Communicative & Integrative Biology 6:6, e27341; November/December; © 2013 Landes Bioscience

The sense is in the fingertips

The distal end controls filopodial mechanics and dynamics in response to external stimuli

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Small hair-like cell protrusions, called filopodia, often establish adhesive contacts with the cellular surroundings with a subsequent build up of retraction force. This process seems to be important for cell migration, embryonic development, wound healing, and pathogenic infection pathways. We have shown that filopodial tips are able to sense adhesive contact and, as a consequence, locally reduce actin polymerization speed. This induces filopodial retraction via forces generated by the cell membrane tension and by the filopodial actin shaft that is constantly pulled rearwards via the retrograde flow of actin at the base. The tip is also the weakest point of actin-based force transduction. Forces higher than 15 pN can disconnect the actin shaft from the membrane, which increases actin polymerization at the tip. Together, this points toward the tip as a mechano-chemical sensing and steering unit for filopodia, and it calls for a better understanding of the molecular mechanisms involved.

Keywords: filopodia, lamellipodia, retrograde flow, membrane tension, pulling force, cytoskeleton

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Submitted: 11/19/2013

Accepted: 11/26/2013

Citation: Bornschlögl T, Bassereau P. The sense is in the fingertips: The distal end controls filopodial mechanics and dynamics in response to external stimuli. Communicative & Integrative Biology 2013; 6:e27341; http://dx.doi.org/10.4161/cib.27341

Addendum to: Bornschlögl T, Romero S, Vestergaard CL, Joanny J, Van Nhieu GT, Bassereau P. Filopodial retraction force is generated by cortical actin dynamics and controlled by reversible tethering at the tip. Proc Natl Acad Sci U S A 2013; 110:18928-33; PMID:24198333; http://dx.doi.org/10.1073/pnas.1316572110

Filopodia can be found in a variety of different cell types where they often create adhesive contact with their environment, both in vivo and in vitro.[1-3] These structures have the ability to pull on the surrounding environment, which was first observed more than 20 y ago in filopodia protruding from neuronal growth cones.[4] The ability to create adhesive contacts followed by force exertion seems to play an important role in embryonic development,[5,6] wound healing,[7] pathogenic infection pathways,[8,9] and during cell migration.[10,11] Although we have a relatively good understanding of the basic molecular components that are involved in filopodial formation and growth,[12,13] filopodial mechanics and especially how they sense adhesive contacts and generate retraction forces is not well understood.[3] There are 2 main sources that can account for rearward force generation in filopodia. First, the cell membrane tension tends to retract the membrane tube surrounding the filopodial actin shaft. This force should depend on the radius of the filopodium,[14] but since filopodia show radii similar to empty membrane tubes directly pulled from the cell membrane,[15] one would expect membrane forces on the order of 5–30 pN.[16] Second, filopodia could use the rearward flux of actin in its shaft to apply retraction forces. This retrograde flow is present in lamellipodia of different cell types, where it is driven by contractile forces of molecular motors in the rear of the lamellipodium together with the push of actin against the cell membrane due to addition of new actin monomers at the front.[17-19] In growth cones, similar speeds of retrograde flow were measured inside filopodia and in the dendritic network adjacent to the filopodial base,[20-22] suggesting a strong interconnection and a high friction between both actin networks. We observed a similar dynamics of both actin networks in HeLa cells, further supporting the idea that retrograde flow in the lamellipodium drives the flow in the filopodium.[23] This could be a common mechanism among different cell types,[3,24] and it would explain the high pulling forces of

