

# pHocal adhesion kinase regulation is on a FERM foundation

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**Increases in intracellular pH (pHi) occur upon integrin receptor binding to matrix proteins and in tumor cells. In this issue, Choi et al. (2013. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201308034>) show that pHi increase activates FAK by causing deprotonation of histidine 58 in its FERM (band 4.1, ezrin, radixin, moesin) homology domain, which exposes a region important for FAK auto-phosphorylation. This model of FAK activation could contribute to motility of tumor cells by promoting focal adhesion turnover.**

Integrins are transmembrane receptors for extracellular matrix that mediate physical cell attachment and also control cell shape, growth, and survival. Integrin signals are generated by the recruitment and activation of protein tyrosine kinases (PTKs) such as Src, Abl, Syk, and FAK that initiate protein phosphorylation signaling cascades. These sites of integrin signal initiation and cell attachment are generally termed focal adhesions. Despite identification of adhesion protein constituents, our understanding of the molecular mechanisms of PTK activation at focal adhesions remains rudimentary. FAK, Src, and Abl signaling contribute to tumor growth and metastasis, and small molecule drugs targeting these PTKs have been approved or are undergoing clinical trials. However, for FAK we really do not fully understand the contributing factors that lead to its elevated activation, and although FAK is an amplified gene in cancer, mutations that increase FAK activation are uncommon.

In this issue, Choi et al. (2013) elucidate a novel connection between increased intracellular pH (pHi) and FAK activation. In the early 1990s, transient pHi elevation upon matrix binding was one of the first integrin-associated signals identified (Schwartz et al., 1991). Pharmacological inhibitors pointed to the importance of sodium–proton antiporters in mediating increased pHi. NHE-1 (sodium–hydrogen antiporter 1) is part of a larger family (NHE1–9) and a ubiquitously expressed transmembrane protein that actively extrudes protons from inside the cell to counter balance acidity and maintain cytosolic pHi (Malo and Fliegel, 2006). NHE-1 can be found at focal adhesions

(Grinstein et al., 1993) and can connect to the actin cytoskeleton via binding to ezrin (Denker et al., 2000). NHE-1 point mutations disrupting either ion translocation or its binding to ezrin prevent cell migration (Denker and Barber, 2002). NHE-1 over-expression in cancer cells elevates pHi and tumor progression (Webb et al., 2011).

