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Single and collective cell migration: the mechanics of adhesions

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**ABSTRACT** Chemical and physical properties of the environment control cell proliferation, differentiation, or apoptosis in the long term. However, to be able to move and migrate through a complex three-dimensional environment, cells must quickly adapt in the short term to the physical properties of their surroundings. Interactions with the extracellular matrix (ECM) occur through focal adhesions or hemidesmosomes via the engagement of integrins with fibrillar ECM proteins. Cells also interact with their neighbors, and this involves various types of intercellular adhesive structures such as tight junctions, cadherin-based adherens junctions, and desmosomes. Mechanobiology studies have shown that cell–ECM and cell–cell adhesions participate in mechanosensing to transduce mechanical cues into biochemical signals and conversely are responsible for the transmission of intracellular forces to the extracellular environment. As they migrate, cells use these adhesive structures to probe their surroundings, adapt their mechanical properties, and exert the appropriate forces required for their movements. The focus of this review is to give an overview of recent developments showing the bidirectional relationship between the physical properties of the environment and the cell mechanical responses during single and collective cell migration.

**INTRODUCTION**

Cells, tissues, and organs must constantly adapt to their surroundings. A cell’s interaction with its environment is crucial for physiological tissue organization and functions during development, as well as for homeostasis, regeneration, and aging. It is also involved in pathological conditions—such as during tumor progression or fibrosis. The cell microenvironment is composed of the extracellular matrix (ECM) neighboring cells and surrounding intercellular medium. The microenvironment varies in composition and organization depending on the tissue or in vitro culture conditions. At the cellular level, when a cell touches a permissive surface, be it a substrate or another cell, it will form adhesive structures that allow it to sense and respond to the properties of its surrounding. Cells can sense two major types of information: chemical signals, such as small molecules and soluble factors, which are read through specific receptors, and physical properties, including substrate stiffness, topography, porosity, and elastic behavior, as well as compressive and traction forces (Figure 1). We focus here on the recent evidence pointing to substrate rigidity as a critical parameter controlling cell mechanical responses. However, it is important to keep in mind that other physical properties of the microenvironment are as likely to affect cell behavior. Each tissue has its own stiffness, which affects cell differentiation or behavior (Swift et al., 2013; Swift and Discher, 2014; Ivanovska et al., 2015). For example, axon elongation in Xenopus depends on a stiffness gradient that affects persistent growth and fasciculation of the retinal ganglion axon in the developing brain (Koser et al., 2016). Variations in tissue stiffness control cell proliferation or cell fate specification (Tse and Engler, 2011; Aleksandrova et al., 2015). Cardiac myocytes need a specific stiffness to become actively beating cells, and muscle cells need muscle-like stiffness to form myotubes; excessive stiffness will impede correct myofibril development and may lead to sclerosis and scars (Engler et al., 2004, 2008). In the case of mesenchymal stem cells, a stiffer environment induces bone-like development, whereas a soft...
The reciprocal relationship between the mechanics of the cell and the physical properties of its surrounding is crucial during cell migration. The cell reads mechanical stimuli (mechanosensing) from the substrate and responds by applying forces to the ECM, which affects cytoskeletal rearrangements and motion. This process involves many genes, such as Yes-associated protein (YAP), which controls nuclear localization. YAP transcription induces matrix stiffening, leading to cytoskeletal rearrangements and cell movement. Collective migration is particularly important during development and tissue shaping, involving many cells, sheets, or chains. Cells can exploit differences in tumor rigidity to promote cell invasion and survival. The interaction between the cell and its environment is complex, involving many factors such as cell density, ECM stiffness, and mechanical cues. The mechanisms of collective migration are still being deciphered, and understanding them is crucial for developing effective treatments.
imposed by the ECM; Doxzen et al., 2013). This implies that the cells must integrate information from the environment, which, in this case, also includes the neighboring migrating cells (Figure 1).

In this review, we focus first on the regulation of forces during single-cell migration and then in collectively migrating cells.

**MECHANOCOUPPING BETWEEN SUBSTRATE RIGIDITY AND TRACTION FORCES DURING MIGRATION**

**Mechanosensing at focal adhesions**

Single migrating cells must sense the physical properties of the ECM and, in response, apply the appropriate forces to generate movement. Adhesion and traction on the ECM mainly rely on integrins. Integrins are heterodimeric transmembrane receptors composed of an α and a β subunit, which bind specific ECM proteins. Their binding to ECM proteins is controlled by a conformational change that can be activated by outside-in signaling upon ECM binding and by inside-out signaling triggered by the association of partner proteins to the integrin cytoplasmic tail (Huttenlocher and Horwitz, 2011). As cells spread on the ECM, a growing number of integrins interact with the ECM proteins, which progressively form clusters, also called nascent adhesions or focal complexes. The initial steps leading to the formation of nascent adhesions occur before mechanosensing, at least during spreading, and are independent of myosin (Choi et al., 2008; Changede et al., 2015; Sun et al., 2016a). However, forces contribute to the maturation of nascent adhesions into focal adhesions (Riveline et al., 2001; Sun et al., 2016a).

The formation of focal adhesions is initiated when the cluster of ECM-engaged integrins is large enough. In NIH3T3 fibroblasts on fibronectin, clusters of integrins smaller than 0.11 μm are unstable and unable to exert forces because the cluster force cannot sustain the cytoskeletal force (Coyer et al., 2012). By developing the tension gauge tether method, Wang and Ha (2013) demonstrated that for CHO-K1 cells, a tension of 40 pN is necessary for integrins to form adhesive structures. During migration, two tension levels can be identified, corresponding to nonclustered integrins (40 pN) and clustered integrins (54 pN). The latter level depends on actomyosin and actin stress fibers that connect focal adhesions together and represents integrins under higher tension, which are found in motile focal adhesions (Wang et al., 2015).

