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Review

Involvement of Rac/Cdc42/PAK pathway in cytoskeletal rearrangements

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The p21-activated kinases (PAKs) are serine/threonine protein kinases interacting with small GTPases — Rac and Cdc42. PAKs are found in most eukaryotes and play an evolutionarily conserved role in many cellular processes. Six human PAKs have been identified, and based on homology, they can be classified into two groups. This review focuses specifically on the role of Rac/Cdc42 regulated PAKs in maintaining and remodeling cytoskeletal structure in various organisms. A list of PAKs substrates and binding partners implicated directly and indirectly in cytoskeletal reorganization is presented. Also perturbations of the Rac/Cdc42/PAK pathway leading to tumorigenesis and neurodegenerative diseases are reviewed.

Keywords: PAK kinase, Rho GTPases, cytoskeletal organization, PAK substrates

INTRODUCTION

p21-Activated kinases (PAKs) are serine/threonine protein kinases involved in multiple biological processes, but a growing list of data shows that PAKs are mainly implicated in cytoskeletal rearrangements. Cytoskeletal organization and dynamics play a central role in cell movement, migration, adhesion, proliferation, differentiation, and vesicle trafficking.

PAKs were first identified as effectors of small GTPases of the Rho family (Manser *et al*., 1994). The Rho GTPases exist in two conformations: inactive, GDP-bound, and active, GTP-bound. These proteins are activated by members of the guanine exchange factors family (GEFs) that increase the GDP/GTP exchange rates, and are inactivated by members of the GTPase activating proteins family (GAPs) (Jaffe & Hall, 2005). Two Rho GTPases, Rac and Cdc42, can directly interact with PAK.

All PAKs share a conserved catalytic domain located at the C-terminus. PAKs have been found in a broad spectrum of eukaryotic organisms from yeast to humans (for references see: Knaus & Bokoch, 1998; Hofmann *et al*., 2004). The six PAK isoforms identified in humans are classified into two groups (I and II) according to the similarity of their catalytic domains, and regulatory mechanisms. While the C-terminal kinase catalytic domain is a region of high homology among all human PAKs, the N-terminal regions are more variable. PAKs belonging to group I (isoforms 1, 2 and 3) contain a con-

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Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ALS2, alsin; BAD, BCL2 antagonist of cell death; CaD, caldesmon; CRIB, Cdc42/Rac-interacting binding domain; DLC1, dynein light chain; FLNa, filamin A; GEF, guanine exchange factor; GAP, GTPase activating protein; GC, guanylyl cyclase; GIT-1, G-protein coupled receptor kinase-interacting protein 1; ILK, integrin-linked kinase; Inca, induced in neuronal crest by activating protein; LIMK, LIM kinase; MARK, MAP/microtubule affinity-regulating kinase; MHC, myosin heavy chain; MLC, myosin light chain; MLCK, myosin light chain kinase; NET1, neuroepithelioma transforming gene 1; PAKs, p21-activated kinases; PIX, PAK-interactive exchange factor; p41-Arc (Arp2/3), 41 kDa subunit actin-related protein 2/3 complex; PRG, PDZ RhoGEF; p35/Cdk5, cyclin-dependent kinase 5 (Cdk5) and its neuron-specific regulator p35; RhoGDI1, Rho GDP dissociation inhibitor; RLC, regulatory light chain; TCoB, tubulin cofactor B.

served Cdc42/Rac-interacting binding domain CRIB (also called p21-binding domain — PBD) and an autoinhibitory domain — AI. The N-terminal region of group II PAKs (isoforms 4, 5 and 6) also have a CRIB sequence, but lack a defined autoinhibitory domain (for references see: Jaffer & Chernoff, 2002; Bokoch, 2003; Eswaran *et al*., 2008). Although both groups of PAKs specifically interact with Rho-family GTPases, PAKs of group I are activated upon binding Rac and Cdc42 whereas PAKs of group II do not require GTPases for their kinase activity, but their interaction with Rac/Cdc42 affects the cellular localization of the protein. The mechanism of PAK activation has been studied in numerous organisms. The extensive and detailed information concerning the biochemical properties of PAKs and their regulation has been reviewed recently (for references see: Bokoch, 2003; Zhao & Manser, 2005).

This review concentrates on the role of Rac/Cdc42 regulated PAKs in maintaining and remodeling cytoskeletal structures in various organisms with a major focus on the newly identified PAK substrates that play an essential function in cytoskeletal rearrangements. Perturbations of the Rac/Cdc42/PAK pathway leading to tumorigenesis and neurodegenerative diseases are also reviewed.

