

Role of Focal Adhesions in Lamellipodia Dynamics

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Abstract

Focal adhesions (FAs) are multi-protein structures containing integrin that serve as a focal point for the association between the extracellular matrix (ECM) and actin cytoskeleton. After cells adhere to the ECM, the cell membrane forms filopodia and lamellipodia. Cells deficient in FA complexes show reduced lamellipodia dynamics and this deficiency influences cell migration. Lamellipodia dynamics have distinguishable stages of lamellipodia protrusion, retraction, and persistence. Particularly, cells with decreasing or absent FA formation commonly show longer persistence time as analyzed using computer-assisted stroboscopic analysis. These results indicate that after cells adhere to the substratum, the reduced lamellipodia dynamics associated with defective FA formation influences cell motility.

Cell migration plays a central role in many physiological and pathological processes including embryogenesis, inflammatory response, wound healing, and metastasis. Cell migration is a distinctive, integrative, multistep process including formation of the cell adhesion, membrane protrusion at the front area, cell body contraction, and tail detachment [1]. Cell adhesion utilizes focal adhesions (FAs), fibrillary adhesions, and podosomes [2] to link the extracellular matrix (ECM) and various cytoplasmic proteins. After cells adhere to the ECM, the membrane begins filopodium formation and lamellipodium extension at the front edge of the cell. These are driven by actin polymerization and microtubule dynamics [3]. During the next steps of cell migration, the cell body moves forward in the migration direction and releases the cell-substrate adhesion at the cell rear [4]. At the cell front, the membrane begins as a flat cellular protrusion that is powered by actin polymerization and can be visualized by phase contrast microscopy as dark waves, which are called membrane ruffles [4,5]. Lamellipodia are sheet-like projections formed at the leading edge of many migrating cells, including fibroblasts, immune cells, neural crest cells, and melanoblasts [5,6]. This review will discuss the role of FA and its effect on lamellipodia dynamics in understanding adhesion-dependent cell migration. FAs are highly dynamic structures that form at sites of membrane contact with the ECM and associate with many cellular proteins known as FA complexes, including vinculin, focal adhesion kinase (FAK), Src family kinases (SFKs), paxillin, p130CAS (Crk-associate substrate), and Crk [2,7]. Deficiency of FA complexes in mouse embryo fibroblasts (MEFs) results in severe defects in cell spreading and culminates in embryonic death. For example, *FAK*-null MEFs show mesodermal defects in the late phase of gastrulation and have a delay in cell migration *in vitro* [8,9]. Deficiency of *p130CAS* causes severe defects in cell spreading [9,10]. In SYF cells (deficient for *Src*, *Yes*, and *Fyn*), MEFs show a decreased formation of FAs, which results in severe developmental defects, lethality, and delayed cell migration [11-13]. Additionally, *Crk*-null mice die during a late stage of embryonic development [14] and present with a defect in cell-spreading *in vitro* [15]. These results imply that FA-associated proteins play primary roles in cell migration.

Lamellipodia and membrane ruffles form when cell adhesions fail or detach from the substrate and retract toward the cell body [4]. Membrane ruffles appear at the edge of cells moving in culture and disappear at the border between the lamella base and the main cell body [16,17]. To elucidate the mechanisms underlying cell motility, a quantitative analysis of lamella dynamics was introduced. This computer-assisted stroboscopic analysis of cell dynamics (SACED) is used for analyzing lamellipodia and ruffle formation after the cell

adheres to the ECM [4,17, 18] (Figure 1). The SACED analysis of lamellipodia dynamics has distinctive stages including lamellipodia protrusion, persistence, and ruffle retraction. Membrane dynamics and ruffling involve many proteins including, $\alpha 4 \beta 1$ integrin [19], Rac1 [20], Arp2/3 [7], and others [21,22].

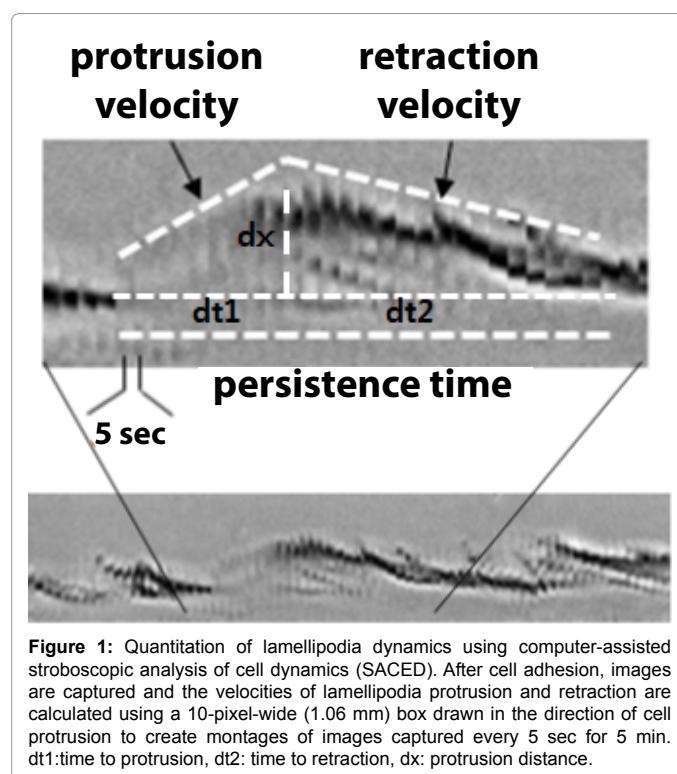


Figure 1: Quantitation of lamellipodia dynamics using computer-assisted stroboscopic analysis of cell dynamics (SACED). After cell adhesion, images are captured and the velocities of lamellipodia protrusion and retraction are calculated using a 10-pixel-wide (1.06 μ m) box drawn in the direction of cell protrusion to create montages of images captured every 5 sec for 5 min. dt1: time to protrusion, dt2: time to retraction, dx: protrusion distance.

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In the comparative studies on defective FA formation in MEFs, these MEFs commonly show longer lamellipodia persistence time than wild type (wt) MEFs. The SACED analysis of persistence time (sec; mean \pm SD) of *Crk*-null MEFs is $40.30s \pm 1.7$ and wt *Crk* is $25.82s \pm 0.66$, SYF MEF is $90.81s \pm 27.79$ and wt *Fyn* (recovered *Fyn* cDNA in SYF MEF) [13] is $65.25s \pm 38.46$, and *p130CAS*-null MEF is $67.2s \pm 10.1083$ and wt *p130CAS* is $36.5455s \pm 6.3303$ [13,15,18]. Furthermore, experiments in *p130CAS*-null MEFs show the lamellipodia protrusion and ruffle retraction velocity decreases compared with wt *p130CAS* MEFs [18]. The *Fyn*-null MEFs also show that the lamellipodia protrusion and ruffle retraction velocity decreases compared to the wt *Fyn* MEFs [13]. These SFK deficient MEFs commonly show less formation of FAs and reduced lamellipodia protrusion and retraction velocity, but membrane persistence times are prolonged compared to wt MEFs. Based on these results, FA-associated proteins modulate membrane dynamics and lamellipodia protrusion, ruffle retraction, and membrane persistence are involved in the cell spreading necessary for cell motility.

Overall, the findings summarized in this article suggest that lamellipodia dynamics control cell migration and membrane persistence time and thus are useful as one criterion of cell migration. However, to fully understand the precise mechanisms in lamellipodia dynamics, it will be necessary to examine the associated cellular factors during lamellipodia protrusion, retraction, and persistence.

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