

Buckle up: Membrane tension drives lamellipodial network compression and adhesion deposition

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Whether to spread on a surface or to crawl, cells must apply traction forces to the underlying substrate via adhesion complexes. In this issue, Pontes et al. (2017. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201611117>) shed new light on how the interplay among membrane tension, the lamellipodial actin network, and adhesions coordinate the dynamics of spreading fibroblasts.

In many types of cells, spreading and crawling motility are driven by a protruding lamellipodium, a thin network of polymerizing actin filaments enveloped by the plasma membrane. Adhesion complexes that transverse the membrane mechanically couple the lamellipodial actin network to the external matrix. In recent years, the fundamental processes underlying lamellipodial motility have become clearer: polymerizing actin filaments push the membrane forward at the leading edge, and the membrane exerts a reciprocal load on the actin network that is transmitted to the substrate via immobile adhesion complexes. Although the role of each of the main characters—the cell membrane, the actin network, and the adhesions—is generally understood, the mechanisms responsible for coordinating their dynamics at the cellular level are still not clear.

Previous work focused on the interplay between membrane tension and actin protrusion (Raucher and Sheetz, 2000; Gauthier et al., 2011; Houk et al., 2012; Lieber et al., 2013; Diz-Muñoz et al., 2016). Yet, adhesion formation plays an equally critical role: for actin polymerization to drive cell movement and spreading, new adhesions have to continually form near the leading edge to facilitate forward translocation of the cell. In this issue, Pontes et al. show that adhesion deposition in spreading fibroblasts is temporally correlated with an increase in membrane tension. Spreading fibroblasts are a convenient model system to study adhesion formation under varying conditions. The characteristic sequence of events during spreading has been studied in detail (e.g., Gauthier et al., 2011). The cells transition from an initial rapid protrusion phase (P1) into a slower protrusion phase characterized by cyclic protrusion cycles (P2; Giannone et al., 2007). By measuring membrane tension (using a tether-pulling assay) during the transition from rapid to slower protrusion (P1 to P2; Gauthier et al., 2011) and during subsequent protrusion cycles (Pontes et al., 2017), Gauthier and coworkers showed that pauses in protrusion are accompanied by a transient increase in membrane tension that temporally coincides with the deposition of a new line of

adhesions. To show that adhesion deposition is triggered by the elevated membrane tension, they induced a tension increase by applying a hypoosmotic shock and demonstrated that cells respond by forming a line of adhesions, as observed during tension peaks in unperturbed cells.

How does an increase in membrane tension induce adhesion deposition? Adhesions could respond directly to the elevated in-plane tension in the membrane. Such behavior is seen in various membrane proteins (e.g., mechanosensitive channels) that modulate their activity as a function of membrane tension levels. Alternatively, adhesion molecules, such as integrins, could be influenced indirectly by the changes in membrane tension, whereby increased tension is translated into an intermediate signal that, in turn, influences the properties and dynamics of the adhesion molecules. Although there is some evidence suggesting that integrin activation can be modulated directly by changes in membrane tension (Wang and Ha, 2013), the formation and maturation of adhesions is thought to depend primarily on mechanical stretching forces across adhesion molecules (Riveline et al., 2001; Oakes and Gardel, 2014). The results in the study by Pontes et al. (2017) suggest that the link between increased membrane tension and adhesion deposition in spreading fibroblasts is mainly indirect; elevated tension increases the load on the lamellipodial actin network at the leading edge, and the compressed actin network mediates this mechanical signal and modulates the stretching forces on adhesion molecules (Fig. 1).

The evidence supporting the role of the lamellipodial actin network as a mechanical link between elevated membrane load and adhesion deposition originates from several independent observations (Pontes et al., 2017). First, the effects of force transduction were the same, regardless of the source of the mechanical load at the leading edge; both increased membrane tension and a load induced by the presence of a physical barrier led to a similar transition in lamellipodial dynamics. Second, the membrane tension values that triggered adhesion deposition were found to vary among cells and between protrusion cycles, arguing against the existence of a direct membrane tension threshold for adhesion deposition. Third, lamellipodial behavior was strongly dependent on coupling between the actin network and the adhesions; when this coupling was weakened, as in vinculin knockout cells or cells containing a mutated vinculin that is deficient in binding to actin, cells were unable to

