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The Pak1 Kinase: An Important Regulator of Neuronal Morphology and Function in the Developing Forebrain

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Abstract The mammalian central nervous system (CNS) represents a highly complex unit, the correct function of which relies on the appropriate differentiation and survival of its neurones. It is becoming apparent that the Rho family of small GTPases and their downstream targets have a major function in regulating CNS development. Among the effectors, the role of the Pak family of kinases, especially Pak1, is becoming increasingly evident. Although highest levels of Pak1 expression and activation are detected in the developing nervous system, much remains undiscovered concerning its function in neurones. This review summarises what is currently known regarding the biological and molecular role of Pak1 in the mammalian forebrain. It emphasises the importance of Pak1 in regulating neuronal polarity, morphology, migration and synaptic function. Consequently, there are also strong indications that Pak1 is required for normal cognitive function. Furthermore, loss of Pak1 has been associated with the progression of neurodegenerative disorders, particularly Alzheimer's disease, while up-regulation and de-regulation may be responsible for oncogenic transformation of support cells within the CNS, especially astrocyte progenitors. Together, these new and exciting findings encourage the future exploration into the function of Pak1 in the nervous system, thus, paving the way for novel strategies towards improved diagnosis and therapeutic treatment of diseases that affect the CNS.

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Introduction

In the developing central nervous system (CNS), neurones often migrate over extensive and challenging routes to reach their correct final destinations where they undergo highly complex morphological changes. It is apparent that signalling pathways that control the organisation and dynamics of cytoskeletal elements, particularly filamentous actin (F-actin) and microtubules, control the morphology, motility and, thus, fate of differentiating neurones. Highly orchestrated cytoskeletal remodelling also accounts for the establishment and plasticity of synaptic communications and, thus, controls cognitive function. Consequently, changes in the signalling pathways that control cytoskeletal organisation can result in developmental defects commonly manifested by cognitive impairments and recurrent seizures. They can also cause pathological changes which characterise neurodegenerative disorders such as Alzheimer's Disease (AD).

The last decade has increasingly revealed the crucial role of the Rho family of small GTPases in the CNS [1]. More recently, research has implicated several downstream effectors of the Rho GTPases as key factors for normal neuronal differentiation and function, among which are the p21-activated kinases (Pak). The Pak serine/threonine kinase family is subdivided into two classes, a division primarily based on levels of homology. In humans, Group I is comprised of Pak1, Pak2 and Pak3, while Group II includes Pak4, Pak5 and Pak6 [2, 3]. A higher degree of homology links the members of Group I than Group II

kinases, although all are characterised by an N-terminal regulatory domain and a C-terminal catalytic domain (Fig. 1 shows domain structure of Pak1) [2, 3]. The distinguishing feature of Group I kinases is the presence of an N-terminal auto-inhibitory domain (AID), which partially overlaps with a Rac/Cdc42 binding domain (RBD). The AID associates in trans with the catalytic domain and, thus, accounts for the homodimerisation and inhibition of Group I kinases. The interaction of activated GTP-bound Rac or Cdc42 acts to disrupt the trans-inhibition, triggering a cascade of Pak phosphorylation and consequential catalytic activation [2, 4–6]. Since phosphorylation accompanies the activation of Group I kinases, it is possible to use specific antibodies raised against particular phosphorylation sites to evaluate the regulation and localisation of their catalytic activation (Fig. 1) [7–11].

Examination of expression patterns has revealed that all six kinases are present in the nervous system [12]. Interestingly, only Pak3 is thought to be exclusive to neurones, while Pak1, Pak5 and Pak6 are highly enriched in the nervous system. The linkage of mutations in the *Pak3* gene with familial cases of X-linked non-syndromic mental retardation (MRX) has confirmed the importance of Pak3 in the CNS [13]. Several studies have attributed this to the role of Pak3 in regulating the morphology and function of small postsynaptic contacts known as dendritic spines [14, 15]. However, deletion of the *Pak3* gene in mice ($Pak3^{-/-}$) did not affect the density or morphology of dendritic spines, suggesting that the abnormalities observed in long-lasting hippocampal long term potentiation (LTP) were due to more complex defects [16]. No abnormalities were observed in CNS development. Similarly, Pak5 does not appear to have a role in CNS development since mouse knockouts did not exhibit any detectable phenotypes [17]. This may, however, illustrate a degree of redundancy among Group II Pak kinases, as well as the possibility that a very local or specific defect remains undetected. Deletion of the *Pak4* gene ($Pak4^{-/-}$) was reported to affect CNS formation, although the early stage of embryonic lethality (11.5 days of gestation; E11.5) prevented a detailed analysis of its CNS function [18]. Unconfirmed reports of $Pak2^{-/-}$ (embryonic lethal) and $Pak1^{-/-}$ (no reported neuronal phenotype) exist, although the lack of published data makes the evaluation of any potential CNS defects difficult [12].

In recent years, the most extensive advance has been made towards understanding the role of Pak1 in neurones. Initially, Pak1 was almost exclusively studied in non-neuronal systems, most likely due to the fact that it was among the first identified Rac- and Cdc42-regulated kinase effectors [19]. Since its discovery, Pak1 has been shown to control directional movement and adhesion of fibroblasts and epithelial cells, have anti-apoptotic effects by promoting the survival of a number of cell types, affect cell division and, thus, be classed as a likely proto-oncogene particularly in breast cancer [2, 20–22]. Consequently, despite being predominantly enriched in neurones, the functional understanding of Pak1 in other cell types and

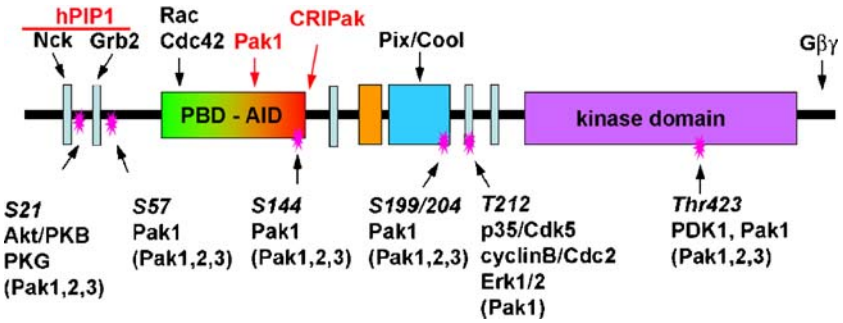

Fig. 1 Schematic diagram depicting Pak1 structure, key binding partners and phosphorylation sites. The N-terminus of Pak1 (amino acids 1–254) contains the majority of protein–protein interaction domains and phosphorylation sites. These include five PXXP motifs (*gray bars*) of which the first two bind SH3 domains of Nck and Grb2, respectively [109, 110]. To date, the putative binding partners of the remaining three PXXP motifs are unknown. A Pak1 inhibitor hPIP1 associates with N-terminal region spanning amino acids 1–70 [111]. The RBD is responsible for Pak1 activation by Rac1,2 and 3, Cdc42, Chp, TC10 and Wrch-1 (*green box*, amino acids 67–113) [2, 112–114]. The autoinhibitory domain—AID (*red box*; amino acids 87–149) overlaps with the RBD; it associates in trans with the catalytic domain of Pak1 [4]. Amino acids 132–270 associate with a Pak1 inhibitor CRIPak, overlapping in part with the AID [115]. An acidic domain (*orange box*) lies immediately upstream of a proline rich region (amino acids 182–203) which associates with PIX/Cool proteins (*light blue box*) [116]. The catalytic domain is shown in purple (amino acids 255–529). The C-terminus of the yeast homologue Ste20, directly associates with Gβγ proteins [117]. This interaction has not been proven for mammalian Group I Pak kinases, however Gβγ proteins can stimulate Pak1 activity in a GTPase independent manner [118]. Pak1 is extensively phosphorylated on Ser, Thr and Tyr residues [119]. Key S/T sites (*pink starbursts*) are labelled in italic with the kinases that target them listed underneath [11, 27, 37, 50, 120–122]. T212 is the only unique site on Pak1; its targeting is dependent on Pak1 activation but has no apparent consequences on its catalytic activity [37]. Phosphorylation of S21 regulates association with Nck, while catalytic activation is accompanied by phosphorylation of S57, S144, S199/204 and T423 [11]

organs has until recently been ahead of the nervous system. The aim of this review is, therefore, to consolidate our current knowledge of the functional role of Pak1 in the formation and function of the CNS, particularly the forebrain, in order to demonstrate its key roles in the development of this complex system.

