

## Small GTP-binding Proteins: A Future for the Treatment of Cognitive Disorders?

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### Abstract

Recent findings indicate that early brain overgrowth could be a key factor in the patho-biology of autism and other disorder with learning disabilities, and that anomalous neuronal wiring might play a role when is affecting brain regions involved in cognition. Thus, precise synaptic connectivity is crucial for normal brain function and a common anatomical pathology associated with autism and cognitive disability is an alteration of that connectivity due to an irregular morphology of the dendritic spines in the neurons. For example, human patients as well as animal models of Fragile X syndrome (FXS), Neurofibromatosis, Tuberous sclerosis, and Rett syndrome, have shown a higher number of immature dendritic spines in certain regions of the brain, phenomena that has been linked with impaired learning and memory functions. However, how this deficiency is produced is not yet well understood. Evidence from our laboratory and others is pointing to a role of small GTP-binding proteins of the Rho family, which mediate actin cytoskeleton reorganization, neuronal morphogenesis and gene expression. We have reported that these proteins are critical for dendritic morphology and plasticity. They act not only in the developing brain but also in the mature nervous system. One of its members, Rac1 is highly expressed in the adult mouse hippocampus, a brain area that exhibits robust synaptic plasticity and is crucial for the acquisition of memories. Moreover, using pharmacological and genetic approaches we and others have demonstrated that Rac1 is necessary for normal long-term plasticity, spine development and learning. Interestingly, glutamate transmission, long-term plasticity and learning behavior are characteristically altered in autistic disorders that present aberrant neuronal development. Therefore, there might be a functional link between small GTP-binding proteins and certain characteristic phenotypes described in cognitive disorders and possibly autism that render interest on the small GTP-binding proteins as possible therapeutic targets for these disorders.

**Keywords:** Autism; Plasticity; Small GTPases; Behavior; Rac1

### Introduction

#### Small GTP-binding proteins: Rho family

Small GTP-binding proteins are involved in many different cellular processes [1], but they are mostly known to have a role in the assembly and organization of the actin cytoskeleton [2-5], which is required for cell remodeling. There are two independent cycles that lead to their activation: (1) cycling between cytosolic and membrane-associated forms through the action of guanine dissociation inhibitor (GDI) proteins; and (2) the removal of bound GDP and loading of GTP [1] (via GEFs and GAPs). Coupling these two cycles appropriately is critical for interaction with their targets [6].

One of the many families of small GTPases, is the Rho family of small GTPases, which include Cdc42, Rho, and Rac. Members of this family have a function in the same signaling systems, but they might either antagonize or activate each other's functions. For example, activation of Rac induces lamellipodium formation, and activation of Cdc42 leads to filopodium development. Cdc42 can activate Rac, what suggests that filopodia might be intimately associated with lamellipodia. Likewise, Rac can activate Rho, which produces stress fibers and fiber retraction [7]. In the brain, activation of Rac supports

neurite outgrowth [8] and activation of Rho results in an opposite reaction, which is neurite retraction.

Rac is a protein of 22-25 kDa molecular weight and nearly 200 amino acid residues, with two closely related isoform: Rac1 and Rac 2. These two forms differ in their C-termini. Rac1 is ubiquitously expressed and contains polybasic amino acid residues. Rac 2 is restricted to hematopoietic cells and is less basic [9]. There is a third form of Rac, Rac 3, which is expressed in a variety of tissues, among them brain, but in very early stages of development. Its subcellular localization and/or binding to regulatory proteins differ from the other two isoforms, possibly due to a differential carboxyl-terminal end [10]. In general, Rac1 is widely known for its role in actin polymerization [11], production of superoxide generation, and gene transcription [5], as well as G1 cell cycle progression [12].

#### Small GTP-binding proteins and neuronal morphology

Very recent studies have proposed a model for Rho GTPase-mediated dendritic arbor growth in which neural activity, through NMDA and AMPA receptors, activates Rac1 and decreases RhoA activation, leading to elaboration of dendritic arbors [13]. In neuronal cultures, Rac1 mediates axonal growth and guidance, and together with activation of Cdc42 promote the formation of lamellipodia and filopodia respectively [14]. In the brain, Rac1 has multiple functions through direct or indirect interactions with many different effector

proteins. Rac1 and other small GTPases regulate neuronal morphogenesis by affecting the stability and assembly of the actin cytoskeleton [15]. Both Rac1-GAPs and -GEFs are enriched in the postsynaptic density, ideally positioned for regulation by glutamate receptors [16,17]. Moreover, Rac1 also functions in the development and structural plasticity of dendrites and dendritic spines [18-24]. As the primary recipients of excitatory inputs in the brain, dendritic spines rely on the actin signaling [25-31] need a period. Spine development and maintenance is an actin dependent event that requires a dynamic cytoskeleton guided by key regulator proteins such as Rac1 [11,15,32]. Using a site-specific Rac1 knockout mouse model, we reported that these Rac1 deficient mice present aberrant dendritic spine morphology in brain areas where Rac1 was genetically removed, indicating that Rac1 is directly involved in the morphogenesis of neuronal spines [33,34].

