

The genetics of Pak

Clemens Hofmann¹, Mikhail Shepelev² and Jonathan Chernoff^{1,*}

¹Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111, USA

²Russian State Medical University, 1 Ostrovitjanova Street, Moscow, 117997, Russia

*Author for correspondence (e-mail: j_chernoff@fccc.edu)

Journal of Cell Science 117, 4343-4354 Published by The Company of Biologists 2004
doi:10.1242/jcs.01392

Summary

p21-activated kinases (Paks) are a highly conserved family of enzymes that bind to and are activated by small GTPases of the Cdc42 and Rac families. With the notable exception of plants, nearly all eukaryotes encode one or more Pak genes, indicating an ancient origin and important function for this family of enzymes. Genetic approaches in many different experimental systems, ranging from yeast to mice, are beginning to decipher the different functions of Paks.

Although some of these functions are unique to a given organism, certain common themes have emerged, such as the activation of mitogen-activated protein kinase (MAPK) cascades and the regulation of cytoskeletal structure through effects on the actin and tubulin cytoskeletons.

Key words: Small GTPases, Protein kinases, Signal transduction

Introduction

Simple eukaryotes such as yeasts and molds encode multiple Paks that, like their orthologs in other systems, act downstream of Rho-family GTPases to regulate cytoskeletal structure and gene transcription. All Paks contain an N-terminal p21 GTPase-binding-domain (PBD), which confers binding to small GTPases such as Cdc42 or Rac, and a C-terminal protein kinase domain. In addition, some Paks from simple eukaryotes contain an N-terminal pleckstrin homology (PH) domain, a feature not found in Paks from more-complex organisms. In higher eukaryotes, the Pak family is divided into two subfamilies: group A and group B (Bokoch, 2003; Dan, I. et al., 2001; Jaffer and Chernoff, 2002) (Fig. 1A). Group A Paks are characterized by the presence of N-terminal proline-rich motifs that mediate association with various Src-homology 3 (SH3)-domain-containing proteins, a PBD, and a C-terminal kinase domain (Fig. 1B). Group A Paks bind both Cdc42 and Rac, and are strongly activated upon binding these GTPases. Group B Paks contain a PBD at the extreme N-terminus of the protein followed by a C-terminal kinase domain (Fig. 1B). The group B Paks bind Cdc42 and, to a lesser extent, Rac, but, unlike group A Paks, are not appreciably activated upon binding. Thus, the term 'p21-activated kinase' is not entirely apt in this case. Instead, the association with Cdc42 is thought to be more important for localization of the group B kinases rather than for their activation per se. Despite these differences, both groups share certain essential functions, such as the regulation of the actin cytoskeleton. However, they are not completely functionally equivalent. For example, mammalian group A Paks can replace the function of the budding yeast Pak Ste20, whereas group B Paks cannot (Cotteret et al., 2003).

Here, we consider genetic analyses of Pak function in yeasts, amoebae, flies and mammals. Although multiple Paks are also present in the nematode *Caenorhabditis elegans*, which is another genetically tractable model system, very little is known of Pak function in this organism.

Budding yeast

For historical and technical reasons, genetic analysis of Pak function has proceeded further in *Saccharomyces cerevisiae* than in any other organism. Although some of the findings are unique to budding yeast, many of the basic signaling pathways in which yeast Paks have been found to participate are also shared in other organisms. *S. cerevisiae* encodes three Paks: Ste20, Cla4 and Skm1. Like all members of the Pak family, these three kinases contain an N-terminal PBD and a C-terminal protein kinase domain; however, Cla4 and Skm1 also contain a PH domain N-terminal to the PBD and, together with Pak2 from *Schizosaccharomyces pombe*, form a distinct subfamily (Fig. 1). As discussed below, these kinases affect cell morphology, polarity, cell-cycle and gene transcription events downstream of the small GTPase Cdc42 and the cell-cycle-dependent kinase Cdc28 (Etienne-Manneville, 2004).

Paks and MAPKs

The founding member of the Pak family, Ste20, was uncovered in a genetic screen for suppressors of the mating defects associated with expression of a dominant-negative form of Ste4, a mating-pheromone-receptor-associated G β subunit. *ste20* cells fail to arrest in G1 phase following pheromone stimulation, fail to form mating projections, and fail to activate transcription of key mating factors, such as Fus1 (Leberer et al., 1992; Ramer and Davis, 1993). In the pheromone mating pathway, Ste20 functions as an activator of mitogen-activated protein kinase (MAPK) cascades, a function that appears to be broadly conserved throughout evolution (Dan, I. et al., 2001).

In budding yeast, the mating MAPK cascade comprises a MAPK (Kss1 or Fus3), a MAPK kinase (MAPKK; Ste7) and a MAPK kinase kinase (MAPKKK; Ste11). Activation of this pathway through the mating pheromone receptor results in changes in transcription and in cytoskeletal structure that are required for mating (Elion, 2000). Epistasis and biochemical experiments have shown that Ste20 operates downstream of

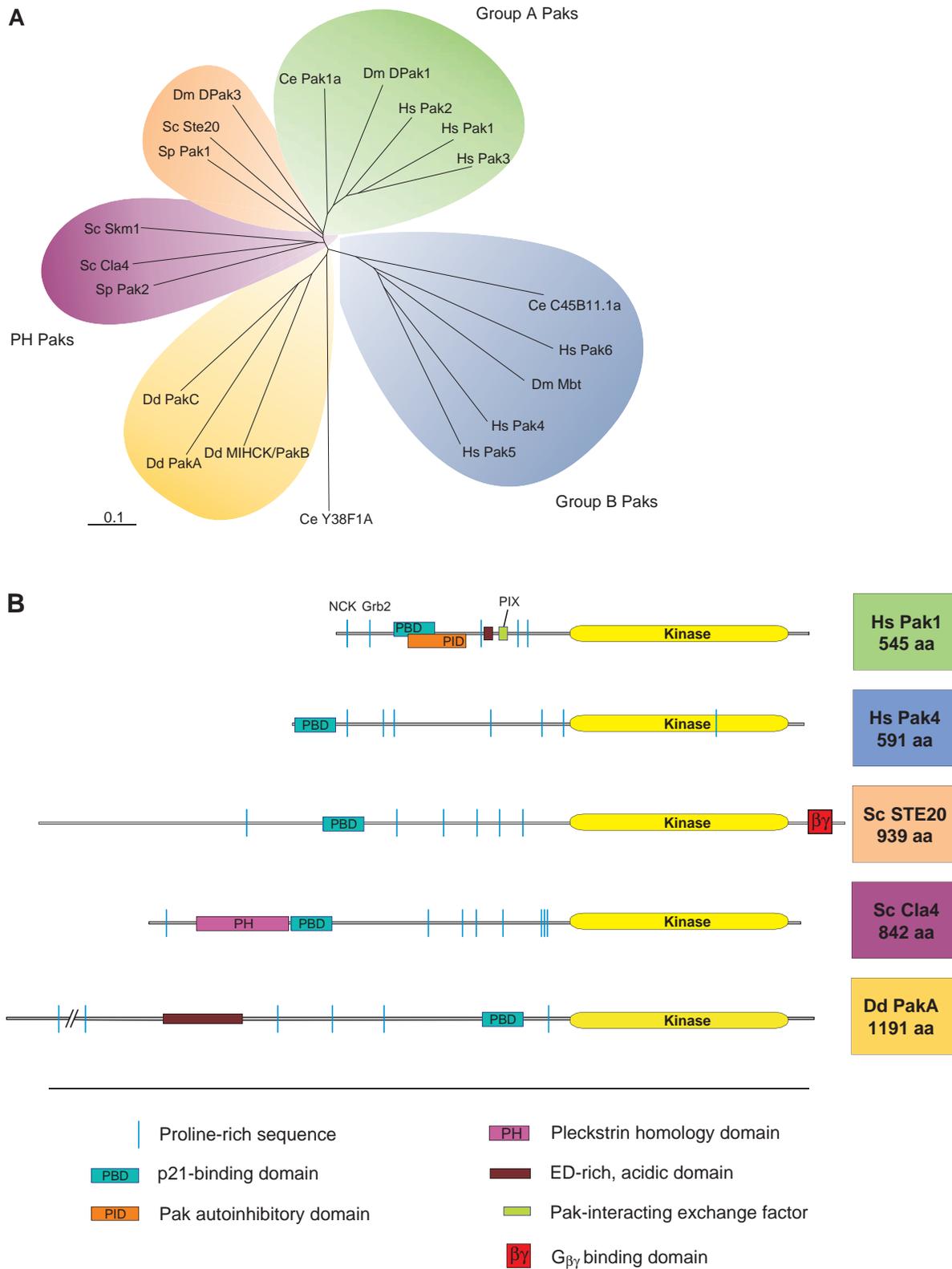


Fig. 1. Phylogenetic tree of the Pak family. (A) Pak subfamilies. In worms, flies and mammals, Paks fall into two structurally and functionally distinct groups, here termed A (green) and B (blue). Both budding and fission yeast contain an evolutionarily related group of Paks that contain a PH domain at the N-terminus (PH Paks; purple). The three Paks from slime mold form a separate group (yellow), as do the remaining Paks from budding and fission yeast (orange). Ce, *Caenorhabditis elegans*; Ds, *Dictyostelium discoideum*; Hs, *Homo sapiens*; Mm, *Mus musculus*; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*. (B) Domain structure of Paks. One representative of each Pak subfamily is shown.

Cdc42 and Ste4 near the top of the mating MAPK cascade (Fig. 2A). Ste20 phosphorylates the MAPKKK Ste11 on residues Ser302 and/or Ser306 and Thr307, and alanine substitutions at these positions abolish Ste11 function (Drogen et al., 2000; Wu et al., 1996). Thus, in this system, Ste20 appears to act as a classic MAP kinase kinase kinase (MAPKKK). As will be discussed, although it is common to extrapolate these findings to other systems, the connections between Paks and elements of the MAPK cascade in other organisms are often more complex than in budding yeast.

Ste20 also functions as an upstream activator of MAPK pathways that regulate invasive growth and the osmotolerance response. As in the pheromone pathway, the main target of Ste20 in both the invasive and the osmotolerance response

pathways is thought to be Ste11 (de Nadal et al., 2002; O'Rourke et al., 2002; Posas et al., 1998). Expression of a dominant-negative form of Cdc42, or of a Ste20 mutant that cannot bind to Cdc42, impairs signaling through the invasive growth pathway, indicating that interaction with Cdc42 is required for proper activation of Ste20 in this pathway (Mosch et al., 1996) (Fig. 2A). Cdc42 also plays an important, albeit ill-defined, role in regulating Ste20 in the osmotolerance response pathway (Raitt et al., 2000). Finally, Ste20 and components of the mating, invasive growth and osmotolerance pathways also participate in a vegetative growth pathway that is implicated in the control of cell wall integrity (Cullen et al., 2000; Elion, 2000; Lee and Elion, 1999).

