Published in final edited form as:
J Cell Biochem. 2009 July 1; 107(4): 579–585. doi:10.1002/jcb.22159.

PAK Thread from Amoeba to Mammals

Anupam Kumar, **Poonam R. Molli**, **Suresh B. Pakala**, **Tri Bui Nguyen**, **Suresh K. Rayala**, and **Rakesh Kumar***
Department of Biochemistry and Molecular Biology, George Washington University Medical Center, Washington DC 20037, USA.

Abstract

The p21-activated kinases (PAKs) are signaling nodes that play a crucial role in cellular processes including cell motility, differentiation, survival, gene transcription and hormone signaling. PAKs are highly conserved family of serine threonine kinases that act as effector for small GTPases Rac and Cdc42. Most of our knowledge about PAK functions has been derived from genetic approaches in lower organisms and many of these functions are similar to that seen in mammalian cells. In this review, we have summarized the extensive information generated in lower eukaryotes and very briefly discussed the current status of PAKs in humans.

INTRODUCTION

The role of p21-activated kinases (PAKs) - the serine/threonine kinases, in basic cellular physiology has been conserved throughout evolution. Mammalian PAKs are categorized into two subgroups: group I includes PAK1, PAK2, and PAK3, and group II includes PAK4, PAK5, and PAK6 (Hofmann et al., 2004;Jaffer et al., 2002). All PAKs share a common structural organization, with an N-terminal regulatory domain and a C-terminal catalytic domain. PAKs are activated by GTPase-dependent and -independent mechanisms. Downstream of activated PAKs, stimulated signaling cascades regulate a wide variety of events, such as cytoskeleton dynamics, cell migration, the cell cycle, cell survival, and apoptosis (Bokoch, 2003). Many of these cellular functions are similar between mammalian cells and lower eukaryotes, which strengthens the importance of PAK's function and its ancient origin (Figure 1). Lower eukaryotes provide an excellent experimental model system that can be easily manipulated to understand the precise role of PAKs in oncogenic progression. In this review, we will discuss the substantial information that has been generated on the phylogenetic organization, structure, regulation and function of PAKs in lower eukaryotes, and only very briefly summarize the current status of PAKs in mammalian cells.

BUDDING YEAST

The budding yeast *Saccharomyces cerevisiae* encodes for three PAK enzymes: sterile 20 (Ste20), Cla4, and Skm1. These kinases form a distinct PAK subfamily. All three Pak members contain an N-terminal PBD and a C-terminal protein kinase domain; while Cla4 and Skm1 also contain a PH domain N-terminal to the PBD. STE20 and CLA4 are redundant in function, but Skm1 is completely dispensable. Ste20 promotes actin polarization and regulates mating, osmotolerance, and vegetative growth. Cla4 is involved in septin ring assembly, actin polymerization, and mitotic entry and exit. Little is known about the function of Skm1 except that Skm1p is only expressed in meiotic cells. Deletion of *STE20* results in sterility, deletion of *CLA4* leads to aberrant cytokinesis, and deletion of both *CLA4* and *STE20* is lethal. These

***Correspondence**: E-mail: E-mail: bcmrxk@gwumc.edu.

kinases are activated by GTPase Cdc42 and by the cell-cycle dependent kinase Cdc28. Once activated, they regulate various downstream effectors that play a crucial role in cytoskeleton dynamics, especially cell polarity, cell cycle, and gene transcription.

Ste20 was identified as a key regulator in the mating pathway and functions as an activator of mitogen-activated protein kinase (MAPK) cascades (Dan et al., 2001b). The MAPK pathway is stimulated through the mating pheromone receptors Ste2–Ste4, which result in activation of the heterotrimeric G protein, followed by activation of the MAPK cascade (Bhattacharjya et al., 2006). This cascade includes the sequential phosphorylation of MAPK kinase kinase (MAP3K), MAPK kinase (MAP2K), and MAPK. The yeast MAPK pathway consists of Ste11p (MAP3K), Ste7p (MAP2K), Fus3p, and Kss1p (MAPKs) (Lee et al., 1999). In addition to mating, Ste20 regulates filamentous growth and osmotolerance by activating MAP3K (de Nadal et al., 2002). Downstream of the activated MAPK pathway, a variety of genes (e.g., FUS1) required for mating are stimulated. Mating, vegetative growth, and filamentous growth all require cell polarization, which is promoted by both Ste20 and Cla4 through a mechanism that is not completely understood. Members of the polarization pathway include type I myosins Myo3 and Myo5 and the forming homolog Bni1 (Sagot et al., 2002;Soulard et al., 2002). During cell polarization, the actin cytoskeleton undergoes reorientation, a process regulated by the cell cycle. Whether Ste20 and Cla4 control cell polarity in G_1 or other phases of the cell cycle remains unclear. However, because Cla4 kinase activity peaks at mitosis, it is believed that it acts late in the cell cycle (Benton et al., 1997;Keniry et al., 2004). PAK enzymes also participate in a negative feedback loop to end the polarized growth phase. This involves a complex interplay of molecules such as guanine-nucleotide-exchange factor Lte1, GTPase Tem1, protein phosphatase Cdc14, and a number of CDK substrates (Simanis, 2003).

FISSION YEAST

The fission yeast *Schizosaccharomyces pombe* encodes for two PAK kinases: Shk1 (also known as AKA, PAK1, and Orb2) and Shk2 (also known as AKA and PAK2). Shk1 most closely resembles budding yeast Ste20 and is an essential gene. It functions in induction of the pheromone response pathway, similar to Ste20. Shk2 is most closely related to Cla4 and Skm1, but is not functionally equivalent to these kinases.

