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Rho GTPases as regulators of morphological neuroplasticity

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SUMMARY

GTPases function as intracellular, bimolecular switches by adopting different conformational states in response to binding GDP or GTP. Their activation is mediated through cell-surface receptors. Rho GTPases act on several downstream effectors involved in cellular morphogenesis, cell polarity, migration and cell division. In neurons, Rho GTPases regulate various features of dendritic and axonal outgrowth during development and regeneration mainly through their effects on the cytoskeleton. This review summarizes the main functions of Rho, Rac and Cdc42 GTPases as key regulators of morphological neuroplasticity under normal and pathological conditions.

1. Introduction

Outgrowing neuronal processes utilize an intrinsic, actin-based treadmilling mechanism to advance the leading edge of their growth cones, which represent highly specialized structures localized at the tips of axons and dendrites. These cones extend finger-like filopodia and veil-like lamellipodia highly enriched in actin polymer chains (Gallo and Letourneau, 2000; Smith and Li, 2004; Letourneau, 2009). An appropriate substratum is required for the generation of traction and tension within the growth cone. Thereby, poorly attached processes are removed while adherent ones are stabilized or move. Cell adhesion molecules, in particular members of the integrin family, are central to our understanding of the underlying molecular mechanisms of these processes which are under tight regulation by neuronal growth factors and guidance cues. Over the recent years, several small GTPases have been identified as key mediators of the interactions between cell adhesion molecules and the cytoskeleton constituting axonal and dendritic morphology. Among them, Rho GTPases are now regarded as major regulators of axonal and dendritic growth.

Rho proteins act as molecular switches integrating signals from the extracellular environment. They cycle between two conformational states (from an active GTP-bound state to an inactive GDP-bound state) by hydrolyzing GTP to GDP. Eukaryotic cells contain hundreds of such low molecular mass GTPase switches (appr. 21 kDa). The Ras superfamily of GTPases falls into five major groups (Ras, Rho, Rab, Arf and Ran). The Rho and Ras families are particularly relevant for cell biology by regulating morphogenesis, polarity, migration and division at the cellular level. Moreover, at the molecular level they are involved in cytoskeletal dynamics, vesicular transport and gene expression (Etienne-Manneville and Hall, 2002). Rho GTPases were first found in fibroblasts as mediators of filopodia and lamellipodia-formation (Nobes and Hall, 1995). Subsequently, RhoA, Rac, and Cdc42 were identified as key regulators of axonal and dendrite morphogenesis in tissue culture and in vivo (Sebok et al., 1999; Hall and Lalli, 2010). The functional

Abbreviations: C3, *Clostridium botulinum* ribosyltransferase; cAMP, cyclic adenosine monophosphate; Cdc42, cell division control protein 42 homolog; CRIB, Cdc42/Rac interactive binding; DRG, dorsal root ganglia; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; FGF-2, fibroblast growth factor 2; GAP, GTPase-activating protein; GDI, guanine nucleotide dissociation inhibitor; GEF, guanine nucleotide exchange factor; GSK-3β, glycogen synthase kinase-3β; JNK, Jun N-terminal kinase; LPA, lysophosphatidic acid; m-Dia, formin mammalian diaphanous; MAP, mitogen-activated protein; MLC, myosin light chain; N-WASP, neural Wiskott–Aldrich syndrome protein; NGF, nerve growth factor; NT3, neurotrophin-3; PAK, partitioning defective-6; PAR6, p21-activated kinase; PI3K, phosphatidylinositol-3 kinase; PIP, phosphatidylinositol 4,5-bisphosphate; PIP-5kinase, phosphatidylinositol-4 phosphate-5 kinase; PKN, protein kinase N; Rac, Ras-related C3 botulinum toxin substrate; Ras, Rat sarcoma; Rho, Ras homologous; ROCK, Rho-associated coiled-coil-containing protein kinase; SAPK, stress-activated protein kinase; Smurf, Smad ubiquitination regulatory factor.

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relevance of these three GTPases is underscored by gene knockouts. Mice lacking RhoB or RhoC exhibit no major developmental defects, but global deficiency of RhoA, Rac1 or Cdc42 is embryonically lethal (Heasman and Ridley, 2008).

In this overview, we focus on the mammalian Rho family that comprises over 20 proteins and can further be divided into several subgroups (Fig. 1). The main members of four of these subgroups, Rho (RhoA, -B, and -C), Cdc42 (Cdc42, TC10, TCL), Rac (Rac1, -2 and -3, RhoG) and Rif (Rif, RhoD), belong to the classical Rho GTPases. These switches are regulated by their GTPase activities and under the control of many switch activators (guanine nucleotide exchange factors, GEFs) and inactivators (GTPase-activating proteins, GAPs). Some of the GEFs and GAPs have been identified as regulators of assembly, disassembly and dynamic rearrangements of the actin and microtubule cytoskeleton (Linseman and Loucks, 2008; Marx et al., 2005; Ng and Luo, 2004; Gad and Aspenstrom, 2010). A recent genome-wide analysis of GAP functions in *Drosophila* identified p190 RhoGAP as essential stabilizer of axons involved in olfactory learning and memory (Billuart et al., 2001). In addition, GDI proteins (guanine nucleotide dissociation inhibitors) prevent binding of Rho GTPases to plasma membranes by stabilizing the GDP-bound form (Siderovski and Willard, 2005). Furthermore, GDIs dissociate from Rho proteins in response to the activation of adhesion receptors of the integrin family (del Pozo et al., 2004).

