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The filopodium

A stable structure with highly regulated repetitive cycles of elongation and persistence depending on the actin cross-linker fascin

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Abbreviations: FA, focal adhesion; FX, focal complex; s.d., standard deviation

The ability of mammalian cells to adhere and to migrate is an essential prerequisite to form higher organisms. Early migratory events include substrate sensing, adhesion formation, actin bundle assembly and force generation. Latest research revealed that filopodia are important not only for sensing the substrate but for all of the aforementioned highly regulated processes. However, the exact regulatory mechanisms are still barely understood. Here, we demonstrate that filopodia of human keratinocytes exhibit distinct cycles of repetitive elongation and persistence. A single filopodium thereby is able to initiate the formation of several stable adhesions. Every single filopodial cycle is characterized by an elongation phase, followed by a stabilization time and in many cases a persistence phase. The whole process is strongly connected to the velocity of the lamellipodial leading edge, characterized by a similar phase behavior with a slight time shift compared with filopodia and a different velocity. Most importantly, re-growth of existing filopodia is induced at a sharply defined distance between the filopodial tip and the lamellipodial leading edge. On the molecular level this re-growth is preceded by a strong filopodial reduction of the actin bundling protein fascin. This reduction is achieved by a switch to actin polymerization without fascin incorporation at the filopodial tip and therefore subsequent out-transport of the cross-linker by actin retrograde flow.

Introduction

Cell migration is a pivotal mechanism in processes such as wound healing, immune response and embryogenesis. Migration is a highly regulated mechanism, involving substrate sensing, adhesion assembly and force generation. The direction of migration is determined by cell polarity, which is in turn regulated by asymmetrical actin polymerization and adhesion formation. At the front of migrating cells, membrane protrusions like the lamellipodium and filopodia are formed and new adhesion sites are created. Conversely, old adhesions become disassembled at the rear of a moving cell.[1] The lamellipodium protrudes via active actin polymerization in an Arp2/3-dependent process leading to a dense meshwork[2,3] and thus filopodia arise through filament-reorganization of this lamellipodial meshwork.[4-6] In contrast to the lamellipodium, filopodia consist of long, parallel actin filaments which are tightly cross-linked via many different proteins such as fascin or VASP.[7,8] The exact mechanism underlying filopodia formation has not yet been clarified. However, recent literature hints at a regulation via formins rather than Arp2/3.[9,10] One of the main functions of filopodia includes environmental sensing. In this regard, transmembrane proteins, such as cadherins, integrins and other receptors are essential to forming cell-cell or cell-matrix interactions and are additionally involved in the response to external guiding cues.[11-14] Recent work focusing on the entire life cycle of filopodia provides strong evidence for another more vital function throughout the entire locomotive process of cells. It has been demonstrated that filopodia define the localization of nearly all focal adhesions (FAs) within the lamellipodium in keratinocytes. These stable adhesions evolve from small filopodial focal complexes (FXs), which enlarge upon lamellipodial contact.[15] The connection between these FXs to force-producing actin bundles derived from filopodia was shown to be crucial for FA maturation. Fascin cross links these filopodial actin bundles

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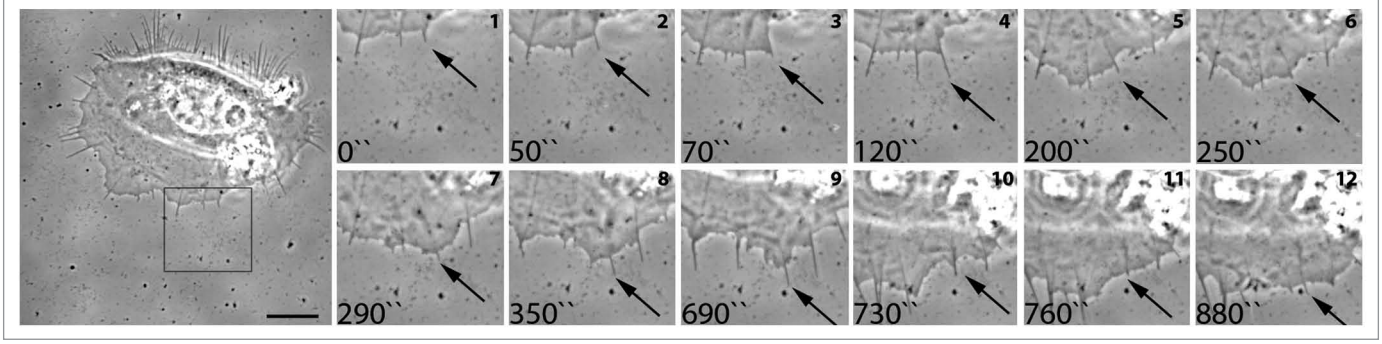

Figure 1. Re-elongating filopodium. Keratinocytes were grown for 24 h, stimulated with EGF for 1 h and subsequently analyzed by phase contrast microscopy. Black box in overview indicates the zoomed area shown in the time series. Time points are given in seconds. The arrow highlights the same filopodium over time. See also **Video S1**. Scale bar = 10 μm.

