 2008, Su et al 2011).

2.1. Application of external forces

The development of various techniques to analyze the transmission of forces through living cells has improved our knowledge on mechanotransduction on the cellular and molecular scales. In fact, understanding the molecular basis of mechanotransduction requires knowledge of the distribution of forces within cells as well as their amplitude. According to the different methods used to mechanically stimulate cells, it appears that responses are heterogeneous in space and time. The techniques used to apply external forces or stresses on cells in a range of stresses ranging from \( pN/\mu m^2 \) up to tens of \( \mu N/\mu m^2 \) include optical tweezers (Choquet et al 1997, Icard-Arcizet et al 2008), magnetic tweezers (Fabry et al 2001, Hu et al 2003), microfabricated actuators...

Historically, fluid shear flow was first used to mimic the influence of blood flow on endothelial cells to study vascular physiology and pathology (Davies 1997). In such a situation, forces acting on blood vessels can be viewed in terms of stresses: normal stresses exerted perpendicular to the wall and frictional forces parallel to the wall that can be described as shear stresses (Davies 1995). Many studies have shown a correlation between endothelial cell shape and the direction of fluid flow as cells align in the direction of the flow (reviewed in Davies 1995 and Su et al 2011). The exposure of endothelial cells to steady flows also induces a reorganization of the cytoskeleton on the time scale of minutes to hours. Most studies have reported a reorientation of actin filaments aligned along the long axis of the cell together with microtubules and intermediate filaments (Takahashi et al 1997). On the time scale of seconds, the application of flows on endothelial cells induces the activation of ion channels such as potassium channels, and intracellular signaling such as the activation of the contractile pathway through Rho GTPases signaling (Nobes and Hall 1999) occurs within minutes. Interestingly, shear stress is applied to the apical membrane of the cells, which is covered by a glycocalyx layer linked to transmembrane receptors. Therefore, the mechanotransduction mechanism should imply a transfer of the outside stress at the apical surface to the cytoskeleton through membrane molecules (Davies 1995). It is still unclear how those cells sense the mechanical stresses and convert them into biochemical pathways. However, plasma membrane proteins at the endothelial surface could act as mechanoreceptors including ion channels, integrins or G protein linked-receptors (Haidekker et al 2000). For instance, previous studies have provided evidence that the mechanotransduction of external stresses could be initiated by an increase in the intercellular calcium concentration (Naruse et al 1998). Membrane tension appears as a plausible candidate for stress transmission (Gauthier et al 2011).

Forces applied at the apical surface of the cell should be transmitted through the cytoskeleton to adhesion sites such as cell–ECM adhesions or cell–cell junctions (Tzima et al 2005). The mechanical interaction of the cytoskeleton with the plasma membrane has been clearly demonstrated. Wang et al (1993) have shown that the resistance to the application of external forces to magnetic beads coated with ECM proteins strongly induced a force-dependent stiffening response. Moreover, the cytoskeletal stiffness (ratio of stress to strain) increased in direct proportion to the applied stress and decreased with the disruption of actin filaments by the application of drugs. The cytoskeleton appears as a structural network that is able to transmit the stress from outside to the cytoplasm. As a result, it affects the dynamics of FAs through a downstream activation of integrins as well as the formation and elongation of cell–cell junctions based on VE-cadherin interactions in the direction of the flow (Orr et al 2006).

Interestingly, FAs also appear as a plausible candidate for mechanotransduction in endothelial cells in response to applied flows. When endothelial cells are subjected to luminal shear flows, FAs undergo a rapid remodeling and alignment in the direction of the flow within a few minutes (Davies 1995). This interesting example of endothelial cells submitted to shear flows highlights the various possible spatio-temporal responses of the cells to external forces. As a result, it appears that several candidates such as plasma membrane tension, FAs, mechanosensitive ion channels and cytoskeleton structures could act as mechanotransduction factors.

Other experimental studies have generalized the observation that the application of external stresses has an impact on cell adhesion in various cell types: fibroblastic (Choquet et al 1997, Riveline et al 2001, Colombelli et al 2009, Heil and Spatz 2010), epithelial (Colombelli et al 2009) and myoblastic (Ahmed et al 2010). In particular, these studies demonstrate that the dynamics of FAs including their growth and stability is affected by external perturbations (Choquet et al 1997, Riveline et al 2001, Colombelli et al 2009, Heil and Spatz 2010). Interestingly, Choquet et al demonstrated the existence of a feedback response between external and internal forces by using optical tweezers and beads coated with ECM proteins to apply a localized force on an adhering cell. When cells pulled on adhesions in such a way that the resisting force could be increased, their adhesions would be reinforced and by doing so, the pulling force exerted by the cell would increase (figures 8(a) and (b)). As a result, it appears that external forces can trigger active modifications in the adhesion sites (Choquet et al 1997, Heil and Spatz 2010) as well as in the cytoskeleton organization (Icard-Arcizet et al 2008) that would balance this force. Along the same lines, a local shear force induced by the tangential displacement of a micropipette on the cell surface towards the cell center can control the assembly and disassembly of adhesion complexes (Riveline et al 2001). Even when...
cells are maintained in a relaxed state using pharmacological treatments, external forces can substitute for spontaneous cell-generated forces to induce an increase and elongation of FAs in the direction of the applied force (figures 8(c) and (d)). Additionally applying forces globally to tissue cells through the deformation of underlying elastic membranes leads to an active cellular response through the remodeling of the actin cytoskeleton. Under static conditions, tissue cells align in the direction of stretching together with an activation of myosin II and a remodel of the stress fibers (Jungbauer et al. 2008, Zhao et al. 2007). Interestingly, cell alignment depends on the strain dynamics and, for high frequencies (∼1 Hz), they could align in the perpendicular direction to the strain. Such behavior has been discussed theoretically in terms of time scales needed for the cytoskeleton to reorganize itself (De et al. 2007). Under slow deformation, the cytoskeleton has enough time to reorient and align in the direction of the strain, whereas internal cell reorganization cannot adjust to high dynamical deformation leading to a perpendicular orientation. Additionally, the application of external forces induces an adaptation of the cell-generated forces (see table 2). Forces produced by the cell itself are generated by various cytoskeletal elements that transmit those forces through the cytoplasm (Wang et al. 2009, Cai et al. 2010). The measurements of traction force are exerted by cells and transmitted to the underlying substrate through FAs and close contacts. Thus, such measurements remain a challenging problem due to the small dimensions of adhesion sites (∼μm²), their density and the range of magnitudes of these traction forces (nano-Newton scale). However, several techniques, based on the high-resolution measurement of the deformation field of elastic ECMs, have succeeded in measuring such forces and correlating them with the remodeling of internal cellular structures such as FAs and cytoskeleton (Balaban et al. 2001, Beningo et al. 2001, Munevar et al. 2001, Butler et al. 2002, Tan et al. 2003, du Roure et al. 2005). Such substrates should allow the detection of forces in the nano-Newton range with a subcellular spatial resolution. They, in addition, can be designed as biocompatible and allow standard surface coatings of ECM proteins to promote cell adhesion.