Ste20 is controlled by specific regulators in the invasive

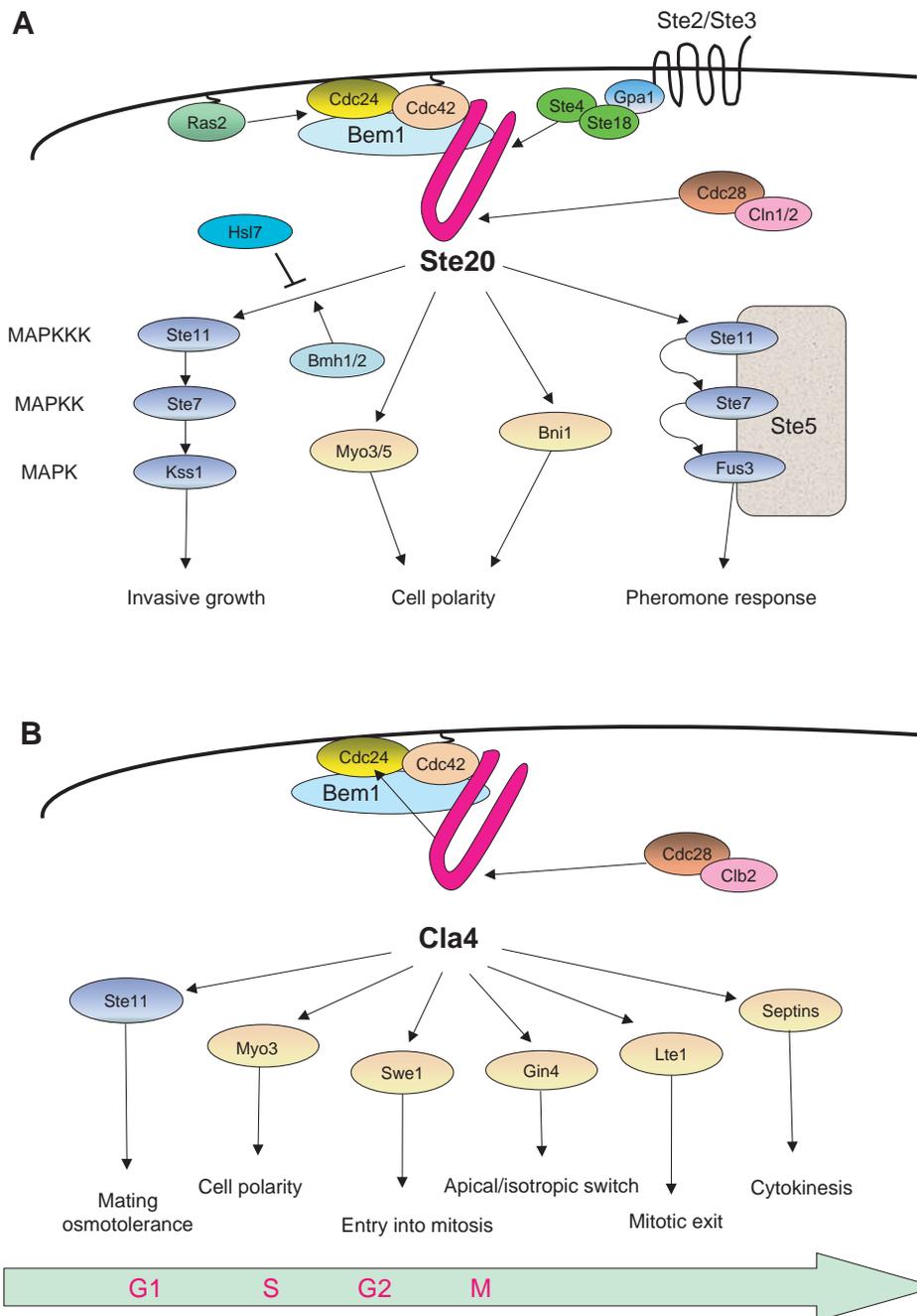


Fig. 2. Pak function in budding yeast. (A) Ste20 function. Ste20 is an upstream signaling element in the invasive growth, mating, osmotolerance (not shown) and vegetative growth/cell wall integrity (not shown) MAPK pathways, composed of Ste11, Ste7 and either Kss1 or Fus3. The mating MAPK components are stabilized and insulated by the adaptor protein Ste5. In this pathway, Ste20 is activated by the pheromone receptors Ste2/Ste3 through Ste4, the G β subunit of the heterotrimeric G protein, composed in addition of Gpa1 (G α subunit) and Ste18 (G γ subunit). The proteins Bmh1/Bmh2 and Hs17 act as positive and negative regulators, respectively, of Ste20 function in the invasive growth pathway. Ste20 associates with its activator Cdc42 as well as the scaffold protein Bem1, which helps anchor Ste20 to a multiprotein signaling complex. Ste20 is regulated in a cell-cycle-dependent fashion by Cdc28-Cln1, and acts upon Myo3 and Bni1 to regulate the actin cytoskeleton during polarized growth. Ras2 is an upstream activator of the invasive growth pathway and acts through Cdc24, a guanine-nucleotide-exchange-factor (GEF) for Cdc42. (B) Cla4 function. Like Ste20, Cla4 is part of a multicomponent complex and can activate MAPK pathways, probably by phosphorylating Ste11. Cla4 has unique functions in mitotic exit and in cytokinesis. As detailed in the text, Cla4 is subject to cell-cycle-dependent activation by Cdc28-Clb2, and is itself a regulator of the cell cycle, phosphorylating Swe1, Lte1, septins and Myo3.

growth signaling pathway. Bmh1 and Bmh2, yeast orthologs of mammalian 14-3-3 adaptor proteins, associate with Ste20 in vivo and are required for invasive growth (Roberts et al., 1997). The methyltransferase Hsl7 negatively regulates Ste20 function in this pathway. Hsl7 binds to the N-terminal region of Ste20, possibly competing with Cdc42 (Fujita et al., 1999). Interestingly, Skb1, a fission yeast homolog of Hsl7, has been shown to have the opposite effect: it positively modulates Pak1 function and binds to a site on Pak1 that is different from that which binds Cdc42 (Gilbreth et al., 1996).

Ste20, Cla4 and cell polarity

Ste20, together with the related protein Cla4, is also required for many of the polarity changes that occur during the vegetative growth cycle, including bud emergence (late G1 phase) and polarized growth of budded cells (G2/M phase), as well as for cell division. The cell-cycle effects are independent of the MAPK pathway, which indicates that Ste20 has one or more additional substrates that are required for cell polarization (Eby et al., 1998; Holly and Blumer, 1999).

During bud emergence, Ste20 is phosphorylated by the CDK-cyclin complex Cdc28-Cln2, and this appears to promote the vegetative morphogenetic functions of Ste20 (Oehlen and Cross, 1998; Wu et al., 1998). Loss of Pak function in late G1 phase (through inactivation of Cla4 in a *ste20* strain) leads to a complete loss of polarization and prevents bud emergence (Holly and Blumer, 1999). One of the relevant Pak targets in this process might be Bem1, because this protein is involved in bud emergence, localizes to sites of polarized growth in G1 phase, co-immunoprecipitates with Ste20, and is phosphorylated in vivo (Chenevert et al., 1992; Leeuw et al., 1995). Myosins such as Myo3 and Myo5 might also be involved, as these proteins are required for budding and are phosphorylated in vitro by Ste20 and Cla4 at sites known to be required for function in vivo (Wu et al., 1996; Wu et al., 1997).

The switch from apical to isotropic bud growth (G2/M phase) is regulated by Cdc28-Cln2. Cla4 is hyperphosphorylated during mitosis in a Cdc28-dependent fashion (Tjandra et al., 1998), and again loss of Pak function during this stage (by inactivation of Cla4 in a *ste20* strain) leads to a complete loss of polarization (Holly and Blumer, 1999). These effects might be in part mediated through the Nim1-related kinase Gin4, which is dependent on Cla4 for activation and regulates septin dynamics, although no direct activation of Gin4 by Cla4 has been reported (Benton et al., 1997; Tjandra et al., 1998), as described below. The formin-homology protein Bni1 represents another likely target for Ste20 in this process. *bni1* is synthetically lethal in a *cla4* background (i.e. in cells that are dependent on Ste20 function for viability), and much of the phosphorylation on Bni1 is dependent on Ste20 (Goehring et al., 2003). Bni1 is a component of the 'polarisome' and many other components of this pathway are also synthetically lethal when mutations in them are combined with *cla4* (Goehring et al., 2003). These results suggest that one function of Ste20 might be to activate the polarisome complex by phosphorylating Bni1.

Paks also participate in a feedback system to signal the end phase of polarized growth. Until the time of bud emergence, Cdc24, which is the activator of Cdc42, resides in the nucleus in a complex with the adaptor protein Far1 (Shimada et al.,

2000; Toenjes et al., 1999). Concomitantly with bud emergence, Cdc28 phosphorylates Far1, inducing its ubiquitin-dependent degradation and promoting the release of Cdc24 from the complex. Cdc24 then translocates to the incipient bud site where, together with the adaptor protein Bem1, it activates Cdc42. Once activated, Cdc42 is able to interact with its effectors, including Cla4. Cla4 phosphorylates Cdc24, inducing its dissociation from the adaptor protein Bem1, thus providing a negative-feedback loop to inactivate Cdc42 and to end the phase of polarized growth. The role in the regulation of Cdc24 seems to be specific for Cla4, because Cdc24 phosphorylation occurs in cells lacking *STE20* (Bose et al., 2001; Gulli et al., 2000).

Cla4 and regulation of cytokinesis

Cla4 was initially identified in a screen for mutants that are not viable in the absence of the G1 cyclins Cln1 and Cln2. In this genetic background, cells carrying a mutation in the *CLA4* gene show defects in cytokinesis: cells bud and their nuclei divide but cytokinesis does not occur. When Cln1 and Cln2 are present, Cla4 is dispensable for viability, although haploid cells still show aberrant cytokinesis. As noted above, viability is lost in *cla4 ste20* double mutants, which suggests that these two kinases share at least one function critical for vegetative growth (Cvrckova et al., 1995).