PAKs ISOFORMS AND EXPRESSION PATTERN

Members of the PAK family have been found in many organisms including *Saccharomyces cerevisiae* (Ste20, Cla4 and Skm1), *Schizosaccharomyces pombe* (Pak1p/Orb2p/Shk1p, Pak2p/Shk2p), *Acanthamoeba castellani* (MIHCK), *Entamoeba histolytica* (EhPAK2 and EhPAK3), *Dictyostelium discoideum* (DPAKa, DPAKb/MIHCK, DPAKc), *Caenorhabditis elegans* (CePAK1a, CeC45BII.1a, CeY38F1A), *Drosophila melanogaster* (DmPAK1, DmPAK3, Mbt/DmPAK2), *Xenopus laevis* (XlPAK1, 2, 3) and *Homo sapiens* (HsPAK1, 2, 3, 4, 5, 6) (Hofmann *et al*., 2004; Mentzel & Raabe, 2005). The presence of PAK homologs in organisms ranging from protozoans to mammals strongly indicates ancient evolutionary origin and high importance of these proteins.

The pattern of mammalian PAKs expression has been studied for different cell types and tissues. For example, mammalian PAK1 is highly expressed in the brain, muscle and spleen, PAK 2 is expressed ubiquitously, PAK4 is present predominantly in the prostate, colon, and testis, PAK6 mainly in the testis, prostate and brain, while PAK3 and 5 are specifically expressed in the brain (Eswaran *et al*., 2008). The intracellular localization of a given PAK depends on its kinase activity, isoform and cell type. The binding partners of a PAK play a crucial role in its distribution in particular compartments. Activation of some PAKs changes their conformation allowing binding of new partners and resulting in different PAK distribution before and after its activation (for references see: Bokoch, 2003; Eswaran *et al*., 2008; Arias-Romero & Chernoff, 2008).

EFFECTS OF Rac/Cdc42/PAK PATHWAY ON CYTOSKELETAL ORGANIZATION

The regulation of cytoskeletal organization by PAKs is very complex and involves both the C-terminal kinase catalytic domain and the N-terminal protein–protein interactions. The effects of PAK on the cytoskeleton can be dependent or independent of its kinase activity (Daniels & Bokoch, 1999; Arias-Romero & Chernoff, 2008). Cdc42 and Rac are the predominant direct upstream signaling molecules of PAKs. PAK activated by Rac/Cdc42 induces formation of lamellipodia, filopodia, the membrane ruffles, stress fibres and remodeling of focal adhesion complexes (for references see: Bokoch, 2003). Proteins involved in the cytoskeletal reorganization include the main components of the actomyosin cytoskeleton, intermediate filaments, microtubules, integrins, and a variety of proteins associated with the above. PAK interacting with Rac/Cdc42 can modify properties of these proteins by their phosphorylation. It should be noted that PAK substrate specificity is similar among many species (Brzeska *et al*., 1997). The next chapter contains a list of substrates and binding partners of PAK directly engaged in cytoskeletal organization and those that are involved indirectly. The multiple regulatory roles of PAK/Rac/Cdc42 in cytoskeletal rearrangements are summarized in Fig. 1.

Substrates and binding partners of PAKs directly implicated in cytoskeletal rearrangement

Several of the PAK — interacting proteins are the main components of thin, thick and intermediate filaments. The myosin motor activity is crucial for the intracellular machinery responsible for cytoskeletal rearrangement implicated in spreading of the cell on a surface, cell migration and cell division, and myosins are targets of PAK.

Myosins constitute a large family of actin-dependent molecular motors that, upon interacting with actin, convert the chemical energy of ATP hydrolysis into mechanical movement. They consist of heavy (MHC) and light chains (MLC) and are present in all eukaryotic cells from yeast and amoeba to mammals. Myosins have been classified into nearly 30 classes (Sellers, 2000; Redowicz, 2007) based on the sequence homology of more than 250 known myosin motor domains. Class II myosins are called conventional myosins since they were first