In focal adhesions, integrin clusters can recruit up to 160 different proteins (Zaidel-Bar et al., 2007; Horton et al., 2015; for a review, see Li et al., 2016). These proteins form a physical bridge between integrins engaged with the ECM and the cell cytoskeleton, and more particularly to actomyosin contractile fibers (Figures 2 and 3B). A key article by the Waterman group defined focal adhesions structurally by three-dimensional (3D) super-resolution. Integrins and actin are separated by a 40-nm focal adhesion core subdivided into a lower group (integrin tails, paxillin, and focal adhesion kinase [FAK]), an intermediate, force-transduction group (talin and vinculin), and a higher, actin-regulatory group (zyxin, α-actinin, and vasodilator-stimulated phosphoprotein [VASP]; Kanchanawong et al., 2010). The intermediate proteins talin and vinculin are fundamental mechanosensors, as they can change conformation and signaling properties upon force-induced stretching (for a review, see Yan et al., 2015; Haining et al., 2016). Ultimately, talin and vinculin allow force transmission through β integrins, regulating migration and detection of stiffness (Austen et al., 2016; Nordenfelt et al., 2016).

Talin was one of the first proteins to be identified as an integrin partner (Horwitz et al., 1986; Figure 2A). In absence of talin, as in absence of integrins, focal adhesions cannot form properly (Zhang et al. 2008). Talin is recruited together with FAK to nascent adhesions (Lawson et al., 2012). Talin is a large protein of 270 kDa composed of an N-terminal head, a neck, and a C-terminal rod domain. It can adopt an autoinhibited, closed conformation and is activated upon release of this autoinhibition and opening into an extended form (Calderwood et al., 2013). Integrin binding to the rod domain activates talin, which reinforces the interaction (Himmel et al., 2009) and promotes the conformational change of the β integrin subunit. Talin binding to the integrin tail can be induced by inside-out signaling. Protein kinase Cα (PKCα), Rap1, Rap-1 GTP-interacting adaptor protein (RIAM), and phosphatidylinositol-4,5-bisphosphate (PIP2) induce talin activation and promote integrin engagement with the ECM (Das et al., 2014). Talin-2 has a particularly high affinity for β integrins, which leads to higher traction forces and faster...

**FIGURE 2:** Tension-sensitive proteins are mechanical players of adhesion sites. (A) Schematic representation of the main tension-sensitive proteins involved in focal adhesions and adherens junctions: talin (purple), vinculin (light blue), and α-catenin (dark blue). The main protein interaction domains are shown, and the known interactors (with their binding sites) are indicated above or below each protein. (B) Top, components of focal adhesions and the structures of talin and vinculin when stretched (talin in pink, vinculin in light blue, PIP2 in purple, and F-actin in orange). Bottom, components of adherens junctions and the structure of the unstretched (closed, top) or stretched (bottom) α-catenin (cadherin in purple, p120 in yellow, β-catenin in coral, α-catenin in purple, vinculin in light blue, and actin in orange).

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Vinculin, a cytoplasmic 117-kDa protein, was initially identified as an actin-binding protein (Geiger et al., 1980) and later found to bind to a high number of partners, including talin, α-actinin, Arp2/3, paxillin, VASP, catenins, and PIP2 (Carisey et al., 2013; for a review, see Peng et al., 2011; Goldmann, 2016; Figure 2A). Vinculin comprises an N-terminal globular head (Vh), which can bind talin, and a C-terminal rod tail (Vt), which can directly or indirectly interact with actin (Cavalheiro et al., 2017). These two major domains are separated by a short, flexible, proline-rich linker. Similar to talin, vinculin can be found in a closed, autoinhibited state in which Vh and Vt bind each other (Johnson and Craig, 1994, 1995a). Vinculin is recruited via talin to adhesion sites. After actin binding to the Vt domain, vinculin stretching dissociates the Vh and Vt (Bakolitina et al., 2004; Izard et al., 2004; Cohen et al., 2005; Chen et al., 2006). At this point, vinculin can induce recruitment, activation, or release of other integrins, talin, focal adhesion proteins, and more actin, promoting the growth of the focal adhesion in a force-dependent manner (Humphries et al., 2007; Carisey et al., 2013). Owing to its activity and localization, vinculin is considered an optimal candidate in mechanotransduction (Atherton et al., 2016). Its loss induces small but dynamic focal adhesions and defects in locomotion (Coll et al., 1995; Saunders et al., 2006; Thievessen et al., 2013). Loss of vinculin is associated with cancer, as well as with developmental diseases such as cardiomyopathies (Olson et al., 2002; Goldmann et al., 2013). Depletion of the vinculin gene in mice leads to embryonic lethality by embryonic day 10, with defects consistent with problems in adhesion, such as neural tube defects and cardiac malformations. Mouse embryonic fibroblasts (MEFs) from vinculin knockout mice are faster but less adhesive, with disrupted focal adhesions (Xu and Baribault, 1998). A recent study showed that hyperactivation of vinculin also causes lethality and muscular defects in Drosophila due to the formation of cytoplasmic aggregates that resemble adhesion subcomplexes, which are bound to talin tail but not to integrins or actin (Maartens et al., 2016). In these complexes, vinculin can ectopically activate talin, mimicking the effect of force.

