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REVIEW

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Initiation of focal adhesion assembly by talin and kindlin: A dynamic view

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Abstract

Focal adhesions (FAs) are integrin-containing protein complexes regulated by a network of hundreds of protein–protein interactions. They are formed in a spatiotemporal manner upon the activation of integrin transmembrane receptors, which is crucial to trigger cell adhesion and many other cellular processes including cell migration, spreading and proliferation. Despite decades of studies, a detailed molecular level understanding on how FAs are organized and function is lacking due to their highly complex and dynamic nature. However, advances have been made on studying key integrin activators, talin and kindlin, and their associated proteins, which are major components of nascent FAs critical for initiating the assembly of mature FAs. This review will discuss the structural and functional findings of talin and kindlin and their immediate interaction network, which will shed light upon the architecture of nascent FAs and how they act as seeds for FA assembly to dynamically regulate diverse adhesion-dependent physiological and pathological responses.

KEYWORDS

focal adhesions, integrin, kindlin, talin

1 | INTRODUCTION

Cell-extracellular matrix (ECM) and cell–cell adhesion are critically regulated by integrins, a class of cell surface receptors. Integrins are heterodimeric and consist of an α and a β subunit with each containing a large extracellular domain, a single-helical transmembrane segment and typically a short cytoplasmic tail. There are 24 different integrins assembled from 18 α and 8 β subunits in vertebrates to control diverse physiological and pathological processes, such as blood clotting, immune response, wound-healing and cancer metastasis.[1,2] Integrin is known to function via unique bi-directional signaling, which has been studied extensively over the past three decades: (i) "Inside-out" signaling where intracellular proteins, such as talin and kindlin, bind to the cytoplasmic face of an inactive integrin to trigger a conformational change of its extracellular domain, switching it to a high-affinity ligand binding state (a process also known as "integrin activation"), and (ii) "Outside-in" signaling where activated (ligand-bound) integrin triggers assembly of large protein complexes called focal adhesions (FAs) that link integrin to the actin cytoskeleton, so as to promote firm cell adhesion and dynamic cell adhesive processes (e.g., cell spreading, migration and proliferation).[1,3-5] FAs have also been defined by different terms such as "integrin adhesome" or "integrin adhesion complexes (IACs)" to reflect the dynamic and complex nature of the protein network.[5–7] More than 150 proteins have been identified in IACs, which are connected by estimated 690 protein–protein interactions (PPIs)[6]. However, a detailed picture of how these proteins engage with one another spatiotemporally to form such complex networks remains obscure.

Talin and kindlin are the key activators of integrins, which also serve as seeds to recruit proteins to initiate FA assembly following their binding to and activation of integrin via "inside-out" signaling. Indeed, talin and kindlin and their associated proteins such as vinculin, paxillin, focal adhesion kinase (FAK), and integrin-linked kinase (ILK) are found in nascent FAs.[7,8] Extensive biophysical studies including structural characterization (via NMR spectroscopy, X-ray crystallography and Cryo-EM), combined with functional analyses (e.g., integrin activation assays, cell adhesion or spreading assays, and mice knock-out and knock-in studies) have been conducted to understand the regulation of these nascent FA proteins. They share some common features: (a) essential for embryonic development and integrin-mediated FA assembly, cell spreading and migration (Table 1, Figure 1); and (b) all contain multiple domains or motifs allowing them to bind multiple proteins to trigger the growth of FAs. In this review, we attempt to summarize the role of talin and kindlin in regulating integrin activation, and important protein–protein interactions (PPIs) and binding interfaces that are mediated by each domain or motif of talin and kindlin. We propose that these interactions are critically involved in the initial network and the core of nascent FAs (Figure 1), which then expand into larger and mature FA complexes via cascades of PPIs in a spatiotemporal manner to promote dynamic cell adhesion processes.

2 | TALIN ACTION AND ITS INTERACTION NETWORK

Talin was discovered in early 1980s[23] as a large cytoskeletal protein (MW: ~ 270 kDa) involved in cell adhesion. However, it only started to draw major attention two decades later when researchers realized that it can bind and activate integrin to trigger cell adhesion.[1,3] Since FAs are formed following integrin activation, talin and its network provide an excellent window for us to understand the initiation of FA assembly. Vertebrates encode two talin isoforms, talin-1 and talin-2, which share ~76% sequence identity.[24] Talin-1 is ubiquitously expressed, whereas talin-2 is tissue-specific and most abundant in heart, brain and skeletal muscle.[25] Here we focus on discussing talin-1 (hereafter talin refers to talin-1), which has been extensively characterized and shown to be vital for life since its ablation in mice causes embryonic lethality (Table 1) and its tissue-specific depletions cause severe phenotypes, such as impaired platelet aggregation and leukocyte trafficking, due to defective integrin activation.[26]

