Cellular functions of FAK kinases: insight into molecular mechanisms and novel functions

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Summary

Focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (Pyk2) are related tyrosine kinases that have important cellular functions, primarily through regulation of the cytoskeleton. Recent studies have identified multiple molecular mechanisms that regulate cytoskeletal responses, and have provided important and exciting insights into how FAK and Pyk2 control cellular processes such as cell migration. Equally exciting are reports of novel and originally unanticipated functions of these kinases, providing the groundwork for future avenues of investigation. This Commentary summarizes some of these recent discoveries that are relevant to the control of biological responses of the cell.

Key words: FAK, Pyk2, Rho, Cell migration, Microtubules

Introduction

Focal adhesion kinase (FAK) is a cytoplasmic protein tyrosine kinase that plays an essential role during embryonic development and in the pathogenesis of human disease, including cancer and cardiovascular disease (Golubovskaya et al., 2009a; Vadali et al., 2007). FAK is expressed in most tissues and its sequence is highly conserved across species. The mouse (97% identity) and frog (90% identity) protein sequences are highly related in sequence to human FAK, and the zebrafish protein is conserved as well (79% identity). Sequence and structural analysis reveals four distinct domains within FAK: an N-terminal FERM domain; a centrally located catalytic tyrosine kinase domain; a C-terminal focal-adhesion targeting (FAT) domain (a four-helix bundle); and an unstructured proline-rich region between the catalytic and FAT domains (Fig. 1). The FERM domain docks with the catalytic domain to autoinhibit kinase activity, but also interacts with other molecules to control FAK signaling. The FAT domain and proline-rich region are also docking sites for binding partners that function in localization and downstream signaling (Fig. 1). Proline-rich tyrosine kinase 2 (Pyk2) is related to FAK in sequence (46% identical and 65% similar, at the protein level) and structure, but is a nonessential gene. Its tissue expression is more restricted compared with that of FAK. Pyk2 shares the four-domain organization with FAK.

FAK expression is required in several tissues for normal development. In endothelial cells, FAK is required for embryonic angiogenesis and FAK expression is necessary for proper development of the heart (Braren et al., 2006; Hakim et al., 2007; Shen et al., 2005). In the adult heart, FAK plays a role in the hypertrophic response, although its precise function has not been resolved (DiMichele et al., 2006; Peng et al., 2006). In neurons, FAK functions downstream of netrins to promote neurite outgrowth and axon guidance (Li et al., 2004; Liu et al., 2004; Ren et al., 2004). FAK is overexpressed (but not mutated) in a number of human malignancies and many lines of investigation support a role for FAK in the development of human cancer (Golubovskaya et al., 2009a). Analysis of *Pyk2*-knockout mice reveals a role for this kinase in macrophages and osteoclasts (Gil-Henn et al., 2007; Okigaki et al., 2003). There is also some evidence suggesting that Pyk2 might control some phenotypes of cancer cells.

FAK responds to extracellular stimuli, including signals from the extracellular matrix (ECM), and regulates cellular processes such as proliferation and cell migration (Mitra et al., 2005). Alterations in these cellular processes are the key events associated with the development and progression of disease. This article focuses on recent findings about the mechanisms used by FAK to control cellular events; selected older findings are only included for context. Topics covered include advances in understanding established functions of FAK (e.g. control of the actin cytoskeleton and adhesion) and emerging functions of FAK (e.g. regulating microtubules and mitosis), and the identification of unanticipated FAK functions (e.g. in the nucleus). A second theme of the article is a comparison of FAK and Pyk2 function in controlling cellular events. Not surprisingly, some functions are shared, but the two kinases can be regulated differently, even in response to the same stimulus, and a similar biological endpoint can be reached by both kinases through distinct mechanisms.

FAK and cell migration

New developments in an old story

FAK has long been known as a regulator of cell migration. In most experimental systems [but not all (Yano et al., 2004)], enhanced FAK signaling promotes cell motility, whereas inhibition of FAK signaling through a variety of approaches impairs cell migration (Mitra et al., 2005). FAK functions in controlling motility in randomly migrating cells and in response to a broad range of stimuli, including chemotactic, haptotactic and durotactic signals. In the simplest terms, the cell must perform three tasks to migrate: first, it must decide where it wants to go (and this applies to even randomly migrating cells); second, cells must generate protrusive activity at the leading edge to move; and third, cells must retract the trailing edge to achieve net migration. FAK has now been implicated as functioning in all three of these tasks (Fig. 2).

In a wounded monolayer of cells in culture, cells at the edge of the wound re-orient and polarize, which is measured by

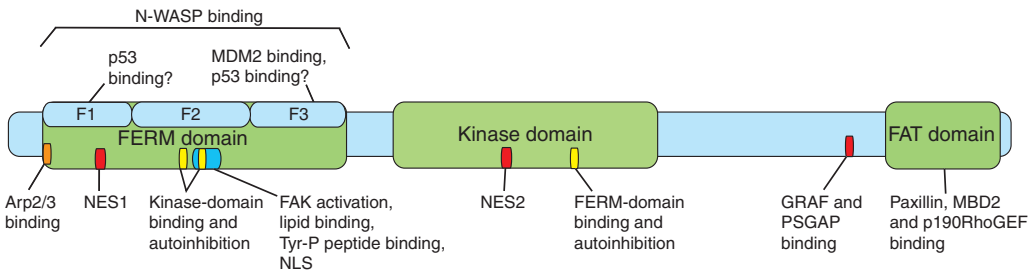

Fig. 1. Molecular structure of FAK. The major domains of FAK (FERM, kinase and FAT domains) are shown. The boundaries of the three subdomains (F1, F2 and F3) of the FERM domain are illustrated. Putative nuclear export sequences (NESs) and the nuclear localization sequence (NLS) are indicated. Regions of FAK implicated in mediating intramolecular and intermolecular protein interactions are also shown.

relocalization of the Golgi to the side of the nucleus closest to the wound. There is some evidence suggesting that this event is important for directional motility into the wound; perturbation of proteins whose primary function is regulation of Golgi structure alters relocalization of the Golgi and impairs cell migration in a wound-healing assay (Yadav et al., 2009). Whether this is a universal event associated with directional motility is clearly questionable, as there is no correlation between Golgi location and direction of movement in randomly migrating fibroblasts (Uetrecht and Bear, 2009). Regardless, there is a defect in relocalization of the Golgi in wounded monolayers of cells lacking FAK (Tilghman et al., 2005; Tomar et al., 2009). In the absence of FAK, cells at the edge of the wound fail to form prominent lamellipodia that protrude into the wound. Interestingly, Pyk2 also functions in controlling cell polarization; macrophages that lack Pyk2 are defective in establishing a polarized morphology in response to a chemotactic stimulus (Okigaki et al., 2003). These findings show a role for FAK and Pyk2 in the establishment of cell polarity in response to a stimulus to migrate (Fig. 2).

