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Rho Family GEFs and Dendritic Spine Structural Plasticity

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Rho family GEFs and Dendritic Spine Structural Plasticity

Dendritic spines are the post-synaptic component of most excitatory glutamatergic synapses and primary site of synaptic structural plasticity[1-4] for modulating synaptic function[3]. Activity-dependent structural plasticity in spines (i.e., spine morphogenesis) depends upon dynamic re-organization of F-actin, the primary structural component of spines[1-7]. Spine morphogenesis is important for normal learning and memory and the development of neurodegenerative diseases and neurological disorders[8-10].

The RhoA, Rac1, and Cdc42 GTPases regulate spine morphogenesis; RhoA inhibits spine growth and stability, whereas Rac1 and Cdc42 exert the opposite effect. In reality, Rho family regulation of spine structural plasticity is much more complex[5,7,11-16]. Precise spatiotemporal regulation of Rho GTPases is with guanine exchange factors (GEFs) triggering GTP/GDP exchange and GTPase activating proteins (GAPs) stimulating intrinsic GTPase activity. At least eight Rho family GEFs regulate spine morphogenesis and these GEFs are activated through a variety of receptor signaling pathways, including glutamatergic NMDA receptors (NMDARs) and receptor tyrosine kinases (RTKs)[5-7]. NMDARs mediate calcium influx and subsequent activation of calcium/calmodulin-dependent kinases (CaMKs), which phosphorylate Rho family GEFs[5,7,17,18], essential for GEF activity. In this newsletter, the regulation of spine structural plasticity by the Rho family GEFs Kalirin7 (Kal7; the most abundant isoform in adult brain), Trio-9 (the most abundant isoform in hippocampus), Tiam1, RasGRF2, DOCK10, DOCK180, ephrexin1, and ephrexin5 is discussed (Fig. 1).

Rac and Cdc42 GEFs

The Rac GEF Kal7 is highly expressed in the spines of mature hippocampal and cortical neurons. Kal7 is essential for NMDAR-dependent long-term potentiation (LTP), the most studied form of synaptic plasticity and the likely cellular basis of learning and memory[19,20]. Calcium-activated CaMKII phosphorylates the Thr95 residue, which enables Kal7-mediated activation of Rac1 and re-organization of the actin cytoskeleton necessary for activity-dependent spine morphogenesis[17,18,21,22]. Elevated Kal7 activity increases spine density and size, whereas Kal7 downregulation decreases spine density *in vitro* and *in vivo*[17,18,21-24]. Similarly, a Kalirin paralog, the GEF Trio-9, is phosphorylated by CaMKII and regulates NMDAR-dependent LTP in hippocampal neurons and presumably the concomitant spine structural plasticity in a manner similar to Kal7[18]. In addition, Kal7 activity is required for spine morphogenesis induced by N-cadherins, a class of trans-synaptic adhesion molecules[5,22](Fig. 1).

The Rac GEF Tiam1 is highly expressed in the developing cortex and hippocampus and also undergoes CaMKII-mediated phosphorylation, resulting in activation of Rac1[17,25]. Selectively blocking Tiam1 function reduces spine density and inhibits NMDAR-dependent formation of new spines, while Tiam1 overexpression increases spine density[5,25]. Interestingly, Tiam1-induced Rac activation, subsequent spine morphogenesis, and Tiam1's restricted localization to spines is regulated by the polarity protein PAR-3 (partitioning-defective gene 3)[26] (Fig. 1).

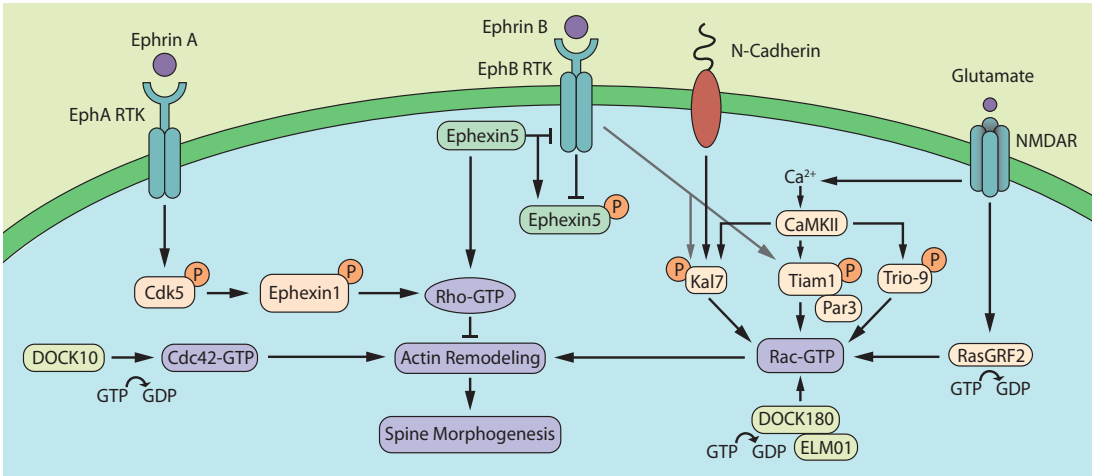

Figure 1. Regulation of dendritic spine structural plasticity by Rho family GEFs.

