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REVIEW

SUBJECT COLLECTION: ADHESION

Emerging evidence for kindlin oligomerization and its role in regulating kindlin function

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ABSTRACT

Integrin-mediated cell–extracellular matrix (ECM) interactions play crucial roles in a broad range of physiological and pathological processes. Kindlins are important positive regulators of integrin activation. The FERM-domain-containing kindlin family comprises three members, kindlin-1, kindlin-2 and kindlin-3 (also known as FERMT1, FERMT2 and FERMT3), which share high sequence similarity (identity >50%), as well as domain organization, but exhibit diverse tissue-specific expression patterns and cellular functions. Given the significance of kindlins, analysis of their atomic structures has been an attractive field for decades. Recently, the structures of kindlin and its β-integrin-bound form have been obtained, which greatly advance our understanding of the molecular functions that involve kindlins. In particular, emerging evidence indicates that oligomerization of kindlins might affect their integrin binding and focal adhesion localization, positively or negatively. In this Review, we presented an update on the recent progress of obtaining kindlin structures, and discuss the implication for integrin activation based on kindlin oligomerization, as well as the possible regulation of this process.

KEY WORDS: Kindlin, Oligomerization, Kindlin-3, Phosphorylation, Integrin, Extracellular matrix

Introduction

Kindlin-1, kindlin-2 and kindlin-3 (also known as FERMT1, FERMT2 and FERMT3) are three evolutionarily conserved focal adhesion proteins comprising a 4.1-ezrin-radixin-moesin (FERM) domain (Rognoni et al., 2016). Despite their high amino acid sequence identities, kindlins are functionally non-redundant. Kindlin-1 is expressed primarily in epithelial tissues (Ussar et al., 2006). Loss of kindlin-1 causes the disease Kindler syndrome, which is characterized by skin abnormalities, including blistering, atrophy and an increased risk of developing squamous cell carcinomas (Rognoni et al., 2014; Siegel et al., 2003). Kindlin-2 is widely expressed in many types of cells except hematopoietic cells (Ussar et al., 2006). There is no report on loss-of-function mutations in kindlin-2 in humans. Nevertheless, gene ablation studies in mice and zebrafish have shown that loss of kindlin-2 is embryonic lethal (Montanez et al., 2008; Dowling et al., 2008). Kindlin-3 expression is restricted to hematopoietic cells and endothelial cells (Bialkowska et al., 2010). Loss of kindlin-3 function causes the disease leukocyte adhesion deficiency type III (LAD III), which is characterized by a propensity to bleed and a compromised immune system (Malinin et al., 2009; Manevich-Mendelson et al., 2009; Svensson et al., 2009). Dysregulated expression of kindlins also contributes to the progression of many types of cancer (Plow et al., 2016; Zhan and Zhang, 2018).

The molecular basis underlying the debilitating effects caused by the loss or dysregulated expression of kindlins involves, in large part, disruption of integrin-mediated cell adhesion and migration (Larjava et al., 2008; Rognoni et al., 2016). Integrins are transmembrane heterodimeric receptors that bind to extracellular matrix (ECM) proteins, counter receptors on another cell and soluble ligands (Hynes, 2002). Each integrin comprises an α and a β subunit that are non-covalently associated. Generally, integrins require activation to bind ligands. This process, which is commonly termed integrin 'inside-out' signaling, involves extensive conformational changes that are induced by the separation of the cytoplasmic tails of α and β integrin, which are clasped in resting integrins (Luo et al., 2007; Tan, 2012). Talin proteins (talin 1 and talin 2 in mammals; hereafter collectively denoted talin), large cytoskeletal proteins that contains a FERM head domain and a long rod segment, are a positive regulator of integrins (Calderwood et al., 2013; Critchley and Gingras, 2008; Tadokoro et al., 2003). Through the F3 subdomain within its head domain, talin binds to a membrane-proximal NxxY/F motif (Garcia-Alvarez et al., 2003), which is highly conserved in the cytoplasmic tails of six integrin β subunits (Hynes, 2002). Following this, a series of electrostatic interactions occur between basic patches in the talin head domain and the negatively charged plasma membrane phospholipids, leading to the separation and re-orientation of the integrin cytoplasmic tails (Elliott et al., 2010; Moore et al., 2012; Wegener et al., 2007).

A key question related to integrin activation by talin is what regulates talin binding to the β cytoplasmic tail of integrin. Three mechanisms, which need not be mutually exclusive, are involved. First, talin can adopt an intra-molecular auto-inhibited conformation in which its rod segment folds back onto its F3 subdomain, thereby masking the integrin-binding site (Goksoy et al., 2008; Goult et al., 2009). Talin auto-inhibition has been shown to regulate its recruitment to sites of adhesion and the maturation of focal adhesions (Haage et al., 2018). Second, talin could be targeted to integrins by the GTPase Rap1 (Rap1a and Rap1b isoforms in mammals) and the Rap1-GTP-interacting adapter molecule (RIAM; also known as APBB1IP) complex (Han et al., 2006; Lee et al., 2009; Lagarrigue et al., 2016). The direct interaction between Rap1 (bound to the GTP analog GMP-PNP) and talin head F0 domain is rather weak with a K_d in the range of 140–162 μM (Goult et al., 2010; Zhu et al., 2017). Mutagenesis experiments based on the interacting interface between Rap1 and talin F0 domain have given somewhat varied outcomes with respect to platelet integrin αIIbβ3 activation, which may be explained by the number of amino acids mutated (Zhu et al., 2017; Lagarrigue et al., 2018; Bromberger et al., 2018). The negative effect of disrupting Rap1–talin F0 domain

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interaction on platelet integrin αIIbβ3 activation also seems less severe as compared to complete ablation of Rap1 proteins (both Rap1a and Rap1b isoforms), suggesting additional contributing factors mediate Rap1 and talin interaction (Stefanini et al., 2018; Bromberger et al., 2018). Indeed, the talin F1 domain and its lysine-rich flexible motif were subsequently demonstrated to function synergistically with the F0 domain to promote Rap1 binding in recent studies (Gingras et al., 2019; Bromberger et al., 2019). Third, the large cytoskeletal protein filamin A prevents integrin activation because its IgFLN21 domain competes with talin for overlapping binding regions in the integrin β cytoplasmic tail (Kiema et al., 2006). Along the same lines, the FERM family protein moesin outcompetes talin for binding to the integrin β1 tail, which leads to the inactivation of and focal adhesion disassembly (Vitorino et al., 2015).

Although talin is a master regulator of integrin activation, as evidenced by knockout and knockdown studies (Wegener et al., 2007; Klapholz and Brown, 2017; Tadokoro et al., 2003; Chinthalapudi et al., 2018), the fact that integrins are not activated in cells and model organisms in which kindlins are lost or have been depleted but retain intact talin expression, suggests that kindlins are crucial regulators of integrin activation (Theodosiou et al., 2016; Plow et al., 2016). Kindlins bind to a NxxY/F motif located at the membrane distal region of the integrin β cytoplasmic tails (Karakose et al., 2010), but kindlins are not known to directly bind to talin (Sun et al., 2019). One suggested mechanism by which kindlins could promote integrin-mediated cell adhesion is by assisting talin in integrin activation through the displacement of the negative regulator filamin A and unmasking the membrane-proximal NxxY/F site in the integrin β cytoplasmic tail to make it available for talin binding. This competitive binding mechanism is largely based on the findings that kindlin-2 binds migfilin (also known as FBLIM1), a LIM-domain-containing protein that associates with filamin A, and migfilin and integrin β cytoplasmic tails share an overlapping binding site on filamin A (Brahme et al., 2013; Das et al., 2011; Lad et al., 2008; Kiema et al., 2006; Liu et al., 2015a,b). Another possible mechanism involves the clustering of integrins. Earlier studies have shown that kindlins promote the clustering of integrins (Feng et al., 2012; Ye et al., 2013). Unlike talin, kindlins do not possess a rod region that can tether F-actin and vinculin, a regulator of F-actin at the leading edge, to form focal adhesions (Thievessen et al., 2013). However, kindlins can interact with F-actin (Bledzka et al., 2016) and focal adhesion scaffold proteins, including paxillin (Zhu et al., 2019; Gao et al., 2017; Theodosiou et al., 2016) and integrin-linked kinase (ILK) (Kadry et al., 2018; Fukuda et al., 2014; Huet-Calderwood et al., 2014; Qadota et al., 2014; Guan et al., 2018). Recently, kindlin-2 has been reported to form a homodimer based on its crystal structure, and this property of kindlins was suggested to have an important role in facilitating integrin clustering (Li et al., 2017). Taken together, it is clear that kindlins play significant roles in the regulation of integrin avidity. In the following sections, we will discuss the structure–function relationships of kindlins and the molecular basis of kindlin oligomerization.

Domain organization and interaction partners of kindlins

In 1998, the name FERM domain was coined, which is based on its initial identification in membrane-cytoskeleton proteins band 4.1 (EPB41), ezrin, radixin and moesin, in order to establish a consistent nomenclature because of the increasing number of proteins reported to contain this domain (Chishti et al., 1998). Generally, the FERM domain contains three subdomains (F1, F2 and F3) (Frame et al., 2010), with linear domain organization (shown in Fig. 1). Like talin, kindlins contain a F0 domain that precedes the F1 domain (Bouaouina et al., 2008; Elliott et al., 2010; Goult et al., 2009; Yates et al., 2012a,b). A unique feature of kindlins among FERM proteins is the presence of a pleckstrin homology (PH) domain that is inserted into the F2 subdomain (Karakose et al., 2010).