A Role for Pak1 in Proliferating Neuronal Precursors

The isolation of Pak1 from rat brain tissue was the first indication that this kinase has a neuronal function [19]. The subsequent examination of mRNA and protein expression in embryonic and adult murine CNS revealed the enrichment of Pak1 throughout development and subsequently in adulthood [23–25]. Within the nervous system, the mammalian embryonic cerebral cortex is an excellent tool to compare proliferating neuronal precursors and postmitotic neurones (Fig. 2). Here, Pak1 was shown to be present throughout all regions, although lowest levels were observed in the ventricular and subventricular zones (VZ/SVZ) where neuronal progenitors proliferate [24, 26]. Regardless of the relatively low expression levels, studies of cortical neuronal progenitors, epithelial and fibroblast cell lines have suggested the requirement for Pak1 in the mitotic phase of the cell cycle which can have important implications on neuronal fate [8, 24, 27, 28].

In early embryogenesis, cortical progenitors proliferate in the VZ and undergo interkinetic nuclear migration. Consequently, cells that enter the M phase of the cell cycle position themselves along the lateral ventricle on the apical side of the VZ. The position of the centrosomes, spindle and, thus, the cleavage plane is key to determining the fate of the two progenitor cells (Fig. 2) [29, 30]. Thus, a cell division that is precisely perpendicular to the surface of the lateral ventricle (vertical) symmetrically divides the precursor into two identical daughter progenitor cells. In contrast, an angled or parallel division (horizontal) will result in unequal, asymmetric distribution of cellular proteins such as β-catenin and numb, thus, resulting in the formation of one progenitor and one immature neurone that will subsequently migrate into the cortical plate [31–34]. Interestingly, in several cell types, different forms of phosphorylated Pak1 were seen to accumulate in the centrosomes during M phase. Thus, centrosomal enrichment of Pak1 targeted by the mitotic Cyclin B/Cdc2 kinase on T212 (Pak1T212(P)) was observed in vivo in cortical M phase progenitors as well as in vitro in Cos7 cells and Swiss 3T3 fibroblasts [24, 28]. Interference with endogenous Pak1T212(P) changed the organisation of astral microtubules, suggesting the possibility that Pak1 may be able to influence spindle organisation and the positioning of the cleavage furrow [28]. To date, this has not been further

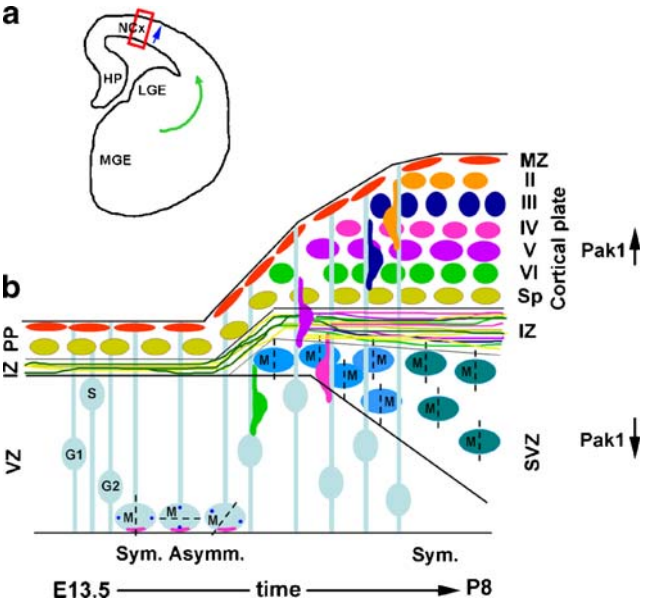

Fig. 2 **A** Schematic diagram showing coronal view of the left hemisphere of a mouse embryo forebrain. *Blue arrow* depicts pathways of radially migrating projection neurones that originate from the dorsal ventricular zone. *Green arrow* represents the trajectories taken by tangentially migrating interneurones that primarily originate from the ventricular zone of the medial ganglionic eminence (*MGE*). Hippocampus (*HP*), neocortex (*NCx*), lateral ganglionic eminence (*LGE*). **B** Diagram depicting cellular behaviour in the developing neocortex marked by *red insert* from (**A**). Time points are related to mouse development. At early embryonic stages, the cortical plate is composed of the preplate (*PP*) and a very thin intermediate zone (*IZ*). In the proliferating ventricular zone (*VZ*), progenitors undergo nucleokinesis where the position of the soma mark cell cycle stages. Progenitors predominantly divide apically at the surface of the lateral ventricle. Here, the plane of division influences the fate of the progenitors, where vertical divisions are symmetrical in relation to apically distributed cellular proteins (*marked in pink*), thus, resulting in two identical progenitors. In contrast, horizontal divisions and those that occur at an angle asymmetrically distribute the fate-determining proteins, thus, generating one progenitor and one committed neurone. Centrosomes are marked in mitotic cells with a *dark blue dot*. As development progresses, secondary progenitors form in the subventricular zone (*SVZ*) where they divide symmetrically to generate neurones. The cortical plate forms in an inside-out manner with the first layer (*VI*) splitting the preplate layer (*PP*) into the marginal zone (*MZ*) and subplate (*SP*). Layer *V* neurones will migrate through the *IZ* and *SP*, and locate immediately above layer *VI*. Subsequent layers are formed in an equivalent manner with younger neurones migrating increasingly greater distances up radial glial progenitors and ultimately arresting beneath the *MZ*. The appearance of the *SVZ* coincides with the progressive reduction in the *VZ*. The *IZ* increases in size with the progressive formation of afferent and efferent axonal tracts

examined in cortical progenitors; thus, no conclusions can be made concerning neuronal fate and Pak1 function. However, recent examination of Cdc42 in proliferating precursors of the VZ revealed a key role for this GTPase in cell fate regulation [35]. Down-regulation of Cdc42 function caused the loss of apically directed nuclear migrations, consequently increasing the number of cell cycle dependent

divisions in the basal part of the proliferating zone which normally occurs in later stages of embryogenesis. Since apical mitotic divisions maintain a proliferating population of neuronal precursors while basal divisions commonly generate two postmitotic neurones, changes in Cdc42 expression affected the fate of cortical progenitors [35]. The consequential implications for Pak1 as a major downstream target of Cdc42 cannot be ignored. It is also interesting to note that in NIH3T3 fibroblasts and HeLa cells recruitment of Pak1 to centrosomes can result in its catalytic activation as determined by phosphorylation of T423 and S199 (T422 and S198, respectively, in Pakα which is the rat orthologue of human Pak1) [8]. Furthermore, in Cos7 cells, active Pak1 serves to induce the function of the Aurora A kinase and is required for centrosome duplication at the late S/G2 phases of the cell cycle [8]. Together, these reports signal the need for further research into the role of Pak1 in proliferating neuronal progenitors.