### Small GTP-binding proteins and synaptic plasticity

Several studies have suggested that some small GTPases involved in the remodeling of the actin cytoskeleton are activated after LTP induction in the CA1 region of the hippocampus via NMDA receptor activation [35]. This suggests that small GTPases play also a role in plasticity at hippocampal synapses during LTP. More recently, Rac1 signaling pathway has been implicated in long term plasticity [36,37]. Both, long-term potentiation (LTP) and long-term depression (LTD) involve formation and elimination of synaptic structures. Rac1 is indeed required and critical for LTP and LTD [38]. Using a pharmacological approach, we demonstrated that NMDA and mGluR receptor activation leads to the activation of Rac1/PAK signaling, which in turn allows LTP and LTD induction respectively. Furthermore, the use of Rac1 inhibitors significantly impaired LTP and LTD. The same effect was observed when using a genetic approach, testing LTP and LTD induction in a site-specific Rac1 knockout mouse model [33,34]

### Small GTP-binding proteins and learning

There is evidence that processes necessary for LTP might also be necessary for memory consolidation in mammals. For example, infusion of a NMDA receptor antagonist interferes with acquisition of spatial memory [35]. Other evidence includes the observations that saturation of LTP *in vivo* impairs spatial learning [39] and that this impairment has a similar time course of decay, as does LTP [40]. As Rac1 has been recently proposed as a critical protein for plasticity [36,37], its signaling pathway has been implicated in learning [36,41,42]. Many Rac1-effector proteins have been associated with cognitive impairment disorders. As example, members of the PAK family have been associated with non-syndromic X-linked mental retardation [43], Fragile X syndrome [44] and Down syndrome [45]. Also, LIMK-1 has been linked to Williams's syndrome [46]. Additionally, other molecules that function downstream from Rac1 have been implicated in the occurrence and progression of other human pathologies that present cognitive problems, such as Alzheimer's (the p35/Cdk5 kinase) [47]. Rac1 itself has been associated to forms of cognitive disorders and X-linked mental retardation syndromes [33,48]. Interestingly, all these cognitive disorders present abnormalities in dendritic spine structure and plasticity, besides their cognitive disability. Thus, a common pathway in cognitive impairment and mental disability may be a perturbation of neural connectivity and plasticity, hindering the development of appropriate connections

between brain regions. Alteration in Rac1 function might be involved in these phenomena.

### Small GTP-binding proteins and their therapeutic potential

Promising manipulations and experiments from our and other laboratories have rescued abnormal effects of Rac1 in neuronal development, synaptic plasticity as well as learning and memory function. Constitutively activated RhoA and Rac1 induced by CNF1 in mice have led to rearrangement of cerebral actin cytoskeleton, enhanced neurotransmission and synaptic plasticity, and improved learning and memory in various behavioral tasks [41]. Similarly, recent examples have involved these proteins in autistic disorders. In animal models of Fragile X syndrome, lack of FMRP protein (causing Fragile X syndrome) appears to correlate with an excessive synthesis of Rac1 [33,34], or its downstream effectors, PAK and CYFIP1 [49], leading to aberrant Rac1-induced actin remodeling, and possibly generating the characteristic aberrant morphology of dendritic spines, resulting in cognitive deficiencies. Consistent with this, it has been demonstrated that double negative PAK mice also yield deficits in memory together with abnormal neuronal morphology in the cortex and altered plasticity [50], suggesting that regulation of this Rac1 effector protein is important for synaptic plasticity and learning. Interestingly, our *in vitro* investigations show that antagonizing Rac1 to reduce its expression/activation levels in Fmr1 KO mice, rescues the exaggerated hippocampal LTD observed [33] as well as their susceptibility to suffer audiogenic seizures (unpublished data). Thus, this signaling pathway involving Rac1/PAK could represent a novel therapeutic site for Fragile X syndrome and possibly autism therapy.

### Conclusion

It is undeniable that available animal models of diseases linked to cognitive impairment as well as the availability of certain specific inhibitory drugs represent optimal methods for studying alteration of neuroplasticity. Because plasticity has been linked to memory function [5], one exciting possibility is that, in these disorders, defects in cognition could be related to an abnormal regulation of pathways that link neuronal connectivity, plasticity and learning; that is, the abnormal regulation of Rac1-dependent actin reorganization. This opens the possibility in the future of learning disability being rescued through pharmacological manipulation of neuronal plasticity via the regulation of Rac1/PAK pathway. As Rac1 activity is important for the regulation of actin dynamics, receptor clustering [2,51] and plasticity, it is not surprising to see that loss of Rac1 in excitatory neurons in the hippocampus results in impaired long-term plasticity [33,36]. Conversely, excess Rac1 expression results in exaggerated long-term plasticity, as reported in FXS [52]. Rac1-dependent alterations in neuroplasticity as well as behavioral abnormalities have been seen in other psychiatric disorders such as depression and schizophrenia [53]. Presently, two agents that specifically inhibit Rac1 have been used in electrophysiological experiments to study the role of Rac1 in long-term plasticity in the mouse brain. One of them has been effective in rescuing aberrant phenotypes observed in FXS related to long-term plasticity [33] and behavior. We consider these findings to be important in view of different psychiatric disorders featuring cognitive impairment, structural deficiencies and aberrant plasticity.

In conclusion, Rac1/PAK modulates spine development, synaptic function and learning in the brain. Dysfunction of Rac1/PAK signaling may be responsible for aberrant phenotypes observed in cognitive disorders and autistic syndromes. These findings suggest that

Rac1/PAK signaling may provide a functional link among altered dendritic spines, aberrant synaptic plasticity and cognition impairment in autistic disorders, pointing to Rac1/PAK as a novel therapeutic target to treat these disorders.

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