A major role of kindlins is to regulate integrin avidity by directly binding to integrin β cytoplasmic tails. Early studies have shown that the phosphotyrosine-binding (PTB)-like region in the F3 domain of kindlins binds specifically to the highly conserved membrane distal NPxY/F motif in the integrin β cytoplasmic tails (Moser et al., 2008; Ma et al., 2008; Montanez et al., 2008; Harburger et al., 2009). In addition to interacting with integrin β cytoplasmic tails, the F3 domain of kindlin-2, for example, binds to clathrin heavy chain and modulates clathrin-dependent expression of the ATP/ADP catabolism enzymes CD39 and CD73 (also known as ENTPD1 and NT5E, respectively) in endothelial cells (Pluskota et al., 2013). Besides the F3 domain, other domains of kindlins are functionally important as well. The F0 domain of kindlin-2 binds negatively charged membrane (Perera et al., 2011), and the F0 domain of kindlin-1 is required for its targeting to focal adhesions (Goult et al., 2009). The F0 domain connects kindlins to the cytoskeleton by binding directly to actin and paxillin (Bledzka et al., 2016; Zhu et al., 2019; Gao et al., 2017; Böttcher et al., 2017). Furthermore, Src phosphorylation of Y193 in kindlin-2 F0 enhances kindlin-2-mediated cell spreading and migration (Liu et al., 2015a, b). Recently, the F0 domain of kindlin-3 has been reported to bind leupaxin, which shares homology with paxillin, and their association is crucial in maintaining podosome stability in preosteoclasts (Klapproth et al., 2019). The F1 domain of kindlins has a long flexible loop containing a poly-lysine stretch, which promotes binding to negatively charged membranes (Bouaouina et al., 2012; Chua et al., 2016). The F2 domain of kindlins is bisected by the PH domain into two halves. In kindlin-2, a 20-residue sequence between its F2 N-terminal half and PH domain is a key binding site for ILK (Fukuda et al., 2014; Guan et al., 2018; Kadry et al., 2018). This sequence is not identical between kindlins, which explains differences in the ability of kindlins to bind ILK and in their colocalization at focal adhesions (Huet-Calderwood et al., 2014).

The PH domain is found in a large number of signaling proteins and serves to localize proteins to membranes or enables protein–protein interactions (Scheffzek and Welti, 2012). As expected, the PH domain of kindlins binds to phosphatidylinositol phosphate (Liu et al., 2011; Yates et al., 2012a,b; Ni et al., 2017). In B cells and neutrophils, the PH domain is required for the recruitment of kindlin-3 to the plasma membrane (Hart et al., 2013; Wen et al., 2020). The interaction between kindlin-2 and paxillin, which involves the kindlin-2 PH domain, at nascent adhesion sites promotes the recruitment and activation of focal adhesion kinase (FAK; also known as PTK2) (Theodosiou et al., 2016). This in turn leads to Rac recruitment (Jung et al., 2011; Sun et al., 2017) and complex formation between kindlin-2 and Arp2/3, which promotes circumferential membrane protrusions (Böttcher et al., 2017). Kindlin-2 has also been shown to form a transcriptional complex via its PH domain with β-catenin and TCF4 to enhance Wnt signaling (Yu et al., 2012). The PH domain of kindlin-3 interacts with the scaffold protein receptor of activated protein C kinase 1 (RACK1) (Feng et al., 2012). RACK1 was first identified to bind and stabilize activated PKCβII, and it was subsequently detected in many signaling complexes, including the small ribosomal subunit (40S) (Gibson, 2012). Kindlin-3, via its interaction with RACK1,

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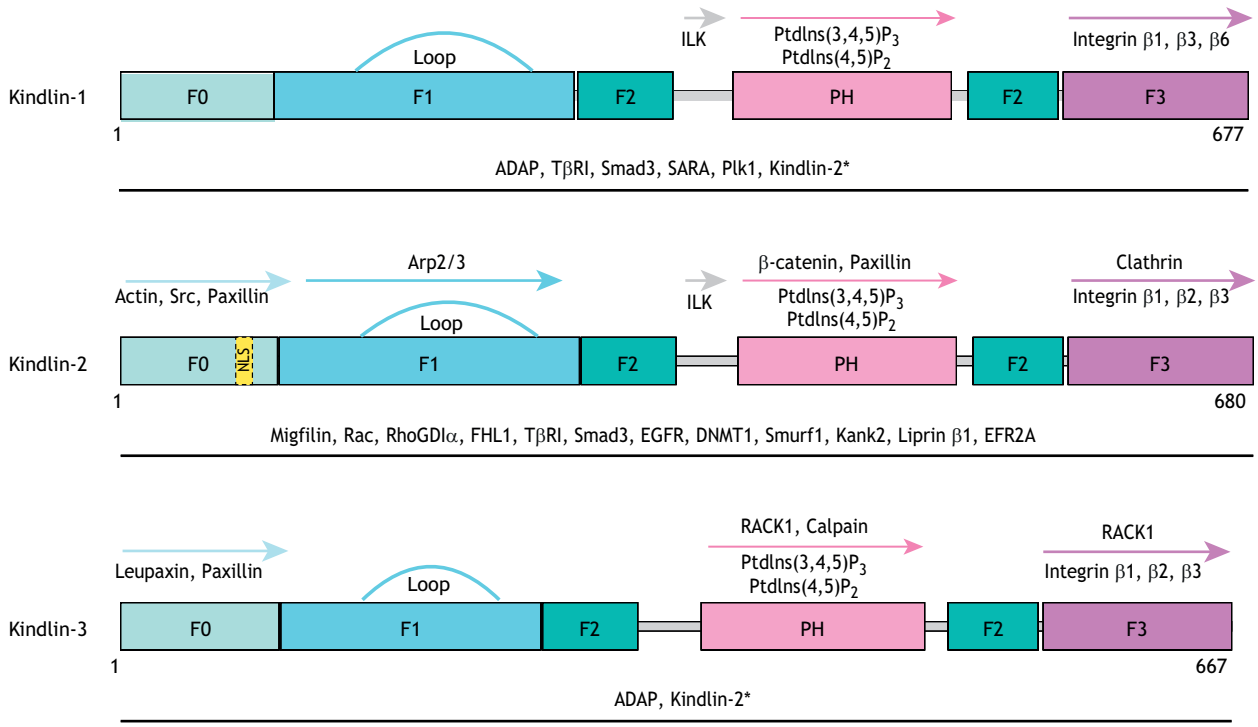

Fig. 1. Domain organization and reported interaction partners of kindlins. The domains F0, F1, the F1 inserted loop, domains F2, PH, and F3 are colored in pale cyan, blue, aquamarine, deep teal, pink and purple are shown for kindlin-1, -2 and -3. The nuclear localization sequence (NLS), located in domain F0 (in yellow), is unique for kindlin-2. Interaction partners reported to interact with specific known subdomains are indicated above or under the corresponding arrows, and those, for which their kindlin interaction domain is not known, are noted below the domain structure. ADAP, adhesion and degranulation promoting adapter protein (also known as FYB1); TβRI, TGF-β receptor I (also known as TGFBR1); SARA, Smad anchor for receptor activation (also known as SAR1A); RhoGDIα, Rho GDP-dissociation inhibitor α (also known as ARHGDIA); FHL1, four-and-a-half LIM protein 1; EGFR, epidermal growth factor receptor; DNMT1, DNA methyltransferase 1.

may be involved in the regulation of protein translation, and this would be an interesting area to expand our structural study on the ribosome and its associated factors into (Qu et al., 2015; Ero et al., 2015, 2016; Selmer et al., 2012). Recently, a molecular complex consisting of kindlin-3, RACK1 and the Ca^{2+} channel Orai1 has been shown to promote Ca^{2+} flux in neutrophils (Morikis et al., 2020). Additional binding partners of kindlins with yet to be fully defined binding regions are also depicted in Fig. 1 (Tu et al., 2003; Kasirer-Friede et al., 2014; Sun et al., 2017; Wang et al., 2018a,b; Wei et al., 2013; Kong et al., 2016; Guo et al., 2015; Patel et al., 2013, 2016; Zhao et al., 2012; Wei et al., 2017; Dong et al., 2016; Huttlin et al., 2017). These interactions could be weak and transient, and additional studies are needed to further characterize them. Collectively, these findings support wide-ranging functions of kindlins via diverse interacting partners.

Kindlin structure and insights into the mechanism underlying kindlin oligomerization

A better understanding of the functions of kindlin requires their structural information. Structural studies of protein 4.1, ezrin, radixin and moesin have revealed that the FERM domain is made up of three subdomains (F1, F2 and F3) which fold into a compact clover-leaf conformation (Han et al., 2000; Smith et al., 2003; Hamada et al., 2000; Pearson et al., 2000). Generally, the F1 domain adopts a ubiquitin-like fold, the F2 domain, which contains primarily α-helices, is similar to the acyl-CoA binding protein, and the F3 domain contains features resembling PTB, PH and enabled/VASP homology 1 (EVH1) domains (Pearson et al., 2000; Frame et al., 2010). Talin and kindlins share high sequence similarity with respect to their FERM domain as compared to other FERM proteins (Ali and Khan, 2014; Frame et al., 2010). The crystal structure of the talin head, which contains the F0 and FERM domains, reveals that it has an extended conformation of its F1, F2 and F3 domains, which is distinct from the typical compact FERM structures (Elliott et al., 2010). Successful fitting of structures of F0, F2 and F3 (from talin) and that of PH and F1 (from kindlin-1) into the full-length kindlin-3 small angle X-ray scattering (SAXS) structure suggests an overall extended kindlin conformation (Yates et al., 2012a,b). However, recent studies have revealed that a clover-leaf conformation is adopted by the FERM domain of talin (Zhang et al., 2020) and that of kindlins (Li et al., 2017; Bu et al., 2020; Sun et al., 2020).

In 2009, the first nuclear magnetic resonance (NMR) structure of the kindlin-1 F0 domain revealed that this domain employs a ubiquitin-like fold with two small hydrophobic α helices surrounded by a five-stranded twisted β-sheet (β1, β2, α1, β3, β4, α2 and β5) with a highly flexible N-terminal region (Goult et al., 2009). Sequence alignment of kindlins F1 domain with other typical FREM proteins identified a long unstructured insertion with a cluster of highly conserved poly-lysine motifs; these positively charged residues are essential for focal adhesion localization and integrin activation (Bouaouina et al., 2012). The overall conformation of the kindlin F1 subdomain together with a long loop (F1 loop) had long been unclear till our group determined the full-length crystal structure of human kindlin-3; the structure demonstrated that the F1 subdomain is an α/β barrel (β1, β2, α1, α2, β3, loop and β4) and the F1 loop (composed of two short helices) involves multilateral contacts with domains F2, PH and F3, respectively (Figs 1 and 2) (Bu et al., 2020). The F2 domain is a

