Lamellipodial wrinkles in fish keratocytes as markers of imperfect coordination between extension and retraction during cell migration

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A R T I C L E   I N F O

A B S T R A C T

Cell migration involves the precise coordination between extension at the front of the cell and retraction at the rear. This coordination is particularly evident in fast moving cells such as fish keratocytes, where it leads to highly stable gliding motion, propelled at the front by broad, 0.1-0.2 µm thick lamellipodia. Transient uncoupling between extension and retraction can occur if the rear is temporarily stuck, which might eventually lead to cell shape instabilities. We have frequently observed in fish keratocytes the presence of lamellipodial radial wrinkles, detected by confocal, scanning electron and side-view microscopy as folds in the lamellipodium up to 2 µm in height. Using a linear finite elements analysis, we simulated the displacement of cells either with perfect coordination between extension and retraction or with the rear transiently stuck while the front continues extending, and we observed that in this last condition compression stresses arise in the lamellipodium which predict the formation of the observed pattern of lamellipodial wrinkles. In support of the numerical modeling findings, we observed that the transient halting of retraction at the rear using micromanipulation induced the formation of lamellipodial wrinkles in previously flat lamellipodia. The obtained results suggest that the conspicuous lamellipodial wrinkles observed in migrating fish keratocytes are the product of transient imbalances between front and rear displacements, and are therefore useful markers of the short scale dynamics of extension and retraction coordination during cell migration.

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1. Introduction

Fish keratocytes are fast moving cells that maintain a stable cell shape as they migrate, for which it is essential that rear retraction and front extension occur in a highly synchronized way [1]. Fish keratocyte locomotion has been described by the graded radial extension (GRE) model, in which front extension and rear retraction occur simultaneously and in a graded manner. Each occurs in directions perpendicular to the cell edge along the front and rear, respectively, ensuring a constant cell shape and predicting the observed circumferential motion along the cell edge of intracellular components [2]. Keratocytes extend broad, fan-shaped and thin lamellipodia, with heights of 0.1-0.2 µm [3]. Actin polymerization occurs near the edge of the lamellipodium, generating protruding forces against the lamellipodial membrane at the front, and actin filament density decreases from the center of the lamellipodial edge towards the margins [4]. Based on this observation, it has been proposed that keratocyte cell shape is maintained by antagonism between actin polymerization and membrane tension, where constant membrane tension limits actin polymerization and extension at the margins of the leading edge [4].

The regulation of cell shape by the plasma membrane is illustrated also by the existence of diverse forms of membrane reservoirs, such as membrane infoldings [5], caveolae [6] and

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vacuole-like dilations [7], which allow cells to withstand mechanically or osmotically induced cell shape deformations, highlighting the variety of ways in which the membrane adapts to physical constraints and sustains cell shape [8]. In the case of fish keratocytes, when observed by phase-contrast microscopy they frequently display phase-dark, transient radial structures that span their lamellipodia and move circumferentially towards the edges, originally termed lamellar ridges [2]. Despite the lack of a detailed description of these structures, initial scanning electron images of keratocyte lamellipodia suggest that lamellar ridges are large wrinkles in the lamellipodium (see for instance Figs. 16–77 in Ref. [9]), pointing to a mechanical process that leads to wrinkling in the thin lamellipodial film as their possible origin [10]. It is important to note that lamellipodial wrinkles are different from membrane ruffles, which are membrane folds that form at the edge of lamellipodia and move centripetally towards the cell body, and which are associated with low lamellipodial adhesion and inefficient migration [11].

Synchronization between rear retraction and front extension in fish keratocytes has been proposed to rely on membrane tension [4,12] or on actomyosin contraction connecting front and rear [13]. However, rear retraction, which requires deadhesion from the substrate, does not always occur in perfect coordination with front extension, as evidenced by retraction fibers trailing behind the cell body [14] and by the occasional abrupt recoil retractions of the rear after being transiently stuck [15].

