Biophysics of possible force generation mechanisms: three-phase force-velocity relation

Sharp drop of velocity at a very small load up to 100 pN/μm

Weak adhesion at the leading edge limiting polymerization.

One of the simplest possible explanations of the sharp velocity drop at very small loads could be mechanically weak adhesions that have to direct actin polymerization at the leading edge parallel to the surface. Indirect evidence for such a mechanism has been reported in Bohnet et al. (2006). In this case, very small loads prevent these weak nascent adhesions from forming, reorienting and buckling the actin filaments. At greater loads, load-dependent adhesion strengthening (Zaidel-Bar et al., 2004) could support slower protrusion.

Dampening of membrane thermal undulations.

Mogilner and Oster (1996) discussed the possibility that a ratchet mechanism in which both the cell membrane in front of the filament undulates thermally away from the filament tip and the filament itself bends thermally away from the membrane together create a gap that is sufficient for monomers to intercalate and assemble onto the tip. This action inhibits the leading edge from moving backward. If the filaments are flexible enough, the membrane thermal undulations are not essential, but if the filaments are more rigid, rapid growth can only be sustained by the membrane undulations. In the absence of hydrostatic pressure at the leading edge, and if the membrane at the leading edge is flexible enough, these undulations can be of significant amplitude; however, they also can be dampened by very small loads. In this case, just tens of piconewtons per 1 μm of the leading edge press the membrane against the filament tips and slow polymerization down significantly. At greater loads, rigid filaments can sustain slow growth by their own thermally driven bending (see section Elastic polymerization ratchet model...filaments).

Small osmotic/hydrostatic pressure at the leading edge.

In the presence of small (approximately ≤10^7 pN/μm^2) osmotic/hydrostatic pressure (Charras et al., 2005) or gel swelling pressure (Herant et al., 2003) at the leading edge, the membrane would be kept away from the growing filament tips, allowing free polymerization. When the very small cantilever load exceeds this pressure, the filament tips could be shrink-wrapped by the leading edge membrane, hindering the access of diffusing actin monomers to the tips and potentially slowing the effective actin growth by an order of magnitude.

Insensitivity of the velocity to low loads and a sharp drop at a greater load

Force-dependent reinforcement of the dendritic actin network.

In principle, insensitivity of the rate of protrusion to the magnitude of the opposing force at low loads could be explained by the autocatalytic branching theory (Carlsson, 2003), which assumed that the rate of filament branching is proportional to the density of the existing leading edge filaments. One of the predictions of this model was that the protrusion rate should not depend on the load; effectively, greater load force causes faster branching and, therefore, greater actin density, so the load per filament remains constant, leaving the growth rate unchanged. Roughly speaking, the number of growing actin filaments (n) impinging on 1 μm of the leading edge can be described by the equation

\[
\frac{dn}{dt} = b - cn
\]

(Mogilner and Edelstein-Keshet, 2002), where b and c are the effective branching and capping rates, respectively. The latter is ~0.1/s (Grimm et al., 2003), so, if in the steady state at zero load ~30 filaments are pushing forward 1 μm of the leading edge (many more filaments can be lagging closely behind), b = cn = \frac{3/2}{(μm × s)}. At large loads, it is feasible for the number of filaments pushing forward 1 μm of the leading edge to increase 10-fold to ~300 (Abraham et al., 1999), which would require the branching rate to increase to ~30/μm×s). In this case, according to equation 1, in the first few seconds, the number of the filaments opposing the load would increase linearly:

\[
\frac{dn}{dt} = b = 30 / (μm×s)
\]

The load also increases linearly in these first few seconds, so the load per filament would stay constant, and the velocity would remain unchanged until ~10 s, when the number of filaments saturates and further load stalls the protrusion.