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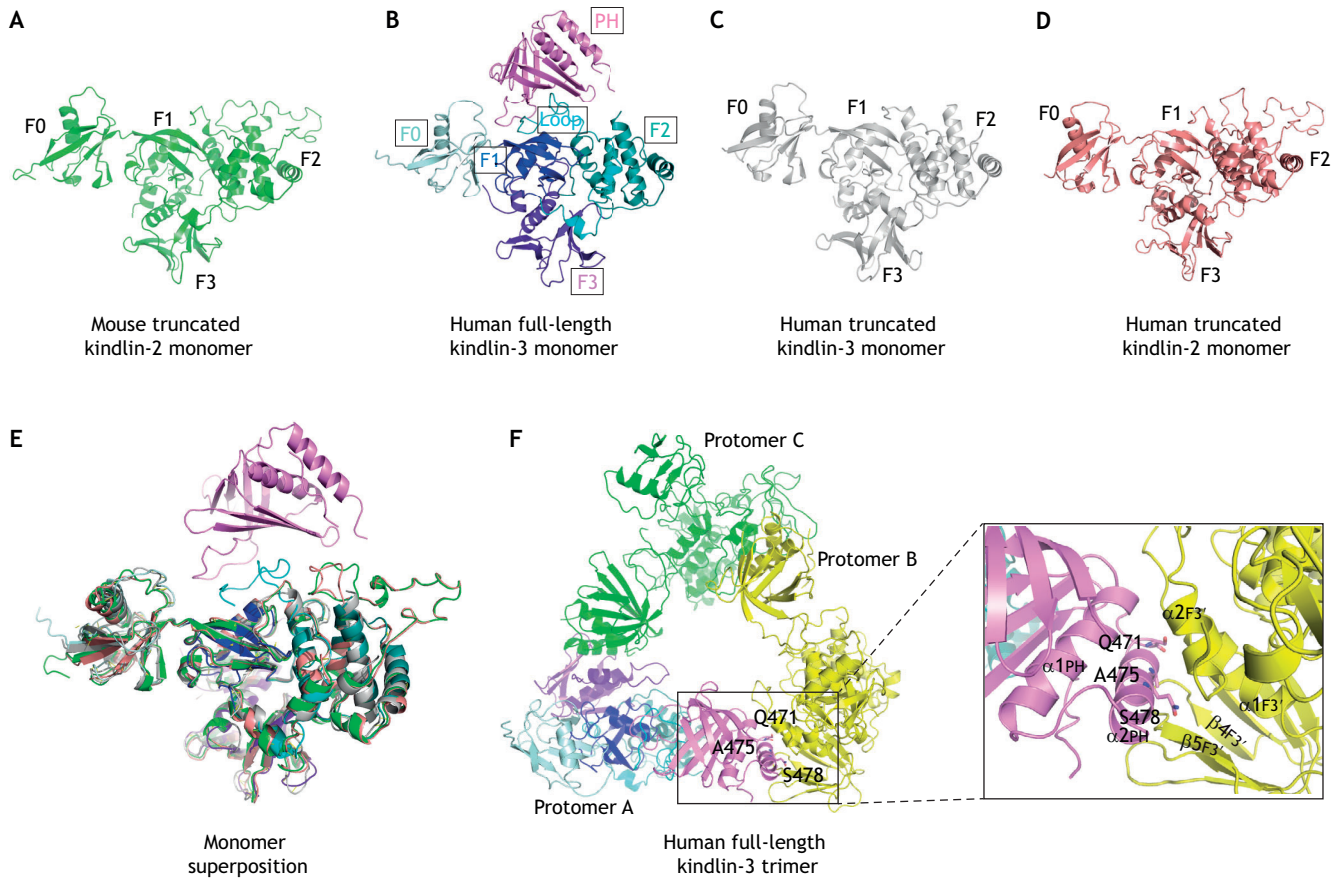

Fig. 2. Kindlins share a similar domain organization. (A) Crystal structure of mouse kindlin-2 monomer with F1 loop and PH domain truncated (PDB: 5XPY). (B) Crystal structure of human full-length kindlin-3 (PDB: 7C3M). (C) Crystal structure of human kindlin-3 monomer with the F1 loop and PH domain truncated (PDB: 6V9G). (D) Crystal structure of human kindlin-2 monomer with the F1 loop and PH domain truncated (PDB: 6XTJ). (E) Superposition of all four monomers indicates a cloverleaf-like conformation of kindlins. (F) Crystal structure of human full-length kindlin-3 trimer (left) and an enlarged view of the trimer interface (right). Full-length kindlin-3 forms a trimer through the interaction between the PH domain of one protomer and the F3 domain of the neighboring protomer. Specifically, the $\alpha 2_{PH}$ helix of one protomer (e.g. promoter A) can form extensive contacts with the two C-terminal helices ($\alpha 1_{F3'}$ and $\alpha 2_{F3'}$) and the β-sheet ($\beta 5_{F3'}$–$\beta 7_{F3'}$) of the neighboring protomer (promoter B). Mutations Q471A, A475F and S478A (referred to as AFA) have been shown to disrupt trimer formation. Note that the truncation strategies employed for kindlin crystallization in PDB 5XPY, 6C9G and 6XTJ were almost identical.

compact α-bundle containing five helices, with the first two helices crossed (α1 and α2), a PH domain inserted and the remaining 3 helices (α3, α4 and α5) forming an arch (Bu et al., 2020). With regard to the PH and F3 domains, they are both composed of a β-barrel and two α-helices (Liu et al., 2011, 2012; Yates et al., 2012a,b; Ni et al., 2017; Li et al., 2017; Bu et al., 2020).

In contrast to the isolated domain structures, the structure of a full-length kindlin at atomic resolution had been a long-standing mystery, likely due to the many disordered regions and relative flexibility of the kindlin sub-domains. To overcome this difficulty, the long F1 loop and PH domain of mouse kindlin-2 were removed to facilitate crystallization (Fig. 2A) (Li et al., 2017). The subsequently solved integrin-bound kindlin-2 structure offered definitive insights into the mechanism by which the F3 subdomain interacts with the integrin β cytoplasmic tail. The same study also proposed the formation of a kindlin-2 dimer as a mechanism by which kindlins induce integrin clustering. However, the truncation strategy employed for crystallization by deleting the PH domain and artificially linking of the two halves of the F2 subdomain, could lead to conformational perturbations in the overall structure, which may also explain the slow process of dimerization (a few days in 4°C) as stated in the study (Li et al., 2017).

More recently, we reported the crystal structure of human full-length kindlin-3 (Bu et al., 2020) (Fig. 2B). This was achieved through extensive large-scale preparations of a highly homogeneous kindlin population based on a bacterial expression system in combination with rational protein crystallization by surface-residue engineering (Derewenda, 2004). To our surprise, instead of a homodimer, we observed three kindlin-3 molecules that form a homotrimer in an asymmetric unit of the crystal (Bu et al., 2020). Almost at the same time, the crystal structure of a truncated human kindlin-3 was reported (Sun et al., 2020) via an experiment that followed the same strategy (PH domain and F1 loop truncation) employed for mouse kindlin-2 (Li et al., 2017) (Fig. 2C). However, in this structure, homodimer formation was neither detected in solution nor in the crystal structure (Li et al., 2017; Sun et al., 2020), nor is it present in a newly released PDB data set (PDB ID: 6XTJ, a truncated human kindlin-2, unpublished thus far) (Fig. 2D). Note that the monomer and dimer structures of another truncated mouse kindlin-2 construct (achieved by cysteine mutation to form a disulphide crosslink) were reported in BioRxiv by the same group that solved the mouse kindlin-2 structure (Li et al., 2017), but the PDB data are not available yet to make any comparison (Li et al., 2020 preprint).

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Despite the differences in kindlin-3 oligomer formation, the overall structures of human kindlin-3 monomer, both the full-length (Bu et al., 2020) and the truncated version (Sun et al., 2020), are similar to that of the truncated mouse and human kindlin-2 monomers (Li et al., 2017) (PDB ID: 6XTJ), all with a cloverleaf-like conformation (Fig. 2E). Looking at the full-length kindlin-3 structure with domains F0–F1–F2 (left to right) sequentially arranged along the horizontal plane, the PH and F3 domains are positioned at the upper and bottom flanks, respectively (Bu et al., 2020). The FERM subdomains (F1 to F3) form a compact core, with the F0 and PH domains surrounded in a clockwise direction (viewed from left to right for F0–F1–F2) (Fig. 2E). Apparently, the PH domain undergoes fewer interactions with the FERM core domain, indicating its flexibility and dynamic features (Bu et al., 2020).

Given human kindlin-3 and truncated mouse kindlin-2 have been reported to be able to form a homotrimer and homodimer, respectively, it is of interest to compare the oligomerization interface. As reported in our study, the structural superposition of human kindlin-3 trimer with the mouse PH-deleted kindlin-2 dimer reveals steric clashes that would disfavor dimer formation through the F2 subdomain in the full-length kindlin-2 (Bu et al., 2020). In line with structural analysis, the formation of PH-deleted kindlin-2 dimer in solution took a number of days, and thus is unlikely to be physiologically relevant (Li et al., 2017). We also analyzed human full-length kindlin-2 and kindlin-3 using the same incubation conditions, but could not detect dimer formation. In contrast, both proteins form trimers in solution when they are expressed in insect cells, but not in bacteria (Bu et al., 2020).

Because the trimeric structure of human full-length kindlin-3 was achieved by crystalizing the monomer that had been obtained by expression in bacteria, it is crucial to demonstrate that kindlin-3 is indeed also able to form a homotrimer in eukaryotic cells. Trimer formation of human full-length kindlin-3 was corroborated by a set of combinatory and complementary approaches, both *in vitro* and *in vivo* (Bu et al., 2020). It is worth mentioning, however, that the trimeric form of kindlin-3 is only a minor population compared with the monomeric pool obtained during the protein preparation procedure (Bu et al., 2020), which explains why it may have been overlooked thus far. Note that oligomerization of human kindlin-3 was also reported in a disuccinimidyl suberate (DSS) crosslinking assay, but the authors interpreted the crosslinked band in their gels as an indication of the presence of a homodimer (Sun et al., 2020). However, the use of a GST–EGFP–kindlin-3 expression construct may not be appropriate for use in a crosslinking assay, given the propensity of GST to dimerize. Moreover, native PAGE is not suitable to estimate a molecular mass as protein migration and separation mainly depend on the intrinsic change (Wittig and Schägger, 2005).