In the 1980s, Harris and colleagues were the first to qualitatively visualize cellular traction forces as visible wrinkles on a deformable silicone rubber substrate, reflecting the strong traction forces exerted by motile fibroblasts parallel to the direction of locomotion (Harris et al. 1980). These sensitive membranes were formed by placing a thin skin of rubber (1 μm thickness) on top of the lubricating fluid silicone layer (Harris et al. 1980, 1981, Burton et al. 1999). This powerful technique has been used with success to show that fibroblasts were able to deform the thin cross-linked layer and thus study the compressive forces exerted by those cells. However, although an elegant approach, the method has several limitations such as the complex relationship between the size of the wrinkles and the amplitude of the generated forces.

Based on the same principle, more advanced techniques have been developed to achieve a better spatial resolution, especially at individual adhesion sites (Dembo et al. 1996, Munevar et al. 2001). The technique, called traction force microscopy, is based on the use of elastic gels or membranes that avoid the formation of wrinkles. For instance, a polyacrylamide (PA) gel with a variable degree of cross-linking allows easy manipulation of substrate rigidity in the kPa range (1 kPa = 1 nN μm⁻² is the scale of rigidity that allows probing the nN cellular forces that are transmitted through μm² adhesion patches (Schwarz 2007)) and a surface onto which ECM proteins can be conjugated to provide optimal cell adhesion (Pelham and Wang 1997, Kandow et al. 2007). Fluorescent beads are embedded into the gel and are used as markers of deformation (figure 9(a)). Quantification of forces can be made through large-scale matrix computation that converts maps of substrate deformation (detected as local bead movements) into maps of traction stress (force per unit area) (Dembo et al. 1996, Dembo and Wang 1999) (see the following equation):

\[ u_i(r) = \int dr' G_{ij}(r - r') F_j(r'), \]

where \( G_{ij} \) is the Green function of an elastic halfspace material, \( u_i \) corresponds to the displacement field and \( F_j \) to the stress
Figure 8. Cell responses to external forces. (a) Optical tweezer experiment on the local cell response to an external force (adapted from Choquet et al. (1997)). Fibronectin-coated beads display increased stiffness of links to the cytoskeleton after application of a force restraining bead movement. (a) and (b) traces) Plots of bead displacement from the leading edge versus time with reference to the point and time at which the bead initially came into contact with the lamellipodia. Traces are from two separate experiments in which (a) the trap was turned off 1.5 s after the initial bead–cell contact, or (b) the trap remained turned on (shaded area labeled (a)) until the bead escaped its radius of action, that is, respectively, for 12 s in (a) and 34 s in (b). After the beads traveled over 2 μm, the stage was moved (broken line) to reposition the bead within 0.7 μm of the trap center. The laser was then turned on again to apply a force on the bead to assess the strength of the bead–CSK link (shaded area labeled (b)). This protocol is illustrated in the inset of (a). With the brief initial trap exposure (a, (a)) the retrapping immediately moved the bead back toward the trap center, but the bead resumed its forward movement within seconds despite the continuous presence of the trap. In contrast, after first experiencing escape from the maximum trap force (b, (a)), retrapping did not move the fibronectin-coated bead (b, (b)), showing a reinforcement mechanism of the linkages after several exposures to an external force. (c) Micropipette experiment to apply external forces on the cell (adapted from Riveline et al. (2001)). Scheme depicting the method of application of external force. The pipette is bent against the coverslip and moved along the chosen lamellipodium as indicated by the yellow arrow. (d) Local formation of focal contacts in response to the application of external force. Fluorescent images of FAs (vinculin) of the cells are shown before ((i), (ii)) and after ((iii), (iv)) application of pulling force produced by micropipette shift. (i), (i’), (iii), (iii’) GFP fluorescence showing the distribution of vinculin; (ii), (iv) phase image of the same cell. (v) Image representing pixel-to-pixel ratio between the intensity of the GFP fluorescence before and after pulling. The values of the ratio between intensities of after and before images are represented by pseudocolors, as indicated in the spectrum scale shown below (v) and (v’). (vi) Ratio between the total intensity of individual focal contacts after and before pulling. The pipette tip is represented by a blue cone, and the direction of the pulling force, by the blue arrow. (vii)–(ix) Graphs illustrating individual FA growth. Changes in the FA area (vii), FA axial ratio (viii) and the fluorescence per pixel of the focal contact (ix) are shown. Bars: (i), (ii), (iii), (iv), (v) shown in (D): 20 μm; (i’), (iii’), (v’) 5 μm.
Table 2. Cell-generated forces.

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Figure 9. Traction force microscopy (TFM) techniques to measure cell-generated forces. (a), (b) Schematic representation of the TFM using fluorescent beads embedded into a PA gel and an example of the substrate deformations and the resulting forces (b). (c), (d) Schematic representation of the TFM using the micropillar method and an example of a MDCK cell lying on a bed of micropillars. This inverse operation is very sensitive to noise, which dominantly originates from the limited spatial resolution of the measured deformation field. Then equation (1) does not lead to unique solutions. Regularization terms must be added to exclude non-physical divergences. Several groups have thus developed various strategies to limit the impact of the noise in computing the traction field from the observed displacement field, such as assuming the force distribution pattern, using a regular array of embedded markers or directly solving the elastic equation together with the inverse problem to circumvent the specific expression of the Green function (Dembo and Wang 1999, Butler et al 2002, Schwarz et al 2002, Ambrosi 2006, Barentin et al 2006). However, the pathologic sensitivity to noise of the calculated force field is inherent to the inversion of the elastic equations on continuous, elastic media, and originates from the high degree of derivation that relates the force field and the deformation field.

Alternative methods can thus be useful in directly measuring traction forces exerted by individual FAs. Instead of using uniformly flexible substrata, an innovative approach has been to use micromachined cantilevers as force transducers on silicon wafers (Galbraith and Sheetz 1997). Cells adhere and...
leads to the following formula:

\[ F = k \cdot \Delta x = \left( \frac{3}{4} \pi E \frac{r^4}{L^3} \right) \Delta x \]  

(2)

where \( E \), \( k \) and \( \Delta x \) are, respectively, the Young modulus, the spring constant and the deflection of the post.

