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THE EMBO JOURNAL

A FAK conundrum is solved: activation and organization of focal adhesion kinase at the plasma membrane

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Focal adhesion kinase (FAK) is a central mediator of cell adhesion, acting both as a scaffold and as catalytically active kinase. Acebrón *et al* (2020) use cryo-electron microscopy (cryo-EM) to visualize the dramatic structural changes that occur upon FAK recruitment to the plasma membrane, which releases FAK autoinhibition and induces its oligomerization. Since activity control via autoinhibition and protein clustering are features also utilized by other focal adhesion (FA) proteins, they have moved center stage in the endeavor to understand the complex process of cell adhesion regulation.

The EMBO Journal (2020) e106234
See also: **I Acebrón *et al*** (2020)

Adhesion of metazoan cells to the surrounding extracellular matrix is mediated by integrins, which are heterodimeric adhesion proteins and bidirectional, allosterically regulated signaling receptors. Integrins assemble large cytoplasmatic complexes called focal adhesions (FA), consisting of hundreds of different proteins, the sum of which is referred to as the adhesome. Proteins of the adhesome are regulated by, and several of them regulate, integrin activity. To allow the minute regulation of FA assembly and disassembly that is paramount for adhesion dynamics and cell motility, the interplay between integrins, their extracellular ligands, and intracellular binding proteins needs to be finely tuned.

Focal adhesion kinase (FAK) is a tyrosine kinase that is found in the cytosol, from where it is either recruited to the adhesome or the nucleus, where it has been implicated in p53 degradation. Cytosolic FAK, which is at the center of the study presented here, is one of the key signaling proteins that facilitate the seemingly spontaneous and rapid assembly of FA proteins distributed throughout the cytosol and the plasma membrane into FAs. FAK is a non-receptor tyrosine kinase, that exists in the cytosol in an autoinhibited state through an intramolecular interaction of the kinase domain (KD) with its N-terminal FERM (band 4.1, ezrin, radixin, moesin homology) domain (Cooper *et al*, 2003). The C-terminal focal adhesion targeting (FAT) domain is involved in FAK recruitment into FAs via interaction with paxillin and appears to have no further regulatory functions (Lietha *et al*, 2007). Activation of FAK is associated with autophosphorylation of a tyrosine residue in the linker between the FERM and kinase domains. This phosphorylated tyrosine then recruits and activates Src, which in return phosphorylates two tyrosine residues in the FAK kinase activation loop (Calalb *et al*, 1995). The now fully activated FAK/Src complex can then phosphorylate numerous downstream targets with roles in cell adhesion, migration, proliferation, differentiation, and survival. Such downstream signaling effectors make it not surprising that FAK is overexpressed in many tumors and is associated with tumor growth, invasion, and resistance to treatment.

Despite the interest in FAK as a target for cancer therapy, it took around 20 years from its initial discovery to starting to understand the molecular basis of the first step of FAK activation. After it was shown that FAK activation is caused by binding of a patch of basic residues in its FERM domain to phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) (Cai *et al*, 2008), Lietha and colleagues demonstrated in 2014 that FAK binding to PI(4,5)P2 induces a multistep activation sequence, which causes FAK oligomerization and an optimal positioning of the linker-tyrosine residue for autophosphorylation (Goni *et al*, 2014). In their new study, they applied cryo-EM to 2D-crystals of FAK bound to PI(4,5)P2 membranes to provide the structural underpinning for their previously described activation mechanism and confirmed it by biochemical and cell biological studies.