Talin consists of an N-terminal FERM (4.1/ezrin/radixin/moesin)-like head domain (talin-H), which is further divided into four subdomains, F0, F1, F2 and F3, and a C-terminal rod domain (talin-R), which is made up of 13 consecutive helical bundles (R1-13), followed by a single-helical dimerization domain (talin-DD) (Figure 2(a)). Structural studies have shown that talin uses its F3 domain, a PTB (phosphotyrosine-binding domain)-like domain, to directly bind to integrin β cytoplasmic tail utilizing the membrane proximal helix and "NPxY" motif (Figure 2(b)).[27–29] It is well understood that such binding results in the separation of integrin α and β subunit at the cytoplasmic face, which further leads to the separation of the transmembrane α and β segments and the "bent-to-extension" conformational change of the extracellular domain leading to a "high-affinity" ligand binding state (Figure 1).[29,30] Notably, the talin-integrin interaction is tightly regulated. In resting-state cells, intact talin is randomly distributed in the cytosol[31] exhibiting an auto-inhibitory conformation and incapable

TABLE 1 Phenotypes caused by deletion of talin and kindlin and critically associated proteins

		FA assembly[b]		Cell function[b]		
	KO mice (day)[a]	Number	Size	Spreading area	Migration	Ref.
Talin-1 (2,541 aa)	E8.5–9.5	Decrease	Decrease	Decrease	Decrease	8–10
Kindlin-2 (680 aa)	E7.5	Decrease	Decrease	Decrease	Decrease	8,11,12
Paxillin (591 aa)	E9.5	Increase	Decrease	Decrease	Decrease	12–14
Vinculin (1,134 aa)	E10.5	Decrease	Decrease	Decrease	Increase	15,16
ILK (452 aa)	E5.5–6.5	Decrease[c]	N/A[d]	Decrease[c]	Decrease	17–19
FAK (1,052 aa)	E8.5	Increase	N/A[e]	Decrease	Decrease	20–22

[a]The embryonic day by which knock-out (KO) mice display embryonic lethality.
[b]In fibroblast system.
[c]ILK-null cells showed delayed spreading and formation of FAs.
[d]See Ref. 18 for detailed analysis.
[e]See Ref. 22 for detailed analysis.

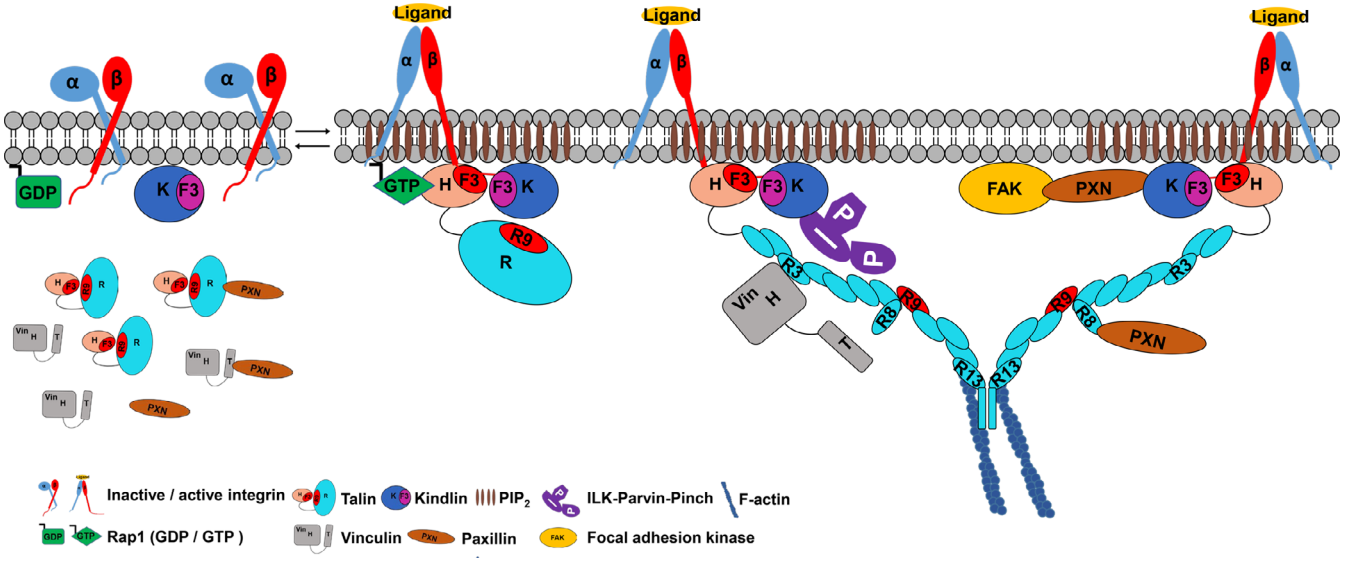

FIGURE 1 Initiation of nascent FAs upon the integrin activation by talin and kindlin via "inside-out" signaling. Activated GTPase Rap1 recruits talin to the membrane site. Talin becomes activated by membrane PIP_2 and exposes talin-F3 domain to bind integrin. Kindlin also binds integrin via its F3 domain and cooperates with talin to fully activate integrin. Talin stretches in the presence of mechanical force and together with kindlin triggers multiple axes including "talin-vinculin", "talin-paxillin" "kindlin-paxillin-FAK" and "kindlin-ILK-parvin-pinch", to initiate FA assembly, and regulate actin cytoskeleton remodeling and dynamic cell adhesion