These approaches have been applied to various situations including single cell mechanics (Lo et al 2000, Balaban et al 2001, Tan et al 2003, Delanoe-Ayari et al 2010), cell–cell interactions (Ladoux et al 2010, Liu et al 2010, Maruthamuthu et al 2011), stem cell differentiation (Fu et al 2010) and multicellular systems (du Roure et al 2005, Nelson et al 2005, Trepat et al 2009). In particular, they provided new insights into the regulation of traction forces, their relationship with the formation of FAs at the single cell level. For instance, as fluorescent beads can be used as markers in the gel, dual channel fluorescence microscopy has permitted the correlation of traction stresses in relation to the spatial localization of either GFP-fused FA protein. Surprisingly, it has been shown by using GFP–zyxin as an adhesion marker that small nascent FAs under the leading edge of migrating cells exert stronger traction stresses than large mature FAs (Beningo et al 2001). This inverse relationship is unique to the leading edge of motile cells and is not observed in the tail. In contrast, in relatively stationary cells, there is a positive correlation between the traction force and the size of the FAs (Balaban et al 2001, Tan et al 2003, Goffin et al 2006). By combining living cell imaging of GFP–paxillin and the micropatterning of elastomer substrates, Balaban et al have demonstrated that traction forces are correlated with the orientation of FAs and also linearly increased with their area, indicating a constant stress (force divided by the FA area) of around 5.5 nN \( \mu \text{m}^{-2} \) (figure 10).

Such a discovery was of great importance, since it showed that FAs in stationary cells would grow or shrink by keeping a

Figure 10. Forces and FAs. (a) Fluorescence image of a human fibroblast expressing GFP–vinculin, which localizes to FAs. Red arrows correspond to forces extracted from the displacements of the patterned elastomer (Young’s modulus = 18 kPa). Forces are aligned with the direction of elongation of large FAs. Inset, phase-contrast image of the upper part of the cell (white rectangle), showing displacements of the dots (green arrows); the pattern that allows measuring substrate deformations consists of small square pits. (b) Fluorescence image of a human foreskin fibroblast stained with antibodies against paxillin, which also localizes at FAs. Red arrows correspond to forces extracted from the displacements of the patterned elastomer (Young’s modulus = 21 kPa). (c) Phase-contrast image of the same cell immediately before fixation. White scale bars represent 4 \( \mu \text{m} \); red scale bars represent 30 nN. (d) Correlation between the area and force of the FAs of the cell shown at various time points. Each symbol represents a different FA. The black line is the correlation line of the linear part of the plot. Its slope defines a stress of 5.5 nN \( \mu \text{m}^{-2} \) at the FAs. Reproduced with permission from Balaban et al (2001). Copyright 2001 Nature Publishing Group.
constant, local ability to transmit a precise level of force to the ECM. Assuming that traction forces are correlated with acto-myosin contractility, these differences in the traction force–FA size relationship under both conditions may be related to the organization of the actin cytoskeleton. In non-motile cells, actin filaments are distributed as stress fibers over several mature FAs, and the force generated by the parallel actin filaments may be distributed to multiple FAs. In motile cells, actin filaments also organize in branched networks at the leading edge of the cell that superimpose to the stress fibers. The combined contraction of both structures may reinforce the stresses that are transmitted to nascent FA. This could explain why a nascent FA may sustain more force transmitted through the actin filament. Consequently, it appears that the relationship between traction forces and adhesion site dynamics could lead to different responses. A recent study (Stricker et al 2011) indicates that similar sizes of adhesion complexes do not exert a constant stress and that a positive correlation between traction forces and FAs does exist but persists only during the initial formation of FAs. After this stage, the stress could still increase or decrease, whereas the adhesion size maintains a constant value.

In general, these studies highlight the role of cell-generated forces in the regulation and maintenance of cellular functions. However, it appears that many questions remain open such as the mechanism(s) that initiates the force production, the regulation in space and time responsible for the modulation of cellular forces and the integration of step mechanisms/signaling pathways in unique or various ways that allow the production of traction forces. Among those questions, the mechanism that converts mechanical stress into biochemical signaling remains a crucial one. This mechanosensitivity is usually accounted for by a generic local mechanism in which a force applied to a FA induces an elastic deformation of the contact that triggers conformational and organizational changes of some of its constitutive proteins, which in turn can enhance binding with new proteins enabling growth of the contact (Nicolas et al 2004, del Rio et al 2009, Kanchanawong et al 2010). ECM proteins could also undergo conformational changes when subjected to external stresses (Smith et al 2007). However, how this local mechanosensitivity can result in the ability of cells to respond over a large scale to modulate their shape and function remains largely unknown (Keren et al 2008, Parsons et al 2010).

2.3. Rigidity sensing mechanism

In this context, the response to the environmental stiffness appears as an important example in the regulation of cell-generated forces. Most of the tissue cells not only apply forces but also respond through cytoskeleton organization to the resistance that they sense with regard to the mechanical properties of the matrix. Different tissues or organs exhibit a wide range of mechanical properties, including their stiffness which can vary from 1 up to more than 100 kPa in vivo (Discher et al 2005). During various processes including tumor progression or tissue development, the stiffness of the environment could also be modified (Georges and Janmey 2005, Paszek et al 2005). Consequently, many cellular functions have been shown to depend on the rigidity of the environment. Cell migration may be directed toward increased substrate adhesiveness ( Carter 1967), stiffness (Discher et al 2005), while cell adhesion depends on substrate rigidity (Pelham and Wang 1997, Giannone et al 2004, Chan and Odde 2008). Even mesenchymal cell fate can be tuned by changing the substrate stiffness (Engler et al 2006).

From these various studies, much conflicting evidence has emerged to understand the interactions between tissue cells and the mechanical properties of their environment. As previously mentioned, FAs play a key role in actively sensing the environment (Pelham and Wang 1997). Not only FAs but also the opening of calcium ion channels (Glogauer et al 1997, Lo et al 2000, Hayakawa et al 2008) could participate in the build-up of cell tension in response to mechanical cues. Moreover, recent indirect observations (Mitrossilis et al 2009, Lam et al 2011) suggest that the contractile acto-myosin apparatus can act as a global rigidity sensor (Kobayashi and Sokabe 2010).

