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# Rho-linked genes and neurological disorders

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## Abstract

Mental retardation (MR) is a common cause of intellectual disability and affects approximately 2 to 3 % of children and young adults. MR has been consistently associated with changes in dendrites and dendritic spine structure. Given that dendritic spine morphology has been tightly linked to synaptic activity, altered spine morphology has been suggested to be the underlying cause of cognitive disabilities observed in MR. The structure and dynamics of dendritic spines is determined by its underlying actin cytoskeleton. Signaling molecules and cascades important for cytoskeletal regulation have therefore naturally attracted a great deal of attention. As key regulators of both the actin and microtubule cytoskeletons, it is not surprising that the Rho GTPases have emerged as important regulators of dendrite and spine structural plasticity. In support of this, mutations in regulators and effectors of Rho GTPases have been associated with diseases affecting the nervous system, including MR and amyotropic lateral sclerosis (ALS). Here we will discuss Rho GTPase related genes and their signaling pathways involved in MR and ALS.

### Keywords

mental retardation; dendritic spines; actin cytoskeleton; Rho GTPases; X-linked mental retardation; amyotrophic lateral sclerosis

## 1. Introduction

Mental retardation (MR) affects approximately 2 to 3 % of children and young adults and is characterized by reduced cognitive functioning, defined by an intelligent quotient (IQ) lower than 70, and severe deficits in basic adaptive and social skills. The severity of cognitive disability can vary among MR patients, and is commonly divided into IQ ranges between 85 and 70 (borderline), 70 and 55 (mild MR), 55 and 40 (moderate MR), 40 and 25 (severe MR) and below 25 (profound MR) [15, 68]. Conventionally, MR has been subdivided into two major classes: syndromic MR which is accompanied with a fixed constellation of other manifestations, such as body and brain malformations, and nonsyndromic MR which is characterized by reduced cognitive function without any other clinical features. The underlying causes of MR are extremely heterogeneous and include non-genetic factors, such as infectious disease, premature birth, and fetal alcohol syndrome, as well as genetic changes that include chromosomal aneuploidies, such as trisomy 21, and single-gene mutations [28]. For monogenic causes of MR, genes have been more frequently found on the X-chromosome (XLMR) than on any other segment of the autosomes [56, 70].

A consistent feature of neurons in patients with various forms of MR is abnormal dendritic spine morphology and/or density [25, 45, 67]. Dendritic spines are highly specialized structures on the dendrites on which most excitatory synapses in the brain are located [38].

They are believed to compartmentalize biochemical responses to the activation of individual synapses [46]. Spines can change their shape within a few seconds. These dynamic changes are determined by the architecture of its actin cytoskeleton [94]. Significantly, increasing evidence indicates that proper regulation of spine shape and size is crucial for processing of information [13, 44, 52, 55, 89]. For instance, spine morphology and/or dynamics have been associated with long-term potentiation (LTP) and long term-depression (LTD), which are two well studied forms of learning and memory [55]. One particular mechanism proposed for some forms of LTP is the addition of new α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) at the postsynaptic site, which has been correlated with an increase in spine size, whereas internalization of AMPARs during LTD has been associated with smaller spines [49]. A recent study provided support for changes in actin polymerization in response to different patterns of synaptic stimulation. They found that LTP induction increases the formation of F-actin filaments in spines, whereas LTD induction, by low frequency stimulation, increases actin depolymerization [62]. Together, these studies link actin dynamics, spine morphogenesis to synaptic function.

Since the shape and size of the dendritic spines are dependent on the fine-tuned regulation of its underlying actin cytoskeleton, and changes in spine morphology are associated with changes in synaptic strength, great efforts have been made in identifying pathways linking synaptic activity to the control of actin dynamics. One group of proteins, small GTP-binding proteins of the Rho subfamily, and in particular Rac1, Cdc42 and RhoA GTPases, have emerged as key modulators of dendritic spine morphogenesis through their regulation of actin organization [31]. Hence, it is not surprising that mutations in genes encoding regulators and effectors of the Rho GTPases have been found to underlie human neurological diseases [60, 83](Fig. 1). In this review, we will discuss how malfunction in Rho GTPase signaling pathways may contribute to defects in neuronal connection, synapse formation and structure underlying the pathology of MR. We will focus on genes reported to be involved in XLMR, such as oligophrenin-1 (*OPHN1)*, *ARHGEF6*, *PAK3* and *FMR1*. In addition we will discuss the Rho GTPase linked genes *MEGAP* and *ALS2*, which are involved in autosomal MR and in the motorneuron disease, amyotrophic lateral sclerosis (ALS), respectively.