The recruitment of talin and vinculin to focal adhesions correlates with the mechanical force applied to the focal adhesion (Golji et al., 2011). The use of a vinculin fluorescence resonance energy transfer (FRET) tension sensor showed that vinculin is recruited to focal adhesions in a force-dependent manner (Grashoff et al., 2010). The Vt binding to actin induces actin fiber bundling to regulate migration and traction (Johnson and Craig, 1995a; Janssen et al., 2006; Thompson et al., 2014; Jannie et al., 2015). Bundling is most likely mediated by the displacement of the first helix (H1) in Vt upon actin binding, partially unfolding vinculin (Ho Kim et al., 2016). Vinculin–actin interaction is also necessary for transmission of forces, mediating myosin contractility, which enhances forces (Dumbauld et al., 2010). The Vt

FIGURE 3: Turnover of focal adhesions. (A) Schematic representation of a migrating cell (pink; nucleus in blue; the arrow shows the direction of migration), highlighting focal adhesion (green) formation, maturation, and disassembly. Different maturation stages (labeled 1–6) can be observed, which can grow (full arrow) or disassemble (dotted arrow) at every step. Inset, summary of the work by Sarangi et al. (2016). The intensity of vinculin and paxillin is analyzed in parallel to vinculin tension (green, high; to white, low) on micropillars. The intensity of vinculin and paxillin is analyzed in parallel to vinculin tension (green, high; to white, low) on micropillars. The intensity of vinculin and paxillin is analyzed in parallel to vinculin tension (green, high; to white, low) on micropillars.
domain is necessary to generate forces (Dumbauld et al., 2010), whereas the Vh domain probably enhances adhesion strength (Dumbauld et al., 2013). Vinculin also plays a role in engaging and stretching talin with the actomyosin system, locking it in an open conformation and stabilizing the talin–integrin complex (Dumbauld et al., 2013) and focal adhesion (Atherton et al., 2016).

**Front-to-rear control of mechanotransduction**

During migration, cells form, use, and dissociate focal adhesions. Much stronger traction forces are applied on mature focal adhesions than on nascent adhesions (Gardel et al., 2008). The newer adhesions at the cell front have a higher tension than the retracting ones at the cell rear (Grashoff et al., 2010), as talin tension is higher in peripheral focal adhesions than in older ones (Kumar et al., 2016). Vinculin appears incorporated in the proximal tip of new focal adhesions with minimal tension in the paxillin-rich lower layer of the focal adhesion. Vinculin then treadmills toward the distal end of the focal adhesion, binding actin and talin, opening and increasing its tension (Case and Waterman, 2015; Figure 3). Vinculin interaction with actin is necessary to regulate the actin retrograde flow, as it slows the flow and leads to higher traction forces (Humphries et al., 2007; Thievesen et al., 2013; Jannie et al., 2015). As tension increases, vinculin progressively detaches from the lower layer and is carried inward and upward by the actin retrograde flow as an open protein but without any tension (Case and Waterman, 2015).

Mechanotransduction through talin and vinculin is continuously influenced by the cytoskeletal dynamics and molecular signaling. As forces increase on new focal adhesions, p130Cas, a protein involved in integrin signaling, is stretched. In this case, the stretching renders phosphorylation sites accessible to the Src kinase (Sawada et al., 2006). The FAT domain of p130Cas appears essential in mechanosensing substrate rigidity and controlling cell speed (Bradbury et al., 2017). The following p130Cas phosphorylation increases integrin signaling to the small GTPase Rap1, which in turn can activate talin via RIAM and promote further integrin engagement. As the focal adhesions mature, vinculin competes with RIAM to bind talin and stabilizes the integrin-talin-actin complex independently of Rap1 (Lee et al., 2013). Vinculin phosphorylation by Src on Y100 and Y1065 promotes vinculin opening and increases adhesion and force transmission (Auernheimer et al., 2015). Phosphoinositide signaling affects both talin and vinculin activities. PIP2 activates autoinhibited talin (Ye et al., 2016). Vinculin also binds PIP2 (Johnson and Craig, 1995b; Izard and Brown, 2016), and vinculin stretching increases its binding to PIP2 (Dwivedi and Winter, 2016). A recent model resolved the structure for a short-chain PIP2 binding to vinculin tail and showed that vinculin dimerizes in the presence of PIP2 (Chinthalapudi et al., 2014, 2015). Vinculin mutants that cannot bind lipids are associated with altered focal adhesion turnover but are still able to reinforce cell stiffness upon mechanical deformation (Thompson et al., 2017).

Disassembly or sliding of focal adhesions can result from the negative regulation of talin (Figure 3). Kank2 was recently identified as a component of focal adhesions that forms a “belt” around more mature focal adhesions. In migrating cells, Kank2 concentrates around most mature focal adhesions and binds talin. This interaction displaces actin but maintains talin active and thereby uncouples integrins from actin fibers, reducing force transmission and promoting the sliding of the focal adhesions (Sun et al., 2016b). Ultimately, the loss of traction can also promote the disassembly of focal adhesions. A decrease in traction forces promotes the association of the clathrin adaptor Dab2 with integrin β3 while excluding talin and thereby promotes clathrin-mediated endocytosis of integrins (Yu et al., 2015).

**Adaptation of forces to substrate rigidity**

During migration, cells use focal adhesion to apply traction forces on the ECM. They respond in a linear manner to the substrate stiffness and change focal adhesion size accordingly (Pelham and Wang, 1997; Saez et al., 2005; Ghibaudo et al., 2008). To measure the traction exerted by the cell, different techniques have been developed over the past 25 years. Initially, cells were plated onto deformable silicon sheets, and the sheet wrinkling was an indirect measure of the cell’s traction onto the substrate (Harris et al., 1980). Toward the end of the 1990s, in a key article, Pelham and Wang (1997) reported that the deformation of a polycrylamide gel could be used to infer the traction force field exerted by a cell, leading to the development of traction force microscopy (TFM; Dembo and Wang, 1999). TFM is based on the measurement of the displacement of fluorescent beads embedded into an inert hydrogel: the amplitude of displacement indicates how much traction the cell exerted. Other techniques, such as micropillars and atomic force microscopy, have also been developed to better study the cells from a mechanical point of view (Polacheck and Chen, 2016). Mechanotransduction at focal adhesions allows the cells to adapt the forces they exert to the physical properties of the substrate (Figure 1). Thus cells tend to exert higher forces on stiffer substrates and lower tractions on softer substrates (Saez et al., 2005; Ghibaudo et al., 2008). On soft gels, focal adhesions usually appear diffuse and dynamic; on stiffer gels (or glass), they are stabler and larger (Pelham and Wang, 1997; Ghibaudo et al., 2008). During branching on soft substrate, human mesenchymal stem cells show small patches of rapidly turning over focal adhesions but longer protrusions. The vinculin head–tail interaction is necessary for this response, as the mutation (T12) of vinculin that prevent this interaction stabilizes the talin–vinculin complex in focal adhesions in amounts that are not rigidity dependent (Liu et al., 2016). The size–force relationship in focal adhesions is not simple, and focal adhesions of the same size can exert different forces, depending on the substrate stiffness (Trichet et al., 2012). The nature of integrins associated with the substrate also influences the mechanical responses. β1 integrins induce Rac1 activation to assemble new small adhesions. αv integrins are involved in rigidity sensing and accumulate in areas of high tension to reinforce adhesions and actomyosin contractility by activating a RhoA-MDia pathway and the formation of additional actin bundles (Schiller et al., 2013). β1 and αv integrins cooperate to promote myosin II contractility and adapt the level of forces to the rigidity of the substrate.