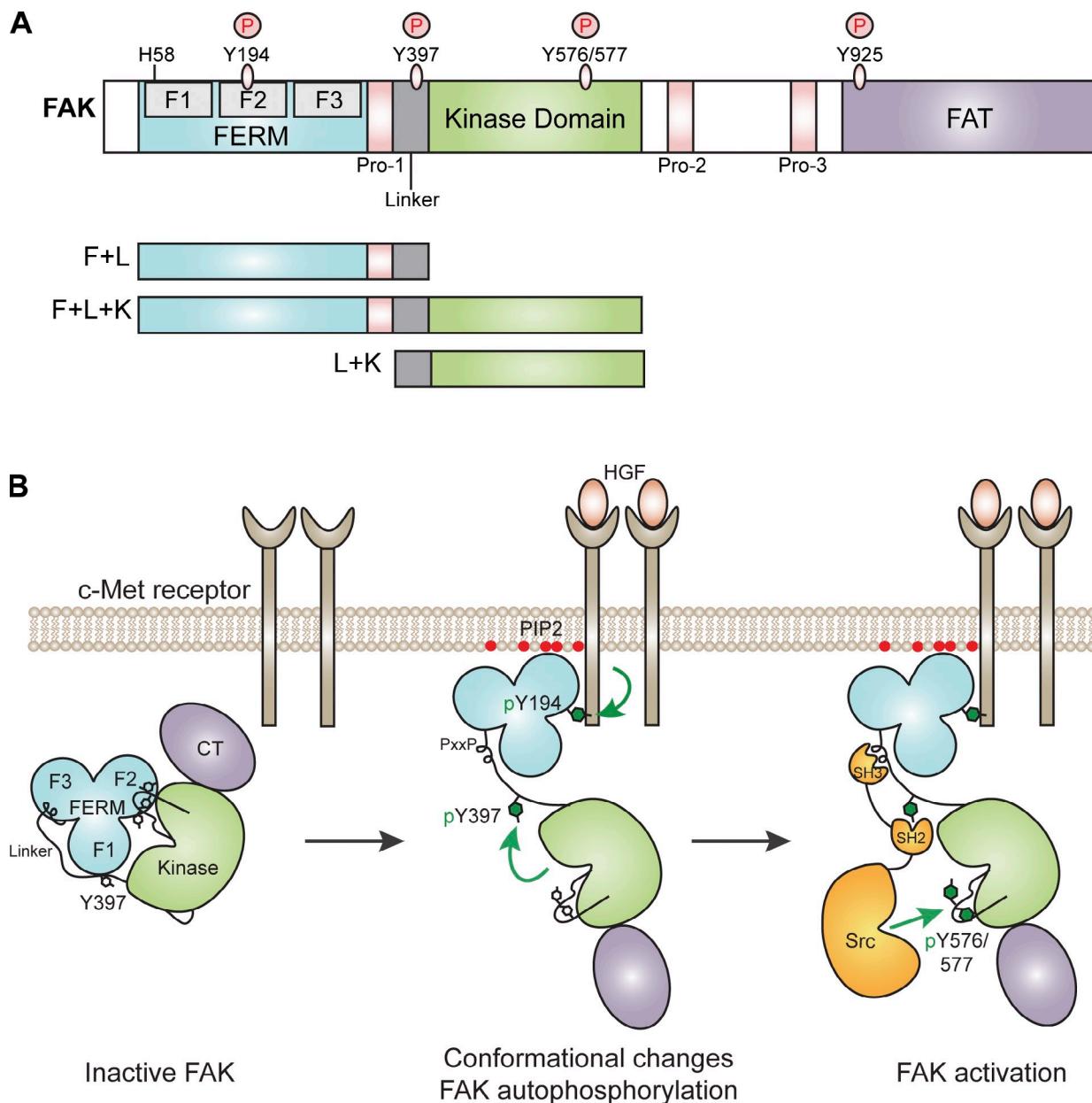
Also in the early 1990s, FAK was the first PTK shown to localize to focal adhesions and to be activated by integrins (Parsons, 2003). FAK and the closely related proline-rich tyrosine kinase 2 (Pyk2) share a common domain structure of an N-terminal FERM domain and an ~40 amino acid linker domain containing an autophosphorylation site (Y397 in FAK) that serves as a Src homology 2 (SH2) binding site for Src-family PTKs. The FAK linker is followed by a central kinase domain, a scaffolding region containing two proline-rich motifs that are SH3 domain binding sites, and a C-terminal focal adhesion–targeting (FAT) domain (Fig. 1 A). It is the FAK FAT domain that binds to integrin-associated proteins (paxillin and talin) and facilitates FAK phosphorylation at Y397 via protein clustering (Toutant et al., 2002). However, the importance of the results of Choi et al. (2013) lies in the role of the FAK FERM domain in the intramolecular regulation of FAK Y397 autophosphorylation.

FERM domains are typically comprised of three lobes (F1, F2, and F3) grouped in a cloverleaf-like structure (Frame et al., 2010). In an inactive conformation, the FAK FERM F2 lobe binds to and blocks the FAK kinase domain active site. The FAK FERM F1 lobe binds to and sequesters FAK Y397 in the linker region (Fig. 1 B). Point mutations of FAK in the F1 lobe, F2 lobe, or within the kinase domain can weaken these inhibitory intramolecular binding interactions and result in elevated FAK Y397 phosphorylation (Lietha et al., 2007). It has been hypothesized that the normal sequence of events for FAK activation starts with the binding of some “activating” factor to the FAK F2 lobe that would trigger FERM lobe displacement and allow FAK cis or trans auto-phosphorylation of Y397 (Fig. 1 B). Subsequent full FAK activation occurs via SH2 domain binding of Src to phosphorylated Y397, resulting in Src-mediated phosphorylation within the FAK kinase domain at Y576 and Y577 to promote catalytic activation and phosphorylation within the FAT domain at Y925 to promote Grb2

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Abbreviations used in this paper: FAT, focal adhesion targeting; FERM, 4.1, ezrin, radixin, moesin; F+L+K, FERM-linker-kinase; FN, fibronectin; MEF, mouse embryo fibroblast; pHi, intracellular pH; PTK, protein tyrosine kinase; SH, Src homology.