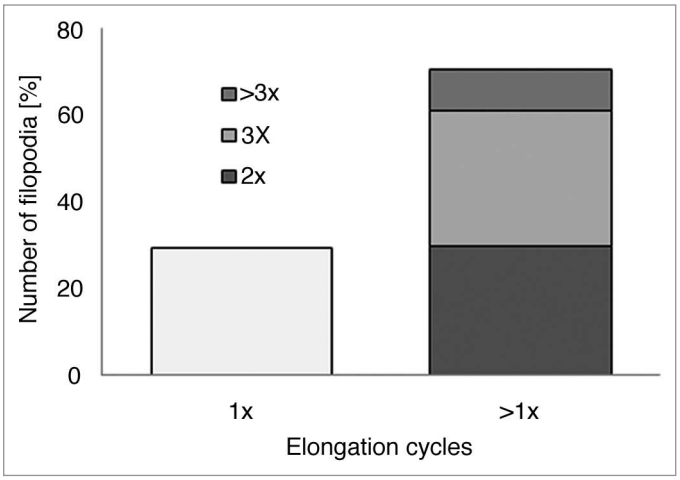

Figure 2. Number of re-elongating filopodia. Only filopodia in migration direction were analyzed. Unstable filopodia were not considered. Furthermore, only filopodia fully disassembled by the end of analysis time were used. Note that most of the filopodia exhibited more than one elongation cycle. n = 10^3 filopodia out of 11 cells.

within the filopodium itself but is subsequently replaced by myosin II and α-actinin within the lamellipodium to form contractile actin fibers. Almost all stable FAs of keratinocytes emerge by this filopodia-dependent mechanism, substantiating the importance of filopodia not only in substrate sensing but also in FAs formation, maturation and force transmission. Here, we demonstrate in human keratinocytes that the majority of filopodia undergo several cycles of elongation and persistence, most probably driven by the same tip complex. Each cycle consist of an elongation phase, a stabilization phase with slight retraction of stable filopodia and a persistence phase. Filopodial tip and lamellipodial leading edge velocities are closely linked to one another but differ in their rates. Cycle interruption was only observed when the filopodium was unable to adhere stably. In this case the filopodium retracted into the lamellipodium and did not re-elongate. Previously it has been demonstrated that filopodial protrusion and retraction is mediated by a balance of actin polymerization at the tip and actin retrograde flow driven by myosin pulling these filaments rearward.[15-20] In this context, we analyzed the actin bundling protein fascin, which is highly expressed in migrating keratinocytes and predominantly located in filopodia. Here, fascin forms densely packed, parallel actin bundles and thereby provides the necessary rigidity to push out membrane tethers upon protrusion. Furthermore, we could show that actin filaments, permanently polymerized at the filopodial tip, are cross-linked by strongly varying amounts of fascin. This fascin concentration correlated with cycles of filopodial elongation and stabilization. In the elongation phase fascin was present along the entire filopodium, whereas upon filopodia stabilization, fascin was removed from filopodia by retrograde actin flow.

Results

Recent literature pointed out that filopodia are not just key players for substrate sensing, but also account for adhesion site formation and force generation.[15] Additionally, it was indicated that once a filopodium is formed it is able to undergo several cycles of elongation and stabilization, thus leading to consecutive formation of FAs.[21] Therefore, we evaluated the life cycle of filopodia in more detail and found that many filopodia re-elongated. In **Figure 1** the life cycle of one filopodium with three re-elongation events is exemplified. It can be seen that the filopodium grows faster than the lamellipodial leading edge (**Fig. 1**, e.g., change from image 3 to 4; see **Vid. S1**). At a certain length the filopodium stops elongation and retracts by some extent (**Fig. 1**, e.g., images 4 to 5 or 9 to 10; see **Vid. S1**). As a result, parts of the stable filopodium are outgrown by the lamellipodial leading edge (**Fig. 1**, e.g., images 10 to 11). Tracking filopodial tips in migrating keratinocytes revealed that 71% (s.d. 17%, n = 103 filopodia out of 11 different cells) of all filopodia pointing toward the direction of migration underwent several elongation cycles. The majority of these filopodia elongated two (30%, s.d. 16%) or three times (31%, s.d. 16%) (**Fig. 2**). In 10% (s.d. 11%) of the filopodia even more than three elongation events were detectable. Only 29% (s.d. 17%) of all filopodia did not elongate more than once.