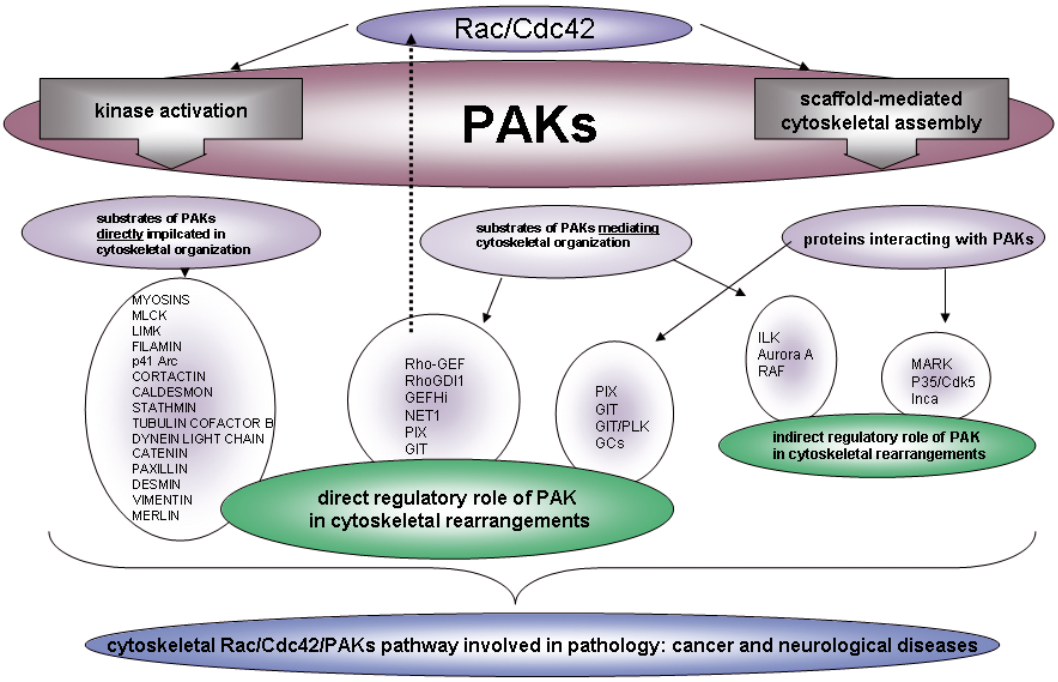

Figure 1. Involvement of Rac/Cdc42/PAK pathway in cytoskeletal rearrangements.
Substrates of PAKs and other proteins that interact with PAKs are indicated. Full names of the proteins are given in the text and in Abbreviations.

discovered. Myosins belonging to the other classes are called unconventional. Most of the conventional non-muscle myosins, as well as smooth muscle myosins II, are regulated by phosphorylation of their regulatory light chains (serine 19 and threonine 18 in smooth muscle myosin RLC). The activity of some myosins I is regulated by phosphorylation of the serine or threonine located in the actin-binding surface loop of the heavy chain (Brzeska & Korn, 1996).

One of the first Cdc/Rac effectors identified by Leberer *et al*. (1992) was Ste20 found in *Saccharomyces cerevisiae*. The Ste20 kinases belong to the PAK family and modulate cell morphology and polarity. The budding yeast *S. cerevisiae* has two myosin-I isoforms encoded by the *MYO3* and *MYO5* genes. Serine 357 residue, an activatory phosphorylation site of Myo3p heavy chain (that is also conserved in Myo5p), was identified as a unique phosphorylation site for Ste20p and Cla4p (another member of PAK family) both *in vivo* and *in vitro* (Wu *et al*., 1997). Phosphorylation of myosin I is required for yeast budding and regulates rearrangements of actin cytoskeleton (Lechler *et al*., 2000). The PAK-related protein Pak1p/Orb2p found in *Schizosaccharomyces pombe* localizes to the actomyosin ring at the cell division site. Pak1p mediates phosphorylation of myosin II regulatory light chain (Rlc1p) at serine 35 and serine 36 *in vivo* and loss of Pak1p activity leads to defective coordination of mitosis and cytokinesis (Loo & Balasubramanian, 2008).

Acanthamoeba PAK-MIHCK catalyses phosphorylation of MHC of three *Acanthamoeba* myosin I isoforms causing their activation (Brzeska *et al*., 1989; 1996). Interestingly, *Acanthamoeba* PAK was probably the first discovered, purified and characterized PAK, long before mammalian PAK became known (Pollard & Korn, 1973). Also in *Dictyostelium*, DdPAKB activates myosin ID by phosphorylation of its heavy chain (Lee & Côté, 1995; Lee *et al*., 1996). In addition, *Acanthamoeba* PAK phosphorylates the regulatory light chain (RLC) of non-muscle myosin *in vitro* and *in vivo* (Brzeska *et al*., 1997; Szczepanowska *et al*., 2006). Expression of the active catalytic domain of amoeba PAK in HeLa cells causes phosphorylation of endogenous myosin II (Brzeska *et al*., 2004). Biochemical studies *in vitro* have demonstrated that *Drosophila melanogaster* PAK phosphorylates the RLC of *Drosophila* nonmuscle myosin II on serine 21 and threonine 20, sites homologous, respectively, to serine 19 and threonine 18 in mammalian smooth muscle myosin RLC (Crawford *et al*., 2001). In *Xenopus laevis*, activated XlPAK1 phosphorylates the regulatory light chain (xMLC) of myosin II on threonine 18 and serine 19 and induces hyperphosphorylation of xMLC *in vivo*. The XlPAK activation is sufficient for apoptotic body formation, and it is strongly suggest that activation of myosin II is essential for this process (Bisson *et al*., 2003). Mammalian PAK phosphorylates MLC on serine 19 (Ramos *et al*., 1997; Chew *et al*., 1998; Van Eyk *et al*., 1998). MLC phosphorylation (by PAK1 and PAK3) in neuronal cells promotes