Shk1, an effector of Cdc42, is essential for the viability of *S. pombe* (Marcus et al., 1995). The loss of functional Shk1 leads to defects in actin and the microtubule cytoskeleton, loss of polarity, and defects in mating (Qyang et al., 2002). Both, Cdc42 and Shk1 function downstream of Ras GTPase-Ras1 (Marcus, Polverino, Chang, Robbins, Cobb, and Wigler, 1995). Shk1 is also regulated by three SH3 domain-containing proteins: Scd2, Skb1, and Skb5. Scd2 is required for the normal cell polarity and mating of *S. pombe* but is not required for cell viability (Chang et al., 1999). Skb1 and Skb5 directly stimulate Shk1 kinase activity and are required for proper cell polarity during hypertonic stress (Bao et al., 2001;Yang et al., 1999b). The cytoskeleton organization and cell polarization of *S. pombe* also requires a kelch repeat protein- Tea1, and the Rho GTPase activation protein- Rga8 (Kim et al., 2003;Yang et al., 2003). Rga8 is a substrate of Shk1. Little is known about the negative regulator of Shk1. Skb15, a WD repeat protein, functions as a negative regulator of Shk1 and is required for the regulation of cytoskeleton dynamics in a cell cycle-dependent manner (Kim et al., 2001).

The signaling pathways downstream of Shk2 are not well defined. Similar to Shk1, Shk2 participates in the Ras1/Cdc42-dependent regulation of morphologic changes and mating response pathways, but Shk2 is not essential for cell viability, morphology, or mating in *S. pombe*. Shk2 is redundant in function with Shk1. Over expression of activated Shk2 in Shk1-deleted cells leads to mating defects, and these defects can be suppressed by over expression of the MAP3K kinase Byr2 (Yang et al., 1998). Similarly, over expression of activated Shk2

in wild-type cells lead to growth defects that can be suppressed by null mutations of Mkh1 and Spm1.

AMOEBAE

To date, three Pak families of genes have been identified in amoebae *Dictyostelium discoideum* that encode DdPAKA, DdPAKB (myosin I heavy chain kinase [MIHCK]), and DdPAKC; one in *Acanthamoeba castellanii* called AcMIHCK; and three in *Entamoeba histolytica*: EhPAK, EhPAK2, and EhPAK3. All these kinases show the presence of an N-terminal p21-binding domain (PBD) and a C-terminal catalytic domain. DdPAKA, DdPAKB, and DdPAKC are approximately 50% and 70% homologous within the PBD and catalytic domains, respectively. AcMIHCK is similar to DdPAKB, and EhPAK3 is homologous to DdPAKC.

Unlike mammalian PAKs, the kinase activity of DdPAKA is regulated by phosphoinositide 3-kinase and protein kinase B (Akt), which phosphorylates DdPAKA at Thr-579. Constitutively active DdPAKA leads to the upregulation of myosin II assembly, which controls cell polarity and chemotaxis. In accordance, DdPAKA-null cells display defects in myosin II assembly and directional motility. DdPAKA-null cells, when in suspension, show defects in cytokinesis. Myosin II is not a physiologic substrate of DdPAKA, but DdPAKA promotes myosin II filament assembly indirectly by regulating the activity of MIHCKs (Muller-Taubenberger et al., 2002).

MIHCK is activated in a GTPase-dependent manner by Rac and Cdc42 but not by sphingosine or any other non-negatively charged lipid. It requires the presence of acidic lipids that are inhibited by Ca (2+)-calmodulin. When inactive, MIHCK is present in a closed conformation, with the auto-inhibitory sequence located within the C-terminal portion of the PBD. Upon activation, it acquires an open conformation, with autophosphorylation at Ser-8, which leads to partial opening of the inactive folded structure. Phosphorylation of Ser-8 primes MIHCK for the second step of activation by promoting the binding of GTP-bound Rac, Cdc42, or acidic lipids such as phosphatidylserine or phosphatidylinositol 4, 5-bisphosphate. Activated MIHCK is enriched at the leading edge of migrating cells and in the macropinocytic and phagocytic cups. Upon activation, MIHCK phosphorylates its downstream effector, myosin I, and enhances the myosin I-dependent process of phagocytosis and pinocytosis. Cells lacking functional MIHCK show no apparent defects in a variety of motile processes, whereas constitutive expression of active MIHCK causes severe defects in cytokinesis by entrapping or sequestering actin filaments (de la et al., 2005).

Similar to MIHCK, the kinase activity of DdPAKC is regulated by GTPases. In response to a chemoattractant, the stimulated phosphoinositide 3-kinase pathway leads to activation of Rac-GTPase-racB, which binds to and activates the GTPase-binding Cdc42/Rac-interactive binding domain of DdPAKC. DdPAKC-null cells exhibit defects in cell polarization and pseudopodia formation in response to a chemoattractant gradient but do not develop defects in cell motility (Lee et al., 2004). Cells lacking both DdPAKC and MIHCK show severe loss of cell movement in a chemoattractant gradient, suggesting that both DdPAKC and MIHCK co-operate to regulate chemotaxis pathways (Lee, Rivero, Park, Huang, Funamoto, and Firtel, 2004). Similarly, the *E. histolytica* PAK-EhPAK plays a role in pseudopod formation and phagocytosis (Labruyere et al., 2003), whereas EhPAK2 regulates receptor capping, a process that helps parasites evade the host's immune system (Arias-Romero et al., 2006). Because EhPAK3 was only discovered recently, little is known about its physiologic role (Dutta et al., 2007).

DROSOPHILA

Drosophila encodes three PAKs: DPAK1, mushroom bodies tiny (Mbt)/DPAK2, and DPAK3. To date, only the functions of DPAK1 and Mbt have been analyzed. Their key functions are in sensory organ development.

In Drosophila, the Nck/Dock signaling pathways regulate the movement of photoreceptor axons under the guidance of a sensorimotor structure called growth cone at the leading edge of the axon. They also form a mechanistic link between the cell surface receptors and the actin cytoskeleton. Dock associates with the fly homolog of human insulin receptor and DPAK1. Upon activation, insulin receptors target the Dock-DPAK1 complex to the plasma membrane, where a second signal increases the local concentration of GTP-bound Rac, leading to the activation of membrane-bound DPAK1. This, in turn, modulates actin dynamics during the growth-cone guidance of the photoreceptor cell. The Dock-DPAK1 complex also regulates the guidance of olfactory axons and axon repulsion. DPAK1 contributes to tissue morphogenesis by mediating communication between the basement membrane and intracellular proteins during epithelial development (Rao, 2005).

