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Molecules in focus

Cdc42: An important regulator of neuronal morphology

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ABSTRACT

Regulation of neuronal morphology and activity-dependent synaptic modifications involves reorganization of the actin cytoskeleton. Dynamic changes of the actin cytoskeleton in many cell types are controlled by small GTPases of the Rho family, such as RhoA, Rac1 and Cdc42. As key regulators of both actin and microtubule cytoskeleton, Rho GTPases have also emerged as important regulators of dendrite and spine structural plasticity. Multiple studies suggest that Rac1 and Cdc42 are positive regulators promoting neurite outgrowth and growth cone protrusion, while the activation of RhoA induces stress fiber formation, leading to growth cone collapse and neurite retraction. This review focuses on recent advances in our understanding of the molecular mechanisms underlying physiological and pathological functions of Cdc42 in the nervous system. We also discuss application of different FRET-based biosensors as a powerful approach to examine the dynamics of Cdc42 activity in living cells.

1. Introduction

Many cellular processes which lead to changes in neuronal morphology are under the control of different small GTPases of the Rho family, including RhoA, Rac1 and Cdc42 (cell division cycle protein) (Nobes and Hall, 1995). These GTPases possess different functions: Rho controls the assembly of actin stress fibers and focal adhesion complexes, Rac regulates actin filament accumulation at the plasma membrane to produce lamellipodia and membrane ruffles, while Cdc42 stimulates the formation of filopodia and neurite outgrowth. Recent studies have also demonstrated that Cdc42 is implicated in a variety of biological activities in the nervous system, such as cell signaling, cytoskeleton organization, establishment of neuron polarity and regulation of cell morphology, motility and cell cycle progression (Etienne-Manneville and Hall, 2003).

The human Cdc42 protein was first purified from the placental membranes and initially named as Gp, in analogue to heterotrimeric Gs and Gi proteins (Evans et al., 1986). Four years later, the human Cdc42 was cloned for the first time and assigned as G25K (Munemitsu et al., 1990; Shinjo et al., 1990). Detailed biochemical analysis revealed that at least one of Cdc42 isoforms undergoes post-translational isoprenylation (Fig. 1, Maltese and Sheridan, 1990) and this isoform is most commonly used for the functional analysis of Cdc42 in different heterologous expression systems. Noteworthy that the brain-specific isoform of Cdc42, which is now known to be palmitoylated (Fig. 1, Kang et al., 2008), was also cloned at the same time and designated as P1′ isoform, which "*codes for a protein which is distinct from, albeit highly similar to, CDC42Hs*" (Shinjo et al., 1990). The brain specific splice variant of Cdc42 has been shown to be preferentially located within dendritic spines as well as at synaptic vesicle membranes and found to play a prominent role in spine induction (Kang et al., 2008).

2. Structure

The human Cdc42 gene is mapped to chromosome 1p36.1. The Cdc42 protein shares structural similarities with Ras and other small GTPases of the Rho family. The common GTPases encompass five α-helices, six β-strands and two highly mobile switch regions (Fig. 1). Switch I region is located between α1 and β2 loops and switch II region can be found within α2 and β3 loops. These regions are thought to be flipped to allow ligand recognition. According to high resolution X-ray crystal structure studies, structure of GTP-bound Cdc42 does not significantly differ from the GDP-bound form. However, effector proteins are able to recognize the activated form and induce conformational changes in Cdc42 (especially the switch I and switch II loops), which are critical for the subsequent signaling pathway (Fig. 1B, Phillips et al., 2008).

3. Expression, activation and turnover

3.1. Cdc42 regulatory proteins

Similar to other small GTPases, activation of Cdc42 can be directly regulated by three classes of proteins: (i) guanine nucleotide exchange factors (GEFs), which catalyze the exchange of

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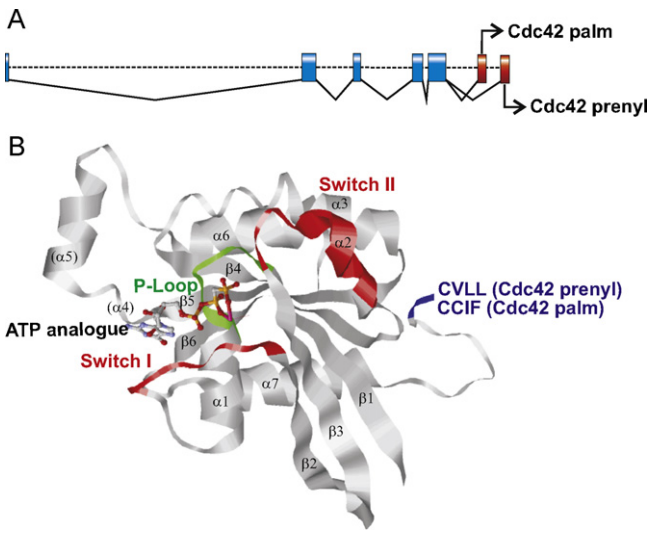

Fig. 1. Schematic representation of the structure of human Cdc42 gene and protein. (A) Cdc42 gene consists of seven exons. Transcripts for palmitoylated (Cdc42 palm) and prenylated (Cdc42 prenyl) isoforms can be produced by the alternative splicing of the last two exons. (B) Crystal structure of the Cdc42 protein (adapted from Phillips et al., 2008). Two flexible switch regions (switch I and switch II) are highlighted in red. P-Loop (in green) is essential for binding phosphate group. The two different C-termini and the last four amino acids of palmitoylated (Cdc42 palm) and prenylated (Cdc42 prenyl) Cdc42 isoforms are colored in purple.

