Amplification of actin polymerization forces

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The actin cytoskeleton drives many essential processes in vivo, using molecular motors and actin assembly as force generators. We discuss here the propagation of forces caused by actin polymerization, highlighting simple configurations where the force developed by the network can exceed the sum of the polymerization forces from all filaments.

Introduction

Mechanical amplification is something we experience every day, in the form of gears, pulleys, and levers. While climbing a hill on a bicycle, for instance, shifting gears increases the force on the wheels while limiting the pressure required on the pedals. However, energy has to be conserved, and because mechanical work is defined as *force* × *displacement*, an increase in force can only be obtained at the expense of displacement. Thus, although shifting gears allows one to develop the additional force needed to go uphill, speed is reduced as each pedal stroke produces a smaller turn of the wheels. Cells have similarly developed microscopic force amplification strategies during evolution. Here, we discuss some amplification schemes for one of the major force generators in the cell—actin polymerization.

Actin plays a ubiquitous role in cell motility and morphogenesis, spanning many scales of space and time. In fission yeast, for example, a miniature actin machinery only ∼100 nm across can induce the invagination of an endocytic vesicle in just a few seconds (Picco et al., 2015). However, to sever the entire yeast cell, a cytokinetic ring forms with an initial perimeter of ∼10 µm and requires ∼30 min to drive division (Proctor et al., 2012). These assemblies differ dramatically in both size and duration. In other species, considerably larger actin assemblies exist that reach the scale of centimeters, such as in muscle cells. Clearly, actin and its associated factors need to be specifically organized to achieve these different functions (Fig. 1). From a functional point of view, a key problem is to understand how the global architecture of an actin network allows forces that are produced at the molecular scale to be productive for the cell. In this respect, we can distinguish two sorts of components. Active components generate forces from chemical sources of energy and include molecular motors, as well as actin itself, which can push by polymerizing (Kovar and Pollard, 2004) and possibly pull while depolymerizing. Passive components, such as actin cross-linkers, are essential but can only transmit forces generated by other elements.

The forces developed by an actin meshwork are determined by the organization of its components. Ultimately, these forces must be sufficient to drive biological processes, and thus their scale depends on the physical characteristics of the cell. For example, in the case of endocytosis in yeast, the turgor pressure pushing the surface of the invagination outward reaches ∼1,000 pN, which the actin machinery must overcome (Basu et al., 2014). During cytokinesis, the actomyosin ring also works against the turgor pressure, which produces high forces on the furrow (Proctor et al., 2012). For both cases, these forces have been calculated from measured cellular parameters, particularly the turgor pressure and the dimensions over which the membrane is deformed. Hence, for these processes at least, the two ends of the problem are known: the forces produced by the molecular components make up the input and the force required for the cellular process to occur represents the output. Yet the force balance within the system must be considered to understand how the actin machinery harvests the input to produce this output.

In this comment, we focus on the transmission of forces produced by the polymerization of actin, setting aside turnover and the contribution of molecular motors. We discuss specifically how the arrangement of the filaments in the system regulates the amount of productive force. In many ways, the actin machinery behaves analogously to a cyclist: though its power is limited, it can "shift gears" to favor either more displacement (high gears) or more force (low gears).

The force generated by actin polymerization

Actin polymerization can produce force. Indeed if an actin monomer in solution binds the barbed end of a filament, there is a change of free energy (ΔG_p) and polymerization will occur if $\Delta G_p < 0$ (Fig. 2 A). This reaction depends on the concentration (C) of monomeric actin and will take place only above a critical concentration (C* of ∼0.14 µM; Table 1; Pollard, 1986). It is associated with $\Delta G_p = -k_B T \ln(C/C^*)$, where k_B is the Boltzmann constant and T is the absolute temperature. If actin is polymerizing against a load and producing work (W), the change in free energy is $\Delta G_p + W$. In this case, polymerization will occur spontaneously if the change is negative, i.e., $\Delta G_p + W < 0$. Consider an actin filament pushing against a force (f) applied parallel to the filament axis (Fig. 2 B). Because the addition of one actin monomer produces a displacement (δ = 2.75 nm; Table 1; Holmes et al., 1990), the mechanical work is $W = f \times \delta$. Forces that are antagonistic to elongation can impede actin assembly (Peskin et al., 1993). The critical force under which the filament would cease to elongate is called the polymerization force (f_a). Using a physiological concentration (C of ∼40 µM; Wu and Pollard, 2005), the polymerization force is thermodynamically limited