Pak1 is Enriched in Differentiating Postmitotic Neurones

In the developing CNS, Pak1 has been most extensively studied in the forebrain where highest levels are evident in postmitotic neurones. Particular enrichment was observed in the cortical plate, hippocampus and major axonal tracts, including the thalamo-cortical and cortico-thalamic projections, corpus callosum, lateral olfactory tract and anterior commissures [24, 36]. A detailed examination into the subcellular distribution of Pak1 using electron microscopy of primate forebrains revealed its enrichment in axon and dendrite terminals indicative of a role in their outgrowth [36]. This distribution pattern of Pak1 strongly suggested its key developmental role for CNS formation. In confirmation, phosphorylated Pak1 is also enriched primarily in postmitotic neurones and major axonal tracts [24, 26, 37, 38]. Thus, accumulation of Pak1 phosphorylated on S199/204 (Pak1S199/204(P)) was seen in the cortical plate and axonal tracts of the developing mouse forebrain, suggesting the need for high levels of its catalytic activation for normal development [26]. It is, however, important to note that the antibody that detects Pak1S199/204(P) can also recognise activated Pak2, phosphorylated on S192/197, suggesting that simultaneous activation of both kinases may be required for normal cortical development. Alternatively, differential activation of Pak1 and Pak2 may be specific to the cell type and stage of differentiation. In support of this, in dissociated cultures of cortical and hippocampal neurones where the presence of glia is minimal, no activated Pak2 was detectable by western blot analysis, while in contrast, high levels of activated Pak1 were evident. Interestingly, Pak1 activation was found to follow a highly polarised pattern with preferential accumulation in the newly forming axon, which indicated a role for Pak1 during neuronal polarisation [26].

Phosphorylation of T212 is unique to Pak1 and in postmitotic neurones is catalysed by the p35/Cdk5 kinase [24, 28]. The importance of p35/Cdk5 in CNS development and function is widely accepted. This proline-directed serine/threonine kinase is required for the correct morphology, migration and synaptic communication of postmitotic neurones [39, 40]. It is, therefore, significant that p35/Cdk5 phosphorylates Pak1 on T212 and that Pak1T212(P) is enriched in the cortical plate and axons of the intermediate zone (IZ) as well as in other major axonal tracts and postmitotic areas of rat or mouse embryos. Interestingly, phosphorylation of T212 occurs only during development and is down-regulated in adulthood, despite the continued presence of Pak1 and p35/Cdk5 [24]. To date, the biological significance of T212 phosphorylated Pak1 is unclear although there are some indications that it is required for cytoskeletal remodelling. In part, this is supported by the microtubule organising function of Pak1T212(P) observed in M phase cells [28]. In postmitotic neurones, Pak1T212(P) is evident throughout axonal growth cones including F-actin rich peripheral lamellipodia. It is also seen to accumulate in areas where microtubule bundles have invaded into the growth cone peripheries, suggesting a role in actin/microtubule coordination during axonal outgrowth and path finding. Crosstalk between microfilaments and microtubules is crucial for the correct navigation of axons and consequential communication between neurones within the CNS. Furthermore, it is increasingly evident that the Rho GTPases can simultaneously affect both cytoskeletal elements in several different cell types [41, 42]. It is, therefore, important to pursue the role of Pak1 during axonal guidance in the developing mammalian CNS. This is emphasised by the previously reported requirement for *Drosophila* Pak kinases during the correct guidance of olfactory and photoreceptor axons to the cognate glomeruli and optic lobe targets, respectively, as well as Roundabout receptor mediated repulsion at the midline [43–46]. Furthermore, signalling pathways that cause growth cone collapse, such as stimulation of the plexin-B1 receptor by Semaphorin 4D cause inhibition of Pak1 kinase activity [47].

Pak1 Functions to Control Neuronal Polarity and Morphology

A number of in vitro studies using primary cortical and hippocampal neurones have provided evidence to support an important function of Pak1 in controlling neuronal morphology. Loss and gain of function in cultured cortical

neurones obtained from rat or mouse embryos suggested its requirement for the initiation, outgrowth and branching of dendrites [25, 38]. Thus, expression of a constitutively active Pak1 mutant (Pak1T423E) promoted the formation of dendrites; in contrast, expression of a Pak1 fragment containing the AID and PBD which may inhibit all three Group I kinases and sequester activated Rac or Cdc42 had an inhibiting effect on dendrite formation. Interestingly, the Pak1 mutants were introduced into the cortical neurones in vivo by in utero electroporation, thus, only targeting progenitors in the VZ/SVZ. Subsequent dissociation and culturing of the neurones allowed the in vitro analysis of morphological maturation that would otherwise have been hard to observe in vivo. The additional advantage of this approach was that the authors avoided the inevitable delay in protein expression associated with the analysis of neurones transfected in vitro and predominantly examined the effects on neurite initiation rather than respecification or regeneration. Subsequent studies of dissociated hippocampal neurones obtained from rat embryos confirmed that altered Pak1 levels and activity affect neuronal morphology and neurite outgrowth, although no significant changes were observed during dendrite initiation [26]. The differences in these results may be accounted for by the use of hippocampal versus cortical cultures, the former of which is a more uniform population of pyramidal neurones, while the latter culture also includes many interneurones. In addition, different approaches were taken to engineer changes in Pak1 expression and activity.

Cultured hippocampal neurones are an ideal tool to study the mechanisms of polarisation in vitro, the use of which was pioneered by Bradke and Dotti 20 years ago (Fig. 3) [48]. The differentiation of these neurones following isolation from late rat or mouse embryos can be separated into five stages based on a series of morphological changes they undergo after attachment to an adhesive substrate such as laminin or poly-lysine (Fig. 3). At stage 1, they resemble flat epithelial cells with a central nucleus and a predominance of ruffling lamellipodia. Stage 2 is marked by the rapid extension of short neurites which are, to a large extent, morphologically indistinguishable from each other. The distinction of one neurite by subsequent rapid outgrowth marks the entry into stage 3 and the specification of an axon. At stages 4 and 5, the neurones continue to extend their axons and dendrites, the latter of which form short stubby post-synaptic contacts referred to as dendritic spines. Stages 1 and 2 generally occur during the first 2–5 days in culture, while spine formation requires prolonged incubation of around 21 days. Much research has been focused at understanding the molecular asymmetry responsible for neuronal progression from stages 1 to 3. A major player is the phosphatidylinositol 3-kinase (PI3K) which accumulates at the tip of the emerging axon and the

