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How to awaken your nanomachines: Site-specific activation of focal adhesion kinases through ligand interactions

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ABSTRACT

The focal adhesion kinase (FAK) and the related protein-tyrosine kinase 2-beta (Pyk2) are highly versatile multidomain scaffolds central to cell adhesion, migration, and survival. Due to their key role in cancer metastasis, understanding and inhibiting their functions are important for the development of targeted therapy. Because FAK and Pyk2 are involved in many different cellular functions, designing drugs with partial and function-specific inhibitory effects would be desirable. Here, we summarise recent progress in understanding the structural mechanism of how the tug-of-war between intramolecular and intermolecular interactions allows these protein 'nanomachines' to become activated in a site-specific manner.

1. Multidomain proteins are versatile nanomachines and promising drug targets

Flexible multidomain proteins are key players in eukaryotic signalling networks. In response to the presence of ligands, post-translational modifications or subcellular conditions, these molecules adopt differently 'assembled' or 'open' conformations, which produce different functions (Vogel et al., 2004). Multidomain proteins are thus versatile nanomachines and their intra- and intermolecular interactions are promising targets for inhibitors of protein:protein interactions (Falchi et al., 2014; Zhang et al., 2014). Given the correlation between structure and function, determining the 3D structures of multidomain proteins and their ligand complexes is important for understanding their different biological roles, and for rationally designing protein-protein interaction inhibitors (PPIIs) against particular subsets of functions. However, multidomain proteins are often too flexible for X-ray crystallography, too large for nuclear magnetic resonance (NMR) and too small for electron microscopy (EM). Obtaining 3D structural information on these molecules consequently requires hybrid approaches, where partial high-resolution information (from X-ray crystallography or NMR) is combined with low-resolution information [for example from small-angle X-ray scattering (SAXS), EM or atomic force microscopy), computational methods (molecular modelling, molecular dynamics simulations) and distance constraints coming from experimental methods [such as Förster resonance energy transfer (FRET), chemical cross-linking, mutation or binding studies]. For recent reviews, see (Adams et al., 2013; Graewert and Svergun, 2013; Kovacs et al., 2015; Lasker et al., 2012; Rambo and Tainer, 2013; Rozycki and Boura, 2014).

Herein we summarise recent advances in our understanding of the structure-function relationship of the non-receptor tyrosine kinase FAK and its close homologue Pyk2. Following the editor's recommendation, this review is based on an oral presentation given at the 2014 EMBO workshop, 'Advances in protein–protein analysis and modulation', and thereby concentrates on how ligands activate kinase-dependent functions of FAK at focal adhesions (FAs). This concept introduces a certain bias, which we have tried to

Abbreviations: FAs, focal adhesions; PR, proline rich region; SH, Src homology; FERM, band 4.1, ezrin, radixin, merlin; FAT, focal adhesion targeting; FAH, FAT homology; PPII, protein-protein interaction inhibitor.

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minimise where possible. We also briefly discuss how this mechanistic framework helps us to understand kinase-independent FAK functions in other environments and how it may support calcium-sensing by Pyk2.

2. Multiple cellular functions of FAK

FAK is essential in embryonic development and wound healing (see (Arold, 2011; Hall et al., 2011; Schaller, 2010)). In adult tissue, FAK is expressed only at low levels. However, FAK is overexpressed in most cancers, and it endows cancer cells with functions that normal adult tissue cells do not have, such as the capacity to survive following detachment from the supporting structure. In so doing, FAK becomes a key player in cancer cell metastasis and tissue invasion (Fu et al., 2012; Sulzmaier et al., 2014; Zhang and Hochwald, 2014).

As indicated by its name, FAK is a central player for the assembly and disassembly of FAs (Arold, 2011; Hall et al., 2011; Mitra et al., 2005; Schaller, 2010). FAs are large and dynamic macromolecular assemblies through which the actin cytoskeleton is connected to the extracellular matrix (ECM). In addition to anchoring the cell, FAs encode the state of the ECM into intracellular biochemical pathways that control cell morphology, migration, differentiation, proliferation, and survival (Gumbiner, 1996; Ridley et al., 2003; Wehrle-Haller and Imhof, 2002). FAK is recruited to FAs in response to integrin-mediated cell adhesion. However, FAK is also involved in signalling of other cell surface receptors (including G protein-coupled receptors, the T cell receptor, the deleted-in-colon-cancer netrin receptor and transmembrane tyrosine kinases) and is acting in many cellular environments, such as lamellipodia, microtubules and the nucleus (Chapman and Houtman, 2014; Schaller, 2010; Zhao and Guan, 2009). FAK can promote different effects in the same subcellular localisation (such as assembly and disassembly of FAs); it can also produce convergent effects at different subcellular localisations (such as cancer cell invasion and metastasis in lamellipodia or the nucleus) (Arold, 2011; Cance and Golubovskaya, 2008; Hall et al., 2011; Schaller, 2010). This functional versatility raises the question of how FAK achieves site-specific and cell state–specific functions.

FAK consists of a central kinase domain, flanked by two non-catalytic domains, the band 4.1, ezrin, radixin, moesin (FERM) domain and the focal adhesion targeting (FAT) domain. These domains are separated by long linkers of about 50 (FERM-kinase) and 220 (kinase-FAT) residues (Fig. 1). More than 50 ligands have been reported for FAK, and each domain and linker region has its own set of ligands. Some ligands bind to FAK in the nucleus, and others in the cytoplasm (Fig. 1; red and black, respectively). A subset of cytoplasmic ligands (green) is incompatible with or counteracts autophosphorylation of FAK tyrosine 397.