dendric spine morphogenesis by local stabilization of the actin network (Zhang *et al*., 2005). In non-neuronal cells, phoshorylated MLC localizes to both the leading edge and the rear end of a migrating cell (Matsumura *et al*., 1998). In addition to phosphorylating the RLC of nonmuscle and smooth muscle myosins II, and heavy chain of myosins I, PAK also phosphorylates heavy chain of myosin VI. Myosin VI plays an important role in membrane trafficking and cell migration (Buss *et al*., 1998).

PAK can also indirectly regulate phosphorylation of myosin by myosin light chain kinase (MLCK). PAK1 phosphorylates MLCK, thereby decreasing its activity (Sanders *et al*., 1999). PAK2 activated by Cdc42 can phosphorylate MLCK on serines 439 and 991, inhibiting its activity and limiting the development of isometric tension in smooth muscle and non-muscle cells (Goeckeler *et al*., 2000).

Another substrate for PAK kinases is LIM kinase (LIMK). LIMK is a serine/threonine kinase involved in the regulation of actin polymerization and microtubule disassembly (Bernard, 2007). PAK1 and PAK4 phosphorylate LIMK on threonine 508 causing LIMK activation (Edwards *et al*., 1999; Dan *et al*., 2001). Activated LIM kinase catalyses phosphorylation of cofilin which acts as an actin capping and severing protein. Phosphorylation of cofilin suppresses its activity, which increases in the amount of cellular filamentous actin (Arber *et al*., 1998; Yang *et al*., 1998). Cofilin is postulated to drive cell protrusion and migration by affecting polymerization of actin filaments. The role of PAK1 in the induction of lamellipodia, filopodia and formation of membrane ruffles as well as in cell motility involves LIMK phosphorylation (Bokoch, 2003). Moreover, PAK1 and PAK4 kinase activation and phosphorylation of LIMK is important for neuronal polarization and differentiation (Kreis & Barnier, 2009).

Filamin A (FLNa) is an actin-binding protein that crosslinks actin filaments into orthogonal networks, and links them to cellular membranes. FLNa is phosphorylated on serine 2152 by PAK1. Filamin is required for PAK1-mediated actin changes and stabilization of membrane ruffling that occurs at the leading edge of motile cells. Moreover, FLNa binds to the CRIB region of PAK1 and stimulates PAK1 kinase activity, which indicates that this interaction is important for the local activation of PAK (Vadlamudi *et al*., 2002).

p41-Arc (Arp2/3) (41 kDa subunit actin-related protein 2/3 complex) is one of Arp2/3 components which have a regulatory role in the assembly and maintenance of the Arp2/3 complex. This complex is required for formation of branched networks of actin filaments at the cell cortex. The Arp2/3 complex consists of seven subunits. Phosphorylation of p41-Arc on threonine 21 by PAK regulates its association with the Arp2/3 complex in the cortical actin nucleation regions of the cell. Moreover, interaction of PAK1 with p41-Arc has an important role in mammalian cell migration and may regulate cell motility and invasiveness (Vadlamudi *et al*., 2004b).

Cortactin is an F-actin binding protein, which is involved in actin polymerization and modulates formation of membrane ruffles, lamellipodia and podosomes. Phosphorylation of cortactin by PAK3 on serine 113 residue regulates actin polymerization and branching (Webb *et al*., 2006a; Ayala *et al*., 2008).

Caldesmon (CaD) is an actin filament regulatory protein, and one of the key regulators of actin dynamics. In the smooth muscle caldesmon controls actin-myosin interactions. Mass spectroscopy data show that PAK phosphorylates CaD at serines 657 and 687 (Foster *et al*., 2000). PAK-catalyzed phosphorylation of CaD is involved in Ca^{2+}-independent smooth muscle contraction. Caldesmon is localized to cell adhesion structures — podosomes. Mutations of caldesmon phosphorylation sites (by PAK1 and PAK2) inhibits cell polarization and leads to defects in membrane extension and cell migration (Eppinga *et al*., 2006; Morita *et al*., 2007).

