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Talins and kindlins; partners in integrin-mediated adhesion

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Abstract

Integrin receptors provide a dynamic tightly-regulated link between the extracellular matrix (or cellular counter-receptors) and intracellular cytoskeletal and signalling networks, enabling cells to sense and respond to their chemical and physical environment. Talins and kindlins, two families of FERM-domain proteins, bind the cytoplasmic tail of integrins, recruit cytoskeletal and signalling proteins involved in mechano-transduction, and synergise to activate integrin binding to extracellular ligands. New data reveal the domain structure of full-length talin, provide insights into talin-mediated integrin activation, and show that RIAM recruits talin to the plasma membrane while vinculin stabilises talin in cell–matrix junctions. How Kindlins' act is less well defined, but disease-causing mutations show that kindlins are also essential for integrin activation, adhesion, cell spreading and signalling.

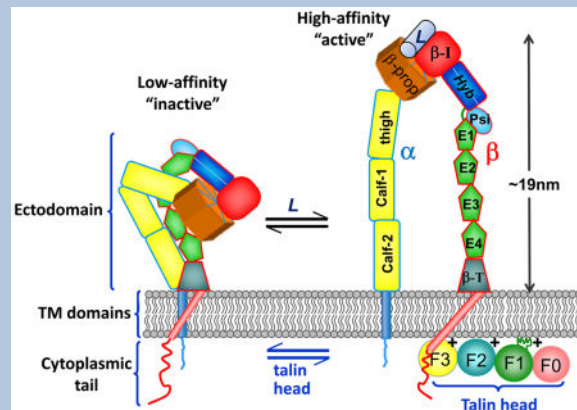
Cell proliferation, cell migration, tissue morphogenesis and homeostasis all depend on cell–cell and cell–extracellular matrix (ECM) interactions, and the integrin family of adhesion receptors play essential roles in these processes. Integrins are heterodimeric type I transmembrane proteins comprised of α and β subunits, both of which have an extracellular ligand-binding region and a generally short cytoplasmic tail that binds multiple cytoskeletal and adaptor proteins that regulate the affinity of integrin for ligands (integrin activation; Box 1). Ligand binding and subsequent integrin clustering lead to the recruitment of additional actin-binding, scaffolding and signalling proteins, resulting in the transmission of mechanical and chemical signals into the cell. Here, we focus on two structurally and functionally related protein families, the talins and kindlins, which bind β -integrin cytoplasmic tails and are essential for integrin activation and signalling.

BOX 1

Integrin structure and activation

Integrin adhesion receptors are obligate $\alpha\beta$ heterodimers. In mammals the 18 α subunits and 8 β subunits combine to form 24 different heterodimeric receptors, each with different ligand-binding specificities and a different tissue distribution¹⁴⁹. The subunits

have large ectodomains that are constructed from various modular units, including Calf and Thigh domains, and the crystal structures of various integrin ectodomains have been solved¹⁵⁰. Each α and β subunit has a single trans-membrane (TM) helix and, usually, a short unstructured cytoplasmic tail²¹. Integrins exist in equilibrium between a bent, low-affinity state and an upright, high-affinity state. They can be activated by the binding of extracellular ligands (L) to integrin ectodomains (known as ‘outside-in’ activation) and by the binding of the talin head (an atypical FERM domain with F0, F1, F2 and F3 domains²⁶) to β -integrin tails and acidic membrane phospholipids (known as ‘inside-out’ activation)^{20–22}. Evidence for a ‘bent’ to ‘upright’ change in the integrin heterodimer on activation includes an electron-microscopy study of α IIb β 3 integrin embedded in membrane nanodiscs, in which talin was observed to cause extension of the integrin conformation¹⁰⁷.



Talin was first identified in membrane ruffles and cell–ECM junctions (focal adhesions (FAs)) 30 years ago¹, and the discovery that it bound two other FA proteins, vinculin and integrins, attracted much interest. Sequencing revealed a highly-conserved, 2541 amino acid protein composed of an N-terminal FERM domain (known as the talin head) linked to a long C-terminal region, known as the talin rod. Initial evidence that talin is important for FA assembly came from antibody microinjection, gene down-regulation and gene deletion experiments (reviewed in ²), while the first mechanistic insights into how talin functions came from the landmark discovery that the talin head binds directly to the cytoplasmic tails of β -integrin subunits³, and performs the key final step in integrin activation⁴. This, combined with structural studies on the integrin–talin head interaction^{5,6}, began to explain how talin activates integrins from within the cell (inside-out activation)⁷. Talin is conserved throughout metazoans, and studies in *D. melanogaster*⁸ and *C. elegans*⁹ confirm its importance in integrin function. Vertebrates have two talin genes encoding closely related proteins with distinct but overlapping functions², and talin2 upregulation in talin1 knockout mouse embryo fibroblasts compensates for loss of talin1¹⁰. However, talin2 knockdown in these cells reveals that talins are essential for linking activated integrins to cytoskeletal actin, for FA assembly and the exertion of force on the ECM¹⁰. Note that endothelial cells only express talin1^{11,12}, making them ideal for talin1 structure–function studies.