Many of the cytokinetic defects observed in *cla4* cells are related to abnormal septin ring assembly. Septins are GTPases that, in normal cytokinesis, form a filamentous ring around the mother bud neck. Morphological studies have shown that the septin ring is severely mislocalized in *cla4* cells (Cvrckova et al., 1995) and that deletion of *CLA4* in strains expressing different combinations of GTP-binding-deficient septins aggravate the aberrant morphology of these cells and, under some growth conditions, lead to lethal effects. Complementation studies have shown that expression of Cla4, but not Ste20 or Skm1, is able to rescue the morphological aberrations and cytokinesis defects of cells expressing GTP-binding deficient septins, suggesting that septins might be a direct and unique target of Cla4. In support of this model, Cla4 phosphorylates at least two septins in vitro (Cdc3 and Cdc10), and phosphorylation of these septins in vivo is largely dependent on Cla4 kinase activity (Versele and Thorner, 2004). Septins, possibly as a result of phosphorylation, are immobilized within the bud neck during S, G2 and M phases, and this immobilization is dependent on both Cla4 and Gin4 (Dobbelaere et al., 2003; Mortensen et al., 2002).

Cla4 also plays at least two roles in the regulation of mitosis linked to its effects on bud neck formation. First, Cla4 is involved in the destruction of the cell-cycle inhibitor Swe1. Swe1 is recruited to the bud neck and is hyperphosphorylated prior to its ubiquitin-mediated degradation (Lew, 2000). Chirolini et al. have shown that cells expressing a dominant-negative form of Cla4 show a delay in the onset of anaphase and this delay requires Swe1 (Chirolini et al., 2003). Sakchaisri et al. have shown that Cla4, along with the Polo kinase Cdc5, phosphorylates and downregulates Swe1, which allows mitosis to proceed (Sakchaisri et al., 2004). Consistent with these data is the observation that Cla4 activity peaks near mitosis and drops as cells complete cytokinesis and enter G1 phase (Benton et al., 1997). Second, Cla4 is required for the phosphorylation

of the guanine-nucleotide-exchange factor (GEF) Lte1. Lte1 is an activator of the GTPase Tem1 which, following inactivation of the Cdc28-Clb complex, promotes the release of protein phosphatase Cdc14 from its inhibitor in the nucleus. Active Cdc14 then reverses phosphorylation of CDK substrates, promoting exit from mitosis (Simanis, 2003). Accordingly, cells lacking Cla4 are severely delayed in telophase, which is consistent with the idea that Cla4 plays a key role in this process (Seshan et al., 2002).

Interestingly, both the PBD and the PH domains of Cla4 appear to be essential for its localization to sites of polarized growth and for execution of mitotic exit. Since Cla4 must retain the ability to bind both Cdc42 and phosphoinositides, Wild et al. have termed it a 'coincidence detector', responding only when two activation signals are both present (Wild et al., 2004). Whether other PH-domain-containing Paks, such as *S. cerevisiae* Skm1 or *S. pombe* Pak2, are also coincidence detectors remains to be determined.

Skm1

Like Cla4, Skm1 contains an N-terminal PH domain, in addition to the PBD and protein kinase domain (Fig. 1B). Disruption of *SKM1* causes no obvious phenotype, even in cells grown under stress stimuli; thus, the protein encoded by this gene is not critical for vegetative growth and morphogenesis. Complementation experiments have shown that the function of *SKM1* is not redundant with that of *STE20* or *CLA4*, because disruption of either *STE20* or *CLA4* in a *skm1* background causes no distinguishable difference between single deletion of *STE20* and *CLA4* and the respective double knockouts. Moreover, overexpression of *SKM1* does not compensate for *STE20* or *CLA4* deletion. When artificially activated by truncation of the N-terminus, Skm1 is able to restore mating to *ste20* cells but does not suppress the morphogenetic defects associated with deletion of *CLA4*. These data suggest that the kinase domain of Skm1 can

potentially phosphorylate at least some of the same substrates as Ste20, but that, in the context of the full-length protein, is unable or unavailable to do so (Martin et al., 1997). Interestingly, the lethal effects of expressing activated Cdc42 in budding yeast can be suppressed by deletion of *CLA4* or *SKM1*, but not by deletion of *STE20* (Davis et al., 1998). These results suggest that, like the other two yeast Paks, Skm1 is a bona-fide effector of Cdc42, but its precise role remains for the moment obscure. Synthetic lethal analyses using *skm1* cells might be useful to clarify its physiological role.

Fission yeast

The fission yeast *S. pombe* encodes two Pak kinases – Pak1/Shk1 and Pak2/Shk2 – both of which, like their counterparts in budding yeast, have been implicated in the regulation of MAPK cascades and cytoskeletal dynamics (Fig. 3). Pak1 most closely resembles budding yeast Ste20, whereas Pak2 contains a PH domain and is most closely related to Cla4 and Skm1 (Fig. 1).

Pak1/Shk1

Disruption of the *PAK1* gene has shown that this kinase is essential for viability (Marcus et al., 1995; Otilie et al., 1995). Promoter-shutoff experiments suggest that this lethality is due to polarity defects, because *PAK1*-null spores germinate but arrest after several rounds of cell division as small spherical cells. This phenotype is similar to that observed in *CDC42*-null cells (Miller and Johnson, 1994) and suggests that Cdc42 and Pak1 function in the same signaling pathway. Deletion of *PAK1* or expression of kinase-dead Pak1 in fission yeast induces a spherical morphology and delocalization of actin patches, which is consistent with an inability to polarize growth towards the cell tips, and severely reduces mating efficiency. Similar defects have been noted in *orb2-32* strains of *S. pombe*, which bear a temperature-sensitive mutation in the *PAK1* gene. Cells

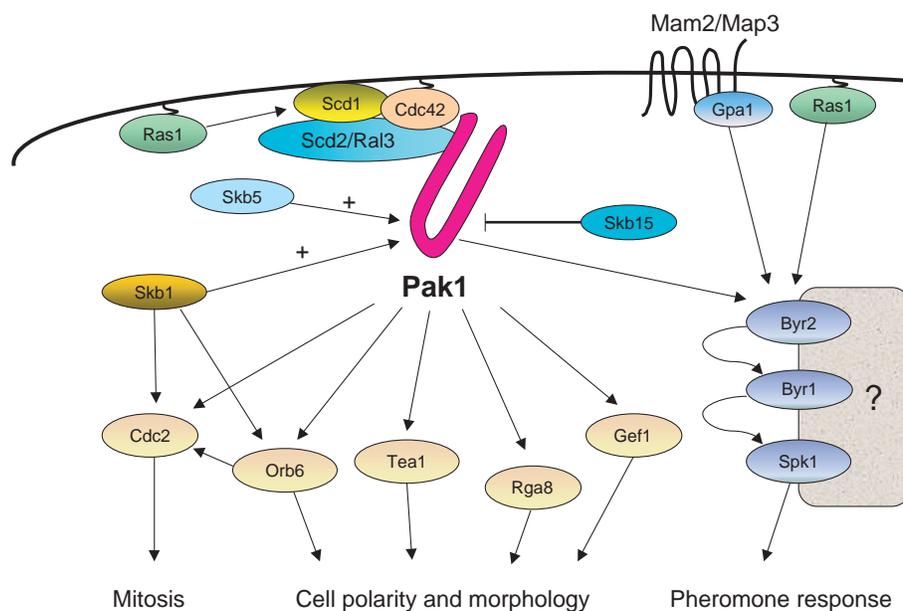


Fig. 3. Fission yeast Pak1. As in budding yeast, fission yeast Pak1 is part of a multicomponent complex, which contains the adaptor Scd2 (Bem1 ortholog), Scd1 (Cdc24 ortholog) and Cdc42. Pak1 is positively regulated by Skb1 and Skb5, and negatively by Skb15. Pak1 activates the mating cascade by physically interacting with Byr2 and affects cell polarity by phosphorylating Tea1 and the Rho-GAP Rga8. Pak1 also genetically interacts with Orb6 as well as with Gef1, a GEF for Cdc42. Byr2 is also regulated by Ras1 and Gpa1 (the G α subunit of the heterotrimeric G protein). Ras1 also signals through Scd1. The link between pheromone receptors Mam2/Map3 and Pak1 is not established yet; also it is not known whether the Byr2-Byr1-Spk1 MAPK cascade associates with a scaffold protein. Pak1 and Skb1 directly bind to Cdc2 and inhibit mitosis. Orb6 delays mitosis in a Cdc2-dependent manner (Verde et al., 1998) and is regulated by Skb1 to control cell polarity (Wiley et al., 2003).

carrying this allele can grow only in a monopolar fashion, because of an inability to recognize their ends as sites for growth (Sawin et al., 1999).

Trans-species complementation experiments showed that Ste20 is able to restore viability, mating and morphology to *PAK1*-null *S. pombe*; likewise, Pak1 is able to induce the mating pheromone response pathway in *S. cerevisiae* lacking *STE20* (Marcus et al., 1995; Otilie et al., 1995b). These observations indicate that, despite the wide evolutionary distance between fission and budding yeasts, these two kinases are functionally related.

Pak1 interacts with the SH3-domain-containing adaptor proteins Scd2/Ral3 and Skb5 (Chang et al., 1999; Yang et al., 1999), the protein methyltransferase Skb1 (a homolog of budding yeast Hs17 (Gilbreth et al., 1996) and the WD-repeat protein Skb15 (Kim et al., 2001) (Fig. 3). Scd2 acts as a scaffold and stimulates Pak1 activity by modulating the interaction between Pak1 and Cdc42 (Chang et al., 1999). Skb1 binds to and activates Pak1, and forms a complex with Pak1 and Cdc42 in vivo (Gilbreth et al., 1996). Together with Skb1, Pak1 directly associates with the cyclin-dependent kinase Cdc2 and negatively regulates mitosis (Gilbreth et al., 1998). By contrast, Skb15 acts as a negative regulator of Pak1. Inhibition of Pak1 activity by Skb15 is essential for the proper regulation of actin remodeling and cytokinetic function (Kim, H. et al., 2003). In addition to these connections, there are genetic interactions between *PAK1* and the genes encoding the mating-pathway kinase Byr2 (a MEKK ortholog) (Tu et al., 1997) and Orb6 (an ortholog of mammalian Rho-activated kinase, p160 ROCK) (Verde et al., 1998). Pak1 appears to act upstream of both Byr2 and Orb6, although neither of these latter proteins has been reported to be a direct substrate of Pak1. Interestingly, like Pak1, Orb6 inhibits mitosis in a Cdc2-dependent manner (Verde et al., 1998). Whether Pak1 and Orb6 constitute a Pak1-Orb6-Cdc2 pathway or act on Cdc2 in parallel remains to be established. In the case of Byr2, it is the physical interaction with Pak1, rather than phosphorylation, that relieves autoinhibition and promotes activation of the downstream MAPK cascade (Tu et al., 1997). Thus, the link between Pak1 and the MAPK mating cascade in fission yeast appears to operate on a different principle to that seen for Ste20 in budding yeast, involving functions of Pak that are independent of protein kinase activity.