A large number of proteins have evolved to adopt particular oligomeric quaternary structures for their function or stability (Frieden, 2019; Gwyther et al., 2019; Griffin and Gerrard, 2012; Hashimoto and Panchenko, 2010). As proposed by Li et al., kindlin-2 could form dimers through F2–F2 interaction and in that way promote integrin clustering and activation (Li et al., 2017). Conversely, we found that kindlin-3 could form trimers through PH–F3 domain interaction, which would inhibit integrin activation as the integrin-binding site was blocked in the trimer (see below) (Bu et al., 2020). Sun et al. have suggested that F1–F3 domain interactions lead to kindlin-3 dimerization and facilitate integrin activation (Sun et al., 2020). Interestingly, a recent study reported a difference in the self-association properties of kindlin-2 and kindlin-3, whereby the PH domain is necessary for kindlin oligomerization (dimers up to tetramers), but the F3 domain is inhibitory (Kadry et al., 2020). Notably, the LS (L327S328) and AQ motifs (A547Q548) were reported to be important for the dimerization of domain-swapped truncated mouse kindlin-2 (Li et al., 2017). Their mutations were recently shown to impair phase separation of kindlin-2 clustered with integrin and to inhibit the assembly of cell adhesion complexes (Li et al., 2020 preprint). In contrast, no effect of the two motifs on the oligomerization of both human kindlin-2 and kindlin-3 expressed in mammalian cells has been observed, despite the fact that the motifs are conserved (Kadry et al., 2020). Therefore, how oligomerization occurs remains controversial due to different protein constructs (particularly truncations) and different

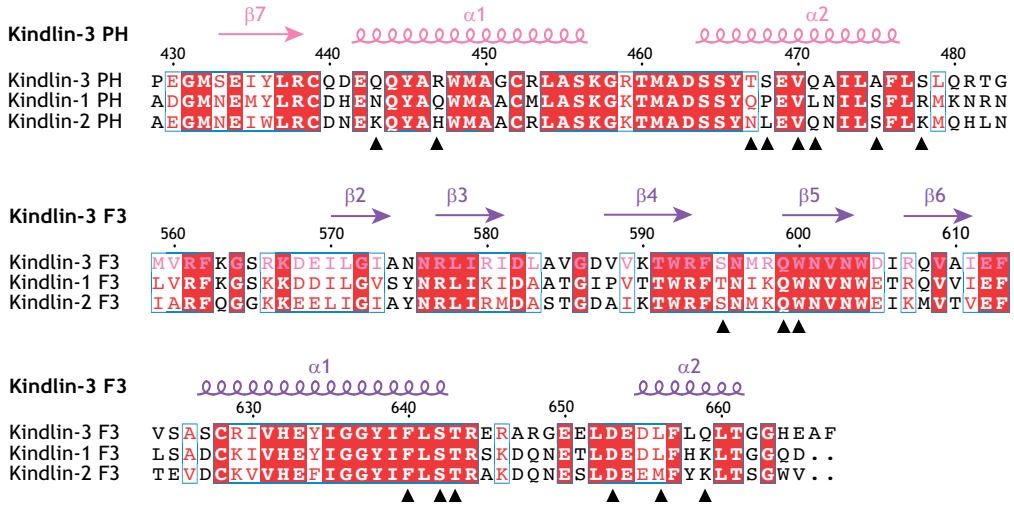

Fig. 3. The residues involving in kindlin-3 trimer interface are highly conserved among kindlins. The top line shows the secondary structures of kindlin-3. Below is the sequence alignment for part of the PH domain and F3 domain. The black triangles indicate 17 residues involved in kindlin-3 trimer formation; this includes the residues in PH domain (Q443 and R447 in $\alpha1_{PH}$, T467, S468, V470 and A475 in $\alpha2_{PH}$ and S478 in the following loop) and F3 domain (S595 between $\beta4_{F3}$ and $\beta5_{F3}$, Q599 and W600 in $\beta5_{F3}$, A610 in $\beta6_{F3}$, I630, F640, S642 and T643 in $\alpha1_{F3}$, D653 between $\alpha1_{F3}$ and $\alpha2_{F3}$, L656 and Q659 in $\alpha2_{F3}$). Sequence analysis shows that seven of these residues (~ 41%) are fully conserved among human kindlins. The secondary structure elements and residue numbering are based on human full-length kindlin-3. Boxes indicate the conserved regions in which the red letters indicate that the residues are conserved between two kindlins, and red shading indicates that the residues are conserved among all kindlins.

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approaches used, and further studies are needed to clarify this issue, as well as to fully understand the oligomerization properties of kindlins.

Relevance of oligomerization for kindlin function

The crystal structure of the kindlin-3 homotrimer shows that it adopts a close triangular form assembled by head-to-tail interaction between the PH domain of one protomer and the F3 domain of the next protomer (Fig. 2F) (Bu et al., 2020). Likewise, the PH domains of protomers B and C forms the same contacts with the F3 domains of neighboring protomers C and A, respectively (Fig. 2F). Trimer formation could be disrupted by mutating three residues, namely Q471A, A475F and S478A (henceforth referred to as AFA) found in the protomer–protomer interaction sites. Indeed, kindlin-3 with AFA mutations expressed in insect cells only exhibited the monomeric form (Bu et al., 2020). Interestingly, sequence alignment shows that almost half of the residues involved in kindlin-3 homotrimer formation are completely conserved (Fig. 3). In particular, the interacting residues in the $\alpha 2_{PH}$ helix (PH domain) and those in $\beta 5_{F3}$, $\alpha 1_{F3}$, $\alpha 2_{F3}$ (F3 domain), which are the major residues that mediate trimer formation, are the most highly conserved. Notably, the residues A475 and S595 in kindlin-3 are not conserved, but they form hydrogen-bonding interaction through their main chains, and the residues for all kindlins at the equivalent positions have similar small side chains. Thereby, it appears that the trimer interface could be conserved for human kindlins.

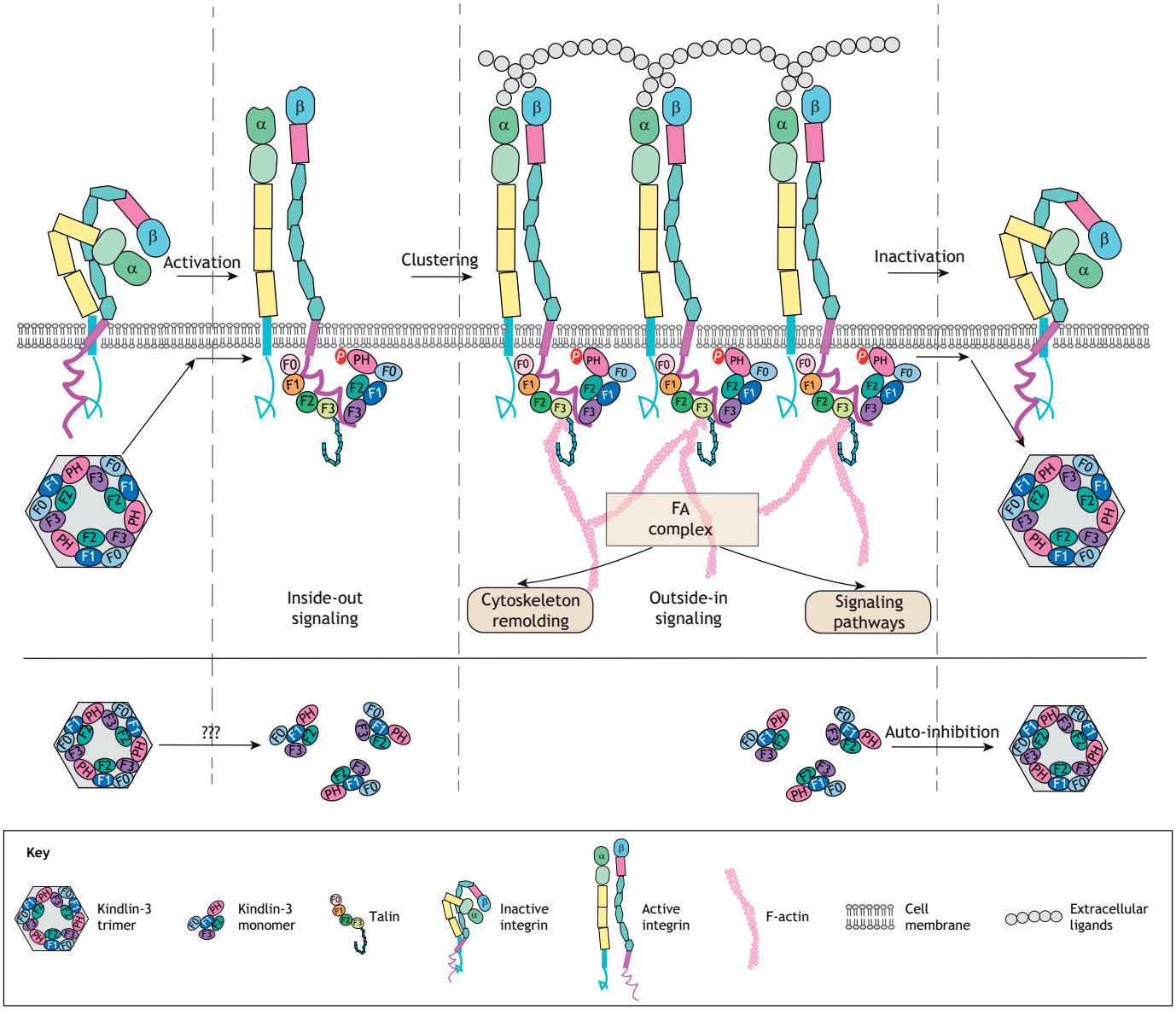

Fig. 4. A model for kindlin-3 regulation involving auto-inhibition by trimer formation. The kindlin-3 trimer (auto-inhibited state) does not activate integrins. In inactive integrins, both the α and β subunits are non-covalently clasped. Talin or kindlin (trimers) are unable to bind to the cytoplastic tail of the integrin β subunit, and thus no linkage to the ECM is formed. An as-yet-unknown mechanism induces the dissociation of the kindlin-3 trimer into active monomers. The kindlin-3 monomer, which could be phosphorylated, then cooperates with talin to separate the integrin α and β cytoplastic tail, which in turn leads to integrin activation, termed 'inside-out' signaling. The engagement of activated integrin with multivalent ligands leads to integrin clustering, which can be stabilized by interactions of talin and kindlin with the plasma membrane and actin, with the subsequent recruitment of focal adhesion proteins in a process termed 'outside-in' signaling. Integrins may revert to the inactive state when the kindlin-3 monomer dissociates from the integrin β cytoplasmic tail and reforms the trimer. Kindlin domains are colored as in Fig. 1.