Given the focus on talin in integrin activation, data showing that a lack of kindlin-3 results in severe integrin activation defects in platelets was unexpected¹³. A role for kindlin in integrin function first emerged when *C. elegans* kindlin (UNC-112) was shown to be essential for the organisation of integrins at muscle attachment sites¹⁴. In 2003, mutations in a human UNC-112 homolog were shown to cause Kindler syndrome^{15,16}, a rare autosomal recessive disease characterized by skin blisters, photosensitivity, mucosal erosion and gastro-intestinal ulcers. Expression of this gene (which was named kindlin-1) is largely restricted to epithelia, and kindlin-1-deficient cells exhibit defects in spreading, polarity, migration, survival and ECM organization¹⁷. Sequence similarity between kindlin and the talin FERM domain, and the ability of both proteins to bind integrin β tails and to localize to FAs¹⁸, heightened the interest in kindlins. Humans have three closely related kindlins: kindlin-1, kindlin-2 and kindlin-3, and all three mammalian kindlins have now been implicated in integrin activation. Mutations in kindlin-3 cause leukocyte adhesion deficiency type III (LAD-III) - reviewed in^{17,19}. While the detailed mechanisms by which kindlins exert their effects on integrin activation remain uncertain, it is clear that kindlins cooperate with talin during integrin activation, and that a direct interaction of kindlin with the integrin β tail is required, but apparently not sufficient, for integrin activation.

Here we focus on recent advances in talin structure, the mechanisms by which the Rap1A-effector RIAM binds and recruits talin to dynamic nascent adhesions at the leading edge of cells, and the way that force-induced conformational changes in the talin rod lead to a switch from talin-RIAM to talin-vinculin complexes, stabilising FAs. We also discuss new information on kindlin domain organization and the possible mechanisms underlying cooperation between kindlins and talin in integrin activation. We refer readers to more in depth reviews on the structural aspects of talin-mediated integrin activation²⁰⁻²², and vinculin structure and function^{23,24}.

STRUCTURAL INSIGHTS INTO TALIN FUNCTION

Understanding the mode(s) of action of talin at the cellular and organismal levels requires information about talin structure, but so far, efforts to crystallize talin have failed, probably because of its inherent flexibility²⁵. However, completion of the structures of all 18 domains in full-length talin represents a significant advance²⁶⁻³⁵, which has allowed a model of full-length talin to be built³⁵ (FIG 1A,B). The structures of individual domains, together with those of domains in complex with binding partners, show that talin has evolved to regulate integrin activity, to couple integrins to cytoskeletal actin and to act as a mechanosensitive protein in which ligand binding to the talin rod is regulated by force.

Structural model of full-length talin

The N-terminal talin head is an atypical FERM domain with an F0 domain in addition to the three domains (F1, F2, F3) that are characteristic of other FERM domains³² (Fig 1). Moreover, the crystal structure shows that it adopts an extended rather than the clover-leaf structure characteristic of other FERM domains²⁶. The F3 phosphotyrosine binding (PTB)-like domain binds β -integrin tails^{6,36,37}, the cytoplasmic tail of the hyaluronan receptor layilin³⁸ and the type 1 PIP-kinase γ -isoform³⁹; structures of these complexes reveal similar modes of talin binding. The F3 domain of talin also reportedly binds focal adhesion kinase

(FAK)⁴⁰, a tyrosine kinase that is phosphorylated in response to integrin ligation, and the Rac1 exchange factor TIAM1⁴¹, although as yet there are no structural details on binding mechanisms.