## 2. Rho GTPases and MR genes

Rho GTPases are guanine nucleotide binding proteins that act as molecular switches, cycling between an active GTP bound and inactive GDP bound state. This cycling between the GTP and GDP bound form is tightly regulated by positive regulators, guanine-nucleotide-exchange factors (GEFs) and negative regulators, GTPase activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDI). Only in their active state, Rho GTPases can bind to their downstream effectors and exert their effect on various important biological activities [82] (Fig. 1). Rho GTPases, among which Rac1, Cdc42 and RhoA have been most extensively studied, are best known for their effects on the actin cytoskeleton, but more recently they have also been shown to influence microtubule organization [22, 33, 93]. As key regulators of the cytoskeleton, the Rho proteins have been implicated in different aspects of neuronal morphogenesis, including dendritic arbor development and spine morphogenesis [31]. For instance, Rac1 and Cdc42 have been shown to promote, whereas RhoA to inhibit the growth and/or stability of dendritic spines [77]. A number of regulators, as well as effectors that mediate the effects of the Rho GTPases on the actin cytoskeleton and spine morphogenesis have been identified over the past few years [31, 60]. The importance of proper Rho GTPase signaling in neuronal development and function has been highlighted by the identification of MR genes that encode regulators and effectors of Rho GTPases such as *OPHN1*, *PAK3*, *ARHGEF6*, *FMR1* and *MEGAP* (Table 1).

## Oligophrenin-1

*OPHN1* is the first identified Rho-linked non-syndromic XLMR (MRX) gene which encodes a Rho-GAP protein. The gene was initially identified by the analysis of a balanced translocation t(X;12) in a female patient with mild MR [6]. Subsequently, its involvement in MRX was reported by the identification of a 1-bp deletion within the *OPHN1* coding sequence, causing a frameshift at the end of the GAP domain in an MRX family (MRX60) [8]. More recently, however, the presence of *OPHN1* mutations have as well been documented in families with syndromic forms of MR. Most of these patients share in common that in addition to cognitive impairment, they display cerebellar hypoplasia with vermian dysplasia and/or epilepsy [5, 66, 79, 90]. In addition, some of these patients showed signs of strabismus, macrocephaly, hypogenitalism, hyperactivity, anxiety, and in some rare cases ataxia. Moreover, a new study indicates that members of the original MRX60 family also suffer seizures and cerebellar dysgenesis [90]. These successive reports have therefore questioned the classification of *OPHN1* as a non-syndromic XLMR gene. Interestingly all *OPHN1* mutations identified to date (3 nonsense mutations, 1 splice mutation, 3 deletions and 1 insertion) are likely to result in OPHN1 loss of function.

OPHN1 is expressed in multiple tissues with the highest expression in the developing brain, where it is found in neurons of all major regions, including hippocampus, cerebellum and cortex, and is present in axons, dendrites and spines [24,30]. The OPHN1 protein negatively regulates RhoA, Rac1, and Cdc42 *in vitro*, in non-neuronal and neuronal cells [8, 24]. Significantly, recent studies by Govek *et al*. showed that knock-down of OPHN1 expression in CA1 pyramidal neurons in hippocampal slices results in a significant decrease in dendritic spine length [30]. As spine morphology is ultimately linked to synaptic function [89], such spine morphological changes are likely to compromise synaptic plasticity, and potentially lead to learning and memory deficits. The above spine length phenotype was mimicked using a constitutive active RhoA (CA-RhoA) mutant and could be largely rescued by inhibiting the RhoA effector, Rho-kinase (Govek et al. 2004). Rho-kinase (also called ROK or ROCK) has been well documented to play a key role in RhoA-induced actin reorganization and to mediate at least in part RhoA's effects on spine morphogenesis [69, 77]. Rho-kinases appear to influence the actin cytoskeleton by acting on LIM Kinase (LIMK), myosin light chain (MLC) and/or MLC phosphatase (see fig 1). In summary the work by Govek et al. supports a model in which loss of OPHN1 causes changes in spine morphology during development as a result of changes in the actin cytoskeleton triggered upon elevation of RhoA and Rho-kinase activities. Noteworthy, a recent study has shown that in non-neuronal cells OPHN1 can directly interact with F-filaments to regulate cytoskeletal dynamics [24].

Interestingly, OPHN1 has also been found to associate with Homer [30], a postsynaptic adaptor protein that links glutamate receptors, such as type-1 metabotropic glutamate receptors (mGluRs), to multiple intracellular targets [23, 85]. Moreover, Homer proteins have been shown to influence dendritic spine morphogenesis and synaptic transmission [26, 71, 72]. Although the function of OPHN1/Homer interaction remains to be established, it raises the intriguing possibility that OPHN1 provides a crucial link between postsynaptic receptors (via Homer) and the actin cytoskeleton to regulate dendritic spine morphogenesis and synaptic function. Together these findings are consistent with the idea that cognitive deficits observed in human patients could be attributed to altered dendritic spine morphology.