Generally, the lamellipodium reaches the filopodial tip until just a short filopodial stub remains present. In 71% of all cases (see above) this stub regains its elongation ability at a distance of approximately 0.6 μm (s.d. 0.4 μm, n = 30), measured from

filopodial tips to the lamellipodial leading edge. The resulting filopodial length after re-elongation averages to 4.8 μm (s.d. 1.5 μm, n = 30) (see **Table 1**).

The detailed analysis of the filopodial life cycle by tracking the filopodial tip over time resulted in a process of fast elongation with velocities of 2.2 μm/min (s.d. 1.3 μm/min, n = 20 elongations of 6 independent filopodia), followed by a significantly slower filopodial tip retraction during a stabilization phase (p-value < 0.001) with velocities of 0.8 μm/min (s.d. 0.5 μm/min, n = 20) (**Table 2**). Such filopodial dynamics are shown exemplarily in **Figure 3A**. To visualize the different phases more clearly and free of noise, sequential tip velocity values with the same algebraic sign were pooled and plotted with their mean velocity. This processing resulted in graphs as given in **Figure 3B** for the same data set as presented in **Figure 3A**. The course of elongation (positive velocity), stabilization (negative velocity) and persistence (zero velocity) was found in all analyzed filopodia. The phase durations were very heterogeneous, ranging from 10 to 250 sec (mean 131 sec, s.d. 73 sec, n = 10) for elongation, 10 to 340 sec (mean 103 sec, s.d. 95 sec, n = 9) for stabilization and 10 to 270 sec (mean 46 sec, s.d. 92 sec, n = 7) for persistence (**Table 3**). In some cases the persistence phase was completely missing. However, the fast elongation and slow retraction was found in all analyzed filopodia.

Next, we followed simultaneously the filopodial tip velocity and the corresponding lamellipodial leading edge velocity of regions right next to the filopodium. The results reveal a close correlation of the velocities of both protrusions (**Fig. 4**). Filopodia and the lamellipodial leading edge undergo the same phases of protrusion and retraction simultaneously with a slight time offset. The elongation velocities differ significantly (p-value < 0.001) whereas the retraction rates are comparable (**Table 2**). This correlation is illustrated in **Figure 4** showing one filopodium and the corresponding lamellipodial region. The lamellipodium also undergoes alternating phases of fast outgrowth (1.8 μm/min, s.d. 1.4 μm/min, n = 20) and slow retraction (0.8 μm/min, s.d. 0.6 μm/min, n = 20) (see **Table 2**). The phases differ in duration with elongation periods ranging from 10 to 460 sec (mean 116 sec, s.d. 139 sec, n = 15) and stabilization phases of 10 to 190 sec (mean 51 sec, s.d. 57 sec, n = 12). Such variations could be caused by analyzing several cells which already display diverse protrusion dynamics among themselves. Their persistence phases were in general very short, ranging from 10 to 20 sec (mean 12 sec, s.d. 4 sec, n = 11) (**Table 3**). Interestingly, each filopodial elongation cycle was normally preceded by the lamellipodial leading edge elongation phase. This resulted in a shortening of the filopodium and thus in a decrease of the filopodial tip to leading edge distance (**Fig. 4**, dashed line).

Since formation and elongation of filopodia strongly depend on actin cross-linkers, here mainly fascin, we analyzed fascin localization and relative fluorescence intensity during filopodial elongation cycles by live cell imaging of migrating, GFP-fascin transfected keratinocytes. We found a homogeneous fascin signal along the complete length of filopodia during elongation (**Fig. 5**, see time point 0"; see **Vid. S2**). This fascin signal remained visible

Table 1. Keratinocytes were treated with EGF and analyzed 1 h after stimulation

	Before re-elongation	After re-elongation
Distance from tip to lamellipodium (μm)	0.6	4.8
s.d. (μm)	0.4	1.5

Filopodial length was measured as distance between filopodial tip and lamellipodial leading edge. n = 30 filopodia.