We also superimposed the structures of mouse kindlin-2 in complex with the integrin cytoplasmic tail and the human kindlin-3 homotrimer (Bu et al., 2020). Both structures share a similar arrangement of the F0, F1 and F3 domains, but they are different with regard to the F3-integrin-binding pocket. In the kindlin-3 homotrimer, the integrin-binding site in F3 of each protomer is blocked by the $\alpha2_{PH}$ helix of the neighboring protomer, indicating that the trimer formation would inhibit the binding of kindlin-3 to the integrin cytoplasmic tail. In line with this structural insight, and unlike the slightly weak binding affinity of the kindlin-3 monomer to the integrin β1 cytoplasmic tail (K_d ∼197 μM) compared with that of kindlin-2 (K_d ∼13.4 μM), the kindlin-3 homotrimer is unable to bind to this integrin, suggesting that trimer formation auto-inhibits kindlin function (Bu et al., 2020). In addition, the mutant full-length AFA kindlin-3 monomer mentioned above exhibited only the monomeric form, which was able to bind to the integrin β1A cytoplasmic tail with a much higher binding affinity (K_d ∼13.8 μM) (Bu et al., 2020). Accordingly, the kindlin-3 AFA mutant enhances cell adhesion and spreading, as compared to wild-type kindlin-3, further pointing to kindlin oligomerization regulating its function (Bu et al., 2020).

In accordance with this notion, mutations in the F3 domain of human kindlin-3 (R595, D601, E641-RAR-G645 mutated to that of kindlin-2) that enhance its affinity for integrin have been shown to impair its oligomerization (Kadry et al., 2020). Interestingly, these residues are involved in the formation of a binding pocket that could be used to interact either with the PH domain of another kindlin-3 molecule for trimerization or with an integrin β tail, which is mutually exclusive (Bu et al., 2020). Therefore, our homotrimer structure can help to rationalize these mutation data and the observed requirement of the PH domain for kindlin self-association. Given the high sequence conservation between kindlins (including those residues involved in homotrimer formation) (Fig. 3), their high structure similarity (Fig. 2) and kindlin-2 trimer formation in solution (Bu et al., 2020), our homotrimer model of an auto-inhibited state is perhaps universal for all kindlins.

With regard to future research, it would certainly be of interest and significance to explore how kindlin oligomerization is regulated, for instance by post-translational modifications (PTMs). Indeed, PTMs, in particularly phosphorylation, which has been the most extensively studied, can induce diverse conformational changes, such as in order–disorder, association and dissociation of complexes and monomer–oligomer transitions, which could be involved in molecular recognition and signal transduction. Phosphorylation of kindlin-1 (Y13 and Y82) and kindlin-2 (T8 and T30) has been reported to be important for their functions (Patel et al., 2013; Qu et al., 2014). An integrated and comprehensive analysis of kindlin PTMs, including their occurrence in the different oligomers from diverse expression systems and their biochemical characterization by *in vitro* phosphorylation and dephosphorylation assays and mutation analysis, in combination with biological and biochemical assays, such as high-throughput screening to identify other PTMs and the respective kinases/phosphatases could offer important insights into the mechanism by which kindlin oligomerization is dynamically modulated.

Conclusions

In summary, recent studies on kindlins by different groups using diverse approaches have greatly boosted our understanding of their structures and functions, notably the reported oligomerization of kindlins. Based on our full-length structure of human kindlin-3 and its detailed characterization, we propose a model in which the kindlin-3 monomer presents an active state and the homotrimer an auto-inhibited state (Fig. 4). Disassembly of the trimer into the monomer, by an as-yet-unknown mechanism, possibly involving phosphorylation and/or other regulatory factors, could convert kindlin-3 into an active state that is able to bind the integrin β cytoplasmic tail and to cooperate with talin to induce integrin activation. Kindlins can also promote integrin clustering by interacting with plasma membrane phospholipids, actin and focal adhesion proteins (Fig. 4). An integrin could be converted from an active into an inactive state upon dissociation of the kindlin-3 monomer from its cytoplasmic tail and the reformation of the trimer. Given the sequence and structure similarity between kindlins, such an auto-inhibition mechanism might apply across the kindlin family. Note that we cannot exclude the possibility that the kindlin-3 homotrimer, which is unable to bind to integrin binding, can associate with other interacting partners to exert other functions, because many of the interaction sites are not masked in the trimer configuration. Furthermore, the precise mechanisms by which kindlin oligomerization is regulated requires further investigation. In the near future, additional high-resolution structures of kindlins alone or in complex with their interacting partners will provide important insights into kindlin biology.

Competing interests

The authors declare no competing or financial interests.

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References

Ali, R. H. and Khan, A. A. (2014). Tracing the evolution of FERM domain of Kindlins. *Mol. Phylogenet. Evol.* **80**, 193-204. doi:10.1016/j.ympev.2014.08.008

Bialkowska, K., Ma, Y.-Q., Bledzka, K., Sossey-Alaoui, K., Izem, L., Zhang, X., Malinin, N., Qin, J., Byzova, T. and Plow, E. F. (2010). The integrin co-activator Kindlin-3 is expressed and functional in a non-hematopoietic cell, the endothelial cell. *J. Biol. Chem.* **285**, 18640-18649. doi:10.1074/jbc.M109.085746

Bledzka, K., Bialkowska, K., Sossey-Alaoui, K., Vaynberg, J., Pluskota, E., Qin, J. and Plow, E. F. (2016). Kindlin-2 directly binds actin and regulates integrin outside-in signaling. *J. Cell Biol.* **213**, 97-108. doi:10.1083/jcb.201501006

Böttcher, R. T., Veelders, M., Rombaut, P., Faix, J., Theodosiou, M., Stradal, T. E., Rottner, K., Zent, R., Herzog, F. and Fässler, R. (2017). Kindlin-2 recruits paxillin and Arp2/3 to promote membrane protrusions during initial cell spreading. *J. Cell Biol.* **216**, 3785-3798. doi:10.1083/jcb.201701176

Bouaouina, M., Lad, Y. and Calderwood, D. A. (2008). The N-terminal domains of talin cooperate with the phosphotyrosine binding-like domain to activate β1 and β3 integrins. *J. Biol. Chem.* **283**, 6118-6125. doi:10.1074/jbc.M709527200

Bouaouina, M., Goult, B. T., Huet-Calderwood, C., Bate, N., Brahme, N. N., Barsukov, I. L., Critchley, D. R. and Calderwood, D. A. (2012). A conserved lipid-binding loop in the kindlin FERM F1 domain is required for kindlin-mediated alphaIIbbeta3 integrin coactivation. *J. Biol. Chem.* **287**, 6979-6990. doi:10.1074/jbc.M111.330845

Brahme, N. N., Harburger, D. S., Kemp-O'brien, K., Stewart, R., Raghavan, S., Parsons, M. and Calderwood, D. A. (2013). Kindlin binds migfilin tandem LIM domains and regulates migfilin focal adhesion localization and recruitment dynamics. *J. Biol. Chem.* **288**, 35604-35616. doi:10.1074/jbc.M113.483016

Bromberger, T., Klapproth, S., Rohwedder, I., Zhu, L., Mittmann, L., Reichel, C. A., Sperandio, M., Qin, J. and Moser, M. (2018). Direct Rap1/Talin1 interaction regulates platelet and neutrophil integrin activity in mice. *Blood* **132**, 2754-2762. doi:10.1182/blood-2018-04-846766

Bromberger, T., Zhu, L., Klapproth, S., Qin, J. and Moser, M. (2019). Rap1 and membrane lipids cooperatively recruit talin to trigger integrin activation. *J. Cell Sci.* **132**, jcs235531. doi:10.1242/jcs.235531

Bu, W., Levitskaya, Z., Loh, Z. Y., Jin, S., Basu, S., Ero, R., Yan, X., Wang, M., Ngan, S. F. C., Sze, S. K. et al. (2020). Structural basis of human full-length kindlin-3 homotrimer in an auto-inhibited state. *PLoS Biol.* **18**, e3000755. doi:10.1371/journal.pbio.3000755

Calderwood, D. A., Campbell, I. D. and Critchley, D. R. (2013). Talins and kindlins: partners in integrin-mediated adhesion. *Nat. Rev. Mol. Cell Biol.* **14**, 503-517. doi:10.1038/nrm3624

Chinthalapudi, K., Rangarajan, E. S. and Izard, T. (2018). The interaction of talin with the cell membrane is essential for integrin activation and focal adhesion formation. *Proc. Natl. Acad. Sci. USA* **115**, 10339-10344. doi:10.1073/pnas.1806275115

Chishti, A. H., Kim, A. C., Marfatia, S. M., Lutchman, M., Hanspal, M., Jindal, H., Liu, S.-C., Low, P. S., Rouleau, G. A., Mohandas, N. et al. (1998). The FERM domain: a unique module involved in the linkage of cytoplasmic proteins to the membrane. *Trends Biochem. Sci.* **23**, 281-282. doi:10.1016/S0968-0004(98)01237-7

Chua, G.-L., Tan, S.-M. and Bhattacharjya, S. (2016). NMR characterization and membrane interactions of the loop region of Kindlin-3 F1 subdomain. *PLoS ONE* **11**, e0153501. doi:10.1371/journal.pone.0153501

Critchley, D. R. and Gingras, A. R. (2008). Talin at a glance. *J. Cell Sci.* **121**, 1345-1347. doi:10.1242/jcs.018085

Das, M., Ithychanda, S. S., Qin, J. and Plow, E. F. (2011). Migfilin and filamin as regulators of integrin activation in endothelial cells and neutrophils. *PLoS ONE* **6**, e26355. doi:10.1371/journal.pone.0026355

Derewenda, Z. S. (2004). Rational protein crystallization by mutational surface engineering. *Structure* **12**, 529-535. doi:10.1016/j.str.2004.03.008

Dong, J.-M., Tay, F. P.-L., Swa, H. L.-F., Gunaratne, J., Leung, T., Burke, B. and Manser, E. (2016). Proximity biotinylation provides insight into the molecular composition of focal adhesions at the nanometer scale. *Sci. Signal.* **9**, rs4. doi:10.1126/scisignal.aaf3572

Dowling, J. J., Gibbs, E., Russell, M., Goldman, D., Minarcik, J., Golden, J. A. and Feldman, E. L. (2008). Kindlin-2 is an essential component of intercalated discs and is required for vertebrate cardiac structure and function. *Circ. Res.* **102**, 423-431. doi:10.1161/CIRCRESAHA.107.161489

Elliott, P. R., Goult, B. T., Kopp, P. M., Bate, N., Grossmann, J. G., Roberts, G. C. K., Critchley, D. R. and Barsukov, I. L. (2010). The Structure of the talin head reveals a novel extended conformation of the FERM domain. *Structure* **18**, 1289-1299. doi:10.1016/j.str.2010.07.011

Ero, R., Dimitrova, V. T., Chen, Y., Bu, W., Feng, S., Liu, T., Wang, P., Xue, C., Tan, S. M. and Gao, Y.-G. (2015). Crystal structure of Gib2, a signal-transducing protein scaffold associated with ribosomes in Cryptococcus neoformans. *Sci. Rep.* **5**, 8688. doi:10.1038/srep08688