Time-lapse video microscopy of individual cells migrating in two dimensions reveals a defect in directional persistence in cells with impaired FAK function (Owen et al., 2007; Wang et al., 2001). Analysis of lamellipodial behavior in macrophages lacking FAK reveals increased protrusion and retraction of lamellipodia, but defective persistence compared with wild-type macrophages (Owen et al., 2007). Pyk2 also functions in the control of macrophage motility; *Pyk2*-null cells also exhibit migration defects (Okigaki et al., 2003). Inhibition of FAK or Pyk2 expression in macrophages impairs colony-stimulating factor-1 (CSF-1)-induced invasion by about 60%, but simultaneous inhibition of the expression of both does not inhibit invasion further (Owen et al., 2007). Thus, both FAK and Pyk2 are required for invasion and, in this scenario, appear to function non-redundantly. These studies demonstrate a role for FAK and Pyk2 in controlling macrophage migration, and a role for FAK in regulating events at the leading edge of migrating cells (Fig. 2).

There is also evidence that FAK functions at the trailing edge of the cell to control tail retraction. In wild-type fibroblasts treated with lysophosphatidic acid (LPA), focal adhesions at the rear are pulled forward as the cell moves (perhaps preceding disassembly). By contrast, in cells with impaired FAK expression owing to RNAi-based knockdown, focal adhesions at the rear of cells are stable (Iwanicki et al., 2008). In some scenarios, for example, in *FAK*-null fibroblasts, Pyk2 can compensate to promote normal cell retraction. Elimination of Pyk2 expression under these circumstances dramatically impairs tail retraction (Lim et al., 2008b). *Pyk2*-null macrophages also exhibit a defect in tail retraction in response to a chemotactic stimulus (Okigaki et al., 2003). These studies demonstrate a role for FAK and Pyk2 at the trailing edge of motile cells (Fig. 2).

FAK and the actin cytoskeleton

Regulation of Rho family proteins – multiple mechanisms

As described above, FAK has a role in regulating cell migration, but what are the molecular mechanisms that are used by FAK to control these cellular responses? One established FAK-dependent mechanism controlling cell migration is regulation of the disassembly of focal adhesions (Ren et al., 2000; Webb et al., 2004). At the rear of the cell, FAK is required for the forward movement of focal adhesions and presumably subsequent disassembly to allow tail retraction (Iwanicki et al., 2008); at the leading edge of the cell, FAK is required for the efficient disassembly of adhesions and also for the assembly of adhesions (Owen et al., 2007) (Fig. 2). One potential mechanism regulating adhesion assembly is regulation of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] generation at sites of adhesion (Ling et al., 2002), which in turn could regulate talin-dependent activation of integrin-ligand binding (Nayal et al., 2004). Other studies exploring the mechanism of regulation of cytoskeletal events by FAK and Pyk2 have focused on the regulation of Rho family proteins.

FAK-null fibroblasts exhibit elevated levels of active Rho (Ren et al., 2000). In macrophages, the absence of FAK leads to a modest

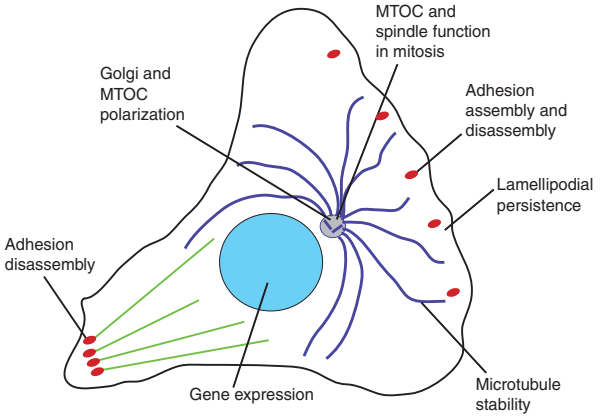

Fig. 2. Major cellular functions of FAK. A migrating cell with the leading edge (right) and trailing edge (left) is shown. Cell-ECM adhesions (red), stress fibers (green), microtubules (dark blue), the MTOC (grey) and nucleus (blue) are illustrated. Black lines denote cellular targets of FAK signaling.

increase in Rac activity (Owen et al., 2007). By contrast, *Pyk2*-null macrophages exhibit a defect in Rho activation in response to chemokines (Okigaki et al., 2003). The role of Pyk2 in controlling Rho activity is clearly context dependent, as *Pyk2*-null osteoclasts exhibit the opposite phenotype: an increase in Rho activity (Gil-Henn et al., 2007). Thus, there appear to be signal-dependent and/or cell-type-dependent mechanisms regulating Rho activity downstream of Pyk2.

Multiple mechanisms are employed by FAK to regulate the activation status of Rac and Rho (Fig. 3). A GTPase-activating protein (GAP) called GRAF (GTPase regulator associated with FAK) binds to the C-terminal domain of FAK and associates with Pyk2 as well (Hildebrand et al., 1996; Ohba et al., 1998). Whereas GRAF promotes hydrolysis of GTP bound to both Rho and Cdc42 in vitro, expression of GRAF in cells blocks the actin-cytoskeleton changes that are associated with Rho activity (formation of stress fibers) and does not affect filopodia formation, which is dependent on Cdc42 activity. This suggests a more restricted substrate specificity in vivo (Taylor et al., 1999). A second GAP that binds FAK and Pyk2 is PSGAP (PH- and SH3-domain-containing RhoGAP protein) (Ren et al., 2001). PSGAP promotes hydrolysis of GTP bound to both Rho and Cdc42 in vitro and in vivo. Interestingly, Pyk2, but not FAK, can phosphorylate PSGAP to impair GAP activity towards Cdc42 (Ren et al., 2001). This suggests different modes of signaling, whereby FAK recruits PSGAP to impair Rho and Cdc42 signaling, and Pyk2 recruits PSGAP for phosphorylation and inhibition of its activity, thus promoting Cdc42 activity. The roles of GRAF and PSGAP in regulating the cytoskeletal events that are controlled by FAK and Pyk2 have not been fully explored. A third GAP, p190RhoGAP, can be recruited into a complex with p120RasGAP and FAK at focal adhesions (Tomar et al., 2009). Both p190RhoGAP and p120RasGAP function in controlling FAK-dependent cytoskeletal changes, as they are both required for proper polarization of cells at the edge of a wound (Tomar et al., 2009).

