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Dynamic coupling between actin network flow and turnover revealed by flow mapping in the lamella of crawling fragments

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ABSTRACT

Dynamic turnover and transport of actin filament network is essential for protrusive force generation and traction force development during cell migration. To elucidate the dynamic coupling between actin network flow and turnover, we focused on flow dynamics in the lamella of one of the simplest but elegant motility systems; crawling fragments derived from fish keratocytes. Interestingly, we show that actin network in the lamella of fragments is not stationary as earlier reported, but exhibits a flow dynamics that is strikingly similar to that reported for higher order cells, suggesting that network flow is an intrinsic property of the actin cytoskeleton that is fundamental to cell migration. We also demonstrate that whereas polymerization mediates network assembly at the front, surprisingly, network flow convergence modulates network disassembly toward the rear of the lamella, suggesting that flow and turnover are coupled during migration. These results obtained using simple motility systems are significant to the understanding of actin network dynamics in migrating cells, and they will be found useful for developing biophysical models for elucidating the fundamental mechanisms of cell migration.

Introduction

Actin-based cell migration is important for various physiological processes including metastasis, wound healing and immune response. During cell migration, the cytoskeletal actin filaments (F-actin) forming the protrusive structures (lamellipodia or filopodia) of migrating cells undergo dynamic turnover, which is characterized by polymerization at the leading edge to generate protrusive forces, and depolymerization to replenish the monomer pool [1,2]. The two processes are highly coupled, and they are tightly regulated by both mechanical and biochemical factors [3,4]. The dynamic turnover is accompanied by network transport of F-actin away from the leading edge toward the center of the lamellipodia, in what is known as retrograde flow [5,6].

F-actin flow continues to generate a lot of research interest owing to its importance to cell migration. Indeed, tremendous progress has been made in the recent past toward understanding the contribution of the flow to traction force development through the molecular clutch mechanism [7,8]. The progress is attributable to the development of new techniques, particularly fluorescent speckle microscopy (FSM) [9], that have made it possible to map F-actin flow at submicron level. Using FSM technique, studies have established a mechanistic relationship between F-actin network dynamics on the one hand, and cytoskeletal and traction forces on the other [10–12]. Thus, actin network flow can be considered as a barometer of force balance in the cytoskeleton.

Therefore, understanding spatiotemporal dynamics of the actin network is crucial to elucidating not only how the actin cytoskeleton achieves self-regulation, but also how cell migration is mechanically regulated. However, cell-level analysis of actin network dynamics still remains a challenging task, partly because migrating cells are complex systems consisting of numerous interacting components within a confined space. Even fish keratocytes that are often considered as simple motility systems still consist of a bulky cell body and numerous other components that do not necessarily take part in motility [13].

In this study, we aim at elucidating the dynamic coupling between actin network flow and turnover by focusing on flow dynamics in the lamella of one of the simplest motility systems; fragments derived from fish keratocytes. We reason that the cytoskeletal actin structure in migrating cells is spatiotemporally self-regulating, meaning that network flow and turnover should be coupled. To test this, we utilize FSM and particle imaging velocimetry (PIV [14]) to quantitatively map actin network flow in the lamella of crawling fragments, and then we use the flow results to determine the spatial distribution of network turnover. Since they lack cell body and most organelles [15], fragments can be

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considered as simple motility systems, a quality that make them suitable for exploring the mechanical and dynamic properties of the actin network as uniquely expressed during cell migration.

Materials and methods

Fragment formation. Fragments were formed from fish keratocytes that had been cultured for 24 h in DMEM (Dulbecco's Modified Eagle's Medium, Sigma) supplemented with 15% FBS (Sigma) according to the procedures described in [16]. Fragmentation was induced by incubating the cells with a culture medium containing 50 nM staurosporine (Cayman Chemical) for 60 min at room temperature. Staurosporine is a well-characterized inhibitor of protein kinase C (PCK) [17] that was previously used successfully to form fragments from fish keratocytes [15]. The progress of fragmentation was monitored using a light microscope under a low magnification lens.

Microinjection. To label actin filament network, fragments were microinjected with an 8 μM conjugate solution of quantum dot-phalloidin (Qdot-phalloidin) prepared in the lab using reagents purchased from Invitrogen. After a sufficient number of fragments were confirmed, a fresh culture medium was added after rinsing three times with PBS and the setup was left undisturbed for 30 min before use. Then, microinjection was performed using a micromanipulator (InjectMan N12, Eppendorf) and a microinjector (Femtojet, Eppendorf) to deliver <1 pL of the dye into a fragment. Since fragments are prone to damage when microinjected due to their small sizes (10–20 μm wide), a low injection pressure (~30 hPa) was used, and the capillary tube was targeted at the lateral edges.

