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Conserved F-actin dynamics and force transmission at cell adhesions

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Adhesions are a central mechanism by which cells mechanically interact with the surrounding extracellular matrix (ECM) and neighboring cells. In both cell–ECM and cell–cell adhesions, forces generated within the actin cytoskeleton are transmitted to the surrounding environment and are essential for numerous morphogenic processes. Despite differences in many molecular components that regulate cell–cell and cell–ECM adhesions, the roles of F-actin dynamics and mechanical forces in adhesion regulation are surprisingly similar. Moreover, force transmission at adhesions occurs concomitantly with dynamic F-actin; proteins comprising the adhesion of F-actin to the plasma membrane must accommodate this movement while still facilitating force transmission. Thus, despite different molecular architectures, integrin and cadherin-mediated adhesions operate with common biophysical characteristics to transmit and respond to mechanical forces in multicellular tissue.

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Introduction

Cellular adhesion to the surrounding extracellular environment is essential to numerous aspects of cell and tissue physiology. The dynamic regulation of adhesions to extracellular matrix (ECM) is crucial to cell proliferation, differentiation and migration [1,2] while adhesions formed between neighboring cells mediate sorting, rearrangement and polarization within multicellular ensembles [3]. The coordination of cell–ECM and cell–cell adhesions is essential for the formation, regulation and maintenance of tissues. These morphological and physical processes all require precise spatiotemporal regulation of force transmission at adhesions that can rapidly adapt and respond to internal or external physical and biochemical stimuli.

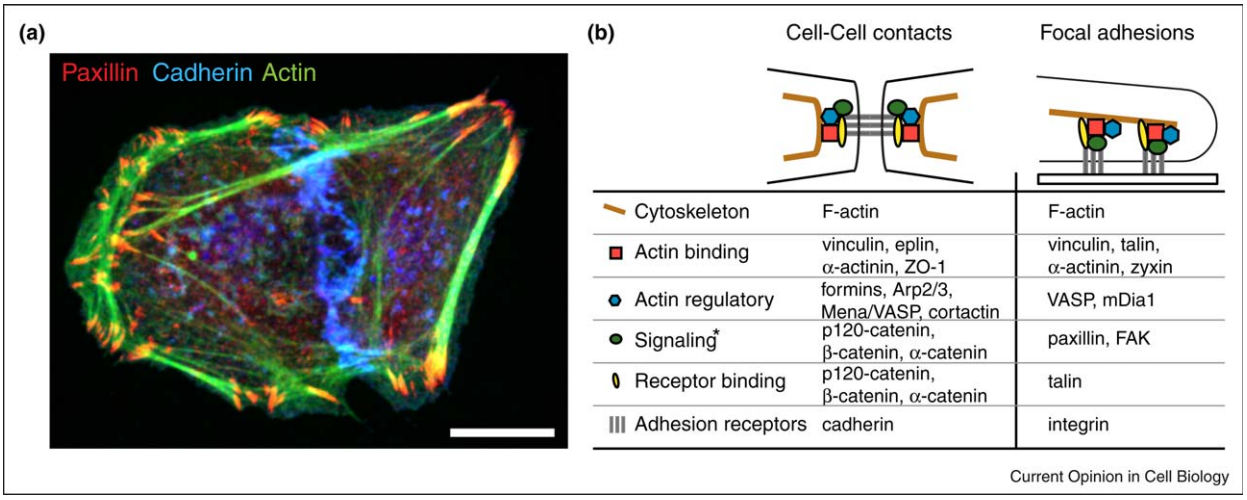
Adhesions are not simply sites of passive mechanical attachment; rather, forces generated within the F-actin cytoskeleton generate active tension that is applied to cellular adhesions. Both the F-actin cytoskeleton and proteins comprising adhesions are highly dynamic, providing the capability to build, maintain and release tension at adhesion sites over physiological time scales. Thus, this dynamic and responsive force transmission is essential for cellular and tissue physiology, but the underlying biophysical mechanisms remain unclear. While significant differences in the molecular composition of cell–cell and cell–ECM adhesions exist, it has recently become evident that these two types of adhesions share remarkable similarities in the nature of mechano-responsiveness and local cytoskeletal dynamics. Here, we review current understanding of the role of F-actin dynamics and forces in the regulation of integrin-mediated cell–ECM adhesion and cadherin-mediated cell–cell adhesion. We also discuss current data and models for the mechanisms of force transmission through a dynamic cytoskeleton at adhesion sites.