GDP with GTP and shift Cdc42 into the activated state, (ii) GTPase activating proteins (GAPs), which accelerate GTP-hydrolysis, rendering Cdc42 inactive, and (iii) guanine nucleotide dissociation inhibitors (GDIs), which compete with GEF/GAP through the overlapped binding site on the switch I region (Fig. 1) and trap the geranyl–geranyl moiety of Cdc42 to dissociate from the membrane (Dovas and Couchman, 2005).

3.1.1. GEFs

Most of GEFs belong to the Dbl (diffuse B-cell lymphoma) and DOCK (Dedicator of cytokinesis) protein family. All Dbl proteins possess DH (Dbl homology), PH (Pleckstrin homology) domains and most of them contain SH3 (Src homology 3) domain. In contrast, DOCK family proteins are featured by two conserved domains: DHR1 (DOCK homology region 1) and DHR2 (DOCK homology region 2). The other well-studied Cdc42 GEFs are intersectin-l (Dbl family), β-Pix (Arhgef7, Dbl family), Zizimin1 (DOCK9), ACG (DOCK11) and collybistin (Arhgef9, Dbl family) (Fig. 2, for a review of the roles of these proteins on synaptic plasticity and development see Kiraly et al., 2010).

3.1.2. GAPs

GAP family proteins accelerate the speed of GTP-hydrolysis of the intrinsic GTPase activity. Many GAPs are known to act unspecifically towards different small GTPases and only a few of them are Cdc42 specific. For example, a Cdc42-selective GAP named dRich was recently found in *Drosophila melanogaster* (Nahm et al., 2010) and this protein has been shown to inhibit the Cdc42-Wsp (Drosophila ortholog of mammalian WASP) pathway by disturbing the post-synaptic localization of Wsp. This inhibition resulted in synaptic undergrowth and impaired neurotransmitter release at neuromuscular junctions. Another Cdc42 specific GTPase activation protein is MEGAP and its overexpression in neuroblastoma cells has been shown to reduce both Cdc42 and Rac1 in their GTP-bound form leading to cell rounding and retraction of protrusions (Yang et al., 2006).

3.1.3. GDIs

These proteins bind to inactive Rho GTPases and thus compete with GEFs. They also keep Rho GTPases in cytoplasm allowing for the GEF-mediated activation in a regulated manner (Olofsson, 1999). Of the three RhoGDI family members, only RhoGDIα and human RhoGDIγ can specifically bind to the Cdc42 (Platko et al., 1995; Adra et al., 1997). Using real-time FRET assays, which monitor the dynamic binding of Cdc42 to liposomes, Johnson and colleagues reported a specific interaction between RhoGDI and Cdc42 in solution (Johnson et al., 2009). They also demonstrated that the dissociation rate of Cdc42/RhoGDI complexes from membranes was independent of the Cdc42 nucleotide-bound state, although RhoGDI possesses much higher affinity for GDP-bound over GTP-bound Cdc42. In addition, the release of Cdc42/RhoGDI complexes from the membrane occurred at a similar rate as the release of Cdc42 alone, suggesting that the major effect of RhoGDI is to prevent the re-association of Cdc42 with the membrane (Johnson et al., 2009).

3.2. Upstream signals

The major upstream signals for the activation of Cdc42 are transmitted via GPCRs (G-protein coupled receptors), RTKs (receptor tyrosine kinases) and integrins (Fig. 2). In addition to extracellular signals, Cdc42 can also be activated through cross-talk between different intracellular signaling cascades (Ponimaskin et al., 2007).

A prominent example of GPCR mediated Cdc42 signaling is the 5-HT_7 receptor-mediated pathway. It has been shown that stimulation of serotonin 5-HT_7 receptor resulted in $G\alpha_{12}$ protein-mediated activation of Cdc42 (Fig. 2). As a consequence of 5-HT_7R/$G\alpha_{12}$/Cdc42 pathway activation, increased formation of filopodia and pronounced extension of neurite length were observed (Kvachnina et al., 2005). Serotonin-mediated activation of Cdc42 also plays an important role in the formation of both functional and structural changes in neurons and thus appears to be a part of neuronal machinery required for the formation of synapses on sensory neurons associated with the storage of long-term memory (Udo et al., 2005).

Cdc42 can also be activated via RTK stimulation. It has been shown that EphB2 (Ephrin B2) RTK receptor is functionally involved in spine morphogenesis by unmasking the Cdc42 specific GEF activity of Intersectin-1. This in turn triggers Cdc42 activation and subsequent actin polymerization in spines via N-WASP and the Arp2/3 complex (Fig. 2, Irie and Yamaguchi, 2002).

In addition to the mechanisms described above, proteo-glycan transmembrane agrin (TM agrin) has been shown to regulate formation and stability of neuronal filopodia via Cdc42- and Rac1-mediated signaling (McCroskery et al., 2006; Lin et al., 2010).

4. Biological functions

4.1. Cdc42 effectors and genetic animal models

Three major classes of Cdc42 effectors are composed of WASP (Wiskott–Aldrich syndrome protein), PAK (p21-activated kinases) and PAR (partitioning-defective) protein families (Fig. 2).

4.1.1. WASP

WASP and its neural isoform N-WASP interact with GTP-bound Cdc42 and are involved in regulation of actin polymerization and filopodia formation both by direct interaction with profilin as well as with actin (Miki et al., 1998). Active N-WASP can interact with Arp2/3 (Actin related protein 2 and 3) and this complex is involved in the control of actin assembly and filopodia outgrowth (Rohatgi