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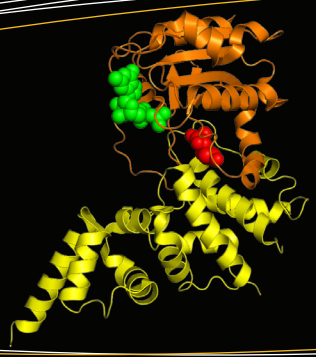

this issue

Upcoming Meetings

SLAS 2013
Orlando, FL, USA
Booth # 2031
Jan. 12-16, 2013

AACR 2013
Washington, DC, USA
Booth # 721
April 6-10, 2013

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Contact Us

P: 1 (303) 322.2254
F: 1 (303) 322.2257
E: cserve@cytoskeleton.com
W: cytoskeleton.com

Distributors

www.cytoskeleton.com/distributors/

Dendritic Spines: Role of Arf6 in Development

Dendritic development encompasses the formation, outgrowth, and branching of dendrites, and in the case of neurons with excitatory synapses, the formation of actin-rich protrusions such as dendritic filopodia (precursor to spines) and spines (Fig. 1). Notably, filopodia are dynamic and while they are spine precursors, not all filopodia become spines. Structurally, spines are primarily composed of F-actin and serve as the site of most excitatory synaptic neurotransmission[1-4]. Dynamic changes in spine structure and/or function have an integral role in normal learning and memory processes, as well as the disease processes associated with neurodegenerative and neurological disorders (e.g., Alzheimer's Disease [AD] and mental retardation)[1,5,6]. Thus, pathways that regulate actin dynamics in dendrites and spines are of immense interest to both basic and clinical neuroscientists. Two processes essential for regulating spine morphology, mobility, and stability (and thereby function) are cytoskeletal actin re-modeling[3,4] and membrane trafficking[7].

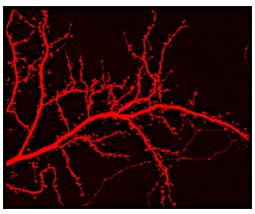

Fig. 1. Dendrites and spines of a DsRed-transfected hippocampal neuron.

Recently, the ADP ribosylation factor (Arf) GTPases, part of the Ras superfamily of small G-proteins, have emerged as critical players in regulating the development of neuronal dendrites and spines because Arfs function in actin re-arrangement and/or trafficking. Like other GTPases, Arfs cycle between a GTP-bound state mediated by guanine exchange factors (GEFs) and a GDP-bound state mediated by guanine activating proteins (GAPs). Of the 6 known isoforms[8,9], Arf6 is the most divergent of the Arfs and is unique in its function and subcellular localization[9]. Arf6 has dual functions in most cells, including neurons: trafficking and actin re-arrangement[7]. GTP-bound Arf6 is localized at the plasma membrane while GDP-bound Arf6 is in endosomal compartments[9] (Fig. 2).

Arfs and Dendritic Branching

Early dendritic developmental studies using hippocampal neurons reported that Arf6 negatively regulates dendritic branching[10-12] by at least two separate pathways, one directly involving Arf effectors and the other involving Rac1[10] (Fig. 2). Over-expression of a dominant-negative (DN) mutant of an Arf6 GEF significantly increases dendritic branching[12], possibly through a mechanism involving alpha-actinin[13]. Likewise, siRNA knockdown of an Arf GAP or a DN version of the same GAP reduces dendritic branching in dissociated hippocampal neurons while over-expression of the wild-type (WT) GAP increases branching[14].

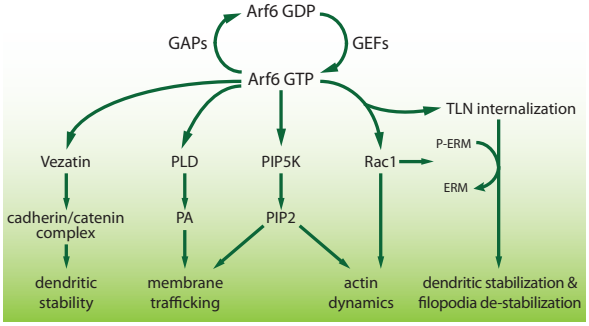

Fig. 2. Arf6 signaling pathways. PLD, phospholipase D; PA, phosphatidic acid; PIP2, phosphatidylinositol bisphosphate; PIP5K, phosphatidylinositol-4-phosphate 5-kinases; TLN, Telencephalin.

