

Central Nervous System Functions of PAK Protein Family

From Spine Morphogenesis to Mental Retardation

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Abstract

Several of the genes currently known to be associated, when mutated, with mental retardation, code for molecules directly involved in Rho guanosine triphosphatase (GTPase) signaling. These include PAK3, a member of the PAK protein kinase family, which are important effectors of small GTPases. In many systems, PAK kinases play crucial roles regulating complex mechanisms such as cell migration, differentiation, or survival. Their precise functions in the central nervous system remain, however, unclear. Although their activity does not seem to be required for normal brain development, several recent studies point to a possible involvement in more subtle mechanisms such as neurite outgrowth, spine morphogenesis or synapse formation, and plasticity. This article reviews this information in the light of the current knowledge available on the molecular characteristics of the different members of this family and discuss the mechanisms through which they might contribute to cognitive functions.

Key Words: Rho GTPases; synaptic plasticity; synaptogenesis; cytoskeleton; brain development.

Introduction

The recent developments of imaging techniques and transfection methodologies applicable to differentiated, non-dividing cells, such as neurons, has opened new avenues to inves-

tigate the role of specific genes and proteins in complex physiological or developmental mechanisms. Furthermore, by combining the expression of several fluorescently tagged molecules or peptides capable of interacting with each other, it has become possible to design rescue-type experiments and thus investigate the role of signaling pathways in the generation of specific phenotypes. These new approaches, mainly applied so far to dissociated cell cultures, have not only brought comple-

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mentary information to that traditionally obtained through studies of transgenic animals, but they have also speeded up and markedly increased the capacity of analysis.

One domain in which these new technologies have revealed particularly interesting information is the study of the complex signaling cascades contributing to synapse development and plasticity, particularly those involving a participation of the family of small Rho guanine triphosphatases (GTPases). These molecules function as molecular switches integrating extra- and intracellular signals to control actin rearrangement (1). Through their critical implication in the complex control of cytoskeleton dynamics, members of the Rho GTPase family revealed crucial for many important aspects of neuronal plasticity including cell migration, axonal growth, protrusion and filopodia elongation, formation of dendritic spines and establishment of synaptic contacts. Consistent with these key functions, recent genetic studies have revealed that several members of these signaling cascades are associated, when mutated in humans, with cognitive defects and mental retardation (2). These observations have raised the possibility that an important cause for mental retardation or diseases such as autism or Rett syndrome might include developmental abnormalities in the formation and function of synaptic networks. Therefore, there is an important need for a better understanding of the molecular events that participate in these regulations, particularly at the level of synapses.

The possible number of interacting genes and proteins involved in Rho GTPases signaling is particularly high and the complexity of their interactions still poorly understood. Among these numerous molecular partners, the PAK protein family is of particular interest as members of this family probably represent important effectors of Rho GTPase activation, mediating, or participating in some of the key aspects of plasticity described so far (3). PAK3 is indeed one of the Rho GTPases-associated X-linked gene for which several mutations have been reported in humans to result in non-syndromic mental retardation (i.e., cases of the

disease in which the only defect is a mental handicap; refs. 2, 4, 5, and 6). This article reviews some of the key molecular features of this protein family, focusing primarily on the possible functional implications of the different members of the family with regard to synapse development and formation.

Structure of Pak Proteins and Mechanisms of Activation

PAKs are a highly conserved family of serine/threonine protein kinases (7). They were first identified in a screen for binding partners of the protein p21, a member of the Rho family of small GTPases (8). A main feature of Rho GTPases is the ability to cycle between guanine triphosphate (GTP)- and guanine diphosphate (GDP)-bound forms, where the GTP-bound form is the activated state and the only one capable of interaction with downstream effectors. Hydrolysis of the GTP by their intrinsic GTPase activity returns them to the inactive stage. The regulation of small GTPase activity is a complex phenomenon involving the participation of a large variety of regulators. Guanine exchange factors (GEFs) activate GTPases by mediating the exchange of GDP for GTP (9). GTPase-activating proteins inactivate them by stimulating the intrinsic GTPase activity of these molecules (10). The activity of GTPases is further regulated by guanine dissociation inhibitors (GDIs), which bind to GDP-bound form and inhibit the dissociation of GDP. However, GDIs also contribute to extract the GTPase from the membrane and sequester it in the cytosol. Membrane association of GTPases is thus believed to represent an important aspect contributing to the regulation of their activity and function and GDIs probably play a dual role by regulating both the GDP/GTP cycling and the membrane association/dissociation characteristics (11). Activated GTPases then bind to various downstream effectors, among which is the PAK protein family. The two main GTPases that usually interact with and activate PAKs are Cdc42 and Rac1.

