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On the edge: modeling protrusion

Alex Mogilner

Actin-based protrusion is the first step in cell crawling. In the last two decades, the studies of actin networks in the lamellipodium and *Listeria*'s comet tail advanced so far that the last goal of the reductionist agenda — reconstitution of protrusion from purified components *in vitro* and *in silico* — became viable. Earlier models dealt with growth of and force generation by a single actin filament. Modern models of tethered ratchet, autocatalytic branching, end-tracking motor action and elastic- and nano- propulsion have recently helped to elucidate dynamics and forces in complex actin networks. By considering these models, their limitations and their relationships to recent biophysical data, progress is being made toward a unified model of protrusion.

Addresses
Department of Mathematics and Center for Genetics and Development, University of California, Davis, California, 95616, USA

Corresponding author: Mogilner, Alex (mogilner@math.ucdavis.edu)

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Introduction

Cell crawling begins with protrusion — the process of actin-based extension of the cell's leading edge [1]. Cell migration involving a flat lamellipodium [2] and intracellular motility of *Listeria* [3] are two model systems that in the past two decades have added considerably to our understanding of how actin polymerization contributes to protrusion. These *in vivo* systems are now complemented by *in vitro* assays using plastic beads and lipid vesicles that, when coated with either ActA or WASP proteins, move much the same way as the pathogens. These systems have several advantages: the number of essential proteins is small; their structures, concentrations and localizations are known; the reaction rates of the actin dynamics have been measured [4]; the actin-based motility can be reconstituted from purified components *in vitro* [5]; and the force generated by the actin comet has just been measured [6••,7••]. Moreover, a 'dendritic nucleation'/'array treadmilling' hypothesis has outlined a qualitative scenario describing how steady protrusion might occur [2].

Thus, we have a rare opportunity to make the final step in the 'reductionist agenda' [2] and to test our understanding by reconstitution of the protrusion *in silico*. Here we review recent protrusion models and their relations to the data. Various aspects of protrusion have been reviewed recently in [2,3,8,9].

Early protrusion modeling

The process of protrusion is based on the polymerization of actin into a two-stranded polar helix with barbed and pointed ends having fast and slow dynamics, respectively [4]. The monomers bind ATP, and ATP hydrolysis results in the filaments' dynamic asymmetry and 'treadmilling' (net depolymerization from the pointed end balanced by net polymerization onto the barbed end with monomers simply being recycled by diffusion). Protrusion is based on the treadmilling of the polar (barbed ends are directed forward) actin arrays, rather than of the individual filaments. What determines the fast rate of treadmilling of these arrays and how do they self-organize? What is the nature of the protrusive force?

Mathematical modeling was used to quantify equilibrium polymerization [10] and to predict treadmilling [11]. Then, T L Hill intuited several ideas, the most important of which was using thermodynamics to demonstrate that a polymerizing filament can generate a force in the piconewton range [12]. A 'Brownian ratchet' theory [13] explained how such force can be generated: even when a resisting force is applied to the object in front of the filament's tip, the object can still diffuse away, creating a gap sufficient for monomers to intercalate and assemble onto the tip, thereby inhibiting the object from diffusing backward. Next, on the basis of observations that the actin filaments are flexible rather than rigid, an 'elastic ratchet' model suggested that a filament's own thermal undulations can create a gap between its tip and the load [14]. Subsequent monomer assembly increases the fiber's length so that when the tip contacts the load the polymer is bent; the resulting elastic force pushes on the load. In these models, the actin binding energy drives protrusion. ATP hydrolysis is not utilized in the force generation but is necessary for treadmilling.

Modern models: from tethered ratchet to elastic propulsion

As often happens, experiments soon revealed earlier models' limitations. First, actin filaments responsible for protrusion are not independent, but are rather parts of the 'dendritic' network [2]. Second, one-filament models cannot adequately describe the complex geometry of the actin network leading edge impinging on the