The different in vitro methods previously described have been extensively used to modify substrate stiffness. On the one hand, the rigidity of PA-based hydrogels can be easily manipulated by varying the concentration of the bis-acrylamide cross-linker (Guo et al 2006, Janney et al 2007, Solon et al 2007). By controlling the extent of polymer cross-linking in the gels, their Young’s modulus, $E$, can be adjusted over several orders of magnitude, from extremely soft to stiff, respectively from 1 kPa to 100 kPa. On the other hand, micropillar substrates can be used as well to quantify the effects of substrate stiffness on cell traction forces (Saez et al 2005, 2010, Ghibaudo et al 2008, Fu et al 2010). Surfaces of different spring constants can be designed by changing the geometrical parameters of the pillars (length and radius) (equation (1)). Interestingly, a rough estimation of the equivalent Young’s modulus, $E_{eq}$, of a micropillar substrate leads to a range of values from 1 up to 150 kPa (Ghibaudo et al 2008). Under both conditions, these values are compatible with the stiffness of in vivo tissues (Discher et al 2005).

Seminal studies on epithelial cells and fibroblasts exploited inert PA-based gels with a thin coating of covalently attached collagen (Pelham and Wang 1997). Images of adhesion proteins such as vinculin revealed that on soft, lightly cross-linked gels ($E \approx 1$ kPa), diffuse and dynamic adhesion complexes were observed whereas stiff, highly cross-linked gels ($E \approx 30–100$ kPa) induced stable FAs, close to those observed in cells cultured on glass substrates (Pelham and Wang 1997). A plausible explanation would be that the reduced stress on soft substrates could impact on the maturation of FAs.

In fact, traction force mapping of fibroblasts on gels proves that they are able to pull harder on relatively stiffer substrates (Lo et al 2000). If a cell can generate higher traction, it has some internal structure that can sense the stiffness of the matrix on which it resides. Tissue cells such as fibroblasts or endothelial cells also preferentially move toward stiffer areas of a gel as well as areas of a gel that are compressed, a phenomenon called ‘durotaxis’ in which a cell can move.
For low rigidities up to around 60 nN epithelial cells) has been determined (Ghibaudo et al 2008). The relationship of different cell types (fibroblasts or MDCK cells on the rigid region remain confined in this region. Fibroblastic L cells move from the soft part to the rigid one, while cells on the rigid region remain confined in this region.

...directionally toward a mechanical signal (Lo et al 2000, Isenberg et al 2009) (figure 11). Additionally, a local change in the substrate stiffness induced by anisotropic micropillars in shape induces a preferential spreading and migration of cells in the stiffest direction of the substrate (Saez et al 2007, Ghibaudo et al 2008). Using the micropillar technique, the force–rigidity relationship of different cell types (fibroblasts or MDCK epithelial cells) has been determined (Ghibaudo et al 2008). For low rigidities up to around 60 nN μm$^{-1}$, a linear variation of the traction forces exerted by adherent cells on their substrate is observed (figure 12), suggesting within a certain range of rigidity cell traction forces are regulated to achieve a given deformation of the matrix between 120 and 160 nm, depending on the cell types. Beyond this regime, a saturation plateau is observed for the highest spring constants of the pillars, characterizing the upper limit of traction forces that cells could exert on the substrate. Recently, it has been shown that the contractile forces generated on short time scales during the initial spreading of cells depend on micrometer-scale and submicrometer pillars. On 2 μm diameter pillars, it appears that adhesions were observed at the pillar periphery with a constant maximum force independent of stiffness whereas on 0.5 μm diameter pillars, adhesions form on the pillar tops, and local contractions between neighboring pillars are observed with a maximum displacement of ~60 nm, independent of stiffness (Ghassemi et al 2012). At these early times, it appears that the geometry of the pillars induces different rigidity sensing processes. Interestingly, for submicrometer pillars, the formation of contractile units leads to a mechanism based on a constant deformation of the substrate.

A spectacular experiment has even shown that mesenchymal stem cells can differentiate toward neurons, myoblasts and osteoblasts on PA gels of different stiffnesses under identical biochemical conditions (Engler et al 2006). It appears that cellular processes such as cell differentiation can occur not only at an optimal stiffness relative to the native tissue environment (Engler et al 2004, Zemel et al 2010). Along the same line, tissue cells tune their internal stiffness to match that of their substrate (Solon et al 2007). It has also been reported that changes in stiffness cause a change in cell shape which exhibits a more rounded morphology on soft substrates and more flattened on stiff ones (Pelham and Wang 1997, Ghibaudo et al 2008, Tee et al 2011). The interplay between cell shape and stiffness remains unclear but it should involve integrin-based mechanisms as well as acto-myosin remodeling since both FAs and actin organization are strongly affected by changes in the substrate stiffness. Recent studies show a stiffness-dependent orientation of the stress fibers in adherent cells showing that cell polarization together with the alignment of stress fiber actin can be tuned by stiffness changes (Zemel et al 2010, Prager-Khoutorsky et al 2011, Trichet et al 2012). It seems that cell contractility could be modulated by the internal cytoskeletal stiffness via a higher polarization of actin filaments.

2.4. Length scales and time scales in stiffness sensing

As previously explained, various mechanisms over different time scales are involved in mechanotransduction processes. The time it takes for living cells to detect the mechanical properties of their environment and the distances over which the signal propagates and is integrated are unclear. One of the plausible mechanisms for stiffness sensing implies the deformation of proteins within the adhesion sites under the stress exerted by acto-myosin contractility (figures 6 and 7). Under such stress, individual proteins within the adhesions could be stretched and undergo conformational changes that expose new cryptic sites and, as such, promote the binding of new proteins (Johnson et al 2007, del Rio et al 2009, Grashoff et al 2010). Such a mechanism is based on a local mechanosensing process since the size of the proteins is in the nanometer range. In this case, substrate stiffness can be probed locally within the submicrometer size of adhesion complexes involving clusters of proteins and small deformations. Previous experiments conducted on fibroblasts (Balaban et al 2001, Riveline et al 2001), which show a constant stress applied within FAs and an active response of FAs in terms of growth and elongation in the direction of the applied force, support this idea. Other hypotheses and experiments demonstrate that mechanosensitive channels coupled to FAs modulate their activity in response to applied forces or stiffness (Glogauert et al 1997, Kobayashi and Sokabe 2010). This mechanism could be used to induce a fast response (millisecond time scale) in the vicinity of FAs to mechanical stimulations but at a larger length scale than the tens of nanometers suggested by protein deformation (Janmey and Miller 2011).