## PAK3

A second non-syndromic Rho-related XLMR gene identified is *PAK3*. Three different mutations in the *PAK3* gene have been identified in several MRX pedigrees. These

mutations have been associated with either loss of PAK3 protein or loss of its kinase activity. The first *PAK3* mutation, R419X, found in family MRX30, introduces a premature stop codon that abolishes the kinase activity of the truncated product [1]. The second *PAK3* mutation, an R67C missense mutation in family MRX47, likely affects GTPase binding and activation of PAK3 [7]. The consequence of the third mutation (A365E), also a missense mutation, on PAK3 function is not yet defined, although it occurs in a highly conserved region of the protein that may affect catalytic kinase domain function [29].

*PAK3* encodes a member of the larger family of p21-activating kinases (PAK). PAK proteins function as Rac1 and Cdc42-specific effector molecules, mediating their effects on the cytoskeleton and gene expression [10, 41]. Activation of PAK kinases by Rac1 or Cdc42 leads to the phosphorylation and activation of LIMK, which in turn phosphorylates and inactivates cofilin, thereby inhibiting cofilin-mediated actin-filament disassembly (Fig. 2) [3, 58, 62]. This results in a regional accumulation of actin filaments which is important for the regulation of spine morphogenesis. PAK3 is highly expressed in the brain, particularly in post mitotic neurons of the cerebral cortex and the hippocampus, where it shows a diffuse distribution throughout the soma and proximal dendrites and is present in dendritc spines [9].

Several lines of evidence have demonstrated a role for PAK3 in the regulation of spine morphogenesis, synapse formation and synaptic plasticity. First, a study using transgenic mice in which the catalytic activity of the PAK family members, PAK1 and 3, is inhibited by expression of a PAK-autoinhibitory domain (AID-PAK) revealed that cortical neurons of these mice have fewer dendritic spines and larger synapses, which is correlated with enhanced LTP and reduced LTD in the cortex. Moreover, these mice showed impaired memory consolidation [36]. Secondly, Boda et al. observed that RNAi mediated suppression of PAK3, or expression of a dominant negative PAK3 mutant carrying the human MRX30 mutation, in rat hippocampal organotypic slice cultures results in the formation of abnormal elongated dendritic spines and filopodia-like protrusions, as well as a decrease in mature spine synapses. They noticed that these defects were associated with reduced expression of AMPARs at the synapse and LTP [9]. In a parallel study, Zhang et al demonstrated that PAK1 and 3 regulate spine morphogenesis by triggering the phosphorylation of MLC, the latter resulting in an increase in dendritic spine size and synapse formation [91]. More recently, mice lacking the *PAK3* gene were generated, and analysis of these mice showed selective impairment in late-phase hippocampal LTP, a distinct form of long-term synaptic plasticity involving new gene expression. Surprisingly, in this mice knockout model, no obvious deficits in neuronal structures were observed [57]. The differences seen with regards to spine morphology between the knockout and RNAi studies could potentially reflect differences between a homogeneous and a heterogeneous cell population, respectively, or could be attributed to compensatory mechanisms in the knockout mice. The *PAK3* knockout mice did however show a dramatic decrease in the levels of the phosphorylated/active form of cAMP-responsive element-binding protein (CREB) in the hippocampus, whereas no changes in the total CREB protein levels were observed. Severeal studies have shown that CREB function is important for synaptic plasticity and memory formation in mice [42, 54]. Therefore, the reduced CREB function may be responsible for the impairment in late-phase hippocampal LTP in these mice. These findings support a model in which mutations in MR genes result in aberrant spine morphology and/or function as a result of altered actin dynamics and /or transcriptional regulation.

Interestingly, defects in PAK signaling have also been associated with cognitive deficits in Alzheimer disease (AD) [92]. AD is characterized by progressive loss of neurons in selected brain regions, extracellular accumulations of amyloid beta (Aß), and intracellular fibrils containing hyperphosphorylated tau [51]. PAK1 and 3 protein levels and their activities are

markedly reduced in brains of AD patients. The latter is accompanied by a prominent cofilin pathology and loss of the actin-regulatory protein drebrin, which cofilin removes from actin. Drebrin is localized in spines in adult brains and is required for active clustering and synaptic targeting of the postsynaptic protein, PSD95 [76]. Aß oligomers, implicated in AD, cause PAK signaling defects which results in a loss of drebrin from the spines. Significantly, expression of an active mutant form of PAK alleviates the effects induced by Aß oligomers, whereas inhibition of PAK kinases in adult mice, using pharmacological drugs, were found to be sufficient to cause drebrin loss and memory impairment.

