

Actin Polymerization: Forcing Flat Faces Forward

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Actin polymerization has been shown to be sufficient to propel curved objects, for example beads and vesicles coated with the *Listeria monocytogenes* protein ActA. Recent studies suggest that actin polymerization on flat surfaces can also provide the propulsive force to push them forward.

Actin polymerization forms the basis of numerous forms of cell motility. Actin is thought to polymerize at the leading edge of eukaryotic cells and push the membrane forward [1]. Due to the biochemical complexity of cells and the intricate networks of proteins that regulate cell motility, understanding the biophysical basis of cell motion has been a challenge. Biomimetic systems have provided a significant advance in our knowledge of how actin-based forces can generate motion [2,3]. The bacterial pathogen *Listeria* uses its surface protein ActA to initiate actin polymerization on its surface and has thus been widely studied as a model for the lamellipodium [4,5]. Objects such as beads and vesicles coated with ActA are propelled by actin polymerization on their surface [6–9]. ActA and similar proteins such as N-WASP activate the Arp2/3 complex which initiates polymerization at the surface and elongates filaments by branching. The polymerizing actin forms a cloud around the object and over time becomes polarized into a cylindrical comet tail consisting of a meshwork of cross-linked, branched filaments. The object is pushed forward by the comet tail which continues to elongate by polymerization at the load surface. Interestingly, until now all experiments have used objects that have curved surfaces as biomimetic cargo in these systems. However, in this issue of *Current Biology*, Schwartz *et al.* [10] show that flat objects can also be pushed by actin polymerization forces.

Experiments with beads and vesicles have shed light on the spatial and temporal distributions of forces [8,9,11], but the exact mechanism of force generation is still unknown. Polymerization of a single filament anchored at one end can, in principle, provide a propulsive force [12]. However, the leading edge of the cell or the comet tail consists of several hundred filaments. The precise biophysical mechanism by which individual filaments work cooperatively to generate a propulsive force is unresolved. Two classes of mathematical models have been proposed to explain polymerization-driven motility: the Brownian ratchet model and the elastic propulsion model. The Brownian ratchet model

is a microscopic description of the polymerization of elastic filaments near a load [13]. To account for the binding between the tail and the load as suggested by experiments [8,11,14,15], some filaments are considered to be tethered to the surface of the load [16]. New filaments attach transiently to the surface, subsequently dissociate and become free to polymerize. Thermal fluctuations bend filament tips away from the load and allow them to grow longer than the available space (Figure 1). As the filaments contact the load again they are compressed and their subsequent relaxation provides the propulsive force that pushes the load forward. The attached fibers, on the other hand, are under tension and oppose forward movement. As the stress on attached filaments increases, they detach and allow the object to move forward. Another model at the microscopic level takes into account the geometry of filaments near the surface, for example branching density and filament orientation with respect to the load [17].

Operating on a much larger length scale than the microscopic models, the elastic propulsion theory treats the actin tail as an elastic continuum [18]. Experimental evidence suggests that the actin mesh around the object behaves like an elastic gel [15]. The polymerization velocity of single filaments provides the boundary conditions at the surface of the object. Growth of actin filaments on a curved surface creates stresses in the gel. As older parts of the gel get displaced by the newly growing layer right next to the surface, they get stretched (Figure 2). As the older layers slip back behind the object, the stress in the gel is relaxed and this deformation leads to a propulsive force that pushes the object forward. Forward motion is opposed by an internal friction force that arises due to bonds between actin and the object. Growth of filaments on a flat surface, on the other hand, will not lead to a build-up of stress, as described in Figure 2. It has been difficult in previous studies to test the relative roles of direct pushing at the molecular level and that of elastic stresses in the gel on generating the propulsive force. The results of Schwartz *et al.* [10] now show that curvature-derived stresses may not be critical for movement.

Schwartz *et al.* [10] use flat disks (made by compression of polystyrene beads) coated uniformly with ActA in cell extracts to reconstitute *Listeria*-like motility. They find that the disks move through extracts with comet tails in a manner similar to *Listeria*, beads and vesicles. Typically, the entire disk is covered by actin, but the comet tails are predominantly positioned on the flat surface. The most common configuration is a tail emerging from each face of a disk. Part of the propulsive force could in principle, arise from actin polymerizing on the curved periphery of the disks. The authors present specific examples of motile disks to demonstrate that actin polymerization can indeed push on a flat surface. Based on the direction of motion of some disks that move with a curved trajectory, they conclude that the largest pushing forces occur on the flat surface

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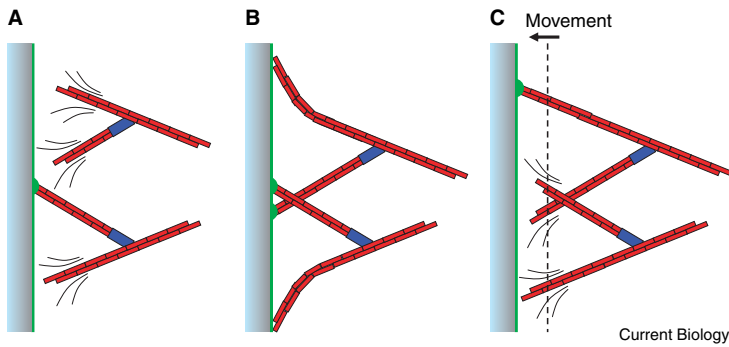


Figure 1. Tethered ratchet model of propulsion.

