Seminars in Cell & Developmental Biology 21 (2010) 350–356

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

I-BAR domains, IRSp53 and filopodium formation

Sohail Ahmed*, Wah Ing Goh, Wenyu Bu

Neural Stem Cell Laboratory, Institute of Medical Biology, 8A Biomedical Grove, #05-37 Immunos, Singapore 138648, Singapore

ARTICLE INFO

Article history:
Available online 11 November 2009

Keywords:
I-BAR
IRSp53
Cdc42
F-actin
Membrane protrusion
Filopodia

ABSTRACT

Filopodia and lamellipodia are dynamic actin-based structures that determine cell shape and migration. Filopodia are thought to sense the environment and direct processes such as axon guidance and neurite outgrowth. Cdc42 is a small GTP-binding protein and member of the RhoGTPase family. Cdc42 and its effector IRSp53 (insulin receptor phosphotyrosine 53 kDa substrate) have been shown to be strong inducers of filopodium formation. IRSp53 consists of an I-BAR (inverse-Bin-Amphiphysin-Rvs) domain, a Cdc42-binding domain and an SH3 domain. The I-BAR domain of IRSp53 induces membrane tubulation of vesicles and dynamic membrane protrusions lacking actin in cells. The IRSp53 SH3 domain interacts with proteins that regulate actin filament formation e.g. Mena, N-WASP, mDia1 and Eps8. In this review we suggest that the mechanism for Cdc42-driven filopodium formation involves coupling I-BAR domain-induced membrane protrusion with SH3 domain-mediated actin dynamics through IRSp53.

Contents

1. Introduction

1.1. Cell morphogenesis and migration

Cells form the fundamental building blocks of all living matter [1]. Thus understanding the form and function of cells will help to reveal the complex biology of tissues, and ultimately whole organisms. Cardinal features of cells are their shape or morphology,

* Corresponding author. Tel.: +65 6407 0165; fax: +65 6464 2048.
E-mail address: sohail.ahmed@imb.a-star.edu.sg (S. Ahmed).

1084-9521/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcdb.2009.11.008

and their ability to migrate. Disease states such as neurodegeneration and cancer can be linked to defects in cell morphology and migration. Two cellular compartments, the membrane and the cytoskeleton, play pivotal roles in regulating cell shape and migration.

1.2. Lamellipodia and filopodia

The actin-based structures at the leading edge – lamellipodia and filopodia – determine cell shape and ability to migrate. Motile cells put forward thin, sheet-like protrusive structures at their leading edge as they crawl across the substratum. The region closest to the leading edge is referred to as the lamellipodium. It is made up of highly branched dendritic microfilaments assembled by the Arp2/3 complex [2].

Filopodia are membrane-based actin-rich finger-like protrusions that are highly dynamic. Filopodia extend and retract rapidly from the cell surface as the cell explores its environment, seeking biological cues. Their movements are not limited to extension and retraction in the horizontal plane; filopodia are also able to swing laterally, as well as lift up away from and down towards the substratum in the vertical plane. Ultimately they form adhesions with the matrix, facilitating lamellae to fill gaps between them and move the cell forward. Filopodia are thought to play important roles in a number of cellular and developmental processes, including (i) neuritogenesis [3,4], (ii) axon guidance in neuronal growth cones [5–7], (iii) receptor–ligand endocytosis [8,9], (iv) dengue virus uptake [10], (v) detection of pathogen targets for phagocytosis [11] and (vi) dorsal closure in *Drosophila* embryos [12].

1.3. Aims of the review

Here, we show how studies of the I-BAR and SH3 domain-containing protein IRSp53 have begun to reveal a mechanism of filopodium formation. Essential to this model for filopodium formation is that the Cdc42–IRSp53 effector complex allows the coupling of membrane protrusion (driven by the I-BAR domain) to actin dynamics (mediated by the SH3 domain).