Ero, R., Kumar, V., Chen, Y. and Gao, Y.-G. (2016). Similarity and diversity of translational GTPase factors EF-G, EF4, and BipA: From structure to function. *RNA Biol.* **13**, 1258-1273. doi:10.1080/15476286.2016.1201627

Feng, C., Li, Y.-F., Yau, Y.-H., Lee, H.-S., Tang, X.-Y., Xue, Z.-H., Zhou, Y.-C., Lim, W.-M., Cornvik, T. C., Ruedl, C. et al. (2012). Kindlin-3 mediates integrin αLβ2 outside-in signaling, and it interacts with scaffold protein receptor for activated-C kinase 1 (RACK1). *J. Biol. Chem.* **287**, 10714-10726. doi:10.1074/jbc.M111.299594

Frame, M. C., Patel, H., Serrels, B., Lietha, D. and Eck, M. J. (2010). The FERM domain: organizing the structure and function of FAK. *Nat. Rev. Mol. Cell Biol.* **11**, 802-814. doi:10.1038/nrm2996

Frieden, C. (2019). Protein oligomerization as a metabolic control mechanism: Application to apoE. *Protein Sci.* **28**, 837-842. doi:10.1002/pro.3583

Fukuda, K., Bledzka, K., Yang, J., Perera, H. D., Plow, E. F. and Qin, J. (2014). Molecular basis of kindlin-2 binding to integrin-linked kinase pseudokinase for regulating cell adhesion. *J. Biol. Chem.* **289**, 28363-28375. doi:10.1074/jbc.M114.596692

Gao, J., Huang, M., Lai, J., Mao, K., Sun, P., Cao, Z., Hu, Y., Zhang, Y., Schulte, M. L., Jin, C. et al. (2017). Kindlin supports platelet integrin αIIbβ3 activation by interacting with paxillin. *J. Cell Sci.* **130**, 3764-3775. doi:10.1242/jcs.205641

Garcia-Alvarez, B., De Pereda, J. M., Calderwood, D. A., Ulmer, T. S., Critchley, D., Campbell, I. D., Ginsberg, M. H. and Liddington, R. C. (2003). Structural determinants of integrin recognition by talin. *Mol. Cell* **11**, 49-58. doi:10.1016/S1097-2765(02)00823-7

Gibson, T. J. (2012). RACK1 research - ships passing in the night? *FEBS Lett.* **586**, 2787-2789. doi:10.1016/j.febslet.2012.04.048

Gingras, A. R., Lagarrigue, F., Cuevas, M. N., Valadez, A. J., Zorovich, M., Mclaughlin, W., Lopez-Ramirez, M. A., Seban, N., Ley, K., Kiosses, W. B. et al. (2019). Rap1 binding and a lipid-dependent helix in talin F1 domain promote integrin activation in tandem. *J. Cell Biol.* **218**, 1799-1809. doi:10.1083/jcb.201810061

Goksoy, E., Ma, Y.-Q., Wang, X., Kong, X., Perera, D., Plow, E. F. and Qin, J. (2008). Structural basis for the autoinhibition of talin in regulating integrin activation. *Mol. Cell* **31**, 124-133. doi:10.1016/j.molcel.2008.06.011

Goult, B. T., Bouaouina, M., Harburger, D. S., Bate, N., Patel, B., Anthis, N. J., Campbell, I. D., Calderwood, D. A., Barsukov, I. L., Roberts, G. C. et al. (2009). The structure of the N-terminus of kindlin-1: a domain important for αIIbβ3 integrin activation. *J. Mol. Biol.* **394**, 944-956. doi:10.1016/j.jmb.2009.09.061

Goult, B. T., Bouaouina, M., Elliott, P. R., Bate, N., Patel, B., Gingras, A. R., Grossmann, J. G., Roberts, G. C. K., Calderwood, D. A., Critchley, D. R. et al. (2010). Structure of a double ubiquitin-like domain in the talin head: a role in integrin activation. *EMBO J.* **29**, 1069-1080. doi:10.1038/emboj.2010.4

Griffin, M. D. W. and Gerrard, J. A. (2012). The relationship between oligomeric state and protein function. In *Protein Dimerization and Oligomerization in Biology* (ed. J. M. Matthews), pp. 74-90. New York, NY: Springer New York.

Guan, S.-Y., Chng, C.-P., Ong, L.-T., Tan, H.-F., Alex Law, S. K. and Tan, S.-M. (2018). The binding interface of kindlin-2 and ILK involves Asp344/Asp352/Thr356 in kindlin-2 and Arg243/Arg334 in ILK. *FEBS Lett.* **592**, 112-121. doi:10.1002/1873-3468.12938

Guo, B., Gao, J., Zhan, J. and Zhang, H. (2015). Kindlin-2 interacts with and stabilizes EGFR and is required for EGF-induced breast cancer cell migration. *Cancer Lett.* **361**, 271-281. doi:10.1016/j.canlet.2015.03.011

Gwyther, R. E. A., Jones, D. D. and Worthy, H. L. (2019). Better together: building protein oligomers naturally and by design. *Biochem. Soc. Trans.* **47**, 1773-1780. doi:10.1042/BST20190283

Haage, A., Goodwin, K., Whitewood, A., Camp, D., Bogutz, A., Turner, C. T., Granville, D. J., Lefebvre, L., Plotnikov, S., Goult, B. T. et al. (2018). Talin autoinhibition regulates cell-ECM adhesion dynamics and wound healing *in vivo*. *Cell Rep.* **25**, 2401-2416.e5. doi:10.1016/j.celrep.2018.10.098

Hamada, K., Shimizu, T., Matsui, T., Tsukita, S. and Hakoshima, T. (2000). Structural basis of the membrane-targeting and unmasking mechanisms of the radixin FERM domain. *EMBO J.* **19**, 4449-4462. doi:10.1093/emboj/19.17.4449

Han, B.-G., Nunomura, W., Takakuwa, Y., Mohandas, N. and Jap, B. K. (2000). Protein 4.1R core domain structure and insights into regulation of cytoskeletal organization. *Nat. Struct. Biol.* **7**, 871-875. doi:10.1038/82819

Han, J., Lim, C. J., Watanabe, N., Soriani, A., Ratnikov, B., Calderwood, D. A., Puzon-Mclaughlin, W., Lafuente, E. M., Boussiotis, V. A., Shattil, S. J. et al. (2006). Reconstructing and deconstructing agonist-induced activation of integrin αIIbβ3. *Curr. Biol.* **16**, 1796-1806. doi:10.1016/j.cub.2006.08.035

Harburger, D. S., Bouaouina, M. and Calderwood, D. A. (2009). Kindlin-1 and -2 directly bind the C-terminal region of β integrin cytoplasmic tails and exert integrin-specific activation effects. *J. Biol. Chem.* **284**, 11485-11497. doi:10.1074/jbc.M809233200

Hart, R., Stanley, P., Chakravarty, P. and Hogg, N. (2013). The kindlin 3 pleckstrin homology domain has an essential role in lymphocyte function-associated antigen 1 (LFA-1) integrin-mediated B cell adhesion and migration. *J. Biol. Chem.* **288**, 14852-14862. doi:10.1074/jbc.M112.434621

Hashimoto, K. and Panchenko, A. R. (2010). Mechanisms of protein oligomerization, the critical role of insertions and deletions in maintaining different oligomeric states. *Proc. Natl. Acad. Sci. USA* **107**, 20352-20357. doi:10.1073/pnas.1012999107

Huet-Calderwood, C., Brahme, N. N., Kumar, N., Stiegler, A. L., Raghavan, S., Boggon, T. J. and Calderwood, D. A. (2014). Differences in binding to the ILK complex determines kindlin isoform adhesion localization and integrin activation. *J. Cell Sci.* **127**, 4308-4321. doi:10.1242/jcs.155879

Huttlin, E. L., Bruckner, R. J., Paulo, J. A., Cannon, J. R., Ting, L., Baltier, K., Colby, G., Gebreab, F., Gygi, M. P., Parzen, H. et al. (2017). Architecture of the human interactome defines protein communities and disease networks. *Nature* **545**, 505-509. doi:10.1038/nature22366

Hynes, R. O. (2002). Integrins: bidirectional, allosteric signaling machines. *Cell* **110**, 673-687. doi:10.1016/S0092-8674(02)00971-6

Jung, G.-Y., Park, Y.-J. and Han, J.-S. (2011). Mediation of Rac1 activation by kindlin-2: an essential function in osteoblast adhesion, spreading, and proliferation. *J. Cell. Biochem.* **112**, 2541-2548. doi:10.1002/jcb.23178

Kadry, Y. A., Huet-Calderwood, C., Simon, B. and Calderwood, D. A. (2018). Kindlin-2 interacts with a highly conserved surface of ILK to regulate focal adhesion localization and cell spreading. *J. Cell Sci.* **131**, jcs221184. doi:10.1242/jcs.221184

Kadry, Y. A., Maisuria, E. M., Huet-Calderwood, C. and Calderwood, D. A. (2020). Differences in self-association between kindlin-2 and kindlin-3 are associated with differential integrin binding. *J. Biol. Chem.* **295**, 11161-11173. doi:10.1074/jbc.RA120.013618

Karakose, E., Schiller, H. B. and Fassler, R. (2010). The kindlins at a glance. *J. Cell Sci.* **123**, 2353-2356. doi:10.1242/jcs.064600

Kasirer-Friede, A., Kang, J., Kahner, B., Ye, F., Ginsberg, M. H. and Shattil, S. J. (2014). ADAP interactions with talin and kindlin promote platelet integrin αIIbβ3 activation and stable fibrinogen binding. *Blood* **123**, 3156-3165. doi:10.1182/blood-2013-08-520627

Kiema, T., Lad, Y., Jiang, P., Oxley, C. L., Baldassarre, M., Wegener, K. L., Campbell, I. D., Ylanne, J. and Calderwood, D. A. (2006). The molecular basis of filamin binding to integrins and competition with talin. *Mol. Cell* **21**, 337-347. doi:10.1016/j.molcel.2006.01.011

Klapholz, B. and Brown, N. H. (2017). Talin - the master of integrin adhesions. *J. Cell Sci.* **130**, 2435-2446. doi:10.1242/jcs.190991

Klapproth, S., Bromberger, T., Türk, C., Krüger, M. and Moser, M. (2019). A kindlin-3-leupaxin-paxillin signaling pathway regulates podosome stability. *J. Cell Biol.* **218**, 3436-3454. doi:10.1083/jcb.201903109