Actin filaments (red) grow by branching as mediated by the Arp2/3 complex (blue). (A) Some filaments are attached to the load surface which is coated with ActA (green). Other unattached filaments are free to polymerize. (B) Thermal fluctuations cause the polymerizing filaments to bend away from the load. (C) Once the elongated filaments attach to the load, they are under compression and their subsequent relaxation provides the propulsive force and can move the object forward. The attached filaments detach and are free to polymerize.

of the disk rather than at the curved periphery. These authors also observe no correlation between surface curvature and actin polymerization (measured from fluorescence intensity along the disk surface) or between tail position and local curvature. Furthermore, particles with tails on their curved perimeters are observed to hop, but disks with face tails move steadily, suggesting that curvature of the load is required for hopping. Hopping has been observed previously for spherical beads in purified protein solutions and was found to be in agreement with a curvature-induced build-up of stress [11]. Schwartz *et al.* [10] also observe that flattened disks move faster than the spheres from which they are manufactured, despite their lower curvature. This is contrary to the results of Bernheim-Groswasser *et al.* [11], who found that larger beads with lower curvature move more slowly than smaller beads with higher curvature. It should be noted that these differences may arise from differences between cell extracts and purified protein systems.

The new results demonstrate that polymerization forces on flat objects can lead to forward motion and imply that curvature-dependent elastic stresses and deformations of the gel are not essential for motion. However, gel elasticity and curvature-dependent stresses could play a role in determining other features of motility such as the time taken to initiate motion of beads by breaking the symmetry of the actin gel and periodic hopping motility. There is thus

a need for experiments that directly test the role of the structural and mechanical properties of the comet tail during motion. In particular, direct visualization of the stress development in the tail and its deformation will be extremely important. The role of parameters such as actin cross-linker density and rigidity, which affect gel mechanical properties, needs to be investigated. Recent results by Briehner *et al.* [19] indicate that tail structure may be an important determinant of motion. They found that *Listeria* can move in an Arp2/3-independent manner by generating hollow comet tails, which consist of parallel actin bundles generated by actin-bundling proteins such as fascin. This is in contrast to the branched meshwork formed by the Arp2/3-dependent comet tail growth. The speed of the bacterium propelled by the hollow tail is higher than during Arp2/3-dependent motion.

The new findings provide interesting constraints for building a comprehensive theoretical understanding of actin-driven motility. The elastic propulsion model successfully predicts many features of moving beads such as effect of load size on time to break symmetry and probability of hopping. These phenomena are not explained by the Brownian ratchet model which does not explicitly consider geometry, such as the size of object or the curvature. The ratchet model, on the other hand, can explain the motion of flat disks. It is plausible that the two mathematical models operate predominantly in different regimes and substrate curvature

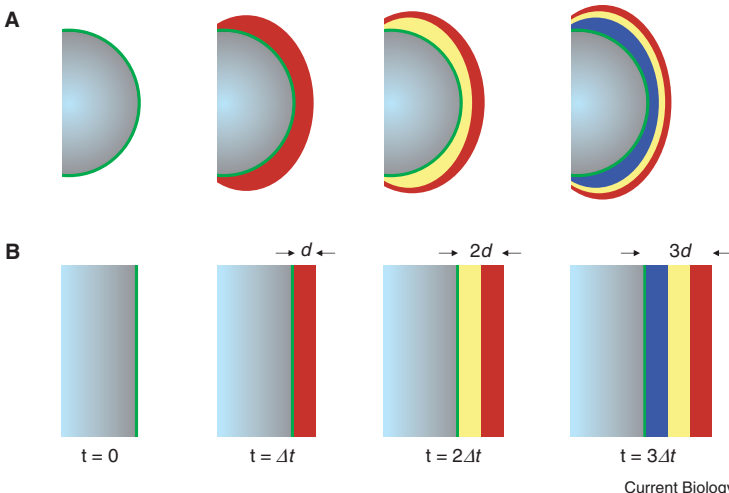


Figure 2. Curvature-induced elastic stress development.

(A) A curved object coated with ActA (green) polymerizes actin on its surface. The first layer of actin is depicted in red. As the subsequent layer (yellow) grows on the surface of the object, it displaces the first layer and causes it to stretch. The growth of the next layer (blue) causes the previous layers to be further stretched. This leads to a compressive force on the bead. (B) Actin polymerizing on a flat object coated with ActA on one surface. In a time interval Δt the thickness of the gel increases by d . Successive layers are not stretched and therefore do not generate a compressive stress. This is in contrast to the curved object where the thickness of older layers decreases as each new layer is added.

determines which model will hold. Is there a critical curvature above which the stresses in the gel start to play a role? The recent results from Schwartz *et al.* [10] underscore the importance of making a unified theory that integrates the experimental observations and can explain phenomena observed at both ends of the scale. A model that incorporates the dynamics and organization of filaments in the actin mesh as well as geometric constraints of the load would be a first step.

Further experiments are also required to improve our understanding of the physical picture of polymerization-driven motility. Better imaging of the actin–substrate interface to visualize single filaments will be useful for understanding the dynamics of binding and dissociation and the distribution of filament lengths and orientations. Such studies will elucidate whether spatial organization of filament attachment and detachment is required to explain the observed motion. The new experimental system using disks can be improved by more precise coating of only the flat faces with ActA in order to eliminate edge effects: methods for ActA coating similar to those used by Marcy *et al.* [20] could be used. A more complete understanding of the biophysics requires further experiments that measure the force–velocity relationship of moving flat disks. Importantly, since the cell membrane at the lamellipodium is a concave surface and the curvature can change as the cell moves, experiments and models must address the role of substrate curvature in actin-dependent propulsion.

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