Stathmin, also called oncoprotein 18 (Op18) is a microtubule-destabilizing protein which binds tubulin dimers, inhibits tubulin polymerization and promotes dynamic instability of microtubules. PAK1 phosphorylates Op18/stathmin specifically at serine 16. Phosphorylation inactivates this protein, which results in stabilization of microtubules at the leading edge of motile cells (Wittmann *et al*., 2004).

A cofactor in the assembly of α/β-tubulin heterodimers — tubulin cofactor B (TCoB) — is a substrate of PAK1 *in vivo* as well as *in vitro*. PAK1 phosphorylates TCoB on serines 65 and 128 and colocalizes with TCoB on newly polymerized microtubules and at centrosomes. This phosphorylation of TCoB plays a role in the growth of new microtubules (Vadlamudi *et al*., 2005).

Dynein light chain (DLC1) is a component of the cytoplasmic dynein complex which moves along microtubules. Dynein is a motor for the retrograde transport of membranous organelles. This intracellular transport is fundamental to cellular morphogenesis and cellular functioning. PAK1 phosphorylation of DLC1 on serine 88 controls vesicle formation and trafficking functions (Vadlamudi *et al*., 2004a; Yang *et al*., 2005).

β-Catenin (homolog in *Drosophila melanogaster* — Armadillo) is linked directly or indirectly to the actin cytoskeleton *via* cadherins and α-catenin. It is a multifunctional protein which regulates both cell–cell adhesion and nuclear transcription. Membrane-localized β-catenin is mostly bound at adherens junctions that maintain cell–cell contact, polar-

ity and communication. In *D. melanogaster* activated Mbt (DmPAK2) is recruited to adherens junctions and phosphorylates Armadillo on Ser 561 and 688. These serine residues are conserved in vertebrate β-catenins. Activation of Mbt leads to destabilization of the interaction of Armadillo with cadherin resulting in a reduction of cadherin-mediated adhesion (Menzel *et al*., 2008).

Paxillin is an adaptor protein which mediates formation of integrin-dependent focal adhesions. Paxillin α isoform directly binds to, and is phosphorylated on serine by PAK3 activated by Cdc42. This indicates that paxillin serves as a link between PAK3 and focal adhesions (Hashimoto *et al*., 2001).

Proteins engaged in the organization of intermediate filaments which are substrates for PAKs are desmin and vimentin. Desmin is expressed in muscle cells, where it plays a critical role in maintaining the structural and functional integrity of myofibers. PAK1 phosphorylates desmin mainly on serine residues located in the head domain of this protein. Phosphorylation affects organization of desmin filaments and inhibits its ability to bind intermediate filaments (Ohtakara *et al*., 2000). Vimentin is one of the most widely expressed intermediate filament proteins. PAK1 regulates the organization of vimentin filaments through direct phosphorylation of this protein on serines 25, 38, 50, 65 and 72 (Goto *et al*., 2002).

Merlin tumor suppressor also called schwannomin is a member of a superfamily of membrane-cytoskeleton linking proteins — ERM. Proteins of this superfamily bind F-actin and modify actin polymerization. Merlin also interacts with many other proteins directly involved in cytoskeletal organization, such as ezrin, radixin, moesin, paxillin, N-WASP, spectrin, tubulin, calpain and many others (a total of 34 interacting proteins have been identified so far) (Scoles, 2008). PAK phosphorylates merlin on serine 518, and this phosphorylation results in inhibition of merlin tumor suppressing activity and its translocation to the plasma membrane in a paxillin-dependent manner (Thaxton *et al*., 2007). Phosphorylation stabilizes this protein in a conformation described as an open state and unmasks binding sites for transmembrane receptors and some actin-associated proteins such as β1 integrin and paxillin (Thaxton *et al*., 2008).

Rho GTPases play a key role in actin cytoskeleton remodeling. Their activity is regulated by guanine exchange factors (GEFs) and by GTPase activating proteins (GAPs). PDZ RhoGEF (PRG) is a GEF. The activity of PRG increases upon binding to heterotrimeric G proteins. PAK4 associates with and phosphorylates PRG thus negatively regulating Rho activity. Therefore, it has been suggested that PAK4 provides a novel cross-talk mechanism between different GTPases (Barac *et al*., 2004).