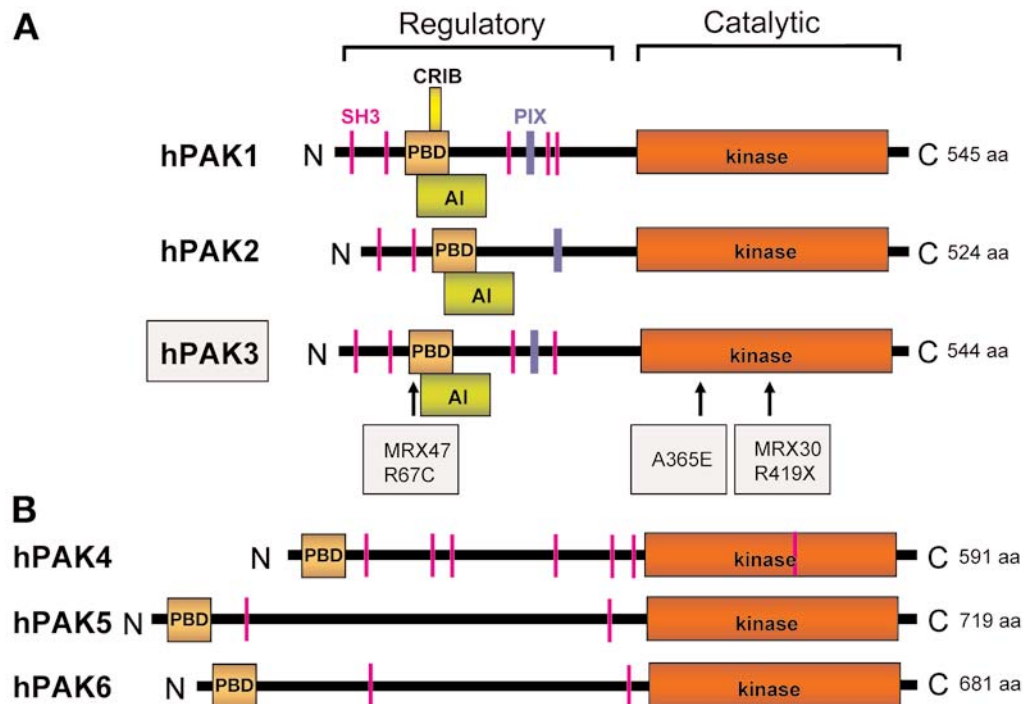


Fig. 1. Molecular structure of human PAK proteins. SH3, proline-rich regions; PBD, p21-binding domain; CRIB, Cdc42 and Rac interactive binding; AI, autoinhibitory domain; Pix, Pix-binding site. The gray squares show the localization of the three mutations identified in human cases of mental retardation.

All PAKs contain a regulatory N-terminal p21 GTPase-binding domain (PBD), which includes a more restricted binding region for Cdc42 and Rac1 referred to as the Cdc42 and Rac interactive binding (CRIB) domain, as well as a highly conserved C-terminal protein kinase domain. In worms, flies, and mammals, PAKs fall into two distinct groups based on sequence conservation and organization of domains. In mammals, group A comprises PAK1 (α PAK), PAK2 (γ PAK), and PAK3 (β PAK), while group B consists of PAK4, PAK5, PAK6 (7). Figure 1 shows the protein structure of PAKs.

Group A PAKs contain an N-terminal regulatory domain whose distinguishing feature is the presence of proline-rich motifs that mediate associations with various proteins containing Src-homology 3 (SH3)-domains. There are

five (PAK1), two (PAK2), or four (PAK3) canonical PXXP SH3-binding motifs for adapter protein interactions and one non-classical (PXP) SH3-binding site for the PIX family of proteins. Another interesting part of the regulatory region is the autoinhibitory domain that is flanking the CRIB or PBD region. This domain binds to and negatively regulates the catalytic site. It functions as an inhibitory switch that controls the kinase basal activity. Group A PAKs further contain an acidic residue-rich region of unknown significance, as well as a conserved binding site for the $\beta\gamma$ -subunit complex of G proteins (1). Recent data indicate that a $G\beta\gamma$ -PAK1 interaction is involved both as a scaffold protein for Cdc42 activation and as an effector of Cdc42 in mediating directional migration of chemotactic leukocytes (12).

All group A PAKs bind Cdc42 and Rac and are strongly activated upon binding these GTPases. However, they can also be activated by a variety of other GTPase-independent mechanisms (1). For example, PAK1 has been shown to be directly activated by Akt, a proto-oncogen kinase (13), and this mechanism has been proposed to account for cell survival action of PAK1 (14).