A recent challenging study analyzed the relationship between focal adhesion internal forces and traction forces by combining micro-pillars of given stiffness and FRET tension sensors (Sarangi et al., 2016). The authors found that the tension inside focal adhesions correlates in space and time with the force exerted on the substrate, depending on the integrity of the stress fibers (Sarangi et al., 2016). Whereas a previous study found that the traction peak localizes distally a few micrometers from where paxillin is most abundant (Plotnikov et al., 2012), Sarangi et al. (2016) demonstrated that both paxillin and vinculin are concentrated at the distal end of the focal adhesions and are less abundant behind the central area (Figure 3A). Vinculin forces are higher in the region that directly contacts the substrate, where vinculin is not at its peak concentration (Sarangi et al., 2016). The coupling between integrins and actomyosin forces was initially explained in neurons by the “molecular clutch” hypothesis (Mitchison and Kirschner, 1988; Schwarz and Gardel, 2012). Actin rapidly polymerizes and pushes the lamellipodia forward, whereas its contraction through myosin II leads to net rearward flow of the actin network. When the retrograde actin flow is coupled to the ECM through integrins and focal adhesion proteins (in other
words, when the clutch is engaged), the force of the actin polymerization at the leading edge is converted into a protrusion force pushing the leading edge forward. The force is transmitted to the ECM (rearward traction), allowing the cell to move forward (Swainathan and Waterman, 2016).

The molecular clutch model allows a better understanding of how cells sense the environment. According to the Odde model, the molecular clutch behaves differently, depending on the substrate stiffness. On stiff matrices, the retrograde flow is fast, with low traction (“frictional slippage”), because the F-actin bundle is continuously disengaged from the clutch. On soft matrices, the retrograde flow is slower, tension can build up, and the clutch remains engaged for longer time, until the load reaches such high levels that some proteins in the clutch are lost and the whole clutch fails (“load-and-fail”; Chan and Odde, 2008). One limitation of the Odde model is that it predicts a biphasic force–rigidity relationship. However, in most conditions, a monotonic increase of traction forces is observed as a function of ECM stiffness. The Roca-Cusachs group (Elosegui-Artola et al., 2016) recently demonstrated that the biphasic curve can be masked by talin. Above a certain rigidity threshold, the force loading becomes fast enough to allow unfolding of talin before integrins disengage, leading to recruitment of vinculin and integrins, reinforcement of integrin binding, adhesion growth, and increase of force transmission. Moreover, when talin unfolds, YAP translocates to the nucleus, possibly through integrin clustering, signaling downstream of vinculin and talin and transmission of forces to the nucleus via actin stress fibers. Below this stiffness threshold, talin is not stretched rapidly enough, integrins disengage, and YAP is not shuttled to the nucleus (Elosegui-Artola et al., 2016). Vinculin, together with FAK and paxillin, is involved in sensing rigidity. Inhibition of FAK and paxillin reduces traction and decreases the rigidity threshold that promotes tugging on softer ECM, involving vinculin recruitment in strengthening the molecular clutch (Mierke et al., 2008; Plotnikov et al., 2012). In line with this, vinculin tends to adopt an inactive conformation on softer ECM and an active one on stiffer ECM, which is important for stiffness-dependent migration (Yamashita et al., 2014). Other proteins involved in the molecular clutch include α3β1 integrin (Schiller et al., 2013; Riaz et al., 2016) and α-actinin (Meacci et al., 2016), the loss of which causes aberrant rigidity sensing.

Adaptation to substrate rigidity is a very fast process (Mitrossilis et al., 2010). The cell must continuously sense and respond in a feedback loop to maintain the situation in a steady state (Roca-Cusachs et al., 2013). How the cell really measures rigidity is still debated; most probably the cell exerts submicrometer contractions and detects the local deformation of the substrate by “measuring” how much force/contraction it needs to generate such deformation. These contraction areas are similar to sarcomeres, as shown by the recruitment of α-actinin on the pillar tips and of myosin-II between pillars. The contraction units involve nanometer-size, myosin-dependent steps with a frequency of ∼2–3 steps/s; when the force reaches a threshold of ∼20 pN, a pause is triggered so that the adhesion can be reinforced by recruiting more α-actinin (Ghassemi et al., 2012; Wolfenson et al., 2015). A recent study on human skin fibroblasts highlighted the role of two receptor tyrosine kinases, AXL and ROR2, which regulate rigidity sensing by respectively modulating the strength or the duration of these contractions (Yang et al., 2016). In the case of MEFs on micropillars, cells measure displacements of 60 nm in early steps of adhesion, until the adhesion itself can grow and couples to the actin retrograde flow (Ghassemi et al., 2012). Moreover, the actin cytoskeleton responds through rheological changes, behaving like a fluid on soft substrates and a nematic solid on stiff ones (Gupta et al., 2015).