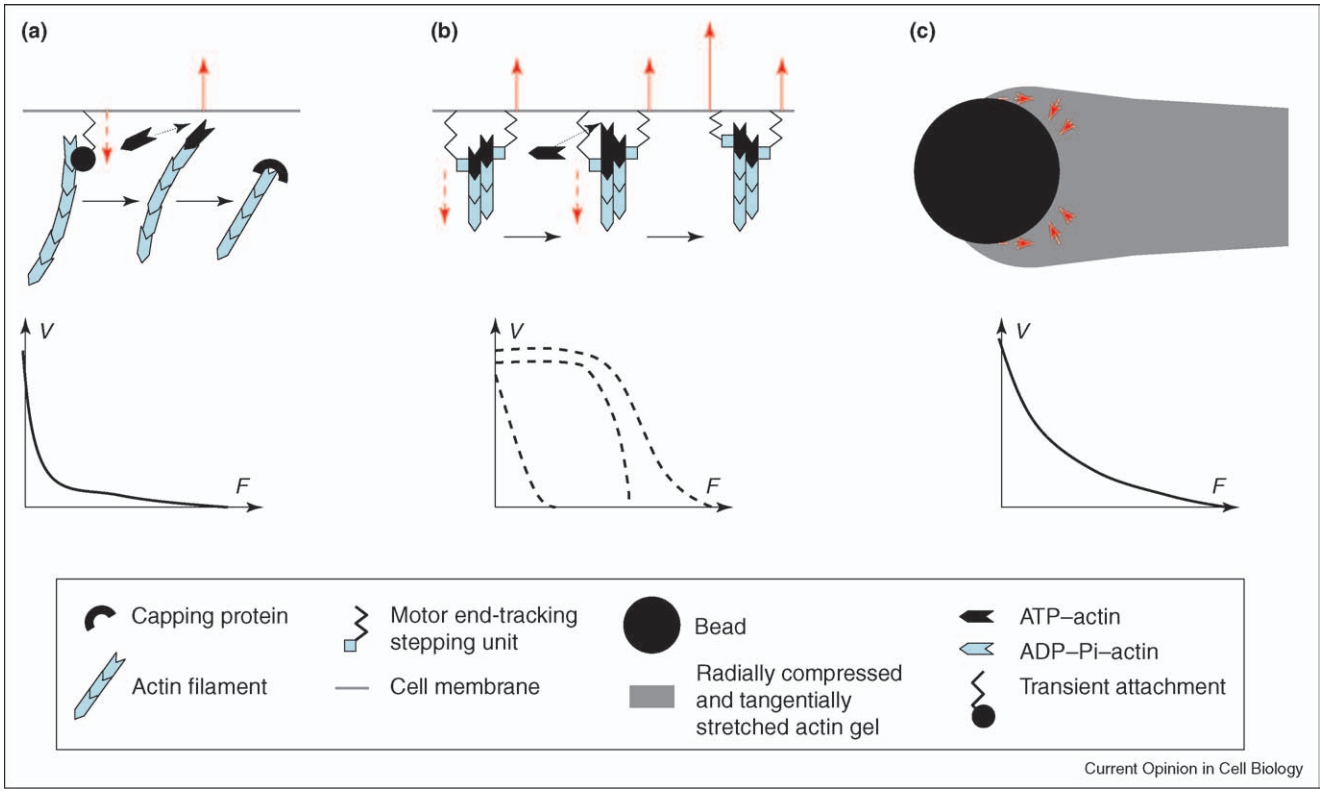
curved cell membrane or bacterial surface. Third, direct [15] and indirect [16] data indicated that the filaments are attached to the surface they push.

How can ratchet models work if the filaments are attached to the surface? The 'tethered ratchet' model answered this question by assuming that the filaments attach to the surface transiently [17]: nascent filaments are associated with protein complexes on the surface. However, they soon dissociate and grow until they lose contact with the surface after capping. The attached fibers are in tension and resist the protrusion, whereas the dissociated fibers are in compression and generate the force of propulsion (Figure 1a).

An alternative model proposed that all filaments are attached to the surface: all pushing barbed ends are clamped in an ATP-dependent fashion to an end-tracking protein (see Box 1) associated with the surface [18,19•]. One of the versions of this model is shown in Figure 1b: two protein subunits have a high affinity for ATP–F-actin, and a low affinity for ADP–F-actin or ADP–Pi–F-actin. Association of ATP–G-actin triggers hydrolysis of ATP on the clamped penultimate actin subunit, causing shifting of the end-tracking protein subunit forward and re-clamping on the terminal ATP–actin subunit. This model suggests the existence of a peculiar 'stepping motor' coupling protrusion to ATP hydrolysis on the filament whose end the motor tracks. Because in this scenario hydrolysis energy is utilized, this model would be able to explain large forces in the range of tens of pN per filament if such forces are ever observed.