Similarly, other plausible factors that have in common the recruitment of more active actin filaments to the leading edge in response to the increased load so its effect is negated could, in principle, explain the velocity insensitivity to the force. First, the filaments at the leading edge have various lengths, and longer filaments would buckle under load and would therefore not contribute to protrusion force generation. As increasing load is applied, the protruding lamellipod slows down, and filaments get capped at shorter lengths so that fewer filaments buckle and a greater proportion can generate force. Second, new filaments that are branched off the
sides of the existing filaments are able to grow freely by polymerization until they catch up with the leading edge, but many of these filaments get capped before reaching the leading edge. After the initial sharp drop of velocity, more uncapped filaments reach the edge, negating the effect of the initial loads. Finally, not all filaments grow at 55° angles to the leading edge (Maly and Borisy, 2001); in fact, some filaments are nearly parallel to the leading edge. At low loads, most of the filaments are lagging behind the leading edge, and only a few—those growing in the direction of protrusion—push the membrane and equilibrate its resistance. After the initial sharp drop of velocity, some of the filaments, which were growing obliquely to the leading edge and lagging behind, catch up with the front and get loaded, effectively decreasing the load per filament and negating the effect of added load (Mogilner et al., 2001).

**Slow force-independent adhesion assembly.**

One of the simplest possible protrusion processes would be sequential steps, each of which is a combination of (1) a force-dependent actin monomer assembly with a rate of \( m \times \exp[-\alpha f] \) (Mogilner and Oster, 1996), where \( m \) and \( \alpha \) are constants and \( f \) is the load force per filament; and (2) a force-independent adhesion assembly with rate \( a \). Then, the effective combined protrusion rate would be

\[
a = \frac{a}{1 + (a/m)\exp[\alpha f]} \quad (3).
\]

In that case, at small loads, the second term in the denominator is negligible compared with one, the force-limited process is much faster than the force-independent process, and the average rate of protrusion is force independent. However, at a greater load, the second term dominates, force-limited process becomes slower than the force-independent one, and the average protrusion rate exponentially decreases with the load.

**Elastic polymerization ratchet model for short and rigid filaments.**

The elastic polymerization ratchet model (Mogilner and Oster, 1996) predicts that if the growing actin filaments are so short and rigid that their thermal bending is very small, the effective growth rate is small, which could be the case after membrane fluctuations are dampened at very small loads. At the same time, loads up to a few piconewtons per filament (that add up to ~0.5 nN for a hundred or so filaments per 1 μm of the leading edge) do not significantly affect the effective growth rate because the load per filament is less than the effective elastic force associated with the significant filament bending necessary to create a gap between the membrane and the filament tip. This could explain the force-insensitive part of the force-velocity curve. At greater loads, the load per filament becomes greater than the effective elastic force associated with significant filament bending, and the growth is rapidly stalled as observed.

**Strong local osmotic/hydrostatic pressure.**

In the presence of a strong (~\( 10^3 \) pN/μm²) osmotic/hydrostatic pressure (Charras et al., 2005) or gel swelling pressure (Herant et al., 2003) at the leading edge, the membrane would be kept away from the growing filament tips until the load is greater than this pressure. Up to such a load, the protrusion rate would be insensitive to the force. At greater loads, actin polymerization would be stalled by the load exponentially.

**Adhesion and elastic deformations of the actin network at the leading edge**

In principle, significant elastic recoil of the lamellipodial actin network at the leading edge could be caused by the deflecting cantilever, which is much stiffer than the actin network (~\( 10^8 \) pN/μm for the cantilever [this paper] versus ~\( 10^3 \) pN/μm for the network [estimated using data from Laurent et al., 2005]). However, the following simple estimate shows that at the leading edge, the actin network should not detach from the substratum. Indeed, if the frontal part of the network of width \( w \) can detach, it would buckle under load \( f \), and the protrusion would be stalled. To estimate the critical width \( w \), we use the following formula (Landau and Lifshitz, 1995): \( f \sim Yh^2/w^2 \), where \( Y \) is the Young modulus of the actin network and \( h \) is the height of the lamellipod. Using the values \( f \sim 1 \) nN/μm, \( h \sim 0.2 \) μm (this paper), and \( Y \sim 10^4 \) Pa (Laurent et al., 2005), we estimate that at most, a region ~300 nm wide at the very front of the actin network is not adhering to the surface. However, at such a small length scale, the network is not continuous but rather consists of individual filaments, and so the filaments in the adherent portion of the lamellipod do not detach from the substratum.