Forces at cell–ECM and cell–cell contacts

The primary sites of force transmission between the cell and the extracellular matrix occur at integrin-mediated adhesions (Figure 1a). Such cellular traction forces can be visualized by adhering cells to compliant, calibrated substrates and visualizing the deformations induced by the cell's substrate-contacting, or basal, surface [4,5]. Cellular traction forces are primarily concentrated at peripheral focal adhesions, directed towards the cell center and are as large as several nano-Newtons [4,5]. In quiescent cells, there is a direct correlation between focal adhesion size and traction force magnitude [4–6], and a feedback between adhesion size and either myosin-II driven or externally applied force exists [7•]. Indeed, application of force leads to enhanced stiffening and force transmission at focal adhesion sites and is required for stabilization of new adhesive contacts [8•,9•].

Similar to focal adhesions, classic cadherin-based adhesions act as force-sensitive and force-bearing mechanical links to maintain cell–cell contact [10•] (Figure 1a). However, it has been difficult to measure the forces sustained at bonafide cell–cell contacts due to the relative inaccessibility of the interface [11]. Laser ablation of cytoskeletal components at cell–cell contacts provides an estimate of the relative magnitude of force sustained [12•]; forces at cadherin-mediated adhesions appear to be tensile and directed either parallel or normal to the

Figure 1



Molecular composition of cadherin-based cell–cell contacts and focal adhesions. **(a)** Immunofluorescence image of two adjoining MDCK cells plated on collagen I, with F-actin stained by phalloidin (green), focal adhesions marked by paxillin (red), and cell–cell contacts stained by E-cadherin (blue). Scale bar is 20 μm. **(b)** Schematic representations of cell–cell contacts and focal adhesions. Classes of F-actin binding (red), F-actin regulatory (blue), signaling (green), and receptor binding (yellow) proteins are indicated, with respective molecular components listed in the table. *For cell–cell contacts, catenins function to recruit signaling proteins. For a more complete table of protein constituents, please see Refs [8•,9•,63].

plane of cell–cell contact [13]. Tensile forces transmitted at cell–cell contacts determine cellular arrangements within a monolayer [12•,14–16] and direct collective migration of epithelial cells [17]. Quantitative measurements of forces transmitted at cadherin-based adhesions have been made with cells adhered to compliant, cadherin-coated substrates wherein cadherin-mediated adhesions form on the cell's basal surface, akin to focal adhesions in 2D culture. Interestingly, the organization, direction and magnitude of traction forces exerted by N-cadherin-mediated adhesions are strikingly similar to those transmitted at focal adhesions [18,19•]. Moreover, similar to focal adhesions, the assembly and stabilization of cadherin-mediated adhesions is force dependent [19•,20,21]. Thus, both cadherin and integrin-based adhesions are mechanosensitive assemblies that transmit significant mechanical cues between a cell and its external environment [10•].

Physical link between F-actin and adhesion receptors

The assembly of integrin-mediated adhesions occurs concomitantly with force-dependent compositional changes and post-translational modifications in a process termed maturation [8•,9•]. These changes are thought to both enhance mechanical coupling between the F-actin and extracellular matrix and regulate the cycle of adhesion assembly/disassembly (Figure 1b). Under low tension, labile connections between F-actin and transmembrane integrin are formed by talin [22]. In turn, talin binding induces conformational changes in integrin to enhance binding to the ECM [23,24•]. Force applied to this linkage results in clustering and activation of more integrins [25•] and recruitment and phosphorylation of focal adhesion kinase (FAK) [26], which initiates integrin-mediated signaling and phosphorylation of other focal adhesion proteins, including paxillin and p130cas [27]. Subsequent recruitment of vinculin likely reinforces the mechanical linkage between F-actin and transmembrane integrin [9•]. These signaling and compositional changes are associated with focal adhesion growth from a sub-micron cluster into an elongated plaque and are accompanied by the recruitment of α-actinin and zyxin, promoting further association with F-actin [9•]. Thus, in cell–ECM adhesions, a hierarchical assembly of structural and signaling proteins regulate mechanical attachment between the F-actin and ECM.

