Does self-organized criticality drive leading edge protrusion?

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Abstract

Arp2/3 complex nucleates dendritic actin networks and plays a pivotal role in the formation of lamellipodia at the leading edge of motile cells. Mouse fibroblasts lacking functional Arp2/3 complex have the characteristic smooth, veil-like lamellipodial leading edge of wild-type cells replaced by a massive, bifurcating filopodia-like protrusions (FLPs) with fractal geometry. The nanometer-scale actin-network organization of these FLPs can be linked to the fractal geometry of the cell boundary by a self-organized criticality through the bifurcation behavior of cross-linked actin bundles. Despite the pivotal role of the Arp2/3 complex in cell migration, the cells lacking functional Arp2/3 complex migrate at rates similar to wild-type cells. However, these cells display defects in the persistence of a directional movement. We suggest that Arp2/3 complex suppresses the formation of FLPs by locally fine-tuning actin networks and favoring dendritic geometry over bifurcating bundles, giving cells a distinct evolutionary edge by providing the means for a directed movement.

Keywords Arp2/3 complex · Lamellipodia · Filopodia-like protrusions · Self-organized criticality · Fractal geometry · Agent-based modeling

Arp2/3 complex is a key component for cell migration (Pollard 2007) that initiates and maintains veil-like lamellipodia at the leading edge of motile cells (Krause and Gautreau 2014). In addition, the Arp2/3 complex also contributes to a large variety of intracellular processes including endosomal sorting, phagocytosis, and endocytosis (Rotty et al. 2013; Goley and Welch 2006). Arp2/3-induced actin morphology is associated with dendritic, branched actin networks. It was shown through RNAi-depletion or gene disruption that the actin cytoskeleton was devoid of branch junctions in the absence of active Arp2/3 complex (Suraneni et al. 2012; Anderson et al. 2016; Wu et al. 2012). We examined the role of the Arp2/3 complex in the formation of the leading edge using mouse fibroblasts without any functional Arp2/3 complex (Suraneni et al. 2012). Wild-type fibroblasts have relatively smooth leading edges while ARPC3^{-/-} fibroblasts lack lamellipodia and instead have leading edges composed of large actin-based protrusions (Suraneni et al. 2012). The lengths and widths of these filopodia-like protrusions (FLPs) can be orders of magnitudes larger than filopodia of wild-type

fibroblasts (Anderson et al. 2016) and protrusion of FLPs in combination with a myosin-based contraction of the arcs between FLPs allow ARPC3^{-/-} fibroblasts to migrate at rates similar to wild-type cells but with defects in persistent directional migration (Suraneni et al. 2015).

One striking feature of the mutant cell's boundaries is their ragged appearance compared to the smooth edges of the wild-type cells (Fig. 1). To quantify the difference, we subjected the cell boundaries extracted from still images of phase-contrast microscopy movies of spreading wild-type or ARPC3^{-/-} fibroblasts to fractal analysis using the box-count method (Smith et al. 1996). We found that the cellular boundaries of ARPC3^{-/-} fibroblasts obey a power law of fractal geometry with a fractal dimension of 1.32 ± 0.05 (Fig. 2) during all stages of spreading. In contrast, the smooth boundaries of wild-type cells do not display fractal properties and have a boundary dimension of 1.02 ± 0.01 , which is essentially the dimensionality of a smooth line ($d = 1.00$). Correlative light microscopy and electron microscopy show that the fractal appearance of the ARPC3^{-/-} fibroblasts is a consequence of a scale-independent self-similar bifurcation process of the FLPs (Fig. 3).

Cryo-tomographic reconstructions of wild-type cells clearly show actin branch junctions in wild-type cells in a narrow zone $< 0.5 \mu\text{m}$ wide between the plasma membrane and bundles of actin filaments running parallel to the membrane while

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Fig. 1 Morphology of isogenic fibroblast cells. Still images from phase-contrast microscopy movies of spreading $\text{ARPC3}^{-/-}$ and wild-type fibroblasts were converted to single-pixel wide boundary images for fractal analysis. Sample outlines of **a** $\text{ARPC3}^{-/-}$ fibroblasts and **b** wild-type fibroblast cell boundaries. Bar is 30 μm

most actin filaments in the mutant $\text{ARPC3}^{-/-}$ fibroblasts are organized in thick bundles either within FLPs or at the periphery of the cell body where they closely approach the membrane (Fig. 4). Measurements of the spacing between parallel filaments yield a minimum distance of about 17 nm for both genotypes, consistent with cross-linking by fascin. Actin filaments occupy 25% of the total volume in FLPs and 19% in filopodia, a highly significant difference in packing density (Anderson et al. 2016).