Mechanotransduction beyond actin

The role of other cytoskeletal networks in the cell mechanical responses is still understudied. However, evidence is accumulating suggesting a possible role of microtubules in the generation of adapted forces. First, microtubules are involved in the regulation of adhesion sites (Akhanova et al., 2009; Etienne-Manneville, 2013), contractility (Kolodney and Elson, 1995; Rape et al., 2011), and RhoA signaling (Heck et al., 2012). In parallel, microtubules can modulate traction through FAK (Rape et al., 2011). Second, microtubule dynamics appear to be controlled by the rigidity of the substrate. In endothelial cells, both actin and microtubules participate in cell branching, and microtubule growth depends on substrate stiffness and myosin (Myers et al., 2011). Microtubules have also been shown to orient toward stiffer areas (Maiuri et al., 2015; Raab and Discher, 2016) and retract from the stiff area when contraction is locally inhibited (Kaverina et al., 2002). On stiff matrices, microtubules are important to regulate cell polarity, while protrusions are mainly generated by actin dynamics (Etienne-Manneville, 2013). However, in soft 3D matrices, microtubules are necessary for fibroblast dendritic-like extensions and endothelial cell migration (Rhee et al., 2007; Bouchet and Akhanova, 2017). Kank family proteins were shown to regulate talin-mediated force transmission (Sun et al., 2016b). Kank1 was recently shown to bind talin, and this interaction is necessary to allow targeting of microtubules near focal adhesions (Bouchet et al., 2016); it will be interesting to see whether this mediates a mechanosensory response.

Detyrosination, a posttranslational modification of microtubules associated with their stability, participates in mechanotransduction of striated muscles. Reduced tubulin detyrosination is associated with decreased cytoskeletal stiffness and faster muscle contraction and relaxation, suggesting a role in mechanotransduction (Kerr et al., 2015). Oncogenes may increase tumor cell stiffness and invasion through HDAC6 (histone deacetylase 6), which inhibits microtubule acetylation and causes the reorganization of the vimentin intermediate filament network (Rathje et al., 2014). In different cell types, solubility of vimentin depends on ECM stiffness and correlates with cell ruffling. On softer substrates, the soluble pool is maintained by microtubules, whereas on stiffer substrates, it depends on contractility (Murray et al., 2014). Intermediate filaments have been shown to participate in and regulate cell migration (Gonzales et al., 2001; Bhattacharya et al., 2009; Mendez et al., 2010; Dupin et al., 2011; Weber et al., 2011; Sakamoto et al., 2013; Leduc and Etienne-Manneville, 2015; Liu et al., 2015a; Vincent et al., 2015). Because of this and their peculiar properties as highly elastic filaments and role in cell mechanics (Herrmann et al., 2007; Block et al., 2015), intermediate filaments are an ideal candidate to mediate mechanotransduction in cells.

THE MECHANICS OF COLLECTIVELY MIGRATING CELL GROUPS

Just as single cells, migrating cell groups are also clearly affected by the biochemical and physical properties of their environment (Figure 1B). However, migrating collectives cannot be simplified as a group of independent cells that happen to move at the same speed and direction. The collective behavior results in a more efficient migration and sometimes in the acquisition of specific features (Mayor and Etienne-Manneville, 2016). This relies on the communication between migrating cells, and the direction of each cell depends on its neighbors (Vicsek et al., 1995; Szabo et al., 2006). During collective migration, cells couple to one another mechanically and chemically through cell–cell contacts and the actin cytoskeleton (for a review, see Mayor and Etienne-Manneville, 2016). This allows the cells...
Each other. In the particular case of contact inhibition of locomotion, which will not be discussed here, cells can also repel each other as a mechanism of collective guidance (Theveneau et al., 2013; Scarpa et al., 2015; Zimmermann et al., 2016). How cells collectively adjust their forces and how they sense and transduce the mechanical properties of their neighbors is currently under intensive investigation.

**Distribution of forces in migrating collectives**

The first attempts to measure forces in collectively migrating Madin-Darby canine kidney (MDCK) cells on micropillars showed that tractions are mainly localized at the cell front and perpendicular to the monolayer edge, with an average force of 5 nN. This demonstrated that migration is due to the pulling of the leader cells onto the substrate and not to the pushing of follower cells (du Roure et al., 2005). The strongest tractions are applied at the leading edge, but, at least in epithelial cell monolayers, traction forces are generated up to several hundreds of micrometers inside the monolayer (Trepat et al., 2009; Tambe et al., 2011; Serra-Picamal et al., 2012; Figure 4B). However, recent measurements obtained with a new silicone wrinkling, temperature-sensitive substrate show that tractions are limited to the first row of MDCK leader cells (Yokoyama et al., 2016). In general, cells far from the leading edge exert smaller forces than leaders, but tractions in the monolayer are heterogeneous and change continuously over time, with small hot spots and fluctuations (Serra-Picamal et al., 2012). Although leader cells give biochemical and mechanical cues to followers, cells inside the monolayer can slow down, move in different directions (sometimes even opposite to the direction of the group), or form swirls (Petitjean et al., 2010; Vedula et al., 2012; Reffay et al., 2014). Thus, the distribution of forces across the monolayer is dynamic and fluctuating, with variations in both the adhesion to the substrate and contractility (Ng et al., 2014). These local variations are likely to induce cells to polarize and exert tractions in a direction that is not necessarily the same as that of the global movement during the entire duration of migration. Thus tractions must be regulated by velocity but also by other local parameters, such as cell polarity (Notbohm et al., 2016).