As mentioned above, PAK and Rho-kinases both stimulate LIMK. LIMK signaling has received a lot of attention since it was found to be one of the genes heterozygously deleted in Williams syndrome (WS: Williams-Beuren syndrome), a rare (1 in 25000) genetic disorder [78]. Patients with this syndrome suffer from a variety of abnormalities, including mild MR, cardiovascular problems, distinctive craniofacial features, poor visual-motor integration, attention deficits, and at times hyperactivity. Of particular interest is that *LIMK1* knockout mice show abnormal spine morphology, abnormal synaptic plasticity and impaired spatial learning [58]. Also, loss of function mutations in *Drosophila LIMK* results in defects in the development of olfactory and neuromuscular synapses [2]. Intriguingly a recent study reported a significant increase in phosphorylated LIMK1 in neurons of brain areas affected with AD pathology. This finding suggests that LIMK signaling may also play a role in the pathology of AD, acting downstream of PAK [37]

## ARHGEF6

The *ARHGEF6* gene, also known as *αPIX* or *Cool-2*, is the third Rho GTPase linked gene shown to be involved in non-syndromic MR. It codes for a Cdc42/Rac1 GEF, which has previously been shown to interact with PAK kinases [18]. The first mutation in *ARHGEF6* associated with MRX was identified in a male carrying a reciprocal X;21 translocation breakpoint located between exons 10 and 11 of the *ARHGEF6* gene [50]. Subsequently, additional mutations have been identified in the first intron of the gene that result in preferential skipping of exon 2 and a predicted protein product lacking the first 28 amino acids in affected males in a large MRX family (MRX46) [50]. Until now, no information is available as to how mutations in *ARHGEF6* contribute to MRX. However, recent studies examining the biological role of a closely related family member, ßPix (or Cool-1), have provided some hints as to the function of ARHGEF6/αPIX/Cool-2 in neurons. Zhang et al. showed that a signaling complex, consisting of ßPix, G-protein-coupled receptor kinase-interacting protein 1 (GIT1), Rac1 and PAK, plays an essential role in spine morphogenesis and synapse formation. GIT1 was shown to target ßPix to the synapse, where it locally activates Rac1 and PAK. Importantly, disruption of the synaptic localization of GIT1 or interfering with ßPix function, perturbs spine morphogenesis and synapse formation [91]. Consistent with these findings, the *Drosophila* homologue, dPIX, has been demonstrated to play a major role in regulating post-synaptic structures and protein localization at the glutamatergic neuromuscular junction [64]. In the future, it will be of interest to investigate whether mutations in αPIX/Cool-2 affect spine morphology and postsynaptic signaling and whether this protein acts together with PAK3 in a common pathway underlying cognitive function.

## Fragile-X syndrome

Recent studies have provided evidence that Rho GTPase signaling also plays a role in Fragile-X syndrome (FRAXA). FRAXA is one of the most common monogenic forms of MR (estimated prevalence of 1:4000 males and 1:8000 females). The manifestations include, besides cognitive deficits, poor eye contact, approach-avoidance behavior, tactile defensiveness, impulsivity, elongated facial features, and machroorchidism. FRAXA is

typically caused by an unstable expansion of a CGG trinucleotide repeat and hypermethylation of CpG dinucleotides in the 5′ untranslated region of the fragile-X mental retardation-1 gene (*FMR1*), which results in transcriptional silencing of *FMR1*[27]. Several studies provided evidence that the *FMR1* mRNA upon activation of mGluRs, becomes localized to dendrites and translated in synaptoneurosomes [4]. The FMRP protein itself associates with actively translating polyribosomes in an RNA-dependent manner via messenger ribonucleoprotein (mRNP) particles and regulates translation and transport of a selective group of mRNAs to which it binds to.

Dendritic spines of both FRAXA patients and *Fmr1* knockout mice were found to be unusually long and irregular [17, 39, 61]. Notably, a similar phenotype was also reported for mutations in some of the Rho-linked genes. It has been suggested that misregulation of target mRNA's could account for the abnormal maturation of dendritic spines (reviewed in [32]). However, subsequent studies also pointed to the involvement of Rac1 signaling in FMRP-controlled actin remodeling and spine morphogenesis. A first link between FRAXA and Rho GTPases was found in *Drosophila*. [53]. Lee et al demonstrated that the mRNA encoding dRac1 is present in the dFmr1-mRNP complexes *in vivo* [53]. Furthermore, they provided evidence that dFmr1 exert its effect on dendritic elaboration and branching at least in part by acting on dRac1. In an independent study, Schenck et al. demonstrated a biochemical interaction between the proteins dRac1 and CYFIP, a dFmr1 interacting protein. Their genetic data support a model in which dRac1 inhibits CYFIP, that in turn negatively regulates dFmr1, implying that dRac1 indirectly activates dFmr1 [73]. Taken together with the studies by Lee et al., these findings suggest that there is a feedback loop between Rac1 and *FMR1*/FMRP function *in vivo.*