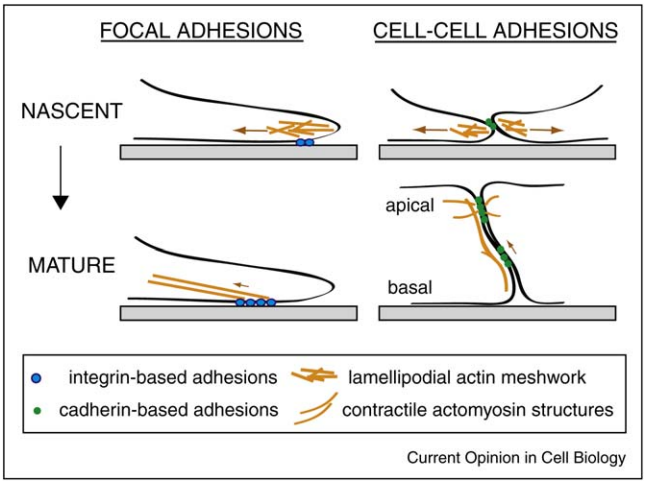
The structural links that associate F-actin to transmembrane cadherins is less clear, although not due to lack of candidates (Figure 1b). While the proximal region of the cytoplasmic domain of cadherin binds to p120-catenin, the distal region binds to β-catenin or plakoglobin, which in turn binds to α-catenin. The linkage between α-catenin and F-actin can be mediated by numerous proteins [28•,29] such as: vinculin, formin [30], α-actinin, eplin [31], afadin [28•,29] and ZO-1. Vinculin may also directly bind to β-catenin [32], but may remain autoinhibited [33]. Even though multiple links may co-exist, some of the linkers may not simultaneously function due to steric or allosteric effects. Delineating which of these different putative links play functional roles in different cells and physiological contexts will be required to better

understand the mechanical regulation of cadherin adhesions by F-actin.

Regulation of adhesions by F-actin dynamics

Throughout their lifecycle, focal adhesions are associated with a dynamic actin cytoskeleton. The assembly of focal adhesions occurs within a branched F-actin meshwork near the cell periphery, termed the lamellipodium, which undergoes a rapid retrograde flow, at approximately 25 nm/s, driven by F-actin polymerization against the cell membrane [34•] (Figure 2). Here, focal adhesion clusters form and flow retrograde with F-actin to the lamellipodial base, 1–3 μm proximally from the cell edge, where they immobilize and become small, sub-micron-sized punctae termed nascent adhesions [34•,35–37]. Nascent adhesions are associated with low traction (~150 pN) exerted on the ECM and F-actin flow on the order 15–25 nm/s; here, the F-actin dynamics and traction originate from F-actin polymerization-generated forces [38•]. Myosin-II-mediated tension applied to F-actin at the lamellipodia base promotes force-dependent focal adhesion maturation [8•,9•]. Mature focal adhesions are associated with myosin-II rich networks or bundles and transmit large traction forces (1–5 nN). Here, myosin-II-mediated retrograde actin flow persists at focal adhesions but is generally on the order of 5–10 nm/s [38•,39•] (Figure 2). VASP, a regulator of F-actin polymerization dynamics, localizes to focal adhesions [27] and can undergo retrograde motion similar to that of actin [40].

Figure 2



F-actin dynamics in nascent and mature adhesions. (top, nascent) In both focal adhesions and cell–cell adhesions, adhesion assembly occurs near the cell periphery within the lamellipodium, a zone of rapid, polymerization-driven F-actin retrograde flow (large brown arrow). (bottom, mature) After tension-dependent stabilization and maturation, adhesions are associated with myosin-II-driven F-actin networks and bundles undergoing slow motion (small brown arrow). In focal adhesions, this movement is retrograde; in cell–cell adhesions, F-actin typically moves from the basal to apical planes.

Formin-dependent polymerization of F-actin plays a crucial role in myosin-dependent stress fiber elongation and force-dependent focal adhesion growth [4,41].

