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this issue

Rho GTPase control of neurite extension
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Rho GTPase control of neurite extension

The Rho family of guanine nucleotide triphosphatases (GTPases) governs neurite extension by regulating the interplay of actin and microtubule (MT) cytoskeletal dynamics. During neurite extension, GTPases are activated by growth factors and guidance cue proteins through receptor binding on the membrane of the growth cone, the motile tip of the extending axonal neurite. The most studied GTPases are RhoA, Rac1 and Cdc42 (1-3).

Neurite extension is a process essential for nervous system development, synaptic plasticity and treatment and repair of brain diseases and injuries. Neurite extension or growth can be considered a three step process (4).

Step 1: Protrusion: Rac1 and Cdc42 induce formation of actin filaments via activation of the downstream effector PAK which activates the WAVE (Rac) or N-WASP (Cdc42) protein that complexes with Arp2/3 to produce actin-rich lamellipodia or filopodia, respectively (1-3). These actin-rich protrusions form and extend from the peripheral domain of the growth cone and act as environmental sensors for axon growth and guidance (Fig. 1). There is also increasing evidence that actin filaments and MTs interact during neurite extension. MTs defined as dynamic (vs stable), based on specific post-translational modifications, are found in the peripheral domain of the growth cone where MTs can interact with F-actin via proteins that can bind or regulate both cytoskeletal elements (5,6). For instance, Rac1 regulates interplay between actin and MT dynamics during protrusion (6-10).

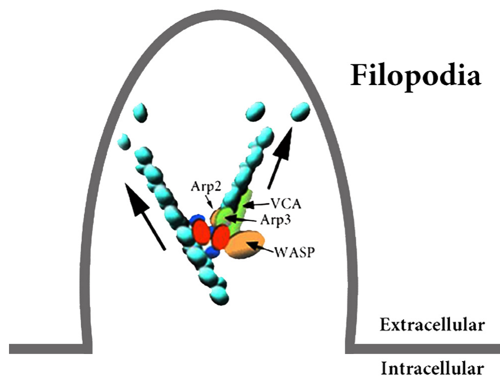

Figure 1. Actin polymerization mediated by Arp2/3/VCA/WASP proteins in filopodia.

Step 2: Engorgement: MTs and organelles move into the protruding regions with MTs providing a scaffold for anterograde and retrograde transport and a structural connection between the stabilized axon shaft and the motile central and peripheral domains of the growth cone (5).

Step 3: Consolidation: RhoA and its downstream effector Rho Kinase (ROCK) activate the myosin II motor protein which mediates actomyosin contraction and the formation of stress fibers and focal adhesions. Activated RhoA/myosin II induces F-actin and MT bundling and re-distribution of F-actin and MTs away from the growth cone periphery, causing the membrane to shrink around MT bundles, consolidating a segment of the developing axon shaft (5,6,11-18).

GTPase signaling is much more complex than initially believed and is influenced by many factors that affect the balance of GTPase activation and inactivation (Fig 2). For instance, RhoA signaling via mDia promotes axon extension and stabilization of MTs (2,3), Rac1 activity is sometimes required for growth cone inhibition (19) and over-expression of the Rac GEF Vav2 inhibits neurite extension (20). Both Rac1 and RhoA/ROCK activate LIM kinases which inhibit the actin depolymerizing factor cofilin, leading to neurite stabilization and retraction (3,21). These paradoxical findings suggest that GTPase control of neurite extension results from a balancing of multiple factors, including GEF/GAP activity, the specific downstream pathways activated (20,22) and the degree to which actin filaments and MTs dynamically interact (Fig. 2).

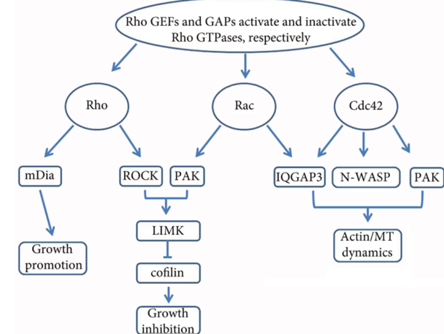

Figure 2. Rho GTPase pathways controlling neurite extension. Adapted from ref. 3

Upcoming Meetings

Neuroscience	Nov 12-16
AACR-NCI-EORTC	Nov 12-16
ASCB	Dec 3-7

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Rho GTPase control of neurite extension

(Cont'd from Pg 1)

For more information on how GTPases regulate neurite extension, please see the references and reviews below.