Qyang and co-workers have established that Pak1 function is required for proper regulation of interphase and mitotic microtubule (MT) dynamics, and that Pak1 activity is reduced by the destabilization of MTs but returns to normal levels as MTs polymerize (Qyang et al., 2002). In this regard, it is interesting to note that mammalian Pak1 phosphorylates and inactivates the MT-associated protein Op18/Stathmin, which promotes destabilization of MTs (Wittmann et al., 2004). Thus, a role for Pak in the regulation of MT stability seems to be common in many organisms.

Pak1 is localized to the cell ends during interphase and to the septum-forming region during mitosis, and associates with mitotic spindle and interphase MTs. The Pak1 inhibitor Skb15 is also associated with mitotic spindles, which suggests this protein has a role in the regulation of Pak1 functions required for spindle/MT dynamics in fission yeast (Sawin et al., 1999). Pak1 is also connected to the cell polarity apparatus through the cell polarity factor Tea1, which is phosphorylated by Pak1

in vivo (Qyang et al., 2002). Phenotypes associated with Pak1 activation caused by the loss of Skb15 (e.g. defects in the actin cytoskeleton, chromosome segregation and cytokinesis) are suppressed by the loss of *TEA1*, which suggests that Tea1 is a mediator of Pak1 polarity functions (Kim, H. et al., 2003).

In fission yeast, Pak1 also regulates a potential regulator of small GTPases, the Rho-GAP Rga8. Pak1 phosphorylates Rga8, and this phosphorylation is required for the proper localization of Rga8, which, like Pak1, is found at cell ends and in the septum-forming region. Interestingly, despite compelling evidence that Rga8 acts as a GAP for Rho1 in cells, Rga8 does not appear to act as a negative regulator of Rho1 function. Instead, these two proteins have a positive functional interaction in *S. pombe* cells: gain of Rga8 function exacerbates phenotypes caused by gain of Rho1 function and vice versa, and both Rho1 and Rga8 antagonize Pak1 function (Yang et al., 2003). This linkage between a member of the Pak family and a regulator of Rho is not unique to fission yeast, because two groups have recently reported that both group A and group B mammalian Paks phosphorylate and thereby alter the localization and/or activity of additional Rho-GEFs (Barac et al., 2004; Zenke et al., 2004).

Pak2/Shk

Pak2, like Cla4 and Skm1 from budding yeast, contains an N-terminal PH domain (Sells et al., 1998). Unlike *PAK1*, the *PAK2* gene is not required for viability or fertility in fission yeast (Sells et al., 1998; Yang et al., 1998). Interestingly, high-level expression of *PAK2* partly suppresses the morphological defects of *PAK1*-null cells but does not restore mating competence. This partial complementation requires the PH domain, the PBD domain and a functional kinase domain. Overexpression of Pak2 also restores rod-shaped morphology to *RAS1*-deleted ovoid cells, indicating that Pak2 participates in the Ras1-dependent morphological pathway (Yang et al., 1998). Such a pathway might be analogous to the Ras/Ste20 pathway for haploinvasive growth in budding yeast discussed previously. In cross-species complementation assays, Sells et al. found that *PAK2* cannot rescue *cla4* morphological defects, *ste20* mating defects or *cla4/ste20* lethality in budding yeast. These observations suggest that, despite its structural resemblance to Cla4, Pak2 is not functionally equivalent to this kinase.

The signaling pathways downstream of Pak2 are not well defined. Overexpression of activated Pak2 leads to morphological defects (Sells et al., 1998) and these defects can be suppressed by loss of the stress-pathway kinases Mkh1 and Spm1 (Merla and Johnson, 2001). Because expression of activated Pak2 does not rescue the growth defects conferred by overexpression of Mkh1, these epistatic data place Pak2 upstream of Mkh1 in this pathway. Pak2, but not Pak1, interacts with Mkh1, which is consistent with a specific function for Pak2 in the Mkh1-Pek1-Spm1 pathway, which regulates cytokinesis and cell division in fission yeast (Merla and Johnson, 2001).

Dictyostelium

In *Dictyostelium*, three Pak genes have been identified, encoding myosin I heavy chain kinase (MIHCK/PakB), PakA

and PakC. The proteins encoded by the first two have been studied in some detail, whereas PakC appears only as a database entry. These three kinases are more closely related to one another than to Paks in other organisms (Fig. 1).

MIHCK

MIHCK is activated by Rac-GTP and also by acidic lipids such as phosphatidylserine, phosphatidylinositol and phosphatidylinositol 4,5-bisphosphate. As in the case of Ste20 in budding yeast, *Dictyostelium* MIHCK phosphorylates and regulates the activity of myosin. MIHCK might thus link small GTPase signaling pathways to motile processes requiring myosin I molecules (Brzeska et al., 1997; Lee et al., 1998). However, a direct test of this model (e.g. by *mihck* gene disruption) has not been reported.

PakA

Chung and Firtel reported that PakA colocalizes with myosin II to the cleavage furrow of dividing cells and to the posterior of polarized, chemotaxing cells (Chung and Firtel, 1999). Upon disruption of cell polarity and rounding up of the cells, PakA becomes uniformly distributed along the membrane cortex of the entire cell. Chung and Firtel found that *paka*-null cells fail to complete cytokinesis in suspension, appear more elongated, and have a less-polarized actin cytoskeleton than wild-type cells. They also observed that PakA is required for maintaining directional cell movement: in their studies, *paka*-null cells or wild-type cells expressing a kinase-dead PakA mutant produced many random, lateral pseudopodia and made wrong turns at a much higher frequency than wild-type cells (Chung and Firtel, 1999). In contrast to these findings, Müller-Taubenberger et al. have found no obvious abnormal phenotype in *paka*-null cells nor could they confirm the localization of PakA to the cleavage furrow of dividing cells (Müller-Taubenberger et al., 2002). It is possible that these discordant results are related to the different genetic strains of *Dictyostelium* used. It is also possible that overexpression of full-length PakA in the Müller-Taubenberger study affected cell morphology and cleavage furrow formation such that PakA became delocalized.

In the experiments of Chung and Firtel, the distribution of PakA and the phenotype of *paka*-null cells were similar to those described for myosin II. Normally, *Dictyostelium* cells increase the level of myosin II in the cytoskeleton in response to cAMP; this response is not observed in *paka*-null cells, which suggests that the assembly of myosin II into the cytoskeleton requires PakA function. Because PakA is activated by cAMP, PakA might thus control chemotaxis by regulating myosin II function. *paka*-null cells also cannot properly retract the rear end of the cell when chemotaxing and exhibit defective myosin II assembly: the myosin II cap in the posterior of chemotaxing cells and myosin II assembly into the cytoskeleton upon cAMP stimulation are both absent in these cells. By contrast, constitutively active PakA leads to an upregulation of myosin II assembly. Interestingly, these effects on myosin II assembly do not appear to result from direct phosphorylation of myosin II by PakA; rather, PakA might indirectly regulate myosin II assembly by negatively regulating myosin II heavy chain kinase (Chung and Firtel, 1999).

PakA is phosphorylated by protein kinase B (PKB/Akt) in vitro at Thr579 between the N-terminal proline-rich motif and the PBD (Chung et al., 2001). Mutation of this site to alanine blocks PakA activation and redistribution in response to chemoattractant stimulation. Conversely, mutation of the same site to the phosphomimic aspartic acid increases the basal level of association with the cytoskeleton (Chung et al., 2001). In cells lacking PKB or phosphoinositide 3-kinase (PI 3-K), PakA does not significantly incorporate into the cytoskeletal fraction upon cAMP stimulation. This suggests that PKB/Akt and PI 3-K regulate cell polarity and chemotaxis at least in part through control of PakA activation and localization.

Drosophila

Drosophila encodes three Paks: one group A (DPak1), one group B (Mbt) and a third that does not fit easily into either classification (DPak3, accession number NP_650545). The functions of DPak1 and Mbt, but not DPak3, have been analyzed by genetic techniques. As will be discussed below, these studies have revealed key functions for Paks in sensory organ development, as well as in organismal morphogenesis.

DPak1

DPak1 binds to the SH3/SH2 adaptor protein Nck (Dock), which is thought to facilitate its translocation to insulin receptors (InRs) at the cell membrane. Mutations in *InR*, *dock* or *dpak1* result in similar errors in photoreceptor axon guidance and targeting (Garrity et al., 1996; Hing et al., 1999; Song et al., 2003). Dock and DPak1 are also necessary for the guidance of olfactory axons (Ang et al., 2003). Interestingly, Kim and co-workers showed that DPak1 is probably not involved in axon outgrowth per se but rather in axon guidance, concluding that it regulates filopodial activity in the growth cone (Kim, M. D. et al., 2003).

In addition to its role in attractive axon guidance, the Dock-DPak1 complex might also be involved in axon repulsion. Dock binds to the cytoplasmic domain of the Roundabout (Robo) receptor, and loss of Dock or DPak1 function compromises Robo-mediated repulsion (Fan et al., 2003).

Mbt

The *mushroom bodies tiny* (*mbt*) gene of *Drosophila* encodes a group B Pak. Mbt was uncovered during a genetic screen for genes involved in the formation of the mushroom body, a structure in the adult fly corresponding to the human hippocampus that is involved in learning and memory. The *mbt*-null mutants have fewer neurons in the brain; this leads to a dramatic reduction in mushroom body volume, which correlates with a reduced number of Kenyon cells in this structure. It is therefore thought that Mbt has a role in cell proliferation, differentiation or survival of these neuronal cells (Melzig et al., 1998).

In addition to mushroom body defects, *mbt* mutant flies display loss of a variable number of photoreceptor cells (R-cells) in many ommatidia of the eye, whereas the innervation pattern in the medulla appears to be normal (Schneeberger and Raabe, 2003). Thus, unlike DPak1, Mbt is believed to be involved in photoreceptor cell morphogenesis rather than

photoreceptor axon guidance. Schneeberger and Raabe showed that Mbt specifically localizes to adherens junctions of photoreceptor cells. In *mbt* mutants, the rhabdomers of the differentiated photoreceptor cells show severe morphological defects, with disorganized adherens junctions. As in the case of other group B Paks, binding of activated Cdc42 has no influence on Mbt kinase activity but instead is required for recruitment of the kinase to adherens junctions. Interestingly, although the PBD appears to be essential for the *in vivo* function of Mbt, kinase activity is not absolutely required, since a kinase-dead Mbt mutant can partially rescue the *mbt* mutant eye phenotype (Schneeberger and Raabe, 2003).

