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Mechanosensation: Capping actin filaments for robustness

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Actin networks adapt to resistance by becoming denser. A recent investigation shows that this mechanosensation relies on a force-sensitive mechanical ratchet of capping actin filaments to reorganize the network. This and other mechanical feedback mechanisms make actin-based protrusion amazingly robust.

Cells are first and foremost chemical machines, but they are also mechanical, both responding to and generating forces. Mechanosensation has preoccupied cell biologists over the last two decades[1], but understanding respective mechanisms has been slow, in large part due to complex intertwined contributions from adhesion, membrane, and cytoskeletal dynamics. Even a much simpler question of how growth and protrusion of *in vitro* actin networks respond to force has been a technical challenge[2–4]. A long-haul effort of the Mullins and Fletcher labs[3,5,6] has now culminated in an elegant demonstration in a recent paper in *eLife*[7] that capping of actin filaments senses force through the same ratchet mechanism that regulates actin network growth, moving us very close to the answer.

One of the most ubiquitous and important types of actin mesh is a branched network (Figure 1), in which actin filaments elongate until their barbed ends are capped by capping proteins. Capped filaments fall behind the network's leading edge and growing barbed ends at the edge are replenished by a complex branching process that requires activation of Arp2/3 complex by a nucleation promoting factor (NPF) bound to the surface (*in vivo*, the membrane) against which the network is growing. Early models, analyzing the conceptually simple equilibrium between nucleation/branching and capping, identified a paradox: as the number of leading barbed ends increases, there are more filaments to which activated Arp2/3 complexes can bind and nucleate even more filaments. This autocatalytic branching[8] cannot be limited by a constant (per filament) capping rate, but how can the capping rate be anything but a constant considering the simple nature of the capping reaction?

One possibility is that something limits the nucleation rate. In the new study, Li *et al.*[7] reconstituted a column of actin growing *in vitro* from a patch coated with NPFs, applied a compressing force to the column by an atomic force microscopy cantilever, and measured the densities of actin, Arp2/3, and capping protein in the network, and growth velocity at different forces. The growth velocity, as expected, was slowed down by force, whereas all three densities increased with force. The net nucleation rate (the number of nascent actin filaments produced per second per square micron of the surface) is proportional to flux, the product of the network's density and velocity. Plotting this product showed that it is a decreasing function of force because velocity decreased with force more quickly than density increased.

But how could the nucleation rate decrease with force while density increases? According to the balance between net capping and nucleation rates, the only answer was that the capping rate decreased with force even more quickly than the nucleation rate. In fact, plotting capping and elongation rates as functions of the force per filament showed that both rates were proportional to each other and exponentially decreased with force. The fact that growth velocity decreased exponentially with force per filament was expected from ratchet models[9,10]: growing filaments thermally tremble, creating small gaps between their tips and the surface being pushed (Figure 1A). The greater the force, the smaller the gaps, and the harder it is for an actin monomer to squeeze into the gap and elongate the filament. However, how could the capping reaction sense force in the same way? Li *et al.*[7] hypothesized that the very same ratchet mechanism limits capping: as with actin monomers, capping protein can only squeeze into the random gap between the barbed end and the surface if the size of the gap is larger than capping protein (Figure 1A). Capping protein is roughly the same size as an actin monomer, which explains why the elongation and capping rates are exactly proportional to each other. The proof of this hypothesis is the most powerful part of the paper: Li *et al.*[7] engineered a larger form of capping protein with the same capping kinetics as the native protein and demonstrated that, as force increased, a relatively smaller fraction of these larger, synthetic capping proteins appeared in the network: according to thermodynamics, the probability of the larger gaps needed for the synthetic capping protein to cap the filament tips decreases with force more quickly than the probability of the smaller gaps necessary for native capping protein to cap the barbed ends. Thus, the network density is increased at greater force by a counter-intuitive mechanism — by decreasing the capping rate, instead of increasing nucleation rate. This mechanism is also purely physical and therefore likely to be universal.