FAK and Pyk2 also associate with regulatory proteins that promote the activation of Rho family signaling (Fig. 3). FAK binds the Rho-activating guanine-nucleotide exchange factor (GEF) PDZRhoGEF, which colocalizes with FAK (Iwanicki et al., 2008). Impairing PDZRhoGEF using a short hairpin RNA (shRNA) approach retards the movement of focal adhesions at the rear of the cell. Expression of exogenous PDZRhoGEF overcomes the defect in the movement of adhesions in cells caused by perturbing FAK expression (Iwanicki et al., 2008). Thus, PDZRhoGEF appears to function with FAK to promote the forward movement of focal adhesions at the rear of migrating cells. FAK also associates with a second exchange factor, p190RhoGEF (Lim et al., 2008b; Zhai et al., 2003). A fraction of p190RhoGEF localizes to focal adhesions, and it also appears to function in conjunction with FAK to control focal-adhesion turnover and migration (Lim et al., 2008b). A mutant of p190RhoGEF that is defective for FAK binding exhibits impaired biological activity, supporting a role for p190RhoGEF in controlling FAK-dependent cytoskeletal responses (Lim et al., 2008b). Pyk2 can also bind to p190RhoGEF to promote Rho activation. The mechanism is slightly different, however, as FAK-p190RhoGEF localization to focal adhesions is important for function, whereas localization of Pyk2-p190RhoGEF to focal adhesions is not required (Lim et al., 2008b). Based on these recent findings that RhoGEFs and RhoGAPs associate with FAK and function in the control of cytoskeletal changes downstream of FAK, the intriguing hypothesis that FAK might alternately control the activation and inactivation of Rho was recently suggested (Tomar and Schlaepfer, 2009).

Recent studies demonstrate that FAK might function in the regulation of cytoskeletal events through the modulation of the paxillin kinase linker (PKL/Git2)–β-pix complex (Fig. 3). β-pix is an exchange factor for Cdc42 that also functions as a scaffold to promote signaling via Rac and PAK (Frank and Hansen, 2008). Thus, β-pix potentially functions to coordinate Cdc42 and Rac signaling. At the cellular level, β-pix is an important regulator of the polarity of migrating cells and lamellipodia formation (Frank and Hansen, 2008). PKL/Git2 targets β-pix to focal adhesions through binding to paxillin (Turner et al., 1999). FAK regulates tyrosine phosphorylation of β-pix, which in turn regulates the

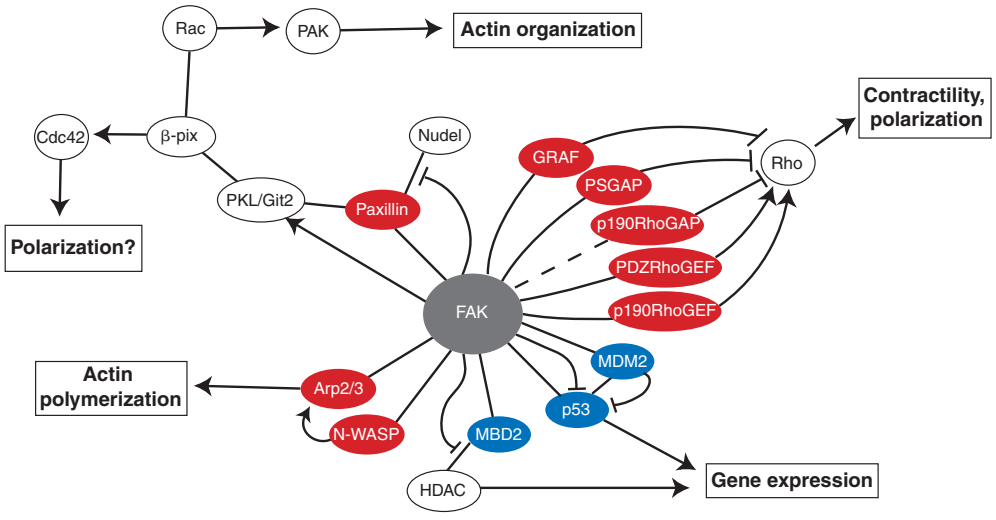

Fig. 3. Networks of FAK signaling that control the actin cytoskeleton and gene expression. Solid lines link proteins that physically interact and dashed lines denote indirect protein-protein interactions. Red proteins are FAK binding partners that are implicated in the regulation of the actin cytoskeleton and blue proteins are FAK binding partners that are implicated in regulating gene expression. Positive (arrows) and negative (bars) regulation of downstream proteins and/or events is indicated.

recruitment of Rac1 into a complex with β-pix (Chang et al., 2007). PKL/Git2 plays a key role in motility by regulating cellular polarization, stabilizing lamellipodia formation and promoting direction persistence in migrating cells (Yu et al., 2009). Tyrosine phosphorylation of PKL/Git2 is required for these functions, and FAK and Src have been implicated as the responsible kinases (Yu et al., 2009). These findings demonstrate additional mechanisms through which FAK can function in the regulation of Cdc42 and Rac activity to control cellular polarization, the extension of lamellipodia and cell migration (Fig. 2).

FAK regulation of the cytoskeleton through other mechanisms

N-WASP (neuronal Wiskott-Aldrich syndrome protein), which functions to activate the Arp2/3 complex to promote both actin branching and nucleation of F-actin assembly, associates with, and is tyrosine phosphorylated by, FAK (Wu et al., 2004). Whereas FAK phosphorylation has little effect on N-WASP activity in an in vitro actin polymerization assay, this observation is interesting in light of the role of FAK in regulating lamellipodia projection (Fig. 2). It is also intriguing that FAK can directly associate with the Arp2/3 complex (Serrels et al., 2007) (Fig. 3). This complex appears to be restricted to the leading edge of cells and is not found in focal adhesions. Furthermore, the Arp2/3 complex fails to bind to autophosphorylated FAK, suggesting it is released from FAK upon its activation (Serrels et al., 2007). These two independent observations raise the possibility that one function of FAK might be the recruitment of Arp2/3 to the leading edge and the coordinated activation of FAK, release of the Arp2/3 complex and the activation of a key regulator of Arp2/3 activity to promote lamellipodia formation. This mechanism is highly speculative and further studies are clearly required to fully test the hypothesis.