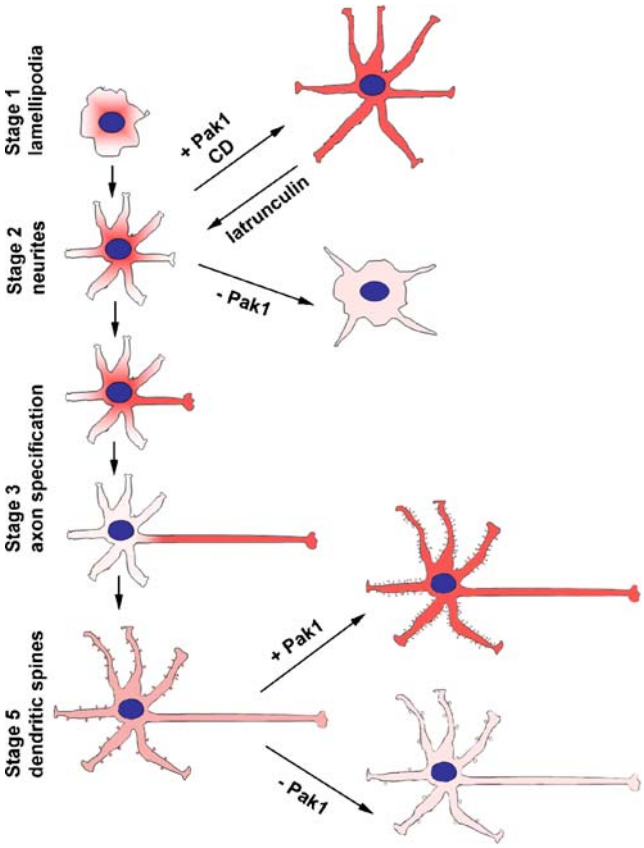

Fig. 3 The distribution and levels of active Pak1 change with neuronal development. Polarisation of cultured mouse or rat embryo hippocampal neurones are depicted diagrammatically illustrating the morphological changes that occur as they progress from *stage 1* to *stage 5*. The distribution and levels of phosphorylated Pak1 are depicted in *shades of pink* and *red* with the latter representing higher concentrations. In *stage 1* and *2* neurones, phosphorylated Pak1 is not polarised and is predominantly seen in perinuclear regions. Progression to *stage 3* is marked by the preferential enrichment of phosphorylated Pak1 to one neurite which subsequently undergoes rapid outgrowth and axonal specification. Activated Pak1 is at the same time down-regulated in the remaining neurites which will subsequently form dendrites. Once the axon is specified, it no longer has preferential enrichment of activated Pak1 which is now seen to localise in dendritic spines. Experimental manipulation of Pak1 expression levels affect neuronal polarisation with gain of function in stage 2 neurones preventing the specification of a single axon and promoting the outgrowth of all neurites. Loss of Pak1 function reduces neurite outgrowth and also affects polarisation. In vitro this has detrimental consequences on neuronal survival. Exposure to cytochalasin D (CD) redistributes phosphorylated Pak1 to all neurites and affects polarisation. The consequences of Pak1 gain of function can be rescued by treatment with latrunculin. Changes in Pak1 activity also affect the formation of dendritic spines where gain of function increases their density and reduces their width, while loss of function has the opposite effect

down-regulation of which prevents axon specification [49]. PI3K is activated by exposure to neurotrophic factors such as BDNF and NT3 and in turn acts to stimulate the phosphoinositide-dependent kinase (PDK1) and consequently the Akt/PKB kinase. Interestingly, both PDK1

and Akt/PKB can directly phosphorylate Pak1 on T423 and S21, respectively, and, thus, control its catalytic activation and subcellular distribution [10, 50]. This signalling has not been fully confirmed in primary neurones, although a recent report suggested that Pak1 mediates the survival of cerebellar granule neurones downstream from the activities of both Akt/PKB and PDK1 [51]. PI3K can also indirectly activate Rac via the stimulation of exchange factors including members of the Vav, Sos, Tiam and PIX/Cool families [52]. Together, these reports strongly indicate that in neurones, Pak1 may function as an important downstream target of the molecular changes that follow PI3K stimulation (Fig. 4). However, to complicate matters, it is increasingly evident that these signalling pathways need not follow a linear progression. Thus, Pak1 has the capacity to function upstream of Rac by affecting its exchange factors of the PIX/Cool family; it can also activate Akt/PKB by promoting its phosphorylation on Ser473 and Thr308 [53, 54]. In addition, evidence exists that Rac can act upstream of PI3K [52]. Therefore, exactly how these molecules interact with each other may be highly specific to cell type, stage of differentiation and the nature of its external stimulation.

Importantly, despite its largely uniform distribution throughout a differentiating hippocampal neurone in vitro, Pak1 activation was observed to specifically mark the formation of an axon [26]. This axonal specific accumulation of activated Pak1 appeared to be temporary, subsiding once the neurones reach the stage 4 or 5 boundary (Fig. 3). The role of Pak1 in neuronal polarisation was subsequently analysed by a loss and gain-of-function approach where uniform hyperactivation of Pak1 in all emerging neurites abolished the specification of a single axon. Neurones either extended what appeared to be multiple axons or arrested in a stage 2-like form where dendritic and axonal markers had not fully segregated to their specific compartments [26]. Loss of Pak1 expression using an shRNA approach caused global inhibition of neurite outgrowth. Polarity was also affected, as determined by the lack of segregated axonal and dendritic markers, although the

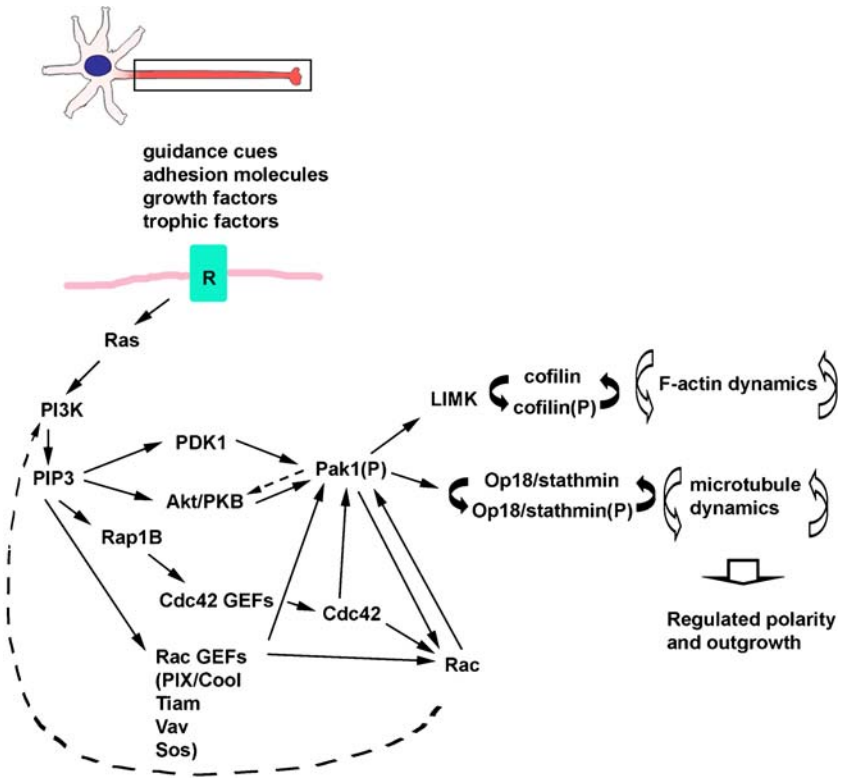

Fig. 4 Signalling pathways that involve Pak1 activation and are required for normal neuronal polarisation. A major regulator of axon specification is the PI3 kinase (*PI3K*) which can be activated by a number of extracellular stimuli. Through phosphatidylinositol 3,4,5-triphosphate (PIP3), PI3K activates kinases including PDK1 and Akt/PKB which can directly phosphorylate Pak1. PIP3 also activates the Ras GTPase Rap1B and subsequent stimulation of Cdc42 GEFs leads to the activation of Cdc42. Activation of Rac is induced though PIP3 stimulation of exchange factors Tiam1, PIX/Cool, Vav and Sos. Exchange factors, particularly PIX/Cool, can directly interact with Pak1 and have been implicated in the capability of Pak1 to act upstream of Rac. Activation of Pak1 in turn affects the function of proteins which regulate F-actin (LIM kinase and cofilin) and microtubule (Op18/stathmin) organisation and dynamic restructuring

severe inhibition of neurite outgrowth together with the increased neuronal death hampered long term analysis of polarisation. Together, these data show that Pak1 is required for neurite outgrowth, in accordance with previous reports [25, 55]. They also strongly suggest the requirement for Pak1 during neuronal polarisation, confirming findings made in *Dictiostelium*, *Saccharomyces cerevisiae* and *Cryptococcus neoformans* [56–59].

