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Review

Filopodia as sensors ☆

C.A. Heckman [a,b,*], H.K. Plummer III [b]

[a] Department of Biological Sciences, Bowling Green State University, Bowling Green, OH, USA
[b] Center for Microscopy & Microanalysis, Bowling Green State University, USA

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ABSTRACT

Filopodia are sensors on both excitable and non-excitable cells. The sensing function is well documented in neurons and blood vessels of adult animals and is obvious during dorsal closure in embryonic development. Nerve cells extend neurites in a bidirectional fashion with growth cones at the tips where filopodia are concentrated. Their sensing of environmental cues underpins the axon's ability to "guide," bypassing non-target cells and moving toward the target to be innervated. This review focuses on the role of filopodia structure and dynamics in the detection of environmental cues, including both the extracellular matrix (ECM) and the surfaces of neighboring cells. Other protrusions including the stereocilia of the inner ear and epididymus, the invertebrate Type I mechanosensors, and the elongated processes connecting osteocytes, share certain principles of organization with the filopodia. Actin bundles, which may be inside or outside of the excitable cell, function to transduce stress from physical perturbations into ion signals. There are different ways of detecting such perturbations. Osteocyte processes contain an actin core and are physically anchored on an extracellular structure by integrins. Some Type I mechanosensors have bridge proteins that anchor microtubules to the membrane, but bundles of actin in accessory cells exert stress on this complex. Hair cells of the inner ear rely on attachments between the actin-based protrusions to activate ion channels, which then transduce signals to afferent neurons. In adherent filopodia, the focal contacts (FCs) integrated with ECM proteins through integrins may regulate integrin-coupled ion channels to achieve signal transduction. Issues that are not understood include the role of Ca^{2+} influx in filopodia dynamics and how integrins coordinate or gate signals arising from perturbation of channels by environmental cues.

Contents

* Corresponding author at: Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403-0212, USA. Tel.: +1 419 372 4833; fax: +1 419 372 2024.
E-mail address: heckman@bgsu.edu (C.A. Heckman).

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1. Introduction

1.1. Protrusions and their properties

1.1.1. Protrusion classes

Cell protrusions are essential for motility, chemotaxis, and haptotaxis. Three protrusion archetypes are commonly recognized in cultured mammalian cells. They differ in size and shape as well as in their physiological characteristics. Pseudopodia-like protrusions called lamellipodia are found at the leading edge of the cell and typically form a shallow arc at the most distant point from the cell center. In the motile cell, the lamellipodium is extended in the direction of travel. However, the movement itself proceeds by periodic protrusion and retraction, with the degree of retraction typically less than that of the protrusion [1]. The edge of the cell, both at the lamellipodium and elsewhere, is characterized by dynamic ruffling activity. In ruffles, the actin filaments extend upward from the edge, i.e., perpendicular to the surface of the culture dish. Previous studies reported that lamellipodia and ruffles are altered in oncogenically transformed cells. Some investigators found that lamellipodia were formed in multiple quarters of the cell [2], and ruffling was generally enhanced in transformed cells [3,4]. A third protrusion archetype is the filopodium, a small, tapering structure with a sharp tip at the distal end. In metazoan cells, filopodia are sensors of the microenvironment [5]. In the growth cone, a single filopodium making contact with a more adhesive substrate is able to set the direction of neurite extension [6].

Since the above features are loosely defined, a single physiological protrusion is inevitably called different names by different investigators. In addition, different protrusions can be named the same thing in different laboratories. The problems posed by this semantic "free-for-all" become apparent when a fourth type of protrusion, the neurite, is considered. Neurites are often defined as protrusions whose length exceeds twice the cell diameter. If this is taken as a physiological definition, however, it gives rise to the *reductio ad absurdum* argument that a short precursor of the neurite cannot exist. The problems have been addressed more recently by giving features a definition based on quantitative data. A method of sampling shape geometries in 3D was used to generate values for many variables including: a) measures of positive curvature, e.g. bending energy, b) measures of contour length in positive and negative curvature, c) relationship of the contour to derived model figures, e.g. ellipse of concentration or a figure formed by connecting the nearest or farthest points of the contour ("shrink-wrap"), d) dimensions of each protrusion modeled as a triangle, and e) measures of the area included in or excluded from the model figures. The methods are described in previous publications [7–12].