In addition to bundles inside filopodia or FLPs, both wild-type and $\text{ARPC3}^{-/-}$ cells also have bundles of actin at the cell periphery that run parallel to the plasma membrane. The filament packing density in the peripheral wild-type bundles was significantly lower than in the wild-type filopodia while the packing density of the peripheral bundles in $\text{ARPC3}^{-/-}$ fibroblasts was statistically indistinguishable from those in FLPs (Anderson et al. 2016). Thus, wild-type cells have at least two different, location-dependent types of actin bundles, while the packing of bundles inside FLPs and in bundles near the cell

periphery is the same for $\text{ARPC3}^{-/-}$ fibroblasts. Filament lengths distributions of mutant and wild-type cells differ significantly with the $\text{ARPC3}^{-/-}$ fibroblasts distribution shifted towards longer filaments (Anderson et al. 2016).

How are these observations at the nanometer scale linked to the macroscopic effect of fractal geometry at the cell boundary? Consistent with a regulatory role of the Arp2/3 complex in processes guiding filopodia length and frequency (Beli et al. 2008; Sarmiento et al. 2008; Korobova and Svitkina 2008), many of our observations indicate that cells lacking functional Arp2/3 complex fail to regulate actin filament growth. The fact that only one bundle type exists in $\text{ARPC3}^{-/-}$ mutants indicates that the location-dependent fine-tuning of actin-bundle morphology is lost in these cells. Instead, they assemble massive FLPs with densely packed, long actin filaments that are orders of magnitude larger than wild-type filopodia.

To shed light on the origin of the bifurcation process that generates the fractal geometry, we devised an agent-based model encompassing the main features of a filament bundle

Fig. 2 Fractal analysis of $\text{ARPC3}^{-/-}$ fibroblasts. The log-log plot of the boundary pixel count versus area size in light micrographs of $\text{ARPC3}^{-/-}$ cells is a straight line, indicating the fractal nature of the cell boundary. The fractal dimension is equal to the negative slope of the fitted line, $d = 1.32$

Fig. 3 Fractal Morphology: Electron microscopy shows that the fractal behavior and bifurcation of FLPs of ARPC3^{-/-} fibroblasts extend to smaller nanoscales. The bars are 5 μ m

growth. In agent-based modeling, a system is modeled as a collection of autonomous entities (Bonabeau 2002). Each entity—or agent—acts individually on the basis of a set of local rules including interaction rules between agents. We used individual actin filaments as agents obeying simple rules encoding their semiflexible nature (Le Goff et al. 2002) and their tendency to be transiently cross-linked in the presence of crosslinkers. The agent-based model was set up in a two-dimensional discrete space composed of lattice points. The dimensionality reduction is only for computational efficiency and simplification of the rules and does not interfere with the generality of the results. The model was initiated with a finite number of parallel actin filaments (the agents) arranged as a cross-linked bundle by occupying neighboring lattice points perpendicular to the bundle growth direction.

Each agent grows exactly by one lattice point each discrete time step (unimpeded growth), subject to the following rules: (i) An agent cannot occupy the same lattice point as another agent, so filaments cannot merge. (ii) An agent can change direction to the left or to the right by no more than a single lattice point. This rule codes the semiflexible nature of actin

filaments. The probability P_S to change direction codes the filament stiffness. (iii) An agent mimics the move of one of its neighbors with probability P_C . This rule codes the propensity of cross-linking two neighboring filaments. Note that two neighboring filaments can still grow in the same direction, even if they are not “cross-linked.”

These three rules lead to the emergence of bifurcation over time for a wide range of parameter values (Fig. 5a). Only in the limiting cases of $P_S = 1$ (no change of direction allowed extreme stiffness) or $P_C = 1$ (always cross-linked), no bifurcation occurs at all. In all other cases, bundle bifurcation occurs eventually. An additional rule (iv) mimics the effect of the Arp2/3 complex: an actin-filament agent will move into a different direction than its neighbor with probability P_A . This rule mimics the effect of the Arp2/3 complex to prevent parallel filament arrangements without adding additional filaments (branching) to the mix. Interestingly, the bifurcation behavior ceases, giving way to a frayed network of diverging filaments (Fig. 5b), even for a relatively small P_A ($\sim 10\%$ of P_C , Fig. 5c), consistent with the observation that the Arp2/3 complex can still fulfill its function in cells even if only 10%

Fig. 4 Nanoscale actin-morphology. **a** Cryotomographic reconstructions show dendritic networks at the edges of spreading wild-type fibroblasts. **b** Actin filaments in spreading ARPC3^{-/-} fibroblasts are organized as tight parallel bundles without branches. Traced actin filaments are shown in red, Arp2/3 branch junctions in orange and cell membranes in blue. Bar is 100 nm