of binding to integrin.[32,33] Membrane-PIP_2 has long been recognized as talin activator that binds and induces conformational change of talin, exposing its F3 domain to bind and activate integrin (Figure 1).[32–34] Talin exists in a monomer/dimer equilibrium in solution and the monomeric form of the inactive talin has been resolved recently by cryo-EM (Figure 2(b)).[33] Consistent with previous domain-based structural studies by NMR[35] and X-ray crystallography,[34] the cryo-EM structure of the intact talin revealed that the integrin binding site of talin-F3 is masked by talin-R9 (Figures 2(b) and S1B). The structure also revealed that the PIP_2 binding site on talin-F2F3, critical for the activation of talin, is occupied by talin-R12 (Figure 2(b) and S1B),[33] thereby providing a basis for understanding how PIP_2 sterically occludes talin-R12 and conformationally activates talin. Talin autoinhibition-activation is delicately balanced in cells. Mutation disrupting the auto-inhibitory interface between talin-F3 and talin-R9 leads to integrin activation, resulting impaired adhesion dynamics and delayed wound healing in vivo.[36]

To activate integrin, talin needs to be recruited to the plasma membrane, a process that was not clearly understood for many years. One previously proposed pathway was that talin is recruited to membrane by a membrane-anchored GTPase called Rap1 via its effector RIAM (referred to the "Rap1-RIAM-talin" pathway).[37] The RIAM binding to talin-F3 (Figure 2(b)) was also shown to contribute to the activation of talin.[38] However, the function of the Rap1-RIAM-talin pathway appears to be leukocyte-specific.[39–41] Recent breakthrough studies revealed that ubiquitin-like talin F0 and F1 domains control the membrane recruitment of talin via direct binding to Rap1 (Figures 1 and 2(b)).[42–46] Mutations that abolished either Rap1/talin-F0 or Rap1/talin-F1 interaction resulted in reduced integrin activation in CHO cells, impaired FA assembly, cell spreading and adhesion in talin-null fibroblast-based assays, as well as defective platelet and leukocyte function in mouse models.[42–46] Disruption of both interactions led to more severe defects in FA assembly, cell spreading and adhesion,[45] and more intriguingly, embryonic lethality in mice and a platelet phenotype resembling that of talin or Rap1 knockout.[46] It is of particular interest to note that talin F0 and F1 both bind to Rap1 very weakly in solution (Table S1), which was initially overlooked. However, in a cellular context, talin-F0 and F1 may bind simultaneously to two membrane-anchored Rap1 molecules, resulting in high affinity binding and effective talin recruitment to membrane.[42–46] Talin-F1 also contains a 30-residue flexible loop, which was suggested to interact with membrane-lipid mainly via R146, R153 and K156 (Figure 2(b)) to assist recruitment of talin to the membrane.[47] It was further suggested recently that the F1 loop may interfere with a salt-bridge formed between the transmembrane domains of integrin α and β subunits to promote integrin clustering and activation.[48] As mentioned above, talin-F2F3 domain bears a PIP_2 binding