The talin head is linked by a large unstructured region (with the potential to span 20nm when fully extended⁴²) to the talin rod, 62 amphipathic α -helices organised into thirteen 4 or 5-helix bundles (R1–R13)³⁵; the single helix at the C-terminal end of the rod serves as the dimerisation domain (DD)²⁷. Crystal structures of the R1R2³⁴, R7R8²⁸ and R11R12³⁰ double domains have been determined, while the structures of other domains have been solved by NMR (FIG 1B). The rod contains multiple vinculin-binding sites (VBSs)³¹, and at least two actin-binding sites², the best characterised of which is in the C-terminal R13 domain and requires talin dimerisation to bind actin²⁷. The rod also contains a second integrin-binding site, IBS^{2,30,43}, and several binding sites for the Rap1A-GTPase effector RIAM³⁵ that is involved in recruiting talin to membranes to activate integrins^{44–46}. The tumour suppressor DLC1 (a RhoGAP)⁴⁷ and synemin (which links intermediate filament proteins to integrin-containing adherens junctions in striated muscle⁴⁸) both bind the R8 domain that is inserted into a loop in the R7 domain (FIG 1). The availability of high-resolution structures for all talin domains should facilitate the detailed mapping of interaction surfaces and therefore studies on talin function.

Mechanism of talin-mediated integrin activation

Biochemical³ and crystallographic studies⁵ established that a complex is formed between the talin F3 domain and the first NPxY motif in integrin β tails (FIG 2A), and that this interaction is required for the talin-mediated inside-out activation of integrins *in vitro*⁴ and *in vivo*⁴⁹. More recent studies of complexes formed with larger β tail fragments and talin subdomains^{6,37} (FIG 2B), and structures of integrin transmembrane (TM) segments⁵⁰, have extended our knowledge of integrin–talin–membrane complexes and have led to plausible models for talin-mediated integrin activation^{20–22} (FIG 2C and Box 1). In brief, the close association of the TM helices of the integrin subunits results in a low-affinity receptor. Structures of the α IIB β 3 TM complex show that, in this state, the α IIB TM helix is roughly perpendicular to the membrane while the β 3 TM helix is tilted^{50,51} (FIG 2C). The regions of the α IIB and β 3 TM helices nearer the extracellular face of these integrin subunits pack closely together, but contact at their intracellular ends involves an unusual folding back of the α -chain that promotes electrostatic interactions between α IIB (R995) and β 3 (D723)⁵⁰ (Fig 2C). Recent evidence indicates that a conserved β 3 TM lysine helps orient the TM helix tilt by placing its positive charge at the membrane–water interface⁵². During physiological activation, talin F3 binds to the first NPxY motif in the β tail; this interaction stabilizes additional interactions between F3 and the membrane-proximal region of the β integrin tail, extending the β TM helix (FIG 2B,C)⁶. Talin F3 also forms a salt bridge with the conserved membrane-proximal Asp in β integrin (D723 in β 3)³⁷, which likely disrupts the inhibitory interaction of the β integrin tail with the conserved R995 residue in α IIB, thereby contributing to integrin activation. These findings are consistent with multiscale molecular dynamics simulations where binding of talin F2F3 to a membrane-embedded α IIB β 3 dimer fragment causes tilting and reorientation of the β TM helix, and dissociation of the α and β TM contacts⁵³ (Fig. 2C and BOX 1). Experiments with fluorophores attached to regions of

the β TM segment at the membrane–water interfaces also show that talin induces changes in β TM segment positioning in the membrane⁵⁴.

Although interactions between talin F3 and β -integrin tails are required for integrin activation they are not sufficient, and interactions between the talin head and the membrane play a key role. The talin head has a series of basic residues along its membrane-proximal surface²⁶ that bind acidic phospholipids, and mutations in basic residues in F2³⁷ and in an inserted loop in F1³² markedly impair membrane binding and integrin activation. Moreover, acidic phospholipids have been shown to increase greatly the affinity of β 3-tails for the talin head⁵⁵, suggesting that acidic plasma membrane phospholipids such as PIP2 play a key role in orientating the talin head such that it can engage and activate integrins²⁶ (BOX 1).

Whether the above model applies to activation of most integrins is controversial, although there is general agreement that the activity of leukocyte⁵⁶ and platelet integrins^{57–59} are regulated by talin, and talin-mediated activation of integrins containing β 1, β 2 and β 3 subunits has been reported in a range of cell types^{4,60}. Integrin activation is generally assessed by measuring the binding of soluble ligands or reporter antibodies that selectively or preferentially bind the active integrin⁶¹. While over-expressing the talin head is sufficient for integrin activation, full-length talin also activates integrins, and mutagenesis confirms that this relies on interactions between the talin head and the integrin β tail^{44,61,62}. Nonetheless, full-length talin is a less potent integrin activator because it can adopt an auto-inhibited conformation^{62–65}. The interaction of full-length talins with integrins is also implicated in integrin clustering^{8,66,67}, and as many integrin ligands are multivalent, this could greatly increase adhesion through avidity modulation⁶⁸. Moreover, clustered talin-bound integrins serve as a link to the actin cytoskeleton and acts as a signalling hub.