These microscopic models did not address the problem of the surface curvature. A macroscopic 'elastic propulsion' model suggested that the curved surface is not merely

Figure 1



Models of protrusion force generation. **(a)** Tethered ratchet model [17]: actin filaments are nucleated in the attached state, and then detach and push the surface according to the elastic polymerization ratchet mechanism until capped. The pushing (solid red arrow; compressed filaments) and pulling (dashed red arrow; filaments under tension) forces are balanced. The model predicts the biphasic force–velocity relation: the velocity decreases rapidly at low loads and slowly at greater loads. **(b)** End-tracking motor model [19•]: in one of the implementations of this model, two end-tracking motor subunits associate with the filament's tip (shown schematically as two parallel strands). Assembly of ATP–actin monomer onto the tip triggers hydrolysis on the clamped penultimate actin subunit, causing shifting of the motor subunit forward. The forces at the surface are illustrated with solid and dashed red arrows. The end-tracking motor theory has many free parameters and predicts a few possible force–velocity relations (dashed). **(c)** Elastic propulsion model [6••] explains the curved objects' propulsion as the balance between the elastic stress 'squeezing' the object forward (solid red arrows) and the effective actin–surface friction (dashed red arrows). The elastic stress is generated by the actin polymerization near the surface and subsequent pushing of the actin shell radially outward. The model predicts a convex force–velocity curve.

Box 1 Can formin be the 'end-stepping motor'?

The end-stepping motor remains but an interesting hypothesis until a protein complex playing this role is identified. Recently, formin — a 'leaky capper' that stays on a barbed end during growth protecting the filament from capping — attracted much attention in this respect. Elegant experiments with growing and buckling filaments tethered to a cover slip at their pointed ends and attached to immobile formin molecules at their barbed ends demonstrated that these filaments generated forces in the pN range [47•]. An interesting theory [48•] explains this formin action by a stair-stepping mechanism, assuming that elastic deformations of forming F-actin complex are coupled to actin monomers' assembly onto the tips of the actin helical strands. One still has to explain how the filaments can grow with their ends attached to the immobile formin's subunits: helical pitch of the filaments, in principle, has to super-coil them, but the filaments do not even twist [47•]. One possible explanation is that from time to time the formin dimer rotates with respect to the filament in the direction opposite to the rotation generated by the stair-stepping mode, preventing persistent torsion strain accumulation [49]. Curiously, rotation of *Listeria* around its long axis during propulsion was observed [50], and the only published quantitative explanation relies on the end-stepping motor translating the single filament twisting into the right-handed rotation of the actin comet [51].

Recent experimental study of formin-coated beads propelled by the actin comet attached to the bead argued that the profilin–actin ATP hydrolysis cycle is coupled to the release of the formin subunit [52], much like in the end-tracking motor model. This opens a tantalizing possibility that in filopodial protrusion based on growth of a tight filament bundle, which is hard to explain with the ratchet models [53], formin (implicated in the filopodial protrusion [54]) is the end-tracking motor. Thus, it is possible that all existent theories are not mutually exclusive, but rather complement each other by describing redundant diverse protrusion mechanisms.

pushed, but is rather 'squeezed' forward by an elastic stress [3,20,21•]. This model treats the actin network as an isotropic elastic continuum and does not consider explicitly the microscopic mechanism of force generation at the surface. The squeezing stress develops when the growth of actin at the surface pushes the actin gel outward, stretching it and generating tangential tension balanced by radial compression at the surface (Figure 1c). This model takes into account the actin–surface attachment by assuming an effective friction between the gel and surface. Similar to the microscopic models, the elastic propulsion model predicts a balance between the pushing elastic and pulling friction forces on the surface.

A few models examined the dynamics and self-organization of the actin network [22–24]. The 'autocatalytic branching' theory [23] (Figure 2a) assumed that the rate of filament branching is proportional to the density of the existing leading edge filaments. An unexpected prediction of this model was that the protrusion rate should not depend on the load (Figure 2a): effectively, greater load force causes faster branching, and therefore greater actin density, so the load per filament remains constant, leaving the growth rate unchanged.