2. Filopodia: diversity, form and composition

In mammalian cells, each individual filopodium is made up of a cylindrical plasma membrane extension enclosing a tight bundle of 15–20 linear actin filaments all oriented in parallel, with their barbed ends distal from the cell body [13]. In addition to actin filaments, a number of proteins are associated with filopodia. The formin Dia2 (Diaphanous 2) nucleates actin filaments and has been found in both mammalian and *Dictyostelium* filopodia, including the tips of these structures [14,15]. Ena/VASP (enabled/vasodilator-stimulated phosphoprotein) proteins are also found at filopodial tips. Ena/VASP and Mena (mouse Ena), together with mDia2 (mouse Dia2), have been proposed to form a 'tip complex'. Ena/VASP facilitates the barbed end growth of actin filaments by protecting them from capping proteins (reviewed in [11]). Myosin X is a VASP-binding protein that localises to both the tips and shafts of filopodia, and appears to transport Ena/VASP and other components to the tips, using its motor domain that travels along actin filaments towards their barbed ends [16]. Along the filopodial shaft, the actin-bundling protein fascin crosslinks individual actin filaments as they polymerise. This gives rise to stiff bundles that are rigid enough not to buckle when they push against the membrane as the filopodium extends [11].

Table 1
Morphological characteristics of mammalian filopodia.

Cell line/cDNA transfection	Length (μm)	Lifetime (s)
Endogenous		
N1E115	15	142
HeLa	14	131
Cos7	10	123
B16F1	8	79
CHO	ND	ND
Transfected		
Cdc42V12, Rac1N17 (fibroblasts)	8.4	157
IRSp53 (N1E115)	6.8	187
N-WASP (N1E115)	7.4	154
Toca-1 (N1E115)[a]	6.6	128
Rif (N1E115)[b]	4.4	155

ND: not detected. Filopodial width 0.6–1.2 μm. Data from [31], except [a][53] and [b](Goh and Ahmed, unpublished observations).

3. Definition of mammalian filopodia

At the outset of this review it is important to define the structure and dynamics of filopodia so that results from different laboratories can be compared and their discrete features investigated. Mammalian filopodia can be followed in cell culture using time-lapse microscopy. Widefield dual channel fluorescence microscopy using sensitive CCD (charged-coupled device) cameras are an ideal set-up to follow filopodia. Individual frames can be acquired in the range of 100 ms each and at a rate of six frames a minute giving a total of 600 frames over a 10 min time course. Generally speaking, this format allows dynamic data of filopodia to be acquired without significant bleaching or toxicity. We have followed filopodium formation in a variety of cell types using GFP-actin to label dynamic actin structures and DIC microscopy to track changes in cell morphology. The use of GFP-actin allows observation of structures in real time. From time-lapse analysis of endogenous structures in N1E115, HeLa, Cos7 and B16F1 cells, the following features of filopodia were determined: lifetime, length, width and morphology (Table 1). Mammalian filopodia are rarely longer than 15 μm and have a lifetime of 79–142 s. They have a uniform width of 0.6–1.2 μm along their length, and are never branched or tapered in appearance. Filopodia usually emerge from the cell periphery or leading edge individually and never in clusters. In contrast, retraction fibres are non-dynamic, tapered in appearance and are found in clusters.

4. Small GTPases as regulators of filopodium formation

Several members of the Ras superfamily of small GTPases have been linked to filopodium formation, with strongest evidence having emerged for Cdc42 [17], Rif (Rho in filopodia) [18] and Rab35 (Ras-like protein in brain 35) [19]. Apart from these, other small GTPases implicated in filopodium formation include RalA (Ras-like A) [20], TC10, Wrch-1 (Wnt-1 responsive Cdc42 homologue-1) and Wrch-2 (Wnt-1 responsive Cdc42 homologue-2) [17].

4.1. Cdc42

In 1995, two studies reported that Cdc42 could regulate the formation of filopodia in mammalian cells. The first study showed that bradykinin could activate Cdc42 leading to three distinct morphological effects – filopodium formation, Rac-mediated lamellipodium formation and inactivation of RhoA [21]. In the other study, Nobes and coworkers demonstrated that Cdc42 induced focal complexes associated with filopodia [22]. In a variety of mammalian cells Cdc42 dominant negative protein inhibits filopodium formation [21–24]. Cdc42 functions as a molecular switch by reg-