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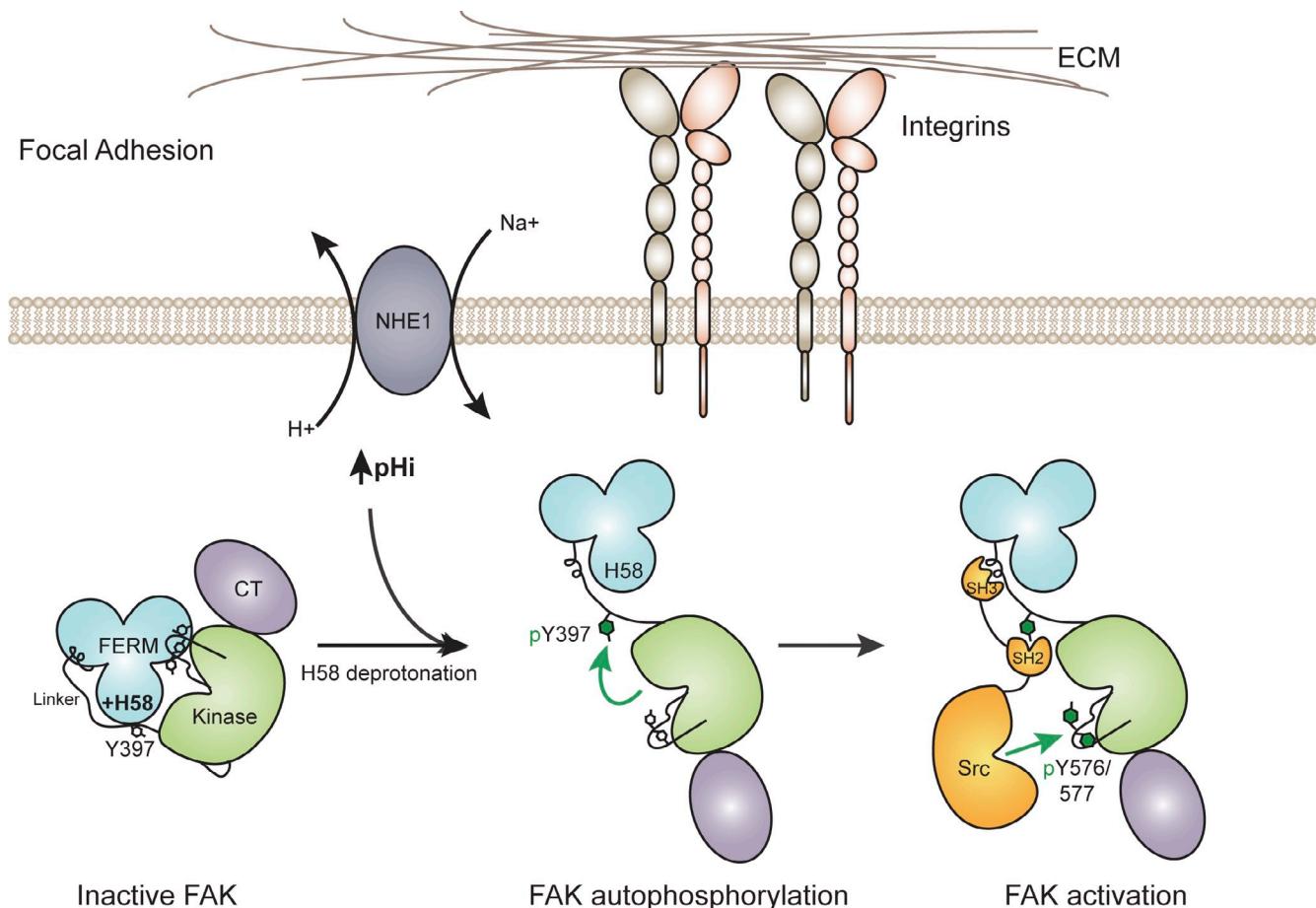