At the leading edge of the crawling cell, actin forms the flat network in which the fibers subtend a $\sim 55^\circ$ angle to the front edge of the cell (Figure 2b) [2]. This angular order is important for effective protrusion, because filaments at other angles do not generate either force or movement [13]. The sterically precise branching mediated by Arp2/3 complex imposes a 70° branching angle between the mother and daughter filaments, but does not explain the symmetric $\pm 55^\circ$ orientation of the filaments relative to the leading edge. The model [22] explains this symmetry on the basis of the idea that the capping rate is very fast everywhere in the cytoplasm except at the leading edge (Figure 2b). Mathematical arguments demonstrate that under this condition the angularly symmetric mother–daughter filament pairs 'survive', whereas the asymmetric (relative to the leading edge) pairs do not (Figure 2b). At the same time, this model provides a plausible explanation for the actin polarization: barbed ends growing away from the leading edge are rapidly capped, whereas those growing forward are not.

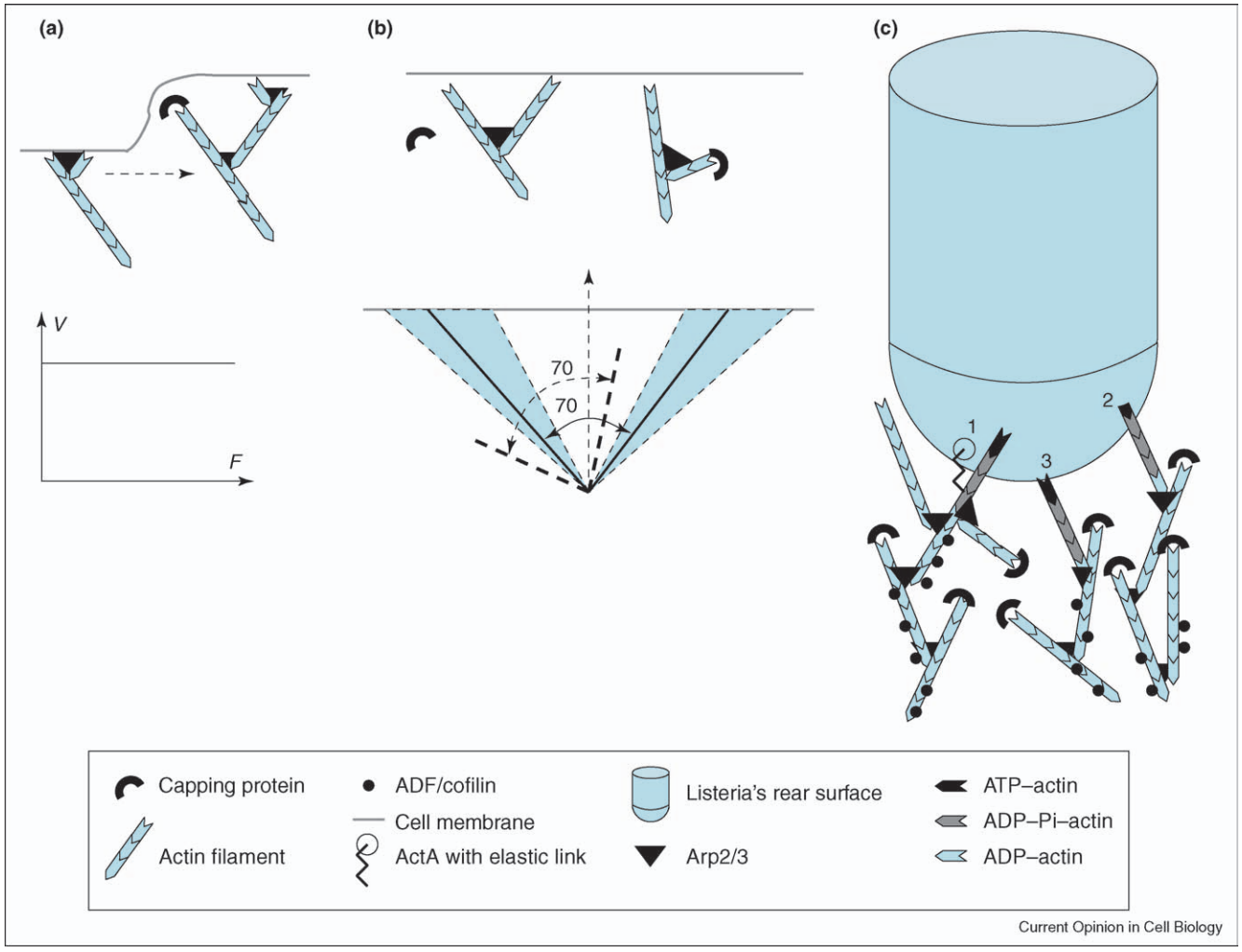
The mechanisms of F-actin self-organization and force generation are only parts of the whole process of protrusion. Other important aspects are coupled cycles of F-actin hydrolysis, array treadmilling and G-actin recycling from the rear to the front. These processes have to be fast to maintain rapid protrusion. Two recent models examined mathematically the conditions necessary to maintain the fast, steady protrusion [25•,26]. By analyzing nucleotide profiles within actin filaments [25•] and diffusion of G-actin and its reactions with actin-binding proteins [26], the models predicted that a combination of enhanced Pi release, an increase in the 'off rate' of ADP-bound subunits at pointed ends, fast G-actin diffusion and optimal levels of capping and profilin function is necessary to accelerate the treadmilling to rates observed *in vivo*.