It is interesting to note that in hippocampal neurones, gain of Pak1 function could be rescued by interfering with the balance of F-actin polymerisation and depolymerisation, such as treatment with low doses of the F-actin destabilising drug latrunculin, or co-expression of a non-phosphorylatable cofilin mutant (Fig. 3) [26]. Pak1 is known to indirectly affect cofilin function via an activating phosphorylation of LIM kinases (LIMK) which in turn phosphorylate and inactivate cofilin [60]. This process is essential for the regulation of F-actin turnover at cell peripheries and thus the formation of leading edge protrusions [61]. There is mounting evidence that LIMK and cofilin play important roles in the developing CNS, especially for neuronal migration, the establishment of functional synaptic contacts and normal cognitive function [62–65]. In *Drosophila*, these three molecules have been shown to signal together in a linear manner, Pak to LIMK to cofilin, which is required for the normal glomerular development in the antennal lobe [43]. The link between Pak1 function and cytoskeletal organisation has further been shown in hippocampal neurones following their exposure to low doses of the F-actin depolymerising drug cytochalasin D (CD). Treatment with CD induced the formation of multiple axon-like neurites and the accompanying loss of asymmetric enrichment of activated Pak1 [26]. Furthermore, reduced or absent Pak1 expression caused the appearance of aberrant ruffling lamellipodia which surrounded the neuronal soma and were populated by disorganised microtubules (Fig. 3) [26]. It remains unclear whether the microtubules were directly affected by the loss of Pak1 signalling or were indirectly changed by alterations of the F-actin filaments. Taking into account the reported role of Pak1 to control the function of the microtubule destabilising proteins of the Op18/stathmin family, it is likely that Pak1 can independently affect both cytoskeletal elements (Fig. 4) [66]. Interestingly, reduced expression of two Op18/stathmin family members (SCLIP or SCG10) in cultured hippocampal neurones has been associated with enhanced axonal branching and increased growth cone spreading, respectively [67]. Recently substrate-bound netrin-1 was shown to promote growth cone spreading of rat spinal commissural neurones by association with its receptor, deleted in colorectal cancer and in turn activating Cdc42, Rac, Pak1 and the neuronal Wiskott–Aldrich syndrome protein (N-Wasp) [68]. Together, these data support a role for Pak1 in controlling neuronal morphology by affecting the organisation and dynamics of microfilaments and microtubules.

In Differentiating Neurones the Subcellular Localisation of Active Pak1 is Biologically Significant

The heterodimerisation and consequential inhibition of Pak1 in the cytoplasm assures tight control of the levels and subcellular localisation of Pak1 activation. It has been shown that recruitment of Pak1 to the plasma membrane stimulates its catalytic activity [2, 4]. It is currently unclear whether, in neurones, this predominantly occurs in a Rho GTPase dependent or independent manner since in non-neuronal cells, Pak1 can be activated by Rac and Cdc42, sphingosine, as well as small adapter proteins Nck and Grb2 which associate with activated receptor tyrosine kinases [2]. Significantly, a membrane-targeted Pak1 protein generated by fusion of a Ras prenilation sequence to its C-terminus (Pak1Caax) results in its constitutive activation in all cell types examined to date. Expression of Pak1Caax in hippocampal neurones caused a pronounced effect on neurite outgrowth, promoting the formation of dendrite-like processes and inhibiting the specification of an axon [26]. Interestingly, a constitutively active mutant which is not actively targeted to the plasma membrane (but can be recruited there through intracellular signalling pathways), Pak1T423E, induced extensive F-actin polymerisation and increased lamellipodia but did not affect neuronal polarisation. These findings suggest that asymmetric targeting of activated Pak1 to the plasma membrane of a single neurite may be crucial for the specification of an axon. Furthermore, a catalytically inactive mutant (Pak1R299Caax) had no effect on neuronal polarisation or morphology despite its membrane localisation [26]. Therefore, it is likely that immature neurones require asymmetric activation of Pak1 at specific regions of the plasma membrane for their correct differentiation. In contrast, overexpression of either Pak1Caax or Pak1R299Caax in PC12 cells was previously reported to promote outgrowth of neurite-like processes, suggesting that in these cells, membrane localisation of Pak1 alone was sufficient to induce outgrowth, irrespective of its catalytic activity [55]. Importantly, despite the fact that PC12 are commonly used to study signalling pathways in neurones, they do not always represent a good model. Specifically, PC12 cells do not have an identifiable axon and are, therefore, not appropriate to study signalling pathways that control neuronal polarisation.

Exploration of Pak1 function in vivo has confirmed its important role in forebrain development, although it remains unclear whether it primarily functions to control neuronal morphology and motility or also directly affects polarisation. Changes in Pak1 levels and activity using in

utero electroporaton of VZ/SVZ neuronal progenitors in E14.5 mouse embryos affected their morphology and motility. Thus, constitutive increases in Pak1 activity (expression of Pak1Caax) as well as its down-regulation (expression of Pak1 shRNAs) arrested neurones in the IZ, in contrast to the controls which migrated to layers II–IV (F. Causeret and M.Nikolic; manuscript under consideration by Cerebral Cortex). Interestingly, loss of Pak1 also caused a significant number of neurones that had entered the cortical plate to over-migrate and invade the normally cell sparse MZ, suggesting their inability to respond to local stop signals or dissociate from guiding radial glia. All neurones with altered Pak1 expression and localisation exhibited altered morphologies. Importantly, expression of Pak1T423E and Pak1R299Caax had far milder consequences than those seen following introduction of Pak1Caax, confirming the findings previously made in vitro. Thus, membrane localisation of catalytically active Pak1 has the most pronounced effect on neuronal morphology and migration in vitro and in vivo. Interestingly, controlled activation of Pak3 was recently revealed to be essential for the timely migration of interneurones into the cerebral cortex [69]. Cortical interneurones originate from ventrally located germinative zones in the basal ganglia from which they migrate tangentially towards the cerebral cortex [70, 71]. In immature interneurones, premature expression of Pak3 due to the down-regulation of transcription factors Dlx1 and Dlx2 induced inappropriate outgrowth of long neurites and caused interneurones to arrest in the basal ganglia [69]. The involvement of Pak kinases in neuronal migration was also demonstrated in the chicken cerebellum. Expression of wild type or a kinase inactive mutant of human Pak1 in granule cell precursors affected the morphology of their leading migratory process suggesting the involvement of Pak1 in migration from the rhombic lip to the surface of the developing cerebellum [72]. Together, these findings reveal that Pak1 has an important function in the developing CNS which may only, in part, overlap with other members of the Group I kinases.

Pak1 Regulates Dendritic Spines

Dendritic spines are an essential postsynaptic structure in the CNS, particularly for excitatory synapses. These small F-actin rich protrusions form on the dendrites of spiny neurones such as pyramidal neurones of the cerebral cortex and hippocampus and Purkinje neurones of the cerebellum. Initially, they resemble long filopodia which subsequently mature into different morphological types. The shape, length and size of the spines determine the effectiveness of the synaptic contacts [73]. Dendritic spines are not static structures, rather they continuously undergo morphological changes that are enabled by the dynamic restructuring of F-actin filaments, the only cytoskeletal elements present in these small protrusions. Dynamic rearrangement of the actin cytoskeleton is mediated in spines, in part, by the localised activation of Rac. This was initially revealed by the use of transgenic mice where overexpression of a constitutively active Rac mutant altered the morphology and density of spines found on cerebellar Purkinje neurones [74]. Subsequent studies in dissociated neurones and slice cultures further extended and confirmed the importance of balanced signalling from Rac and Rho to maintain the normal formation and function of dendritic spines [75–77]. Specific targeting of activated Rac to young spines may at least, in part, be achieved through the actions of its exchange factor kalirin which is, in turn, translocated to spines following activation of ephrinB-EphB receptor signalling [78]. Rac may also be recruited to spines by the G-protein-coupled receptor kinase-interacting protein 1 (GIT1) and the exchange factors of the PIX/Cool family [79, 80]. Since PIX/Cool proteins directly interact with Pak kinases, as does Rac, this strongly suggests a role for Pak1 in controlling spine morphology and function (Fig. 5). In support, constitutively active mutants of either Pak1 or Pak3 were able to efficiently rescue the consequences of GIT1 down-regulation on spine formation [80]. Furthermore, a large adapter protein Shank that localises to postsynaptic densities (PSDs) of hippocampal neurones can recruit both PIX/Cool and Pak1 to the PSDs [81]. Moreover, activated forms of the Group I Pak kinases were found to localise to postsynaptic densities in cultured hippocampal neurones and mouse forebrains [38, 80, 82]. Pak1 was also show to be an essential downstream component of ephrinB-EphB signalling during the morphogenesis and maturation of dendritic spines [78]. In addition, Pak1 and Pak3 induced the generation of spines at least in part through the regulation of myosin light chain and consequently acto-myosin contractility [80].