According to this mechanism, the locally increased concentration of FAK in FAs, triggered by its recruitment into FAs through the interaction of FAT with paxillin, causes the formation of FAK dimers through FERM-FERM interactions that were previously shown to be able to form in solution (Brami-Cherrier *et al*, 2014). Binding of dimerized FAK to PI(4,5)P2 via the charged amino acids in the FERM domain causes a steric clash between the lipid bilayer and the KD, resulting in dissociation of the intramolecular FERM-KD interaction. This leaves the KD with an increased degree of special freedom allowing it to establish further contact to negatively charged lipids in the membrane via two lysine residues in the kinase C-lobe. It is interesting to note that these lysine residues were already implicated as a possible membrane binding site with an impact on FAK activation by a coarse-grained molecular dynamics simulation 5 years ago (Feng &

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DOI 10.15252/embj.2020106234 | The EMBO Journal (2020) e106234

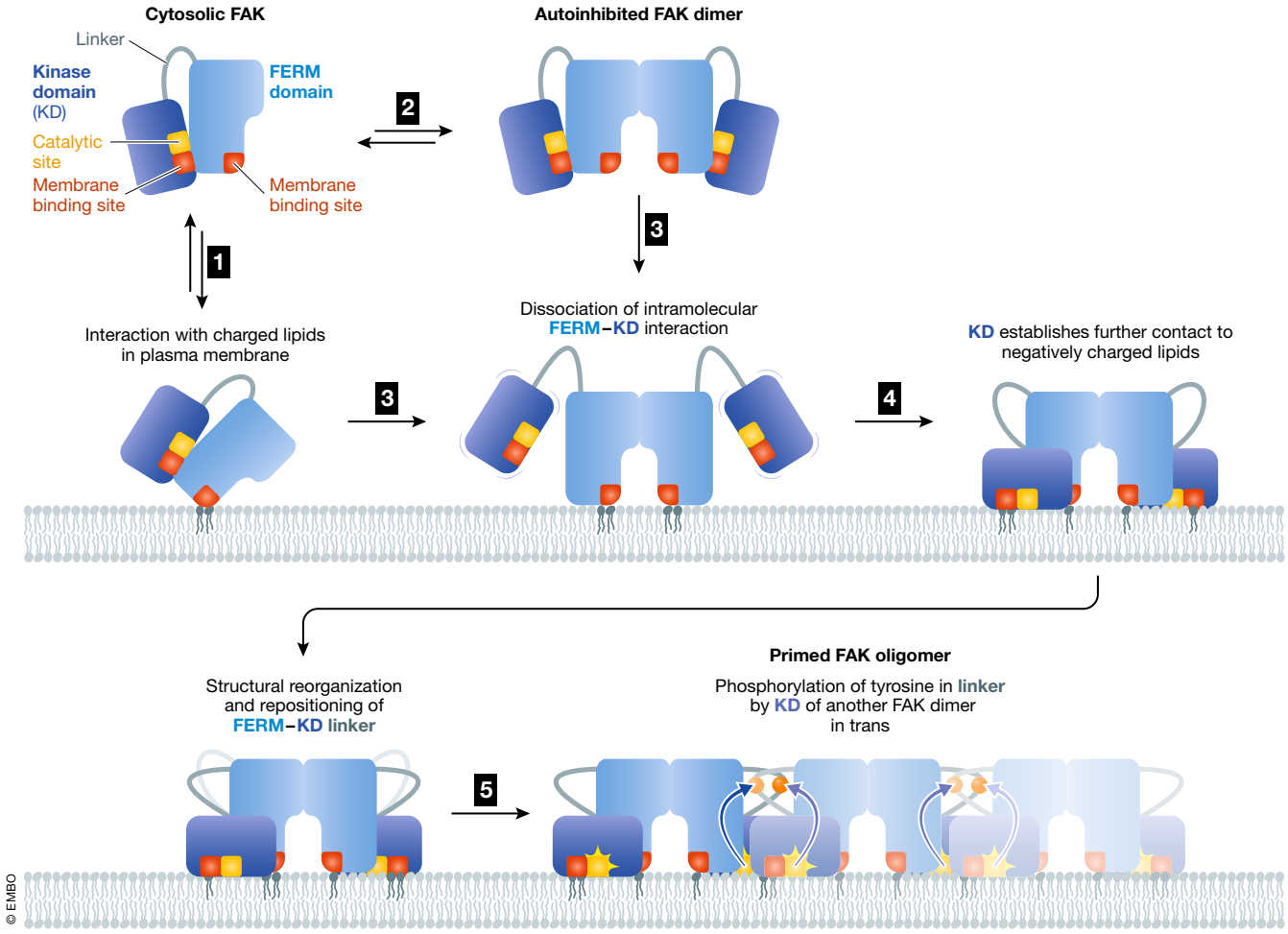

Figure 1. Activation of FAK at the plasma membrane.