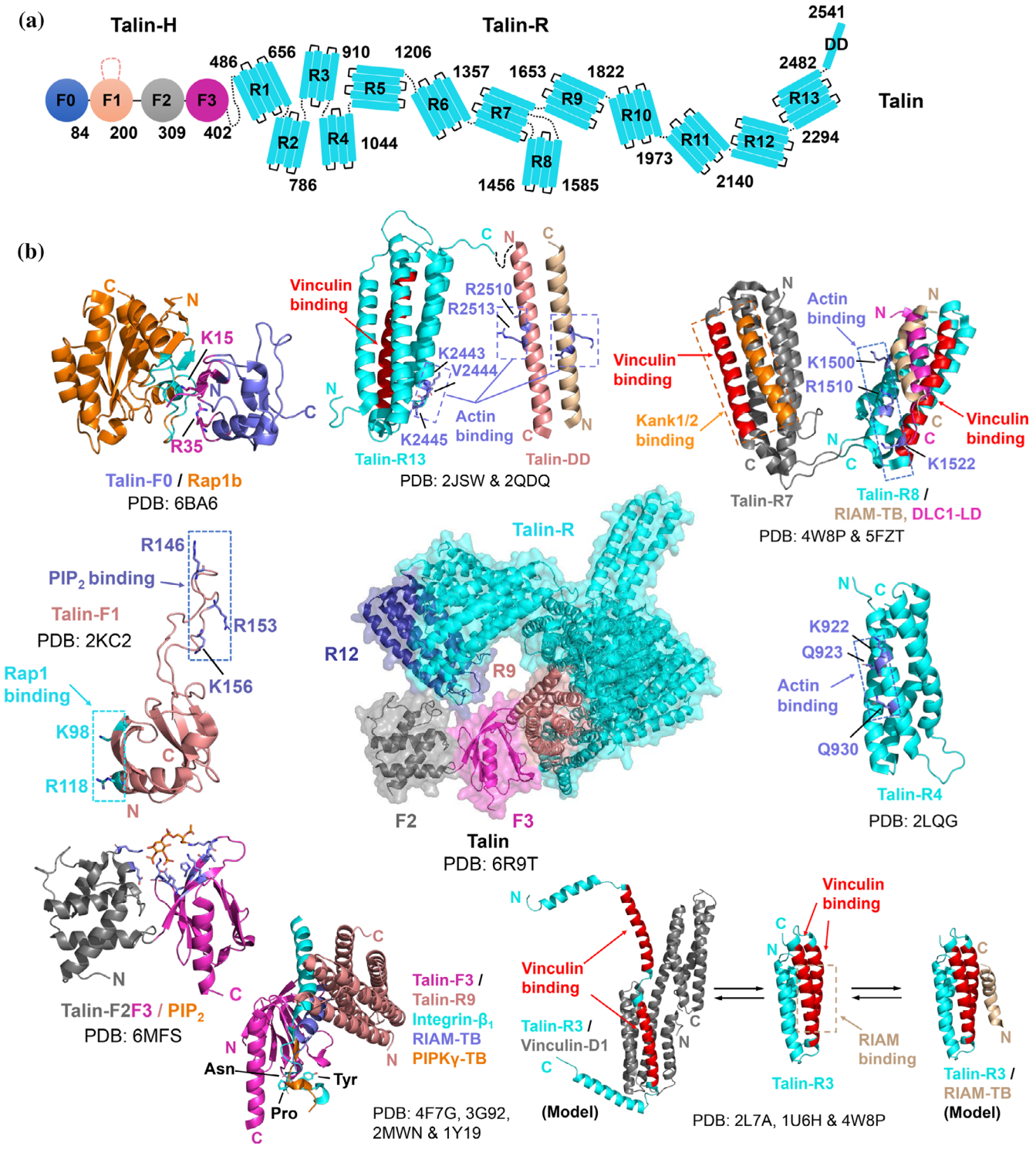

FIGURE 2 The binding interfaces of talin-mediated interactions in integrin and FA signaling. (a) Domain organization of talin. (b) Reported structures of individual domains of talin in apo or complex form. The binding interfaces/sites for target proteins are indicated in the figure. Important residues involved in the binding interfaces or targeted in mutagenesis studies are shown in stick representation. The interface residues of talin-F0 and Rap1b (within a cut-off of 5 Å) are colored in magenta and cyan, respectively. The interface residues of talin-F2F3 (within a cut-off of 5 Å) that interact with PIP_2 are colored in blue and shown in stick representation. N and C indicate the N-terminus and C-terminus of the protein molecule, respectively. PDB codes used to generate the figures are indicated

pocket formed by K272 of F2, K316, K324 and K343 of F3 (Figure 2(b)).[49] The PIP_2 binding may not only activate talin[32–34] but also cooperate with Rap1 to enhance the association of talin-H with the cell membrane.[42,45] Interestingly, talin-F3 also binds to the C-terminal segment "WVYSPLHY" of PIPKIγ (Type I phosphatidylinositol

phosphate kinase gamma) in a similar mode as it binds integrin-β "NPxY" motif (Figure 2(b)).[50,51] The interaction allows PIPKIγ to be co-localized with talin to promote local production of PIP_2 at the membrane site that binds and activates talin.[52,53] It is important to clarify here conceptually that although PIPKIγ seems to block integrin from binding talin-F3 (Figure 2(b)), only a very small fraction of talin, which is extremely abundant in cells, is needed to recruit PIPKIγ that is typically at very low concentration as a kinase but can enzymatically produce a large amount of PIP_2 to bind and activate the large portion of PIPKIγ-free talin.

We note that although previous crystallographic studies of talin-H revealed a linear conformation different from cloverleaf-like folding of a canonical FERM domain (Figure S1A),[49,54] SAXS data suggested that talin-H in solution is not as linear as in the crystal structure.[48,54] In line with the latter, Rangarajan et al. recently solved the crystal structure of intact talin2-H in which the F0-F1-F2 domain adopted a cloverleaf-like fold (Figure S1A).[55] However, both of these conformations contrast with the recent cryo-EM structure of full-length talin wherein the densities of F0 and F1 domains were not observed (Figure 2(b)).[33] These findings suggest that talin-H, which is critical for binding and activating integrin, is highly dynamic in nature likely due to the existence of a long linker between F1 and F2 subdomain (Figure S1A). It remains to be investigated if the presence of membrane-anchored Rap1 and membrane-PIP_2 would stabilize talin-H to a rigid conformation that favors integrin binding.

While talin-H is directly involved in talin membrane recruitment and integrin activation, talin-R is thought to be indispensable for transmitting mechanical force between integrin and actin cytoskeleton to support cell adhesion and spreading. Expression of talin-H in talin-null fibroblasts was sufficient to support integrin activation but not cell spreading and migration.[10,56] All 13 talin-R subdomains are an assembly of 4–5 alpha-helices (Figure 2(a)) and bear binding sites for a variety of adhesion proteins, such as vinculin, paxillin, RIAM, DLC-1 (deleted in liver cancer 1) and Kank-1/2, as well as two major actin binding sites, ABS2 and ABS3 (Note that talin-H also contains one actin binding site ABS1 in talin-F2F3, see ref. [57,58]). ABS2 and ABS3 locate at R4-R8 (R4 and R8 are the major binding sites) and R13-DD respectively (Figure 2(b)).[59,60] The binding of ABS3 to actin is crucial for initial force transduction, which promotes the talin binding to vinculin and initiates FA assembly. Deletion or mutations of ABS3 to block actin binding resulted in loss of FA formation, and defective cell spreading, polarization and migration.[10,59] In contrast, mutations of ABS2 led to smaller size of FAs and reduced number of actin stress fibers, suggesting its role in traction force generation for FA maturation and stabilization.[59]