Other experimental models propose that large-scale mechanisms could coordinate the cellular responses of tissue cells to substrate stiffness (Mitrossilis et al 2009, Kobayashi and Sokabe 2010, Trichet et al 2012). These experiments show that acto-myosin apparatus can act as a global rigidity sensor. Even the experiments carried out at early times during...
Figure 12. Traction forces as a function of substrate rigidity using the micropillar technique (adapted from Ghibaudo et al. (2008)). (a), (b) Mean and maximal forces exerted by individual fibroblasts. For each panel, the straight line represents the linear fit and the dashed the saturation plateau. These results exhibit two regimes: first a constant deformation for small stiffnesses and then a constant force regime for higher stiffnesses.

the initial contact of fibroblastic cells with their substrate (Ghassemi et al. 2012) suggest that substrate deformation is induced by local contractile units, which could be the precursors of large-scale structures such as stress fibers observed at later times. Although indirect observations, the work by Mitrossilis et al. (2009, 2010) analyzes the global response of cells spread between two microplates, one of them being flexible and acting as a force sensor (Thoumine and Ott 1997). Their results suggest that the interaction between molecular motors such as myosin II and actin filaments could drive the rigidity response of cells. Additionally, recent studies on flexible micropillars show that rigidity sensing requires a large-scale mechanical feedback that involves the reorganization of actin stress fibers instead of an only local mechanism based on FA mechanosensitivity. It has been observed that similar sizes of FAs on soft and stiff substrates can lead to very different force magnitudes. Moreover, the organization of actin filaments appear isotropically distributed on soft whereas they are highly polarized on a stiff substrate, as also observed in stem cells (Zemel et al. 2010). Such observations are in favor of a global sensing mechanism. Moreover, in the particular case of micropillars, as suggested by Janmey and Miller (2011), the local stiffness probed by integrins and other adhesion proteins is that of PDMS at the top of the pillar and, as such, exhibits a high stiffness (around 2 MPa). This suggests that cells do not probe the substrate rigidity over distances smaller than a few micrometers and, hence, probe the global stiffness of the micropillar substrate. As a result of these various observations, it appears that stress fibers may act as a force sensor that transmits a mechanical tension to FAs over a time scale of minutes.

Based on these different experiments, the time scale over which cells respond to substrate stiffness is a difficult question. On the one hand, recent studies (Mitrossilis et al. 2010) show that a lower limit for stiffness response could be of the order of 0.1 s, which is also in the same range as the cycling time of a molecular motor. Additionally, similar time scales are observed for the activation of ion channels in response to external forces (Kobayashi and Sokabe 2010). On the other hand, the cell response to cross the boundary between soft and stiff substrates and the build-up of traction forces in response to substrate stiffness could be observed in a range of several minutes.

These various experiments have led to the development of various theoretical models that aim at capturing these different length scales and time scales.

3. Theoretical aspects of cell adhesion

From a naive point of view, the growth of FAs could look similar to a nucleation and growth process, in the presence of an external force field (that mimics cell contractility or any external stress). That is the way pioneering works first tried to describe the specific response of cellular FAs to force (anisotropic growth, growth in response to force).

Cell adhesion was initially modeled by analogy to the adhesion of vesicles that contain mobile ligand molecules embedded into their membrane (figure 13). In the absence of force, ligands are attracted toward the adhesion sites by physical forces, such as short-range molecular attraction between the ligands (Zuckerman and Bruinsma 1998, Bruinsma and Sackmann 2001, de Gennes et al. 2003). The
finite size of the adhesion patches originates from the entropic fluctuations of the membrane, which move the membrane away from the substrate and prevent the ligands from attaching to the surface receptors. Normal forces pulling off the vesicle modify the fluctuation spectrum of the membrane by making it tensed. The bound molecules then concentrate toward a unique, central adhesion patch, and the region that adheres to the substrate extends (Smith et al 2008). In this model system, local shear forces applied to the adhesion patches, by stretching the ligand–receptor bonds, increase the elastic energy stored in them. This energy increase leads to the disruption of the adhesion patches. Such a prediction is in contradiction with the observation that, within cells, the size of the adhesion patches grows in response to such a local shear force (Riveline et al 2001). Thus, these models point out the need of including more specific cellular mechanisms, such as the contribution of cell contractility, in order to account for the adhesion growth in response to shear forces. As a consequence, models were elaborated, which contain more biological inputs.

Several approaches have been proposed, which can be divided into three categories: (i) molecular models that focus on the sensitivity of individual ligand–receptor bonds to force, (ii) condensation models that focus on the dynamics of clustering of proteins that are mechanically stressed at the adhesion site, and (iii) cell scale models that emphasize the contribution of the acto-myosin cytoskeleton to produce a mechanosensitive response.

3.1. Molecular scale modeling

The very first models that aimed at describing cell adhesion focused on the response of individual ligand–receptor bonds to mechanical cues (Bell et al 1984). Ligand–receptor bonds are non-covalent bonds, and therefore the resistance of the bond is an issue of time. Application of Kramers’ theory to the stretched bond showed that the most probable rupture force of the bond depends on the loading rate it is stressed with in a logarithmic way (Evans 1998). Regarding the complexity of the energy landscape, the history of the formation of the adhesion complex also impacts the resistance of the bond, the time of contact influencing the degree of entanglement of the partners (Pincet and Husson 2005). Thus, the complexity of the interaction energy between ligands and receptors makes the bond mechanosensitive, in the sense that its resistance is enhanced when loaded with increased force rate. In the context of cell adhesion, ligand–receptor bonds are connected to the acto-myosin cytoskeleton that stresses the bond. The stress originates from the sliding of actin filaments with respect to others, triggered by a change in the conformation of the myosin motor. The myosin-generated intracellular forces are also sensitive to the resistance they face, in a similar way to muscles (Howard 2001). Rigid ECMs favor faster loading of the bond than soft ones (Schwarz et al 2006) since it costs less elastic energy to increase the force on a more rigid substrate. Therefore, increased resistance of the loaded ligand–receptor bond to forces is expected on more rigid ECMs. As a consequence, ligand–receptor bonds on a rigid ECM are expected to be more stable in time and allow further condensation (Bruinsma et al 2005).

Figure 14. Local models based on molecular dynamics on the scale of FAs. (a) Acto-myosin-induced deformation of the mechanosensitive adhesion protein complexes triggers signaling cascades that promote the aggregation of new proteins. Aggregation and disruption occur at the edges of the FA, where gradients of stress take place (from Deshpande et al (2006), Olberding et al (2010) and Nicolas et al (2004)). (b) FAs are viewed as aggregates of adhesion proteins that are unstable in the absence of tension. Heterogeneities of the acto-myosin force field leads to a local depletion of proteins in the FA. Free protein complexes are thermodynamically attracted toward the depleted area to restore the equilibrium concentration (from Shemesh et al (2005)).