Interestingly, the mammalian homologues of *Drosophila* CYFIP, CYFIP1 and CYFIP2, have also been demonstrated to interact with mammalian FMRP. In mammals, CYFIP1 (also known as p140/Sra-1) was initially identified as a target of Rac1 [48], whereas CYFIP2 (also called PIR121) was found to be part of the inactive WAVE protein complex [20], that is responsive to Rac signaling. In its inactive state, this complex includes WAVE1, which is localized to spines in hippocampal neurons, along with four other proteins: HSPC 300, Nap125, Abi2 and PIR121. Nap125 and PIR121 are both direct Rac1 targets [20]. When active Rac-GTP is added, the complex dissociates, freeing WAVE1 and HSPC 300, allowing WAVE1 to activate the actin-related protein 2/3 (Arp2/3) complex to induce actin polymerization. Interestingly, recent studies have demonstrated a critical role for phosphorylated WAVE1 in dendritic spine morphogenesis through its effect on the actin cytoskeleton [47].

In analogy to the mechanism of WAVE activation, a model was proposed in which CYFIP dissociates from FMRP/dFmr1 upon interaction with activated Rac1, allowing released FMRP/dFmr1 to regulate local protein translation. Interestingly, in this regard, is the observation that Rac1 activation in murine fibroblasts leads to relocalization of four FMRP-interacting proteins (CYFIP1, FXR1P, NUFIP and 82-FIP) to actin-containing domains [14]. Taken together, these data suggest a model in which CYFIP and FMRP act together in a dynamic signaling complex to regulate actin dynamics and protein translation, processes that are critical for neuronal morphogenesis and connectivity.

## MEGAP

An example of a Rho-linked gene associated with syndromic MR on the autosome is the *MEGAP* (mental-disorder-associated GAP) gene which encodes a Rho GAP. *MEGAP* was identified as a gene which is disrupted by a translocation breakpoint in a female patient who shares some clinical manifestations, such as hypotonia and severe MR, with the $3p^{-}$ syndrome [21, 74]. The gene resides on chromosome 3p25. Subsequent studies also found

*MEGAP* deleted in 3p⁻ syndrome patients that present MR. These patients exhibit in addition to MR, microcephaly, growth failure, heart and renal defects, hypotonia and facial abnormalities [59].

The *MEGAP* gene is predominantly expressed in fetal and adult brain, and this protein is enriched in the neurons of the hippocampus, cortex and amygdala [21]. Biochemical studies demonstrated that MEGAP possesses GAP activity towards both Rac1 and Cdc42 [21]. Consistent with this, a recent study showed that expression of MEGAP in the neuroblastoma SHSY-5Y cell line leads to a loss of filopodia and lamelliopodia protrusions, which could be rescued by expressing constitutive active forms of Cdc42 or Rac1 [88].

The specific cellular effects brought about by mutations in *MEGAP* remain to be largely defined. However, it is of particular interest that MEGAP was identified as a WAVE-interacting protein, called WRP, as well as a SLIT-ROBO interacting protein, called srGAP3 [74, 84]. Information on the function of these proteins provided some hints towards the mode of action of MEGAP. As mentioned before, WAVE proteins act downstream of Rac1 to regulate actin polymerization by acting on the Arp 2/3 complex and to mediate the effect of Rac1 on spine morphogenesis. Hence MEGAP may be part of a negative feedback loop to regulate Rac1 activity and modulate Rac1-induced actin reorganization. Of particular interest is that loss of WAVE1 causes defects in balance and coordination, reduced anxiety, and deficits in hippocampus-dependent learning and memory in mice [75]. These phenotypes are strikingly similar to those of 3p⁻ syndrome patients. In the case of srGAP3, this protein is one of three srGAPs (srGAP1-3) shown to interact with the repulsive guidance receptor Robo1. Among the srGAPs, srGAP1 was demonstrated to interfere with Slit-triggered repulsion of migratory cells from the subventricular zone of the forebrain [84]. This observation suggests that MEGAP may have a role in neuronal migration and guidance as well. Thus, aberrations in MEGAP function are likely to affect actin nucleation, which in turn may impact synaptic connections and neuronal responses.

## 3. Rho GTPase and motor neuron disease

Whereas the above described Rho-linked genes have been associated with disorders of the central nervous system, more recent studies suggested that aberrations in Rho signaling may also contribute to disorders of the peripheral nervous system, such as ALS. In this context we will discuss alsin, a Rho GEF protein involved in the pathology of ALS.