Kong, J., Du, J., Wang, Y., Yang, M., Gao, J., Wei, X., Fang, W., Zhan, J. and Zhang, H. (2016). Focal adhesion molecule Kindlin-1 mediates activation of TGF-β signaling by interacting with TGF-βRI, SARA and Smad3 in colorectal cancer cells. *Oncotarget* **7**, 76224-76237. doi:10.18632/oncotarget.12779

Lad, Y., Jiang, P., Ruskamo, S., Harburger, D. S., Ylänne, J., Campbell, I. D. and Calderwood, D. A. (2008). Structural basis of the migfilin-filamin interaction and competition with integrin β tails. *J. Biol. Chem.* **283**, 35154-35163. doi:10.1074/jbc.M802592200

Lagarrigue, F., Kim, C. and Ginsberg, M. H. (2016). The Rap1-RIAM-talin axis of integrin activation and blood cell function. *Blood* **128**, 479-487. doi:10.1182/blood-2015-12-638700

Lagarrigue, F., Gingras, A. R., Paul, D. S., Valadez, A. J., Cuevas, M. N., Sun, H., Lopez-Ramirez, M. A., Goult, B. T., Shattil, S. J., Bergmeier, W. et al. (2018). Rap1 binding to the talin 1 F0 domain makes a minimal contribution to murine platelet GPIIb-IIIa activation. *Blood Adv.* **2**, 2358-2368. doi:10.1182/bloodadvances.2018020487

Larjava, H., Plow, E. F. and Wu, C. (2008). Kindlins: essential regulators of integrin signalling and cell-matrix adhesion. *EMBO Rep.* **9**, 1203-1208. doi:10.1038/embor.2008.202

Lee, H.-S., Lim, C. J., Puzon-Mclaughlin, W., Shattil, S. J. and Ginsberg, M. H. (2009). RIAM activates integrins by linking talin to ras GTPase membrane-targeting sequences. *J. Biol. Chem.* **284**, 5119-5127. doi:10.1074/jbc.M807117200

Li, H., Deng, Y., Sun, K., Yang, H., Liu, J., Wang, M., Zhang, Z., Lin, J., Wu, C., Wei, Z. et al. (2017). Structural basis of kindlin-mediated integrin recognition and activation. *Proc. Natl. Acad. Sci. USA* **114**, 9349-9354. doi:10.1073/pnas.1703064114

Li, Y., Zhang, T., Li, H., Yang, H., Lin, R., Sun, K., Wang, L., Zhang, J., Wei, Z. and Yu, C. (2020). Kindlin2-mediated phase separation underlies integrin adhesion formation. *bioRxiv*, 2020.07.10.197400. doi:10.1101/2020.07.10.197400

Liu, J., Fukuda, K., Xu, Z., Ma, Y.-Q., Hirbawi, J., Mao, X., Wu, C., Plow, E. F. and Qin, J. (2011). Structural basis of phosphoinositide binding to kindlin-2 protein pleckstrin homology domain in regulating integrin activation. *J. Biol. Chem.* **286**, 43334-43342. doi:10.1074/jbc.M111.295352

Liu, Y., Zhu, Y., Ye, S. and Zhang, R. (2012). Crystal structure of kindlin-2 PH domain reveals a conformational transition for its membrane anchoring and regulation of integrin activation. *Protein Cell* **3**, 434-440. doi:10.1007/s13238-012-2046-1

Liu, J., Das, M., Yang, J., Ithychanda, S. S., Yakubenko, V. P., Plow, E. F. and Qin, J. (2015a). Structural mechanism of integrin inactivation by filamin. *Nat. Struct. Mol. Biol.* **22**, 383-389. doi:10.1038/nsmb.2999

Liu, Z., Lu, D., Wang, X., Wan, J., Liu, C. and Zhang, H. (2015b). Kindlin-2 phosphorylation by Src at Y193 enhances Src activity and is involved in Migfilin recruitment to the focal adhesions. *FEBS Lett.* **589**, 2001-2010. doi:10.1016/j.febslet.2015.05.038

Luo, B.-H., Carman, C. V. and Springer, T. A. (2007). Structural basis of integrin regulation and signaling. *Annu. Rev. Immunol.* **25**, 619-647. doi:10.1146/annurev.immunol.25.022106.141618

Ma, Y.-Q., Qin, J., Wu, C. and Plow, E. F. (2008). Kindlin-2 (Mig-2): a co-activator of β3 integrins. *J. Cell Biol.* **181**, 439-446. doi:10.1083/jcb.200710196

Malinin, N. L., Zhang, L., Choi, J., Ciocea, A., Razorenova, O., Ma, Y.-Q., Podrez, E. A., Tosi, M., Lennon, D. P., Caplan, A. I. et al. (2009). A point mutation in KINDLIN3 ablates activation of three integrin subfamilies in humans. *Nat. Med.* **15**, 313-318. doi:10.1038/nm.1917

Manevich-Mendelson, E., Feigelson, S. W., Pasvolsky, R., Aker, M., Grabovsky, V., Shulman, Z., Kilic, S. S., Rosenthal-Allieri, M. A., Ben-Dor, S., Mory, A. et al. (2009). Loss of Kindlin-3 in LAD-III eliminates LFA-1 but not VLA-4 adhesiveness developed under shear flow conditions. *Blood* **114**, 2344-2353. doi:10.1182/blood-2009-04-218636

Montanez, E., Ussar, S., Schifferer, M., Bosl, M., Zent, R., Moser, M. and Fassler, R. (2008). Kindlin-2 controls bidirectional signaling of integrins. *Genes Dev.* **22**, 1325-1330. doi:10.1101/gad.469408

Moore, D. T., Nygren, P., Jo, H., Boesze-Battaglia, K., Bennett, J. S. and Degrado, W. F. (2012). Affinity of talin-1 for the β3-integrin cytosolic domain is modulated by its phospholipid bilayer environment. *Proc. Natl. Acad. Sci. USA* **109**, 793-798. doi:10.1073/pnas.1117220108

Morikis, V. A., Masadeh, E. and Simon, S. I. (2020). Tensile force transmitted through LFA-1 bonds mechanoregulate neutrophil inflammatory response. *J. Leukoc. Biol.* **108**, 1815-1828. doi:10.1002/JLB.3A0520-100RR

Moser, M., Nieswandt, B., Ussar, S., Pozgajova, M. and Fässler, R. (2008). Kindlin-3 is essential for integrin activation and platelet aggregation. *Nat. Med.* **14**, 325-330. doi:10.1038/nm1722

Ni, T., Kalli, A. C., Naughton, F. B., Yates, L. A., Naneh, O., Kozorog, M., Anderluh, G., Sansom, M. S. and Gilbert, R. J. C. (2017). Structure and lipid-binding properties of the kindlin-3 pleckstrin homology domain. *Biochem. J.* **474**, 539-556. doi:10.1042/BCJ20160791

Patel, H., Zich, J., Serrels, B., Rickman, C., Hardwick, K. G., Frame, M. C. and Brunton, V. G. (2013). Kindlin-1 regulates mitotic spindle formation by interacting with integrins and Plk-1. *Nat. Commun.* **4**, 2056. doi:10.1038/ncomms3056

Patel, H., Stavrou, I., Shrestha, R. L., Draviam, V., Frame, M. C. and Brunton, V. G. (2016). Kindlin1 regulates microtubule function to ensure normal mitosis. *J. Mol. Cell Biol.* **8**, 338-348. doi:10.1093/jmcb/mjw009

Pearson, M. A., Reczek, D., Bretscher, A. and Karplus, P. A. (2000). Structure of the ERM protein moesin reveals the FERM domain fold masked by an extended actin binding tail domain. *Cell* **101**, 259-270. doi:10.1016/S0092-8674(00)80836-3

Perera, H. D., Ma, Y.-Q., Yang, J., Hirbawi, J., Plow, E. F. and Qin, J. (2011). Membrane binding of the N-terminal ubiquitin-like domain of kindlin-2 is crucial for its regulation of integrin activation. *Structure* **19**, 1664-1671. doi:10.1016/j.str.2011.08.012

Plow, E. F., Das, M., Bialkowska, K. and Sossey-Alaoui, K. (2016). Of kindlins and cancer. *Discoveries (Craiova)* **4**, e59. doi:10.15190/d.2016.6

Pluskota, E., Ma, Y., Bledzka, K. M., Bialkowska, K., Soloviev, D. A., Szpak, D., Podrez, E. A., Fox, P. L., Hazen, S. L., Dowling, J. J. et al. (2013). Kindlin-2 regulates hemostasis by controlling endothelial cell-surface expression of ADP/AMP catabolic enzymes via a clathrin-dependent mechanism. *Blood* **122**, 2491-2499. doi:10.1182/blood-2013-04-497669

Qadota, H., Luo, Y., Matsunaga, Y., Park, A. S., Gernert, K. M. and Benian, G. M. (2014). Suppressor mutations suggest a surface on PAT-4 (Integrin-linked Kinase) that interacts with UNC-112 (Kindlin). *J. Biol. Chem.* **289**, 14252-14262. doi:10.1074/jbc.M114.556308

Qu, H., Tu, Y., Guan, J.-L., Xiao, G. and Wu, C. (2014). Kindlin-2 tyrosine phosphorylation and interaction with Src serve as a regulatable switch in the integrin outside-in signaling circuit. *J. Biol. Chem.* **289**, 31001-31013. doi:10.1074/jbc.M114.580811

Qu, J., Ero, R., Feng, C., Ong, L.-T., Tan, H.-F., Lee, H.-S., Ismail, M. H. B., Bu, W.-T., Nama, S., Sampath, P. et al. (2015). Kindlin-3 interacts with the ribosome and regulates c-Myc expression required for proliferation of chronic myeloid leukemia cells. *Sci. Rep.* **5**, 18491. doi:10.1038/srep18491

Rognoni, E., Widmaier, M., Jakobson, M., Ruppert, R., Ussar, S., Katsougkri, D., Böttcher, R. T., Lai-Cheong, J. E., Rifkin, D. B., Mcgrath, J. A. et al. (2014). Kindlin-1 controls Wnt and TGF-β availability to regulate cutaneous stem cell proliferation. *Nat. Med.* **20**, 350-359. doi:10.1038/nm.3490

Rognoni, E., Ruppert, R. and Fässler, R. (2016). The kindlin family: functions, signaling properties and implications for human disease. *J. Cell Sci.* **129**, 17-27. doi:10.1242/jcs.161190