The 'nano-propulsion' model [27••] is the first *in silico* reconstruction of *Listeria*'s movement (Figure 2c). In this model, the filaments propel the virtual bacterium by the tethered ratchet mechanism, and a realistic geometry and actin network architecture are also simulated stochastically. The model also takes into account the reaction–diffusion process of actin recycling and vectorial hydrolysis of actin subunits. The simulations result in a vivid and realistic mimicking of *Listeria*'s propulsion. The nano-propulsion model is a very promising step toward a comprehensive mesoscopic protrusion model. Some of the assumptions used in the first generation of this model are dubious: for example, the virtual filaments are rigid. Introducing elasticity and judiciously combining large-scale simulations with mathematical analysis will undoubtedly lead towards the ultimate model of protrusion.

Models versus data

Protrusion models can be tested by comparing predicted force–velocity relations with those measured experimentally. Two groups used methylcellulose as a viscous

Figure 2



Models of actin networks growing against a surface. **(a)** 'Autocatalytic branching' model [23] assumes that filaments branch off the sides or ends of existent filaments with the rate proportional to the number of the existent filaments. This model predicts that the protrusion velocity is independent of the load force. **(b)** Top: 'mother and daughter' filaments grow at 70° relative to each other as a result of the sterically precise Arp2/3-mediated branching. Barbed ends of the pairs of filaments growing at approximately 35° relative to the protrusion direction stay close to the cell membrane and are protected from capping. On the other hand, if the mother filament is almost normal to the membrane, then the daughter filament growing almost normal to the protrusion direction lags behind the leading edge, is capped rapidly, and does not branch out the next generation 'mother' filament. Bottom: mathematical arguments [22] demonstrate that these processes cause angular selection of the filaments such that most of the filaments grow at $\approx \pm 35^{\circ}$ relative to the protrusion direction (shadowed regions), while the mother-daughter filament pairs growing in the unshaded angular regions lose the competition for growth to the symmetric filament pairs and go to extinction. **(c)** Detailed computational model of *Listeria* propulsion [27••] reconstitutes *in silico* a 3D treadmilling actin array. The growing filaments either attach elastically to the *Listeria*'s surface (1) or collide with the surface generating pushing force (2), or become so close to the surface that their assembly rate becomes inhibited (3). The model takes into account the reaction-diffusion process of actin recycling and vectorial hydrolysis of actin subunits.

