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 \( \text{force} \times \text{displacement} \), 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 \( \sim 100 \text{ nm} \) across can induce the invagination of an endocytic vesicle in just a few seconds (Pico et al., 2015). However, to sever the entire yeast cell, a cytokinetic ring forms with an initial perimeter of \( \sim 10 \text{ µm} \) and requires \( \sim 30 \text{ min} \) 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 \( \sim 1,000 \text{ pN} \), 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 (\( \Delta G_p \)) 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 \( \sim 0.14 \text{ µM} \); 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 (\( \delta = 2.75 \text{ nm} \); 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_0). Using a physiological concentration (C of \( \sim 40 \text{ µM} \); Wu and Pollard, 2005), the polymerization force is thermodynamically limited.

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we will thus consider that f_a is within 1 and 9 pN. We further theoretical maximum under the experimental conditions. Here, in vivo, and the measured forces were in fact close to the the- concentration of actin was an order of magnitude lower than tion force using single-molecule techniques are scarce. A first addi- tion of assembly. Direct measurements of the polymeriza- tion force developed by polymerization will depend on the con- 

Assume that an actin filament is able to elongate as long as end remains lower than f_a, irrespective of the perpendicular component of the antagonistic force at its barbed end. 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.

**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 θ ≈ 54°) at which actin filaments encounter the membrane (Fig. 2 D). A force f_p parallel to the axis of a filament corresponds to a proportional force perpendicular to the membrane (f_p/sinθ). The total pushing force (F) on the membrane, then, is the sum of such perpendicular forces applied by n filaments (F = n × f_p/sinθ). 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 (θ × sinθ). 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 (sinθ) 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.
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 ($\epsilon$) 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

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<th>Characteristic</th>
<th>Measurement</th>
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<tr>
<td>Length increment per actin monomer</td>
<td>$\delta = 2.75$ nm</td>
<td>Holmes et al., 1990</td>
</tr>
<tr>
<td>Diameter of filamentous actin</td>
<td>$\epsilon = 7–9$ nm</td>
<td>Holmes et al., 1990</td>
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<td>Polymerization force of actin</td>
<td>$f_a$ between 1 and 9 pN</td>
<td>See Fig. 2</td>
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<td>Concentration of actin monomers</td>
<td>C = $\sim$15–500 µM in nonmuscle cells; C = $\sim$30–60 µM in fission yeast</td>
<td>Wu and Pollard, 2005; Footer et al., 2007</td>
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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 $\sim 9$ 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 $k_B T$, where $k_B$ is the Boltzmann constant; at room temperature, the associated force ($k_B T/\delta$) corresponds to $1.5$ pN. Hence, if $f_a$ is truly $\sim 1$ 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 thermal fluctuations. It is to be hoped that future experimental studies, possibly closer to in vivo conditions, will reveal higher thermal fluctuations. It is to be hoped that future experimental studies, possibly closer to in vivo conditions, will reveal higher thermal fluctuations.

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.

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