To find out why nucleation decreased with force, Li *et al.*[7] counted Arp2/3-mediated branching events and observed that a fraction of nascent branches broke off and failed near the surface (Figure 1A) and that this fraction increased with force. Even more significant insights were gleaned from using a cocktail of drugs to fix the number of stable uncapped filaments

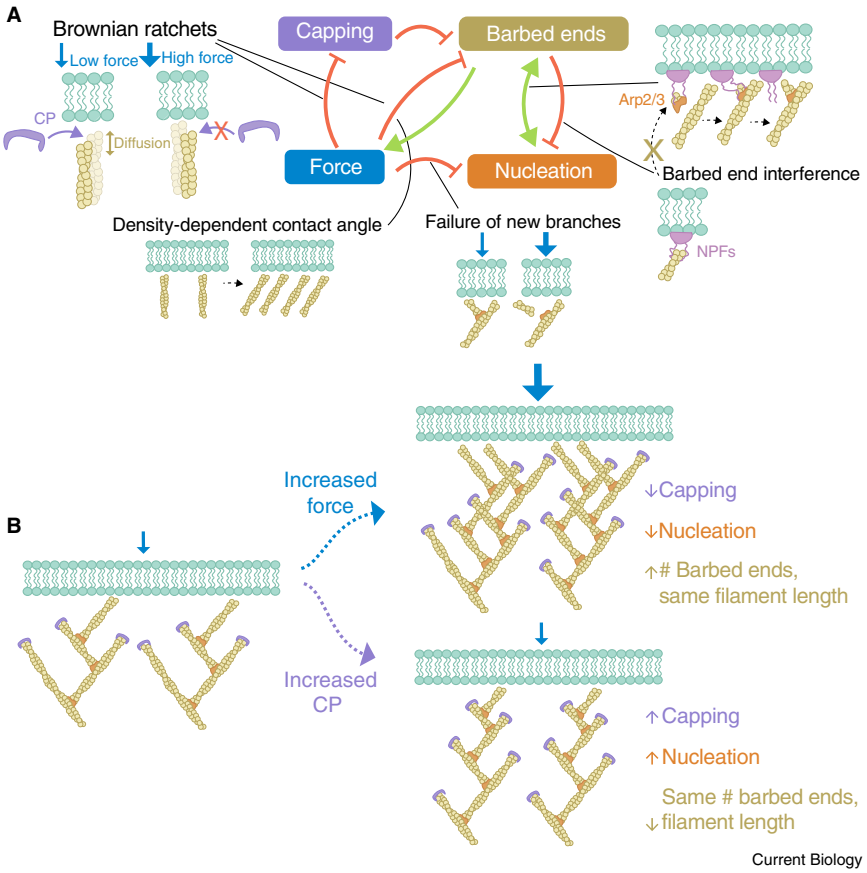

Figure 1. Multiple mechanical feedbacks make actin-based protrusion robust.
(A) The main discovery of the new work by Li *et al.*[7] (left) is that the load force makes it harder for capping protein (CP) to squeeze into the gap between the growing actin filament and the loaded surface, so the capping rate goes down with force, prolonging the 'life' of pushing filaments and increasing their density. Another finding reported by Li *et al.*[7] (right) is that transient binding of the growing filament tips to NPFs competes with the NPF nucleation activity, so increasing the number of growing filaments damps the branching of new filaments. This negative feedback counters the autocatalytic property of branching (double arrow). Two additional feedback mechanisms are shown at the bottom: the force breaks some of the nascent branches, creating a direct negative effect of force on actin density; and the force causes geometric reorganization of the actin network, effectively bending the filaments away from the direction of protrusion. The positive/negative mechanical feedback mechanisms are shown as arrows with pointed/blunt ends, respectively. (B) Two examples of the mechanosensing response. (Top) When the network is resisted by greater force, nucleation decreases moderately, but decreasing capping rate is a dominant factor, and on balance the number of the growing barbed ends pushing against the surface increases. The average filament length is unchanged because, despite filaments growing longer before getting capped, the growth is slower because load force per filament increases. (Bottom) When the concentration of the capping protein increases, the filaments become shorter, and capping reduces their number; however, fewer filaments means less interference with NPFs, therefore nucleation rate goes up and restores the filament density.

and measuring the frequency of interactions of actin monomers with NPFs. It turned out that a negative feedback mechanism operates at the level of barbed end interference (Figure 1A): transient binding of the barbed ends to NPFs means that these NPFs cannot be involved in branching, so increasing filament density attenuates the NPF nucleation activity.