REGULATION OF TALIN FUNCTION

Since talin plays a key role in integrin activation, its function is tightly regulated. Mechanisms involved include changes in talin conformation and localisation, which are induced by signalling pathways and mechanical force, the action of competitor proteins and limited proteolysis of talin by calpain2.

Talin conformations and auto-inhibition

Biochemical data and electron microscopy^{25,69} show that talin exists in both globular and extended conformations (~60 nm long), and a structural explanation for this has recently begun to emerge. Specifically, the talin F3 domain binds the R9 domain in the talin rod^{62,64,65}, which sterically inhibits binding of the F3 domain to the membrane-proximal region of β -integrin tails (compare FIG 2B and D). Further insights into the structure of auto-inhibited talin are provided by a 3D model of full-length talin derived from EM reconstruction studies, and the shapes of individual domains and inter-domain angles determined by small angle X-ray scattering (SAXS)⁶³. The model indicates that talin can form a compact dimer (12.5nm \times 11nm \times 9.5nm) in which the two talin rods form a donut-shaped structure, with the talin heads packed in the centre of the donut. The model recapitulates the high affinity interaction between the F3 head and R9 rod domains, plus several weaker inter-subunit interactions that have been detected by NMR.

The concept of auto-inhibited talin is consistent with observations that much of talin is cytosolic⁷⁰, and that it translocates to the membrane in response to activation of the Rap1A GTPase, a key regulator of integrin activity^{45,46}. The positively-charged residues on F3 that interact with the negatively-charged face on R9 also interact with acidic membrane phospholipids (Box 1). PIP2 has been shown to relieve the F3–R9 interaction⁶², and could therefore activate talin. Note: the interactions that stabilise auto-inhibited talin⁶² appear much weaker than those in auto-inhibited vinculin⁷¹.

As predicted by the structural data, mutations that compromise the talin F3–R9 interaction accelerate the rate of FA assembly¹¹. However, cell fractionation studies show that, on its own, disrupting the F3–R9 interaction is not sufficient to drive talin from the cytosol to the plasma membrane; additional interactions between the F2F3 FERM domains and R1R2 rod domains mask membrane-targeting sequences in the FERM domain⁷⁰. Interestingly, R1R2 contains 3 VBSs (FIG 1A), and expression of the talin-binding domain of vinculin (Vd1)²⁴ in cells was sufficient to drive talin to the plasma membrane. Thus, vinculin could play a significant role in talin activation, a conclusion supported by the observation that Vd1 stabilises FAs and locks integrins into the activated state^{72,73}. Moreover, vinculin Vd1 increases binding of α IIB β 3 integrin to recombinant talin *in vitro* and in cells⁷⁴.

Rap1A–RIAM in talin-mediated integrin activation

Elegant reconstitution studies of α IIB β 3 integrin activation in Chinese hamster ovary cells have defined a pathway in which activated Rap1A and its effector RIAM recruit talin to the cytoplasmic domain of integrins, which leads to integrin activation^{44–46}. A short amphipathic helix (residues 6–30) in the N-terminal region of RIAM binds talin, and a protein containing this helix fused to the membrane-targeting sequence of Rap1A recruits talin to the plasma membrane, and supports integrin activation⁴⁵. Talin also binds the N-terminal helix of lamellipodin⁴⁵, another member of the MRL family of proteins⁷⁵. RIAM and lamellipodin contain adjacent RA and PH domains, and the structure of the RIAM RA–PH double domain suggests that they act as a proximity detector for activated Rap1A and PIP2⁷⁶; that is, RIAM and therefore talin is only recruited to Rap1-GTP embedded in PIP2 rich microdomains. Interestingly the N-terminal region of RIAM inhibits its recruitment to membranes, raising the possibility that talin might contribute to RIAM activation. This leaves open the question as to whether RIAM activates talin.

