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Review

P21-activated kinase 4 – Not just one of the PAK

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ABSTRACT

P21-activated kinase 4 (PAK4) is a member of the p21-activated kinase (PAK) family. Historically much of the attention has been directed towards founding family member PAK1 but the focus is now shifting towards PAK4. It is a pluripotent serine/threonine kinase traditionally recognised as a downstream effector of the Rho-family GTPases. However, emerging research over the last few years has revealed that this kinase is much more than that. New findings have shed light on the molecular mechanism of PAK4 activation and how this kinase is critical for early development. Moreover, the number of PAK4 substrates and binding partners is rapidly expanding highlighting the increasing amount of cellular functions controlled by PAK4. We propose that PAK4 should be considered a signalling integrator regulating numerous fundamental cellular processes, including actin cytoskeletal dynamics, cell morphology and motility, cell survival, embryonic development, immune defence and oncogenic transformation. This review will outline our current understanding of PAK4 biology.

Introduction

PAK4 is a member of the p21-activated kinase (PAK) family of serine/threonine kinases. This family contains six mammalian PAK proteins, PAK1-6 that have been subdivided into two groups based on domain structure, sequence homology and regulation: group I PAKs (PAK1-3) and group II PAKs (4–6) (Arias-Romero and Chernoff, 2008). PAK4 was the first Group II PAK to be cloned and characterised. It was originally identified as a cytoskeletal regulatory protein, specifically controlling filopodia formation downstream of activated Cdc42 (Abo et al., 1998).

Similarly to other PAK isoforms, PAK4 contains an N-terminal PBD (p21-GTPase-binding domain) and a highly conserved C-terminal catalytic serine/threonine kinase domain. Unlike its Group I counterparts, PAK4 has much less sequence N-terminal to the PBD and distinct from other Group II PAKs does not harbour a nuclear localisation signal in this region (Fig. 1A). The central region of PAK4 also differs significantly from all other PAKs, containing three putative proline-rich SH3 (Src homology 3) domain binding sites. However as of yet, no binding partners have been identified that interact specifically with these proline-rich regions in the PAK4 sequence. There is also the presence of a RhoA GEF binding site upstream of the PAK4 kinase domain which binds the substrates GEF-H1 (Callow et al., 2005) and PDZ-RhoGEF (Barac et al., 2004), as well as the interacting partner Gab1 (Paliouras et al., 2009) (Fig. 1B). Through sequence homology it has been established that all the PAK proteins possess a potential integrin binding site within the kinase domain. However, only a direct interaction between PAK4 and an integrin cytoplasmic tail has been demonstrated (Zhang et al., 2002) (Fig. 1). Interestingly, despite differences in sequence homology between the kinase domains, and considerable sequence variation outside the kinase domain PAK1 and PAK4 share a number of substrates (Table 1) and PAK4 specific substrates have not been formally identified.

PAK4 is highly expressed throughout development (Qu et al., 2003) and is ubiquitously expressed at low levels in many adult tissues (Callow et al., 2002). Indeed, *Pak4* gene deletion results in embryonic lethality (Qu et al., 2003). Human PAK4 has orthologues in all metazoans but is not present in plants, fungi and protozoa. Interestingly, a splice variant of PAK4 lacking 154 residues (exon 4) of the full length PAK4 (591 residues) has also been identified in human HeLa and U20S cells but is absent from mouse B16 cells (Baskaran et al., 2012). At this time, the physiological significance of this shorter variant remains undetermined (Fig. 1B).

Regulation of PAK4 kinase activity

PAKs are a highly conserved group of effector proteins that are recognised as crucial regulators of Rac and Cdc42 function. Yet, the part played by the Rho GTPases in activating Group II PAKs has been a subject of much debate. The interaction between PAKs and

Abbreviations: MRLCs, myosin regulatory light chains; MLC, myosin light chain; NK, natural killer; NES, nuclear export signal; NLS, nuclear localisation signal; CRM-1, chromosome region maintenance-1; UV, ultraviolet; ECM, extracellular matrix; miRNA, microRNA; SH3, Src homology domain; GEF, guanine nucleotide exchange factor; IRES, internal ribosome entry site; EGFR, epidermal growth factor receptor; PI3K, phosphatidylinositol 3-OH kinase; ATM, ataxia telangiectasia mutated.