Table 2. Mean velocities of filopodia and corresponding lamellipodia movement

	Filopodium	Lamellipodium
Mean elongation velocity (μm/min)	2.2	1.8
s.d. (μm/min)	1.3	1.4
Mean retraction velocity (μm/min)	0.8	0.8
s.d. (μm/min)	0.5	0.6

n = 20 elongation/retraction cycles out of 6 filopodia and lamellipodia.

over time, as long as filopodia extended in length. Interestingly, a strong decrease in fascin intensity was found in stably adhered, not growing filopodia. Analyses with high temporal resolution showed that fascin intensity did not decrease homogeneously within filopodia. Instead, the intensity decreased continuously as a sharp border moving retrogradely from the filopodial tip to the lamellipodial leading edge (**Fig. 5**, see time points 40"–120"; see also **Vid. S2**). As soon as the lamellipodial leading edge nearly reached filopodial tips, remaining filopodial stubs started to re-elongate at the same time new fascin molecules became incorporated, leading to fully recovered GFP-fascin fluorescence intensities within new outgrowing filopodia (**Fig. 5**, see time points 380"–500").

To illustrate the continuous removal of fascin and to quantify the velocity of fascin removal we analyzed fascin intensities along filopodia using kymographs (**Fig. 6A**). A line of 3 pixels in width was drawn from the filopodial tip toward the lamellipodial leading edge and the intensity over time was displayed. For the mean velocity of fascin retrograde flow we calculated 1.2 μm/min (s.d. 0.5 μm/min, n = 6). This velocity is highly comparable with actin retrograde flow rates (1.1 μm/min) within stable filopodia associated with FA formation determined in our earlier studies by tracking laser generated bleach lines over time in filopodia of GFP-actin transfected cells.[15]

To clarify whether the observation of fascin fluorescence intensity reduction may be attributable to impaired free diffusion of labeled fascin molecules in filopodia combined with photo bleaching, we studied the fluorescence intensity within filopodia over time at areas close to the leading edge as indicated in **Figure 6A** with a red box (**Fig. 6A and B**). Here, the fascin fluorescence signal is intense and stable for 100 sec. Only when the fascin intensity border reaches this area, the fluorescence intensity drops dramatically by approximately 60% (**Fig. 6B**). As we do not see a linear or exponential decrease in fluorescence intensity over time in this region, we can exclude photo bleaching as a cause. Comparative analysis of filopodia tip velocity with fascin fluorescence intensity within the same filopodium over time showed

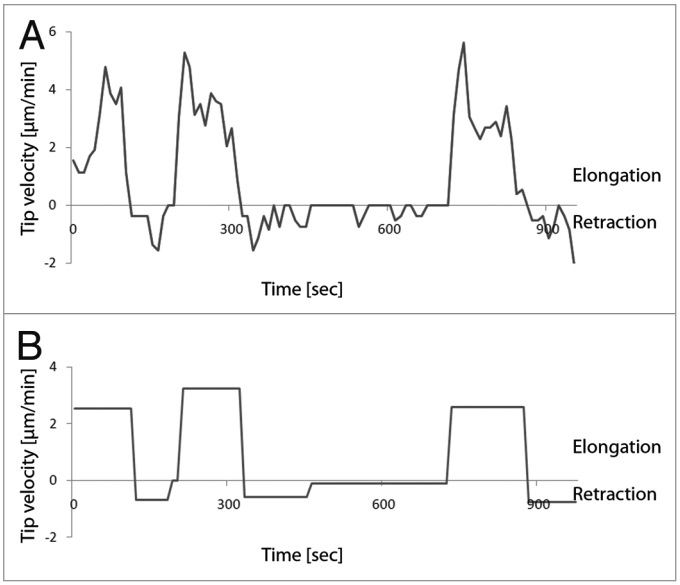

Figure 3. Phases of elongation, stabilization and persistence during the life cycle of one filopodium. A motility stimulated keratinocyte was analyzed by tracking a filopodial tip over time. Positively signed tip velocity represents elongation and negatively signed numbers show retraction. Zero equals persistence without movement in either direction. (A) Presentation of raw velocity values averaged over three consecutive values. (B) For better phase visualization sequential values of A with the same algebraic sign were pooled, averaged and the resulting average value was plotted for each time point within this phase. In total, six independent filopodia were analyzed all characterized by the same phase behavior.