Similar to focal adhesions, cadherin-based adhesions also form at lamellipodia or at filopodia, where cells initiate contact [42–44] (Figure 2). E-cadherin adhesions initiate as sub-micron sized puncta, but grow into elongated plaques [42], dependent on local and global actin motion [45]. In well-developed contacts, there is no F-actin retrograde flow perpendicular to cell–cell contacts [46]. However, there is considerable F-actin motion within the plane of the cell–cell contact [47•], wherein myosin-II dependent basal to apical movement of cadherin clusters and associated actin is observed at a rate of 5 nm/s (Figure 2). Two distinct populations of actin are associated with E-cadherin clusters: a stable pool that is localized with the clusters and a contractile, dynamic pool that controls the position of the clusters [48,49•]. Several actin binding and regulatory proteins such as myosin VI, Arp 2/3, ena/VASP and cortactin are necessary for proper junction formation [43,44,50]. Disparate dynamics of the cadherin–catenin complex and actin at cell–cell contacts also suggests dynamic coupling between F-actin and cadherin [33]. The overall architecture of F-actin at sites of cell–cell contact, however, depends on the cell type and may even vary between different epithelial cell lines [51]. Cells plated on N-cadherin-coated coverslips form cadherin-mediated adhesions at the cell periphery near the lamellipodia. After appearance, cadherin-mediate adhesions elongate in a myosin-dependent manner and are associated with dynamic actin [52]. Thus, several similar features of F-actin dynamics regulate the assembly and growth of both cadherin and integrin-based adhesions, in spite of differences in structural links.

How can a dynamic cytoskeleton sustain mechanical load?

Forces generated by myosin-II motors and F-actin polymerization drive coherent movements of the actin cytoskeleton. To reconcile how adhesions harness such actin dynamics to mediate force transmission to the extracellular environment, it has long been hypothesized that adhesions function as a ''molecular clutch'' between the F-actin cytoskeleton and extracellular ligands [53]. In this model, retrograde F-actin flow is treated as an 'engine' running at a certain speed with a certain stall force. When an adhesion is assembled to engage the F-actin to extracellular ligands, resistive forces from the extracellular matrix stall F-actin movement. In models of cell migration, this 'stalled' retrograde motion, corresponding to a high tension state, would then enable de novo assembly of F-actin at the cell front to result in efficient cell protrusion. Indeed, observed inverse correlations between protrusion rate and F-actin retrograde flow in fast moving cells support this model [54,55].

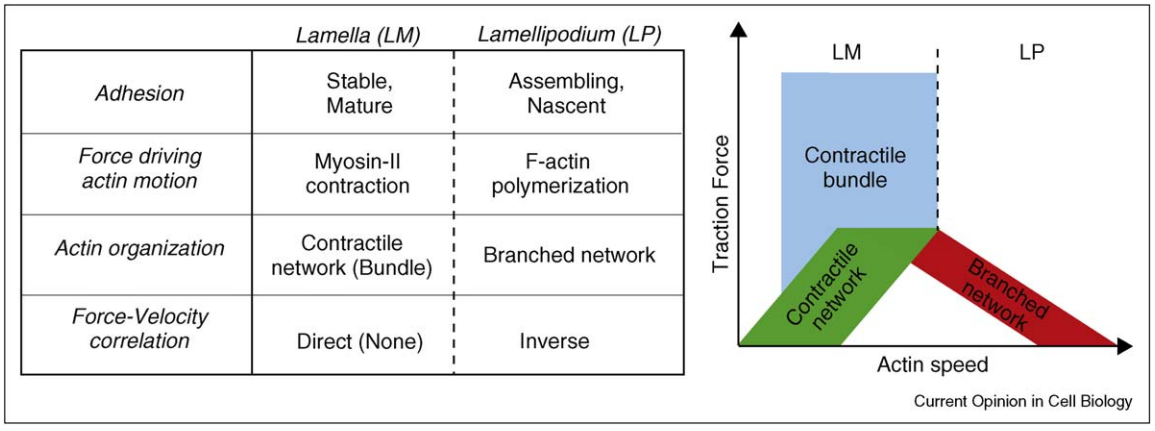
The most natural way to conceptualize a molecular clutch would be for it to be a binary switch, either ''on/engaged'' or ''off/disengaged''. However, recent data have shown that this simplistic picture does not accommodate the rich interplay between cytoskeletal dynamics and traction forces at adhesion sites. For instance, during focal adhesion assembly in epithelial and fibroblast cells, increased traction stress occurs concomitantly with decreased F-actin retrograde flow speed [37,38•] (Figure 3); thus, a continuous transition between an ''off'' and an ''on'' state exists. Furthermore, in focal adhesions that exert high tension on the ECM, retrograde flow of F-actin persists. In other words, a fully engaged clutch must accommodate F-actin motion while still transmitting tension. Similar dynamic links between N-cadherin-mediated adhesions to moving F-actin are also likely [56•].