Based on what has been found for PAK1, it is believed that group A PAKs form homodimers in solution and in cells. In this configuration, the N-terminal regulatory domain of one PAK1 molecule binds and inhibits the C-terminal catalytic domain of the other. In this way, dimerization allows overlap of the PBD/CRIB and inhibitory switch domains (15,16), thus maintaining the kinase inactive. Activation of the enzyme is then believed to involve GTPase binding, which disrupts dimerization and leads to a series of conformational changes that destabilize the folded structure of the inhibitory switch domain. This induces its dissociation from the catalytic domain resulting in kinase activation.

Group B PAKs show important similarities with group A PAKs. They also contain a PBD region at the extreme N-terminus of the protein followed by a C-terminal kinase domain (17). This group binds Cdc42 and to a lesser extent Rac1, but, unlike group A PAKs, binding does not lead to a consistent activation of the kinase. Rather, it would seem that association with Cdc42 contributes to regulate the localization of group B kinases and not their activation *per se* (7). However, the precise mechanisms involved in group B PAKs activation remain unclear.

Signaling Through PAKs

PAKs are believed to produce most of their effects through their activity of serine/threonine phosphorylation. They have been implicated in a broad range of biological functions, including cell migration and motility, actin reorganization, gene transcription, cell prolif-

eration, and cell survival. Their substrates are diverse and numerous, affecting the signaling of various pathways.

In mammals, as in simpler eukaryotes, PAKs play an important role by regulating mitogen-activated protein kinase cascade. They appear to do this through the phosphorylation of Raf1 and Mek1 (18,19), a step that is required for the activation of these two molecules and the subsequent stimulation of transcription that they regulate. However, there is also recent evidence that PAK1 may directly associate with Erk1/2, allowing the recruitment of Raf1, Mek1, and Erk1/2 to adhesion complexes (20). It is probably through activation of this pathway that PAK1 contributes to its mitogenic activity and participates in the regulation of cell proliferation or differentiation. Several lines of evidence also suggest that integrins and growth factors might promote Erk signaling through an activation of Rac and PAKs, in this way mediating adhesion-dependent processes, such as migration and motility (20–23).

Another group of mechanisms in which PAKs appear to be involved is the control of the cytoskeleton and the regulation of actin dynamics. The pathways through which PAKs participate in these processes also appear to be diverse and remain poorly understood. In many cases, activation of PAKs results from signaling mediated through either adhesion molecules or growth factors (21,24–26). PAKs then probably act through different pathways. One possibility involves the activation of LIM-kinase (LIMK), which in turn could phosphorylate cofilin and thus control actin rearrangement (27–29). PAK1 can, however, also activate myosin light chain kinase (MLCK), involved in myosin–actin interactions (30), or directly regulate microtubule-associated proteins and affect microtubule dynamics (31). Furthermore, PAKs may additionally interact with or activate various exchange factors that may feed back on Rac1 activity or mediate coordination with other GTPase signaling pathways (32–34). Overall, the role of PAKs in regulating cytoskeleton dynamics is believed to be important for PAKs functions in the brain.

Role of PAKs in Invertebrates

PAKs homologs exist in invertebrates and several models have provided interesting information about their neuronal implication. *Drosophila* encodes one group A PAK, DPAK1; one group B, referred to as mushroom bodies tiny (Mbt); and a third group that does not fit easily into either classification, DPAK3 (7). The functions of DPAK1 and Mbt, but not DPAK3, have been analyzed by genetic techniques. Loss-of-function mutation in DPAK1 indicates a role for the protein in axon guidance (35–37) and the regulation of postsynaptic proteins localization and structure at the glutamatergic neuromuscular junctions (38). Mbt was uncovered in a genetic screen for genes involved in the formation of the mushroom body, a structure in the adult fly corresponding to the human hippocampus and involved in learning and memory. Mbt-null mutant have defects in cell proliferation, differentiation, or survival of the mushroom body neurons leading to a dramatic reduction of its volume (39). In addition, Mbt mutants display defects of photoreceptor morphogenesis (40). These data clearly indicate an important role of PAK protein family in brain development.

In *Aplysia*, a mollusc widely used as a model for studies of sensory learning, stimulation of the siphon results in a gill-withdrawal reflex that can be mapped to specific synaptic connections between identified sensory and motor neurons. Long-term sensitization or facilitation of this reflex has been shown to be associated with the growth of new sensory neuron varicosities. Repeated applications of serotonin reproduce the phenomenon. Recently, it was found that serotonin-induced synaptic growth requires the activation of several proteins including ApCdc42, the *Aplysia* homolog of Cdc42, neuronal Wiskott-Aldrich syndrome protein (N-WASP) and PAK (41). They showed that repeated serotonin administration activates ApCdc42, recruiting N-WASP and PAK, thereby leading to the outgrowth of filopodia. The PAK protein involved in these mechanisms was recognized by a PAK1 antibody and

thus probably represents a mammalian PAK1 homolog.