A noncatalytic mechanism of regulation of the strength of cell-matrix adhesion by FAK was recently proposed. This mechanism involves Nudel, a dynein-associated protein that is linked to the regulation of membrane trafficking, mitosis and cell migration (Liang et al., 2004; Liang et al., 2007; Shen et al., 2008). Nudel binds to, and co-localizes with, paxillin at the leading edge of the cell in nascent adhesions, but not in focal complexes and adhesions (Shan et al., 2009) (Fig. 3). Interestingly, Nudel binds to the LIM-domain-containing C terminus of paxillin, the region of the protein that is required for focal-adhesion localization (Brown et al., 1996). Artificial targeting of Nudel to focal adhesions by the creation of a paxillin-Nudel chimeric protein produces large focal adhesions and increases cell adhesion to the ECM. The binding of FAK to N-terminal LD motifs of paxillin somehow displaces Nudel from the C terminus of paxillin. Although the molecular mechanism of displacement is not clear, this is proposed as a mechanism to modulate the adhesiveness of cell-matrix adhesions as they form at the leading edge of the cell and mature (Shan et al., 2009).

The emerging theme from studies examining the control of cell migration by FAK is that multiple downstream targets are involved in regulating cytoskeletal responses (Fig. 3). This is not surprising, but it does present the next challenge in the discovery of the mechanisms of FAK-dependent regulation of cell motility. The questions of whether different mechanisms are dominant in specific situations and how different signaling events might be coordinated remain to be addressed. Furthermore, additional mechanisms controlling the temporal and spatial regulation of these different downstream signaling events will undoubtedly become the focus of future mechanistic studies.

FAK, mechanosensation, migration and proliferation

In addition to its role in regulating cell migration, FAK regulates cell proliferation through a number of mechanisms that impinge on central cell-cycle regulatory molecules (Cox et al., 2006). For example, FAK promotes expression of cyclin D1 and can control the levels of $p27^{Kip1}$ and $p21^{Cip1}$, in part by controlling SKP2-dependent ubiquitination and degradation. FAK also responds to mechanical stimulation, for example, becoming activated in endothelial cells in response to shear stress and mechanical stretch (Vadali et al., 2007). Recent studies have implicated FAK in controlling changes in migration and proliferation in response to mechanical stimulation. Fibroblasts sense the stiffness of an underlying ECM and, at a boundary of stiff and soft matrix, they migrate onto the stiff matrix. Fibroblasts that lack FAK fail to sense the difference in stiffness and these cells continue to migrate indiscriminately onto softer or stiffer matrices (Wang et al., 2001). Cancer cells respond to the stiffness of the ECM to produce invadopodia for invasion into the matrix and FAK has been implicated in this response (Alexander et al., 2008). In three-dimensional matrices, the proliferation of both fibroblasts and epithelial-derived cells depends on the stiffness of the ECM. Proliferation is low in soft matrix and high in stiff matrix. In the absence of FAK, both cell types exhibit defects in proliferation in stiff matrices (Klein et al., 2009; Provenzano et al., 2009). These observations might be physiologically and/or pathologically relevant, as injuries that promote proliferative responses cause increases in tissue stiffness (Klein et al., 2009).

FAK kinases, cadherins and cell-cell junctions

In addition to its well-established role in cell-ECM signaling events, a role for FAK in regulating cell-cell junctions has emerged. FAK has been implicated in Src-induced epithelial-mesenchymal transition (EMT), a process that involves dissolution of cell-cell junctions, loss of epithelial markers and gain of mesenchymal markers. Expression of a FAK mutant in this model results in increased surface expression of E-cadherin and an increase in E-cadherin-based junction-like structures during a calcium switch (i.e. when cells are switched to a high-calcium-containing medium) (Avizienyte et al., 2002). In a transforming growth factor-β (TGFβ)-induced model of EMT, inhibition of FAK using a dominant-negative approach seems to impair loss of E-cadherin from the cell surface (Cicchini et al., 2008). These findings are consistent with a role for FAK in regulating the disassembly of E-cadherin-based junctions and internalization of E-cadherin. In contrast to these findings, however, FAK has been implicated in promoting efficient cell-cell junction formation. Inhibition of FAK by short interfering RNA (siRNA) in HeLa cells retarded N-cadherin-dependent cell-cell junction formation; this impaired junction formation was attributed to elevated Rac activity in the absence of FAK (Yano et al., 2004). Similarly, a role for FAK in promoting E-cadherin-dependent cell-cell junction formation in NBT-II cells was established using a number of approaches to perturb FAK signaling; however, in this case, impaired junction formation was apparently due to elevated Rho activity in the absence of FAK (Playford et al., 2008). These discordant results are yet to be reconciled, but it seems apparent that, in different contexts, FAK signaling can elicit different outcomes at cell-cell junctions. Additional factors impacting these outcomes are yet to be identified.

Pyk2 has also been implicated in regulating cell-cell junctions. In endothelial cells, Pyk2 can control disassembly of vascular

endothelial (VE)-cadherin-mediated cell-cell junctions. Two different mechanisms have been reported that appear to operate in response to different stimuli. VE-cadherin antibodies can induce a signaling event through Rac that results in the production of reactive oxygen species, Pyk2 activation and phosphorylation of β-catenin (van Buul et al., 2005); this leads to dissolution of VE-cadherin junctions. Endothelial-cell Pyk2 also functions in facilitating transendothelial migration of leukocytes (Allingham et al., 2007). Engagement of cell-surface intercellular adhesion molecule 1 (ICAM-1) results in activation of Pyk2, but the key event in this scenario is phosphorylation of the cytoplasmic tail of VE-cadherin (Allingham et al., 2007). In each case, the consequence is presumed to be abrogation of the VE-cadherin–β-catenin complex. Why different Pyk2 targets might be selected in response to different stimuli remains unclear.

In addition to regulating proximal signaling events that control cell-cell junction assembly and disassembly, FAK and Pyk2 might also contribute to alterations in cell-cell junctions by means of an indirect mechanism, that is, by regulating expression of N-cadherin. Upon attachment to collagen I, N-cadherin expression is upregulated at the protein level, cell-cell adhesion is reduced and cells scatter. Two collagen receptors, the $\alpha_2\beta_1$ integrin and DDR1 (discoidin domain receptor 1), regulate the activation of FAK and Pyk2, respectively (Shintani et al., 2008). Both signaling pathways contribute to elevation of N-cadherin protein expression and the change in cellular phenotype. Although it is clear that $p130^{cas}$ and Rap1 function downstream of FAK and Pyk2 to promote N-cadherin expression and cell scattering (Shintani et al., 2008), the mechanism regulating N-cadherin protein levels remains to be established.