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The Rockefeller University Press $30.00
J. Cell Biol. Vol. 212 No. 7 763–766
www.jcb.org/cgi/doi/10.1083/jcb.201512019

THE JOURNAL OF CELL BIOLOGY

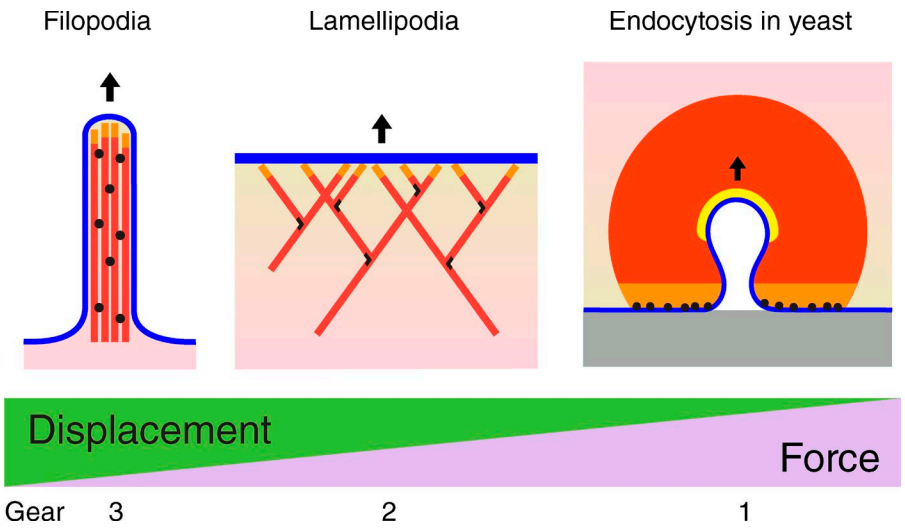

Figure 1. **Different actin networks.** Networks of actin filaments are essential for many biological processes at the cellular level, and the organization of the filaments in space must be adapted to the task. Here, polymerization force (orange) of actin filaments (red) occurs near the plasma membrane (blue). Linear filopodia bundles with fascin (black) can produce high speeds, but represent a weak configuration for force generation. Lamellipodia are thin cellular extensions in which filaments are nearly parallel to the substrate on which the cell is crawling. The 2D branched network, created by Arp2/3 actin-nucleating complexes (black), can produce higher forces at the expense of displacement. During endocytosis in yeast, actin forms a 3D network at the site of the invagination that appears roughly spherical, but the organization of actin filaments in space is not known. The coat structure (yellow) enables actin to pull the membrane inward and actin polymerizes near the base of the structure, where Arp2/3 nucleators are shown in black (Picco et al., 2015). Endocytosis requires strong force amplification to pull the invagination against the turgor pressure.

to $k_BT \ln(C/C^*)/\delta = \sim 9$ pN (Hill, 1981). Within such limits, the force developed by polymerization will depend on the conditions of assembly. Direct measurements of the polymerization force using single-molecule techniques are scarce. A first study used optical traps on bare filaments, giving a force of ~1 pN (Footer et al., 2007). By monitoring the buckling of filaments capped with formins, a second study found the force to be ~1.3 pN (Kovar and Pollard, 2004). In both cases, the concentration of actin was an order of magnitude lower than in vivo, and the measured forces were in fact close to the theoretical maximum under the experimental conditions. Here, we will thus consider that f_a is within 1 and 9 pN. We further assume that an actin filament is able to elongate as long as the parallel component of the antagonistic force at its barbed end remains lower than f_a, irrespective of the perpendicular components (Fig. 2 C). We discuss various examples of force amplification in which the network develops forces that exceed f_a per filament, without breaking the thermodynamic requirement for actin polymerization ($\Delta G_p + W < 0$).