Unlike DPAK1, Mbt is involved in photoreceptor cell morphogenesis rather than photoreceptor axon guidance. Mbt was found to be key in the formation of a structure called mushroom body in adult Drosophila; this structure corresponds to the hippocampus in the human brain. Mbt has a role in the proliferation, differentiation, and survival of neuronal cells; Mbt-null mutants have defects in the central brain structure and have fewer neurons. In addition, Mbt mutant flies have a reduced number of photoreceptor cells in the eye and disorganized adherens junctions. Similarly, constitutive activation of Mbt disturbs the actin cytoskeleton and affects adherens junction organization (Melzig et al., 1998). One target of Mbt signaling that plays a role in cytoskeleton organization is the actin depolymerization factor Twinstar/Cofilin (Menzel et al., 2007). Mbt interacts with Cdc42, but the binding of activated Cdc42 has no influence on Mbt kinase activity and is required for recruitment of the kinase to adherens junctions (Schneeberger et al., 2003).

CAENORHABDITIS ELEGANS

Caenorhabditis elegans expresses three PAK isoforms: CePAK1, CePAK2, and MAX-2. CePAK-1 is most closely related to group I PAKs, whereas CePAK-2 is closely related to group II PAKs and MAX-2 is similar to *Drosophila* DPAK3. CePAK-1 is expressed in the hypodermal cell boundaries during embryonic body elongation and co-localizes with CeRac1 and CeCdc42 (Chen et al., 1996). CePAK1 and MAX-2 function with the rac GTPases during axon guidance. Immediately after *C. elegans* hatches, the 12P cells migrate from their lateral positions down into the ventral cord and subsequently divide, generating the P cell lineage. Both CePAK1 and MAX-2 function redundantly in P cell migration, along with the rac activator UNC-73/Trio and the rac GTPases CED-10 and MIG-2. MAX-2 is also required for motor neuron axon guidance, which functions in a rac-independent pathway. During development, the ventral cord commissural motoneurons control the forward and reverse locomotion of the animal, and MAX-2's functions are required for the dorsal guidance of ventral cord commissural motoneuron axons (Lucanic et al., 2006).

XENOPUS

Xenopus PAKs have been studied extensively. There are four PAK genes in the *Xenopus* genome, encoding xPAK1, xPAK2, xPAK3, and xPAK5. Accumulating evidence indicates that *Xenopus* PAKs have roles in oocyte maturation and embryonic development. In *Xenopus*, the trigger from the steroid hormone progesterone is required to stimulate the signal transduction pathway, leading to oocyte maturation (Hammes, 2004). Oocytes naturally arrest

for long periods of time during G_2/M transition in the ovary. During the G_2 phase, translation of the proto-oncogene c-Mos (an MAP3K) leads to activation of MAPK and the maturation-promoting factor (MPF), a heterodimeric protein complex composed of $p34^{cdc2}$ kinase and cyclin B (Palmer et al., 2000). This MPF complex is negatively regulated by the Myt1 protein kinase that phosphorylates Cdc2 on threonine and tyrosine residues (Wells et al., 1999). Cdc2 is eventually activated to cause the oocyte to progress to meiosis; this step is catalyzed by the phosphatase Cdc25C. Phosphorylation of Cdc25C on Ser-287 by Chk1 and cAMP-dependent protein kinase causes Cdc2 to remain inactive in G_2-arrested oocytes through binding to 14-3-3 proteins (Yang et al., 1999a). To continue to mitosis, Cdc25C phosphorylation and activation is required. Cyclin B-Cdc2 can phosphorylate Cdc25C at the activating sites (Margolis et al., 2006). However, at the G_2/M transition, the initial phosphorylation of Cdc25C is triggered by polo-like kinase Plx1 prior to cyclin B-Cdc2-mediated activation (Abrieu et al., 1998). Cdc25C is also phosphorylated and activated by MPF, forming a positive feedback loop that contributes to G_2/M transition (Hoffmann et al., 1993). The eggs arrest again at metaphase of the second meiosis, with high MPF activity until fertilization.

xPAK1 promotes oocyte arrest in the G_2/prophase of the cell cycle, thereby negatively regulating oocyte maturation. xPAK1 prevents the accumulation of c-Mos and activation of both MAPK and MPF, which keeps the MPF amplification loop in an inactive state and prevents further maturation of the oocyte. Recent evidence indicates that the small GTPase Cdc42, not Rac1, has a role in progesterone-induced oocyte maturation. GDP-bound Cdc42 facilitates progesterone-induced maturation, whereas the GTP-bound Cdc42 completely blocks this process. Cdc42 regulates the maturation pathway via phosphorylation and inactivation of xPAK2 (Cau et al., 2000). xPAK2 is inactivated during maturation upon stimulation of the MPF and MAPK pathways and is reactivated upon fertilization. Apart from a role in arresting oocytes at G_2/prophase of the first meiotic prophase, xPAK1 also regulates cell survival and death. Ectopic expression of a dominant negative form of xPAK1 promotes apoptosis in *Xenopus* oocytes, indicating a role for xPAK1 in survival signals (Faure et al., 1997). xPAK1 can be activated by caspases via release of the catalytic domain from an N-terminal inhibitory sequence that promotes apoptosis. Activated xPAK1 phosphorylates the regulatory light chain of myosin II at Thr-18 and Ser-19 and induces its hyperphosphorylation, which helps in the formation of apoptotic bodies for the safe disposal of damaged or dead cells by phagocytosis (Bisson et al., 2003).

For normal development, embryogenesis requires the proper coordination of cell proliferation and differentiation, both of which require the *Xenopus* Tumorhead gene. Tumorhead over expression results in expansion of the neural plate at the neurula stage and abnormal growth in the head region during the tailbud and tadpole stages. Tumorhead overexpression also causes apoptosis during gastrulation. Tumorhead's functions are regulated by its subcellular localization. It translocates from the cell periphery into the nucleus during *Xenopus* development. Ectopic overexpression of Tumorhead mutant localizes to the plasma membrane; this results in neural plate expansion and inhibition of neuronal differentiation, which leads to embryonic lethality. xPAK1 mediates the phosphorylation of Tumorhead and enhances its binding to the apical cortex and lateral cell membrane of neural plate epithelial cells, resulting in neural plate expansion and inhibition of neuronal differentiation; organisms without xPAK1 activity show loss of the Tumorhead phenotype (Wu et al., 2007).