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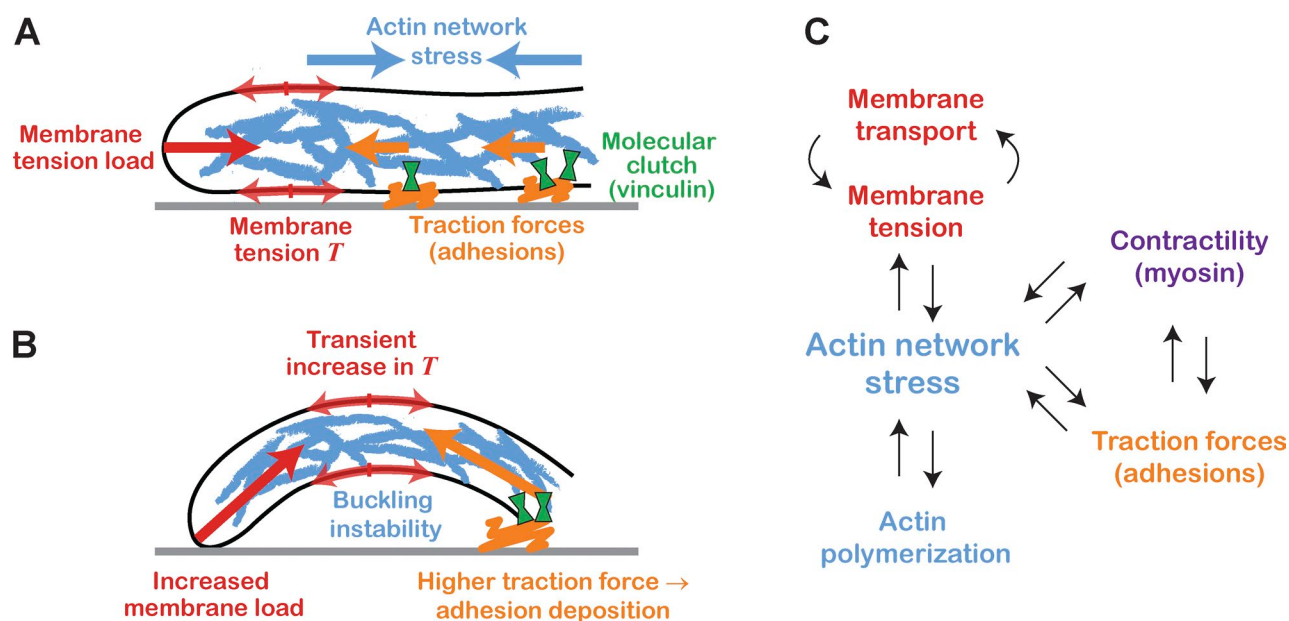


Figure 1. The lamellipodial actin network as a mechanical force transducer linking membrane tension variations with adhesion deposition. (A) The membrane tension load at the leading edge and the traction forces generated at adhesion sites are mechanically coupled by the lamellipodial actin network. (B) The transition in lamellipodial dynamics is triggered by an increase in membrane tension. The compressive stress on the actin network rises because of the higher membrane load at the leading edge, leading to a buckling instability, which is associated with a narrowing of the lamellipodial width and the positioning of an adhesion row. (C) Schematic illustration of the processes involved in lamellipodial dynamics in spreading fibroblasts and the interplay between them.

position new rows of adhesion during the slow-spreading phase, despite higher membrane tension values. In this case, the lamellipodium could not efficiently transmit the load at the leading edge to the adhesions, and the reduced traction resulted in a dramatic increase in actin retrograde flow rates, as well as widening of the lamellipodium.

Collectively, these findings illustrate how the large-scale behavior of the lamellipodium arises from the coupled collective dynamics of cytoskeletal proteins, adhesion molecules, and the plasma membrane (Fig. 1). At the leading edge, the balance between protrusive forces generated by the polymerizing actin and the load imposed by the tensed membrane determines the protrusion rate of the lamellipodium. At the rear of the lamellipodium, the actin network is coupled to the substrate through integrins that bind to the cytoskeleton via vinculin and additional adapter proteins, determining the rate of retrograde actin flow, and the traction forces that are applied against the substrate. The actin network serves as an intermediary, mechanically linking the load at the leading edge with the traction forces on the substrate. Thus, variations of the membrane tension at the leading edge are transduced to changes in the forces that are applied to adhesion complexes and, thereby, affect adhesion dynamics.