Table 3. Mean phase duration of filopodia and lamellipodia

	Filopodium	Lamellipodium
Mean elongation duration (sec)	131 (n = 10)	116 (n = 15)
s.d. (sec)	73.1	139
Mean stabilization duration (sec)	103 (n = 9)	51 (n = 12)
s.d. (sec)	95	57
Mean persistence duration (sec)	46 (n = 7)	12 (n = 11)
s.d. (sec)	92	4

Numbers of analyzed phase durations were taken from three independent filopodia with connected lamellipodia.

a clear correlation between fascin intensities and the filopodial phases described above (**Fig. 7**; see also **Fig. 5**). Here, the filopodial elongation phase was always characterized by high GFP-fascin intensities. In turn, fascin retrograde flow correlated with the filopodial stabilization phase. Fascin intensity remained low during the persistence phase. As soon as filopodia reentered into a new elongation phase, fascin fluorescence intensity increased as well in parallel or even slightly before.

Discussion

Filopodia have long since been under investigation. A number of recent studies, investigating the entire life cycle of filopodia highlight filopodia's significance in formation of stable focal adhesion sites, as well as force generation and transmission.[11,15,21,22] The functionality and integrity of these processes are essential for cells to migrate. Thus, interfering with filopodia formation leads to complete inhibition of motility or even to cell death.[21]

It was recently hypothesized that the formation of new filopodia depends on previously existing filopodia through a distinct order of repetitive elongation and adherence.[21] Here we analyzed the entire life cycle of filopodia and in fact show for the first time that the majority of filopodia do emerge from already pre-existing filopodia. Once a filopodium is formed, the cell is able to recycle already built up filopodial actin fibers containing all signaling and scaffolding proteins. This is consistent with other studies, which also pointed out filopodial contribution to the lamellipodial actin cytoskeleton in migrating cells.[22-25] Furthermore, our study showed that only stable filopodia re-elongate and that this re-elongation is very likely dependent on the re-incorporated actin cross linker fascin. The mechanism of sequential elongation and stabilization resulted in a very distinct order of rapid forward and slow rearward movements as well as a persistence phase before the commencement of the next cycle.

During the elongation phase the fascin signal remained constant within filopodia. Previous studies have shown that filopodial protrusions are driven by fascin incorporation into filopodial actin,[26] and that active, dephosphorylated fascin promotes the formation of very long filopodia in vivo.[7] In this manner fascin stabilizes actin filaments and therefore stiffens them so that these filaments are able to overcome the stiffness of the plasma membrane and grow.[27,28] In keratinocytes, the maximum length of growing filopodia of appears to be limited to approximately 5 µm. As active transport and/or diffusion of proteins to the filopodial tip are required for filopodial growth, the limitation in length might be due to limitations in protein diffusion and/or transport mediated by myosinX along the thin filopodium.[5,27,29-34] In other cell types, different maximal lengths of filopodia were reported. For example the filopodia of filopodia-forming sea urchin cell types can reach lengths of 5 µm to 35 µm.[35] The varying lengths of filopodia in different cell types are likely due to the diverse functions and are probably affected by a distinct set of involved molecules.[36] Thus, the observed maximal length of filopodia in our cells most likely depends on filopodial architecture and dynamics and is closely related to cell type and function.

Unlike in the elongation phase, the stabilization phase is characterized by a slow rearward movement of the filopodium. Presumably binding to the ECM by filopodial FX formation takes place during this phase. The establishment of FXs is associated with an enhanced force generation due to the connection to contractile actin fibers,[15] with which the filopodium stabilizes itself.[37] The whole process of adherence, contraction and maturation is a prerequisite for filopodia to enter the elongation phase once again. The duration of this stabilization can vary from filopodia to filopodia. This will affect the filopodial dynamics and therefore the duration of the different phases.

During the stabilization phase, the tip complex must be functionally inhibited, leading to a decrease in actin polymerization so that polymerization and retrograde flow compensate each other and the filopodia stop growing. How the tip complex is regulated