Embryogenesis involves the migration of embryonic cells to form new structures via a complex interplay of intercellular signaling and cytoskeletal remodeling processes. The large Eph tyrosine kinase receptor family and their ephrin ligands play key roles in regulating cell migration and cell adhesion. *Xenopus* embryos express several Eph receptors and ephrin ligands. Activation of EphA4 in early *Xenopus* embryos induces a loss of blastomere adhesion, leading to occlusion of the blastocoel (Bisson et al., 2007). xPak1 has been identified as an

upstream activator of EphA4 receptor, which is required for loss of adhesion. Ectopic expression of xPAK1 leads to the loss of blastomere adhesion, disruption of the blastocoel and blastocoel roof, and disassembly of cortical actin, all characteristic of EphA4 activation. Interestingly, both the xPAK1- and EphA4-induced phenotypes can be rescued by enhanced C-cadherin expression, by transfer to a low-salt medium, or by the constitutively activated GTPases Cdc42, Rac, and Rho (Bisson, Poitras, Mikryukov, Tremblay, and Moss, 2007). In addition to a role in embryonic cell migration, xPAK1 also play a role in the differentiation of the mechano-sensors of the auditory system (Islam et al., 2000).

Another member of *Xenopus* PAK family, xPAK3, is expressed in the area of primary neurogenesis in the developing embryo. In *Xenopus*, neuronal differentiation is governed by a basic helix-loop-helix transcription factor called neurogenin-related-1. Neurogenin-related-1 activates a set of genes that regulate cell differentiation. xPAK3 functions downstream of neurogenin to allow differentiation of neuronally programmed cells (Souopgui et al., 2002).

Like xPAK1, xPAK5 regulates cellular movement during early embryonic development in *Xenopus*. It is mainly expressed in regions of the embryo that undergo extensive cell movement during gastrula. xPAK5 can bind to both actin and microtubule networks and can regulate their coordinated dynamics during cell motility. Endogenous xPAK5 co-localizes with adherens junction proteins, and its activity is regulated by extracellular calcium (Faure et al., 2005). xPAK5 also cooperates with Inca (induced in neural crest by AP2) protein, which is upregulated in *Xenopus* embryos in cytoskeletal restructuring and cell adhesion regulation during early embryo development (Luo et al., 2007).

ZEBRA FISH

Zebra fish express only four PAK genes: PAK1, PAK2a, PAK2b, and PAK7. The physiologic functions of these PAK family members were not known until recently, when PAK2 was found to have a role in vascular development (Liu et al., 2007).

HUMANS

In human, six members of Pak have been identified which are classified in two groups each comprising of three members. Group I consists of Pak1-3 and group II consists of Pak4-6 (Arias-Romero et al., 2008). The classification is based on the domain structure and regulator mechanisms of the Paks. Similar to lower eukaryotes, all human Pak isoforms contain an N-terminal regulatory domain and a highly conserved C-terminal kinase domain. The regulatory domain of group I Pak consists of a GTPase binding domain (PBD) and an autoinhibitory domain (AID). In its inactive state, the group I Paks exists in a dimeric conformation. But on activation, the autoinhibitory domain uncouples from the kinase domain enabling autophosphorylation of the Pak. Group I Paks can be activated in both GTPase dependent (involving Cdc42 and rac) and GTPase independent manner (involving sphingolipids or caspases). In contrast, the group II Paks exist as monomers in crystal structure and the kinase domain of the group II Paks are found to be constitutively active (Eswaran et al., 2007;Eswaran et al., 2008). PAK4-6 bind to activated Cdc42 and, to a lesser extent, to Rac, but the activity of these kinases is not appreciably enhanced upon binding to the GTPases. In fact, interaction with Cdc42 induces the translocation of group II PAKs to different cellular compartments. Binding to Cdc42 results in PAK4 translocation to the Golgi apparatus (Abo et al., 1998;Cotteret et al., 2003;Cotteret et al., 2006). Thus, unlike group I PAKs, the interaction of group II PAKs with GTPases has no influence on kinase activity.

Paks are implicated in a variety of processes including cytoskeletal dynamics, motility, cell cycle, apoptosis and tumorigenesis. The Group I Paks regulate cytoskeleton dynamics by phosphorylating multiple downstream effectors including myosin light-chain kinase (MLCK),

paxillin, filamin A, cortactin, the PIX/COOL guanine nucleotide exchange factors, the LIM-kinases, Arpc1b, stathmin, tubulin cofactor B, paxillin-α and -β isoforms (Kumar et al., 2006). The nuclear translocation of PAK in response to stimulus has been associated with PAK-dependent gene transcription and translation. Some of the transcription factors and transcriptional co-regulators identified so far, includes the forkhead transcription factor (FKHR), estrogen receptor α (ERα), SHARP, C-terminal binding protein 1 (CTBP1), and Snail homologue 1 (SNAI1). Pak, a significant player of cell cycle progression undergo mitotic phosphorylation at Thr-212 by cyclin B1/Cdc2 which alters its association with histone H3 and promotes chromosome condensation and cell division. PAK1 also regulates centrosome dynamics through regulation of Aurora A activation. Recently, Pak mediated phosphorylation of polo like kinase 1 (Plk1) on Ser 49 is identified to be required for metaphase-associated events. In addition to cell cycle, the group I Paks plays an important role in cell survival and apoptosis. Pak1 function as cell survival signal by phosphorylating and inhibiting the pro-apoptotic protein Bad. In addition, PAK1 interacts with dynein light chain 1 (DLC1) and BimL and triggers their degradation, leading to blockage of the pro-apoptotic signal of BimL. PAK1 also inhibits apoptosis by phosphorylating and inactivating FKHR and by activating cell survival signals such as mitogen-activated protein kinases (p42/44 MAPK, p38 MAPK), Jun N-terminal kinase (JNK), and nuclear factor κB (NFκB). Unlike PAK1, PAK2 regulates both cell survival and cell death pathways, depending on the signals. In response to cellular stress, PAK2 generates a proteolytic fragment, the PAK-2p34. Activation of full-length PAK2 promotes cell survival by phosphorylating Bad and inhibiting its interaction with Bcl-2 or Bcl-x(L), thus promoting cell survival. In contrast, the proteolytic activation of PAK-2p34 leads to apoptosis (Kumar, Gururaj, and Barnes, 2006). Although, it is hypothesized that the functions of group I Paks are redundant but the members of group I Paks are involved in different biological processes as indicated by the respective knockout mice (Arias-Romero and Chernoff, 2008).