Rho GDP dissociation inhibitor (RhoGDI1) negatively regulates the activity of Rho GTPases. PAK1 phosphorylates RhoGDI1 on serines 110 and 174, leading to activation of Rac1 (DerMardirossian *et al*., 2004). PAK2 binds to and phosphorylates RhoGDI1 on serines 34 and 101 (both *in vitro* and *in vivo*). This phosphorylation results in activation of Rac1/Cdc42 GTPases during neurite outgrowth (Shin *et al*., 2009).

GEF-H1 is another guanine nucleotide exchange factor controling Rho activity. PAK1 phosphorylates *in vivo* GEF-H1 on serine 885. Phosphorylated GEF-H1 binds 14-3-3 protein causing its translocation to microtubules. This phosphorylation may also coordinate Rho, Rac and Cdc42-mediated signaling pathways (Zenke *et al*., 2004). PAK4 forms a stable complex with GEF-H1 and phosphorylates it on serines 67 and 810. Phosphorylation of both sites plays an important role in controlling the localization and function of GEF-H1. The activity of GEF-H1 plays a role in the crosstalk between actin and microtubules networks, which is crucial for cell morphology and motility (Callow *et al*., 2005).

PAK1 also regulates the activity of RhoA-specific guanine nucleotide exchange factor — NET1 (neuroepithelioma transforming gene 1). PAK-dependent phosphorylation of NET1 on serines 152, 153 and 538 reduces its activity as a GEF and stimulates actin stress fiber formation (Alberts *et al*., 2005).

Integrin-linked kinase (ILK) is a major signaling integrator in mammalian cells. It plays a critical role in cell motility, cytoskeleton reorganization and tumor progression and invasion. PAK1 phosphorylates ILK on threonine 173 and serine 246 *in vitro* and *in vivo* (Acconcia *et al*., 2007). Mutation of these phosphorylation sites inhibited cell growth and migration.

Other PAK substrates indirectly invoved in cytoskeletal rearrangements

Among the great number of molecules which are substrates of PAK kinase, some of them are indirectly implicated in cytoskeletal organization. Also many of proteins are indirectly involved in cytoskeletal rearrangements only by their interaction with a PAK molecule.

PIX (PAK-interactive exchange factor) also called COOL-1 is a specific guanine nucleotide exchange factor. Binding to PIX activates PAK and influences its localization (Rennefahrt *et al*., 2007; Manser *et al*., 1998). PAK1 phosphorylates βPIX on serines 340 and 525 (Shin *et al*., 2002; Ballif *et al*., 2004; Olsen *et al*., 2006). Activated PAK and PIX translocate from focal complexes to cell–cell contact

sites during wound closure (Zegers *et al*., 2003). In addition to PAK binding, PIX also binds to synaptic adaptor protein GIT/PKL (G protein-coupled receptor kinase-interacting protein/paxillin kinase linker). Interactions between GIT1 and PAK3/PIX/Rac play a particularly important role during spine morphogenesis and in mental retardation (Zhang *et al*., 2005). In this case, activated PAK (in a signaling complex consisting of GIT1, PIX, and Rac) acts through phosphorylation of myosin II regulatory light chain (MLC). Phosphorylated and activated MLC causes an increase in dendritic spine and synapse formation (Zhang *et al*., 2005). Another type of interaction between PIX and PAK was found in a mouse macrophage-like cell line (RAW274) during chemotaxis. Subunits released from heterotrimeric G proteins ($\beta\gamma$) bind to PAK1 and indirectly cause its activation through α-PIX and Cdc42. This G$\beta\gamma$ /PAK1/PIX/Cdc42 pathway influences localization of F-actin at the leading edge of the cell and regulates directional cell migration during chemotaxis (Li *et al*., 2003).

GIT-1, G-protein coupled receptor kinase-interacting protein 1 is GAP of Arf protein that binds PIX and paxillin. PAK1 phosphorylates GIT1 *in vitro* on serine 517 (Zhao *et al*., 2005) and on serine 709 (Webb *et al*., 2006b). GIT1 phosphorylation on serine 709 increases its binding to paxillin and is necessary for a GIT-dependent increase in protrusive activity. The PAK/PIX/GIT1/paxillin signaling pathway has a role in regulating focal adhesion turnover. PAK, PIX and GIT also have another function, they participate in regulation of centrosome maturation (Zhao *et al*., 2005).

Activation of the cell cycle normally requires cytoskeletal remodeling involving cooperation and coordination of numerous cellular events. PAKs are indirectly engaged in cell cycle regulation *via* their involvement in microtubule network rearrangement and formation of microtubule organizing center. Aurora-A (known as centrosomal-located kinase) is involved in cell cycle. Only activated PAK1 can bind to Aurora-A at the centrosome. PAK1 phosphorylates Aurora-A on threonine 288 and serine 342, and this phosphorylation is responsible for Aurora kinase activation in mitosis. Inhibition of PAK (or βPIX depletion) causes delay in centrosome maturation (Zhao *et al*., 2005).

