Filopodial focal complexes direct adhesion and force generation towards filopodia outgrowth

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Filopodia are key structures within many cells that serve as sensors constantly probing the local environment. Although filopodia are involved in a number of different cellular processes, their function in migration is often analyzed with special focus on early processes of filopodia formation and the elucidation of filopodia molecular architecture. An increasing number of publications now describe the entire life cycle of filopodia, with analyses from the initial establishment of stable filopodium-substrate adhesion to their final integration into the approaching lamellipodium. We and others can now show the structural and functional dependence of lamellipodial focal adhesions as well as of force generation and transmission on filopodial focal complexes and filopodial actin bundles. These results were made possible by new high resolution imaging techniques as well as by recently developed elastomeric substrates and theoretical models. The data additionally provide strong evidence that formation of new filopodia depends on previously existing filopodia through a repetitive filopodial elongation of the stably adhered filopodial tips. In this commentary we therefore hypothesize a highly coordinated mechanism that regulates filopodia formation, adhesion, protein composition and force generation in a filopodia dependent step by step process.

Cell protrusion depends on collaborative interactions of lamellipodia and filopodia. Although filopodia cannot drive cell migration alone, in contrast to lamellipodia, they are essential for many cell biological functions such as guidance of neuronal growth cones or during angiogenesis. Furthermore, filopodia are vital to cell-cell contact establishment as described for epithelial cells or during dorsal closure in Drosophila, and are also implicated in cancer cell metastasis. Lamellipodia as well as filopodia can be formed independently from each other, and recent results implicate independent basic mechanisms of cytoskeletal regulation for their formation. While lamellipodia protrusion is a well accepted Arp2/3-dependent process where actin branches constantly form the protrusive force at the leading edge of the lamella, the details of filopodia formation are far from being understood. Although earlier experiments indicated Arp2/3 was also involved in filopodia formation, recent results point to a machinery that is far less dependent, or even possibly independent, of Arp2/3 with formins being the central regulating molecules instead.

As soon as filopodia start to form, they constantly sense their environment upon elongation. Transmembrane proteins such as cadherins or integrins connect filopodia to surrounding cells, extracellular matrix, or even pathogens to form stable contacts. When filopodial adhesion fails, retraction takes place. Although integrins and talin have been shown to be initially present at these sites in an unclustered but active state, many additional adhesion proteins take part in filopodial focal complexes (filopodial FXs). Starting from a small VASP-containing adhesion spot at the tip of filopodia, proteins such as vinculin, paxillin, talin, tensin and even zyxin form an elongated

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Here, filopodial actin bundles were clearly shown to be the origin of nearly 85% of all actin bundles found in the lamella. These actin filaments typically pointed towards the direction of migration. Additionally, myosin II was associated with these filopodial derived actin filaments to form polarized actin bundles. Of equal importance are findings presented by Schäfer et al. in this issue. The authors analyzed the fate of stably adhered filopodia and identified a stepwise exchange of filopodial fascin against the actin cross-linker proteins palladin and α-actinin in areas where filopodia were just overgrown by the lamellipodial leading edge (schematically presented in Figure 1).

α-Actinin further induced incorporation of myosin II into filopodial actin bundles in the lamellipodium. The authors additionally found that FAs displayed an enhanced lifetime when adhered to these myosin containing actin filaments. Therefore, these findings could also explain the unusual stability of filopodial actin filaments in neuronal growth cones observed by Mallavarapu and Mitchison. For keratinocytes, filopodia-dependent actin bundles are the only myosin containing filopodial FXs were responsible for only a sub-fraction of FAs in fish fibroblasts. Stable FAs of human keratinocytes were formed almost exclusively by enlargement of existing filopodial FXs (see scheme, Fig. 1).

The structural dependency of lamellipodial complexes on filopodial protein aggregates could be also shown for actin bundles. Here, parallel oriented actin filaments become cross-linked by proteins such as fascin or IRSp53-Eps8-complex upon filopodia formation. These tightly packed bundles of 15–30 single actin filaments originate from the lamellipodial actin meshwork. Interestingly, filopodial actin bundles in turn also affect lamellipodial actin structures independent of whether the filopodium adheres in a stable manner or loses contact. Nemethova et al. described the contribution of non-adhering filopodia to the construction of concave bundles of actin filaments within the lamellipodium of fish fibroblasts. These bundles often extended in length and interconnected with adjacent bundles. Similar observations were found for fibroblasts of chicken embryos and neuronal growth cones. Here, filopodial actin bundles were clearly shown to be the origin of nearly 85% of all actin bundles found in the lamella. These actin filaments typically pointed towards the direction of migration. Additionally, myosin II was associated with these filopodial derived actin filaments to form polarized actin bundles. Of equal importance are findings presented by Schäfer et al. in this issue. The authors analyzed the fate of stably adhered filopodia and identified a stepwise exchange of filopodial fascin against the actin cross-linker proteins palladin and especially α-actinin in areas where filopodia were just overgrown by the lamellipodial leading edge (schematically presented in Fig. 1). α-Actinin further induced incorporation of myosin II into filopodial actin bundles in the lamellipodium. The authors additionally found that FAs displayed an enhanced lifetime when adhered to these myosin containing actin filaments. Therefore, these findings could also explain the unusual stability of filopodial actin filaments in neuronal growth cones observed by Mallavarapu and Mitchison. For keratinocytes, filopodia-dependent actin bundles are the only myosin containing...
actin structures oriented in the direction of movement within the lamellipodium and the lamella. Sensitivity and resolution improvements in cell force analyses further proved that these actin bundles were responsible for almost the entire force transmitted from the lamellipodium of migrating keratinocytes to the substrate. These forces were transferred at FA sites emerging from filopodial FXs, proving the importance of filopodia in lamellipodial structures and functions. Although filopodia-independent adhesion sites are also formed in keratinocytes right behind the leading edge, these sites are neither connected to detectable actin filament bundles nor do they transmit significant forces (see scheme, Fig. 1). Consequently, their sizes and life spans are strongly reduced (Schäfer et al., this issue).