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Other Rac GEFs that regulate actin-based spine plasticity are RasGRF2 and DOCK180. RasGRF2 is required for NMDAR-dependent LTP and the correlated rapid spine enlargement[27]. Similarly, DOCK180, in a mandatory complex with ELMO1, also positively regulates spine morphogenesis via an activation of Rac GTPases in neurons. Loss of DOCK180 reduces spine density with no effect on spine head size, while over-expression enhances density[28] (Fig. 1).

Rac GEFs are also phosphorylated following activation of EphB RTKs[5-7,17,22,29] (Fig. 1). Kal7 is necessary for EphB-mediated increases in spine density[30] and Kal7 phosphorylation could be mediated by either EphB or associated kinase. A noteworthy candidate is cyclin-dependent kinase 5 (Cdk5), which phosphorylates Kal7 on Thr1590 and affects Kal7's regulation of spine morphogenesis[31]. Similar to Kal7, Tiam1 is necessary for EphB-mediated increases in spine density and is activated via phosphorylation of Tyr829, also by either EphB or associated kinase[32]. EphB RTKs also regulate the coordinated activities of Tiam1 and the Rac GAP Bcr, which form a complex essential for spatially-regulated EphB RTK-mediated control of spine morphogenesis[33]. Athough DOCK10 is a Cdc42/Rac1 GEF, the positive regulation of spine number and head size in cerebellar and hippocampal neurons is mediated by its activation of Cdc42[34] (Fig. 1).

Rho GEFs

As opposed to Rac and Cdc42 GEFs, which upregulate spine plasticity, Rho GEFs exert the opposite effect. For instance, GEF-H1 (a.k.a. ARHGEF2 or Lfc), negatively regulates spine density and length[35]. Following the sequential activation of the RTK EphA4, Cdk5, and the Rho GEF ephexin1 by Src kinase-mediated phosphorylation of Tyr87 and Cdk5-mediated phosphorylation of Thr41 and Ser139 residues on ephexin1, activated RhoA induces spine retraction in hippocampal neurons[29] (Fig. 1). Another Rho GEF, ephexin5, also induces spine retraction via RhoA activation. Ephexin5 directly binds and inhibits EphB-mediated spine morphogenesis while activating RhoA. Upon ligand-induced activation of EphB, the RTK inactivates ephexin5 through tyrosine phosphorylation (Tyr361), which induces ubiquitin-mediated degradation. Upon disinhibition, EphB can positively regulate spine morphogenesis[36] (Fig. 1).

Summary

The dendritic spine is the post-synaptic component of most excitatory neurotransmission and site of synaptic structural plasticity. Spine structural plasticity relies upon re-modeling of the actin cytoskeleton, which is regulated by GEF-mediated activation of Rho family GTPases. Thus, it is imperative to better understand how GEFs function in neurons and how the same GTPase is controlled by different GEFs for precise spatiotemporal regulation. To assist scientists in these studies, Cytoskeleton, Inc. offers SiR-actin live cell imaging probe and multiple purified GEFs and GTPases, along with activation and exchange assays, antibodies, and activators and inhibitors.

Related Products

Products	Purity	Cat. #	Amount
NEW **Ras-GRF GEF protein Cdc25 domain** Human recomb., MBP tagged	>85%	CS-GE03	1 x 100 µg
NEW **Tiam1 GEF protein, GEF for Rac** Human recomb. DHPH domain, MBP tag	>85%	CS-GE04	1 x 100 µg
NEW **Vav1 GEF protein, GEF for Rac** Human recomb. DHPHC1 domain Y174D mutant, 6xHis tagged	>85%	CS-GE05	1 x 100 µg
NEW **Vav2 GEF protein , GEF for Rac** Human recomb. DH domain, 6xHis tagged	>85%	CS-GE06	1 x 100 µg
RhoGEF Exchange Assay Biochem Kit™	n/a	BK100	60-300 assays
SiR-Actin Kit Includes SiR700-Actin and Verapamil	n/a	CY-SC001	50 nmol

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