### Alsin

ALS is a progressive neurodegenerative disease caused by a preferential loss of motor neurons, resulting in progressive weakness of skeletal muscles, atrophy, and death due to respiratory muscle paralysis. The pathology of ALS is characterized by a loss of upper and lower motor neurons and degeneration of pyramidal tracts. Mutations in Cu/Zn superoxide dismutase 1 (*SOD1*) and *ALS2* were identified as the most frequent causes of inherited ALS [65]. Mutations in *SOD1* cause the classical form of ALS. Mutations in *ALS2*, which encodes the protein alsin, also give rise to a rare juvenile form of ALS [35,87], an infantile onset ascending hereditary spastic paralysis, and a form of complicated hereditary spastic paraplegia [11, 16]. All mutations in *ALS2*, identified to date are missense mutations that result in unstable truncated alsin proteins implying that loss of alsin function induces the disease phenotypes [35, 87].

Alsin is most abundant in the brain, with lower levels found in the spinal cord and other tissues [63, 86]. The structure of alsin predicts that it functions as a GEF. It contains 3 putative GEF domains: an amino-terminal domain that displays homology to the Ran GEF RCC1; a central region containing Dbl and pleckstrin homology (DH/PH) domain that is

present in GEFs for Rho, Rac and Cdc42; and a carboxyl-terminal vacuolar protein-sorting 9 (VPS9) domain that is found in GEFs for Rab5. Subsequent studies have indeed demonstrated that alsin acts *in vitro* as a GEF for Rab5 and Rac1 [43, 63, 80,81]. At present, it is very unclear how the Rac1 and Rab5 exchange activities of alsin influence motor neuron maintenance and survival and contribute to the disease of ALS. However recent studies have begun to shed some light on the function of alsin.

Rab5 is known to play an important factor in early stages of endocytosis and early trafficking of signaling molecules. Alsin and Rab5 localize to early endosome in HeLa cells and cortical neurons. Coexpression of alsin and Rab5 results in enlarged endosomes, suggesting a role of alsin in endosome fusion or trafficking [63]. Recent studies on *Als2*$^{-/-}$ mice have confirmed the role of alsin in endocytosis. Hadano et al. reported that alsin knock-out mice exhibit a slowly, but progressive, loss of cerebellar Purkinje cells, together with a subclinical motor dysfunction and altered endosome trafficking [34]. Consistent with these observations, Devon et al showed that *Als2*$^{-/-}$ mice reveal Rab5-dependent fusion of early endosomes, and a disturbance in endosomal transport of insuline-like growth factor 1 and brain-derived neurotrophic factor receptors [19]. Surprisingly, these *Als2*$^{-/-}$ mice did not show any obvious clinical, histopathological, or electrophysiological signs of neuronal degeneration [12, 19, 34]. The latter could be due to compensations during development.

With respect to alsin's role as a Rho family GEF, experiments have shown that alsin is an exchange factor for Rac1 [43, 80, 81]. Alsin specifically interacts with Rac1 and shows little, if any, association with Rac3, RhoA, or Cdc42. Alsin colocalizes with Rac1 in membrane ruffles and lamellipodia in NIH3T3 cells. In neurons, Tudor et al. showed that alsin and Rac1 colocalize in growth cones of hippocampal neurons and that alsin increases neurite outgrowth in cortical neurons by stmulating the Rac1-PAK signaling pathway [81]. Recently, RNAi mediated alsin knockdown in motorneurons has been shown to induce cell death and to inhibit axon growth in the surviving neurons. Both cellular phenotypes were mimicked by expression of a dominant-negative Rac1 mutant and were completely blocked by expression of a constitutive-active Rac1 mutant[40]. These observations suggest that alsin signaling can contribute to the disease of ALS by its unique dual exchange factor activity that may couple endocytosis (via Rab5 activation) to cytoskeletal modulation (via Rac1 activation).