Raf family protein kinases are key effectors of Ras-mediated adhesion-dependent signaling that are also implicated in cytoskeletal organization. PAK1-3 phosphorylate Raf-1 on serine 338 *in vivo* and *in vitro* and this phosphorylation stimulates the kinase activity (King *et al*., 1998).

Proteins interacting with PAKs and indirectly implicated in cytoskeletal rearrangements

MARK (MAP/microtubule affinity-regulating kinase) is involved in establishing embryonic polarity. In neurons, MARK phosphorylates tau protein (microtubule associated protein) thus destabilizing microtubules. PAK5 binds to and inhibits the activity of MARK2, which affects microtubule dynamics (Matenia *et al*., 2005; Timm *et al*., 2006).

Cyclin-dependent kinase 5 (Cdk5) and its neuron-specific regulator p35 are essential for neuron migration. p35/Cdk5 concentrates at the leading edges of axonal growth cones and regulates neurite outgrowth. PAK1 is present in the Rac-p35/Cdk5 complexes. Active p35/Cdk5 kinase causes PAK1 hyperphosphorylation and affects the reorganization of the actin cytoskeleton in neurons, thus promoting neuronal migration and neurite outgrowth (Nikolic *et al*., 1998; Banerjee *et al*., 2002). Moreover, p53/Cdk5 phosphorylates PAK1 in cells undergoing mitosis. Activated PAK1 localizes to microtubule-organizing centers and along parts of the spindles and causes microtubules lengthening (Banerjee *et al*., 2002).

PAKs are also implicated in the guanylyl cyclases (GC) pathway. GCs catalyze the conversion of GTP to cGMP. cGMP is a ubiquitous second messenger mediating cellular responses to various exogenous and endogenous signaling molecules. Transmembrane GCs are directly stimulated by PAK kinases. Activation of GCs leads to increased cellular cGMP levels. Rac-PAK-GC signaling is important for the formation of lamellipodia (Guo *et al*., 2007).

Inca (induced in neuronal crest by activating protein) is a newly identified protein required for morphogenesis of the neural crest (NC). *Xenopus* PAK5 binds to and cooperates with Inca in restructuring cytoskeletal organization and the regulation of cell adhesion in the early embryo and in neural crest cells during craniofacial development. Mammalian PAK4 binds Inca thereby reorganizing the cytoskeleton and directing cell adhesion during craniofacial development (Luo *et al*., 2007).

IMPLICATIONS OF PAKs IN PATHOPHYSIOLOGY

Deregulation of PAKs has been reported in several human tumors and neurodegenerative diseases. Particularly the Rac/Cdc42/PAKs pathway, which is mainly implicated in cytoskeletal rearrangement, is involved in complex processes such as carcinogenesis (tumorigenesis) and neurodegeneration.

Cancerogenesis

PAKs play an important role in numerous types of tumor (for references see: Vadlamudi & Kumar, 2003; Kumar *et al*., 2006). A crucial step in tumor progression is rearrangement of the cytoskeleton which is necessary for cell migration and inva-

sion. Cell movement requires a defined cell polarity, membrane protrusion at the leading edge, adhesion and retraction of the rear end of the cell. Because PAKs regulate cytoskeletal organization, their overexpression or dysfunction of the Rac/Cdc42/PAK pathway in tumor cells can potentially alter cytoskeletal remodeling. PAK1, 4 and 6 are the only PAK family members that are directly oncogenic (Callow *et al*., 2002; Kumar *et al*., 2006), and expression of these proteins has been shown to be increased in a variety of cancer cell lines. PAK1 is overexpressed in ovarian and breast cancers, bladder transitional-cell carcinoma, T-cell lymphoma and glioblastomas (Kumar & Vadlamudi, 2002). Moreover, overexpression of PAK4 and PAK6 as well as hyperactivation of PAK2 have been reported in prostate cancer (Kumar *et al*., 2006). In addition, PAK1, PAK2 and PAK4 are activated by cellular cues which stimulate cell migration. PAK1 and 2 cooperate in a carcinoma cell line to ensure optimal focal adhesion generation and maturation during migration (Coniglio *et al*., 2008). In breast cancer cells, expression of active PAK1 results in abnormal organization of the mitotic spindle characterized by the appearance of multiple spindle orientation (Vadlamudi & Kumar, 2003). A PAK1 mutant in the highly invasive breast cancer cells causes enhanced cell spreading, stabilization of stress fibres and reduced invasiveness. Activated PAK1 can also promote cancer progression by phosphorylation and inactivation of a proapoptotic protein — BAD (BCL2 antagonist of cell death) — thereby inhibiting the proapoptotic effects of BAD (Schürmann *et al*., 2000). The presence of activated forms of PAK1 in the cytoplasm of glioblastoma cells is correlated with shorter survival of patients probably due to enhanced invasiveness of these cancer cells (Aoki *et al*., 2007). PAK4 directly interacts with an integrin cytoplasmic subunit and has an effect on cell motility in an integrin-specific manner in breast carcinoma cells (Zhang *et al*., 2002).