At least 9 talin-R subdomains (R1, R2, R3, R6, R7, R8, R10, R11 and R13) interact with vinculin (~116 kDa), which contains a head domain (vinculin-H) composed of D1-D4 subdomains, followed by a flexible proline-rich neck region and a tail D5 domain (vinculin-T).[61] The binding of vinculin to talin is essential for force transmission and mechanosensing, which are important for the growth and maturation of FAs.[59,62,63] As many as 11 potential single-helical vinculin binding sites (VBS) were identified in talin-R (Figures 2(b) and S1B, Table S1), and they are all capable of binding to the hydrophobic pocket of the D1 domain (Vd1) of vinculin-H (Figure 2(b)),[64] which is otherwise masked by vinculin-T via an auto-inhibitory mechanism (Figure 1).[65] However, these binding sites are not accessible in a folded rod subdomain (Figures 2(b) and S1B). Studies showed that mechanical force can unfold talin-R subdomains into a linear conformation to allow vinculin binding (Figure 2(b)).[66–68] Among the talin-R subdomains, talin-R3 is the first domain to unfold to bind vinculin due to its having the highest susceptibility to mechanical force.[67,69] This interpretation was also supported by mutagenesis studies which showed that the initial talin/vinculin engagement is highly dependent on the stability of talin-R3.[62,70] The binding of vinculin to talin-R2R3 triggered a conformational change that regulates actin binding to ABS2 to promote maturation of FAs linked to actin filaments.[59] The FA localization of vinculin is highly dependent on talin-R subdomains, as the intensity of FA-localized vinculin correlates with the number of VBS in talin constructs expressed in talin-null fibroblasts.[10,59] Lack of VBS in talin severely impairs cell spreading, adhesion and migration.[10,71] Likewise, a vinculin mutant (A50I) that does not bind talin failed to promote the growth of FAs.[62,63] The unfolding of talin-R to bind vinculin may be assisted by mechanical force but initial engagement of talin and vinculin is likely to be independent of mechanical force as supported by several lines of evidence: (a) Atherton et al. showed that talin and vinculin are able to co-localize in force-free mitochondria,[62] (b) Kelley et al. applied an in-vitro synthetic membrane system to show that membrane PIP_2 is sufficient to trigger the activation of talin and its engagement with vinculin to induce actin bundling in the absence of force,[72] (c) active vinculin or vinculin-H can form complex with open-conformation of talin or talin-R fragments in solution,[33,71] and (d) vinculin-H directly causes the unfolding of single talin-R subdomains in NMR studies.[73] On the other hand, the stoichiometry of active vinculin binding to open-conformation talin in

solution is 1:1,[33] suggesting that the engagement of all 11 VBS of talin-R subdomains with vinculin may be assisted by mechanical force. Overall, both force-independent and dependent processes are involved in "talin-vinculin" engagement.

In addition to vinculin binding, talin was also shown to interact with other target proteins (e.g., RIAM, DLC-1, Kank-1/2, and paxillin). Unlike vinculin, these proteins bind to folded talin-R subdomains via a similar interacting mode. The groove formed by two helices of a talin-R subdomain provides a potential binding site for a helical LD or LD-like motif of the target proteins (Figures 2(b) and S1B). Talin-R2, R3, R8, and R11 (R3 and R8 are most potent) are all capable of binding to the N-terminal talin binding (TB) region of RIAM (Figures 2(b) and S1B), which assists talin membrane recruitment for integrin activation[37,71,74] and forms "sticky fingers" with talin to regulate leukocyte migration.[75] Talin-R8 also interacts with paxillin and DLC-1 via the LD1 and LD-like motif respectively in a similar fashion but with different binding affinities (Figure 2(b), Table S1).[76] Talin-R8/DLC-1 interaction is required for the FA targeting and tumor suppressor activity of DLC-1,[77] and mechanistically, acts like a force-controlled molecular switch to regulate the mechanotransduction of talin so as to regulate FA assembly and cell migration.[76,78] Paxillin binding to talin-R8 is not well characterized at structural level but may act similarly to DLC-1.[76,78] This interaction may account for the association of talin/paxillin to form initial adhesion complex, as suggested recently.[62] In addition, paxillin could also engage vinculin in the absence of talin to form initial adhesion complex,[62] suggesting an interesting interplay among these three proteins that remains to be further explored. Talin-R7 specifically interacts with the KN domain of Kank-1/2 which also contains a LD-like motif (Figure 2(b)). The interaction was suggested to regulate talin and integrin activation, the force transduction between integrin-talin and actomyosin, as well as the recruitment of cortical microtubule stabilizing complexes to FAs.[79,80] Interestingly, these binding events (vinculin included) mediated by talin-R subdomains are mutually exclusive and likely regulated spatiotemporally or in a cell-type specific manner.