Scheffzek, K. and Welti, S. (2012). Pleckstrin homology (PH) like domains - versatile modules in protein-protein interaction platforms. *FEBS Lett.* **586**, 2662-2673. doi:10.1016/j.febslet.2012.06.006

Selmer, M., Gao, Y.-G., Weixlbaumer, A. and Ramakrishnan, V. (2012). Ribosome engineering to promote new crystal forms. *Acta Crystallogr. D Biol. Crystallogr.* **68**, 578-583. doi:10.1107/S0907444912006348

Siegel, D. H., Ashton, G. H. S., Penagos, H. G., Lee, J. V., Feiler, H. S., Wilhelmsen, K. C., South, A. P., Smith, F. J. D., Prescott, A. R., Wessagowit, V. et al. (2003). Loss of kindlin-1, a human homolog of the Caenorhabditis elegans actin-extracellular-matrix linker protein UNC-112, causes Kindler syndrome. *Am. J. Hum. Genet.* **73**, 174-187. doi:10.1086/376609

Smith, W. J., Nassar, N., Bretscher, A., Cerione, R. A. and Karplus, P. A. (2003). Structure of the active N-terminal domain of Ezrin. Conformational and mobility changes identify keystone interactions. *J. Biol. Chem.* **278**, 4949-4956. doi:10.1074/jbc.M210601200

Stefanini, L., Lee, R. H., Paul, D. S., O'shaughnessy, E. C., Ghalloussi, D., Jones, C. I., Boulaftali, Y., Poe, K. O., Piatt, R., Kechele, D. O. et al. (2018). Functional redundancy between RAP1 isoforms in murine platelet production and function. *Blood* **132**, 1951-1962. doi:10.1182/blood-2018-03-838714

Sun, Y., Guo, C., Ma, P., Lai, Y., Yang, F., Cai, J., Cheng, Z., Zhang, K., Liu, Z., Tian, Y. et al. (2017). Kindlin-2 association with Rho GDP-dissociation inhibitor α suppresses Rac1 activation and podocyte injury. *J. Am. Soc. Nephrol.* **28**, 3545-3562. doi:10.1681/ASN.2016091021

Sun, Z., Costell, M. and Fässler, R. (2019). Integrin activation by talin, kindlin and mechanical forces. *Nat. Cell Biol.* **21**, 25-31. doi:10.1038/s41556-018-0234-9

Sun, J., Xiao, D., Ni, Y., Zhang, T., Cao, Z., Xu, Z., Nguyen, H., Zhang, J., White, G. C., Ding, J. et al. (2020). Structure basis of the FERM domain of kindlin-3 in supporting integrin αIIbβ3 activation in platelets. *Blood Adv.* **4**, 3128-3135. doi:10.1182/bloodadvances.2020001575

Svensson, L., Howarth, K., Mcdowall, A., Patzak, I., Evans, R., Ussar, S., Moser, M., Metin, A., Fried, M., Tomlinson, I. et al. (2009). Leukocyte adhesion deficiency-III is caused by mutations in KINDLIN3 affecting integrin activation. *Nat. Med.* **15**, 306-312. doi:10.1038/nm.1931

Tadokoro, S., Shattil, S. J., Eto, K., Tai, V., Liddington, R. C., De Pereda, J. M., Ginsberg, M. H. and Calderwood, D. A. (2003). Talin binding to integrin beta tails: a final common step in integrin activation. *Science* **302**, 103-106. doi:10.1126/science.1086652

Tan, S.-M. (2012). The leucocyte β2 (CD18) integrins: the structure, functional regulation and signalling properties. *Biosci. Rep.* **32**, 241-269. doi:10.1042/BSR20110101

Theodosiou, M., Widmaier, M., Böttcher, R. T., Rognoni, E., Veelders, M., Bharadwaj, M., Lambacher, A., Austen, K., Müller, D. J., Zent, R. et al. (2016). Kindlin-2 cooperates with talin to activate integrins and induces cell spreading by directly binding paxillin. *eLife* **5**, e10130. doi:10.7554/eLife.10130

Thievessen, I., Thompson, P. M., Berlemont, S., Plevock, K. M., Plotnikov, S. V., Zemljic-Harpf, A., Ross, R. S., Davidson, M. W., Danuser, G., Campbell, S. L. et al. (2013). Vinculin-actin interaction couples actin retrograde flow to focal adhesions, but is dispensable for focal adhesion growth. *J. Cell Biol.* **202**, 163-177. doi:10.1083/jcb.201303129

Tu, Y., Wu, S., Shi, X., Chen, K. and Wu, C. (2003). Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation. *Cell* **113**, 37-47. doi:10.1016/S0092-8674(03)00163-6

Ussar, S., Wang, H.-V., Linder, S., Fässler, R. and Moser, M. (2006). The Kindlins: subcellular localization and expression during murine development. *Exp. Cell Res.* **312**, 3142-3151. doi:10.1016/j.yexcr.2006.06.030

Vitorino, P., Yeung, S., Crow, A., Bakke, J., Smyczek, T., West, K., Mcnamara, E., Eastham-Anderson, J., Gould, S., Harris, S. F. et al. (2015). MAP4K4 regulates integrin-FERM binding to control endothelial cell motility. *Nature* **519**, 425-430. doi:10.1038/nature14323

Wang, P., Chu, W., Zhang, X., Li, B., Wu, J., Qi, L., Yu, Y. and Zhang, H. (2018a). Kindlin-2 interacts with and stabilizes DNMT1 to promote breast cancer development. *Int. J. Biochem. Cell Biol.* **105**, 41-51. doi:10.1016/j.biocel.2018.09.022

Wang, X., Wei, X., Yuan, Y., Sun, Q., Zhan, J., Zhang, J., Tang, Y., Li, F., Ding, L., Ye, Q. et al. (2018b). Src-mediated phosphorylation converts FHL1 from tumor suppressor to tumor promoter. *J. Cell Biol.* **217**, 1335-1351. doi:10.1083/jcb.201708064

Wegener, K. L., Partridge, A. W., Han, J., Pickford, A. R., Liddington, R. C., Ginsberg, M. H. and Campbell, I. D. (2007). Structural basis of integrin activation by talin. *Cell* **128**, 171-182. doi:10.1016/j.cell.2006.10.048

Wei, X., Xia, Y., Li, F., Tang, Y., Nie, J., Liu, Y., Zhou, Z., Zhang, H. and Hou, F. F. (2013). Kindlin-2 mediates activation of TGF-β/Smad signaling and renal fibrosis. *J. Am. Soc. Nephrol.* **24**, 1387-1398. doi:10.1681/ASN.2012101041

Wei, X., Wang, X., Zhan, J., Chen, Y., Fang, W., Zhang, L. and Zhang, H. (2017). Smurf1 inhibits integrin activation by controlling Kindlin-2 ubiquitination and degradation. *J. Cell Biol.* **216**, 1455-1471. doi:10.1083/jcb.201609073

Wen, L., Marki, A., Roy, P., McArdle, S., Sun, H., Fan, Z., Gingras, A. R., Ginsberg, M. H. and Ley, K. (2020). Recruitment of kindlin-3 to plasma membrane through its PH domain precedes high affinity β_2 integrin activation and neutrophil arrest. *J. Immunol.* **204**, 220.7. doi:10.1096/fasebj.2020.34.s1.02524

Wittig, I. and Schägger, H. (2005). Advantages and limitations of clear-native PAGE. *Proteomics* **5**, 4338-4346. doi:10.1002/pmic.200500081

Yates, L. A., Füzéry, A. K., Bonet, R., Campbell, I. D. and Gilbert, R. J. C. (2012a). Biophysical analysis of Kindlin-3 reveals an elongated conformation and maps integrin binding to the membrane-distal β-subunit NPXY motif. *J. Biol. Chem.* **287**, 37715-37731. doi:10.1074/jbc.M112.415208

Yates, L. A., Lumb, C. N., Brahme, N. N., Zalyte, R., Bird, L. E., De Colibus, L., Owens, R. J., Calderwood, D. A., Sansom, M. S. P. and Gilbert, R. J. C. (2012b). Structural and functional characterization of the kindlin-1 pleckstrin homology domain. *J. Biol. Chem.* **287**, 43246-43261. doi:10.1074/jbc.M112.422089

Ye, F., Petrich, B. G., Anekal, P., Lefort, C. T., Kasirer-Friede, A., Shattil, S. J., Ruppert, R., Moser, M., Fässler, R. and Ginsberg, M. H. (2013). The mechanism of kindlin-mediated activation of integrin αIIbβ3. *Curr. Biol.* **23**, 2288-2295. doi:10.1016/j.cub.2013.09.050

Yu, Y., Wu, J., Wang, Y., Zhao, T., Ma, B., Liu, Y., Fang, W., Zhu, W. G. and Zhang, H. (2012). Kindlin 2 forms a transcriptional complex with β-catenin and TCF4 to enhance Wnt signalling. *EMBO Rep.* **13**, 750-758. doi:10.1038/embor.2012.88

Zhan, J. and Zhang, H. (2018). Kindlins: Roles in development and cancer progression. *Int. J. Biochem. Cell Biol.* **98**, 93-103. doi:10.1016/j.biocel.2018.03.008

Zhang, P., Azizi, L., Kukkurainen, S., Gao, T., Baikoghli, M., Jacquier, M.-C., Sun, Y., Määttäa, J. A. E., Cheng, R. H., Wehrle-Haller, B. et al. (2020). Crystal structure of the FERM-folded talin head reveals the determinants for integrin binding. *Proc. Natl. Acad. Sci. USA* **117**, 32402-32412. doi:10.1073/pnas.2014583117

Zhao, Y., Malinin, N. L., Meller, J., Ma, Y., West, X. Z., Bledzka, K., Qin, J., Podrez, E. A. and Byzova, T. V. (2012). Regulation of cell adhesion and migration by Kindlin-3 cleavage by calpain. *J. Biol. Chem.* **287**, 40012-40020. doi:10.1074/jbc.M112.380469

Zhu, L., Yang, J., Bromberger, T., Holly, A., Lu, F., Liu, H., Sun, K., Klapproth, S., Hirbawi, J., Byzova, T. V. et al. (2017). Structure of Rap1b bound to talin reveals a pathway for triggering integrin activation. *Nat. Commun.* **8**, 1744. doi:10.1038/s41467-017-01822-8

Zhu, L., Liu, H., Lu, F., Yang, J., Byzova, T. V. and Qin, J. (2019). Structural basis of paxillin recruitment by kindlin-2 in regulating cell adhesion. *Structure* **27**, 1686-1697.e5. doi:10.1016/j.str.2019.09.006