The high gear: actin pushing forward

A clear example of pushing by actin is found in filopodia (Fig. 1), which are thin tubular actin-rich cytoplasmic projections extending forward and orthogonally to the leading edge of motile cells. Extending a filopod should require a force (F) >10 pN (Mogilner and Rubinstein, 2005) to overcome membrane tension and rigidity. In a filopod, actin is organized as a bundle of n parallel filaments. If the load is distributed over all barbed ends, then each end sustains a fraction of the total force (F/n). Extension will then be possible only if the polymerization force is larger than the fraction of force experienced by each filament ($F/n < f_a$) and thus requires sufficient barbed ends to distribute the force. Therefore, ten filaments are theoretically sufficient to extend a filopod. This quasi 1D organization maximizes growth speed for a given amount of added monomers; i.e., it is the highest gear of the actin machinery. Assembling more filaments can increase the force, but because the molecular forces are always equal to the productive force, there is no mechanical amplification.

Intermediate gears: actin pushing with an angle

In lamellipodia, actin filaments form a branched meshwork rather than a bundle. If each filament can produce the same amount of force parallel to its axis, the push on the membrane can be higher as a result of the contact angle (usually $\theta = \sim 54°$) at which actin filaments encounter the membrane (Fig. 2 D). A force f_a parallel to the axis of a filament corresponds to a proportional force perpendicular to the membrane ($f_a/\sin\theta$). The total pushing force (F) on the membrane, then, is the sum of such perpendicular forces applied by n filaments ($F = n \times f_a/\sin\theta$). Because $\sin(54°) < 1$, the productive force is increased. This occurs at the detriment of displacement achieved by each actin monomer, which is also proportional to the contact angle ($\delta \times \sin\theta$). Importantly, the contact angle is not solely determined by the branching angle imposed by Arp2/3, the primary nucleating complex for branched actin filaments, because the branched network can adopt different orientations with respect to the leading edge (Weichsel and Schwarz, 2010). Thus, this quasi-2D system works like a gearbox, where the coefficient ($\sin\theta$) can vary, allowing a lamellipod to generate nanonewton scale forces (Prass et al., 2006).

This idea can be extended to other architectures with various amplification factors. Consider, for example, the configuration illustrated in Fig. 2 E, in which two asymmetrically branched filaments engage the membrane, but only the long branch polymerizes whereas the short branch provides support by transmitting force between the membrane and the filament network. Upon polymerization, the whole construction rotates around a pivot point at the base of the supporting branch, and the contact angle of the polymerizing filament becomes shallower in comparison to the symmetrically polymerizing configuration. Strikingly, this configuration can develop more force than the symmetric case, as an additional amplification $(x + y)/x$ is associated with the lever arms (compare Fig. 2, D and E). This illustrates that the network force is not solely proportional to the number of polymerizing barbed ends. The geometry of the system, particularly the angle at which the filaments contact the membrane, and the lever arms can further affect and amplify the total forces generated by the network.