This argument suggests that the lamellipodial elasticity does not appreciably redistribute the locally applied cantilever load to adjacent parts of the leading edge in the first few seconds of contact. The tight adhesions localize the load to the length of the leading edge in immediate contact with the cantilever. However, it is possible that viscoelastic deformations of the lamellipodial actin network and/or slippage of adhesions in addition to the effect of force on actin polymerization contribute to the observed slowing of the protrusion on longer time scales.

Another hypothetical possibility for the elastic recoil is that although actin filaments do not detach from adhesions, they can slip relative to the surface (Jurado et al., 2005). In this case, theoretically, the first sharp drop of velocity could be explained by rapid recoil of the softer lamellipodial network when it encounters the cantilever. In fact, the ratio of the rate of such recoil to the rate of cantilever deflection would be of the same order of magnitude as the ratios of the stiffness of the cantilever to that of the lamellipod. Because the latter ratio is close to 10, the initial rate of cantilever deflection would be an order of magnitude less than the cell speed before the contact. This would be a simple explanation for the apparent sudden velocity decrease before any observed cantilever deflection, suggesting that it is not a feature of the network but rather a result of the measurement technique. One problem with such an explanation is that the localized load applied
to the edge of an elastic shell generates significant long-range deformations that decay logarithmically away from the load (Landau and Lifshitz, 1995). For our experimental setup, there would be significant deformations even 10 μm away from the load, which is not observed in the first few seconds. On the other hand, if the deformations grow for a very short time, they may not be observable, so this explanation cannot be ruled out. Future experiments with cantilevers of different stiffness can help to resolve this issue.

The curved leading edge gets in contact with the cantilever rapidly

The leading edge has a characteristic parabolic shape with a radius of curvature on the order of 100 μm (Grimm et al., 2003; unpublished data). Mathematically, this means that the leading edge can be described with the function \( f(x) = \frac{x^2}{2R} \), where \( R \approx 100 \) μm is the radius of curvature, \( x \) is the distance from the center of the leading edge toward the side along the line normal to the direction of locomotion, and \( f(x) \) is the distance from this line to the point on the leading edge with coordinate \( x \). After the center of the cantilever touches the center of the leading edge, the sides of the cantilever with coordinates \( f(x) \pm 1.5 \) μm are \( f(\pm 1.5 \) μm) \approx (1.5 \) μm) \( \approx 200 \) μm \( \approx 0.01 \) μm away from the corresponding points on the leading edge. It would take less than a second (0.01 "s") for the leading edge to travel this distance.

Time and spatial scale considerations

On short second-range time scales, the reaction of the lamellipod to the load is likely to be local. Actin–myosin interactions are significant only at the rear of the lamellipod in these cells (Svitkina et al., 1997), and the rear of the lamellipod cannot react to processes at the leading edge in a few seconds (the respective time scale is tens of seconds; Giannone et al., 2004). Also, at these time scales, the actin network behaves mechanically as an elastic body; the applied force cannot break Arp2/3-mediated cross-links in the lamellipodial network because these links are not likely to react in a few seconds to forces amounting to a few piconewtons per cross-link (Fujiwara et al., 2002). Although elastic deformations of the lamellipodial network induced by a localized load are generally not local, strong adhesions localize them to the immediate vicinity of the cantilever on the short time scale (see section Adhesion and elastic deformations...edge). Finally, signaling is also unlikely to become global on the short time scale because diffusion of the signaling proteins is limited to a few square micrometers per second.

On the other hand, on the longer time scale of tens of seconds, signals from the leading edge reach the cell body, triggering significant changes in the contractility patterns (Giannone et al., 2004). Also, on these time scales, the actin gel is likely to behave as a highly viscous fluid rather than as an elastic solid body (Janmey, 1998), and the lamellipodial network is likely to undergo drastic global remodeling. This is consistent with our observation of the lamellipod remodeling itself to accommodate the load delivered by the cantilever a few tens of seconds after initial contact.

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