Image acquisition. For analyzing F-actin dynamics, sequential FSM images (15–30 frames per fragment) with a spatial sampling of 120 nm per pixel were captured using a microscope (Zeiss Axiovert 200M, Carl Zeiss) with a Plan-APOCHROMAT 100×, N.A 1.4 oil immersion objective lens at an exposure time of 0.8 s and an interval of 4 s. The acquired FSM images were preprocessed prior to analysis using both low and high pass Gaussian filters (Image Pro Plus, Media Cybernetics) to suppress image noise and to enhance speckle appearance, respectively.

Analysis of actin network flow. Preliminary estimation of actin network flow in the lamella of crawling fragments was performed based on kymographs constructed from the acquired FSM images using the montage function of Image J (NIH). For quantitative analysis of F-actin flow, however, vector maps of F-actin flow were obtained by tracking numerous actin speckles on sequential FSM images using image correlation-based PIV with a sub-pixel resolution, essentially as described previously [16]. The obtained flow maps were further processed for presentation using image processing software (MicroAVS, KGT).

Results

Fragment formation

The process of fragment formation from fish keratocytes is illustrated in Fig. 1A, and the obtained fragment is shown in Fig. 1B. Keratocytes began to elongate 2 min after staurosporine addition, indicating that the drug had immediate effect on the cells. Actual fragmentation, however, was observed 10 min later when portions of the lamella, usually at opposite ends of the cell, began to separate from the cell body and move in opposite directions (Fig. 1A, middle panel). The fragmented pieces of the lamella remained connected to the cell body through elongated thin stalks, but these began to break after 30 min of drug treatment, giving rise to stand-alone fragments (Fig. 1A, lower panel). Two types of fragments were formed, namely, polarized crescent-shaped fragments that were freely moving (white arrow in Fig. 1A, bottom panel), and non-polarized discoid-shaped fragments that were stationary.

Since our interest was to correlate F-actin flow with network turnover during migration, we focused on the polarized fragments (Fig. 1B) and analyzed their movement before and after microinjection. We found that fragments moved persistently at a speed ranging from 9.0 to 13.0 μm/min, comparable with that of fish keratocytes [18]. Furthermore, to visualize the distribution of F-actin network, we obtained fluorescent images of fragments that had been fixed and stained with rhodamine-phalloidin. As shown in Fig. 1C, appearance of F-actin was found to be typically similar to that of keratocytes [19]; F-actin density decreases from the leading lamella toward the rear. Taken together, these results confirm that fragments retain the motility properties of keratocytes, but since they are mainly composed of the cytoskeletal actin network, they present a minimal system for quantitative analysis network dynamics during migration.

Actin network dynamics in the lamella of crawling fragments

To correlate flow with turnover, we determined the spatiotemporal dynamics of F-actin flow in the lamella of crawling fragments by analyzing the motion of actin speckles on sequential FSM images. Both kymographs and PIV were used for the analysis. An FSM image of a representative fragment is shown in Fig. 2A. The figure displays distinct actin speckles that can serve as material points for tracking network flow. At the bottom of Fig. 2A is a kymograph constructed using slices taken from the region indicated by the rectangle in the figure. The kymograph displays diagonally tilted streaks whose slopes decrease toward the rear of the fragment, indicating that F-actin flows retrogressively, and that flow velocity decreases toward the rear. By analyzing these slopes, we estimated retrograde F-actin flow for fragments moving at various speeds, and found it to be in the range 1.0–5.0 μm/min, comparable with that of keratocytes [7], but slightly above those of other cells [20,21].

Since the information obtainable from kymographs is only limited to the selected region, more quantitative flow maps were obtained by cross-correlating the sequential FSM images using PIV. This technique was previously applied successfully to map the dynamics of actin network in keratocytes [16,22]. The flow vector map shown in Fig. 2B corresponds to the FSM image in Fig. 2A, and below it is a magnification of the flow region indicated by the rectangle in the flow map. As can be noticed from Fig. 2B, F-actin flow in the lamella was centripetally organized, and decreased in magnitude toward the center of the lamella, consistent with the flow organization reported for keratocytes [23]. Specifically, retrograde flow dominated the anterior region of the lamella, and its intensity decreased toward the center where it merged with anterograde flow emanating from the posterior region (Fig. 2B). The convergence of the two flows resulted in zones of markedly reduced flow at the center of the lamella. Such zones of flow convergence were previously associated with increased network depolymerization [21]. Furthermore, F-actin flow intensity was relatively high at the lateral rear edges of fragments (Fig. 2B), which may indicate the presence of pinching traction forces similar to that reported for fish keratocytes [24,25].

We further examined the spatiotemporal dynamics of actin network flow in the course of a fragment's movement, and obtained the flow intensity color maps shown Fig. 2C. By comparing the time-lapse images shown in the figure, we can notice that local flow intensity varied considerably with time. For instance, the first image (t = 4 s) shows relatively strong anterograde flow at the lateral rear edges, whereas the last image (t = 52 s) shows a compar-