The group II Paks are recently identified members of Pak family of kinases. Although the mechanism of activation of group II Paks differs from group I Paks, they share functional similarities. Activation of PAK4 in fibroblasts leads to filopodia formation, dissolution of stress fibers, and loss of focal adhesions (Dan et al., 2001a;Callow et al., 2005). Of all the Pak family members, only PAK4 show a direct role in oncogenic transformation in primary fibroblasts (Callow et al., 2002;Cammarano et al., 2005). Similar to Pak1, PAK4 too protect cells against apoptosis by phosphorylating Bad and by antagonizing the death receptor induced activation of initiator caspase 8 (Gnesutta et al., 2001;Gnesutta et al., 2003). The second member of the group II Pak family - Pak5 express exclusively in brain and plays a crucial role in neuronal development and spine morphogenesis (Dan et al., 2002). Depending on the sub-cellular localization of PAK5, it interacts with different effectors. In the cytosol, PAK5 activates the JNK kinase pathway, while in the mitochondria promotes cell survival signals (Cotteret, Jaffer, Beeser, and Chernoff, 2003;Cotteret and Chernoff, 2006). The third member of the group II Paks- Pak6, play a crucial role in steroid-hormone-mediated signal transduction. PAK6 interaction with ERα and represses ERα transcriptional activity. Similarly, interaction of PAK6 to androgen receptor (AR) inhibits its transactivation function (Lee et al., 2002).

CONCLUDING REMARKS

The structure and functions of PAKs have been conserved across all eukaryotes, but mammalian PAKs are still not completely understood and continue to offer new insights and surprises. Genetic manipulation of *Xenopus* and yeast has increased our understanding of PAK signaling tremendously, but these organisms are different from mammalian PAKs in certain aspects, such as the constitutive activation of group II PAKs. With the availability of molecular tools and much needed reagents, it is expected that a combination of lower eukaryotes,

mammalian cell cultures, and knockout mice will help us understand the functional complexity of PAK enzymes.

ACKNOWLEDGMENT

AK is from MD Anderson Cancer Center, Houston, Tx 77030 and PRM is from Rutgers University, New Jersey.

Reference List

1. Abo A, Qu J, Cammarano MS, Dan C, Fritsch A, Baud V, Belisle B, Minden A. PAK4, a novel effector for Cdc42Hs, is implicated in the reorganization of the actin cytoskeleton and in the formation of filopodia. EMBO J 1998;17:6527–6540. [PubMed: 9822598]
2. Abrieu A, Brassac T, Galas S, Fisher D, Labbe JC, Doree M. The Polo-like kinase Plx1 is a component of the MPF amplification loop at the G2/M-phase transition of the cell cycle in Xenopus eggs. J.Cell Sci 1998;111(Pt 12):1751–1757. [PubMed: 9601104]
3. Arias-Romero LE, Chernoff J. A tale of two Paks. Biol.Cell 2008;100:97–108. [PubMed: 18199048]
4. Arias-Romero LE, Jesus Almaraz-Barrera M, Diaz-Valencia JD, Rojo-Dominguez A, Hernandez-Rivas R, Vargas M. EhPAK2, a novel p21-activated kinase, is required for collagen invasion and capping in Entamoeba histolytica. Mol.Biochem.Parasitol 2006;149:17–26. [PubMed: 16716419]
5. Bao S, Qyang Y, Yang P, Kim H, Du H, Bartholomeusz G, Henkel J, Pimental R, Verde F, Marcus S. The highly conserved protein methyltransferase, Skb1, is a mediator of hyperosmotic stress response in the fission yeast Schizosaccharomyces pombe. J.Biol.Chem 2001;276:14549–14552. [PubMed: 11278267]
6. Benton BK, Tinkelenberg A, Gonzalez I, Cross FR. Cla4p, a Saccharomyces cerevisiae Cdc42p-activated kinase involved in cytokinesis, is activated at mitosis. Mol.Cell Biol 1997;17:5067–5076. [PubMed: 9271384]
7. Bhattacharjya S, Gingras R, Xu P. An NMR-based identification of a peptide fragment from the beta-subunit of a G-protein showing specific interactions with the GBB domain of the Ste20 kinase in budding yeast. Biochem.Biophys.Res.Commun 2006;347:1145–1150. [PubMed: 16870141]
8. Bisson N, Islam N, Poitras L, Jean S, Bresnick A, Moss T. The catalytic domain of xPAK1 is sufficient to induce myosin II dependent in vivo cell fragmentation independently of other apoptotic events. Dev.Biol 2003;263:264–281. [PubMed: 14597201]
9. Bisson N, Poitras L, Mikryukov A, Tremblay M, Moss T. EphA4 signaling regulates blastomere adhesion in the Xenopus embryo by recruiting Pak1 to suppress Cdc42 function. Mol.Biol.Cell 2007;18:1030–1043. [PubMed: 17215521]
10. Bokoch GM. Biology of the p21-activated kinases. Annu.Rev.Biochem 2003;72:743–781. [PubMed: 12676796]
11. Callow MG, Clairvoyant F, Zhu S, Schryver B, Whyte DB, Bischoff JR, Jallal B, Smeal T. Requirement for PAK4 in the anchorage-independent growth of human cancer cell lines. J.Biol.Chem 2002;277:550–558. [PubMed: 11668177]
12. Callow MG, Zozulya S, Gishizky ML, Jallal B, Smeal T. PAK4 mediates morphological changes through the regulation of GEF-H1. J.Cell Sci 2005;118:1861–1872. [PubMed: 15827085]
13. Cammarano MS, Nekrasova T, Noel B, Minden A. Pak4 induces premature senescence via a pathway requiring p16INK4/p19ARF and mitogen-activated protein kinase signaling. Mol.Cell Biol 2005;25:9532–9542. [PubMed: 16227603]
14. Cau J, Faure S, Vigneron S, Labbe JC, Delsert C, Morin N. Regulation of Xenopus p21-activated kinase (X-PAK2) by Cdc42 and maturation-promoting factor controls Xenopus oocyte maturation. J.Biol.Chem 2000;275:2367–2375. [PubMed: 10644687]
15. Chang E, Bartholomeusz G, Pimental R, Chen J, Lai H, Wang L, Yang P, Marcus S. Direct binding and In vivo regulation of the fission yeast p21-activated kinase shk1 by the SH3 domain protein scd2. Mol.Cell Biol 1999;19:8066–8074. [PubMed: 10567532]
16. Chen W, Chen S, Yap SF, Lim L. The Caenorhabditis elegans p21-activated kinase (CePAK) colocalizes with CeRac1 and CDC42Ce at hypodermal cell boundaries during embryo elongation. J.Biol.Chem 1996;271:26362–26368. [PubMed: 8824291]