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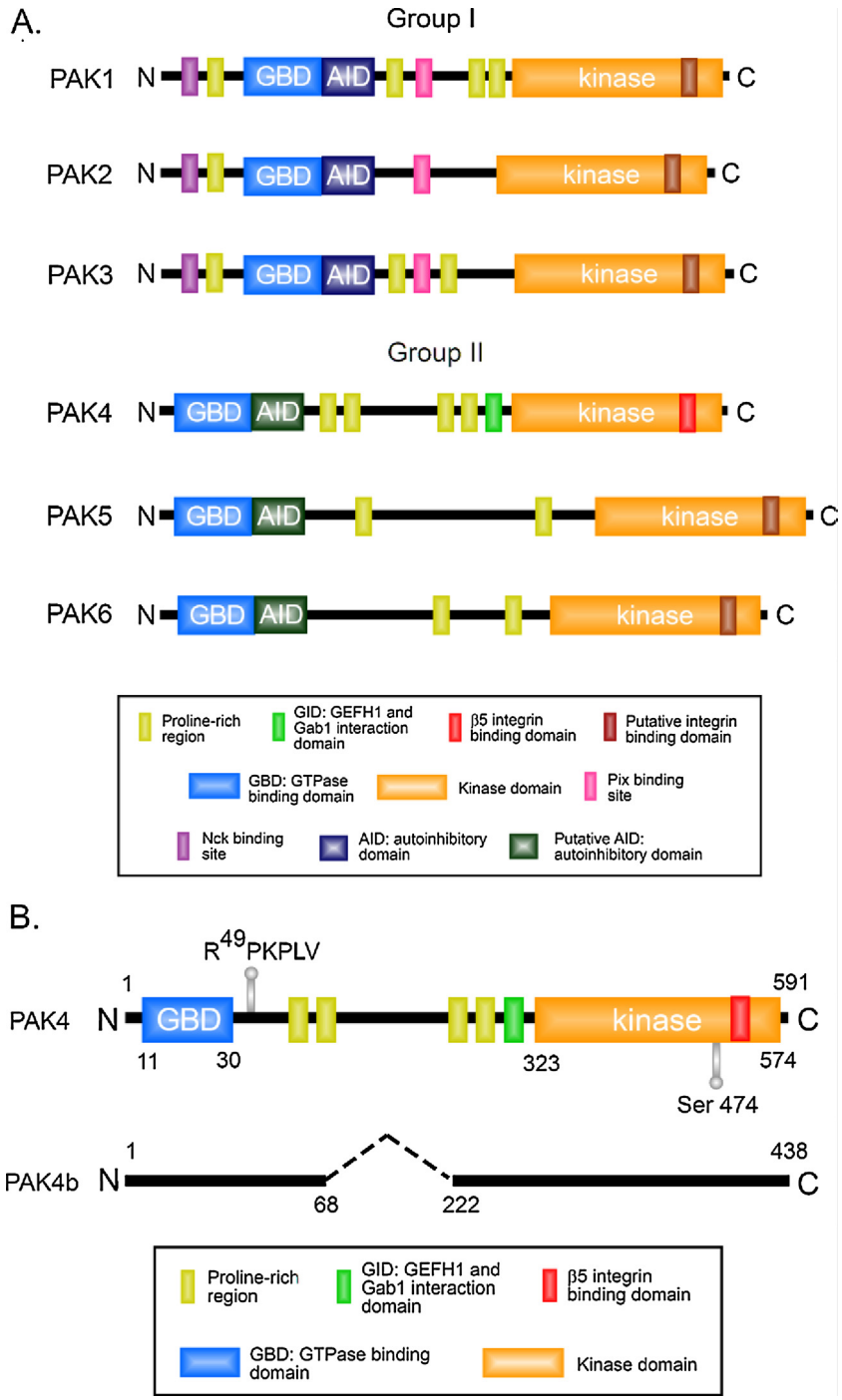

Fig. 1. Domain structure of PAK family proteins. (A) All members of the p21-activated kinase (PAK) family share a common domain architecture consisting of an N-terminal p21-GTPase binding domain (GBD) and a C-terminal serine/threonine kinase domain. Within the Group I PAKs, the GBD contains a Cdc42/Rac interactive binding region (CRIB) which overlaps with an autoinhibitory domain (AID). The Group II PAKs contain a GBD that binds Rho GTPases and a putative sequence-related AID (likely to be present in PAK5 and 6 by virtue of conserved sequences with PAK4). Variable numbers of core PxxP motifs, ligands for SH3 domain-containing proteins, are enclosed within the sequences of all the PAKs. The N-termini of Group I PAKs bind directly to the SH3 domains of Nck1/2 (indicated in purple) and Grb2 whilst a proline-rich motif in the central portion of the Group I PAKs binds PIX (indicated in pink). These consensus binding sites are not present in the Group II PAKs. A β5 integrin binding domain has been identified in the PAK4 sequence (indicated in red) and this region is highly homologous amongst PAK family members (indicated in brown). (B) The mammalian PAK4 protein comprises of an N-terminal GBD and a C-terminal kinase domain. The central region of PAK4 includes three SH3 binding sites and a GEF-H1- and Gab1-interacting domain (GID) adjacent to the kinase domain. Situated within the kinase domain resides a β5 integrin binding region. PAK4 autophosphorylation occurs at the activation loop phospho residue Ser 474 and is independent of its activation now believed to be regulated by a pseudosubstrate sequence (R^{49}PKPLV) within the N-terminal. The PAK4b splice variant lacks exon 4.

the Rho GTPases occurs *via* the p21-binding domain (alternatively known as the GTPase binding domain (GBD)) and specifically for PAK4, it has been identified to bind Cdc42 and Cdc42-like GTPases, TC10, TCL and Chp (Abo et al., 1998; Aspenstrom et al., 2004). To date, no functional significance of the interaction of PAK4 with the Cdc42-like GTPases has been revealed. In addition, whilst it has been shown that PAK4 can bind Rac, it is to a much lesser extent than that of Cdc42 and on the basis of this weak interaction, is not