3.2. Condensation models

Condensation models focus on the adsorption of proteins into an existing adhesion patch that is stressed by the acto-myosin cytoskeleton. The proposed mechanisms at the origin of adsorption are diverse. Most of them assume that bound proteins increase their affinity with neighbors in response to a mechanical deformation, as is expected when proteins change conformation and unveil interaction sites (figure 14). Thus, some models propose that the condensation is then driven by the minimization of the energy of the adsorbing proteins (Deshpande et al 2007), the intermolecular attraction between the ligands and the receptors being favored by the change in conformation. This physical scenario resembles the historical description of ligand/receptor interaction in vesicle systems, with the additional consideration of a two-state potential of interaction between the ligands and the receptors that favors the interaction of stressed molecules. In these approaches, the probability that the adhesion proteins change conformation and
become activated is only marginally dependent on the rigidity of the ECM. More specifically, it is determined by the elastic properties of the adhesome itself. In the presence of an intrinsic shear modulus, a coupling that is expected if the adhesome has a gel-like structure (figure 5), the deformation of the adhesome is enhanced when the ECM is soft (Nicolas and Safran 2006). The attractiveness of nascent adhesion is therefore favored on a soft ECM. The matrix-mediated elastic interaction favors the condensation of proteins on a deformable ECM. Considering that the adhesome consists of point-like units at position \( \vec{x}_i \) that are stressed by identically oriented stresses \( f(\vec{x}_i) \), the elastic energy between them is written

\[
F_{\text{int}} = \frac{1}{2} \sum_{i \neq j} \vec{f}(\vec{x}_i) \cdot \vec{u}(\vec{x}_i, \vec{f}(\vec{x}_j))
\]

with \( \vec{u}(\vec{x}_i, \vec{f}(\vec{x}_j)) \) the deformation field caused by the inclusion at position \( \vec{x}_j \). In the simplest case, where \( \vec{f} \) is tangential to the surface and the ECM can be considered as semi infinite, the interaction energy is proportional to

\[
F_{\text{int}} \propto -\sum_{i \neq j} \vec{f}(\vec{x}_i) f(\vec{x}_j) E|\vec{x}_i - \vec{x}_j|
\]

with \( E \) the Young’s modulus of the ECM. This contribution, which is the ECM-sensitive dominant term in the free energy of the condensing proteins, shows that such an approach favors the formation of large aggregates of adhesion proteins on deformable ECMs. This opposes the observation that large adhesion patches are observed on rigid ECMs and not on soft ones (Pelham and Wang 1997, Georges and Janmey 2005, Saez et al 2005).

To solve this issue, a second category of models suggests that condensation of molecules at the adhesion patch is driven by the minimization of the energy the cell must provide to maintain a constant stress on the growing adhesive patch (Nicolas et al 2004, Olberding et al 2010) (figure 14(a)). These models emphasize the central role of actin contractility in the dynamics of the adhesions, and suggest that their regulation, which indeed influences cell fate, is performed by the large-scale, energy-consuming, acto-myosin stress fibers. Although considering the contribution of the actin cytoskeleton, these models remain local: they take for granted the experimental observation that adhesion growth or disruption takes place with the maintenance of a constant stress, or equivalently a constant force per molecule, pulling on it (Balaban et al 2001). The contribution of the acto-myosin cytoskeleton then enters the free energy of the adhesion patch through its effect: the energy it costs to deform the adhesion molecules, and possibly the ECM, by the application of a constant stress. The energy associated with the elastic interaction between adsorbing proteins is then to be taken from the point of view of the acto-myosin stress fibers, which maintain the stress on the growing domain:

\[
F_{\text{int}} = \frac{1}{2} \sum_{i \neq j} \vec{f}(\vec{x}_i) \cdot \vec{u}(\vec{x}_i, \vec{f}(\vec{x}_j)) \approx \frac{1}{2} \sum_{i \neq j} \vec{f}(\vec{x}_i) f(\vec{x}_j) E|\vec{x}_i - \vec{x}_j|.
\]

It undoubtedly costs less energy to maintain a constant stress on a stressed domain on a rigid ECM than on a soft one since the ECM is less deformed. For this reason, these models predict a preferred growth on rigid ECMs.

Both descriptions are based on the minimization of free energy, in the frame of different thermodynamic ensembles. Stochastic analyses were proposed, which base the dynamics of the adhesion patch on the stress-dependent lifetime of the bond between the cellular receptor and the ligand on the ECM (Bruinsma 2005, Walcott et al 2011) (figure 13). Former observations (Merkel et al 1999) and analysis (Evans 1998) of the detachment rate of individual, mechanically pulled away, ligand/receptor bonds showed that (i) the rupture force of the bond increases with increasing loading rate, and (ii) depending on the molecular structure of the proteins, the lifetime of the bond could be either decreased with increasing amplitude of the pulling force (slip bonds) (Merkel et al 1999) or more surprisingly increased (catch bonds) (Marshall et al 2003) (figure 15). Linkage of adhesion proteins to the ECM, such as integrin–fibronectin linkage, indeed shows catch bond behavior (Kong et al 2009). The presence of stress is therefore expected to stabilize the adhesion patch. Further condensation of the complex architecture of proteins at the base of the FA structure is then kinetically favored. In addition, independently of the catch or slip behavior of the stressed bond, increasing the rigidity of the visco-elastic ECM, to which the adhesion patch is anchored, was shown to increase the lifetime of the bond and its resistance to stress (Bruinsma and Sackmann 2001).

All these approaches are based on conformation-induced change in the affinity of proteins. An exception to this molecular description is the work by Shemesh et al (2005). They propose to adapt the theory of surfactant adsorption at an interface to the description of cell adhesion mechanosensitivity (figure 14(b)). In the presence of a heterogeneous, directional force field, such as the stress field due to actin stress fibers, the adhesion patch is distorted. The variation of the internal density in the patch drives the adsorption of new proteins in the depleted regions, so that the energy of the adsorbed proteins is minimal. The simplicity of this approach is very attractive. It proposes some ‘universal’ mechanism for cell adhesion mechanosensitivity, fairly independent of cell adhesion details and complexity.