17. Cotteret S, Chernoff J. Nucleocytoplasmic shuttling of Pak5 regulates its antiapoptotic properties. Mol.Cell Biol 2006;26:3215–3230. [PubMed: 16581795]
18. Cotteret S, Jaffer ZM, Beeser A, Chernoff J. p21-Activated kinase 5 (Pak5) localizes to mitochondria and inhibits apoptosis by phosphorylating BAD. Mol.Cell Biol 2003;23:5526–5539. [PubMed: 12897128]
19. Dan C, Kelly A, Bernard O, Minden A. Cytoskeletal changes regulated by the PAK4 serine/threonine kinase are mediated by LIM kinase 1 and cofilin. J.Biol.Chem 2001a;276:32115–32121. [PubMed: 11413130]
20. Dan C, Nath N, Liberto M, Minden A. PAK5, a new brain-specific kinase, promotes neurite outgrowth in N1E-115 cells. Mol.Cell Biol 2002;22:567–577. [PubMed: 11756552]
21. Dan I, Watanabe NM, Kusumi A. The Ste20 group kinases as regulators of MAP kinase cascades. Trends Cell Biol 2001b;11:220–230. [PubMed: 11316611]
22. de la RM, Mahasneh A, Lee SF, Rivero F, Cote GP. Cellular distribution and functions of wild-type and constitutively activated Dictyostelium PakB. Mol.Biol.Cell 2005;16:238–247. [PubMed: 15509655]
23. de Nadal E, Alepuz PM, Posas F. Dealing with osmostress through MAP kinase activation. EMBO Rep 2002;3:735–740. [PubMed: 12151331]
24. Dutta S, Sardar A, Ray D, Raha S. Molecular and functional characterization of EhPAK3, a p21 activated kinase from Entamoeba histolytica. Gene 2007;402:57–67. [PubMed: 17761392]
25. Eswaran J, Lee WH, Debreczeni JE, Filippakopoulos P, Turnbull A, Fedorov O, Deacon SW, Peterson JR, Knapp S. Crystal Structures of the p21-activated kinases PAK4, PAK5, and PAK6 reveal catalytic domain plasticity of active group II PAKs. Structure 2007;15:201–213. [PubMed: 17292838]
26. Eswaran J, Soundararajan M, Kumar R, Knapp S. UnPAKing the class differences among p21-activated kinases. Trends Biochem.Sci 2008;33:394–403. [PubMed: 18639460]
27. Faure S, Cau J, de Santa BP, Bigou S, Ge Q, Delsert C, Morin N. Xenopus p21-activated kinase 5 regulates blastomeres' adhesive properties during convergent extension movements. Dev.Biol 2005;277:472–492. [PubMed: 15617688]
28. Faure S, Vigneron S, Doree M, Morin N. A member of the Ste20/PAK family of protein kinases is involved in both arrest of Xenopus oocytes at G2/prophase of the first meiotic cell cycle and in prevention of apoptosis. EMBO J 1997;16:5550–5561. [PubMed: 9312014]
29. Gnesutta N, Minden A. Death receptor-induced activation of initiator caspase 8 is antagonized by serine/threonine kinase PAK4. Mol.Cell Biol 2003;23:7838–7848. [PubMed: 14560027]
30. Gnesutta N, Qu J, Minden A. The serine/threonine kinase PAK4 prevents caspase activation and protects cells from apoptosis. J.Biol.Chem 2001;276:14414–14419. [PubMed: 11278822]
31. Hammes SR. Steroids and oocyte maturation--a new look at an old story. Mol.Endocrinol 2004;18:769–775. [PubMed: 14630999]
32. Hoffmann I, Clarke PR, Marcote MJ, Karsenti E, Draetta G. Phosphorylation and activation of human cdc25-C by cdc2--cyclin B and its involvement in the self-amplification of MPF at mitosis. EMBO J 1993;12:53–63. [PubMed: 8428594]
33. Hofmann C, Shepelev M, Chernoff J. The genetics of Pak. J.Cell Sci 2004;117:4343–4354. [PubMed: 15331659]
34. Islam N, Poitras L, Moss T. The cytoskeletal effector xPAK1 is expressed during both ear and lateral line development in Xenopus. Int.J.Dev.Biol 2000;44:245–248. [PubMed: 10794083]
35. Jaffer ZM, Chernoff J. p21-activated kinases: three more join the Pak. Int.J.Biochem.Cell Biol 2002;34:713–717. [PubMed: 11950587]
36. Keniry ME, Kemp HA, Rivers DM, Sprague GF Jr. The identification of Pcl1-interacting proteins that genetically interact with Cla4 may indicate a link between G1 progression and mitotic exit. Genetics 2004;166:1177–1186. [PubMed: 15082539]
37. Kim H, Yang P, Catanuto P, Verde F, Lai H, Du H, Chang F, Marcus S. The kelch repeat protein, Tea1, is a potential substrate target of the p21-activated kinase, Shk1, in the fission yeast, Schizosaccharomyces pombe. J.Biol.Chem 2003;278:30074–30082. [PubMed: 12764130]
38. Kim HW, Yang P, Qyang Y, Lai H, Du H, Henkel JS, Kumar K, Bao S, Liu M, Marcus S. Genetic and molecular characterization of Skb15, a highly conserved inhibitor of the fission yeast PAK, Shk1. Mol.Cell 2001;7:1095–1101. [PubMed: 11389855]