Role of PAK1 in Mammalian Central Nervous System

PAK1 is expressed in the brain, muscle, and spleen. However, its function has mainly been studied in fibroblasts or other dissociated cell lines (3,42). In these cells, PAK1 has been localized to focal adhesion complexes, which mediate signaling and contact with the extracellular matrix (43). Activated PAK1 has been found to induce polarized filopodia and membrane ruffles (42). An important role of PAK1 has thus been proposed in the regulation of cell motility and migration.

In rat brain, PAK1 has a high level of expression, as shown by *in situ* hybridization techniques in cerebral cortex and piriform cortex, ventral and lateral thalamic nuclei, CA1 of the hippocampus, subiculum, cerebellum, and medulla (8,44). So far, the major role reported for PAK1 in neurons is in neurite formation (33,45,46), although the data point also to a possible role of PAK1 in modulating growth cone behavior (47). In cortical primary neurons, PAK1 was found to be responsible for dendrite initiation (48) and, in hippocampal primary neurons, overexpression of a GEF (GEFT) inducing PAK1 and PAK5 activation also resulted in neurite outgrowth (33,45). Transfection studies carried out on dissociated hippocampal neurons further revealed that expression of constitutively active PAK1, but also PAK3, promoted the formation of dendritic spines and protrusions, an effect that was correlated by an increase in the number of excitatory synapses (30). This phenotype corresponded to that observed with Rac1 overexpression and could be reproduced by activation of MLCK. Furthermore, they could link this phenotype to the activation of two other synaptic proteins, β Pix, a GEF for Rac and GIT1, its adaptor-binding partner. They found that expression of a dominant-negative GIT1, disrupting its synaptic localization, resulted in

numerous dendritic protrusions and a significant decrease in the number of synapses and normal mushroom-shaped spines. Conversely, knockdown of GIT1 by short interfering RNA (siRNA) techniques caused a decrease in spine and synaptic density. Furthermore, they found that Rac1 is locally activated in dendritic spines and is regulated by β PIX. Taken together, they proposed the interesting hypothesis that a GIT1/ β PIX/Rac/PAK1,3 signaling complex would regulate the mechanisms of spine formation in hippocampal neurons through activation of MLCK and myosin/actin interactions.

Curiously, and in contrast to this finding, genetic analyses of PAK1 functions revealed that PAK1-null mice are viable and show no detectable neuronal phenotype, except for some immune defects (7). In another recent approach, transgenic mice were generated that expressed a dominant-negative PAK peptide under the control of the α calcium-calmodulin-dependent protein kinase II promoter, the expression being thus limited to the forebrain and post-developmental stages (48). This 68 amino acid peptide corresponded to the autoinhibitory domain of PAKs, which binds to the catalytic domain of all three PAKs of group A family to block their autophosphorylation and consequently their activation. However, it is likely that PAK1 is the major contributor to the phenotype of these mice, as the level of PAK1 expression in the postsynaptic density fraction is much higher than that of PAK2 and PAK3.

Interestingly, analyses of these mice showed that there were no significant alterations in dendrite formation, and no differences in dendritic length or dendritic branch points. However, cortical, but not hippocampal, neurons displayed fewer dendritic spines and an increased proportion of larger spines compared with wild-type controls. These alterations of cortical synapse morphology were associated with enhanced glutamate receptor-mediated synaptic transmission, enhanced long-term potentiation (LTP), but reduced long-term depression. These mice also exhibited specific deficits in the

consolidation phase of hippocampus-dependent memory, although the level of phosphorylated PAK activity (PAK1/2/3) was high in this structure and there were no other morphological or functional hippocampal defects. A possible explanation for these puzzling observations is that a 40% reduction in overall PAK levels is possibly not sufficient in the hippocampus to result in a phenotype or, alternatively, that the suppression of PAK under the control of the α calcium-calmodulin-dependent protein kinase II promoter occurred too late to generate defects. There might also be compensation mechanisms activated in transgenic mice models that are more easily avoided upon transient transfection.

Together and despite some inconsistencies, our current knowledge of the role of PAK1 in the brain suggests that the kinase is probably an important molecule for the morphogenesis of dendritic spines and the development of synaptic networks.