remains to be shown in future experiments. Most likely, the tip complex will either be disassembled or inactivated by an on/off mechanism. Fascin, during this stabilization phase, undergoes retrograde flow from the tip to the base of filopodia. Since fascin stiffens filopodial fibers, removal of filopodial fascin could result in slowing of actin filament elongation thus reducing filopodial growth as observed during this phase. The velocity of the retrograde fascin flow averaged to 1.2 μm/min and is highly comparable to retrograde actin flow rates determined in stable filopodia associated with FAs formation.[15] We therefore assume that fascin removal is associated with filopodial retrograde actin flow. Such removal might additionally go along with alterations in protein kinase C activity, a main regulator of fascin activity and exchange dynamics.[32,38,39] So far we can just speculate about the role of fascin activators controlling its filopodial removal observed here, but it will be a challenge in the future to test this possibility. Former studies on melanoma and neuroblastoma cells analyzed the exchange dynamics of fascin in filopodia and showed that fascin is constantly exchanged.[7,32] However, there was no indication of fascin retrograde flow that might have been primarily attributable to analyzing mainly stable filopodia which already reached persistence phase. Alternatively, cell type specific effects are possible. In this study we focused only on filopodia formed in the direction of migration. It is conceivable that filopodial tip velocities, and therefore also fascin dynamics, are altered or even increased in migrating cells since migration strongly depends on accurate filopodia formation.[15] Finally, in the persistence phase of the filopodium, the lamellipodial leading edge nearly outgrows filopodia. We measured the distance between filopodial tips and the leading edge before re-elongation to be about 0.6 μm. Here, presumably an optimal distance is reached for the interaction between lamellipodium and filopodium. The lamellipodium displayed the same order of sequential movements with protrusion and retraction during the persistence phase as well as during the stabilization phase. At this state the tip complex is presumably reassembled or re-activated to initiate filopodial growth. The latter case would have the advantage that all proteins needed to increase actin polymerization in order to initiate the next elongation cycle are already in place.[40-42] Recent investigations favor the idea of an inactivation/activation process, which might involve VASP and the formin mDia2.[40,43,44] This is supported by the fact that one and the same filopodium grows out several times and that VASP signal in the tip remains stably visible over time along the elongation cycles.[21,33]

In summary, we demonstrate for migrating keratinocytes a very organized phase behavior of elongation, stabilization and persistence for filopodia, which highly correlates with lamellipodia behavior. For the first time we show that fascin displays a retrograde flow during filopodia stabilization and persistence and that its dynamic is strongly linked to the filopodial phases.

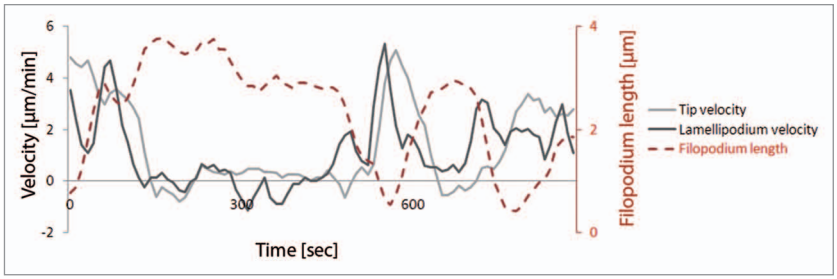

Figure 4. Correlation of filopodium length and the tip velocity of a filopodium and corresponding lamellipodial leading edge. Keratinocytes were grown for 24 h and stimulated with EGF for 1 h. Filopodium length from tip to leading edge was measured in time lapse phase contrast movies and plotted in red against the filopodial tip and lamellipodial velocity determined for the same filopodium at the same time points. Positively signed velocity represents protrusion in migration direction, whereas negatively signed velocity shows retraction. Presented values were averaged over three consecutive image values. Analysis of three filopodia with corresponding lamellipodial leading edge velocities revealed comparable results.

Material and Methods

Cell culture. Normal human epidermal keratinocytes from neonatal foreskin were purchased from Lonza (Verviers, Belgium) and cultured in complete keratinocyte growth medium (KGM, Lonza) at 37°C and 5% CO_2. For experiments cells from passage two to four were seeded in self made glass bottom Petri dishes (bottom thickness 170 μm). Dishes were coated with 2.5 μg/cm^2 fibronectin (BD, Bioscience, Palo Alto, CA) in phosphate buffered saline (137 mM NaCl, 4.2 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4 in H_2O) for 45 min at 37°C. Cells were stimulated with 50 nM human epidermal growth factor (EGF, Sigma) 1 h prior experimental start to induce motility.

Plasmids. GFP-fascin was kindly provided by Dr. Josephine C. Adams (Department of Cell Biology, Cleveland Clinic Foundation, Cleveland, OH USA).

Transfection. Transient transfection was performed with Transit Keratinocyte reagent (Mirus, Madison, WI USA) according to manufacturer's instructions.

Microscopy. All images were acquired with a confocal microscope (LSM510) mounted on an Axiovert 200 (Carl Zeiss MicroImaging, Germany) using a 63x/1.25 Ph3 plan-neofluar oil objective (Zeiss). All time lapse and fascin flow analyses were performed at 37°C and 5% CO_2. For GFP-imaging and phase contrast the 488 nm argon ion laser line was used.