The major role of the FAK kinase domain appears to be the autophosphorylation of FAK Y397 (Ciccimaro et al., 2006). Y397 is situated in the FERM-kinase linker, adjacent to a proline-rich motif (PR1), and autophosphorylation of Y397 has to proceed in *trans* in the full-length standard form of FAK (Brami-Cherrier et al., 2014; Toutant et al., 2002). Once phosphorylated, this region constitutes a bi-dentate binding site for the Src-homology 2 (SH2) and SH3 domains of Src family kinases Fyn and Src (which bind to *p*Y397 and PR1, respectively) (Arold et al., 2001; Schaller et al., 1994; Thomas et al., 1998) (Fig. 1). The interaction with FAK activates these Src-family kinases, which then provide most kinase activity associated with FAK, including phosphorylation of tyrosines within FAK (including Y576/Y577 in the kinase activation loop, Y861 in the kinase-FAT linker, and Y925 in the FAT domain) (Schaller et al., 1999, 1994; Schlaepfer et al., 1994; Xie et al., 2008). Thus, autophosphorylation of Y397 triggers the kinase-dependent functions of the Src:FAK complex. In the absence of Y397 autophosphorylation, FAK binds to different ligands and performs different functions (Corsi et al., 2009). Hence FAK can be seen as a scaffolding protein with a Y397-phosphorylation switch between different conformational and functional states (Fig. 1).

3. New insights into the structure and regulation of individual FAK domains

The atomic-resolution 3D structures of the individual domains of FAK have been previously described [(Fig. 2); for a detailed review, see (Alam et al., 2014; Arold, 2011; Hall et al., 2011)]. The FAK FERM domain is composed of three lobes (F1–F3) and is structurally similar to the FERM domains of those proteins after which it was named (band 4.1, ezrin, radixin and moesin) (Ceccarelli et al., 2006; Girault et al., 1999). However, the FAK FERM domain has adapted this scaffold to engage different interactions (such as binding to transmembrane receptor tails, or intramolecular interactions), or to engage in the same type of interactions differently (for example binding to phospholipid head groups) (Arold, 2011; Ceccarelli et al., 2006; Hall et al., 2011). The crystal structure of a FAK fragment comprising the FERM and kinase domains (residues 31-686) revealed that the FERM domain can dock onto the kinase domain (Fig. 2) (Lietha et al., 2007). This assembled FERM-kinase conformation is stabilised by binding of ATP to the kinase domain, yet is incompatible with phosphorylation of Y576/Y577 in the kinase activation loop (Goni et al., 2014; Lietha et al., 2007; Zhou et al., 2015). The FAK kinase domain cannot efficiently autophosphorylate its activation loop tyrosines Y576/Y577, but requires Src kinases for this modification (Ciccimaro et al., 2006). Binding of the Src kinase SH2 domain to *p*Y397 requires dissociation of the contacts between *p*Y397 and the FERM F1 lobe (Fig. 2A and data not shown), suggesting that this event weakens the FERM:kinase interaction. Hence Y397 autophosphorylation, which leads to phosphorylation of Y576/Y577 by Src kinases, ultimately promotes an open, disassembled FAK conformation, as experimentally observed using FRET sensors (Cai et al., 2008; Goni et al., 2014; Lietha et al., 2007). Thus, the presence of ATP (which is expected to be constitutive, given the high ATP concentrations in the cell) and phosphorylation of the kinase activation loop stabilise structurally and functionally distinct structural states.

Despite forming a simple four-helix bundle structure, the ~140 residue FAT domain also possesses several layers of regulation, linked to conformational plasticity (Fig. 2B) (Alam et al., 2014; Arold et al., 2002; Dixon et al., 2004; Hayashi et al., 2002; Kadare et al., 2015; Prutzman et al., 2004). The interaction between FAT and paxillin LD motifs results in FAK being recruited to FAs (Fig. 2C) (Brown et al., 1996). FAT binds two helical LD motifs, one between helices H1 and H4 (site 1/4), the other one between helices H2 and H3 (site 2/3) (Bertolucci et al., 2005; Gao et al., 2004; Hoellerer et al., 2003), whereas FAT-homology domains (FAHs) of other FA-localising proteins (CCM3, GIT1/2 and vinculin) bind only one LD motif (Alam et al., 2014; Brown et al., 1996; Li et al., 2011; Schmalzigaug et al., 2007; Turner et al., 1990; Zhang et al., 2008). FAT binds also two CD4 endocytosis motifs, in a structurally similar way to LD motifs, allowing CD4 to recruit FAK for T cell receptor signalling (Garron et al., 2008). Conversely, the FAT helix 4 is sufficient to bind to the talin FERM F3 lobe, and the FAT four-helix bundle structure is not required for this interaction (Hayashi et al., 2002; Lawson et al., 2012).

Phosphorylation and subsequent interaction of Y925 [located on helix 1 (H1) of FAT] with the Grb2 SH2 domain requires opening of H1 (Arold et al., 2002; Schlaepfer et al., 1994). H1-opening is promoted by the P^{944}XPP motif between H1 and H2 (Fig. 2B and D), which functions as a molecular spring (Arold et al., 2002; Kadare

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