39. Kumar R, Gururaj AE, Barnes CJ. p21-activated kinases in cancer. Nat.Rev.Cancer 2006;6:459–471. [PubMed: 16723992]
40. Labruyere E, Zimmer C, Galy V, Olivo-Marin JC, Guillen N. EhPAK, a member of the p21-activated kinase family, is involved in the control of Entamoeba histolytica migration and phagocytosis. J.Cell Sci 2003;116:61–71. [PubMed: 12456716]
41. Lee BN, Elion EA. The MAPKKK Ste11 regulates vegetative growth through a kinase cascade of shared signaling components. Proc.Natl.Acad.Sci.U.S.A 1999;96:12679–12684. [PubMed: 10535982]
42. Lee S, Rivero F, Park KC, Huang E, Funamoto S, Firtel RA. Dictyostelium PAKc is required for proper chemotaxis. Mol.Biol.Cell 2004;15:5456–5469. [PubMed: 15483055]
43. Lee SR, Ramos SM, Ko A, Masiello D, Swanson KD, Lu ML, Balk SP. AR and ER interaction with a p21-activated kinase (PAK6). Mol.Endocrinol 2002;16:85–99. [PubMed: 11773441]
44. Liu J, Fraser SD, Faloon PW, Rollins EL, Vom BJ, Starovic-Subota O, Laliberte AL, Chen JN, Serluca FC, Childs SJ. A betaPix Pak2a signaling pathway regulates cerebral vascular stability in zebrafish. Proc.Natl.Acad.Sci.U.S.A 2007;104:13990–13995. [PubMed: 17573532]
45. Lucanic M, Kiley M, Ashcroft N, L'etoile N, Cheng HJ. The Caenorhabditis elegans P21-activated kinases are differentially required for UNC-6/netrin-mediated commissural motor axon guidance. Development 2006;133:4549–4559. [PubMed: 17050621]
46. Luo T, Xu Y, Hoffman TL, Zhang T, Schilling T, Sargent TD. Inca: a novel p21-activated kinase-associated protein required for cranial neural crest development. Development 2007;134:1279–1289. [PubMed: 17314132]
47. Marcus S, Polverino A, Chang E, Robbins D, Cobb MH, Wigler MH. Shk1, a homolog of the Saccharomyces cerevisiae Ste20 and mammalian p65PAK protein kinases, is a component of a Ras/Cdc42 signaling module in the fission yeast Schizosaccharomyces pombe. Proc.Natl.Acad.Sci.U.S.A 1995;92:6180–6184. [PubMed: 7597098]
48. Margolis SS, Perry JA, Weitzel DH, Freel CD, Yoshida M, Haystead TA, Kornbluth S. A role for PP1 in the Cdc2/Cyclin B-mediated positive feedback activation of Cdc25. Mol.Biol.Cell 2006;17:1779–1789. [PubMed: 16467385]
49. Melzig J, Rein KH, Schafer U, Pfister H, Jackle H, Heisenberg M, Raabe T. A protein related to p21-activated kinase (PAK) that is involved in neurogenesis in the Drosophila adult central nervous system. Curr.Biol 1998;8:1223–1226. [PubMed: 9811608]
50. Menzel N, Schneeberger D, Raabe T. The Drosophila p21 activated kinase Mbt regulates the actin cytoskeleton and adherens junctions to control photoreceptor cell morphogenesis. Mech.Dev 2007;124:78–90. [PubMed: 17097274]
51. Muller-Taubenberger A, Bretschneider T, Faix J, Konzok A, Simmeth E, Weber I. Differential localization of the Dictyostelium kinase DPAKa during cytokinesis and cell migration. J.Muscle Res.Cell Motil 2002;23:751–763. [PubMed: 12952073]
52. Palmer A, Nebreda AR. The activation of MAP kinase and p34cdc2/cyclin B during the meiotic maturation of Xenopus oocytes. Prog.Cell Cycle Res 2000;4:131–143. [PubMed: 10740821]
53. Qyang Y, Yang P, Du H, Lai H, Kim H, Marcus S. The p21-activated kinase, Shk1, is required for proper regulation of microtubule dynamics in the fission yeast, Schizosaccharomyces pombe. Mol.Microbiol 2002;44:325–334. [PubMed: 11972773]
54. Rao Y. Dissecting Nck/Dock signaling pathways in Drosophila visual system. Int.J.Biol.Sci 2005;1:80–86. [PubMed: 15951852]
55. Sagot I, Rodal AA, Moseley J, Goode BL, Pellman D. An actin nucleation mechanism mediated by Bni1 and profilin. Nat.Cell Biol 2002;4:626–631. [PubMed: 12134165]
56. Schneeberger D, Raabe T. Mbt, a Drosophila PAK protein, combines with Cdc42 to regulate photoreceptor cell morphogenesis. Development 2003;130:427–437. [PubMed: 12490550]
57. Simanis V. Events at the end of mitosis in the budding and fission yeasts. J.Cell Sci 2003;116:4263–4275. [PubMed: 14514882]
58. Soulard A, Lechler T, Spiridonov V, Shevchenko A, Shevchenko A, Li R, Winsor B. Saccharomyces cerevisiae Bzz1p is implicated with type I myosins in actin patch polarization and is able to recruit actin-polymerizing machinery in vitro. Mol.Cell Biol 2002;22:7889–7906. [PubMed: 12391157]