The cryo-EM structure of monomeric inactive talin reveals that talin-R subdomains and talin-H fold into a compact conformation.[33] It is conceivable that upon activation, talin would engage with the above-mentioned target proteins in a spatiotemporal manner to trigger the formation of nascent FAs. It is important to note that fully active talin likely exists as a dimer at physiological condition,[33] so one can imagine that dimeric talin may provide a broad platform for initiating the networking of nascent FAs (Figure 1). Future structural and functional studies may provide more detailed insights into the operation of such a talin-based platform.

3 | KINDLINS AND KINDLIN NETWORK

Kindlins have also attracted extensive attention since a decade ago due to their essential ability to enhance talin-mediated integrin activation.[4,26,81] There is growing evidence that kindlins may be bound to integrin prior to integrin activation,[8,82] but how the integrin-bound kindlins cooperate with talin to activate integrin is still not well understood. Kindlin family contains three members, namely kindlin-1, kindlin-2, and kindlin-3, which are encoded by separate genes. Kindlin-2 is ubiquitously expressed, while kindlin-1 and 3 are primarily expressed in epithelial and hematopoietic cells, respectively,[81,83,84] although there are exceptions to this as kindlin-3 is found in endothelial cells[85]and some tumor cells.[86] All kindlins are physiologically important. While kindlin-2 is essential for embryonic development, loss of kindlin-1 in humans and in mice causes skin fragility and blistering (known as "Kindler Syndrome"), and kindlin-3 deficiency can lead to bleeding disorder and immune defects (a disease known as "leukocyte adhesion deficiency type III, LAD-III").[81,83,84] Kindlins (MW: $\sim$ 77 kDa) adopt a FERM (4.1/ezrin/radixin/moesin)-like domain which consists of F1, F2, and F3 subdomains with a pleckstrin homology (PH) domain inserted into F2, and a preceding ubiquitin-like F0 domain (Figure 3(a)). Similar to talin-H, kindlins also use its F3 domain to bind integrin β cytoplasmic tail but at a distinct site known as distal "NxxY" or "NPxY/F" motif (Figure 3(a)). Despite only the involvement of the F3 domain in direct binding to integrin, the integrity of kindlin is required for its integrin activation function.[87] All the other domains of kindlin have been shown to mediate different binding events to regulate FA assembly.

The F0 domain of kindlin was initially suggested to contribute to the membrane association of kindlin via a cluster of positive charged residues binding to negatively charged membrane lipid PIP_2 (Figure 3(a)), so as to regulate integrin activation.[88] Recently, kindlins have drawn a lot of attentions due to their newly identified ability to bind paxillin. Studies by different groups gave rise to an interesting "kindlin-paxillin-FAK" pathway where kindlin recruits paxillin to regulate FAK activation, FA assembly and dynamic cell adhesion.[8,12,89] NMR studies revealed a modest affinity ($\sim$200 μM) of kindlin-2/paxillin interaction mediated by a unique interface between kindlin-2 F0 and paxillin LIM4 (Figure 3(a)).[12]