There are methods of aggregating variables that do not rely upon any prior assumptions about the statistical distribution of the data. When data are collected about cell geometry and then evaluated by factor analysis, one obtains unbiased classes or features. The resulting variables, latent factors, corresponded to different cell features including four protrusions. The relationship between factors' values and protrusion characteristics is defined in Table 1. The smallest features, indexed by factor #4, corresponded to filopodia [8,13]. Although the ruffle is recognizable by eye in most cultured cells, this feature was not extracted from the primary data by unbiased classification. When correlations among the features, including ruffles, were analyzed, the results showed that ruffles could originate from multiple factors. Factor #4 values were inversely correlated to ruffling, whereas ruffling tended to increase with increases in factor #5 [13]. By definition, variables generated by factor analysis are independent, and indeed, the data show that the formation and turnover of each of the protrusion types are distinct, suggesting that they are irreducible features [9,10,12,14]. Since the ruffle lacked such independence, it would not have been extracted as a latent factor.

How do the mathematical factors correspond to features known from conventional morphological studies, such as the above archetypes? Here, we reveal distinctions among the unbiased classes by illustrating cells in which one protrusion class is high and the others low, as shown in Fig. 1. To simplify the presentation, only the three classes that account for a high proportion of overall variance in the data are illustrated. Factor #4 clearly corresponds to filopodia. The classification also yielded a strap-shaped or triangular-shaped feature with bulkier dimensions. This feature, factor #7, was identified as a nascent neurite [12,14]. The remaining factors, #5 and #16, are less well understood. Factor #5 values depend on mass distribution around the cell center. Despite their distinct identities, there is little doubt that both #5 and #7 features would be called lamellipodia on a descriptive basis. The physiological distinction is important, however. A neurite is formed by the cell without cycles of protrusion and retraction and rarely involves locomotion of the whole cell. In contrast, the lamellipodium is associated with recurrent extensions and retractions of the leading edge, and it facilitates locomotion of the whole cell.

1.1.2. Subjectively defined classes

Both lamellipodia and filopodia depend on the polymerization and depolymerization of actin filaments. The filaments in filopodia intersect with the membrane at angles greater than 60° and are formed into a paracrystalline array by the actin-binding protein, fascin [15,16]. In contrast, filaments in lamellipodia are thought to intersect the plasma membrane at a mean angle of 50° [17], although angles up to 90° have been reported [18]. In dynamic studies, the two types of protrusions are distinguished by the speed of process formation and retraction. The larger protrusions move outward by 30–100 nm/s (1.8–6.0 μm/min) and the smaller by 100–200 nm/s. In the nerve growth cone, the filopodia may protrude at 500–700 nm/min (~0.1 μm/s). Speeds ranging as high as 3 μm/min or more have been observed, with most of the variation caused by differences in the rates of actin filament assembly [19,20]. The protrusions respond differently to osmolarity, as lamellipodia expand faster in hypotonic and filopodia faster in hypertonic media [21] (reviewed in [22]). Lamellipodia expansion occurs some 20 s before maximal actin subunit addition [23], which supports previous suggestions that lamellipodia are driven by cycles of solation, osmotic expansion, and re-gelation of the actin filament network [22]. Filopodia dynamics depend on diffusible cues from the environment as well as the nature of the substrate to which they adhere, as discussed below in Filopodia dynamics and Filopodial FCs. There is a possibility that cues such affect other protrusions but, if so, there is little indication of how the cues regulate their dynamics.

The knowledge of actin filament dynamics can be fit into a theoretical model that is consistent with physiological aspects discussed above.

Table 1
Relation of factor values for protrusions to morphology of the cell edge.

Unbiased class	Value increases with increases in
Factor #4	Prevalence of filopodia
Factor #5	Centripetal mass distribution
Factor #7	Prevalence of nascent neurites
Factor #16	Features more massive than filopodia but pointed

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