Arfs and Spine Formation

The final stage of dendritic development is synapse formation, which in the case of excitatory synapses, consists of dendritic filopodia forming and developing into spines. Over-expression of WT or constitutively-active (CA) Arf6 reveals that dendritic filopodia formation is negatively regulated by Arf6[11]. Similar constructs reduce spine density in cultured hippocampal neurons, while over-expression of a DN Arf6 has the opposite effect[15]. Likewise, over-expression of a DN Arf GAP decreases spine density in hippocampal slice cultures[14]. Conversely, Choi et al.[16] reported that over-expression of a fast-cycling Arf mutant (i.e., CA) or WT Arf GEF increases spine number while the Arf mutant decreases filopodia number in maturing neurons. A DN Arf GEF increases filopodia number[16]. Moreover, the authors[16] found that siRNA knock-down of Arf6 or an Arf GEF decreases spine number while increasing filopodia number and live cell imaging confirmed that this change is due to reduced

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conversion of filopodia to spines as well as stabilization of young spines. Rac1 activation is implicated in the development of filopodia into spines[16]. Raemaekers et al.[17] confirmed the increase in spine number (concomitant with decreased filopodia number) in hippocampal neurons over-expressing the same fast-cycling Arf mutant. In all of these studies, WT, CA, or DN Arf6, Arf GEFs, and Arf GAPs were exogenously expressed in cultured neurons. Confounding factors are that different mutants were used and the GEFs and GAPs regulate multiple Arfs, which may have opposing functional effects. Additionally, how the exogenously expressed proteins interact with their endogenous binding partners or WT counterparts can alter the functional outcome of over-expressing these proteins. Finally, expression levels of transfected proteins can differ. These factors may explain the conflicting data regarding how Arf GTPases regulate the formation of dendrites and spines in neurons.

Identification of the downstream Arf effectors mediating these changes in dendrite and spine formation is unfulfilled (Fig. 2). Candidates include phosphatidylinositol-4-phosphate 5 kinases, phospholipase D, vezatin, and the Rac1 GTPase[7,18]. Telencephalin (TLN; a.k.a intercellular adhesion molecule-5) is also likely invoved. TLN inhibits spine development and its internalization and trafficking is mediated by Arf6[17]. Arf6-mediated endocytosis of TLN initiates a signaling cascade that culminates in increased dendritic spine stability regulated at least in part by Rac1-mediated de-phosphorylation of ERM actin binding proteins, allowing dissociation of TLN from the plasma membrane[17].

Besides Arf6, Arf4 also regulates spine development and in a positive manner[19]. Knockdown of Arf4 (Arf+/- mice) decreases spine density in cultured hippocampal neurons, whereas over-expression of WT or CA Arf4 has the opposite effect. Clinically, Arf4 over-expression rescues spine loss induced by expression of apolipoprotein E4 in hippocampal neurons of a murine AD model[19].

Despite these advances, many questions remain, including the specificity and redundancy of the various Arf4 and 6 GEFs and GAPs as well as resolving exactly how Arfs regulate dendritic development. To help researchers answer these questions, Cytoskeleton, Inc. offers multiple Arf activation assays and Arf reagents.

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Arf Protein Research Tools

Activation Assays	Cat. #	Amount
NEW **Arf1 G-LISA® Activation Assay, colorimetric**	BK132	96 assays
NEW **Arf1 Activation Assay Biochem Kit™, Pull-down format**	BK032-S	20 assays
NEW **Arf6 G-LISA® Activation Assay, colorimetric**	BK133	96 assays
NEW **Arf6 Activation Assay Biochem Kit™, Pull-down format**	BK033-S	20 assays
Rac1,2,3 G-LISA® Activation Assay, colorimetric	BK125	96 assays
Rac1 G-LISA® Activation Assay, colorimetric	BK128	96 assays
Rac1 Activation Assay Biochem Kit™, Pull-down format	BK035	50 assays

Proteins	Purity	Cat. #	Amount
Rac1 His Protein, constitutively-active (Q61L)	>90%	R6101-A R6101-C	1 x 10 µg 4 x 10 µg
Rac1 GST Protein, dominant-negative (T17N)	>90%	R17G01-A R17G01-C	1 x 25 µg 4 x 25 µg
Rac1 GST Protein, wild-type	>90%	RCG01-C	8 x 25 µg
Rac1 His Protein, wild-type	>90%	RC01-A RC01-C RC01-XL	1 x 100 µg 3 x 100 µg 1 x 1 mg
Rac2 His Protein, wild-type	>90%	RC02-A RC02-B	1 x 100 µg 3 x 100 µg

Small G-protein Antibodies	Host	Type	Species Reactivity	Cat. #	Amount
Rac1 Specific Antibody Human C-terminal Peptide	Mouse	mAb	Hu, Ms, Rt, other extracts	ARC03-A ARC03-B	2 x 50 µg 6 x 50 µg

Phalloidin	Excitation	Emission	Signal stability * ($T_{1/2}$ in secs)	Cat. #	Amount
Acti-stain™ 488 phalloidin	480 nm	535 nm	57	PHDG1-A	300 Slides
Acti-stain™ 535 phalloidin (Rhodamine Phalloidin)	535 nm	585 nm	27	PHDR1	300 Slides
Acti-stain™ 555 phalloidin	535 nm	585 nm	46	PHDH1-A	300 Slides
Acti-stain™ 670 phalloidin	640 nm	670 nm	8	PHDN1-A	300 Slides

* Stability measured without antifade. For comparison, fluorescein phalloidin has a T1/2 of 6 secs.
** One slide equals enough phalloidin to stain a 25 mm^2 coverslip