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Stressing the limits of focal adhesion mechanosensitivity

Patrick W Oakes and Margaret L Gardel

Focal adhesion assembly and maturation often occurs concomitantly with changes in force generated within the cytoskeleton or extracellular matrix. To coordinate focal adhesion dynamics with force, it has been suggested that focal adhesion dynamics are mechanosensitive. This review discusses current understanding of the regulation of focal adhesion assembly and force transmission, and the limits to which we can consider focal adhesion plaques as mechanosensitive entities.

Addresses
James Franck Institute, Institute for Biophysical Dynamics and Physics Department, University of Chicago, Chicago, IL, United States

Corresponding author: Gardel, Margaret L (gardel@uchicago.edu)

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Introduction

Cells utilize focal adhesions to facilitate and regulate mechanical coupling with the extracellular matrix (ECM) in physiological processes such as migration, proliferation and differentiation [1–3]. Focal adhesions are complex organelles which span the actin cytoskeleton and the ECM, and are comprised of more than 150 different proteins [1–4]. They assemble with a stereotypical sequence of protein recruitment [5,6] and exhibit a distinct nanoscale architecture [7•]. Situated at the interface between the cytoskeleton and ECM, adhesions are superbly positioned to act in a variety of signaling pathways [1,3,8].

In their most prominent role, focal adhesions function as sites of force transmission between stresses generated within the cytoskeleton and the ECM [9,10]. In complex shape changes during cell migration, adhesion dynamics are coordinated with and guide tension redistribution across the cell [11]. To coordinate focal adhesion assembly with mechanical stimuli, the concept of adhesion mechanosensitivity was introduced almost 15 years ago [12•,13••]. Mechanosensitivity implicates force-dependent processes within the adhesion plaque in overall growth and compositional maturation. Experimental evidence for this concept was supported by findings that myosin II activity and ECM stiffness impact focal adhesion size and maturation [14•,15], as well as the observed growth of focal adhesion plaques in response to external forces [12•,13••]. Subsequent studies have sought to identify the molecular bases of force-dependent processes within focal adhesions [16,17•]. This transformative concept was a major intellectual advance for the integration of mechanics with cytoskeletal processes.

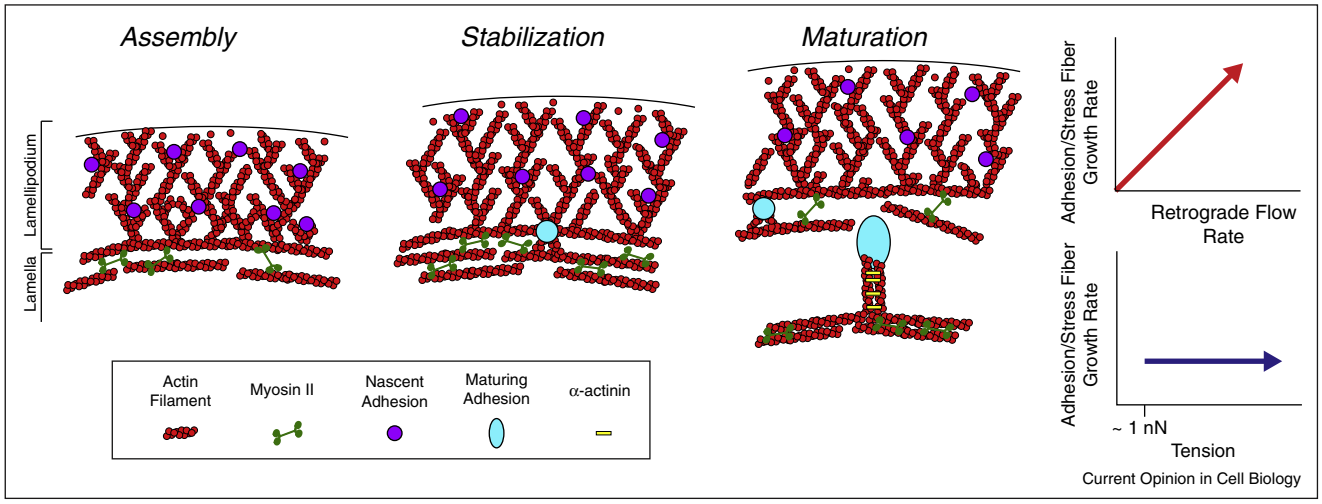
Since the introduction of focal adhesion mechanosensitivity, great progress has been made on the understanding of adhesion assembly and in the development of tools to make mechanical measurements of adhesion strength and cellular traction stresses [18–20]. The studies have also demonstrated the limitations to which we can consider focal adhesions to be mechanosensitive. Here we summarize the current understanding of focal adhesion assembly and force transmission.