Recent results in keratinocytes additionally close the circle from stably adhered filopodia to the generation of new ones. Our original observations indicated that new filopodia were mainly formed in a direct extension of focal adhesions. Since these adhesion sites also depended on previously adhered filopodial FXs, a closer look revealed a consecutive outgrowth of the same filopodia. These cycles were only interrupted when outgrowing filopodia did not adhere in a stable manner between outgrowth cycles. Present results suggest that the same tip complex is present in all subsequently formed filopodia with a VASP tip signal remaining in place during successive filopodial elongations. As a result, well aligned, consecutive elongated focal adhesions can be found in keratinocytes. We can only speculate whether such an Arp2/3-independent mechanism describes a basic principle in filopodia formation at this point, but similar results have been observed for fish fibroblasts with a repetitive and alternating transition between filopodia and microspikes as filopodia-like structures barely extending over the lamellipodial leading edge.

The strong interdependency between lamellipodial FAs and stably adhered filopodia is also highlighted by actin retrograde flow analyses in keratinocytes (Schäfer et al. this issue). Retrograde actin flow is generated by actin polymerization at the cell front and myosin activity pulling the filaments rearwards. The interaction of actin with FAs is known to dampen flow rates in front of lamellipodial FAs. Furthermore, filamentous-actin dynamics measured in lung epithelial cells showed a fast retrograde actin flow at the leading edge compared to rates within the lamellae. The highest flow rates were in the range of 0.3–0.5 µm/min. Interestingly, keratocytes exhibited ten times slower flow rates at the leading edge, indicating that retrograde flow strongly depends on the cell type analyzed. Actin filaments polymerizing at the tips of filopodia also undergo retrograde flow, but these flow rates are much faster compared to those found in lamellipodia, as shown by bleaching experiments in chick embryo fibroblasts with flow rates approximately two-fold faster in filaments derived from filopodia compared to flow rates measured within the lamellipodium. These flow rates of approximately 1.3 µm/min were similar to those found for filopodia in other studies. Furthermore, we could show that this retrograde flow rate strongly depends on stable FAs formed behind the filopodium (Schäfer et al. this issue and Fig. 1). In the absence of these FAs, actin retrograde flow is doubled once more to rates of approximately 2.5 µm/min in filopodia. Therefore, although rates of FAs containing filopodia are still much higher than those found in lamellipodia, these rates are still slowed down indicating an effective connection between FAs and filopodial actin. These results further imply that myosin II incorporation into filopodial-originated actin bundles is responsible for enhanced retrograde flow rates in filopodia compared to rates found in the lamellipodium and that myosin II incorporation does not depend on stably adhered FAs directly behind filopodia. These data also strongly support the hypothesis that new filopodia form in front of stable lamellipodial FAs. It will be an intriguing question for future studies to analyze whether the reduced retrograde flow speeds in front of lamellipodial FAs might even be a prerequisite for efficient assembly and stable adhesion of small filopodial FXs, or perhaps even for filopodia formation in general.

Taking into account all the currently known functions of filopodia, the presented results finally indicate that filopodia might be characterized best not only by one but actually two main functions. The first function is environmental sensing. Various transmembrane proteins can be involved leading to various roles for filopodia such as formation of cell-cell or cell-matrix interactions. Although these functions in environmental sensing seem to be highly diverse, force generation along filopodial-originated actin bundles as the second function for filopodia might be of universal importance independent of the cell type that forms them. Force transmission along cell-pathogen interacting filopodia have been observed, and the formation of adherens junctions after filopodia mediated cell-cell interaction is also a cell force dependent process. Therefore, these observations fit well to the currently presented data by Schäfer et al. (this issue) proving the importance of filopodia-dependent cell matrix interactions in cell force generation in the direction of migration (see scheme, Fig. 1).

Present in almost every moving cell type, filopodia are therefore much more than just sensors for environmental conditions. In fact, these needle-like structures are the starting point for essential structures of adhesion and movement. Independent of whether they adhere stably or not, filopodia define the position of cellular adhesion sites, actin bundles, cell force generation and application, and, finally, the new filopodia to be formed.

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