FAK and microtubules

There is a slowly emerging and intriguing link between FAK and microtubules (Fig. 2). When dynamic microtubules make contact, focal adhesions disassemble and FAK plays a role in this process (Ezratty et al., 2005). This is not surprising given the role of FAK as a regulator of focal-adhesion disassembly; however, the mechanism is unexpected. Phosphorylation of tyrosine 925 and the subsequent recruitment of Grb2 and dynamin into a complex with FAK are required for microtubule-induced disassembly of focal adhesions (Ezratty et al., 2005). A more surprising finding is the link between FAK and the regulation of microtubule structure and organization. In cells lacking FAK or expressing dominant-negative FAK mutants, microtubules exhibit defects in tyrosination and acetylation, two post-translational modifications that are associated with stabilization of microtubules (Palazzo et al., 2004). FAK is proposed to modulate the organization of lipid rafts to promote mDia signaling and microtubule stabilization. Interestingly, *Pyk2*-null osteoclasts are defective for bone resorption owing to a defect in the formation of podosomes and the sealing zone abutting the underlying bone (Gil-Henn et al., 2007). Microtubules play a role in the organization of podosomes in osteoclasts and *Pyk2*-null cells exhibit a defect in stabilization of microtubules (Gil-Henn et al., 2007). Thus, Pyk2 also appears to control stabilization of microtubules. Although the mechanism remains to be established, levels of Rho activity are elevated in the absence of Pyk2 and aberrant regulation of Rho is implicated in the perturbation of microtubule stabilization in osteoclasts (Gil-Henn et al., 2007). In this regard, there are some parallels between microtubule stability and aberrant Rho activity in *FAK*-null fibroblasts and *Pyk2*-null osteoclasts, although the precise mechanistic details are yet to emerge.

FAK also seems to function at the microtubule-organizing center (MTOC) (Fig. 2). This was first observed in neurons, where FAK was identified as a substrate for phosphorylation by Cdk5 (Xie et al., 2003). Serine 732 in the C-terminal domain of FAK is the site of phosphorylation and the phosphorylated form of FAK colocalizes with γ-tubulin in the perinuclear region of the cell, that is, at the MTOC. A mutant defective for phosphorylation localizes strongly to the MTOC, suggesting that phosphorylation is important for movement of FAK away from the MTOC. The mutant functions in a dominant-negative manner and perturbs the organization of the microtubules at the MTOC (Xie et al., 2003). FAK might also play a role in organizing the MTOCs in mitotic endothelial cells. *FAK*-null endothelial cells exhibit mitotic defects, specifically, alterations in the mitotic spindle and centrosome organization (Park et al., 2009). The defects are rescued by re-expression of wild-type FAK, but not by a mutant defective for phosphorylation at serine 732. As in neurons, Cdk5 regulates phosphorylation of FAK at serine 732 and the phosphorylated form of FAK co-localizes with γ-tubulin at the centrosome (Park et al., 2009). Phosphorylation of serine 732 seems to regulate the association of FAK with dynein in the mitotic centrosome. These studies clearly link FAK and Pyk2 to the regulation of microtubule structure and function, which in turn controls biologically significant events. Although some insight into mechanism has been gained, this is an underdeveloped area of investigation that warrants additional studies.

FAK functions in the nucleus

Initial reports first suggested a nuclear function for FAK when N-terminal fragments of FAK were found to localize in the nucleus (Jones et al., 2001; Lobo and Zachary, 2000). Additional studies revealed that full-length FAK and Pyk2 could localize to the nucleus under appropriate conditions and in a number of different cell types (Aoto et al., 2002; Yi et al., 2003). Recent evidence has shed some light on the mechanisms regulating nuclear localization and nuclear function. Consistent with the initial finding that the N-terminal domain of FAK could be found in the nucleus, basic sequences within the N-terminal FERM domain have been implicated as a nuclear localization signal in FAK (Lim et al., 2008a) (Fig. 1). However, both full-length FAK and the C-terminal domain of FAK can be localized to the nucleus in cardiomyocytes (Yi et al., 2003). This implies an alternative mechanism because the C-terminal domain lacks the N-terminal nuclear localization sequences, but a potential mechanism has not been defined. Export from the nucleus might be regulated by two nuclear export sequences, one of which lies in the N-terminal FERM domain and the other in the catalytic domain (Ossovskaya et al., 2008) (Fig. 1).

Recent studies suggest multiple important nuclear functions for FAK. Two groups provide evidence that FAK functions to impair transcription of p53-dependent genes (Golubovskaya et al., 2005; Lim et al., 2008a). This is mediated by FAK binding to p53 in the nucleus, potentially resulting in attenuation of its transactivating potential (Golubovskaya et al., 2005). FAK also regulates nuclear p53 through a second mechanism by scaffolding the p53-MDM2 complex, leading to p53 ubiquitination and degradation (Lim et al., 2008a) (Fig. 3). These results support a role for FAK in controlling the expression of specific genes, but there is also evidence that FAK regulates gene expression at a more global level by controlling chromatin structure. Nuclear FAK binds to MBD2 (methyl CpG-binding protein 2) (Fig. 3), which binds methylated DNA sequences to coordinate acetylation and changes in chromatin structure (Luo et al., 2009). FAK binding reduces histone deacetylase 1 (HDAC1)

binding to MBD2, which might be important for inducing myogenin expression in differentiating muscle cells. It is also interesting to note that FAK is required for the expression of genes that encode mesenchymal markers in the TGFβ-induced EMT model described above, although no mechanistic details are available (Cicchini et al., 2008). These findings implicate FAK in regulating gene expression by more direct mechanisms than originally expected (Fig. 2) and will provide the impetus for studies to identify FAK-regulated genes, which are beginning to appear in the literature (Golubovskaya et al., 2009b; Provenzano et al., 2008).

Concluding remarks

FAK and Pyk2 are the focus of attention of many laboratories that are trying to determine how FAK and Pyk2 regulate the actin cytoskeleton, particularly through Rho family GTPases. As outlined above, these efforts have yielded strong evidence supporting multiple molecular mechanisms that link FAK and Pyk2 to Rho family signaling. Presented individually, they appear as competing means to accomplish the same goal. Unique responses to different stimuli or differential spatial and temporal regulation of each signaling event might provide one reason for the evolution of different mechanisms of regulation. Alternatively, these different downstream signaling molecules might provide nodes for input from other signaling pathways, allowing enhancement or dampening of FAK-dependent signals based on other stimuli experienced by the cell.