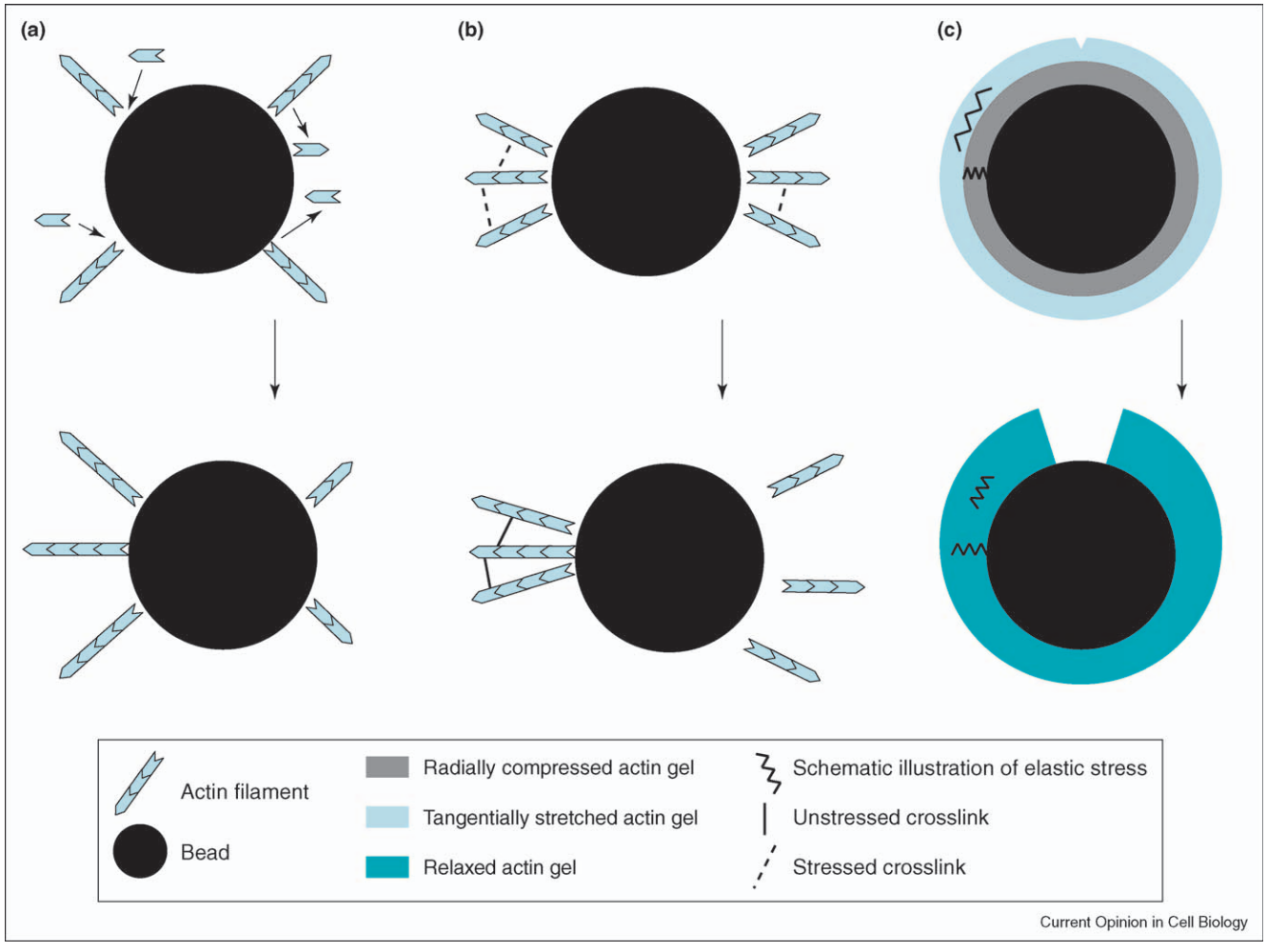
medium in which to measure *Listeria*'s force–velocity relations [28,29]. The details of the results obtained were different. The first experiment showed that the bacterium's velocity decreases rapidly at increasing load of tens of pN and then more slowly at a greater load [28], in agreement with the tethered ratchet model (Figure 1a). However, the second experiment showed that velocity is independent of the load [29], which is consistent with the autocatalytic branching model. It is impossible to say which force–velocity relation is right until we have quantitative data on concentrations of F-actin, Arp2/3 and capping protein at the leading edge of the actin tail to compare with the models.

The first direct measurement of the steady velocity of the actin 'comet' growing from a coated bead at constant loads [6••] resulted in a convex force–velocity curve consistent with the predictions of the elastic propulsion theory

(Figure 1c), hence differing from both other experiments and from the predictions of the microscopic theories. Astonishingly, another recent experiment in which force was applied to a transiently growing actin comet resulted in a concave force–velocity curve ([7••]; the trapezoidal comet's geometry in this experiment is different from the cylindrical one in [6••]). Moreover, in the latter experiment the growth rate depends on the history of loading of the actin network, hinting at complex actin dynamics.