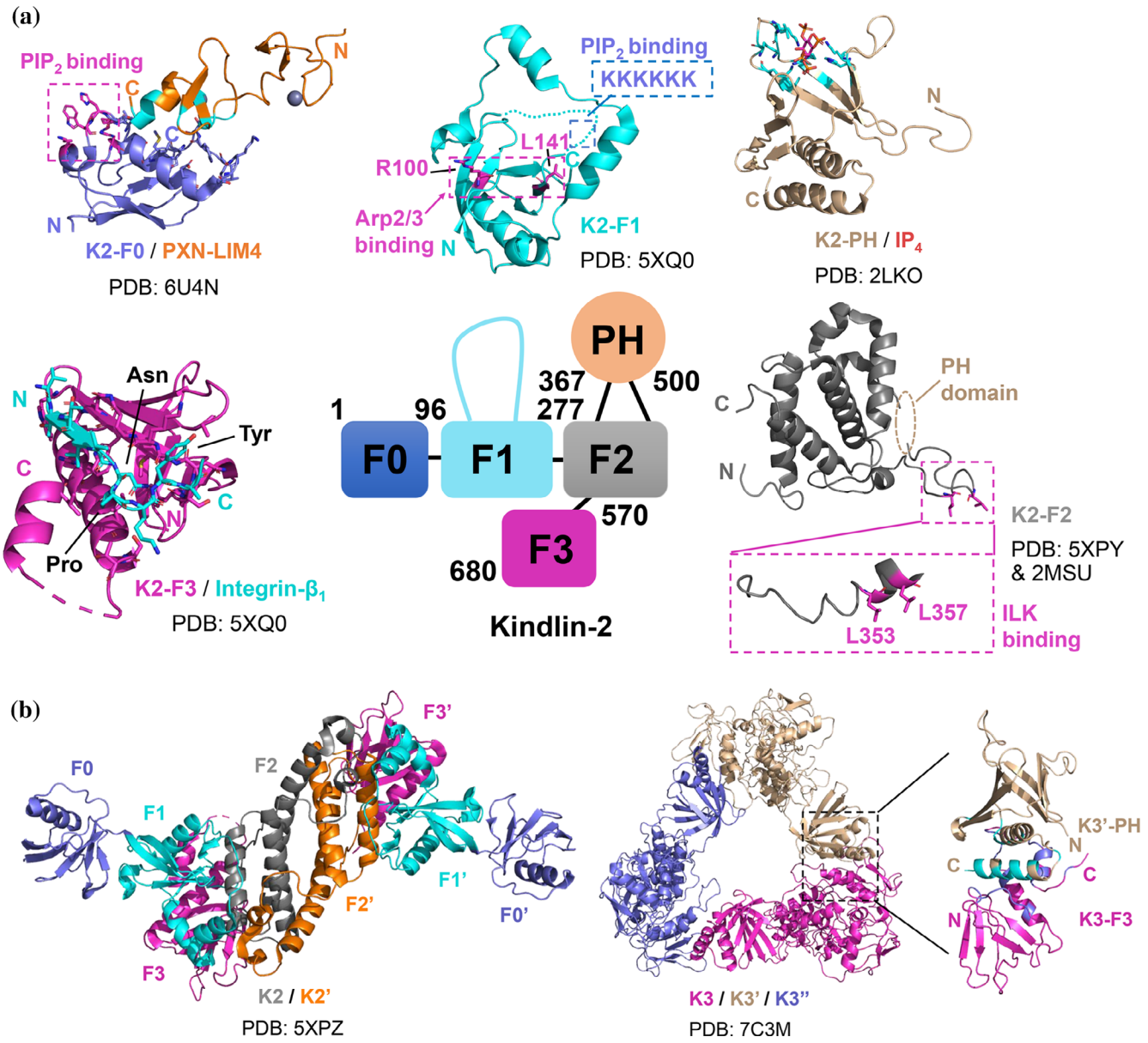

FIGURE 3 The binding interfaces of kindlin-mediated interactions in integrin and FA signaling. (a) Domain organization of kindlin-2 and reported structures of individual domains of kindlin-2 in apo or complex form. The binding interfaces/sites for target proteins are indicated in the figure. Important residues involved in the binding interfaces or targeted in mutagenesis studies are shown in stick representation. The interface residues of paxillin LIM4 (within a cut-off of 5 Å) that interact with kindlin-2 F0 are colored in cyan. The interface residues of kindlin-2 PH (within a cut-off of 5 Å) that interact with IP_4 are colored in cyan. (b) Reported crystal structures of truncated kindlin-2 and full-length kindlin-3 in respective dimeric and trimeric conformation. The interface residues of kindlin-3 F3 and kindlin-3' PH (within a cut-off of 5 Å) are colored in blue and cyan, respectively. PXN, paxillin. K2, kindlin-2. K3, kindlin-3. N and C indicate the N-terminus and C-terminus of the protein molecule, respectively. PDB codes used to generate the figures are indicated

The interaction is highly conserved between kindlin family and paxillin superfamily,[12,90] but the functional importance could be cell-type specific. Disruption of kindlin-3 binding to paxillin and leupaxin in macrophages resulted in enhanced cell spreading and migration,[90] in sharp contrast to the effects of kindlin-2/paxillin interaction in fibroblasts.[12,89] Moreover, Klapproth et al. showed that the recruitment of leupaxin but not paxillin to podosomes (another type of adhesive structure) in hematopoietic cells is dependent on kindlin-3,[91] suggesting the binding of kindlin-3 to paxillin or leupaxin is spatiotemporally regulated. It is via this interaction that led Gao et al to suggest that paxillin may bridge kindlin and talin (paxillin also binds talin-H shown in the study) to promote integrin activation.[92] Since paxillin was found in nascent FAs, these studies

highlight the physiological importance of the F0 domain of kindlins binding to paxillin superfamily in regulating nascent FA assembly in different types of cells upon integrin activation by talin and kindlins. To further understand the potential phenotype caused by the deficiency of the interactions between the two protein families, mutagenesis-based knock-in mice could be generated for detailed investigation in the future.

F1 domain of kindlin adopts similar folding to F0 but contains a surprisingly long unstructured loop, 108 residues for kindlin-1/2 and 83 residues for kindlin-3.[93,94] A conserved stretch of six positive charged lysines (polylysine motif) locates at the beginning of the loop and was shown to be capable of associating with membrane lipids (Figure 3(a)).[93] Deletion or mutation of this polylysine motif diminished the FA targeting of kindlin as well as the integrin activation in CHO cells.[93] Interestingly, the replacement of the kindlin F1 loop by talin F1 loop which is also known to bind membrane lipids resulted in the same defects,[93] suggesting that the intact F1 loop is required for kindlin's action and some other unknown function of F1 loop may also be important. For example, the tyrosine 193 of F1 loop could be phosphorylated by tyrosine kinase Src, and the phosphorylation was shown to be important for the FA localization of kindlin and migfilin as well as cell spreading.[95] Note that this residue is not conserved in kindlin-3, indicating the function of F1 loop may vary among kindlins. Other than the loop region, the binding partners of F1 domain are less identified, except a recent study suggesting that kindlin F1 can complex with Arp2/3 (Figure 3(a)) to regulate cell spreading.[89]