PAKs are also involved in genetic disorders responsible for tumors of the central and peripheral nervous system. PAK1 mediates phosphorylation and inactivation of Merlin, which may play a role in tumor cell spreading and metastasis (Kumar *et al*., 2006). Rac/Cdc42/PAK pathway regulates many key cellular processes which are affected during tumor development and metastasis. Many substrates of PAK play an indirect roles in this process. LIMK has been found to be overexpressed in breast and prostate tumors. p41-ARC phosphorylated by PAK mediates actin nucleation and filament assembly and regulates invasiveness of breast cancer cells. Another PAK substrate, stathmin, is overexpressed in several malignancies, and TCoB upregulation has been found in human breast tumors (Kumar *et al*., 2006). The Rac/Cdc42/PAKs pathway is also implicated in modulation of endothelial cell motility and morphology in tumor angiogenesis (Fryer & Field, 2005).

Neurodegenerative diseases

AD — Alzheimer's disease. Alzheimer is the most often occurring human progressive neurodegenerative disorder. It is characterized by accumulation of β-amyloid protein aggregates. This accumulation causes abnormal spine morphology and synaptic defects like synaptic loss and synaptic dysfunction (Small, 2008). PAK 1, 2 and 3 are important regulators of synaptic plasticity, because it depends on the actin cytoskeletal organization in dendric spines. Some studies show that β-amyloid oligomers inhibit PAK, and this may result in disassembly of synaptic actin filaments. Recent data suggest that the loss of neurons and synapses observed in AD is related to deregulation of PAK1 and 3 expression and enzymatic activities (Nguyen *et al*., 2008; Salminen *et al*., 2008).

ALS — amyotrophic lateral sclerosis. A neurite outgrowth defect is associated with some subtypes of amyotrophic lateral sclerosis disease. Although the etiology of ALS is not fully understood, it seems that at least the juvenile form of this disease is caused by a mutation in alsin (ALS2), which is a GEF for Rac. PAK1 activated by alsin/Rac promotes neurite extension in hippocampal neuron growth cones, thus an impairment of the alsin/Rac/PAK1 pathway may contribute to motor neuron disease (Kreis & Barnier, 2009).

Huntington disease. Aggregation of a protein called huntingtin is implicated in the development of this neurodegenerative disease. PAK1 binds to huntingtin *in vitro* and *in vivo*, leading to an enhanced oligomerization of this protein (Luo *et al*., 2008).

Mental retardation

PAK3 plays a specific role in synaptic plasticity, and is implicated in dendritic spine morphogenesis. Five mutations affecting different domains in the PAK3 molecule that correlate with mental retardation have been identified so far (for references see: Kreis & Barnier, 2009). Three mutations associated with mental retardation have different effects on the biological functions of PAK3. Two of them completely abrogate the kinase activity, and the third one decreases the binding of PAK3 to Cdc42 and impairs PAK activation. Expression of kinase-"dead" mutants of PAK3 alters spine morphology, whereas expression of a mutant with impaired activation by Cdc42 drastically decreases spine density. These data indicate that Cdc42/PAK3 participates in dendritic spine formation and synaptic plasticity (Kreis *et al*., 2007). Overexpression of constitutively

active PAK3 also potently rescues abnormal spine morphology caused by mutation of αPIX (Nodé-Langlois *et al*., 2006).

CONCLUSIONS

All the data presented here show that the Rac/Cdc42/PAK pathway plays a fundamental role in the regulation of the cytoskeleton in a variety of organisms by phosphorylation and interaction with numerous proteins implicated directly and indirectly in cytoskeletal rearrangements (Fig. 1). This in turn implies that PAKs may ultimately be linked to various pathologies, including neuronal degeneration, cancer, and mental retardation. Therefore, understanding of the molecular mechanisms responsible for PAK activation and regulation as well as identification of their multiple interacting partners and kinase substrates seems to be very important.

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