FAK can bind negatively charged phospholipids in the plasma membrane via a patch of positively charged amino acids in its FERM domain (1). Upon an increase in local concentration (as encountered in FAs), FAK dimerizes via FERM-FERM contacts (2). Interaction of the FAK dimer with the plasma membrane can, for steric reasons, only occur upon release of the autoinhibitory KD-FERM contact (3). The released KD can bind the membrane via its own membrane binding site (4). In this membrane bound state, the FAK dimer can form large oligomers in which a tyrosine in the linker region between KD and FERM domain can be phosphorylated in trans by the KD of a neighboring FAK dimer (5). This is followed by Src recruitment and subsequent full activation of the FAK/Src complex (not shown here).

Mertz, 2015). Binding of the KD to the membrane is associated with dramatic structural reorganization, exposing new intermolecular binding sites, and positioning the FERM-KD linker that contains the tyrosine residue responsible for Src recruitment to allow its phosphorylation by the KD of another FAK dimer in trans. Interaction between FAK dimers is mediated by a large contact surface comprising direct KD-KD and FERM-KD interactions (Fig 1). Intriguingly, the kinase active site faces toward the membrane in this multimer of dimers configuration, limiting the accessibility of Src to the activation loop as well as the access of substrates to the FAK catalytic site. The authors speculate that this could present another level of regulation, in which mechanical force is required to lift the KD off the membrane to make the active site readily accessible. This force could potentially be transmitted via the FAT domain, which is however unfortunately missing from the structure.

Autoinhibition as a recurrent feature in adhesion regulation has recently been highlighted in a review by Khan and Goult (2019). They note that a large number of adhesome key components, including talin, vinculin, and FAK, are regulated via intramolecular inhibitory interactions, allowing for the assembly of primed pre-complexes that can be rapidly activated and interact with other proteins upon receiving a stimulus. Such stimuli could be, among others, intermolecular interactions, post-translation modifications, or mechanical forces. All of these play a role in the activation sequence proposed for FAK (Fig 1), which is, in order of events, dimerization and interaction with charged lipids in the plasma membrane (intermolecular interactions) followed by autophosphorylation (post-translational modifications). Full activation is finally achieved by mechanical lifting of the KD off the membrane (mechanical forces) and binding to and phosphorylation by Src (intermolecular interactions and

post-translational modifications). This complex activation sequence highlights how the convergence of multiple regulatory stimuli within FAs allows for a highly nuanced regulation of adhesion, motility, and cell survival.

Protein clustering has been recognized early on as one of the mechanisms that allow the rather weak interactions of integrins with their extracellular ligands as well as with their intracellular interactors to withstand the forces they transmit (Hato *et al*, 1998). With the emergence of the concept of catch bonds, whose lifetime increases when the force that acts on them increases, and the realization that integrins engage in catch bonds with intracellular as well as extracellular ligands, protein clustering temporarily shifted from focus, but has returned there with a number of publications since. It has particularly regained attention as a possible mechanism of how kindlin contributes to integrin activation. Following the observation that kindlin-2 can form dimers *in vitro* and that the ability to dimerize is of importance for integrin activation *in vivo*, it was hypothesized that kindlin and talin dimers act together to cluster integrins in FAs (Ye *et al*, 2013; Li *et al*, 2017). The observation that FAK, which interacts with talin, kindlin, and other essential FA components such as ILK via paxillin, can form regular oligomers adds another dimension to this hypothesis.

Many of the conceptual foundations of cell adhesions were laid more than 20 years ago. Now, where the structural data on the vast number of proteins involved in adhesion are becoming more and more complete, it is very interesting to revisit these old concepts and begin to fill in all the questions left unanswered two decades ago. The structural analysis of FAK activation presented here does exactly this, thereby not only answering some of these questions, but also providing new impulses to address the remaining gaps in our understanding of this process, so fundamental to all metazoan life.

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