59. Souopgui J, Solter M, Pieler T. XPak3 promotes cell cycle withdrawal during primary neurogenesis in Xenopus laevis. EMBO J 2002;21:6429–6439. [PubMed: 12456650]

60. Wells NJ, Watanabe N, Tokusumi T, Jiang W, Verdecia MA, Hunter T. The C-terminal domain of the Cdc2 inhibitory kinase Myt1 interacts with Cdc2 complexes and is required for inhibition of G (2)/M progression. J.Cell Sci 1999;112(Pt 19):3361–3371. [PubMed: 10504341]

61. Wu CF, Delsert C, Faure S, Traverso EE, Kloc M, Kuang J, Etkin LD, Morin N. Tumorhead distribution to cytoplasmic membrane of neural plate cells is positively regulated by Xenopus p21-activated kinase 1 (X-PAK1). Dev.Biol 2007;308:169–186. [PubMed: 17560976]

62. Yang J, Winkler K, Yoshida M, Kornbluth S. Maintenance of G2 arrest in the Xenopus oocyte: a role for 14-3-3-mediated inhibition of Cdc25 nuclear import. EMBO J 1999a;18:2174–2183. [PubMed: 10205171]

63. Yang P, Kansra S, Pimental RA, Gilbreth M, Marcus S. Cloning and characterization of shk2, a gene encoding a novel p21-activated protein kinase from fission yeast. J.Biol.Chem 1998;273:18481–18489. [PubMed: 9660817]

64. Yang P, Pimental R, Lai H, Marcus S. Direct activation of the fission yeast PAK Shk1 by the novel SH3 domain protein, Skb5. J.Biol.Chem 1999b;274:36052–36057. [PubMed: 10593886]

65. Yang P, Qyang Y, Bartholomeusz G, Zhou X, Marcus S. The novel Rho GTPase-activating protein family protein, Rga8, provides a potential link between Cdc42/p21-activated kinase and Rho signaling pathways in the fission yeast, Schizosaccharomyces pombe. J.Biol.Chem 2003;278:48821–48830. [PubMed: 14506270]

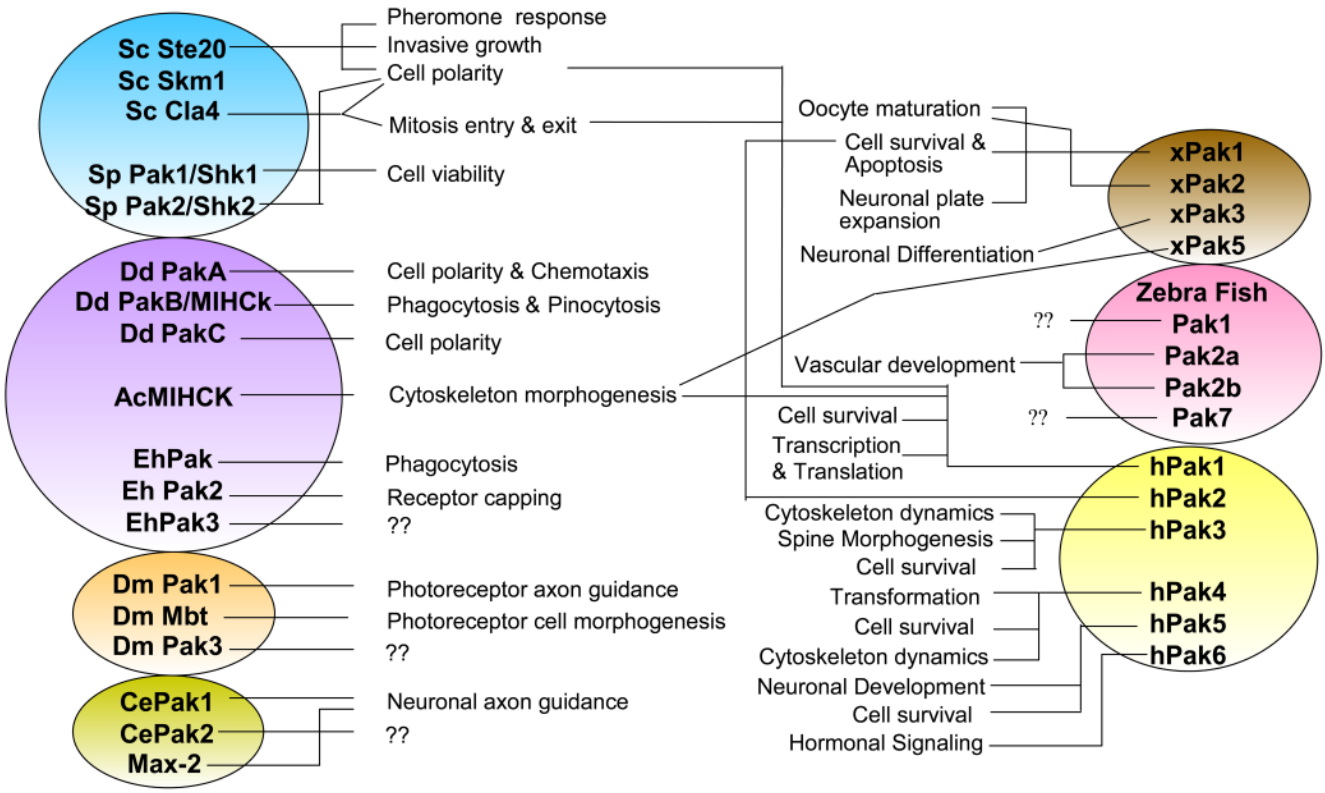

Figure 1. Pak mediated regulation of signaling cascades

p21 activated kinases (PAKs) play important role in a wide variety of cellular processes such as cytoskeleton dynamics, cell migration, survival, , apoptosis, gene transcription, neuronal development and hormone signaling. Although all Paks share structural similarities, some of the functions of Pak are unique to a given organism and a few functions are common across lower eukaryotes and mammals including regulation of cell morphology and cell survival.