Mammalian Paks

Mammals encode six Paks: three group A and three group B (Fig. 1A). As in simpler eukaryotes, Paks in mammalian cells regulate MAPK pathways and cytoskeletal organization (Fig. 4). The connection to the ERK MAPK pathway is particularly interesting, because Paks appear to phosphorylate both Raf1 (at Ser338) and Mek1 (at Ser298); that is, they behave both as MAPKKKs and as MAPKKs. These phosphorylations are in themselves not sufficient to activate Raf1 or Mek1, but are required for the activation of these kinases by Ras and Raf, respectively (Frost et al., 1997; King et al., 1998). Paks also activate the stress-activated JNK and p38 MAPK cascades, but these effects are modest in most cell types (Bagrodia et al., 1995; Brown et al., 1996).

Microinjection of activated Pak1 protein into quiescent Swiss 3T3 cells induces the rapid formation of lamellipodia, filopodia and membrane ruffles (Sells et al., 1997), which is similar to the effect produced by microinjection of Cdc42 (Nobes and Hall, 1995). Expression of various constitutively active forms of Pak1 induces disassembly of stress fibers and

focal adhesion complexes (Manser et al., 1997; Sells et al., 1997). The basis for these activities is not completely understood, but involves the phosphorylation of multiple substrates that affect cytoskeletal structure, including LIM kinase, myosin light chain kinase, merlin, filamin, p41^{Arc}, Rho-GEFs and stathmin. Paks also phosphorylate the apoptotic protein BAD and the estrogen and progesterone hormone receptors (reviewed by Bokoch, 2003) (Fig. 4). It is important to note that, as in *Drosophila*, some of the signaling functions of mammalian Paks, particularly certain cytoskeletal effects, are independent of its kinase function (Daniels et al., 1998; Frost et al., 1998; Sells et al., 1999; Sells et al., 1997). For example, Pak1 plays a key role in the polarization of chemotaxing cells; however, it appears that the main role of Pak1 in this context is not as a kinase per se but as a scaffold protein, bringing the GEF PIX alpha to the plasma membrane, where it encounters and activates Cdc42 (Li et al., 2003). It is also becoming apparent that mammalian Paks have GTPase-independent functions. For example, Pak1 can be directly activated by Akt (Tang et al., 1999; Tang et al., 2000), and Pak2 can be activated by cleavage by caspase 3 (Lee et al., 1997; Rudel and Bokoch, 1997).

The genetic analysis of Pak function in mammals is ongoing. In mice, the *Pak1*, *Pak2*, *Pak4* and *Pak5* genes have been disrupted (Table 1). *Pak1*- and *Pak5*-null mice are viable and healthy, whereas loss of *Pak2* or *Pak4* results in embryonic lethality. In humans, mutations in the *Pak3* gene are associated with an X-linked mental retardation syndrome (see below).

Group A Paks

Pak3

Pak3 is the only member of the Pak family known to be associated with a human genetic disease. Mutations in the *Pak3* gene are associated with X-linked, nonsyndromic mental retardation (MRX) syndromes, which are forms of mental retardation accompanied by grossly normal brain development and few other signs or symptoms.

The cause of MRX30 is a mutation in *pak3* that generates a truncated, kinase-dead mutant (R419Stop) (Allen et al., 1998). Two more independent MRX kindreds have been found to have *pak3* mutations. One of these (R67C mutation) is expected to affect GTPase binding, whereas the other (A365E mutation) affects a highly conserved region within subdomain VIA of the protein kinase domain (Bienvenu et al., 2000; Gedeon et al., 2003). Affected males bearing this latter mutation show mild-to-borderline mental retardation with no additional clinical manifestations other than mental impairment and relatively long ears, and occasionally neuropsychiatric problems.

The absence of severe brain defects in these MRX patients suggests that Pak3 function is not absolutely required for neuronal proliferation, migration or cortical gyration. However, the observation that *pak3* mutations result in mental retardation might reflect a later requirement for Pak3 function in the adult cortex. Perhaps, by analogy with the function of DPak1 in *Drosophila*, Pak3 is necessary for the normal development of axonal connections and hence there are aberrant or absent axonal connections in these MRX patients. Alternatively, because Rac and Pak signaling also appears to be important for dendritic spine morphogenesis, Pak3 might be

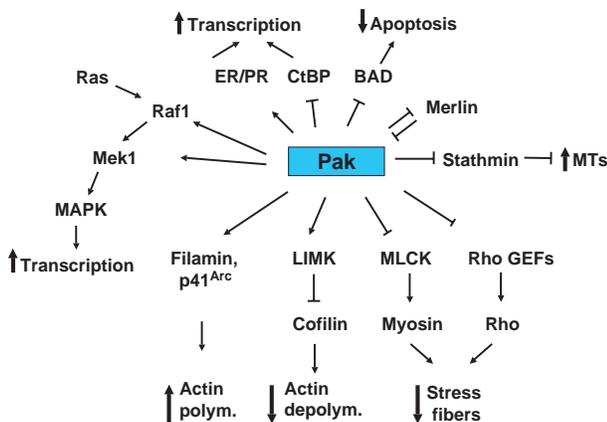


Fig. 4. Pak functions in mammalian cells. Paks target the MAPK pathway by phosphorylating Raf1 at Ser338 and Mek1 at Ser298. These phosphorylations are necessary but not sufficient for MAPK activation. Paks also act upon a number of regulators of the cytoskeleton, including filamin, p41^{Arc}, LIMK, merlin, myosin light chain kinase (MLCK) and stathmin. The net effect of these phosphorylations is to increase actin and tubulin polymerization. In addition, Paks phosphorylate steroid receptors such as the estrogen (ER) and progesterone (PR) receptors, which results in their activation, and the transcriptional co-repressor CtBP and the pro-apoptotic protein BAD, which results in their inactivation.

Table 1. Phenotypes associated with Pak loss-of-function mutations

Species/mutation	Phenotype	References
<i>Saccharomyces cerevisiae</i>		
Ste20	Sterility; cell polarity defects	Leberer et al., 1992a; Ramer and Davis, 1993
Cla4	Cytokinesis defects; cell polarity defects	Cvrckova et al., 1995b
Skm1	No phenotype	Martin et al., 1997
<i>Schizosaccharomyces pombe</i>		
Pak1	Lethality; cell polarity defects	Marcus et al., 1995; Otilie et al., 1995a
Pak2	No phenotype	Sells et al., 1998; Yang et al., 1998
<i>Dictyostelium discoideum</i>		
PakA	Cell polarity and motility defects	Chung and Firtel, 1999
<i>Drosophila melanogaster</i>		
DPak1	Lethality; axon guidance defects	Hing et al., 1999
Mbt	Mushroom body defects; photoreceptor morphogenesis defects	Melzig et al., 1998
<i>Mus musculus</i>		
Pak1	Immune defects	C.H., Z. M. Jaffer and J.C., unpublished
Pak2	Embryonic lethality	C.H., Z. M. Jaffer and J.C., unpublished
Pak4	Embryonic lethality; cardiac and neuronal defects	Qu et al., 2003a
Pak5	No phenotype	Li and Minden, 2003
<i>Homo sapiens</i>		
Pak3	Mental retardation	Allen et al., 1998; Bienvenu et al., 2000; Gedeon et al., 2003

necessary for dendritic development or for the rapid cytoskeletal reorganizations in dendritic spines associated with synaptic plasticity (Park et al., 2003; Penzes et al., 2003). Pak3 might have additional functions in the adult brain that involve its interaction with the amyloid precursor protein. Indeed, it might be one of the proteins that mediate the increased DNA synthesis and apoptosis found in neuronal cells of patients with familial Alzheimer's disease (McPhie et al., 2003). Through interference with these and/or similar pathways, mutation of *pak3* might cause mental retardation.

To date, a *pak3*-knockout mouse model has not been reported. Considering the phenotype of the MRX patients, the expression pattern of mouse *pak3* and pathways in which the Pak3 protein is known to be involved, one might expect a *pak3*-knockout mouse to develop normally, but to show some brain abnormalities and/or signs of memory and learning defects. Such a model would be invaluable as a tool to investigate the molecular basis for cognitive function. Indeed, loss of all group A Pak function in the brain, induced by transgenic expression of a Pak-inhibitory peptide in mice, induces aberrant synaptic morphology in cortical neurons and is associated with defects in hippocampus-dependent, long-term memory consolidation (Hayashi et al., 2004).

Group B Paks

Pak4

Pak4 was the first member of the group B Paks to be identified (Abo et al., 1998). It binds preferentially to activated Cdc42 and promotes filopodium formation in response to activated Cdc42 in fibroblasts and other cell types (Abo et al., 1998; Qu et al., 2001). Expression of activated Pak4 in fibroblasts decreases adhesion to the extracellular matrix and promotes proliferation, leading to anchorage-independent growth and increased cell migration (Callow et al., 2002; Qu et al., 2001). Pak4 substrates include the cytoskeletal regulatory kinase LIMK1, the pro-apoptotic protein BAD and probably PDZ-

Rho-GEF (Barac et al., 2004; Dan, C. et al., 2001; Gnesutta et al., 2001).

Mouse *pak4*-null embryos die around embryonic day 10.5 (Qu et al., 2003). The most likely cause of death is a cardiac defect, although its basis is not yet known. *Pak4*-null embryos also show severely abnormal development and migration of neurons. 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 areas. Neuroepithelia of *pak4*-mutant embryos are abnormally thin in the hindbrain and forebrain, which results in the appearance of a nearly translucent head and neural tube. Neuronal differentiation and migration is also defective in motor neurons of the developing spinal cord and neural tube. In addition to these abnormalities, the neural tube in these mice is improperly folded (Qu et al., 2003). The development of the neural tube depends on proper cell migration and therefore dynamic cytoskeletal reorganization in these cells. Interference with the underlying signaling pathways may therefore lead to defects in neural tube development. Previous studies have shown that Pak4 is indeed involved in regulation of focal adhesions and filopodia, both of which are structures that are important for cell migration (Callow et al., 2002; Qu et al., 2001). Neural tube defects have previously been found in mice lacking other proteins that are involved in the regulation of the cytoskeleton (Brouns et al., 2000; Snapper et al., 2001).

Pak4-deficient cultured cells have increased numbers of focal adhesions under serum-starved conditions, whereas cells overexpressing activated Pak4 have fewer focal adhesions compared with wild-type cells (Qu et al., 2001; Qu et al., 2003). Therefore, increased adhesion of neuronal cells might contribute to the observed defects in the knockout mice.

Pak5

Like Pak3, Pak5 is expressed preferentially in the brain (Pandey et al., 2002). Pak5 has a high basal kinase activity that

is not significantly increased by binding Cdc42, and overexpression of Pak5 activates the JNK MAPK pathway but not the p38 or ERK MAPK pathways (Dan et al., 2002; Pandey et al., 2002). In N1E-115 cells, overexpression studies suggest that Pak5 has a role in filopodium formation and neurite outgrowth, and probably acts downstream of Cdc42 and Rac (Dan et al., 2002). In common with other family members phosphorylating BAD, Pak5 is thought to have a role in the regulation of apoptosis (Cotteret et al., 2003). In CHO and neuronal HMN1 cells, overexpressed Pak5 localizes at mitochondria, independently of Cdc42 binding or its kinase activity (Cotteret et al., 2003).