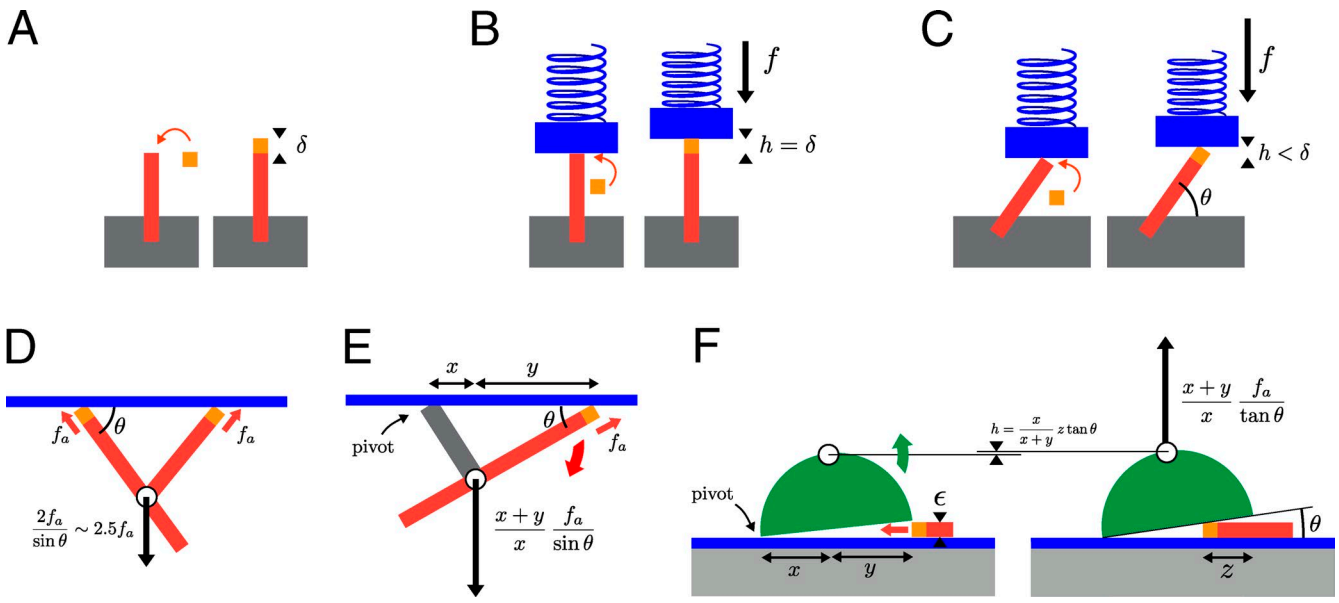

Figure 2. **Polymerization mechanics.** (A) During polymerization, the addition of one actin monomer (orange) corresponds to an elongation (δ) at the barbed end of an actin filament (red) and is associated with a change of free energy ($\Delta G_p = -k_b T \ln(C/C^*)$). (B) The work required to push a load over a distance (h) with a force (f) is f × h, and thus assembly remains favorable as long as $\Delta G_p + f \times h < 0$. In the case where polymerization occurs straight against a load (h = δ), the maximal force (f_a) is $f_a = k_b T \ln(C/C^*)/\delta$ (Hill, 1981). (C) If the filament encounters the load with an angle (θ), then h = δ sinθ and the maximal force is consequently increased: $f_\theta = f_a/\sin\theta$. (D) In the branched network of a lamellipod, actin grows against the leading membrane at an angle (θ = ∼54°). In the absence of friction, the force between the polymerizing tip (orange) of the actin and the membrane (blue) is perpendicular to the membrane. It can then reach a maximum magnitude of $f_a/\sin\theta$. The sum of the forces produced by the two filaments is then ∼2.5 f_a. (E) Higher forces arise by polymerizing with shallow angles. The device illustrated here is composed of a growing actin filament with a "leg" on its side. By elongating, the filament will induce rotation around the pivot point, where the leg is contacting the membrane. High forces can be exerted on a load supported at the branch point, as a result of the amplification achieved by the lever arm and contact angle. (F) The highest forces are generated if a filament polymerizes parallel to the surface. In the illustrated configuration, elongation of the filament will cause a load (green dome) to separate from the membrane. The maximal force is calculated as in E, except that anchoring has to be assumed at the pivot point to balance forces horizontally. The device can sustain high forces applied on the top of the dome because the upward movement is small compared with the elongation of the filament.