**Figure 1. Overview of FAK structure and activation.** (A) FAK schematic. Depicted is the FAK N-terminal FERM domain comprised of three lobes (F1, F2, and F3), a linker domain, central kinase domain, and a C-terminal focal adhesion–targeting (FAT) domain. Shown are histidine (H) residue 58 and tyrosine (Y) residues 194, 397, 576/577, and 925, and proline-rich domains (Pro-1, Pro-2, and Pro-3) that are sites for SH3 domain binding. FERM plus linker (F+L), FERM-linker-kinase (F+L+K), and linker-kinase (L+K) are constructs used by Choi et al. (2013). (B) Model of growth factor–stimulated FAK activation. Upon binding of the FAK FERM F2 lobe to c-Met receptor and/or phosphatidylinositol 4,5-P<sub>2</sub> (PIP2) lipid, FAK undergoes conformational changes and Y194 phosphorylation, leading to FAK Y397 autophosphorylation in the linker region. Src binding to and phosphorylation of FAK within the kinase domain leads to full FAK activation. HGF, hepatocyte growth factor.

SH2 binding (Schaller, 2010). Recently, growth factor receptor phosphorylation of FAK at Y194 within the FERM F2 lobe was shown to promote FAK activation (Fig. 1 B; Chen et al., 2011). Conformational changes triggered by FAK kinase activity also regulate FAK FERM–mediated binding to targets such as VE-cadherin (Chen et al., 2012). Outside of FAK phosphorylation at Y194, and a potential role for lipid binding to FAK FERM (Cai et al., 2008), additional “initiators” of FERM conformational changes remain undefined.

Choi et al. (2013) provide new and important insights in FERM-mediated FAK activation by changes in pH<sub>i</sub>. By using a

chimeric paxillin construct fused to the pH biosensor pHluorin and to mCherry, Choi et al. (2013) showed that pH<sub>i</sub> increases within peripheral adhesions in mouse embryo fibroblasts (MEFs) spreading on fibronectin (FN) at 30 to 50 min after plating. Stable NHE-1 knockdown resulted in the lowering of pH<sub>i</sub> and the inhibition of FAK but not Src activation at 30 and 60 min during MEF spreading on FN. Interestingly, NHE-1 knockdown or pharmacological NHE-1 inhibition resulted in MEFs with a rounded morphology and an increased number of small focal adhesions. This adhesion and spreading phenotype is similar to that of FAK-null MEFs (Sieg et al., 1999).



**Figure 2. Simplified model of FAK activation via histidine 58 (H58) deprotonation.** The FAK FERM F1 lobe sequesters FAK Y397 in the linker region keeping FAK in an inactive and closed conformation. Integrin engagement at focal adhesions results in transient and local increases of pH through NHE-1 activity. Changes in pH result in H58 deprotonation within the FERM F1 lobe, leading to FAK conformational changes that expose the FAK linker region and enabling FAK Y397 autophosphorylation. Src binding to and phosphorylation of FAK within the kinase domain leads to full FAK activation.

To determine if there was a connection between increased pH and FAK activation, Choi et al. (2013) performed recombinant FAK *in vitro* phosphorylation assays. The FAK FERM-linker-kinase (F+L+K) fragment but not the linker-kinase (L+K) (Fig. 1 A) exhibited increased Y397 phosphorylation as a function of pH (pH 7.5 > pH 6.5). Intriguingly, regulation of FAK Y397 phosphorylation by pH was dependent upon the presence of the FAK FERM domain, which was shown by using the FERM-linker (F+L) as a substrate in trans (Fig. 1 A). These results support the hypothesis that exposure of the FAK linker region for phosphorylation is pH dependent (Fig. 2). Changes in amino acid protonation can be considered a post-translational modification, as ionic interactions contribute to secondary and tertiary protein structure (Schönichen et al., 2013). The  $pK_a$  of histidine is  $\sim 6.5$ , and variations from this value depend upon the local protein environment. The challenge is to determine which sites functionally serve as pH sensors *in vivo*.