Role of PAK3 in Mammalian Central Nervous System

As a result of its involvement in mental retardation in humans, PAK3 is clearly one of the interesting members of the PAK protein family. It is so far the only PAK protein associated with mental retardation and its mutation results in a non-syndromic form of the disease, in which the mental handicap is the unique clinical manifestation. It is also interesting that the human genes encoding the six isoforms are situated on six different chromosomes: five of them are on the somatic chromosomes (PAK1: chrom. 11, PAK2: chrom. 3, PAK4: chrom. 19, PAK5: chrom. 20, and PAK6: chrom. 15) and one, PAK3, is on the chromosome X. Three types of point mutations have been described for PAK3: MRX30 is an R419-Stop mutation that generates a truncated form of the PAK3 protein without kinase activity (5), MRX47 is a R67C missense mutation near CRIB domain (4), and, lastly there is an A365E missense mutation that affects the kinase subdomain (6).

The absence of severe brain defects in these MRX patients suggests that PAK3 function is not absolutely required for neuronal proliferation, migration, and/or cortical gyration. However, the observation that PAK3 mutation results in mental retardation might reflect a later requirement for PAK3 function in the developing or adult cortex for synapse development and/or synaptic plasticity. Much recent evidence indeed suggests that various genetic forms of mental retardation could be linked to abnormalities in the mechanisms that regulate dendritic spine morphogenesis or synapse formation (49,50).

Dendritic spines are specialized morphological structures that host excitatory synapses in the central nervous system of mammals. These small protrusions are actin-rich, highly motile, and share different sizes and forms (51,52). Dendritic spine morphology and function are tightly associated. Dendritic spines show very dynamic properties; they undergo a turnover that is developmentally regulated and modulated by activity and express properties of plasticity both in terms of function and structural organization. It is believed that these properties of plasticity underlie some of the higher cognitive functions, such as learning and memory (53–55). An involvement of mental retardation proteins in these mechanisms could therefore provide a plausible pathophysiological explanation of the disease.

PAK3 is reported to be a brain-specific isoform, although it is also expressed in the testis (5,56). It is interesting that genes, expressed simultaneously in the brain and testis, have been proposed to be relevant for human speciation (57). In the adult rat brain, PAK3 is expressed at high levels in the piriform cortex, medial preoptic nucleus, hippocampus, amygdala, hypothalamus, thalamus, and dorsal raphe nucleus (8,56).

Experimental data on the role of PAK3 in PC12 cells showed that overexpression of PAK3 induced cell spreading, membrane ruffling, and increased lamellipodia formation at growth cones and shafts of neurites, an effect mediated through interaction with β PIX (58).

Transfection of dissociated hippocampal neurons with constitutively active PAK3 resulted, as for PAK1, in an increase in dendritic protrusions and increased spine density, while conversely, expression of kinase-dead mutants decreased spine density and PSD-95 clusters. The role of PAK3 in hippocampus was further analyzed in hippocampal slice cultures, an *in vitro* model that is more closely related to *in vivo* networks. In this study, we found that PAK3 significantly contributes to synapse formation and plasticity (59). Pyramidal neurons within organotypic slice culture were transfected with different PAK3 constructs using a biolistic approach. The results showed that overexpression of wild-type PAK3 did not affect spine morphology, whereas interference with PAK3 function, either through expression of a PAK3 construct carrying the human MRX30 mutation, through knockdown of the protein by antisense or siRNA approaches, resulted in the formation of abnormally elongated dendritic spines and filopodia-like protrusions and a decrease in mature, mushroom-type spines, thus reproducing the phenotype described in human cases of mental retardation (Fig. 2A; ref. 60). Closer analysis at the electron microscopy level of these modified protrusions revealed that many of them (up to 30%) failed to contact presynaptic terminals (Fig. 2B). Furthermore, those spines that formed synaptic contacts expressed postsynaptic densities of reduced size, as measured on electron microscopy sections. These abnormalities were further associated with a reduced spontaneous activity, altered expression of AMPA-type glutamate receptors, and defective LTP (Fig. 2C,D). Together, this study showed that transient suppression of PAK3 in pyramidal neurons resulted in an increased proportion of immature, non-functional spines, a defect in establishment of synaptic contact, and stabilization of the postsynaptic density as well as altered properties of synaptic plasticity. As such, these alterations are likely to profoundly affect information processing by these neurons and could provide a mechanism explaining the cognitive handicap. Somewhat