Adhesion assembly and maturation is regulated by distinct actin organelles

The initial stages of adhesion assembly typically occur in actin-rich regions at the cell periphery within lamellipodia (Figure 1) [21••] or filopodia [22,23]. Polymerization-driven retrograde flow drives rearward movement of activated integrins from the tip of the lamellipodium [24•]. Clusters of integrins are thought to be potentially brought together through directed actin polymerization [24•] or local high densities of F-actin [21••] and require the addition of proteins such as talin [6,25] and α-actinin [21••,26] to form adhesions. Such 'nascent' adhesions are myosin II-independent and thus are only exposed to small forces arising from membrane tension and actin polymerization (Figure 2) [27].

The engagement of the adhesion to the ECM occurs in the lamellipodium within a few micrometers of the cell periphery [21••] (Figure 1) and coincides with the recruitment of additional focal adhesion proteins such as vinculin, phosphorylated paxillin and FAK [28]. Adhesion stabilization requires both a sufficiently high ECM density [29•] and rigidity [30], and occurs at a constant force [31]. Bands of nascent adhesions exist in the lamellipodium but only a small fraction persists within the lamella, with the majority rapidly disassembling at the

Figure 1

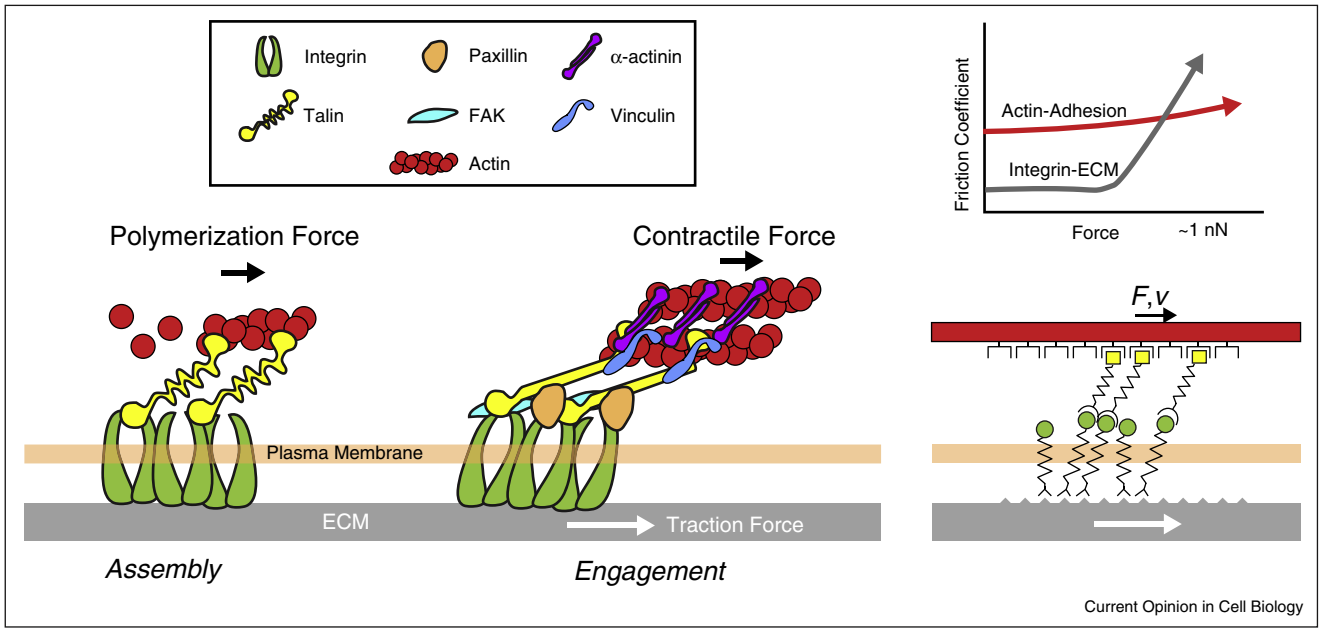


Schematic of the stages of adhesion assembly from (left) nascent adhesion formation within the lamellipodium (middle) adhesion stabilization at the lamellipodium/lamella interface and (right) adhesion maturation associated with stress fiber assembly at the adhesion plaque. Focal adhesion growth rate is identical to stress fiber growth rate and both are correlated to actin retrograde flow. Above a critical tension threshold of ~1 nN, adhesion growth rate is independent of changes to intracellular tension.

interface between the lamellipodium and lamella [21••]. Feedback from nascent adhesion assembly can promote Rac1 activation and additional lamellipodial protrusion through focal adhesion proteins such as β-pix [32].