Confirmation that the Group I Pak kinases are important regulators of dendritic spine morphology in vivo came from the generation of a mouse transgenic model where expression of the AID was driven by the αCAMK-II promoter assuring expression in neurones of the postnatal forebrain. The AID sequence used was derived from Pak3, although it has the potential to inhibit all three Group I kinases. Due to the late expression of the inhibitory construct, no alterations were evident in the normal anatomical organisation of the forebrain. Interestingly, no changes were observed in dendrite initiation or branching. However, in layers II–III of the cerebral cortex, a significant reduction in the number of dendritic spines was observed [82]. This was accompanied with a change in the overall population of spines present which were shorter and larger than those seen in the wild-type controls. They

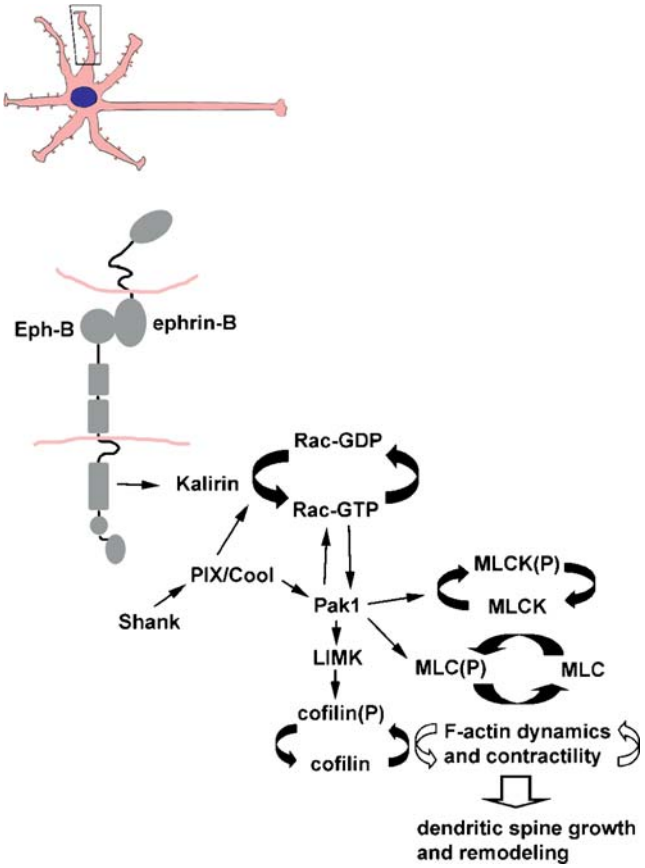

Fig. 5 Signalling pathways involving Pak1 that affect dendritic spines. The ephrin-B–Eph-B interaction can stimulate changes in dendritic spines by association with and consequential activation of the Rac exchange factor kalirin. Increases in GTP bound Rac can also be stimulated through the functions of the postsynaptic density enriched protein Shank and the exchange factors of the *PIX*/*Cool* family directly or via activation of Pak1. Downstream targets of Pak1 that have been implicated in spine formation and restructuring include the regulatory myosin light chain (*MLC*), myosin light chain kinase (*MLCK*), LIM kinase and cofilin. They affect the dynamic organisation and contractility of F-actin and consequently the number and morphology of dendritic spines

also displayed perforated rather than continuous PSDs [82]. Accordingly, expression of the Pak3 AID in cultured hippocampal neurones caused the formation of fewer spines with widened heads. In contrast, expression of a constitutively active Pak1T423E mutant increased the number and density of spines which were narrower (Fig. 3) [38]. Furthermore, overexpression of wild-type Pak1 resulted in an increased number of dendritic spines, while in contrast a dominant negative mutant had the opposite effect [38, 80]. Similar results were observed following changes in Pak3 function, suggesting an overlapping role with Pak1. It is important to note that the size of the spine head correlates with the number of available postsynaptic receptors and thus has the capacity to affect synaptic strength [83]. Furthermore, studies have shown that filopodia and dendritic spines can stimulate the maturation and docking of presynaptic terminals [84]. Accordingly, close examination using electron microscopy revealed that transgenic mice expressing the Pak3 AID displayed an increased number of docked presynaptic vesicles, which positively correlated with the sizes of the dendritic spines. Biochemical confirmation that Group I kinases can affect presynaptic function in the CNS has been provided by the discovery that synapsin I is phosphorylated on S603 by Paks 1–3 [85]. Synapsin I is an important mediator of vesicle docking and recruitment to active zones, which it performs in a phosphorylation state dependent manner. To date it is not entirely clear whether differences exist in the specific functions of Pak1 and 3 in controlling synaptogenesis, or whether this biological function enjoys a significant degree of signalling redundancy.

Fragile X syndrome (FXS) is a commonly inherited form of moderate to severe mental retardation and autism in humans. It is caused by mutations of the *fragile X mental retardation 1* gene (*FMR1*$^{-/-}$) and is associated with defects in dendritic spines and synaptic plasticity. Mouse models of FXS display an increased number and length of dendritic spines, a phenotype also observed in FXS individuals. Interestingly, the dendritic spines seen in mice expressing the Pak3 AID displayed largely opposite morphological changes to those observed in the *FMR1*$^{-/-}$ mice [86–88]. An indirect link between the functions of FMR1 and Group I Pak kinases was subsequently demonstrated in vivo by crossing these two mouse models, resulting in a significant rescue of the FXS and Pak3 AID phenotypes [86]. Specifically, down-regulation of Group I kinase activity in a *FMR1*$^{-/-}$ background restored spine length and density to the levels observed in wild-type controls. Furthermore, the morphological rescue was accompanied with a recovered LTP magnitude which had been reduced in *FMR1*$^{-/-}$ mice. The rescued mice also displayed a reduction in hyperactivity, repetitive behaviour and hypoanxiety, which are characteristic traits of *FMR1* loss. Thus, an intriguing hypothesis was proposed where Group I Pak kinases and FMR1 may antagonise each other to regulate spine morphology and synaptic function. It is not clear how much of this function could be attributed to individual members of the Group I family since the Pak3 AID may inhibit all three. However, it is important to note that to date, Pak2 has not been directly linked with the regulation of dendritic spines, in contrast to Paks1 and 3. Significantly, in humans, only mutations in the *Pak3* gene have been associated with MRX which are thought to be caused by defective dendritic spine morphology and synaptic plasticity in the hippocampus [13–15]. Collectively, these studies suggest that Pak3 may have the predominant role in controlling dendritic spines; however, they also suggest

distinct as well as overlapping functions of the Group I Pak kinases in regulating synaptic function and cognitive behaviour.

Pak1 and Cognitive Function

The ability to store information directly correlates with synaptic strengthening. Furthermore, a positive link is believed to exist between the size of a synapse and the strength of synaptic transmission. Transgenic mice expressing the Pak3 AID were characterised by spines with larger heads and increased docking of presynaptic vesicles; therefore, the finding that they also displayed an enhancement of mean AMPA and NMDA receptor mediated synaptic transmission was in accordance with published literature [82]. In addition, extracellular field recordings of synaptic transmission from layer IV to layers II–III in the temporal cortex of control and transgenic animals revealed enhanced LTP and reduced long-term depression following expression of the Pak3 AID. The transgenic mice also displayed defects in memory consolidation which was determined by their impaired ability to remember the location of a platform or retain long-term context-dependent fear conditioning [82].