Provocative new studies have also emerged that solidify roles for FAK in controlling microtubules and in the nucleus. Studies of nuclear FAK have now established functional relevance for its observed localization in the nucleus. Whether these functions of FAK are universal and as important as its established role in the regulation of the actin cytoskeleton remain to be established, but they are certainly novel areas of focus for future investigation.

References

Alexander, N. R., Branch, K. M., Parekh, A., Clark, E. S., Iwueke, I. C., Guelcher, S. A. and Weaver, A. M. (2008). Extracellular matrix rigidity promotes invadopodia activity. *Curr. Biol.* **18**, 1295-1299.

Allingham, M. J., van Buul, J. D. and Burridge, K. (2007). ICAM-1-mediated, Src- and Pyk2-dependent vascular endothelial cadherin tyrosine phosphorylation is required for leukocyte transendothelial migration. *J. Immunol.* **179**, 4053-4064.

Aoto, H., Sasaki, H., Ishino, M. and Sasaki, T. (2002). Nuclear translocation of cell adhesion kinase beta/proline-rich tyrosine kinase 2. *Cell Struct. Funct.* **27**, 47-61.

Avizienyte, E., Wyke, A. W., Jones, R. J., McLean, G. W., Westhoff, M. A., Brunton, V. G. and Frame, M. C. (2002). Src-induced de-regulation of E-cadherin in colon cancer cells requires integrin signalling. *Nat. Cell Biol.* **4**, 632-638.

Braren, R., Hu, H., Kim, Y. H., Beggs, H. E., Reichardt, L. F. and Wang, R. (2006). Endothelial FAK is essential for vascular network stability, cell survival, and lamellipodial formation. *J. Cell Biol.* **172**, 151-162.

Brown, M. C., Perrotta, J. A. and Turner, C. E. (1996). Identification of LIM3 as the principal determinant of paxillin focal adhesion localization and characterization of a novel motif on paxillin directing vinculin and focal adhesion kinase binding. *J. Cell Biol.* **135**, 1109-1123.

Chang, F., Lemmon, C. A., Park, D. and Romer, L. H. (2007). FAK potentiates Rac1 activation and localization to matrix adhesion sites: a role for betaPIX. *Mol. Biol. Cell* **18**, 253-264.

Cicchini, C., Laudadio, I., Citarella, F., Corazzari, M., Steindler, C., Conigliaro, A., Fantoni, A., Amicone, L. and Tripodi, M. (2008). TGFbeta-induced EMT requires focal adhesion kinase (FAK) signaling. *Exp. Cell Res.* **314**, 143-152.

Cox, B. D., Natarajan, M., Stettner, M. R. and Gladson, C. L. (2006). New concepts regarding focal adhesion kinase promotion of cell migration and proliferation. *J. Cell Biochem.* **99**, 35-52.

DiMichele, L. A., Doherty, J. T., Rojas, M., Beggs, H. E., Reichardt, L. F., Mack, C. P. and Taylor, J. M. (2006). Myocyte-restricted focal adhesion kinase deletion attenuates pressure overload-induced hypertrophy. *Circ. Res.* **99**, 636-645.

Ezratty, E. J., Partridge, M. A. and Gundersen, G. G. (2005). Microtubule-induced focal adhesion disassembly is mediated by dynamin and focal adhesion kinase. *Nat. Cell Biol.* **7**, 581-590.

Frank, S. R. and Hansen, S. H. (2008). The PIX-GIT complex: a G protein signaling cassette in control of cell shape. *Semin. Cell Dev. Biol.* **19**, 234-244.

Gil-Henn, H., Destaing, O., Sims, N. A., Aoki, K., Alles, N., Neff, L., Sanjay, A., Bruzzaniti, A., De Camilli, P., Baron, R. et al. (2007). Defective microtubule-dependent podosome organization in osteoclasts leads to increased bone density in Pyk2(–/–) mice. *J. Cell Biol.* **178**, 1053-1064.

Golubovskaya, V. M., Finch, R. and Cance, W. G. (2005). Direct interaction of the N-terminal domain of focal adhesion kinase with the N-terminal transactivation domain of p53. *J. Biol. Chem.* **280**, 25008-25021.

Golubovskaya, V. M., Kweh, F. A. and Cance, W. G. (2009a). Focal adhesion kinase and cancer. *Histol. Histopathol.* **24**, 503-510.

Golubovskaya, V. M., Zheng, M., Zhang, L., Li, J. L. and Cance, W. G. (2009b). The direct effect of focal adhesion kinase (FAK), dominant-negative FAK, FAK-CD and FAK siRNA on gene expression and human MCF-7 breast cancer cell tumorigenesis. *BMC Cancer* **9**, 280.

Hakim, Z. S., DiMichele, L. A., Doherty, J. T., Homeister, J. W., Beggs, H. E., Reichardt, L. F., Schwartz, R. J., Brackhan, J., Smithies, O., Mack, C. P. et al. (2007). Conditional deletion of focal adhesion kinase leads to defects in ventricular septation and outflow tract alignment. *Mol. Cell Biol.* **27**, 5352-5364.

Hildebrand, J. D., Taylor, J. M. and Parsons, J. T. (1996). An SH3 domain-containing GTPase-activating protein for Rho and Cdc42 associates with focal adhesion kinase. *Mol. Cell Biol.* **16**, 3169-3178.

Iwanicki, M. P., Vomastek, T., Tilghman, R. W., Martin, K. H., Banerjee, J., Wedegaertner, P. B. and Parsons, J. T. (2008). FAK, PDZ-RhoGEF and ROCKII cooperate to regulate adhesion movement and trailing-edge retraction in fibroblasts. *J. Cell Sci.* **121**, 895-905.

Jones, G., Machado, J., Jr and Merlo, A. (2001). Loss of focal adhesion kinase (FAK) inhibits epidermal growth factor receptor-dependent migration and induces aggregation of nh(2)-terminal FAK in the nuclei of apoptotic glioblastoma cells. *Cancer Res.* **61**, 4978-4981.

Klein, E. A., Yin, L., Kothapalli, D., Castagnino, P., Byfield, F. J., Xu, T., Levental, I., Hawthorne, E., Janmey, P. A. and Assoian, R. K. (2009). Cell-cycle control by physiological matrix elasticity and in vivo tissue stiffening. *Curr. Biol.* **19**, 1511-1518.

Li, W., Lee, J., Vikis, H. G., Lee, S. H., Liu, G., Aurandt, J., Shen, T. L., Fearon, E. R., Guan, J. L., Han, M., et al. (2004). Activation of FAK and Src are receptor-proximal events required for netrin signaling. *Nat. Neurosci.* **7**, 1213-1221.