Owing to unequal distribution of tractions between the leaders and the followers, the cell sheet is under global tensile stress, and forces are transmitted at large scales (Trepat et al., 2009; Tambe et al., 2011; Serra-Picamal et al., 2012; Figure 4A). The leaders and the rest of the cells in the monolayer play a tug-of-war: the monolayer...
manages to migrate forward because the leader cells are stronger. However, leaders exert forces only up to ~100 nN, which is not strong enough to pull the whole monolayer. They might nevertheless succeed because of a mechanical “X-wave” that propagates from the front to the back of the monolayer, communicating information on mechanics and polarity to the followers. This wave is continuously repeated so that the flow of information is maintained during the whole migration process (Serra-Picamal et al., 2012). The coordinated behavior of the followers may be simply explained by the fact that single cells in the group align their traction forces with the general velocity of the cell group (Basan et al., 2013). Development of monolayer stress microscopy (MSM) has allowed a better understanding of the distribution of stresses (σ) in the monolayer. Stresses are again heterogeneous, with large areas of tensile (positive) stresses alternating with regions of weak compressive (negative) stresses that vary over time. Stresses are defined by their components, shear stress (σxy and σyx) tangent to the surface and normal stress (σxx and σyy) perpendicular to the surface. In biological terms, these correspond respectively to the tangential and perpendicular forces exerted on cell–cell junctions by neighboring cells. During migration, cells in the monolayer migrate in the direction that maintains the shear stress minimum and the normal stress maximum (Figure 4A). The collective tendency of cells in a monolayer to migrate along the orientation of maximal principal stress is called plithotaxis (Zaritsky et al., 2015). Merlin, a tumor suppressor and regulator of the Hippo pathway, has been proposed to play a role in plithotaxis of epithelial cells through Rac1 modulation (Das et al., 2015). When leader cells start to migrate, Rac1 is activated toward the cell front. Leader cells pull the followers, which in turn release merlin from junctions to promote the polarized activation of Rac1 in the followers.

The transmission of forces within the cell group is mainly mediated by adherens junctions (Tambe et al., 2011; Trepat and Fredberg, 2011). Leader cells induce traction forces on followers and shear stress on neighbors, transforming local forces into coordinated and polarized traction forces, ensuring plithotaxis (Zaritsky et al., 2015). Merlin, a tumor suppressor and regulator of the Hippo pathway, has been proposed to play a role in plithotaxis of epithelial cells through Rac1 modulation (Das et al., 2015). When leader cells start to migrate, Rac1 is activated toward the cell front. Leader cells pull the followers, which in turn release merlin from junctions to promote the polarized activation of Rac1 in the followers.

The transmission of forces within the cell group is mainly mediated by adherens junctions (Tambe et al., 2011; Trepat and Fredberg, 2011). To obtain direct information of the mechanical role of adherens junctions between neighboring cells migrating collectively on ECM, cells can be plated as doublet, the smallest possible group on adhesive micropatterns. Even if this is clearly not an example of collective cell migration per se, it still provides useful information. For example, a pair of endothelial cells sustains forces at cell–cell junctions of ~100–120 nN perpendicular to the cell–cell contact (Liu et al., 2010; Maruthamuthu et al., 2011). However, the different adherens junction proteins can differently affect the physical parameters controlling either the monolayer kinematics or forces. For example, in the case of MCF10A cells, P-cadherin and E-cadherin show different responses to mechanical stress in genetic tweezers experiments. E-cadherin allows the cells to adapt to an extracellular force by activating a mechanotransduction pathway via vinculin, whereas P-cadherin cannot reinforce junctions. However, the two proteins seem to compete for the same mechanotransduction pathway because P-cadherin can rescue the absence of E-cadherin (Bazellières et al., 2015). The specific role of each cadherin in mediating intercellular stresses during collective migration is still unclear. The expression of different levels of cadherins during epithelial-to-mesenchymal transition or tumor invasion might also help the cells migrate and invade collectively through a cadherin-dependent regulation of forces (Friedl and Mayor, 2017). In a carcinoma model, CAFs are able to pull the tumor cells to drive collective invasion. This is due to a heterophilic interaction between the N-cadherin of CAFs and the E-cadherin of the tumor cells, which actively responds to forces and allows polarization of CAFs (Labernadie et al., 2017).

Sensing and adjusting forces between adjacent migrating cells
Adherens junctions are the main cell–cell adhesion structures that mediate tissue mechanical integrity. Adherens junctions are typically composed of cadherins—transmembrane proteins that interact homotypically. Cadherins bind intracellularly to catenins (p120 catenin, α-catenin, and β-catenin) and can activate different signaling pathways to influence the cytoskeleton, differentiation, and the cell cycle (Gumbiner, 2005; Leckband and de Rooij, 2014). Cadherin cytoplasmic partners also associate with actin directly or indirectly via vinculin and zyxin. Thereby, they couple cell–cell interactions to the actin cytoskeleton.

In the Drosophila ovary, border cells migrate as a cohesive and coordinated group through the nurse cells that compress them. Migrating border cells express E-cadherin, which on one hand contributes to their migration—E-cadherin expressed by the immobile surrounding nurse cells being used as a substrate—and on the other hand mediates the communication between the leaders to follower cells of the moving cluster. To resist compression, the migrating border cell cluster activates cycles of myosin II contraction to promote cortical tension (Aranjuez et al., 2016). Moreover, the common direction of migration is controlled through E-cadherin and Rac. E-cadherin is under higher tension at the front of the border cells, where it activates Rac to increase E-cadherin tension (Cai et al., 2014).

Information on the forces exerted at the level of cell–cell junctions can be obtained from assays easier to interpret than whole migrating monolayers. As for TFM or micropillar experiments, where cells are plated on ECM substrates, cells can also be plated onto cadherin patterns. These experiments demonstrated that cell spreading and force transmission on N-cadherin–coated substrate is stiffness dependent. Cadherin adhesions are larger and stronger on stiff gels, whereas cells have smaller adhesions and a disorganized actin network on softer gels (Ladoux et al., 2010). Moreover, increasing forces on adherens junctions leads to a force-dependent reinforcement of their structure and of the associated actomyosin system (Lambert et al., 2007; le Duc et al., 2010). These observations imply that cadherin-mediated adhesions possess a mechanosensor and serve as a major site of mechanotransduction.