All these models account quite well for the observations reported on a rigid ECM: favored growth of the adhesion
patch in the presence of a force, anisotropy of the dynamics of growth, in the direction of the force (Nicolas et al 2004, Shemesh et al 2005, Walcott et al 2011), treadmill dynamics at the edges of the adhesion patch (Nicolas et al 2004, Besser and Safran 2006, Olberding et al 2010, Walcott et al 2011) and the existence of distinct regimes of growth (Besser and Safran 2006, Nicolas et al 2008, Olberding et al 2010). However, the preferred growth of the adhesion patches on rigid versus soft ECM questions the relevance of passive theories (Shemesh et al 2005, Deshpande et al 2007), which are based on the minimization of the energy of the adsorbing proteins. As described above, under the assumption that the condensation is driven by the minimization of the energy of the adsorbing proteins, larger adhesions are expected onto deformable substrates, in contradiction with experiments. On the other hand, active, thermodynamic approaches (Nicolas et al 2004, Olberding et al 2010), which include the work done by the acto-myosin cytoskeleton to maintain a constant stress on the growing adhesion patch, predict a limitation of the size of the adhesion patches that is inversely proportional to the rigidity of the ECM (Nicolas and Safran 2006, Nicolas et al 2008). Sensitivity to the rigidity in these models originates from the increased elastic energy it costs to maintain a constant stress on proteins bound to the deformable matrix, the invested energy then being stored in the elastic deformation of the ECM. Stochastic models predict the reinforcement of the cell ligand–ECM receptor bond with increasing rigidity too, either because the loading rate of the force increases with increasing rigidity of the visco-elastic ECM and thus the lifetime of the bond (Bruinsma 2005), or because the probability that a neighboring ligand binds to a receptor on a soft ECM is smaller due to the increased distance between the deformed surface of the ECM and the ligand at the cell membrane (Walcott et al 2011).

3.3. Models on the scale of the entire cell

Apart from molecular models, another possibility is that cells respond to the mechanical properties of their environment by adapting their contractility to the resistance they oppose. In this approach, cellular adhesions are only stress transmitters, and play a secondary role. The dominant role is played by the actin cytoskeleton. Then the complex, biochemical responses of the FAs we described earlier, such as the adaptation of the FA size to the rigidity of the ECM, result from chemical reactions controlled by the rearrangement of the acto-myosin cytoskeleton. In fact, the acto-myosin cytoskeleton consists of actin filaments cross-linked by myosin molecular motors, whose changes in conformation make actin filaments slide with respect to the others.

Such a ‘living’ assembly of fibers was shown to have a muscle-like response to load (Hill 1938), meaning that contractility and rate of force generation are enhanced when a resistance is introduced to the contraction of the cytoskeleton (Mitrossilis et al 2009). Perturbation of myosin motors’ sliding ability in the presence of a load is at the origin of such behavior. Stochastic models have been proposed that base cell sensitivity to the mechanical properties of its environment on the mechanosensitivity of the acto-myosin complex (Schwarz et al 2006, Chan and Odde 2008, Walcott and Sun 2010) (figures 16(a) and (b)). The elastic resistance to deformation of the ECM indeed influences the rate at which the myosins can generate forces in the actin bundle. In turn, the lifetime of the actin anchorage to the ECM (through the adhesion proteins) results from the balance between the detachment rate of the actin/ECM bond and the load-dependent force generation rate by the myosins. Since the time that myosins require to build a certain level of force is inversely proportional to the rigidity of the ECM (Schwarz et al 2006), myosin displacements rapidly generate the maximal force they can bear when actin filaments are bound to a rigid ECM. Then the rupture of the actin/ECM bond is driven by entropy (Bell 1978), and the lifetime of the bond is determined by the probability to escape the energy barrier of the potential of the stressed bond. Individual linkages often break (short lifetimes) but each of them withstands the maximal forces that myosins can generate (high strength). Adhesion spots, therefore, appear as a very dynamic, moving assembly of proteins, with an approximately constant size (figure 16(a)). When the ECM is soft, the load generated by myosin displacements increases on a time scale comparable to the lifetime of the actin/ECM bond in the absence of force. Then the lifetime of the actin/ECM bond is increased compared with the rigid ECM. But individual linkages cannot bear important forces. Therefore, as greater tension builds in the adhesion cluster, thermal fluctuations are enough to generate a cascade of failure events (Chan and Odde 2008) (figure 16(b)).

Predictions on the dynamics of the adhesions as a function of the rigidity of the ECM differ from experiments, which observe an increased apparent sliding of the adhesions on a soft ECM (Discher et al 2005). The former models were therefore reconsidered by limiting the maximal force the adhesion can be submitted to (equivalently, by limiting the amount of working myosins) (Walcott and Sun 2010). Then soft ECM indeed favors the fast renewal and apparent sliding of slightly stressed adhesions, while rigid ECM favors stationary adhesions subjected to larger traction forces, as experimentally observed. Including the kinetics of the formation of the stress fibers can account for the observed dynamics of the actin/ECM linkages on soft versus rigid ECM. Deterministic numerical simulations were performed, which, in addition to the muscle-like response of the acto-myosin cytoskeleton, assume a stress-dependent rate of self-assembly of the actin filaments into stress fibers (Deshpande et al 2006). In the absence of resistance to the contraction of the cytoskeleton (for instance, on a very soft ECM), the stress generated into the cytoskeleton decreases (muscle-like response), and stress fibers dissolve. Although stochastic models would predict an increase in the lifetime of the actin/ECM linkage in such a situation, including stress-dependent kinetics of the self-assembly of the actin stress fibers indeed leads to the conclusion that the stress fibers are dissolved on soft ECMs, and therefore the actin/ECM linkage is also dissolved, due to the disappearance of the actin scaffold. This assumption of stress-dependent self-assembly of the stress fibers is consistent with the observation of thickening of stress fibers in the presence of externally applied tensions (Riveline et al 2001).
The model developed by Schwarz et al (2006) introduces the concept of a ‘two-spring’ model, the external stiffness and the global cellular stiffness acting as springs in series. This model leads to the prediction that stiffer environments induce stronger forces. The recent observations that stress fibers within adherent stem cells can respond to substrate stiffness by exhibiting a higher degree of polarization (Zemel et al 2010) have led to the formulation of a ‘three-spring model’ (figures 16(c) and (d)). This model thus includes both the passive forces arising from the elasticity of the cell and the environment as well as the active cell-generated forces. A phenomenological cell polarizability is introduced to exhibit this coupling. This study suggests the existence of a mechanical coupling between the cell shape, the rigidity of the surroundings and the organization of stress fibre in the cytoskeleton of stem cells.