F2 domain of kindlin associates tightly (~110 nM) with another essential adhesion protein, integrin-linked kinase (ILK), which forms complex with pinch and parvin, namely "ILK-pinch-parvin (IPP)" complex, to regulate actin cytoskeleton remodeling and dynamic cell adhesion.[96,97] Kindlin F2 binds to the kinase-like or pseudokinase domain (KLD) of ILK. Although the exact nature of the binding interface is not yet defined, the key residues of both proteins involved in the binding have been identified via mutagenesis (Figure 3(a)).[96,98] Mutations that disrupt the interaction resulted in significant defects in the FA localization of kindlin and cell spreading, but interestingly had little effect on integrin activation in CHO cells,[96] suggesting the interaction is likely involved in early integrin "outside-in" signaling. Like canonical PH domains, kindlin PH domain harbors a binding site for membrane-lipids including PIP_2 and PIP_3 (Figure 3(a)) to assure the membrane localization of kindlin for integrin activation and cell adhesion.[99,100] The cooperative membrane binding of kindlin and talin likely promotes a stable active conformation of integrin allowing potent ligand binding and clustering (Figure 1).

Recent structural studies have suggested that kindlins may undergo oligomerization to regulate the integrin function, but the precise mechanism remains controversial. Li et al. in 2017 first reported crystal structures of truncated kindlin-2 with deletion of PH domain and F1 loop, which revealed a monomeric conformation as well as a domain-swapped dimer conformation (Figure 3(b)). The dimer interface is mediated by F2 domain (Figure 3 (b)) and was shown to contribute to integrin activation in CHO cells (20%–30% reduction by mutations that disrupt dimer formation).[101] However, in sharp contrast, the crystal structure of full-length kindlin-3 solved by Bu et al. revealed an auto-inhibitory homotrimer where the integrin binding site on F3 domain of one molecule is masked by the PH domain of another (Figure 3(b)).[102] The authors also provided evidence to show that disruption of the trimer interface promotes integrin-mediated cell adhesion and spreading.[102] Moreover, Kadry et al. in a recent study suggested that both kindlin-2 and kindlin-3 can self-associate into 2–4 molecule oligomers via their F2PH domains but with an interface different from that in the truncated kindlin-2 dimer.[103] The F3 domain is clearly not involved since deletion of F3 domain increased the self-association of kindlin molecules.[103] Given that the majority of either isolated kindlin-2 or kindlin-3 is monomeric as shown in all these studies, careful investigation is needed to examine if or when kindlin oligomerization occurs in physiological context.

While all kindlins share conserved domain architecture, they are not fully compensatory to one another. Kindlin-1 and 2 are more alike by sharing 64% identity and 79% similarity in primary sequence, while kindlin-3 and 2 share 52% identity and 72% similarity. In adherent cells, overexpressed kindlin-1 or 2 but not kindlin-3 targets to FAs and also kindlin-3 is not able to substitute for kindlin-2 in supporting the activation of β_1 integrin.[104] One explanation for these differences may arise from the observation that kindlin-3 binds much less potently to ILK than kindlin-2. However, such explanation cannot explain why kindlin-1 or 2 but not kindlin-3 robustly enhances integrin activation when co-expressed with talin-H in CHO cells.[96,105] Interestingly, kindlin-3 fused to a GST tag, which forms an artificial dimer, is able to enhance talin-H mediated integrin activation,[105] suggesting that some unknown factor(s) may play a role in the oligomerization of kindlin1/2 which is not operative for kindlin-3 in non-hematopoietic cells. This hypothesis remains to be tested in the future. In addition, some phosphorylation sites are not fully conserved among kindlins. As mentioned earlier, the Y193 of kindlin-2 F1 loop is conserved in kindlin-1 but not kindlin-3. Conversely, kindlin-3 possesses a unique

phosphorylation site (T482 or S484) that is not conserved in kindlin-1 and 2. The phosphorylation is crucial for regulating integrin activation and cell spreading in hematopoietic cells.[106]

4 | CONCLUSION

As summarized in this review, talin and kindlin are centrally involved in the regulation of integrin activation and early FA assembly via mediating dozens of binding events. The affinities of the interactions range from nM to mM (Tables S1 and S2), indicating the dynamic nature of interactions during cell adhesion. Since one single domain or motif of the proteins is often capable of mediating diverse binding events, we envision that these interactions occur in a spatiotemporal or tissue-specific manner. Our analysis also suggests that domain-deletion based functional assays are not sufficient as an exclusive approach to pinpoint the function of a specific interaction as deletion of a domain may disrupt many interactions and potentially the overall folding of these proteins. Continuous efforts by employing combination of structural tools (Cryo-EM, X-ray crystallography and NMR spectroscopy) and interface-based mutagenesis studies will allow us to gain more precise insight into the regulation of such complex and dynamic network. Currently, many drugs including monoclonal antibodies, peptide-mimetics and small compounds have been developed to target the extracellular domains of integrin receptors to treat related human diseases.[107,108] Intracellular interactions summarized here also play pivotal roles in the regulation of integrin signaling. Therefore, membrane permeable therapeutics could be developed to target these intracellular interfaces to intervene integrin-related human diseases.

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AUTHOR CONTRIBUTIONS

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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