Liang, Y., Yu, W., Li, Y., Yang, Z., Yan, X., Huang, Q. and Zhu, X. (2004). Nudel functions in membrane traffic mainly through association with Lis1 and cytoplasmic dynein. *J. Cell Biol.* **164**, 557-566.

Liang, Y., Yu, W., Li, Y., Yu, L., Zhang, Q., Wang, F., Yang, Z., Du, J., Huang, Q., Yao, X. et al. (2007). Nudel modulates kinetochore association and function of cytoplasmic dynein in M phase. *Mol. Biol. Cell* **18**, 2656-2666.

Lim, S. T., Chen, X. L., Lim, Y., Hanson, D. A., Vo, T. T., Howerton, K., LaRocque, N., Fisher, S. J., Schlaepfer, D. D. and Ilic, D. (2008a). Nuclear FAK promotes cell proliferation and survival through FERM-enhanced p53 degradation. *Mol. Cell* **29**, 9-22.

Lim, Y., Lim, S. T., Tomar, A., Gardel, M., Bernard-Trifilo, J. A., Chen, X. L., Uryu, S. A., Canete-Soler, R., Zhai, J., Lin, H. et al. (2008b). PyK2 and FAK connections to p190Rho guanine nucleotide exchange factor regulate RhoA activity, focal adhesion formation, and cell motility. *J. Cell Biol.* **180**, 187-203.

Ling, K., Doughman, R. L., Firestone, A. J., Bunce, M. W. and Anderson, R. A. (2002). Type I gamma phosphatidylinositol phosphate kinase targets and regulates focal adhesions. *Nature* **420**, 89-93.

Liu, G., Beggs, H., Jurgensen, C., Park, H. T., Tang, H., Gorski, J., Jones, K. R., Reichardt, L. F., Wu, J. and Rao, Y. (2004). Netrin requires focal adhesion kinase and Src family kinases for axon outgrowth and attraction. *Nat. Neurosci.* **7**, 1222-1232.

Lobo, M. and Zachary, I. (2000). Nuclear localization and apoptotic regulation of an amino-terminal domain focal adhesion kinase fragment in endothelial cells. *Biochem. Biophys. Res. Commun.* **276**, 1068-1074.

Luo, S. W., Zhang, C., Zhang, B., Kim, C. H., Qiu, Y. Z., Du, Q. S., Mei, L. and Xiong, W. C. (2009). Regulation of heterochromatin remodelling and myogenin expression during muscle differentiation by FAK interaction with MBD2. *EMBO J.* **28**, 2568-2582.

Mitra, S. K., Hanson, D. A. and Schlaepfer, D. D. (2005). Focal adhesion kinase: in command and control of cell motility. *Nat. Rev. Mol. Cell Biol.* **6**, 56-68.

Nayal, A., Webb, D. J. and Horwitz, A. F. (2004). Talin: an emerging focal point of adhesion dynamics. *Curr. Opin. Cell Biol.* **16**, 94-98.

Ohba, T., Ishino, M., Aoto, H. and Sasaki, T. (1998). Interaction of two proline-rich sequences of cell adhesion kinase beta with SH3 domains of p130Cas-related proteins and a GTPase-activating protein, Graf. *Biochem. J.* **330**, 1249-1254.

Okigaki, M., Davis, C., Falasca, M., Harroch, S., Felsenfeld, D. P., Sheetz, M. P. and Schlessinger, J. (2003). Pyk2 regulates multiple signaling events crucial for macrophage morphology and migration. *Proc. Natl. Acad. Sci. USA* **100**, 10740-10745.

Ossovskaya, V., Lim, S. T., Ota, N., Schlaepfer, D. D. and Ilic, D. (2008). FAK nuclear export signal sequences. *FEBS Lett.* **582**, 2402-2406.

Owen, K. A., Pixley, F. J., Thomas, K. S., Vicente-Manzanares, M., Ray, B. J., Horwitz, A. F., Parsons, J. T., Beggs, H. E., Stanley, E. R. and Bouton, A. H. (2007). Regulation of lamellipodial persistence, adhesion turnover, and motility in macrophages by focal adhesion kinase. *J. Cell Biol.* **179**, 1275-1287.

Palazzo, A. F., Eng, C. H., Schlaepfer, D. D., Marcantonio, E. E. and Gundersen, G. G. (2004). Localized stabilization of microtubules by integrin- and FAK-facilitated Rho signaling. *Science* **303**, 836-839.

Park, A. Y., Shen, T. L., Chien, S. and Guan, J. L. (2009). Role of focal adhesion kinase Ser-732 phosphorylation in centrosome function during mitosis. *J. Biol. Chem.* **284**, 9418-9425.

Peng, X., Kraus, M. S., Wei, H., Shen, T. L., Pariaut, R., Alcaraz, A., Ji, G., Cheng, L., Yang, Q., Kotlikoff, M. I. et al. (2006). Inactivation of focal adhesion kinase in

cardiomyocytes promotes eccentric cardiac hypertrophy and fibrosis in mice. *J. Clin. Invest.* **116**, 217-227.

Playford, M. P., Vadali, K., Cai, X., Burridge, K. and Schaller, M. D. (2008). Focal adhesion kinase regulates cell-cell contact formation in epithelial cells via modulation of Rho. *Exp. Cell Res.* **314**, 3187-3197.

Provenzano, P. P., Inman, D. R., Eliceiri, K. W., Beggs, H. E. and Keely, P. J. (2008). Mammary epithelial-specific disruption of focal adhesion kinase retards tumor formation and metastasis in a transgenic mouse model of human breast cancer. *Am. J. Pathol.* **173**, 1551-1565.

Provenzano, P. P., Inman, D. R., Eliceiri, K. W. and Keely, P. J. (2009). Matrix density-induced mechanoregulation of breast cell phenotype, signaling and gene expression through a FAK-ERK linkage. *Oncogene* **28**, 4326-4343.

Ren, X. D., Kiosses, W. B., Sieg, D. J., Otey, C. A., Schlaepfer, D. D. and Schwartz, M. A. (2000). Focal adhesion kinase suppresses Rho activity to promote focal adhesion turnover. *J. Cell Sci.* **113**, 3673-3678.

Ren, X. R., Du, Q. S., Huang, Y. Z., Ao, S. Z., Mei, L. and Xiong, W. C. (2001). Regulation of CDC42 GTPase by proline-rich tyrosine kinase 2 interacting with PSGAP, a novel pleckstrin homology and Src homology 3 domain containing rhoGAP protein. *J. Cell Biol.* **152**, 971-984.