Altogether, the findings from this[7] and previous studies[3,5,6] reveal a complex web of mechanochemical feedback mechanisms (Figure 1A) that collectively make the protruding branching actin network remarkably robust. Indeed, at larger forces, filaments grow more slowly, but they also show a proportional reduction in capping rate, so the average filament length becomes force independent (Figure 1B), which Li *et al.*[7] confirmed experimentally. Keeping filament length constant is very important for effective pushing: it cannot be too short because short filaments are too stiff and do not bend enough to create gaps allowing monomer assembly onto the barbed ends[10], and it also cannot be too long because then filaments bend too much and start growing along the surface rather than pushing it[10]. Note that this length robustness works only if the capping protein and monomer sizes are the same, hinting at evolutionary pressure on capping protein structure, considering the likely universality of the force–capping feedback[7]. Another demonstration of robustness is that the network density (and growth velocity) is not very sensitive to capping protein concentration (Figure 1B): elevated capping tends to decrease the filament number, but this weakens the barbed end interference feedback, thereby restoring filament density. The concept that robustness of biochemical and genetic regulation networks relies on the coordination of multiple feedback mechanisms is well known[11]; it now seems likely that mechanochemical networks following similar principles evolved to ensure the robust mechanical properties of the cytoskeleton.

The feedback mechanisms operating within the actin leading edge are not even limited to the ones investigated by Li *et al.*[7]. As was first proposed by Maly and Borisy[12], barbed ends that are not contacting the surface are capped much more quickly than actively pushing filaments. Their idea from 20 years ago is extremely close to the main idea put forward by Li *et al.*[7]. For filaments to 'survive', their growth must keep up with the surface. Slowing of protrusion that results from greater load allows filaments growing more parallel to the surface to catch up with the surface; thus, the geometry of the actin network is force sensitive (Figure 1A), making the protrusion robust in a subtle way. This effect was observed at the lamellipodial protruding edge of keratocytes[13]. Other feedback mechanisms include 'monomer gating' (faster capping redirects monomers to NPFs, thereby accelerating branching)[5], 'funneling' (faster capping redirects monomers to growing barbed ends, thereby accelerating protrusion)[14], 'local monomer depletion' (slower capping increases the density of growing barbed ends, which consume more monomeric actin, in turn locally depleting the concentration of actin monomers and slowing down protrusion)[15,16].

Over three decades, the saga of actin-based protrusion has become one of the most exciting and consequential stories of quantitative cell biology, and it is by no means over. The particularly quantitative nature of this field emerged early[17]. In keeping with this tradition, Li *et al.*[7] used mathematical modeling effectively and measured nearly all model parameters. Now the field is ready to harness this quantitative knowledge to start answering the following questions: how do the kinetics and geometry of the growing actin leading edge determine bulk mechanical properties of the actin network and how does force-induced mechanical recoil of this network contribute to the quality of the protrusion[2,3]? How are all of the feedback mechanisms integrated at the leading edge of flat, protruding lamellipodia[13,16,18,19]? More importantly, how are they integrated within more geometrically and mechanically complex 3D actin networks at the front of protrusions of cells crawling in extracellular matrix[20]? Above all, how can cells switch between different types of actin network architecture and dynamics needed for different tasks (e.g., endocytosis and migration) if the combination of feedback mechanisms makes the network so robust[4]?

DECLARATION OF INTERESTS

A.M. is a member of the journal's advisory board.

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Photosynthesis: Compatibility between incompatible pathways explained

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A recent study has overturned a long-held view that two distinct modifications to photosynthesis are incompatible. The findings provide interesting new insights into trade-offs associated with photosynthetic metabolism, as well as likely routes by which evolution altered one of the most fundamental processes in biology.

When plants moved onto land, they inherited a photosynthetic system first elaborated in photosynthetic bacteria. Their biochemistry allows CO_2 to be fixed and generates the three-carbon compound phosphoglyceric acid, and so these plants became known as C_3 plants. However, at higher temperatures, or when CO_2 supply is limited, this process becomes less efficient, and so many lineages of plants evolved so-called carbon-concentrating mechanisms