Extinction of contextual fear in animal models can be used to experimentally ascertain the molecular and cellular pathways required to forget unpleasant experiences. It is most applicable to study the pathogenesis of post-traumatic stress disorders. The approach in animals involves repeated exposure to a conditioning context in the absence of the unpleasant stimulus that had previously been used during fear conditioning and the subsequent measurement of the time required for the averse freezing behaviour to decline. Interestingly, the use of this experimental strategy recently suggested that Group I Pak kinases, particularly Pak1, are involved in promoting extinction [89]. Thus, expression of the Pak1 AID in the dorsal hippocampus of adult mice resulted in significantly impaired extinction when compared to GFP-expressing controls. A similar approach was previously used to demonstrate that Group I Pak kinases are required for normal memory in adult mice where their inhibition affected social recognition memory tasks [90]. Changes in Pak levels and activation were associated with altered functions of the F-actin regulating proteins cofilin and drebin, suggesting consequential cytoskeletal abnormalities in postsynaptic sites [90]. Significantly, since the Pak1 AID may also inhibit Pak2 and Pak3, these findings did not prove the exclusive requirement for Pak1. However, the involvement of Pak1 during learned reduction of fear was supported by its signalling links with the Cdk5 kinase which specifically phosphorylates Pak1 on T212, a residue that is not conserved in Pak2 or Pak3. Extinction was found to correlate with the reduction of membrane associated Cdk5 activity and decreased phosphorylation of Pak1 on T212 [89]. Furthermore, inhibition of Cdk5 or Rac facilitated extinction, revealing their inhibitory effects on this process. Surprisingly, membrane associated Pak1 activity was found to be upregulated during extinction while inhibition of Rac or Cdk5 resulted in the redistribution of total Pak1 from the membrane to the cytosol of hippocampal neurones. Significantly, the immunological measure of Pak1 activity was determined using an antibody raised against phosphorylated T423 which has equal affinity towards activated Pak1, 2 and 3 and may also cross-react with other related kinases. Together these results highlight the likely involvement of Pak1 in memory storage and reduction of fear, however further research is required to fully understand its function and evaluate its potential for therapeutic targeting in post-traumatic stress disorders.

Cognitive impairment can occur due to signalling defects in the developing embryo. Down syndrome (DS) is the most common disorder occurring in live human births. It is associated with mental retardation as all individuals with DS have some level of cognitive dysfunction. DS results from the trisomy of chromosome 21q, leading to the overexpression of at least 170 known proteins. Understanding the molecular mechanisms of this disorder has consequently been highly complex. Among the upregulated genes, a significant number are known to affect cytoskeletal organisation. Therefore, the phenotypic characteristics of the disorder include anatomical defects of the CNS such as reduced volumes of the hippocampus, cerebellum and prefrontal cortex. At a cellular level, reduced neuronal densities are evident in the hippocampus and basal forebrain, while morphologically neurones of the hippocampus display reduced branching and lower densities of dendritic spines [91]. One of the proteins linked to DS is the Down syndrome cell adhesion molecule (DSCAM) which is thought to function as a cell surface receptor, mediating axonal growth and pathfinding as well as dendritic morphology [92–94]. Interestingly, DSCAM was found to directly interact with Pak1 and stimulate its activation by Rac which further promoted phosphorylation of the c-jun N-terminal kinase (JNK) [95]. Similar results were originally observed in *Drosophila* where DSCAM regulates axonal pathfinding of the Bolwig's nerve in a Dock (homologue of Nck) and DPak dependent manner [96]. It is likely that the diverse pathology associated with DS occurs due to direct and indirect consequences of genetic duplication of 21q. Consequently, it is highly possible that changes in Pak1-associated signalling may contribute to its complexity and may ultimately be considered in future therapeutic strategies.

Changes in Pak1 Activation Accompany Neurodegeneration

Recent evidence suggests that signalling molecules which are essential for cytoskeletal remodelling and correct forebrain development are frequently altered during neurodegeneration. For instance, molecular changes which underlie forms of refractory epilepsy in humans, such as focal cortical dysplasia, may increase the susceptibility of these individuals to neuronal death and neurodegeneration [97, 98]. DS is currently one of the best known examples where approximately 50% of affected individuals will develop dementia associated with AD by the age of 40 [99]. The most obvious cause of this is genetic duplication and consequential overexpression of the amyloid precursor protein (APP) causing early accumulation of amyloid plaques. However, the contribution of other factors such as defective synaptic function is also likely [100]. In confirmation, in AD, the loss of dendritic spines, dendrites and synaptic function correlates more tightly than neuronal death with the emergence of cognitive defects [101, 102]. Interestingly, examination of forebrain samples obtained from AD patients suggested that changes in the expression and activation of Pak 1 or Pak3 accompany the pathology of AD [90, 103]. Thus, significant loss of Pak1 and Pak3 expression was observed in the hippocampi and temporal lobes of individuals with AD, when compared to age, gender and post mortem delay matched controls. Further decreases were also evident in the levels of phosphorylated or activated forms of the Group I kinases which exceeded the reductions expected purely from protein down-regulation [90]. These results suggested that molecular changes occur in AD that affects Pak kinase activation as well as their expression. Altered Pak kinase activation was subsequently seen to correlate with the progression of the disease. Thus, individuals with a clinical diagnosis of early AD (Braak stages I and II) exhibited temporary hyperactivation of the Group I Pak kinases in their hippocampi, which subsequently declined as the disease progressed. Consequently, individuals with clinically severe AD (Braak stages V and VI) had the lowest levels of activated Pak1–3 kinases [103]. Interestingly, the appearance of pathogenic intracellular inclusion bodies of unphosphorylated cofilin closely correlated with the alterations in Pak levels and activity, indicative of corresponding molecular consequences and changes in F-actin organisation [90].

Studies using in vitro setups and animal models have further confirmed that altered Group I Pak kinase expression may account for the synaptic loss and neuronal death which mark the degenerative processes associated with AD (Fig. 6). Interestingly, Pak1 has been shown to be required for the survival of a number of cell types including

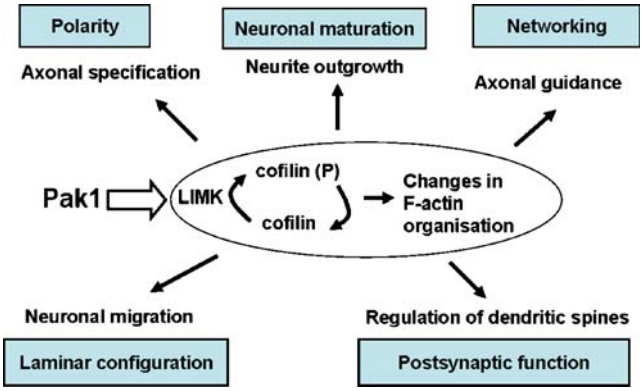

Fig. 6 Activation of Pak1 affects a number of processes in the developing CNS. Many of these processes have been shown to result from the consequential activation of LIM kinase (LIMK) and phosphorylation of cofilin. These include the specification of a single axon and consequential neuronal polarisation; the promotion of neurite outgrowth and thus morphological maturation; the ability of axons to navigate through complex territories and thus guidance and connectivity; the morphological changes required for neurones to migrate from their points of origin to their final destinations; the formation and shapes of dendritic spines and, thus, the functionality of postsynaptic sites

cerebellar granule neurones. Thus, its constitutive activation protects against apoptosis induced by exposure of granule neurones to low potassium media, while expression of a dominant negative mutant enhances cell death [51]. In contrast, Pak3 was found to interact with APP in vitro and in cultured neurones via a domain that lies adjacent to it Rac/Cdc42 binding region [104]. All Group I Pak kinases are highly conserved in this region suggesting that Pak1 and Pak2 likely associate with APP. The interaction was proposed to contribute to the increased neuronal death caused by expression of mutated APP that in humans strongly predisposes to familial AD. Pathological cleavage products of APP such as the insoluble Aβ aggregates can affect the activation and localisation of activated Group I Pak kinases both in vivo in transgenic mice as well as in cultured hippocampal neurones [90]. Similar to the observations made in human brain samples, a transient increase and subsequent down-regulation of Group I Pak kinase activation was observed in transgenic mice overexpressing APP [103]. This was, however, not apparent upon expression of a mutated APP that was resistant to pathological cleavage by caspases, suggesting a direct link between the effects of Aβ and changes in the catalytic function of the Pak kinases. In confirmation, some of the molecular consequences of Aβ exposure, such as the decrease in drebin levels were prevented by the overexpression of active Pak1, suggesting that reduced Pak function may enhance neurodegenerative cytoskeletal pathologies [90].