Li and Minden have reported that *pak5*-null mice develop normally and are fertile. Although Pak5 is expressed at high levels in neurons in the brain and the eye, all parts of the nervous system studied develop normally in the mice, and histological analysis showed no obvious differences between them and wild-type mice (Li and Minden, 2003). One possible explanation is functional redundancy, because all members of the Pak family are expressed in the central nervous system. Although other members of the Pak family were not found to be upregulated in whole brain extracts of 8-week-old *pak5*-knockout mice (Li and Minden, 2003), it is nevertheless possible that these can compensate for the deficiency in Pak5. Interestingly, the expression patterns of Pak5 and Pak6 are very similar (Lee et al., 2002; Li and Minden, 2003). In addition, Pak6 mRNA levels in the adult mouse brain are significantly higher than those of Pak4 (Li and Minden, 2003). Although Pak5 does not appear to be required for brain development, it may yet prove to be important for cognitive function of the adult brain, as is thought to be the case for Pak3. Detailed behavioral and learning/memory studies of the *pak5* knockouts could reveal such defects, if they indeed exist.

Concluding remarks

Paks are named for their ability to bind and become activated by small GTPases, and their functions are usually considered in relation to their roles as GTPase effectors. It is clear from the wealth of biochemical, cell biological and genetic data that the regulation of MAPK cascades and regulation of cytoskeletal organization are ancient and conserved functions of Paks. However, the mechanisms by which they carry out these functions differ considerably, and the existence of kinase-independent effects further complicates the analysis of Pak signaling. It is also becoming evident that Paks shoulder an increasingly heavy signaling burden with increased organismal complexity and that, in higher eukaryotes, some of these signaling tasks are independent of small GTPase function. In other words, Paks have evolved into something more than simple GTPase effectors. A vigorous and systematic search for such GTPase-independent activities is likely to yield new insights, and the ongoing genetic analysis of Paks in mammalian systems should help clarify the cellular and developmental signaling pathways regulated by this fascinating family of protein kinases.

Work in the Chernoff lab is funded by the National Institutes of Health and the American Cancer Society, as well as by a center-wide CORE grant and an appropriation from the Commonwealth of Pennsylvania. We are grateful to our many colleagues who provided

information and insights into Pak function, as well as E. Golemis and J. Peterson for comments on the manuscript. We regret the inevitable omissions that were necessary to keep to the designated word limits.

References

- Abo, A., Qu, J., Cammarano, M. S., Dan, C., Fritsch, A., Baud, V., Belisle, B. and Minden, A. (1998). PAK4, a novel effector for Cdc42Hs, is implicated in the reorganization of the actin cytoskeleton and in the formation of filopodia. *EMBO J.* **17**, 6527-6540.
- Allen, K. M., Gleeson, J. G., Bagrodia, S., Partington, M. W., MacMillan, J. C., Cerione, R. A., Mulley, J. C. and Walsh, C. A. (1998). PAK3 mutation in nonsyndromic X-linked mental retardation. *Nat. Genet.* **20**, 25-30.
- Ang, L. H., Kim, J., Stepensky, V. and Hing, H. (2003). Dock and Pak regulate olfactory axon pathfinding in *Drosophila*. *Development* **130**, 1307-1316.
- Bagrodia, S., Derijard, B., Davis, R. J. and Cerione, R. A. (1995). Cdc42 and PAK-mediated signaling leads to Jun kinase and p38 mitogen-activated protein kinase activation. *J. Biol. Chem.* **270**, 27995-27998.
- Barac, A., Basile, J., Vazquez-Prado, J., Gao, Y., Zheng, Y. and Gutkind, J. S. (2004). Direct interaction of p21-activated kinase 4 with PDZ-RhoGEF, a G protein-linked Rho guanine exchange factor. *J. Biol. Chem.* **279**, 6182-6189.
- Benton, B. K., Tinkelenberg, A., Gonzalez, I. and Cross, F. R. (1997). Cla4p, a *Saccharomyces cerevisiae* Cdc42p-activated kinase involved in cytokinesis, is activated at mitosis. *Mol. Cell. Biol.* **17**, 5067-5076.
- Bienvenu, T., des Portes, V., McDonell, N., Carrie, A., Zemni, R., Couvert, P., Ropers, H. H., Moraine, C., van Bokhoven, H., Fryns, J. P. et al. (2000). Missense mutation in PAK3, R67C, causes X-linked nonspecific mental retardation. *Am. J. Med. Genet.* **93**, 294-298.
- Bokoch, G. M. (2003). Biology of the p21-activated kinases. *Annu. Rev. Biochem.* **72**, 743-781.
- Bose, I., Irazoqui, J. E., Moskow, J. J., Bardes, E. S., Zyla, T. R. and Lew, D. J. (2001). Assembly of scaffold-mediated complexes containing Cdc42p, the exchange factor Cdc24p, and the effector Cla4p required for cell cycle-regulated phosphorylation of Cdc24p. *J. Biol. Chem.* **276**, 7176-7186.
- Brouns, M. R., Matheson, S. F., Hu, K. Q., Delalle, I., Caviness, V. S., Silver, J., Bronson, R. T. and Settleman, J. (2000). The adhesion signaling molecule p190 RhoGAP is required for morphogenetic processes in neural development. *Development* **127**, 4891-4903.
- Brown, J. L., Stowers, L., Baer, M., Trejo, J., Coughlin, S. and Chant, J. (1996). Human Ste20 homologue hPAK1 links GTPases to the JNK MAP kinase pathway. *Curr. Biol.* **6**, 598-605.
- Brzeska, H., Knaus, U. G., Wang, Z. Y., Bokoch, G. M. and Korn, E. D. (1997). p21-activated kinase has substrate specificity similar to *Acanthamoeba* myosin I heavy chain kinase and activates *Acanthamoeba* myosin I. *Proc. Natl. Acad. Sci. USA* **94**, 1092-1095.
- Callow, M. G., Clairvoyant, F., Zhu, S., Schryver, B., Whyte, D. B., Bischoff, J. R., Jallal, B. and Smeal, T. (2002). Requirement for PAK4 in the anchorage-independent growth of human cancer cell lines. *J. Biol. Chem.* **277**, 550-558.
- Chang, E., Bartholomeusz, G., Pimental, R., Chen, J., Lai, H., Wang, L., Yang, P. and Marcus, S. (1999). Direct binding and in vivo regulation of the fission yeast p21-activated kinase shk1 by the SH3 domain protein scd2. *Mol. Cell. Biol.* **19**, 8066-8074.
- Chenevert, J., Corrado, K., Bender, A., Pringle, J. and Herskowitz, I. (1992). A yeast gene (BEM1) necessary for cell polarization whose product contains two SH3 domains. *Nature* **356**, 77-79.
- Chiroti, E., Fraschini, R., Beretta, A., Tonelli, M., Lucchini, G. and Piatti, S. (2003). Budding yeast PAK kinases regulate mitotic exit by two different mechanisms. *J. Cell Biol.* **160**, 857-874.
- Chung, C. Y. and Firtel, R. A. (1999). PAKa, a putative PAK family member, is required for cytokinesis and the regulation of the cytoskeleton in *Dictyostelium discoideum* cells during chemotaxis. *J. Cell Biol.* **147**, 559-576.
- Chung, C. Y., Potikyan, G. and Firtel, R. A. (2001). Control of cell polarity and chemotaxis by Akt/PKB and PI3 kinase through the regulation of PAKa. *Mol. Cell* **7**, 937-947.
- Cotteret, S., Jaffer, Z. M., Beeser, A. and Chernoff, J. (2003). p21-activated kinase 5 (Pak5) localizes to mitochondria and inhibits apoptosis by phosphorylating BAD. *Mol. Cell. Biol.* **23**, 5526-5539.
- Cullen, P. J., Schultz, J., Horecka, J., Stevenson, B. J., Jigami, Y. and Sprague, G. F., Jr (2000). Defects in protein glycosylation cause SHO1-dependent activation of a STE12 signaling pathway in yeast. *Genetics* **155**, 1005-1018.
- Cvrckova, F., de Virgilio, C., Manser, E., Pringle, J. R. and Nasmyth, K.