## 4. Summary

Rho GTPase signaling pathways modulate actin cytoskeleton dynamics and gene expression, which are critical for neuronal morphogenesis and dendritic plasticity (including changes in dendritic spine number and morphology) in the developing and mature nervous system. Such synaptic remodeling and dendritic plasticity are thought to underlie the anatomic basis for learning and memory formation and normal cognitive function. Consistent with this are the findings demonstrating an association between various MR conditions and mutations in Rho-linked genes. MR is in many cases associated with abnormalities in dendritic branching and spine morphology and since spine shape and function are intricately linked, the observed changes are likely to impair neuronal connectivity and synaptic plasticity, leading to reduced cognitive function. The current view of how mutations in Rho-linked genes result in MR is by disrupting the normal development, structure, and/or plasticity of neuronal networks via perturbations in the regulation of the actin cytoskeleton and gene regulation. Evidence supporting such a view has come from MR patients, mouse models of MR and RNAi studies in hippocampal slices. The findings outlined in this review clearly demonstrate an association between various MR conditions that result from, or are in some way linked to, aberrant Rho GTPase signaling. Defects in Rho signaling pathways have recently also been linked to motor neuron diseases, such as ALS, and to Alzheimer disease

(AD). Significantly, these neurological diseases all share in common aberrations in the actin cytoskeleton of the neuron's structure. Of interest is that aberrant PAK-signaling was reported for some forms of MR and AD, suggesting that the prominent changes in PAK signaling associated with AD could contribute to the synaptic and cognitive deficits observed in AD. Further elucidation of the molecular mechanisms by which Rho signaling contribute to the above disorders will not only shed light on the epidemiology of these diseases, but also on basic mechanisms of neuronal development and function and may provide candidates for therapeutic intervention.

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## List of abbreviations

| | |
|---|---|
| **AD** | Alzheimer disease |
| **ALS** | amyotropic lateral sclerosis |
| **AMPAR** | α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor |
| **ARHGEF6** | Rho guanine nucleotide exchange factor 6 |
| **Arp2/3** | actin-related proteins 2 and 3 |
| **Aß** | amyloid ß |
| **CREB** | cAMP-responsive element-binding protein |
| **CYFIP** | cytoplasmic FMR1 interacting protein |
| **FMRP** | fragile-X mental retardation protein |
| **FMR1** | fragile-X mental retardation-1 gene |
| **FRAXA** | Fragile-X syndrome |
| **GAP** | GTPase activating proteins |
| **GDI** | guanine nucleotide dissociation inhibitor |
| **GEF** | guanine-nucleotide-exchange factor |
| **LIM** | Lin-11, Isl-1 and Mec-3 kinase |
| **LTD** | long term depression |
| **LTP** | long term potentiation |
| **MEGAP** | MEntal disorder-associated GAP protein |
| **mGluR** | metabotropic glutamate receptor |
| **MLC** | myosin light chain |
| **MLCK** | myosin light chain kinase |
| **MLCP** | myosin light chain phosphatase |
| **MR** | mental retardation |
| **MRX** | non-syndromic X-linked mental retardation |
| **MRXS** | syndromic X-linked mental retardation |

| | |
|---|---|
| **OPHN1** | oligophrenin-1 |
| **PAK** | p21-activated kinases |
| **ROCK** | Rho-Kinase |
| **WASP** | Wiskott-Aldrich-syndrome protein |
| **WAVE** | WASP family Verprolin-homologous protein |
| **WS** | Williams-Beuren syndrome |
| **XLMR** | X-linked mental retardation |

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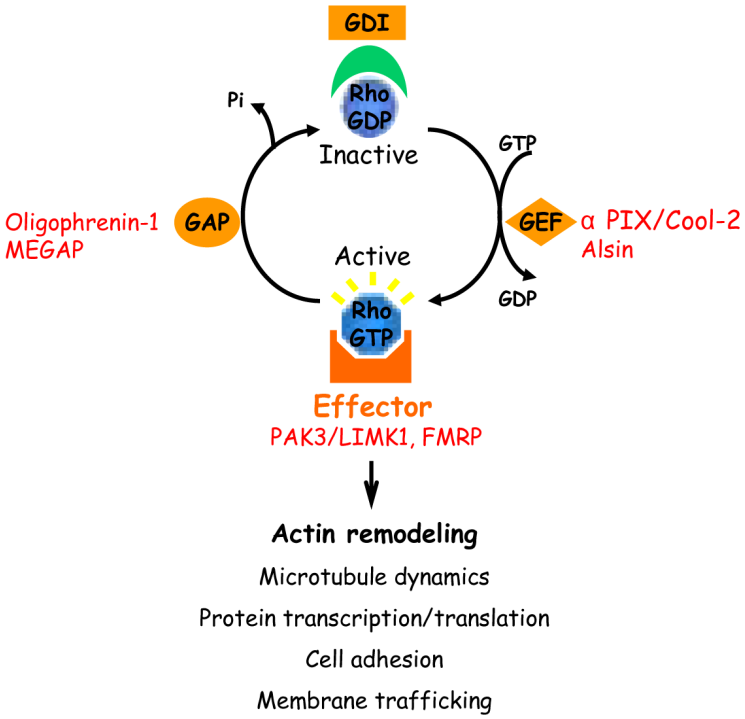