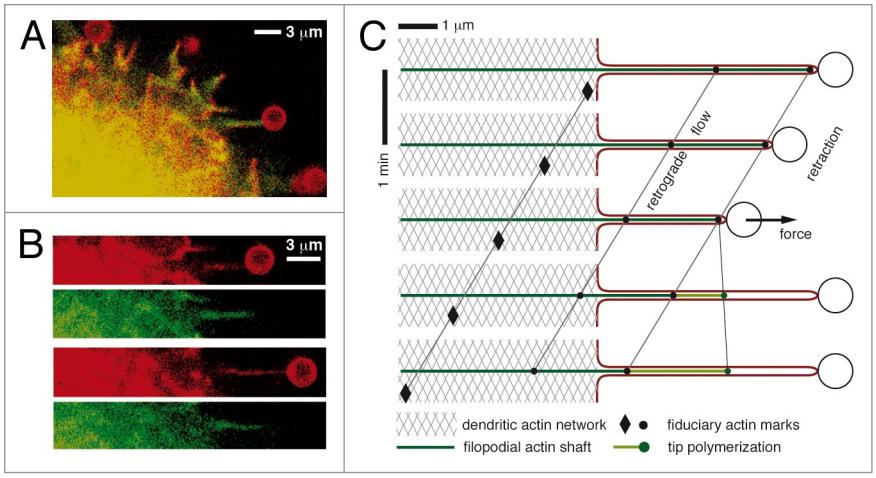

Figure 1. (**A**) Merged confocal image of a filopodium in contact with an optically trapped bead. Red shows the fluorescence of a membrane marker, green of fascin-GFP (actin marker). (**B**) Snapshots of the different color channels before forced elongation of the filopodium (upper 2 images) and after (lower 2 images). (**C**) Schematics of filopodial dynamics. Retrograde flow with similar speeds is observed in the dendritic actin network of the lamellipodium and the filopodial actin shaft. Retrograde flow velocity can be measured, e.g., by detecting the motion of bleached areas on fluorescent actin used as fiduciary marks. After adhesion of a bead to the tip, actin polymerization is locally reduced (not necessarily zero, as shown in the example) leading to filopodial retraction. External force application disconnects the actin shaft from the membrane, which induces enhanced actin polymerization at the tip.

1 nN observed for filopodia.[4,25] How the friction coefficient between lamellipodial and filopodial actin networks depends on the network organization at the filopodial base still needs clarification. For example, one could imagine a higher friction coefficient for filopodia rooted in the broad dendritic actin network of lamellipodia as compared with filopodia rooted in a thin cell cortex.[26] Not much is known about the ultrastructure and the dynamics of the cell cortex,[26,27] but its small size and a more direct interaction of molecular motors might lead to a distinct actin-based force application at filopodial roots as it was proposed for macrophages.[28]

In the situation of continuously rearward flowing actin, the actin polymerization speed at the tip becomes an important regulator for filopodial dynamics. In growth cones, most changes from filopodial retraction to elongation are due to changes in the actin polymerization speed at the tip.[22] We observed a similar behavior in unbound filopodia of HeLa cells,[23] also pointing toward the tip as a regulator for filopodial dynamics in this distinct cell line.

Since the tip is often the first to make contact with distant objects, a fast way to respond on detected signals such as adhesion would be the direct control of local actin polymerization speed. To test this idea, we approached optically trapped beads to the tip of a filopodium (**Fig. 1A-C**), while simultaneously measuring the retrograde flow of actin. With this approach we showed that non-specific adhesion leads to a local reduction of actin polymerization at the tip and thereby to filopodial retraction (**Fig. 1C**). Most interestingly, besides detecting adhesion, the tip can also sense if the actin shaft becomes mechanically disconnected from the membrane. By applying forces up to 50 pN that are only slightly higher than the membrane force of 15 pN, we could induce such a mechanical disruption (**Fig. 1B**). This demonstrates that the connection between membrane and actin core is fragile compared with the force produced by the retrograde flow of actin.This rupture was in all probed cases (n = 16) followed by an increase in actin polymerization at the tip that allows the filopodial actin shaft to keep its length (**Fig. 1C**), or in some cases (n = 6) to even reconnect to the membrane and pull again. Thus the filopodial tip changes actin dynamics upon mechanical stimuli and could also control forces exerted to the substrate by controlling the strength of actin–membrane links.

Moving forward, it is important to develop a molecular level understanding of how the tip senses chemical and mechanical signals and how it subsequently translates them into a coordinated cytoskeletal response. The filopodial tip can contain many actin – and membrane-related proteins,[13] and formins are among the best-studied ones.[1] Recently, these proteins have been shown to processively polymerize actin in a force-dependent manner, speeding up if pulling forces up to 3 pN are applied.[29] Although not known yet, a similar mechanism might also occur during actin polymerization mediated via VASP, another filopodial tip protein that can processively polymerize actin filaments when clustered.[30] However, until now, no acceleration of elongating, non-ruptured filopodia was observed when pulling forces were increased.[23,31] This could be due to the large variation in observed elongation speeds, or due to other proteins such as myosin X[32] or cofilin[33] that have been observed at the tip and that might interfere with the force dependent elongation behavior of formins. Therefore, an important objective of future research is to carefully unravel how the different tip-proteins are involved in the filopodial response to external mechano-chemical stimuli and if different cell types use a different tip machinery for their specific tasks.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank S Romero, G Tran Van Nhieu, CL Vestergaard and J-F Joanny for the productive collaboration and stimulating discussions, and W Ahmed for proofreading. Bassereau P thanks the Agence Nationale de la Recherche (Grant 08-MIEN-011–02) for funding. Bornschlögl T thanks the Human Frontier Science Program for funding.

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