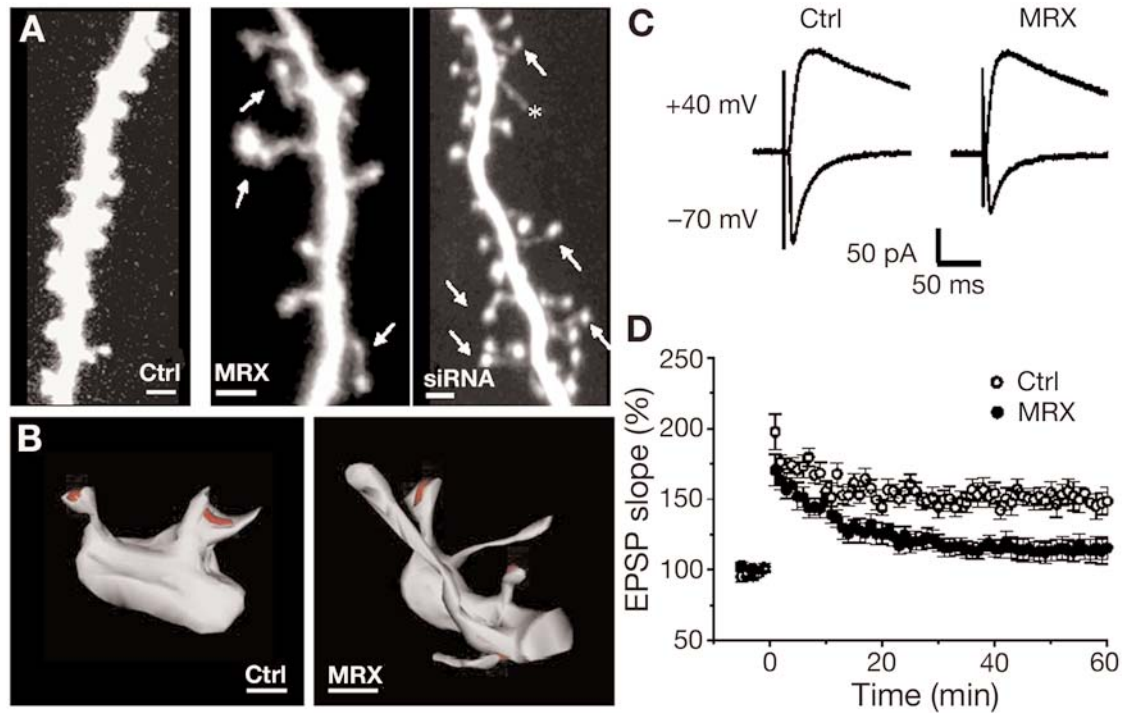


Fig. 2. Spine and synaptic defects associated with expression of PAK3 carrying the human MRX30 mutation. (A) Illustration of the alterations in spine morphology observed with confocal microscopy in CA1 pyramidal cells transfected with EGFP (ctrl) or PAK3 carrying the MRX30 mutation (MRX30) or PAK3 siRNA oligos. Note the increased number of filopodia-like, elongated spines and the reduction in mushroom-type spines. Scale bars: 2 μ m. (B) Electron microscopy three-dimensional reconstructions of dendritic spines in cells transfected with EGFP (Ctrl) or PAK3 carrying the MRX30 mutation (MRX30). Many of filopodia-like, elongated spines failed to establish synaptic contacts and when this occurred, the postsynaptic density was smaller. Scale bars: 0.5 μ m. (C) Cells transfected with PAK3 carrying the MRX30 mutation showed reduced AMPA/N-methyl-D-aspartate receptor ratios, suggesting the presence of immature spines. (D) Cells transfected with PAK3 failed to express long-term potentiation (filled circles) in contrast to what was observed in control cells (open circles).

in contrast with these data, a very recent study of PAK3 knockout mice showed defects in long-lasting synaptic plasticity and impaired learning of a taste aversion associative task, but, surprisingly, no detectable spine abnormalities and no deficit in hippocampus-dependent spatial memory (61). An interesting finding, however, was that these PAK3 knockout mice exhibited defects in the regulation of cyclic adenosine monophosphate-responsive element-binding (CREB) function, suggesting a possible novel mechanism through which PAK3 might affect synaptic plasticity.

Another interesting aspect of PAK3 function has also been illustrated recently in the context

of Alzheimer's disease. It was found that PAK3 binds the amyloid precursor protein (APP) on a site adjacent to the CRIB domain and that it participates in this way in APP-mediated apoptosis and DNA synthesis (62). This mechanism is probably the result of a miss-regulation between mutant APP and PAK3. Binding of APP to PAK3 may draw it into a complex of proteins, or move it into a compartment of the cell where it might be exposed to new substrates, illustrating the importance not only of the mechanisms of PAK3 activation, but probably also of the localization of this activity. Together these data about PAK3, and also PAK1, it is suggested that these two kinases are important modulators of synapse

formation and plasticity mechanisms. However, it remains unclear up to which point PAK3 and PAK1 have specific and/or different roles and how exactly they produce these effects.