**Figure 1. Regulatory cycle for the activation and inactivation of the Rho-GTPases**

Rho GTPases cycle between an inactive GDP and an active GTP bound form which is regulated by positive regulators, GEFs and negative regulators, GAPs and GDIs. Only in their active state, Rho GTPases bind to their downstream effectors and exert their effect on various important biological activities The Rho-associated mental retardation genes are indicated at the appropriate positions. Abbreviations: ALS: amyotropic lateral sclerosis; ARHGEF6, a Rho guanine nucleotide exchange factor 6; FMRP: fragile-X mental retardation-1 protein, GAP: GTPase activating proteins , GDI: guanine nucleotide dissociation inhibitor, GEF: guanine-nucleotide-exchange factor MEGAP, MEntal disorder-associated GAP protein; OPHN1, oligophrenin-1; PAK, p21-activated kinases;

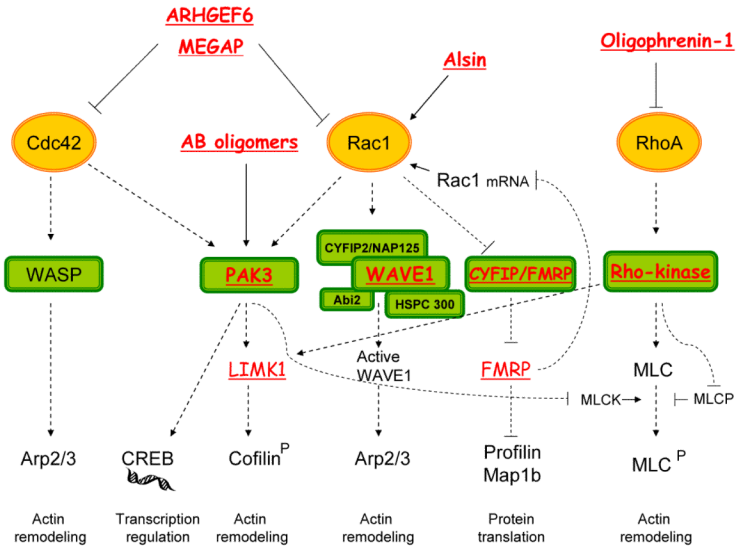


**Figure 2. Rho-linked mental retardation proteins and effector pathways connecting Rho GTPases to actin dynamics**

Proteins encoded by Rho-linked genes involved in different forms of MR are highlighted in red text. See main text for explanation. Abbreviations: ARHGEF6, a Rho guanine nucleotide exchange factor 6; Arp2/3, actin-related proteins 2 and 3; Aß, amyloid ß; CREB, cAMP-responsive element-binding protein; CYFIP, cytoplasmic FMR1 interacting protein; LIM, Lin-11, Isl-1 and Mec-3 kinase; MEGAP, MEntal disorder-associated GAP protein; MLC, myosin light chain; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; OPHN1, oligophrenin-1; PAK, p21-activated kinases; WASP, Wiskott-Aldrich-syndrome protein; WAVE, WASP family Verprolin-homologous protein.

**Table 1**

Mental retardation genes involved in Rho GTPasesignaling

| Gene/ Locus | Protein | Function | Clinical manifestation s | Spine/synapse phenotypes |
|---|---|---|---|---|
| *OPHN1* Xq12 | Oligophrenin -1 | Rho family GAP for RhoA/ Rac1/Cdc42 | MR, cerebellar hypoplasia, epilepsy strabismus, macrocep haly , ataxia hypogenitalism, hyperactivity | Reduced spine length |
| *PAK3* Xq22 | PAK3 | Ser/Thr kinase, effector of Rac1/Cdc42 | MR, AD | Abnormal elongated spines, decreasein maturesynapses and impaired LTP |
| *ARHGEF6* Xq26 | _Pix/C ool -2 | Rho family GEF for Rac1/ Cdc42, Interacts with PAK | MR | ND |
| *FMR1* Xq27 | FMRP | RNA binding protein (Ra c1), interacts with CYFIP, downstream of Rac1 | MR, Macrocephaly, long face, long ears, machroorchidism | Long and irregular dendriticspines |
| *MEGAP* 3p25 | MEGAP,WRP, srGAP3 | Rho family GAP for Rac/Cdc42 | 3p- syndrome: microcephaly,growth failure heart and renal defects, hypotonia and facial abnormalities | Loss of filopodiaand lamelipodia |
| *LIMK1* 7q11 | LIMK1 | Ser/Thr kinase, downstream of Rac1/Cdc42 | Williams Syndrome, AD | Decreased spinehead sizeand thicker spine necks, enhanced LTP |
| *ALS2* 2q33 | Alsin | GEF for Rac1 and Rab5 | Motor neuron degeneration | Reduced axon growth, increased cell death |