Cadherins form nanoclusters that associate with the actin cytoskeleton to mediate mechanotransduction (Changede and Sheetz, 2016; Cosgrove et al., 2016). The understanding of the role of cadherins in mechanical intercellular coupling has progressed significantly since the α-catenin/vinculin modulus has been involved in mechanotransduction. α-Catenin is a 102-kDa protein that possesses roles in different signaling pathways involved in proliferation and size, such as YAP, MAPK (mitogen-activated protein kinase), and Wnt (Figure 2A). It is also a key molecule in adherens junctions, where it is recruited through its association with β-catenin on one side and can bind actin filaments and vinculin on the other side to reinforce cell–cell junctions (Figure 2). Loss of α-catenin results in alterations of adherens junctions and loss of its connection to the actin cytoskeleton (Hiirano et al., 1992; Vasioukhin et al., 2000). α-Catenin contains three main domains called vinculin homology domains (VH1, VH2, and VH3), which, as their names suggest, possess high homology with vinculin domains (27, 31, and 34%, respectively). VH1 is important for both β-catenin binding and α-catenin homodimerization. The VH2 domain contains binding sites for many of its partners, including vinculin and the actin-binding proteins α-actinin and formin-1. Part of the VH2 domain contains an adhesion modulation domain (M) of four α-helix bundles. The VH3 C-terminal domain binds actin (Kobielak and Fuchs, 2004). α-Catenin can be found in an autoinhibited conformation, where M1 and M2-3
domains interact. In a key study, Yonemura et al. (2010) showed that α-catenin is a mechanosensor. Stretching forces induce a change in α-catenin conformation that un masks the vinculin-binding site. Disruption of the intramolecular inhibitory interaction requires only ~5 pN and leads to an open catenin conformation (Yao et al., 2014). The interaction of α-catenin with vinculin reinforces the junction in a force-dependent manner by promoting actin recruitment (Yonemura et al., 2010). Vinculin binding stabilizes catenin in an intermediate conformation, which allows its activity as a mechanotransducer without excessively opening it. Forces >30 pN induce vinculin dissociation and junction disassembly (Ishiyama et al., 2013; Yao et al., 2014; Maki et al., 2016). A recent study described the nanoscale architecture of cadherin-based cell–cell junctions (Bertocci et al., 2016). Similar to the structure of focal adhesions (Kanchanawong et al., 2010), cell–cell junctions are divided into compartments. The cytoplasmic tails of cadherins bound by catenins are separated from the actin and actin-regulatory protein compartment by vinculin, which bridges the two compartments and separates them by ~30 nm. In this model, the conformation and position of vinculin depend on α-catenin and tension. Vinculin opening can also be induced by Abl kinase–mediated phosphorylation of Tyr-822, which can be dephosphorylated by protein tyrosine phosphatase 1B. Once open, vinculin recruits proteins such as VASP, probably promoting further actin polymerization and a feedback loop (Bertocci et al., 2016; Figure 2).

Ultimately, α-catenin and vinculin cooperate to link cadherins and actin and allow a proper force response and junction reinforcement over time (Le Duc et al., 2010; Borghi et al., 2012; Thomas et al., 2013; Figure 2). Defects in the connection to actin impair cell coordination and increase migration (Staale et al., 2015). This is also demonstrated by the fact that endothelial cells expressing a mutant α-catenin (ΔVBS) that cannot recruit vinculin show defects in junction reinforcement and mechanosensing (Twiss et al., 2012). The importance of the α-catenin–vinculin modulus in mechanotransduction has also been confirmed in vivo in Drosophila (Desai et al., 2013; Jurado et al., 2016) and zebrafish (Han et al., 2016). Although most reports focused on the α-catenin and vinculin modules in mechanotransduction, recent work in endothelial cells suggests a possible role for other proteins, such as zyxin, VASP, and testin, in the mechanical responses of adherens junctions (Oldenburg et al., 2015). In addition to actin, microtubules are probably involved in the strengthening of cadherin adhesions (Plestant et al., 2014) and could therefore influence mechanotransduction in adherens junctions. Moreover, the interaction of keratin intermediate filaments with desmosomal cadherin is also involved in mechanotransduction at cell–cell contacts (Weber et al., 2012). Finally, an elegant study in nontumorigenic breast epithelial cells (MCF10A) showed that many proteins involved in cell–cell junctions (not limited to adherens junctions) are important for force transmission (Bazellière et al., 2015). In the case of hepatocyte growth factor–stimulated MDCK cells plated on N-cadherin substrates, depletion of the cytoplasmic domain does not completely abolish tractions (Lee et al., 2016), suggesting that alternative mechanisms may also contribute.

### Mechanical cross-talk between focal adhesions and cell–cell junctions

Focal adhesions and adherens junctions share similar structures and connection to the cytoskeleton, as well as similar mechanosensing mechanisms and mechanotransduction pathways (Han and de Rooij, 2016; Mui et al., 2016). It is thus tempting to speculate that these two major adhesive structures influence each other. Mui et al. (2016) addressed the most recent findings on adhesion cross-talk from the mechanical point of view. Several studies suggest that increasing forces in one compartment decreases them in the other; in other words, strong adhesion to the ECM decreases the strength of cell–cell junctions and vice versa (Guo et al., 2006; Wang et al., 2006). However, the relationship between the two structures is clearly more complex.