- (1995). Ste20-like protein kinases are required for normal localization of cell growth and for cytokinesis in budding yeast. *Genes Dev.* **9**, 1817-1830.
- Dan, C., Kelly, A., Bernard, O. and Minden, A.** (2001). Cytoskeletal changes regulated by the PAK4 serine/threonine kinase are mediated by LIM kinase 1 and cofilin. *J. Biol. Chem.* **276**, 32115-32121.
- Dan, I., Watanabe, N. M. and Kusumi, A.** (2001). The Ste20 group kinases as regulators of MAP kinase cascades. *Trends Cell Biol.* **11**, 220-230.
- Dan, C., Nath, N., Liberto, M. and Minden, A.** (2002). PAK5, a new brain-specific kinase, promotes neurite outgrowth in N1E-115 cells. *Mol. Cell Biol.* **22**, 567-577.
- Daniels, R. H., Hall, P. S. and Bokoch, G. M.** (1998). Membrane targeting of p21-activated kinase 1 (PAK1) induces neurite outgrowth from PC12 cells. *EMBO J.* **17**, 754-764.
- Davis, C. R., Richman, T. J., Deliduka, S. B., Blaisdell, J. O., Collins, C. C. and Johnson, D. I.** (1998). Analysis of the mechanisms of action of the *Saccharomyces cerevisiae* dominant lethal cdc42G12V and dominant negative cdc42D118A mutations. *J. Biol. Chem.* **273**, 849-858.
- de Nadal, E., Alepuz, P. M. and Posas, F.** (2002). Dealing with osmolarity through MAP kinase activation. *EMBO Rep.* **3**, 735-740.
- Dobbelaere, J., Gentry, M. S., Hallberg, R. L. and Barral, Y.** (2003). Phosphorylation-dependent regulation of septin dynamics during the cell cycle. *Dev. Cell* **4**, 345-357.
- Drogen, F., O'Rourke, S. M., Stucke, V. M., Jaquenoud, M., Neiman, A. M. and Peter, M.** (2000). Phosphorylation of the MEKK Ste1p by the PAK-like kinase Ste20p is required for MAP kinase signaling in vivo. *Curr. Biol.* **10**, 630-639.
- Eby, J. J., Holly, S. P., van Drogen, F., Grishin, A. V., Peter, M., Drubin, D. G. and Blumer, K. J.** (1998). Actin cytoskeleton organization regulated by the PAK family of protein kinases. *Curr. Biol.* **8**, 967-970.
- Elion, E. A.** (2000). Pheromone response, mating and cell biology. *Curr. Opin. Microbiol.* **3**, 573-581.
- Etienne-Manneville, S.** (2004). Cdc42 – the centre of polarity. *J. Cell Sci.* **117**, 1291-1300.
- Fan, X., Labrador, J. P., Hing, H. and Bashaw, G. J.** (2003). Slit stimulation recruits Dock and Pak to the roundabout receptor and increases Rac activity to regulate axon repulsion at the CNS midline. *Neuron* **40**, 113-127.
- Frost, J. A., Steen, H., Shapiro, P., Lewis, T., Ahn, N., Shaw, P. E. and Cobb, M. H.** (1997). Cross-cascade activation of ERKs and ternary complex factors by Rho family proteins. *EMBO J.* **16**, 6426-6438.
- Frost, J. A., Khokhlatchev, A., Stippes, S., White, M. A. and Cobb, M. H.** (1998). Differential effects of PAK1-activating mutations reveal activity-dependent and -independent effects on cytoskeletal regulation. *J. Biol. Chem.* **273**, 28191-28198.
- Fujita, A., Tonouchi, A., Hiroko, T., Inose, F., Nagashima, T., Satoh, R. and Tanaka, S.** (1999). Hsl7p, a negative regulator of Ste20p protein kinase in the *Saccharomyces cerevisiae* filamentous growth-signaling pathway. *Proc. Natl. Acad. Sci. USA* **96**, 8522-8527.
- Garrity, P. A., Rao, Y., Salecker, I., McGlade, J., Pawson, T. and Zipursky, S. L.** (1996). *Drosophila* photoreceptor axon guidance and targeting requires the dreadlocks SH2/SH3 adapter protein. *Cell* **85**, 639-650.
- Gedeon, A. K., Nelson, J., Geetz, J. and Mulley, J. C.** (2003). X-linked mild non-syndromic mental retardation with neuropsychiatric problems and the missense mutation A365E in PAK3. *Am. J. Med. Genet.* **120**, 509-517.
- Gilbreth, M., Yang, P., Wang, D., Frost, J., Polverino, A., Cobb, M. H. and Marcus, S.** (1996). The highly conserved skb1 gene encodes a protein that interacts with Shk1, a fission yeast Ste20/PAK homolog. *Proc. Natl. Acad. Sci. USA* **93**, 13802-13807.
- Gilbreth, M., Yang, P., Bartholomeusz, G., Pimental, R. A., Kansra, S., Gadiraju, R. and Marcus, S.** (1998). Negative regulation of mitosis in fission yeast by the shk1 interacting protein skb1 and its human homolog, Skb1HS. *Proc. Natl. Acad. Sci. USA* **95**, 14781-14786.
- Gnesutta, N., Qu, J. and Minden, A.** (2001). The serine/threonine kinase PAK4 prevents caspase activation and protects cells from apoptosis. *J. Biol. Chem.* **276**, 14414-14419.
- Goehring, A. S., Mitchell, D. A., Tong, A. H., Keniry, M. E., Boone, C. and Sprague, G. F., Jr** (2003). Synthetic lethal analysis implicates Ste20p, a p21-activated protein kinase, in polarisome activation. *Mol. Biol. Cell* **14**, 1501-1516.
- Gulli, M. P., Jaquenoud, M., Shimada, Y., Niederhauser, G., Wiget, P. and Peter, M.** (2000). Phosphorylation of the Cdc42 exchange factor Cdc24 by the PAK-like kinase Cla4 may regulate polarized growth in yeast. *Mol. Cell* **6**, 1155-1167.
- Hayashi, M. L., Choi, S. Y., Rao, B. S., Jung, H. Y., Lee, H. K., Zhang, D., Chattarji, S., Kirkwood, A. and Tonegawa, S.** (2004). Altered cortical synaptic morphology and impaired memory consolidation in forebrain-specific dominant-negative PAK transgenic mice. *Neuron* **42**, 773-787.
- Hing, H., Xiao, J., Harden, N., Lim, L. and Zipursky, S. L.** (1999). Pak functions downstream of Dock to regulate photoreceptor axon guidance in *Drosophila*. *Cell* **97**, 853-863.
- Holly, S. P. and Blumer, K. J.** (1999). PAK-family kinases regulate cell and actin polarization throughout the cell cycle of *Saccharomyces cerevisiae*. *J. Cell Biol.* **147**, 845-856.
- Jaffer, Z. M. and Chernoff, J.** (2002). p21-activated kinases: three more join the Pak. *Int. J. Biochem. Cell Biol.* **34**, 713-717.
- Kim, H. W., Yang, P., Qyang, Y., Lai, H., Du, H., Henkel, J. S., Kumar, K., Bao, S., Liu, M. and Marcus, S.** (2001). Genetic and molecular characterization of Skb15, a highly conserved inhibitor of the fission yeast PAK, Shk1. *Mol. Cell* **7**, 1095-1101.
- Kim, H., Yang, P., Catanuto, P., Verde, F., Lai, H., Du, H., Chang, F. and Marcus, S.** (2003). 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.* **278**, 30074-30082.
- Kim, M. D., Kamiyama, D., Kolodziej, P., Hing, H. and Chiba, A.** (2003). Isolation of Rho GTPase effector pathways during axon development. *Dev. Biol.* **262**, 282-293.
- King, A. J., Sun, H., Diaz, B., Barnard, D., Miao, W., Bagrodia, S. and Marshall, M. S.** (1998). The protein kinase Pak3 positively regulates Raf-1 activity through phosphorylation of serine 338. *Nature* **396**, 180-183.
- Leberer, E., Dignard, D., Hargus, D., Thomas, D. Y. and Whiteway, M.** (1992). The protein kinase homologue Ste20p is required to link the yeast pheromone response G-protein beta gamma subunits to downstream signalling components. *EMBO J.* **11**, 4815-4824.
- Lee, B. N. and Elion, E. A.** (1999). The MAPKKK Ste11 regulates vegetative growth through a kinase cascade of shared signaling components. *Proc. Natl. Acad. Sci. USA* **96**, 12679-12684.
- Lee, N., MacDonald, H., Reinhard, C., Halenbeck, R., Roulston, A., Shi, T. and Williams, L. T.** (1997). Activation of hPAK65 by caspase cleavage induces some of the morphological and biochemical changes of apoptosis. *Proc. Natl. Acad. Sci. USA* **94**, 13642-13647.
- Lee, S. F., Mahasneh, A., de la Roche, M. and Cote, G. P.** (1998). Regulation of the p21-activated kinase-related *Dictyostelium* myosin I heavy chain kinase by autophosphorylation, acidic phospholipids, and Ca²⁺-calmodulin. *J. Biol. Chem.* **273**, 27911-27917.
- Lee, S. R., Ramos, S. M., Ko, A., Masiello, D., Swanson, K. D., Lu, M. L. and Balk, S. P.** (2002). AR and ER interaction with a p21-activated kinase (PAK6). *Mol. Endocrinol.* **16**, 85-99.
- Leeuw, T., Fourrest-Lieuvin, A., Wu, C., Chenevert, J., Clark, K., Whiteway, M., Thomas, D. Y. and Leberer, E.** (1995). Pheromone response in yeast: association of Bem1p with proteins of the MAP kinase cascade and actin. *Science* **270**, 1210-1213.
- Lew, D. J.** (2000). Cell-cycle checkpoints that ensure coordination between nuclear and cytoplasmic events in *Saccharomyces cerevisiae*. *Curr. Opin. Genet. Dev.* **10**, 47-53.
- Li, X. and Minden, A.** (2003). Targeted disruption of the gene for the PAK5 kinase in mice. *Mol. Cell Biol.* **23**, 7134-7142.
- Li, Z., Hannigan, M., Mo, Z., Liu, B., Lu, W., Wu, Y., Smrcka, A. V., Wu, G., Li, L., Liu, M. et al.** (2003). Directional sensing requires G beta gamma-mediated PAK1 and PIX alpha-dependent activation of Cdc42. *Cell* **114**, 215-227.
- Manser, E., Huang, H. Y., Loo, T. H., Chen, X. Q., Dong, J. M., Leung, T. and Lim, L.** (1997). Expression of constitutively active alpha-PAK reveals effects of the kinase on actin and focal complexes. *Mol. Cell Biol.* **17**, 1129-1143.
- Marcus, S., Polverino, A., Chang, E., Robbins, D., Cobb, M. H. and Wigler, M. H.** (1995). 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. USA* **92**, 6180-6184.
- Martin, H., Mendoza, A., Rodriguez-Pachon, J. M., Molina, M. and Nombela, C.** (1997). Characterization of SKM1, a *Saccharomyces cerevisiae* gene encoding a novel Ste20/PAK-like protein kinase. *Mol. Microbiol.* **23**, 431-444.
- McPhie, D. L., Coopersmith, R., Hines-Peralta, A., Chen, Y. Z., Ivins, K. J., Manly, S. P., Kozlowski, M. R., Neve, K. A. and Neve, R. L.** (2003). DNA synthesis and neuronal apoptosis caused by familial Alzheimer disease mutants of the amyloid precursor protein are mediated by the p21 activated kinase PAK3. *J. Neurosci.* **23**, 6914-6927.
- Melzig, J., Rein, K. H., Schafer, U., Pfister, H., Jackle, H., Heisenberg, M. and Raabe, T.** (1998). A protein related to p21-activated kinase (PAK) that is involved in neurogenesis in the *Drosophila* adult central nervous system. *Curr. Biol.* **8**, 1223-1226.
- Merla, A. and Johnson, D. I.** (2001). The *Schizosaccharomyces pombe* Cdc42p GTPase signals through Pak2p and the Mkh1p-Pek1p-Spm1p MAP kinase pathway. *Curr. Genet.* **39**, 205-209.