Central Nervous System Functions of Other PAK Proteins

PAK2 is a ubiquitously expressed member of group A family of PAKs. It has the characteristic of being activated through proteolytic cleavage by caspases or caspase-like proteases. Full-length PAK2 is localized in the cytoplasm and stimulates cell survival, while the proteolytic fragment (PAK2p34) obtained by cleavage translocates to the nucleus and is involved in the cell death response of non-neuronal cell lines (63). PAK2 also contributes to the pathogenicity of the human immunodeficiency virus infection (64). PAK2 knockout mice are not viable (7) and the only information available about PAK2 in neurons concerns its involvement in a signaling pathway for basic fibroblast growth factor. In PC12 cells, it was found that activation of a PAK2/ β PIX complex via the ERK cascade induced neurite outgrowth (65). Effects on spine morphogenesis were not yet reported for PAK2.

PAK4 was the first described group B family isotype. It was identified from a polymerase chain reaction screen with degenerate primers based on the PAK2 kinase domain. It is implicated in actin cytoskeleton reorganization and in the formation of filopodia in different cell lines (66). These cytoskeletal changes are mediated by LIMK1 and cofilin (67). PAK4 also participates in cell death signaling, cell growth, and migration (68,69). It was found to be expressed in most tissues examined, with the highest levels in the prostate, testis, and colon, but abundant in all tumor cell lines analyzed (66,68). Mouse PAK4-null embryos die at an early embryonic stage (E10.5) by an unknown mechanism (70). They display cardiac and neuronal defects. Neuronal progenitors form normally, but differentiation of these cells is mostly inhibited, axonal outgrowth is impaired, and neurons do not

migrate to their correct target area. A synapse-specific role of PAK4 was not studied yet.

PAK5 is the brain-specific member of the group B PAKs. First it was described in *Xenopus* to act on microtubule stabilization, then it was cloned from human testis and brain, and demonstrated to promote neurite outgrowth in N1E-115 cells (71–73). PAK5 is also thought to have a role in the regulation of apoptosis (74). PAK5 knockout mice develop normally, they are fertile and no phenotype was found until now (75). It seems that, in vivo, PAK5 is not implicated in brain development, but it could participate in more subtle neuron-specific functions.

PAK6 was discovered in a yeast two-hybrid screen for androgen receptor-interacting proteins (76). It is mainly expressed in brain, testis, prostate, and breast tissues, and specifically represses androgen and estrogen receptor-mediated transcriptions (77). Specific functions in the brain have not yet been reported.

Conclusion

PAK proteins form a family of kinases that are highly implicated in several important processes in mammalian tissue. As reported earlier, the neuronal functions of these kinases remain unclear for most of them, although recent data point to a clear implication of PAK1 and PAK3 in the regulation of spine morphogenesis, synapse formation, and plasticity. Whether the role of PAK1 and PAK3 in these mechanisms is specific and how it differs from that of the other members of the family is still an intriguing question. One might wonder, for instance, what is the significance of all these different isoforms? It has been proposed that evolution of higher complexity requires acquisition of pleiotropy: the same genes are redeployed to different functions within a network of epistatic interactions (78). Thus the specificity of the different PAK proteins might not be so much in the different functions to which they participate and which indeed share similarities, but in their localization and distribution in different cell types.