One possibility is that transient connections between proteins within adhesions could foster a dynamic molecular clutch [57•]. For example, vinculin, α-actinin, zyxin, VASP and talin undergo retrograde flux correlated to the actin motion at large focal adhesions [39,40,58•]. On the other hand, integrin, FAK and paxillin are predominantly stationary with respect to the ECM over similar time scales. Force transmission through such a dynamic interface can be modeled by considering a population of dynamic bonds formed between a moving and stationary interface with individual bonds undergoing cycles of attachment and force-assisted detachment. Thus, the dynamics of bond association/dissociation facilitate F-actin motion and force transmission simultaneously [57•,59–61]. These models are consistent with the observed relationships between F-actin flow speed and traction force [60,61]; at high retrograde flow rates, force transmission is limited by bond breakage whereas at low rates, the magnitude of displacement or force within the actin cytoskeleton is limiting. Furthermore, these models have elucidated how such a dynamic clutch could facilitate adhesion assembly [59] and mechanosensing [57•]. This mechanism allows for both the build-up of tension at locations of rapid F-actin flow to promote adhesion assembly and reduce tension at sites of low F-actin flow to promote adhesion disassembly.

The role of F-actin dynamics in regulating force transmission in mature focal adhesions is less well understood. In some cell types, where adhesions are associated with contractile actomyosin networks, traction forces diminish as the F-actin flow speed decreases below a critical threshold [38•,57•,62] (Figure 3). This direct correlation is consistent with the picture that retrograde movement of F-actin is a manifestation of myosin forces and diminished rates of F-actin movement is an indicator of reduced myosin-II force. Alternatively, in cells that form organized stress fibers, forces exerted at focal adhesions can be modulated significantly without changes in retrograde flow speed (Figure 3). Thus, at large adhesions, local organization of F-actin may dominate over F-actin dynamics in determining the magnitude of force transmitted. While F-actin dynamics and organization are likely to play similar roles in force transmission at cadherin-based adhesions, their roles are much less clear.

In conclusion, there exists a strong interdependence between F-actin dynamics, adhesion assembly and force transmission occurring at both cell–ECM and cell–cell adhesions despite dramatic differences in the molecular

Figure 3



The correlation between F-actin and traction force during adhesion assembly in the lamellipodium (LP) and in stable adhesions found in the lamella (LM). In the lamellipodium, F-actin polymerization drives a rapid retrograde flow. During adhesion assembly in the lamellipodium, there is an inverse correlation between F-actin retrograde flow and traction force (red region). By contrast large, stable adhesions in the lamella are associated with a slower, myosin-II dependent F-actin retrograde flow. In cells where adhesions are associated with contractile networks, a direct relationship between F-actin flow speed and traction force is observed (green region). By contrast, in cells where adhesions are associated with contractile actomyosin bundles, there is no strong correlation between traction force magnitude and F-actin flow speed (blue region).

components that link F-actin to extracellular ligands in these two different types of cell adhesions. This suggests that the origins of cellular mechano-responsiveness may be dominated by generic physical features of the actin cytoskeleton coupled to a dynamic clutch rather than specific molecular components of adhesions. Moreover, the generality of these behaviors to two very different types of cell adhesions suggests that there may be common underlying physical principles relating adhesion assembly, actin dynamics and force transmission. Elucidating such general physical principles will enable predictive understanding of the nature of adaptive force transduction in the cytoskeleton and its transmission to the external environment that facilitates complex processes such as cell migration and multicellular organization.

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