Taylor and colleagues [16] have provided a comparative of available numerical approaches for the determination of location, geometry and evolution of wrinkles of elastic membranes. The numerical methods range from static post-buckling analysis to a dynamic relaxation approach, showing that the wrinkling simulation problem is still considered as a theoretically and numerically challenging task. Recently, a nonlinear wrinkling model considering large strains has been described and applied to obtain results about the influence of various factors [17]. Although the wrinkle formation process corresponds to a complex nonlinear mechanism, a simplified approach can be considered assuming that wrinkles are formed at points with compressive and tension principal stresses.

In this study, we analyzed the radial structures frequently observed in the lamellipodia of fish keratocytes by different microscopy approaches. We have shown that these structures are folds in the lamellipodium, reaching up to 2 μm in height, which we termed lamellipodial wrinkles. We have also studied their formation, both by numerical modeling using linear finite elements analysis and by micromanipulation experiments. Our results point to an imperfect coordination between rear retraction and front extension as the source of the mechanical perturbation that leads to lamellipodial wrinkling, and suggest therefore that lamellipodial wrinkles are markers of the short scale balance between retraction and extension during cell migration.

2. Materials and methods

2.1. Cell culture

Keratocytes from goldfish (*Carassius auratus*) scales were cultured as previously described [18], with the following modifications: scales were placed in 12 mm coverslips, covered with a small amount of medium (DMEM supplemented with 10% fetal bovine serum, both from Invitrogen) and left overnight at RT in a humidified chamber, by which time most scales showed outgrowths of keratocytes adhered to the coverslips, which were then used for experiments.

2.2. Time-lapse imaging

Time-lapse images of migrating keratocytes were acquired at 2–5s intervals by phase-contrast microscopy, using a 20× objective and a Nikon Diaphot inverted microscope. For side-view microscopy, coverslips with attached keratocytes near their margins were held upright with binder clips and imaged as previously.

2.3. Fixed cell staining and confocal microscopy

Keratocytes were fixed 10 min with 4% paraformaldehyde, washed in PBS, permeabilized with 0.1% Triton X-100 and stained for 30 min with Alexa Fluor 488 phalloidin (Invitrogen). Alternatively, cells were also blocked with 3% BSA, incubated overnight at 4 °C with anti-vinculin monoclonal antibody (ab18058, Abcam), washed in PBS, incubated 1 h at RT with Alexa Fluor 555 anti-mouse secondary antibody and mounted with Prolong Antifade (both from Invitrogen). Cells were visualized by confocal microscopy with 60× objectives, either with an Olympus FV300 confocal microscope, or with a Zeiss LSM 800 confocal microscope equipped with an Airyscan module.

2.4. Scanning electron microscopy

For scanning electron microscopy, keratocytes were prefixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetraoxide, dehydrated in a series of increasing ethanol concentrations and submitted to critical point drying using carbon dioxide in a Denton SPC-1 Critical Point Vacuum Drying Apparatus. Next, samples were gold plated in a Denton Vacuum Desk-II and visualized in a Jeol-5900 LV scanning electron microscope.

2.5. Micromanipulation

Microneedles with 1–5 μm tip diameter were pulled from borosilicate glass capillaries with a P-97 Sutter pipette puller. Using an MX7600 Siskiyou micromanipulator, and in the same conditions as described above for time-lapse imaging, microneedles were carefully lowered so as to press and pin down the rear region of migrating keratocytes.

2.6. Finite elements modeling analysis

The methodology considered to simulate the deformation of the membrane is given by the Linear Elasticity Problem and the Finite Element Method [19]. The membrane is considered as a linear elastic solid occupying a two-dimensional region submitted to a plane stress state. The boundary conditions are given by known displacements or tensions. The hypothesis of small strains is considered and forces per unit volume are disregarded. The constitutive parameters considered are: Young Elastic Modulus $E = 50$ kPa [3], and Poisson's ratio $\nu = 0.45$. The geometry of the considered lamellipodium is given by three circular arcs with radii of 20 μm (front) and 10 μm (left and right rear corners). The considered frontal displacement is 4 μm. The finite elements analyses were performed using FreeFem++ software [20].

3. Results

3.1. Characterization of lamellipodial wrinkles

Studying goldfish keratocyte migration by phase-contrast, we frequently observed prominent phase-dark radial structures in the lamellipodia of keratocytes, which appeared and disappeared dynamically, often moving circumferentially along the cell edge