The low gear: actin like a wedge

To interpret in vitro experiments in which actin polymerizes around beads (Achard et al., 2010; Démoulin et al., 2014), it has been suggested that resistance from a load could cause actin to polymerize parallel to the surface. In this simple configuration, a filament is confined between a base and a load, which is pushed upward as the filament grows (Fig. 2 F). The upward displacement of the load is determined by the thickness of the actin filament (ε) and by the lever arms *x* and *y*, relative to the pivot point. The result is nearly identical to the configuration in Fig. 2 E, but the new device offers better performance; whereas the long filament in Fig. 2 E can bend all the more as it elongates, this configuration works well even with flexible filaments. In the geometry suggested by Fig. 2 F, the load is lifted by the filament thickness once the filament has polymerized over the entire base. In a more realistic 3D network, the relationship between polymerization and displacement will not be as simple, because the arrangement of filaments in 3D networks is intricate. Nevertheless, the mechanical concepts remain valid and, in particular, polymerization parallel to a surface could lead to strong orthogonal forces. In yeast endocytosis, actin polymerizes at the bottom of the network in a configuration resembling the wedge (Picco et al., 2015). This may perhaps resolve the apparent mismatch between the number of polymerizing filaments and the force resulting from pressure (Basu et al., 2014). The force generated by the network depends critically on the network architecture, as this determines the constraints under which filaments grow (Carlsson and Bayly, 2014). In general, the force that can be exerted on a load will also depend on the mechanics of the entire structure. Network elasticity allows the polymerization force to be stored as stress, whereas stress relaxation by disassembly and turnover will decrease the force the network can exert (Zhu and Mogilner, 2012).

Conclusion

In 1D structures, such as filopodia, force balance forbids mechanical amplification; however, in 2D structures, the contact angle between the barbed end and the membrane provides a

Table 1. **Physical characteristics of actin**

Characteristic	Measurement	Reference
Length increment per actin monomer	δ = 2.75 nm	Holmes et al., 1990
Diameter of filamentous actin	ε = 7–9 nm	Holmes et al., 1990
Polymerization force of actin	f_a between 1 and 9 pN	See Fig. 2
Concentration of actin monomers	C = ∼15–500 µM in nonmuscle cells; C = ∼30–60 µM in fission yeast	Wu and Pollard, 2005; Footer et al., 2007

mechanism for tradeoff between force and displacement, and thus allows for force amplification. Configurations in which filaments grow parallel to the membrane, and thus act like wedges, produce the highest forces. Of course, energy conservation dictates that displacement is reduced as force is increased, such that there is a "cost" for force amplification.

A key parameter of our considerations is the force that a polymerizing actin filament can support (f_a). Energetic consideration provides an upper bound of ∼9 pN, but so far direct measurements have yielded lower values, around 1 pN. Thermal fluctuations provide a scale to which this can be compared. At a given temperature (T), the characteristic energy associated with thermal fluctuations is k_BT, where k_B is the Boltzmann constant; at room temperature, the associated force (k_BT/δ) corresponds to 1.5 pN. Hence, if f_a is truly ∼1 pN, it would imply that actin polymerization is hardly more efficient than thermal fluctuations. It is to be hoped that future experimental studies, possibly closer to in vivo conditions, will reveal higher forces, as it would be truly astonishing if actin used only 10% of the available energy.

In conclusion, the architecture of a network determines the productive force, often in a nonintuitive manner. Hence, once a system has been well characterized experimentally, mechanical theory should be used to balance the forces within the network. When this cannot be done, energetic considerations, in which the mechanical work of the forces are summed and compared, are informative. A thorough analysis of force transduction in the system makes it possible to predict the most efficient architecture for performing a given task (Ward et al., 2015), which is of outstanding value when comparing different modus operandi across species.

Acknowledgments

We thank Andrea Picco, Markus Mund, Marko Kaksonen, Jonas Ries, Ulrich Schwarz, Peter Lenart, Marija Burdyniuk, Karin Sasaki, and Laurent Blanchoin for critically reading the manuscript.

This work was funded by the European Molecular Biology Laboratory, Center for Modelling and Simulation in the Biosciences, and European Commission under FP7 grant agreement number 258068.

The authors declare no competing financial interests.

Submitted: 4 December 2015
Accepted: 17 February 2016

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