Another test for the theories is provided by observations of nano- and micro-saltation of *Listeria* and coated beads. *Listeria* appeared to advance in discrete steps of 5.5 nm, similar to the size of an actin monomer [30], suggesting some intrinsic molecular-scale mechanism at the interface between filaments and the surface, which is most easily explained by the stepping motor theory; there are, however, doubts about the observations of the nano-saltation [18,19•]. The tethered ratchet theory also predicts movement in small yet irregular steps resulting from the breaking of individual actin-surface bonds [17]. Interestingly, the nano-propulsion model also predicts small and irregular stepping movement of *Listeria* originating from cooperative actin–surface bond breaking [27••]. Such cooperative de-adhesion seems to be the only explanation for the recently measured temperature dependence of the bacterial velocity [31]. On a very different scale, micron-size saltatory movements are most naturally explained by

Figure 3



Models of symmetry breaking. **(a)** Stochastic polymerization model [36]: stochastic fluctuations increasing one filament polymerization at one side of the bead cause the autocatalytic polymerization process, in which the filaments on that side assist each other by pushing the bead to the other side and creating gaps for other filaments to grow. At the other side, depolymerization of one filament increases the force on another catalyzing disassembly. **(b)** Autocatalytic crosslink-breaking model [17]: breaking of a crosslink stressed by actin growth at one side of the bead causes the autocatalytic breaking of other crosslinks at that side, because the same stress is distributed between lesser number of the crosslinks. **(c)** Elastic cracking model [37•]: growth of the actin filaments at the actin-bead interface leads to radial compression of the inner layer of the actin gel and tangential stretching of its outer layer. An initial crack at the outer surface of the gel expands rapidly as a result of effective stress concentration in the crack's vicinity leading to the symmetry breaking.

the elastic propulsion theory [21•], assuming a nonlinear friction–velocity relation: elastic stress grows without propelling the surface attached to the actin tail until the stress exceeds a threshold friction, resulting in a 'jump' forward and stress relief, upon which the new propulsion cycle starts.

Experiments with coated lipid vesicles demonstrated the separation of forces: pushing at the sides, pulling at the rear [32,33]. The tethered ratchet theory could explain this effect if the mobile actin–surface links are simply swept to the rear of the moving vesicle and the growing, pushing filaments are concentrated at the sides. The end-tracking motor model also can explain this behavior by taking into account a G-actin concentration gradient toward the tail center resulting from actin assembly at the actin–surface interface and subsequent force differential accumulation between the outer pushing filaments and the inner pulling filaments (R Dickinson, personal communication). Another experiment demonstrates that curved surfaces are propelled more slowly than flat ones, which is another critical test for the role of actin gel elasticity in protrusion [34].

Finally, the protrusion models have to explain the 'symmetry breaking' phenomenon [35], in which a 'cloud' of actin growing around coated beads loses its symmetry by 'melting' away at one side of the bead. The actin comet then develops at the other side, and the bead's motility ensues. The ratchet models explain this phenomenon as cooperative acts of filament growth at one side and disassembly at the other side of the bead assisted by stochastic fluctuations [36] (Figure 3a) or as a similar process of breaking crosslinks in the rigid actin cloud [37•] (Figure 3b). Elastic models are more successful in describing the sequence of events for large beads where the stochastic fluctuations are less significant [38] (Figure 3c): growth of actin at the bead's surface pushes the outer actin layer outward, stretching it and generating growing tangential stress. When critical tangential stress is reached, a crack at the gel outer surface develops and propagates to the bead's surface.

None of the models can explain all the existent data. The tethered ratchet model, in its simple (mathematical) original [17] and advanced (computational) [27••] forms, seems to fit more data than other models, but so far there are not enough quantitative data, especially on the actin network structure and dynamics, to condense the multiple models to the extent of developing an ultimate protrusion model. However, detailed biophysical data [39] are rapidly accumulating and will soon fine-tune the existent models into solid theories.

Conclusions

Understanding dendritic actin arrays will not be enough. We will have to clarify the role of other plausible force generation mechanisms, such as filament bundling [39], myosin-driven hydrostatic pressure [40], gel swelling [41] and processes involving non-dendritic actin structures [42]. More realistic actin rheology [43] and membrane adhesion [44] and regulation pathways [45] associated with protrusion have to be quantified and incorporated into a unified model of protrusion, which eventually will serve as a boundary condition for multi-scale models of migrating cells [46].

In the dictionary of idioms, 'on the edge' is defined as being 'in a precarious position' or 'in a state of keen excitement'. In both senses, the life of a modeler trying to understand what is going on at the leading edge of the cell is truly on the edge: the models are short-lived, making a wrong turn once in a while inevitable, yet very few areas of biology are as exciting.

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