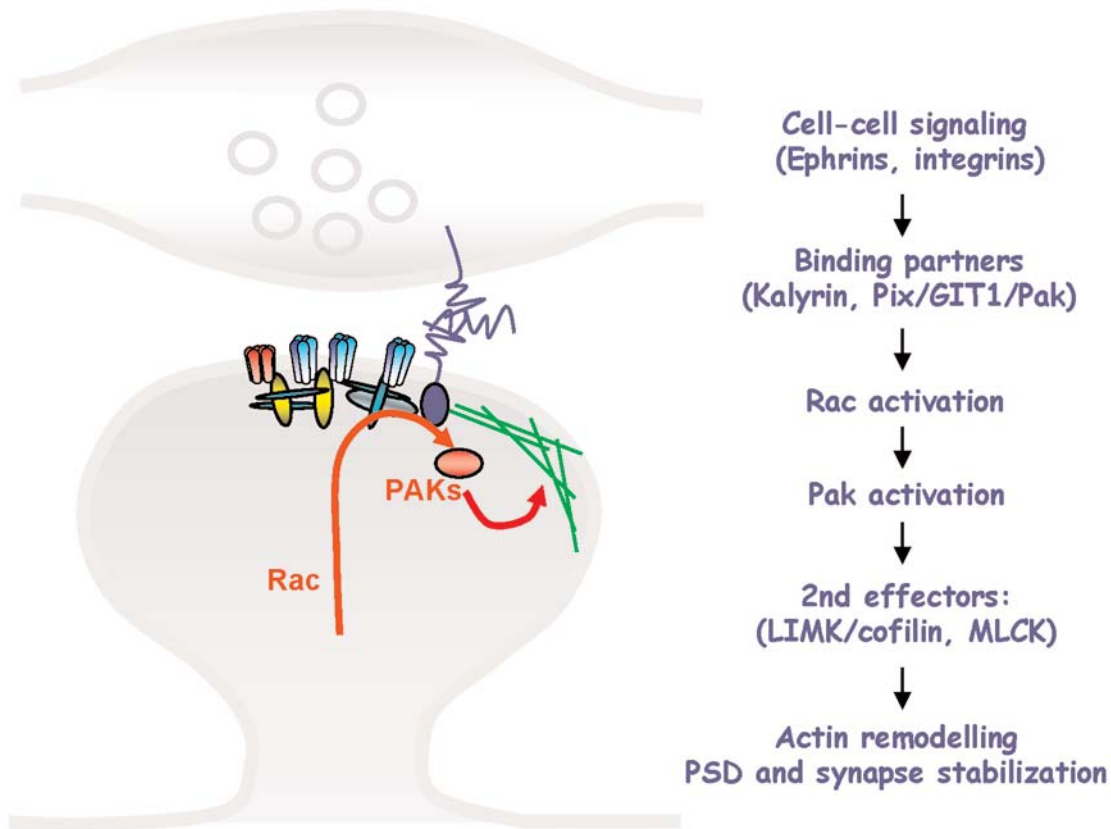


Fig. 3. Hypothetical model to account for the role of PAK1 and PAK3 in spine morphogenesis and synaptic plasticity. Communication between pre- and postsynaptic structures is required to establish a functional, mature synaptic contact. This communication could involve the participation of adhesion molecules (integrins, CAMS), growth factors, or Ephrin receptors that would signal to guanine exchange factors molecules (kalirin, GIT1/Pix/PAK complex) localized in spines and postsynaptic densities. These in turn might result in Rac1 activation, which, through PAK activation and downstream effectors (LIM kinase, myosin light chain kinase), would regulate actin dynamics and actin–myosin interactions. This reorganization of the cytoskeleton is known to be necessary for the establishment of mature synaptic contacts and for properties of synaptic plasticity, such as long-term potentiation.

In the brain, the information currently available mainly suggests an important role for PAK1 and PAK3. In both cases, an implication as effectors of Rac1 signaling appears the most likely in view of the similarities in phenotype observed when interfering with Rac1 (79) and/or PAKs: the main defects concern spine formation mechanisms and spine morphology. However, how the signaling cascade is initiated remains unclear. Work by Penzes et al. (25) suggests that activation of Ephrin receptors EphB2

may induce synaptic recruitment of the Rho-GEF kalirin, resulting in local activation of Rac1 and PAKs. There is also evidence from other cell types that integrin signaling may result in activation of a Pix/Rac/PAK complex. Accordingly, a possible working model that could account for the effects of PAKs on spine morphogenesis is summarized in Fig. 3. Intercellular signaling through adhesion molecules, growth factors, or Ephrin receptors could lead to GEFs activation, which could include kalirin

or a G_{it1}/Pix/PAK complex. This would recruit and activate Rac1 in spines and result in PAK activation, which, in turn, would act on a number of potential substrates (MLCK, LIMK, mitogen-activated protein kinase cascade) regulating actin organization or actin–myosin interaction. An interesting hypothesis would be to consider that this signaling pathway would be critically involved in the communication between pre- and postsynaptic structures and contribute to the formation and stabilization of the postsynaptic density and eventually also in its capacity for plasticity. Much recent evidence has indeed shown that actin dynamics and reorganization of the spine cytoskeleton are required for the expression of properties of synaptic plasticity, such as LTP (80). This attractive possibility is supported by several recent studies and in particular the observation that expression of PAK3 mutants is associated with defects in synaptic contact formation and the existence of immature postsynaptic densities. There are, however, numerous questions that remain unanswered, particularly with regard to the molecular partners involved and the numerous possibilities of interactions with other signaling pathways. In particular, the very recent evidence for abnormalities in CREB phosphorylation obtained from mice lacking PAK3 gene (61) raises the possibility that the kinase contributes not only to cytoskeletal control, but also to more complex regulations, including modulation of gene expression. In view of the importance of PAK protein for the understanding of the molecular events underlying cognitive mechanisms, it is very likely that this new exciting field will progress rapidly in the coming years and bring new light on the complexity of the machinery that controls synaptic structures.

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