The mechano-coupling between adhesions and the cytoskeleton is bidirectional (Fig. 1 C); the forces generated depend on the properties of the actin network and the adhesions. Yet, at the same time, cytoskeletal dynamics and adhesion properties are highly dependent on the forces they experience. Similarly, the tension in the membrane, which applies a load on the growing network, is also influenced by the dynamics of the cytoskeleton (Lieber et al., 2013), as well as by additional processes that modulate the amount of available membrane surface (e.g., membrane transport). Thus, the compressive stress in the lamellipodial network is both affected by and affecting adhesion dynamics and membrane tension, generating

feedback loops between the components of the lamellipodium (Fig. 1 C). Theoretical analysis (Shemesh et al., 2012) showed that such coupled dynamics can lead to different regimes of lamellipodial behavior, ranging from a rapidly protruding regime characterized by a broad lamellipodium with distributed adhesions, as observed during the initial spreading phase, to an oscillating regime with a narrow lamellipodium delimited by a row of adhesions positioned behind it, as observed during the slower protrusion phase.

The shape and dynamics of the actin network are directly related to its function as a transducer and generator of mechanical forces. Yet, what is the reciprocal effect that external loads have on the network shape? Pontes et al. (2017) showed that increased membrane tension is followed by an upward, out-of-plane buckling of the actin network (Fig. 1 B). The forward contact points of the buckled gel serve as precursors for formation of new adhesions, whereas the gel at the rear of the buckled region subsequently disintegrates. Importantly, the buckling observed is essentially a bending deformation of the entire sheet of cross-linked actin gel, caused by the load applied by the membrane at one end and the friction force with adhesions at the other. This is elegantly shown by plating cells on adhesive islands surrounded by a nonadhesive substrate that prevented formation of adhesions beyond the island's edge (Pontes et al., 2017). An adhesion-free protruding lamellipodium formed between the edge of the adhesive island and the cell's periphery. Consistent with the relationship between the size of a thin sheet and the critical load for its buckling, the width of the adhesion-free lamellipodium that underwent buckling was shown to decrease with increasing membrane tension load. This elastic deformation of the lamellipodial network as a whole is distinct from the buckling of individual filaments seen within actomyosin networks (Lenz et al., 2012).

Altogether, the observations by Pontes et al. (2017) describe a simple mechanism for how the membrane load on the

actin network triggers narrowing of the lamellipodium and deposition of new adhesions, placing the buckling instability at the heart of the large-scale protrusion cycles observed during the slow-spreading phase (Pontes et al., 2017). Whereas previous works proposed that these cyclic lamellipodia shortenings are caused by myosin contractility (Giannone et al., 2007), Pontes et al. (2017) demonstrate that myosin activity is not required for this characteristic dynamic behavior of lamellipodia. Alternatively, the origin of the protrusions cycles was hypothesized to be related to the coupling between the actin network and adhesion complexes switching from a stick to a slip mode (Shemesh et al., 2012). Although the observed buckling does not preclude the possibility that a stick-slip friction mechanism contributes to the protrusion cycles, further work is required to clarify the dynamics of actin flow during buckling events and to establish the velocity dependence of the adhesion-network friction.

Recent work has shown that changes in membrane tension are not merely responding to the underlying cellular dynamics. Rather, membrane tension should be viewed as a master regulator (Gauthier et al., 2011; Houk et al., 2012; Lieber et al., 2013; Diz-Muñoz et al., 2016), a global physical variable that equilibrates rapidly and effectively coordinates local dynamics across cellular scales. The work by Pontes et al. (2017) reveals an additional aspect of this coordination, illustrating how temporal change in membrane tension can be coupled to adhesion deposition. In this case, the mechanical signal that triggers adhesion deposition does not follow directly from the increase in membrane tension levels but, rather, indirectly because of the increased membrane load at the leading edge, which is relayed to the adhesions via the lamellipodial actin network. As such, this work nicely demonstrates how different forms of mechanical signaling, initially mediated by the tension in the membrane and subsequently by the stress in the actin network, shape the large-scale dynamics of cells.

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