Fascin flow measurements. GFP-fascin transfected migrating keratinocytes were recorded one day post transfection with phase contrast and fluorescence simultaneously. Time lapse movies were taken over a period of 8 to 21 min with a frame rate of 6 images/min. Images were drift corrected using image processing routines of the MatLab software (The MathWorks, version 7.6, Natik, MA USA) before evaluation. For this purpose rectangular patches in the background were defined and the drift was calculated by cross-correlation over time. To determine fascin retrograde flow within filopodia a kymograph of the filopodia was sampled and the average speed was determined with the "read

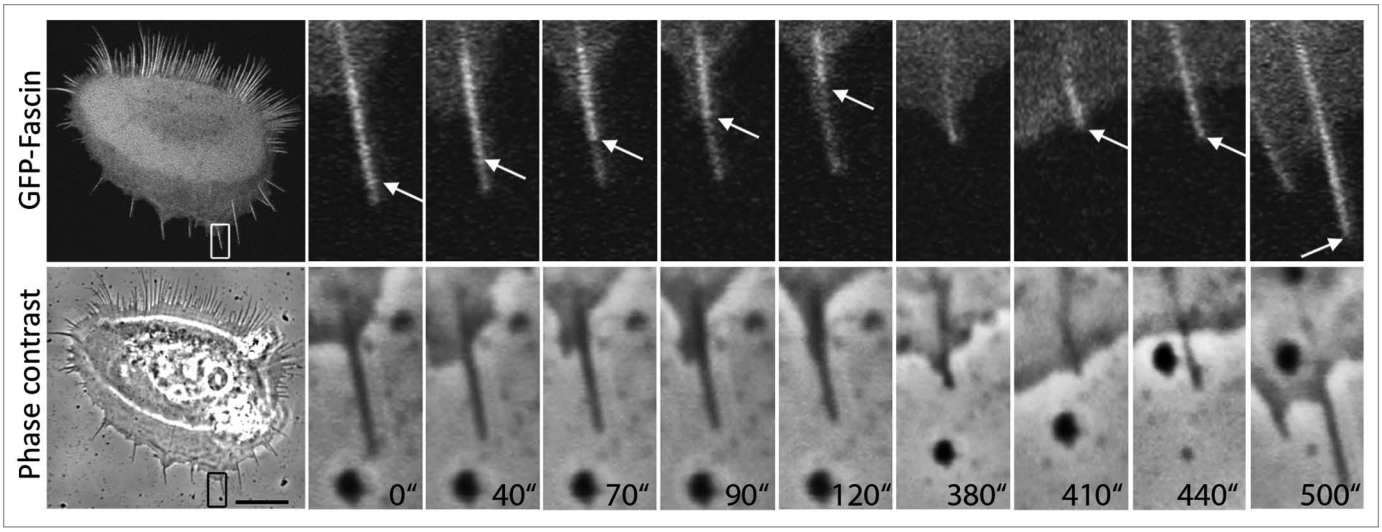

Figure 5. Fascin flow in filopodia over time. Cells were grown for 24 h and transfected with GFP-fascin. Cells were stimulated with EGF one day after transfection and analyzed 1 h later by confocal microscopy. Boxes indicate the zoomed areas shown in the corresponding time series. Time points are given in seconds. The arrow highlights the position of the sharp transition in GFP-fascin intensity. See also **Video S2**. Scale bar = 10 μm.

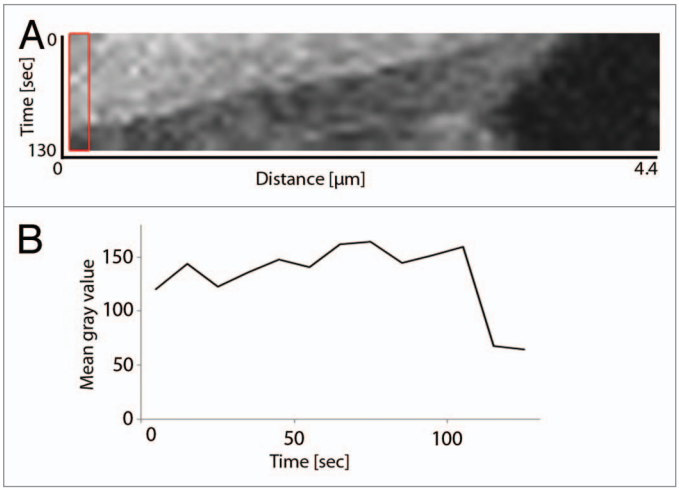

Figure 6. Fascin flow is continuous. (A) Kymograph of a filopodium shows that the fluorescence intensity of GFP-fascin (given in gray value) moves continuously retrograde from the tip (right) to the base (left) of the filopodium. Red box [2 pixel in width (120 nm) and 13 pixel in length (130 sec)] indicates the area of gray value analyzed in (B). The plot clearly shows that there is no reduction in fluorescence intensity due to bleaching. The drop in fluorescence intensity at time point 100 sec correlates with the time point at which the retrograde fascin flow reaches the analyzed area.