1.1. Regulation

Activation of Rho GTPases is mediated predominantly through cell surface receptors (cytokine-dependent, tyrosine kinase- or G-protein-coupled). Receptor tyrosine kinases (RTKs) are activated by their respective ligands, which lead to the dimerization and autophosphorylation of the receptor and to the stimulation of various signaling pathways including small Rho GTPases (Fig. 2). Most RTKs influence more than one Rho GTPase in a time course similar to the activation of the Ras/Raf/ERK (extracellular signal-regulated kinase) signaling cascade, i.e., within minutes (Schiller, 2006). Some of these Rho proteins, in turn, activate MAP kinase pathways, e.g. c-Jun N-terminal kinase (JNK). Rac1 is activated by various RTKs and induces phosphorylation of Raf (Coles and Shaw, 2002). RhoB is involved in growth factor stimulated RTK trafficking, thus playing a role in modulating RTK signaling from endosomes (Gampel et al., 1999). The link between RTKs and Rho switches is often constituted by Rho GEFs. Some of them mediate signals from several RTKs, while other Rho GEFs appear to be more specific for certain RTKs. The Rnd proteins represent atypical Rho family members that lack intrinsic GTPase activity. Therefore, they remain constitutively active and probably represent another link between RTKs and Rho GTPases. For example, activated fibroblast growth factor receptor (FGFR) type 1 phosphorylates FRS2β, which recruits Shp2 and releases Rnd1 from FRS2. Liberated Rnd1 then inhibits RhoA and promotes neurite outgrowth in FGF-stimulated PC12 (pheochromocytoma) cells (Greene and Tischler, 1982; Harada et al., 2005).

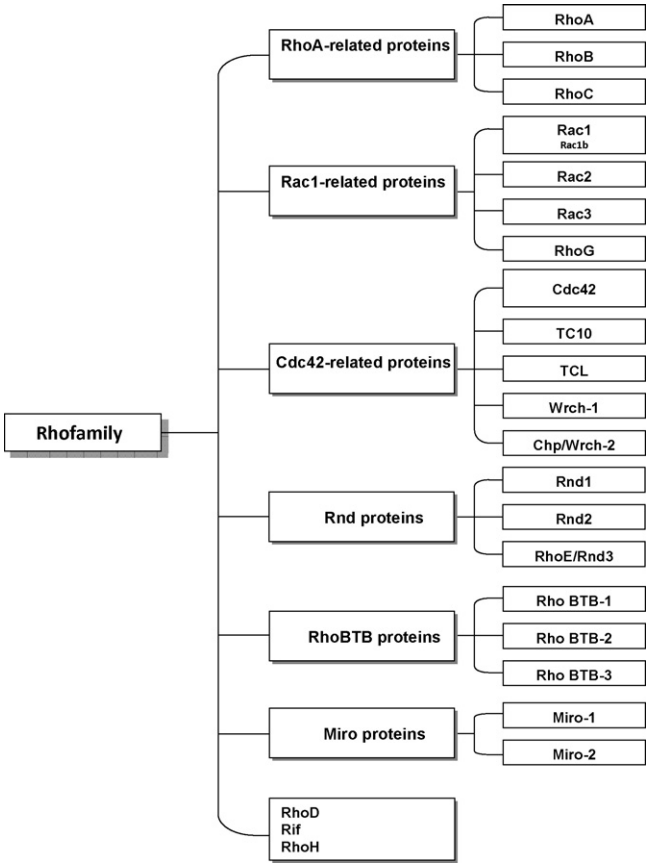

Fig. 1. Structure of the Rho protein family.

1.2. Effectors

Rho proteins act on several downstream effectors involved in the stabilization, contraction, polymerization and capture of cytoskeletal building blocks. Among the critical associations are RhoA binding to mDia (formin mammalian diaphanous), Rac1 binding to WAVE (WASP-family verprolin-homologous protein) and Cdc42 binding to N-WASP (neural Wiskott–Aldrich syndrome protein) which all induce protein assemblies required for actin polymerization (Fig. 2). Microtubule stabilization is regulated by RhoA, Rac1 and Cdc42 through the actions of mDia, PAK (p21-activated kinase) or PAR6 (partitioning defective-6) (Iden and Collard, 2008). Moreover, RhoA activates several other effector proteins, among them the Rho-associated coiled-coil-containing protein kinases, ROCKI and ROCKII, which in turn phosphorylate myosin light chain (MLC) and its phosphatase resulting in enhanced actomyosin-based contractility (Kimura et al., 1996; Amano et al., 1996). Inhibition of ROCK in semaphorin-treated embryonic hippocampal neurons reverses the stimulatory effect on axonal branching and increases axonal length. In contrast, dendritic branching is not markedly altered by ROCK inhibition (Hunt et al., 2003; Vodrazka et al., 2009). Other downstream signaling molecules of Rho proteins are not directly related to the cytoskeleton, such as p38α, which is required for calcium-dependent excitotoxic cell death (Semenova et al., 2007).

1.3. Degradation

Ubiquitylation and proteasomal degradation of Rho GTPases has been demonstrated as the decisive mechanism to limit and spatially restrict GTPase signaling. The E3 ubiquitin ligase Smurf1 is responsible for the elimination of RhoA-GDP. Its overexpression reduces RhoA protein levels in Neuro2a cells during dibutyric cyclic AMP (cAMP) induced neurite outgrowth suggesting that localized regulation of different subsets of Rho GTPases regulates neurite outgrowth and guidance (Bryan et al., 2005). Furthermore Smurf is required for neuronal polarity. Smurf2 ubiquitinates the small GTPase Rap1B, which is under the control of PI3K (phosphatidylinositol-3 kinase). Degradation of Rap1B results in restriction to a single neurite and thereby ensures that neurons extend a single axon only (Schwamborn et al., 2007).