Fig. 5 Agent-based modeling of the growth of actin filament bundles. The model encompassed the semiflexible nature of actin filaments, their tendency to cross-link in the presence of crosslinkers, and the fact that filaments cannot occupy the same space at the same time. Parallel actin filaments originated at the top of the field of view and grew towards the bottom. **a** Actin filaments in the presence of crosslinkers. The semiflexible nature of the filaments in conjunction with their tendency to form crosslinks leads to a bifurcation of bundles as an emergent property. **b**

Actin filaments in the presence of crosslinkers and an agent that induces neighboring filaments to deviate from a parallel arrangement. This agent mimics the effect of Arp2/3 complex but does not create physical branches. The network is frayed rather than bifurcating into distinct bundles. **c** Same as **b** but with 10% of the concentration of the deviating agent in **b**. The concentration of the agent mimicking Arp2/3 complex fine-tunes the network architecture

are still active (Nolen et al. 2009). The model shows that the fact that filaments can bend in a semiflexible fashion but cannot occupy the same space, combined with a tendency to be linked to each other by crosslinkers, leads to bifurcating bundles. Cryo-tomographic reconstructions of reconstituted actin-fascin bundles show clear evidence of this bifurcation behavior (Fig. 6), adding experimental evidence to the modeling. But how can this type of bifurcation behavior result in the fractal geometry of the cell edge?

The presence of a power-law behavior, such as that describing fractal geometry, has often been linked to self-organized criticality (SOC). SOC refers to the tendency of large, non-equilibrium systems with many degrees of freedom to drive themselves to a

state close to the critical point of some phase transition (Bak et al. 1988). Because power laws are present virtually in every critical system, the existence of power laws is a fundamental prerequisite for criticality, but as such is not sufficient to prove that a system is in a critical state (Marković 2014). SOC is an appealing hypothesis to link the bifurcation behavior of the actin bundles to the fractal geometry of ARPC3^{−/−} cell boundaries, because self-organization is a hallmark of actin-based cytoskeletal systems (Huber and Käs 2011) and modeling based on SOC has been successfully used to explain power-law behavior in Arp2/3-complex driven motility of keratocytes (Cardamone et al. 2011). SOC does not require regulation or parameter tweaking and often couples one threshold-dependent and one threshold-

Fig. 6 Actin-fascin assemblies. Slices through three-dimensional cryo-tomogram of reconstituted bundles of actin filaments cross-linked by fascin. Areas of paracrystalline order show evidence for bifurcation. Arrowheads in **b** mark some bifurcation points where actin bundles split into smaller sub-bundles. Slices are 3.8 nm thick. Bars are 100 nm

independent process to evolve towards a metastable state from multiple starting points.

The simplicity and robust self-organization features make SOC an attractive model for cell migration in early evolution. In *ARPC3*^{-/-} cells, membrane tension provides a threshold-dependent process and unregulated actin polymerization with bundle bifurcation could provide the coupled threshold-independent process to lead to SOC in the system. Similarly, other processes depending on the Arp2/3 complex such as endocytosis or endosomal sorting may rely on a similar mechanism in the mutant cells. The fact that the *ARPC3*^{-/-} cells are viable suggests that cellular processes that normally depend on Arp2/3 complex and are crucial for cell survival are still functional in the absence of functional Arp2/3 complex. For directed motility, the tendency to self-organize is strong enough to overwrite the signaling pathways that lead to directed movement in wild-type cells. One may then speculate that Arp2/3 complex would have appeared later in evolution in order to optimize the performance of the system in terms of directed movement, building on a simpler prototype based on SOC like the one proposed here for the *ARPC3*^{-/-} cells. It appears that Arp2/3 complex also may provide an advantage for other cellular processes because it is also present in non-moving cells such as yeast or moss. There are a number of early branches of the eukaryotic phylogenetic tree, including Diplomonadida, Rhodophyta, and some of the Apicomplexa, that do not express Arp2/3 complex (Kollmar et al. 2012). In this scenario, in the context of cell movement, Arp2/3 complex acts as a regulatory element to suppress the formation of extensive, bifurcating filopodial protrusions, possibly by directly and locally breaking the natural tendency of filaments to cross-link into parallel bundles. The ability to fine-tune actin networks by the presence of Arp2/3 complex overrides the SOC system of cells that lack functional Arp2/3-complex and are defective in persistent directional migration (Suraneni et al. 2015). Thus, the Arp2/3 complex will give cells a distinct evolutionary edge, explaining why Arp2/3 complex is ubiquitous in higher eukaryotes.

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Compliance with ethical standards

Conflict of interest Karen L. Anderson declares that she has no conflict of interest. Mark F. Swift declares that he has no conflict of interest. Dorit Hanein declares that she has no conflict of interest. Niels Volkmann declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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