velocities from tsp" tool of the ImageJ software (Wayne Rusband, US. National Institute of Health). This velocity basically represents the slope of the fluorescence intensity border exemplarily shown in **Figure 6A**. To exclude photobleaching the fascin fluorescence intensity was analyzed in an area of 2 x 13 pixels (corresponds to 120 nm and 130 sec) close to the leading edge in the kymograph with the plot profile tool of the ImageJ software. Gray values in width were averaged and plotted against time. For filopodial overall fascin fluorescence intensity analyses over time, a line of 3 pixels in width was analyzed along the whole length of the filopodia. Line length varied and reflected the length of the filopodia over time. Gray values for all pixels within the line were averaged resulting in a single gray value for each time point.

Analysis of re-elongating filopodia. Phase contrast time lapse images were used. Only stable filopodia in migrating direction of polarized migrating cells were analyzed. Filopodia not completely in focus over their life time or still growing at the end of the movie were not considered. Every filopodium was interactively designated and followed over time. Elongation phases of stable filopodia that were in retraction or persistence phase before for at least 20 sec were considered as re-elongation.

Measurement of filopodial length. The distances between lamellipodial leading edge and filopodial tip were determined from phase contrast time lapse images with the ImageJ line tool. Every distance was measured 5 times to estimate measurement inaccuracies by determining the standard deviation of the 5 measurements of the same filopodium. The mean value of these 5 measurements was used for each filopodium to then calculate the mean length of all analyzed filopodia.

Calculation of filopodial tip and lamellipodial leading edge velocity. Keratinocytes were stimulated with EGF and time lapse images were taken over a time period of 10 to 20 min. Images were drift corrected using the MatLab software (The MathWorks, version 7.6, Natik, MA) before evaluation. Filopodial tip velocity was detected by interactively tracking the tip over time. The lamellipodial leading edge velocity was detected directly next to the filopodium shaft using the point tool of the ImageJ software in phase contrast time lapse images. For presentation of filopodial and lamellipodial velocity the tracked movement values were averaged over three values. Velocity direction was identified by kymograph display of the analyzed area.

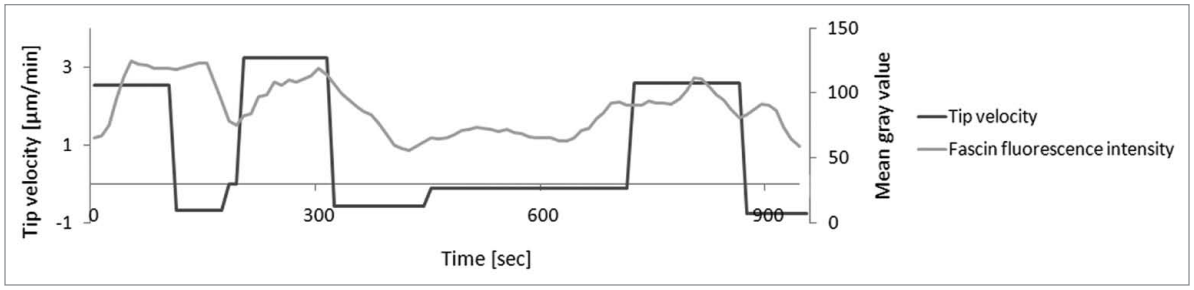

Figure 7. Fascin fluorescence intensity over time. The fascin intensity was measured as mean gray value of the GFP-fascin signal in single filopodia over time. Filopodial tip velocity was detected for the same filopodium in parallel and is depicted in µm/min with positively signed numbers indicating movement in migration direction and negatively signed numbers indicating retraction. The presented graph for tip velocity was processed as described in **Figure 4**. Note that the fascin signal reduces when a filopodium is retracting, but increases upon elongation. Analysis of three filopodia with corresponding fascin intensity revealed comparable phase correlations.

Elongation was signed positive, retraction movement was signed negative. For even better visualization of filopodial phases, averaged values were further processed by combining all sequential values of identical algebraic sign. Single outliers were not considered as phase end but included into the phase. The mean value of all data points within one phase was calculated and subsequently used as value for each time point within the phase. Values with repetitively changing algebraic signs were combined as phase of persistence.

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Note

Supplementary material can be found at:
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