Within the lamella, myosin II activity drives focal adhesion growth and recruitment of focal adhesion proteins such as zyxin, pY31 Paxillin and pY397 FAK [28,33] in a process termed 'maturation' [1] (Figures 1 and 2). As adhesions

Figure 2



Schematic of force transmission by focal adhesion plaques both during nascent adhesion assembly (left) and after engagement to the ECM and production of traction force (middle). The complex macromolecular interactions can be modeled as a dynamic friction with labile bonds between multiple interfaces (right). The effective friction coefficients change as a function of force applied to the focal adhesion, with the strength of the integrin–ECM interface increasing dramatically compared to the strength of the actin-focal adhesion interface during adhesion engagement.

grow they elongate centripetally, typically to a length of a few micrometers, while maintaining a constant width of ~1 μm. This growth rate is identical to the rate of myosin II-dependent actin retrograde flow [34•,35••] and occurs simultaneously with the assembly of a radial stress fiber at the adhesion plaque [36••,37]. Interestingly, the rate of lamellar retrograde flow, radial stress fiber assembly and adhesion growth is unaffected even when myosin II activity is partially inhibited such that intracellular tension is reduced by 80% [35••]. Thus, focal adhesion growth rate is strongly correlated to retrograde flow rate (Figure 1), but not force. Similarly, tension is insufficient for focal adhesion maturation when radial stress fiber assembly is impaired [36••]. Thus, myosin II-mediated maturation depends on the structure and dynamics of the lamellar actin cytoskeleton, but is not sensitive to changes to intracellular force.

Focal adhesion lifetimes within the lamella are ~20 min, and contribute to cell shape maintenance and tension sustained on the ECM [11,38]. Mature focal adhesions are precursors to fibrillar adhesions, which are essential for remodeling fibronectin [1,39]. Fibrillar adhesions can exist under a large range of intracellular tensions but fail to form in the absence of stress fibers [36••].

While less studied than the processes occurring during assembly, focal adhesion disassembly is an equally vital component of cell migration and is its own distinct process [40–43]. Though adhesions can suffer mechanical rupture from the substrate [44], it is not believed to be the dominant mechanism of adhesion disassembly [45].

Force transmission at focal adhesions

Focal adhesions do not actively generate forces, but rather serve to regulate force transmission between the cytoskeleton and ECM. Stresses generated within the cytoskeleton drive the rearward movement of actin known as retrograde flow. Engagement of focal adhesions results in a reduction of actin retrograde flow rates around adhesion sites [46–48] and an increase in traction stresses [27] and thereby can be thought of as a 'molecular clutch' [49,50]. The 'clutch engagement' at a focal adhesion involves increases in density and affinity of binding interactions spanning the actin cytoskeleton and the ECM. At a minimum, this stabilization requires the presence of talin, which is thought to act as a slip-bond (i.e. bond lifetime decreases with applied force) between the integrin and F-actin [51]. During adhesion stabilization, integrin–ECM bonds [31] and actin–vinculin [34•] interactions assist with adhesion strengthening. Because of the actin retrograde flow and binding dynamics, force transmission during early adhesion stabilization can be modeled as a dynamic friction [52–54] (Figure 2). The stabilization of focal adhesions involves an increase in the friction coefficient at the interface between the integrin and ECM, as compared to the actin/focal adhesion interface and occurs around tensions of 1 nN [31] (Figure 2). Once stabilized, mature adhesions can transmit a wide range of forces generated in the cytoskeleton [55•].

Superresolution light microscopy has revealed a distinct stratification of layers of proteins within the adhesion [7•], with the most interesting proteins being those which stretch within the adhesion such as talin [7•,56] and vinculin [57]. In the case of talin, stretching exposes additional vinculin binding sites, and thus a mechanism for force-dependent recruitment [17•]. Vinculin stretching has been implicated in adhesion stability, force transmission and adhesion strength [34•,57,58]. While these molecules are often referred to as mechanosensors, the exact mechanisms regulating their stretching within the adhesion remain to be elucidated.

Recent works have also served to establish the rich internal dynamics of proteins within the adhesion. At the nanoscale it has been shown that both β1 and β3 integrins exhibit different diffusive behavior inside and outside of focal adhesions [59••], consistent with findings that tension on integrins within focal adhesions is inhomogenous [60••]. Similarly, transient stretching of individual talin molecules within adhesions has been reported, suggesting repeated binding and unbinding events of single proteins within the adhesion [61]. Stretching of molecules within the focal adhesion could also potentially trigger downstream signaling pathways, such as that observed for p130Cas [16].