During development, mesenchymal cells have to adapt from a cell–cell adhesion-based system to one that relies more on cell–substrate interactions. A method has recently been developed to decouple the presentation of RGD (fibronectin) from that of HAVD1 (N-cadherin) ligand peptides at different stiffnesses and assess mesenchymal stem cell mechanosensing (Cosgrove et al., 2016). On keeping RGD constant and presenting HAVD1, the cells read ECM stiffness as softer than it actually is. This is coupled to inhibition of Rac1, which reduces cell contractile forces and YAP nuclear localization and leads to errors in proliferation and differentiation (Cosgrove et al., 2016). A recent study showed that E-cadherin mediates force transmission by downstream activation of PI3K (phosphoinositide 3-kinase) in an epidermal growth factor receptor–dependent manner in epithelial cells, leading to integrin activation, probably by inside-out signaling, which in turn induces cell stiffening through ROCK and myosin II (Muhamed et al., 2016). The direct role of the cytoskeleton in the mechanical coupling between adherens junctions and focal adhesions is not entirely clear, and more complex biochemical signaling pathways are likely to be involved. In migrating astrocytes, which mainly express N-cadherin, loss of N-cadherin or alteration of its dynamics results in the faster and less-directed migration of the leader cells, which detach from their followers (Camband et al., 2012). Cadherin-mediated adherens junctions are necessary to regulate the lamellipodia activity, cell polarization, and the direction of migration (Borghi et al., 2010; Dupin et al., 2011). They control the position of focal adhesions and the recruitment of the β1-PIX/Cdc42/Par6/aPKC pathway proteins that promote cell polarity and persistent migration (Dupin et al., 2009; Camband et al., 2012). In C2C12 myoblasts, expression of P-cadherin, but not other cadherins, induces efficient collective cell migration and polarization. In this system, activation of Cdc42 by the guanine nucleotide exchange factor β1-PIX, recruited by P-cadherin, controls polarity and cadherin-dependent forces, leading to increased traction and intracellular stresses in the monolayer (Plutoni et al., 2016). The maintenance of adherens junctions between actively migrating cells is crucial for the collective behavior.

In astrocytes, as well as in endothelial cells or fibroblasts, adherens junctions located on lateral contacts dynamically flow backward during collective migration (Peglion et al., 2014). This ensures that cells keep stable yet malleable interactions as they migrate through a complex environment. Given that lateral adherens junctions link the actin transverse arcs of adjacent cells, they also likely contribute to the coordination of the actin retrograde flow between cells migrating next to each other (Etienne-Manneville, 2014). It will be interesting to test whether the retrograde flow of adherens junctions is involved in the transmission of forces through the monolayer.

The cross-talk between focal adhesions and adherens junctions is bidirectional. The ECM also affects the localization and the forces exerted on cell–cell junctions. The physical proximity between ECM and junctions results in higher intercellular and intracellular forces, which control the position of the junctions (Tseng et al., 2012), supporting the fact that integrins and ECM regulate cell–cell adhesions (Marsden and DeSimone, 2003; De Rooij et al., 2005). This phenomenon may explain how durotaxis can be acquired during the collective migration of cells that do not normally durotact (Sunyer et al., 2016). Leader cells sense substrate rigidity and communicate mechanical information to their followers through actomyosin contractility. The efficiency of this mechanical signal decays over large distances in a stiffness-dependent manner (Ng et al., 2012). Changes in substrate rigidity modulate...
forces at the level of cell–cell junctions, demonstrating that tension at focal adhesions correlates with tension at junctions (Maruthamuthu et al., 2011). During convergent extension movements—for instance, during Xenopus development—β1 integrin modulates cell–cell adhesion. Blocking fibronectin or β1 integrins alters cadherin-mediated adhesion, aggregation, cell intercalation, and axial extension during gastrulation (Marsden and DeSimone, 2003).

CONCLUSIONS

Many open questions remain to be answered in the expanding field of mechanobiology and migration. First, reports have concentrated on proteins that act as mechanosensors by stretching and allowing the binding of other binding partners. It will be crucial to understand whether stretching of proteins can also induce enzymatic activity in addition to the unmasking of protein-binding sites. Stretching might not be limited to protein structure but could also be related to the deformation of a membrane (e.g., pulling forces, curved membranes, or protrusions), which is especially crucial during migration. A recent study describes cadherin fingers—polarized VE-cadherin–rich protrusions between leaders and followers—during endothelial migration (Hayer et al., 2016). These fingers are formed by convex curved membranes that recruit curvature-sensing proteins that might induce specific signaling pathways. The primary candidates are BAR proteins, which contain domains for reading membrane curvatures and are likely to be essential players in mechanosensing (McMahen and Boucrot, 2015). Much work still needs to be done to better understand the molecular mechanisms involved in mechanotransduction and the coupling between mechanotransduction sites.

More attention will have to be devoted to understanding how the cytoskeleton—not only actin, but also microtubules, intermediate filaments, and septins—regulates mechanotransduction in migration. It is crucial to decipher which GTPases affect the cytoskeleton during mechanosensing. How exactly does a cell integrate information from both junctions and focal adhesions? Mechanically it is the same type of signal (a force), but biochemically, different signaling pathways are involved. From a biological point of view, adherens junctions and focal adhesions share similar molecular organization and common mechanosensing mechanisms but differ in their downstream signaling (Han and de Rooij, 2016). Key questions are whether and when one type of adhesion site predominates over the other, although it is probable that the two systems form a feedback loop. Finally, many different physical parameters other than substrate rigidity can affect migration. These include substrate topography, porosity, elasticity, and other physical constraints (Nelson and Tien, 2006; Liu et al., 2015b). There are influences of both nanoscale and microscale cues, although the nanoscale geometric cues tend to dominate (Nam et al., 2016). How these physical properties are sensed by cells and affect mechanotransduction to control cell migration needs to be further investigated. More generally, we do not understand how cells process the multiple physical inputs in a robust and coherent manner, clearly pointing to the need for a systems-level investigation of mechanobiology.

The majority of reports have given information on in vitro conditions. With the advent of new technologies and intravital imaging, studies will focus on the in vivo situation during morphogenesis and pathological conditions. Some studies have already started to describe what happens in vivo during migration (Koser et al., 2016) or fibrosis (Kai et al., 2016). A key question to answer is whether mechanobiological signals and cues are druggable. For example, during fibrosis or tumor growth and invasion, modifying the mechanobiological properties of the cell or its surrounding could be a valid treatment option that has not yet been approached.

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