Along the same lines, simple models (Marcq et al 2011) from active gel theory (Kruse et al 2005, Joanny and Prost 2009), where the active contribution is generated within the cytoskeleton through acto-myosin contractility, can capture the cellular responses to substrate stiffness experimentally observed (Ghibaudo et al 2008, Mitrossilis et al 2009, 2010, Ladoux et al 2010) (figure 16(c)). In particular, a linear relationship between the force generated by the cell and the stiffness of the substrate at low external stiffness is obtained, as experimentally observed (figure 12). In this case, the degree of cellular contractility could be an important parameter to explain the cellular adaptation to the external stiffness. Moreover, a similar modeling could explain why tension mediated through the actin cytoskeleton induces a higher polarization of actin fibers on stiffer substrates (Zemel et al 2010, Trichet et al 2012) (figures 16(c) and (d)). Such responses could be due to a large-scale mechanical feedback that involves the reorganization of actin stress fibers that could act as a force sensor that transmits a mechanical tension to FAs. However, this description ignores the coupling with the local scale and as such, with the dynamics of adhesive complexes. Another possibility would be that stress fiber reinforcement is regulated by the mechanosensitivity of the proteins in the adhesion patch.

4. Discussion of the physical understanding of adhesion mechanosensitivity

Whether cell sensitivity to its mechanical environment occurs on a molecular or a cellular scale is still an open debate. The propagation of a mechanical signal in between FAs reveals propagation times of a few hundred milliseconds (Na et al 2008). Such a short time is neither consistent with a diffusion process, of the order of seconds, nor with a cascade of chemical reactions, of the order of minutes. Only a fast, mechanical propagation of the solicitation, for instance through elastic waves, combined with the mechanical deformation of the probe that is in between the actin stress fibers and the ECM (the activation of Src, a mechanosensitive protein that is part of the complex of adhesion (Wang et al 2005)), could account for such short times. In addition, it was shown that the propagation of the mechanical signal would only occur when the transmembrane, integrin receptors are activated. This experiment supports the assumption that adhesion proteins,
which are localized in the adhesion patch, are major players in the cell’s ability to sense its mechanical environment. It also points out that mechanisms that circumvent the load-dependent contraction of the acto-myosin cytoskeleton are at work in the regulation of the adhesion patches, in response to external forces or to the elasticity of the ECM.

Apart from these observations, a recent work proved that cells pull on the ECM with a stress that does depend on the elastic properties of the ECM (Trichet et al 2012). Independence of the stress with the size of the adhesions (Balaban et al 2001) is confirmed on an ECM with a uniform compliance. But the value of this stress is shown to adapt to the rigidity of the ECM. This result emphasizes the existence of a large-scale mechanosensitive apparatus that regulates the amplitude of the stress used by the cell to pull on the ECM through each of its FAs. All the condensation models assume that this stress is an intrinsic property of the cell. In these models, the value of stress measured in Balaban et al (2001) is used as a constant when predicting the dynamics of FAs as a function of the rigidity of the ECM. Consistency of these models with the experiments on deformable ECMS is therefore surprising. For instance, condensation models account for the linear dependence of the saturation size of the FAs on the Young’s modulus of the ECM (Nicolas and Safran 2006, Nicolas et al 2008), as observed experimentally (Saez et al 2005). Inserting a phenomenological dependence of the value of stress on the Young’s modulus makes the theories match more precisely with the more resolved experiments reported in Trichet et al (2012). Assuming this phenomenological relationship between the acto-myosin stress and the elastic properties of the ECM indeed involves assuming that the time scale for the stabilization of the value of stress by which the acto-myosin fibers pull on the adhesion is much shorter than the maturation time of the adhesion itself. This means that a decoupled analysis of the sensitivity of the acto-myosin stress fibers to the mechanical properties of their environment, and of the mechanosensitivity of the adhesion proteins could be relevant. However, the saturation of the acto-myosin contractility to a stationary value was shown to take place on several minutes (Mitrossilis et al 2009, 2010). This time is of the same order as the time that is needed for cells with inhibited contractility to grow micrometric adhesions in the presence of an external force (Riveline et al 2001). Thus both time scales, acto-myosin stress adaptation and aggregation of adhesion proteins, seem to be comparable. Then, the apparent agreement between the condensation theories and the experiments cannot originate from decoupled time scales. It more probably originates from (i) the dispersion of the measurements, and (ii) from the fact that the stress dependence on the rigidity of the ECM might be a second-order contribution when quantifying the size of the FAs for instance. Anyhow, this result emphasizes the existence of a large-scale mechanosensitive apparatus that regulates the amplitude of the stress used by the cell to pull on the ECM through each of its FAs.

The assumption that a global probing of the mechanical properties of the ECM, for instance through the mechanosensitive contractility of the acto-myosin cytoskeleton, takes place first that regulates the dynamics of the adhesions, or the inverse scenario, can probably not be resolved. Coupling the dynamics of contraction of the stress fibers to a condensation model that accounts for the sensitivity of the FAs to the mechanical properties of the ECM, such as deterministic or stochastic ‘active’ models that include the cellular work that is spent to pull on the adhesions, might give indications whether distinct time scales for the response of the local and global machineries are necessary to reproduce the experimental observations. A tentative study was performed in this direction (Deshpande et al 2008), but the passive model used to describe the condensation of the proteins to FAs fails in accounting for the favored growth of the adhesion on a rigid ECM.

5. Conclusions and outlook

Cell responses to physical cues appear as important regulators of various biological functions. Evidence accumulates that force sensing is crucial during the different steps of cellular life. As pointed out in this review, the transduction of mechanical inputs into biochemical signals as cells probe their substrate could involve various mechanosensitive components that act on various length scales, locally or more globally on the cell scale. However, the interplay between these different mechanosensitive units remains unclear. Both theoretical and experimental studies should now focus on the coupling between these different length-scale processes in order to unravel the different sequential steps leading to appropriate cellular responses.

Here, we focus on the mechanosensitive players that directly interact with the surrounding tissue. Ultimately, since substrate elasticity can govern cell fate (Engler et al 2006), the mechanotransduction pathways seem to be coupled to different expression of genes, raising crucial questions about the physical coupling between the nucleus and the elastic components of the cytoplasm (Mazumder and Shivashankar 2010).

Finally, our review focuses on the interaction of individual cells with a 2D substrate but most of the in vivo aspects of cell adhesion and migration should take into account the three-dimensional (3D) environment. Many processes are altered when comparing cells in their native 3D environments or maintained on 2D substrates. Cells encapsulated in a 3D matrix exhibit dramatically different morphology, cytoskeletal organization and focal adhesion structure from those on 2D substrates (Cukierman et al 2002). Recent studies have shown that measuring forces in a 3D context is possible (Legant et al 2010, Ghibaudo et al 2011) but even though the impact of the various physical parameters such as rigidity, porosity and topography remains important in 3D (Zaman et al 2006), the inter-dependence between these parameters make the observations more difficult to interpret in terms of mechanotransduction.

Acknowledgments

The authors would like to thank Axel Buguin, Marion Ghibaudo, Leyla Kocegolu, Jimmy Le Digabel, Sam Safran,
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