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Pull-down Activation Assays

Pull-down Activation Assays	Effector Protein	Cat. #	# of Assays
Cdc42 Activation Assay Biochem Kit™	p21 activated kinase 1	BK034	50
Rac1 Activation Assay Biochem Kit™	p21 activated kinase 1	BK035	50
Ras Activation Assay Biochem Kit™	Kinase Raf1	BK008	50
RalA Activation Assay Biochem Kit™	Ral-BP1	BK040	50
RhoA Activation Assay Biochem Kit™	Rhotekin	BK036	80

Fluorescent Probes

Product	Excitation	Emission	Cat. #	Amount
Acti-stain™ 488 phalloidin	480 nm	535 nm	PHDG1-A	300 Slides
Acti-stain™ 555 phalloidin	535 nm	585 nm	PHDH1-A	300 Slides
Acti-stain™ 670 phalloidin	630 nm	680 nm	PHDN1-A	300 Slides
Rhodamine phalloidin	535 nm	585 nm	PHDR1	1 x 500 µl

Neuroscience Tools

G-LISA® Activation Assays

Product	Cat #	# of Assays
RhoA G-LISA® Activation Assay, colorimetric	BK124	96
RhoA G-LISA® Activation Assay, luminescence	BK121	96
Cdc42 G-LISA® Activation Assay, colorimetric	BK127	96
RalA G-LISA® Activation Assay, colorimetric	BK129	96
Rac1,2,3 G-LISA® Activation Assay, colorimetric	BK125	96
Rac1 G-LISA® Activation Assay, colorimetric	BK128	96

Cytoskeletal Proteins

Labeled Actins	Source	Purity	Cat. #	Amount
Rhodamine Actin Protein	Human platelet, non-muscle	>99%	APHR-A APHR-C	4 x 10 µg 20 x 10 µg
Rhodamine Actin Protein	Rabbit skeletal muscle	>99%	AR05-B AR05-C	10 x 20 µg 20 x 20 µg
Arp2/3 Protein Complex	Bovine Brain	>95%	RP01-A RP01-B	2 x 50 µg 6 x 50 µg
WASP VCA domain GST Fusion Protein	Recombinant human	>95%	VCG03-A VCG03-B	1 x 500 µg 5 x 500 µg
AMCA Labeled Tubulin	Porcine Brain	>99%	TL440M-A TL440M-B	5 x 20 µg 20 x 20 µg
HiLyte Fluor™ 488 Labeled Tubulin	Porcine Brain	>99%	TL488M-A TL488M-B	5 x 20 µg 20 x 20 µg
TRITC Rhodamine Labeled Tubulin	Porcine Brain	>99%	TL590M-A TL590M-B	5 x 20 µg 20 x 20 µg
X-Rhodamine Labeled Tubulin	Bovine Brain	>99%	TL620M-A TL620M-B	5 x 20 µg 20 x 20 µg
HiLyte Fluor™ 647 Labeled Tubulin	Porcine Brain	>99%	TL670M-A TL670M-B	5 x 20 µg 20 x 20 µg

G-switch™ Activators and Inhibitors

G-Protein Modulator	Cell Entry Mechanism	Rho Family Protein Modulation	Cat. #	Amount
Rho Activator II Deamidation of Rho Gln-63	Cell permeable	Direct	CN03-A CN03-B	1 x 20 µg 5 x 20 µg
Rho Inhibitor I ADP ribosylation of Rho Asn-41	Cell permeable	Direct	CT04-A CT04-B	1 x 20 µg 5 x 20 µg
Rho/Rac/Cdc42 Activator I Deamidation of Rho Gln-63 & Rac/Cdc42 Gln-61	Cell permeable	Direct	CN04-A CN04-B	1 x 20 µg 5 x 20 µg
Rho Pathway Inhibitor I Rho kinase (ROCK) inhibitor Y-27632	Cell permeable	Direct	CN06-A CN06-B	5 x 10 units 20 x 10 units
Rho Activator I SHP-2 phosphatase mediated Rho activation	Cell permeable	Indirect	CN01-A CN01-B	5 x 10 units 20 x 10 units
Rac/Cdc42 Activator II EGF receptor mediated Rac/Cdc42 activation	Receptor mediated	Indirect	CN02-A CN02-B	5 x 10 units 20 x 10 units