The FAK FERM domain contains seven histidine residues, three of which (H41, H58, and H75) are within the FERM F1 lobe. Mutation of these sites individually to alanine revealed that H58A mutation selectively enhanced F+L and F+L+K Y397 phosphorylation at pH 6.5. This approach allowed Choi et al. (2013) to conclude that H58 deprotonation confers some

type of change within the F+L region to facilitate Y397 auto-phosphorylation (Fig. 2). Using the crystal structure of FAK F+L+K as a template, molecular dynamic simulations with H58 (neutral or positively charged) revealed conformational differences within residues of the linker region around Y397 despite the lack of direct binding to H58. Although Choi et al. (2013) did not identify key partner electrostatic interactions that contribute to conformational changes upon H58 deprotonation, recent studies by (Ritt et al. (2013) proposed that E466 within the FAK kinase domain may be important for this regulation within full-length FAK. However, this does not explain results from Choi et al. (2013) for the pH dependence of Y397 phosphorylation within F+L.

Lastly, full-length H58A FAK exhibited elevated Y397 phosphorylation upon re-expression in FAK-null MEFs in combination with NHE-1 shRNA knockdown. H58A FAK promoted spreading and adhesion changes in both control and NHE-1 shRNA FAK-null MEFs, whereas wild-type FAK did not rescue FAK-null phenotypes in the absence of NHE-1 expression. Choi et al. (2013) note that pH-regulated FAK Y397 phosphorylation required combined integrin stimulation and elevated pH to activate FAK. In tumor cells, NHE-1 inhibition prevents elevated FAK Y397 phosphorylation and recent studies show that FAK

can phosphorylate cortactin to promote adhesion turnover (Tomar et al., 2012). Cortactin tyrosine phosphorylation facilitates the recruitment of NHE-1 to tumor cell invadopodia (Magalhaes et al., 2011), leading to the pH-dependent release of actin-depolymerizing factor cofilin from cortactin (Frantz et al., 2008). As FAK activity promotes ovarian and breast tumor metastasis (Walsh et al., 2010; Ward et al., 2013), it is possible that FAK may serve as a pH-dependent sensor to initiate cell spreading.

In the control of cell motility, NHE-1 is postulated to create pH nanodomains at focal adhesions to control protein–protein interactions (Ludwig et al., 2013). A simplistic model is that integrin clustering facilitates rapid FAK recruitment to focal adhesion where increases in pH trigger FERM conformational changes, release of the FAK linker region, and allow for FAK Y397 phosphorylation in cis or trans (Fig. 2). Adhesion turnover is increased at alkaline pH, consistent with leading edge cell spreading and extension. At nascent adhesions FAK recruits talin (Lawson et al., 2012), and at alkaline pH FAK signaling activity may be enhanced over talin binding to filamentous actin needed for adhesion maturation (Srivastava et al., 2008). However, as pH falls, pH sensor residues within the talin rod domain confer enhanced actin binding and this may be part of a signaling switch to promote a cycle of focal adhesion maturation. Additionally, pH changes may alter phosphorylation site specificity by Src within the FAK FAT domain (Cable et al., 2012). Thus, pH can affect FAK activity and FAK phosphorylation. Moreover, the FAK-related Pyk2 PTK is activated by acidic pH within cells of the kidney (Li et al., 2004). Although the molecular mechanism is not known as to how Pyk2 is regulated by acidity, clearly there is much more to discover about the role of pH changes at adhesions and invadopodia and how this may alter PTK activation in the control of cell movement and invasion.

This work was supported by National Institutes of Health grants CA103210 and CA180769.

Submitted: 6 August 2013

Accepted: 20 August 2013

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