Ren, X. R., Ming, G. L., Xie, Y., Hong, Y., Sun, D. M., Zhao, Z. Q., Feng, Z., Wang, Q., Shim, S., Chen, Z. F. et al. (2004). Focal adhesion kinase in netrin-1 signaling. *Nat. Neurosci.* **7**, 1204-1212.

Serrels, B., Serrels, A., Brunton, V. G., Holt, M., McLean, G. W., Gray, C. H., Jones, G. E. and Frame, M. C. (2007). Focal adhesion kinase controls actin assembly via a FERM-mediated interaction with the Arp2/3 complex. *Nat. Cell Biol.* **9**, 1046-1056.

Shan, Y., Yu, L., Li, Y., Pan, Y., Zhang, Q., Wang, F., Chen, J. and Zhu, X. (2009). Nudel and FAK as antagonizing strength modulators of nascent adhesions through paxillin. *PLoS. Biol.* **7**, e1000116.

Shen, T. L., Park, A. Y., Alcaraz, A., Peng, X., Jang, I., Koni, P., Flavell, R. A., Gu, H. and Guan, J. L. (2005). Conditional knockout of focal adhesion kinase in endothelial cells reveals its role in angiogenesis and vascular development in late embryogenesis. *J. Cell Biol.* **169**, 941-952.

Shen, Y., Li, N., Wu, S., Zhou, Y., Shan, Y., Zhang, Q., Ding, C., Yuan, Q., Zhao, F., Zeng, R. et al. (2008). Nudel binds Cdc42GAP to modulate Cdc42 activity at the leading edge of migrating cells. *Dev. Cell* **14**, 342-353.

Shintani, Y., Fukumoto, Y., Chaika, N., Svoboda, R., Wheelock, M. J. and Johnson, K. R. (2008). Collagen I-mediated up-regulation of N-cadherin requires cooperative signals from integrins and discoidin domain receptor 1. *J. Cell Biol.* **180**, 1277-1289.

Taylor, J. M., Macklem, M. M. and Parsons, J. T. (1999). Cytoskeletal changes induced by GRAF, the GTPase regulator associated with focal adhesion kinase, are mediated by Rho. *J. Cell Sci.* **112**, 231-242.

Tilghman, R. W., Slack-Davis, J. K., Sergina, N., Martin, K. H., Iwanicki, M., Hershey, E. D., Beggs, H. E., Reichardt, L. F. and Parsons, J. T. (2005). Focal adhesion kinase is required for the spatial organization of the leading edge in migrating cells. *J. Cell Sci.* **118**, 2613-2623.

Tomar, A. and Schlaepfer, D. D. (2009). Focal adhesion kinase: switching between GAPs and GEFs in the regulation of cell motility. *Curr. Opin. Cell Biol.* **21**, 676-683.

Tomar, A., Lim, S. T., Lim, Y. and Schlaepfer, D. D. (2009). A FAK-p120RasGAP-p190RhoGAP complex regulates polarity in migrating cells. *J. Cell Sci.* **122**, 1852-1862.

Turner, C. E., Brown, M. C., Perrotta, J. A., Riedy, M. C., Nikolopoulos, S. N., McDonald, A. R., Bagrodia, S., Thomas, S. and Leventhal, P. S. (1999). Paxillin LD4 motif binds PAK and PIX through a novel 95-kD ankyrin repeat, ARF-GAP protein: A role in cytoskeletal remodeling. *J. Cell Biol.* **145**, 851-863.

Uetrecht, A. C. and Bear, J. E. (2009). Golgi polarity does not correlate with speed or persistence of freely migrating fibroblasts. *Eur. J. Cell Biol.* **88**, 711-717.

Vadali, K., Cai, X. and Schaller, M. D. (2007). Focal adhesion kinase: an essential kinase in the regulation of cardiovascular functions. *IUBMB. Life* **59**, 709-716.

van Buul, J. D., Anthony, E. C., Fernandez-Borja, M., Burridge, K. and Hordijk, P. L. (2005). Proline-rich tyrosine kinase 2 (Pyk2) mediates vascular endothelial-cadherin-based cell-cell adhesion by regulating beta-catenin tyrosine phosphorylation. *J. Biol. Chem.* **280**, 21129-21136.

Wang, H. B., Dembo, M., Hanks, S. K. and Wang, Y. (2001). Focal adhesion kinase is involved in mechanosensing during fibroblast migration. *Proc. Natl. Acad. Sci. USA* **98**, 11295-11300.

Webb, D. J., Donais, K., Whitmore, L. A., Thomas, S. M., Turner, C. E., Parsons, J. T. and Horwitz, A. F. (2004). FAK-Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly. *Nat. Cell Biol.* **6**, 154-161.

Wu, X., Suetsugu, S., Cooper, L. A., Takenawa, T. and Guan, J. L. (2004). Focal adhesion kinase regulation of N-WASP subcellular localization and function. *J. Biol. Chem.* **279**, 9565-9576.

Xie, Z., Sanada, K., Samuels, B. A., Shih, H. and Tsai, L. H. (2003). Serine 732 phosphorylation of FAK by Cdk5 is important for microtubule organization, nuclear movement, and neuronal migration. *Cell* **114**, 469-482.

Yadav, S., Puri, S. and Linstedt, A. D. (2009). A primary role for Golgi positioning in directed secretion, cell polarity, and wound healing. *Mol. Biol. Cell* **20**, 1728-1736.

Yano, H., Mazaki, Y., Kurokawa, K., Hanks, S. K., Matsuda, M. and Sabe, H. (2004). Roles played by a subset of integrin signaling molecules in cadherin-based cell-cell adhesion. *J. Cell Biol.* **166**, 283-295.

Yi, X. P., Wang, X., Gerdes, A. M. and Li, F. (2003). Subcellular redistribution of focal adhesion kinase and its related nonkinase in hypertrophic myocardium. *Hypertension* **41**, 1317-1323.

Yu, J. A., Deakin, N. O. and Turner, C. E. (2009). Paxillin-kinase-linker tyrosine phosphorylation regulates directional cell migration. *Mol. Biol. Cell* **20**, 4706-4719.

Zhai, J., Lin, H., Nie, Z., Wu, J., Canete-Soler, R., Schlaepfer, W. W. and Schlaepfer, D. D. (2003). Direct interaction of focal adhesion kinase with p190RhoGEF. *J. Biol. Chem.* **278**, 24865-24873.