Is focal adhesion maturation mechanosensitive?

Historically, the role of myosin II in focal adhesion maturation has been interpreted as evidence of focal adhesion mechanosensitivity. However, myosin II activity also impacts the structure and dynamics of the actin cytoskeleton. Myosin II cross-links F-actin into stress fibers and drives retrograde flow. Recent data has shown that the internal tension required to maintain lamellar retrograde flow and radial stress fiber assembly is quite small [35••]. Over a wide range of intracellular tensions and perturbations, focal adhesion growth rate is correlated to the retrograde flow speed [34•,35••,36••]. Moreover, actin crosslinking is sufficient for adhesion stabilization, growth and compositional changes in the absence of myosin II activity and when intracellular stress is reduced by ~90% [21••]. Finally, in the absence of radial stress fibers, tension is insufficient to drive focal adhesion maturation [36••]. These results have led to the idea that the actin architecture is the important component and that stress fibers serve as templates for focal adhesion growth [36••].

While there exists some evidence for focal adhesion growth under applied external stress [13••], it should be noted that the timescale of growth is consistent with

the time it takes to form new stress fibers [62]. On shorter time scales, during which the cell establishes a mechanical equilibrium, adhesions exhibit no change in length [55•]. Stress fiber assembly at the focal adhesion site could also account for some of the force-dependent recruitment of proteins, such as zyxin and α-actinin [63].

Adhesion size does not regulate local cell traction force

The connection between adhesion size and the local traction stress has been a longstanding question in the field. Various data have indicated strong positive correlations [14•,64], an inverse correlation [65] as well as more complicated relationships [66]. Our own work showed that a correlation between adhesion size and traction stress is limited to the initial period during adhesion growth [55•]. In the absence of growth history, adhesion size is a poor predictor of traction stress [55•]. Moreover, this correlation does not imply causality, as perturbations can facilitate large forces at small adhesions and low forces at large adhesions [36••,55•,62]. Thus, focal adhesion size is not a strong predictor of local force.

When considering the role of adhesions in regulating overall cell traction, previous studies have reported positive correlations with the total number of focal adhesions and total contractile stress generated by cells [67–69]. An increase in the number of adhesions, however, is often accompanied by simultaneous changes in other regulatory parameters such as spread area and cell shape [70,71]. Additional work is needed to isolate these individual regulatory pathways.

Finally, it is also important to distinguish between traction forces and adhesion strength. Traction force is the product of cell-generated forces that are transmitted to the substrate. Adhesion strength is the level of force required to physically disrupt the adhesion plaque from the ECM. All data indicate that adhesion strength is weakly dependent on adhesion size and is at least an order of magnitude larger than the traction forces generated by the cell [19,55•,72].

A broader lesson from these studies is the importance of consistent benchmarks to attain when querying the role of physical perturbations and parameters in cell biological problems. For instance, it is important not to deduce causal relationships from purely correlative measurements (e.g. a correlation between focal adhesion size and force does not imply causation). It is also essential to consider and test alternate interpretations of mechanical perturbations, controlling for concomitant changes when possible (e.g. changes in substrate stiffness may concomitantly impact spread area and geometry). And third, it is helpful to consider the scale, whether local or global, of the interaction when determining the proper quantitative measurements (e.g. force-sensitive processes at the molecular scale may not directly scale to the organelle or cell level).

Implications for environmental sensing and global force regulation

How these new insights into the regulation of focal adhesions impact current models of cell environmental sensing and tension regulation are not yet fully clear. One important implication is that focal adhesion size should not be used as a predictor of the overall tension state of the cell. For instance, the lack of prominent focal adhesions by cells in 3D or on soft matrices may not reflect altered cytoskeletal contractility, but rather altered cytoskeletal organization. Similarly, the number of focal adhesion plaques will not be a strong predictor of the overall level of cell contractility.

The mechanosensitivity of focal adhesions has played a large role in current models of cell sensing of environmental stiffness and external forces. It is important to consider the possibility that the extent of mechanosensitivity may differ depending on the type of adhesion or its composition [73] and only occur under a limited range of tension. Alternately, mechanosensitivity could arise within other parts of the cytoskeleton which then feed back into adhesion assembly indirectly. Moreover, in addition to myosin II generated forces, we must consider the consequences of other external mechanical perturbations such as strain and local curvature. Finally, recent evidence has suggested dynamics of internal tension may play an important role in a ECM rigidity sensing mechanism [74]. Dissecting the physical, temporal, and molecular contributions that regulate the dynamic control of adhesion assembly and maturation will provide interesting lines of research for many years to come.

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