A clear demonstration that developmental and degenerative processes are linked come from the studies of

amyotrophic lateral sclerosis (ALS), a debilitating neurodegenerative disorder associated with spastic paralysis and early mortality. Much research has been carried out to determine the genetic causes and molecular pathways responsible for motor neuronal death in ALS, recently revealing the link between deletion of the *Als2*/*Alsin* gene and the occurrence of rare juvenile forms of ALS [105, 106]. Als2/Alsin encodes a Rac and Rab5 activator, the GTPase exchange factor Alsin. Interestingly, Alsin was seen to co-localise with Rac in the growth cones of cultured hippocampal neurones. Furthermore, expression of Alsin in cortical neurones stimulated neurite outgrowth while in vitro Alsin promoted Pak1 activation [107]. Together, these data suggest that some of the consequences of Alsin loss may be due to the impaired activation of Pak1 in the developing spinal cord motor neurones. However, due to the multitude of Rac effectors, it remains to be determined exactly to what extent reduction of Pak1 contributes to the pathology of ALS.

Together, these findings clearly place the Group I Pak kinases, including Pak1, as likely targets for future investigations into novel strategies for the prevention and treatment of neurodegenerative pathologies. It is, however, clear that much research is needed before we could consider Pak1 as a therapeutic target in AD or ALS.

Pak1 and Cancers of the CNS

The majority of primary CNS tumours originate in glial cells or their precursors and can occur throughout life. Thus, the drive to understand the molecular mechanisms that account for cellular transformation in the nervous system has been primarily directed at examining astrocytomas, oligodendrogliomas and ependiomas. The last few years have revealed an increasing interest in the oncogenic potential of the Group I Pak kinases, particularly Pak1 [21]. Pak1 expression was found to be upregulated in a number of breast, colon, bladder, pancreatic and primary brain tumours where its levels and activity can correlate with a poorer prognosis [21]. This was recently demonstrated in tumour specimens obtained from patients with glioblastomas. Examination of more than a 100 samples both by immunohistochemical and western blotting techniques revealed an upregulation of Pak1 phosphorylation on T212 which has the potential to be targeted by the Cdc2, Cdk2 or Cdk5 kinases [108]. Increased phosphorylation correlated with a reduced survival rate of the patients suggesting that Pak1 promoted tumour progression and invasiveness. In confirmation, reduction of Pak1 expression reduced the motility and invasiveness of two glioblastoma cell lines. Interestingly, in transfected cell lines, the tumour suppressor protein merlin can inhibit the Rac-mediated activation of Pak1, while loss of merlin correlates with increases in Pak1 catalytic activation. Importantly, mutations of the gene encoding for merlin (*Nf2*) is linked to the onset of neurofibromatosis, a cancer of Schwann cells in the peripheral nervous system (PNS). It is, therefore, likely that consequential changes in Pak1 activity contribute towards the oncogenic transformation of these cells. Furthermore, similar alterations in signalling may account for oncogenic transformation in both the PNS and CNS.

Conclusions and Perspective

It has become apparent that Pak1 can have important roles in multiple stages of CNS development. These range from very early processes such as fate specification to late events including the morphological maturation and synaptic connection between neurones. The common factor in all of these biological events is the control that Pak1 exerts on the dynamic organisation of the F-actin and microtubule cytoskeleton. In many cases the upstream and downstream signalling pathways involved with Pak1 signalling in the CNS are currently poorly characterised. A theme is emerging where the indirect regulation of cofilin is commonly involved (for instance in the control of neuronal morphology and in AD; Fig. 7) [61, 100]. Nevertheless, this may only reflect the highly established nature of the signalling pathway and consequential availability of good reagents for the analysis of cofilin activity. It, therefore, remains highly likely that Pak1 affects a multitude of cytoskeletal regulators and that the balance of these

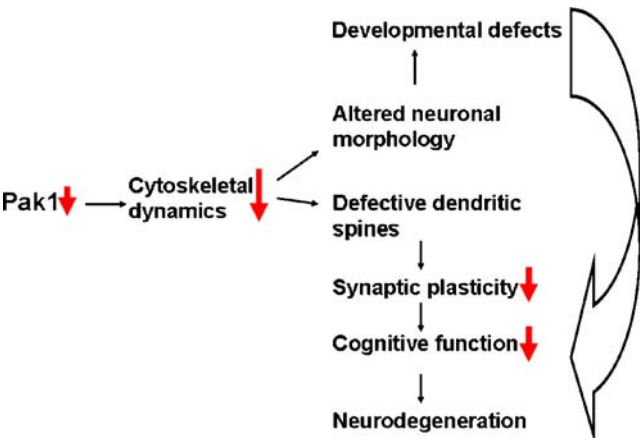

Fig. 7 The known consequences of loss of Pak1 expression in the CNS. Pak1 is required for regulated restructuring of the F-actin and microtubule cytoskeleton. In its absence, alterations take place that affect neuronal migration, morphology and the elaboration of dendritic spines. The consequences on development, postsynaptic function and cognition are considerable and can be associated with predisposition to neurodegenerative pathologies, particularly Alzheimer's Disease

effects varies in different systems and is developmentally specific. Subsequent molecular studies are therefore required as well as the continued use and development of mouse models to study particular stages of CNS development, especially neurogenesis, neuronal migration and morphological maturation.

Another important question regards the functional redundancy between the three members of Group I kinases; Pak1, Pak2 and Pak3. Research has strongly indicated the presence of overlapping roles particularly between Pak1 and Pak3 during the control of dendritic spine morphology and consequential cognitive function [82]. However, these are difficult issues to address in a truly in vivo context and so far have not been completely clarified. Where it is evident that both Pak1 and Pak3 control dendritic spines, from the genetic studies, in humans, it is apparent that Pak3 has a more important and likely unique role in certain areas of the hippocampus which cannot be compensated for by Pak1. On the other hand, evidence is mounting that Pak1 has important functional roles during early stages of CNS development which may not be shared with Pak2 or Pak3. This is confirmed by the presence of unique phosphorylation sites on the individual kinases (for instance T212 on Pak1) [37]. Thus, specific targeting of Pak1 on T212 by Cdk5 has clearly demonstrated the potential for its unique function in higher cognitive function as demonstrated by the effects on extinction in mice [89]. The consequential potential for specifically targeting particular phosphorylated forms of Pak1 to for instance treat depression or post traumatic disorders is considerable. It also provides clear evidence for unique non-overlapping roles of the three Group I kinases in the CNS.

Since Pak1 function is required for continued cognitive function, it is likely that the enhancement of localised Pak1 hyperactivation may be beneficial in the treatment of post-traumatic disorders and for the prevention of progressive neurodegeneration. The fascinating and highly challenging aspect of these investigations is the delicate balance that will be required to ensure the appropriate levels and localisation of Pak1 activation and inactivation which will have to be adjusted to specific clinical circumstances. Thus, where it may be beneficial to enhance Pak1 activity to promote our ability to deal with unpleasant memories and slow down cognitive impairment, we must also take care not to induce inappropriate cell behaviour as illustrated in the oncogenic potential of upregulated Pak1. The considerable levels of homology between Group I kinases will also require the design and availability of highly specific compounds and small molecule inhibitors that can be efficiently produced and administered. These challenges will have to be met prior to our ability to assess the real clinical potential for specifically targeting Pak1. Whether this is achievable remains an open question.

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