- Miller, P. J. and Johnson, D. I. (1994). Cdc42p GTPase is involved in controlling polarized cell growth in *Schizosaccharomyces pombe*. *Mol. Cell Biol.* **14**, 1075-1083.
- Mortensen, E. M., McDonald, H., Yates, J., III and Kellogg, D. R. (2002). Cell cycle-dependent assembly of a Gin4-septin complex. *Mol. Biol. Cell* **13**, 2091-2105.
- Mosch, H. U., Roberts, R. L. and Fink, G. R. (1996). Ras2 signals via the Cdc42/Ste20/mitogen-activated protein kinase module to induce filamentous growth in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **93**, 5352-5356.
- Müller-Taubenberger, A., Bretschneider, T., Faix, J., Konzok, A., Simmeth, E. and Weber, A. (2002). Differential localization of the *Dictyostelium* kinase DPAKA during cytokinesis and cell migration. *J. Muscle Res. Cell Motil.* **23**, 751-763.
- Nobes, C. D. and Hall, A. (1995). Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* **81**, 53-62.
- Oehlen, L. J. and Cross, F. R. (1998). Potential regulation of Ste20 function by the Cln1-Cdc28 and Cln2-Cdc28 cyclin-dependent protein kinases. *J. Biol. Chem.* **273**, 25089-25097.
- O'Rourke, S. M., Herskowitz, I. and O'Shea, E. K. (2002). Yeast go the whole HOG for the hyperosmotic response. *Trends Genet.* **18**, 405-412.
- Ottlie, S., Miller, P. J., Johnson, D. I., Creasy, C. L., Sells, M. A., Bagrodia, S., Forsburg, S. L. and Chernoff, J. (1995). Fission yeast pak1+ encodes a protein kinase that interacts with Cdc42p and is involved in the control of cell polarity and mating. *EMBO J.* **14**, 5908-5919.
- Pandey, A., Dan, I., Kristiansen, T. Z., Watanabe, N. M., Voldby, J., Kajikawa, E., Khosravi-Far, R., Blagoev, B. and Mann, M. (2002). Cloning and characterization of PAK5, a novel member of mammalian p21-activated kinase-II subfamily that is predominantly expressed in brain. *Oncogene* **21**, 3939-3948.
- Park, E., Na, M., Choi, J., Kim, S., Lee, J. R., Yoon, J., Park, D., Sheng, M. and Kim, E. (2003). The Shank family of postsynaptic density proteins interacts with and promotes synaptic accumulation of the beta PIX guanine nucleotide exchange factor for Rac1 and Cdc42. *J. Biol. Chem.* **278**, 19220-19229.
- Penzes, P., Beeser, A., Chernoff, J., Schiller, M. R., Eipper, B. A., Mains, R. E. and Huganir, R. L. (2003). Rapid induction of dendritic spine morphogenesis by trans-synaptic ephrinB-EphB receptor activation of the Rho-GEF kalirin. *Neuron* **37**, 263-274.
- Posas, F., Takekawa, M. and Saito, H. (1998). Signal transduction by MAP kinase cascades in budding yeast. *Curr. Opin. Microbiol.* **1**, 175-182.
- Qu, J., Cammarano, M. S., Shi, Q., Ha, K. C., de Lanerolle, P. and Minden, A. (2001). Activated PAK4 regulates cell adhesion and anchorage-independent growth. *Mol. Cell Biol.* **21**, 3523-3533.
- Qu, J., Li, X., Novitsch, B. G., Zheng, Y., Kohn, M., Xie, J.-M., Kozinn, S., Bronson, R., Beg, A. A. and Minden, A. (2003). PAK4 kinase is essential for embryonic viability and for proper neuronal development. *Mol. Cell Biol.* **23**, 7122-7133.
- Qyang, Y., Yang, P., Du, H., Lai, H., Kim, H. and Marcus, S. (2002). The p21-activated kinase, Shk1, is required for proper regulation of microtubule dynamics in the fission yeast, *Schizosaccharomyces pombe*. *Mol. Microbiol.* **44**, 325-334.
- Raitt, D. C., Posas, F. and Saito, H. (2000). Yeast Cdc42 GTPase and Ste20 PAK-like kinase regulate Shol-dependent activation of the Hog1 MAPK pathway. *EMBO J.* **19**, 4623-4631.
- Ramer, S. W. and Davis, R. W. (1993). A dominant truncation allele identifies a gene, STE20, that encodes a putative protein kinase necessary for mating in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **90**, 452-456.
- Roberts, R. L., Mosch, H. U. and Fink, G. R. (1997). 14-3-3 proteins are essential for RAS/MAPK cascade signaling during pseudohyphal development in *S. cerevisiae*. *Cell* **89**, 1055-1065.
- Rudel, T. and Bokoch, G. M. (1997). Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of PAK2. *Science* **276**, 1571-1574.
- Sakchaisri, K., Asano, S., Yu, L. R., Shulewitz, M. J., Park, C. J., Park, J. E., Cho, Y. W., Veenstra, T. D., Thorner, J. and Lee, K. S. (2004). Coupling morphogenesis to mitotic entry. *Proc. Natl. Acad. Sci. USA* **101**, 4124-4129.
- Sawin, K. E., Hajibagheri, M. A. and Nurse, P. (1999). Mis-specification of cortical identity in a fission yeast PAK mutant. *Curr. Biol.* **9**, 1335-1338.
- Schneeberger, D. and Raabe, T. (2003). Mbt, a *Drosophila* PAK protein, combines with Cdc42 to regulate photoreceptor cell morphogenesis. *Development* **130**, 427-437.
- Sells, M. A., Knaus, U. G., Bagrodia, S., Ambrose, D. M., Bokoch, G. M. and Chernoff, J. (1997). Human p21-activated kinase (Pak1) regulates actin organization in mammalian cells. *Curr. Biol.* **7**, 202-210.
- Sells, M. A., Barratt, J. T., Caviston, J., Ottlie, S., Leberer, E. and Chernoff, J. (1998). Characterization of Pak2p, a pleckstrin homology domain-containing, p21-activated protein kinase from fission yeast. *J. Biol. Chem.* **273**, 18490-18498.
- Sells, M. A., Boyd, J. T. and Chernoff, J. (1999). p21-activated kinase 1 (Pak1) regulates cell motility in mammalian fibroblasts. *J. Cell Biol.* **145**, 837-849.
- Seshan, A., Bardin, A. J. and Amon, A. (2002). Control of Lte1 localization by cell polarity determinants and Cdc14. *Curr. Biol.* **12**, 2098-2110.
- Shimada, Y., Gulli, M. P. and Peter, M. (2000). Nuclear sequestration of the exchange factor Cdc24 by Far1 regulates cell polarity during yeast mating. *Nat. Cell Biol.* **2**, 117-124.
- Simanis, V. (2003). Events at the end of mitosis in the budding and fission yeasts. *J. Cell Sci.* **116**, 4263-4275.
- Snapper, S. B., Takeshima, F., Anton, I., Liu, C. H., Thomas, S. M., Nguyen, D., Dudley, D., Fraser, H., Purich, D., Lopez-Illasaca, M. et al. (2001). N-WASP deficiency reveals distinct pathways for cell surface projections and microbial actin-based motility. *Nat. Cell Biol.* **3**, 897-904.
- Song, J., Wu, L., Chen, Z., Kohanski, R. A. and Pick, L. (2003). Axons guided by insulin receptor in *Drosophila* visual system. *Science* **300**, 502-505.
- Tang, Y., Yu, J. and Field, J. (1999). Signals from the Ras, Rac, and Rho GTPases converge on the Pak protein kinase in Rat-1 fibroblasts. *Mol. Cell Biol.* **19**, 1881-1891.
- Tang, Y., Zhou, H., Chen, A., Pittman, R. N. and Field, J. (2000). The Akt proto-oncogene links Ras to Pak and cell survival signals. *J. Biol. Chem.* **275**, 9106-9109.
- Tjandra, H., Compton, J. and Kellogg, D. (1998). Control of mitotic events by the Cdc42 GTPase, the Clb2 cyclin and a member of the PAK kinase family. *Curr. Biol.* **8**, 991-1000.
- Toenjes, K. A., Sawyer, M. M. and Johnson, D. I. (1999). The guanine-nucleotide-exchange factor Cdc24p is targeted to the nucleus and polarized growth sites. *Curr. Biol.* **9**, 1183-1186.
- Tu, H., Barr, M., Dong, D. L. and Wigler, M. (1997). Multiple regulatory domains on the Byr2 protein kinase. *Mol. Cell Biol.* **17**, 5876-5887.
- Verde, F., Wiley, D. J. and Nurse, P. (1998). Fission yeast orb6, a ser/thr protein kinase related to mammalian rho kinase and myotonic dystrophy kinase, is required for maintenance of cell polarity and coordinates cell morphogenesis with the cell cycle. *Proc. Natl. Acad. Sci. USA* **95**, 7526-7531.
- Versle, M. and Thorner, J. (2004). Septin collar formation in budding yeast requires GTP binding and direct phosphorylation by the PAK, Cla4. *J. Cell Biol.* **164**, 701-715.
- Wild, A. C., Yu, J. W., Lemmon, M. A. and Blumer, K. J. (2004). The PAK-related kinase Cla4 is a coincidence detector of signaling by Cdc42 and phosphatidylinositol 4-phosphate. *J. Biol. Chem.* **279**, 17101-17110.
- Wiley, D. J., Marcus, S., D'Urso, G. and Verde, F. (2003). Control of cell polarity in fission yeast by association of Orb6p kinase with the highly conserved protein methyltransferase Skb1p. *J. Biol. Chem.* **278**, 25256-25263.
- Wittmann, T., Bokoch, G. M. and Waterman-Storer, C. M. (2004). Regulation of microtubule destabilizing activity of Op18/stathmin downstream of Rac1. *J. Biol. Chem.* **279**, 6196-6203.
- Wu, C., Lee, S. F., Furmaniak-Kazmierczak, E., Cote, G. P., Thomas, D. Y. and Leberer, E. (1996). Activation of myosin-I by members of the Ste20p protein kinase family. *J. Biol. Chem.* **271**, 31787-31790.
- Wu, C., Lytvyn, V., Thomas, D. Y. and Leberer, E. (1997). The phosphorylation site for Ste20p-like protein kinases is essential for the function of myosin-I in yeast. *J. Biol. Chem.* **272**, 30623-30626.
- Wu, C., Leeuw, T., Leberer, E., Thomas, D. Y. and Whiteway, M. (1998). Cell cycle- and Cln2p-Cdc28p-dependent phosphorylation of the yeast Ste20p protein kinase. *J. Biol. Chem.* **273**, 28107-28115.
- Yang, P., Kansra, S., Pimental, R. A., Gilbreth, M. and Marcus, S. (1998). Cloning and characterization of shk2, a gene encoding a novel p21-activated protein kinase from fission yeast. *J. Biol. Chem.* **273**, 18481-18489.
- Yang, P., Pimental, R., Lai, H. and Marcus, S. (1999). Direct activation of the fission yeast PAK Shk1 by the novel SH3 domain protein, Skb5. *J. Biol. Chem.* **274**, 36052-36057.
- Yang, P., Qyang, Y., Bartholomeusz, G., Zhou, X. and Marcus, S. (2003). 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.* **278**, 48821-48830.
- Zenke, F. T., Krendel, M., DerMardirossian, C., King, C. C., Bohl, B. P. and Bokoch, G. M. (2004). p21-activated kinase 1 phosphorylates and regulates 14-3-3 binding to GEF-H1, a microtubule-localized Rho exchange factor. *J. Biol. Chem.* **279**, 18392-18400.