The talin–PIPkinase type 1 γ interaction

Cells lacking PIP-kinase type 1 γ , the isoform of PIP-kinase that binds talin, show reduced initial rates of attachment to ECM, impaired recruitment of both talin and vinculin (but not kindlin-2 or paxillin) to FAs, and reduced ability of β 1 integrins to exert force on ECM⁷⁷. A similar phenotype was obtained with wild-type cells expressing a K274E mutation in F2 of the talin FERM domain that inhibits PIP2 binding and dramatically reduces integrin activation³⁷. The authors conclude that the major role of PIP-kinase type 1 γ in FAs is the local synthesis of PIP2 that is required to orientate the talin head such that it can activate integrins⁷⁷. However, PIP2 also likely plays a role in the RIAM-mediated recruitment of talin to the membrane⁷⁶ and talin activation⁶². Moreover, PIP-kinase type 1 γ and talin associate with the exocyst complex to deliver integrins to the leading edge⁷⁸. Thus, PIP-

kinase type 1 γ can modulate talin function and cell adhesion in several ways. Interestingly, ubiquitination of PIP-kinase type 1 γ has recently been shown to regulate FA turnover⁷⁹.

FAK-mediated recruitment of talin to nascent adhesions

Evidence that FAK also plays a key role in recruiting talin to nascent adhesions has recently emerged⁴⁰, challenging the conventional model of FA assembly in which integrin–talin–actin complexes precede the recruitment of other FA proteins. Although talin and paxillin colocalised in FAs formed 15 min after plating mouse embryo fibroblasts on fibronectin, talin was not detectable in early paxillin-positive FAs formed by FAK null cells even though it was present in mature FAs. Moreover, talin co-localised with FAK in early FAs in cells expressing a β 1-integrin tail mutation (Y783A) that abrogates talin binding, suggesting that talin can be recruited to FAs independently of integrin binding. What recruits FAK to these early adhesions has not been established although this might involve p190RhoGEF (also known as Rgnef), which binds FAK⁸⁰. Recent developments in single-protein tracking and super-resolution imaging⁸¹ may help clarify the kinetics of FAK and talin recruitment during adhesion initiation and maturation. Interestingly, talin-deficient adhesions in FAK null cells stain with the 9EG7 antibody that detects activated β 1 integrin, and mammary epithelial cells depleted of talin1 also contain activated β 1 integrins and assemble FAs, although these are deficient in vinculin, paxillin and ILK⁸². Whether integrin activation, and also the early stages of cell spreading¹⁰, in talin knockdown cells is driven by ECM binding, via other tail-binding proteins, or by residual talin below the limits of detection, is unclear.

Inhibitors of talin–integrin interactions

Several mechanisms have the potential to modulate the talin–integrin interaction and therefore integrin activation (FIG 3), even if talin itself remains activated and membrane-bound^{7,83}. Phosphorylation of the membrane-proximal NPxY motif in integrin β tails by Src family kinase (SFK) directly inhibits binding of the talin F3 PTB-like domain^{84,85}, and so impairs integrin activation. However, this tail phosphorylation also promotes the binding of other PTB domain proteins, such as the scaffold protein DOK1^{84–86}; competition of these proteins with talin is likely to further suppress integrin activation (FIG 3). Other proteins that bind β integrin tails can also interfere with talin binding, the best characterised of which is filamin. Structural studies reveal that filamin binds β -integrin tails at a site overlapping that for talin, and hence filamin competes with talin for β integrin binding⁸⁷. Filamin also likely competes with kindlin for β integrin tail binding (FIG 3). Consistent with this, loss of filamin expression enhances integrin activation⁸⁷, and expression of the filamin-binding protein migfilin, which occupies the integrin-binding site on filamin^{88,89}, enhances integrin activation⁹⁰. However, migfilin knockout mice either exhibit no phenotype⁹¹ or the effects are restricted to bone remodeling by osteoblast progenitors⁹², raising questions about the wider relevance of migfilin in regulating integrin activation. Filamin binding to β 2-integrins is also inhibited by 14-3-3 proteins that bind to β 2 tails that are phosphorylated on Thr758⁹³.

Another well-characterized integrin inhibitor is integrin cytoplasmic domain-associated protein 1 (ICAP1)⁹⁴ (FIG 3). The PTB-domain in ICAP1 binds the membrane-distal kindlin-binding NPxY motif in β 1 integrins, but also inhibits talin binding to integrin⁸³. The crystal structure of the integrin β 1–ICAP1 complex has now been solved⁹⁵ and talin-binding

residues are not involved in the interface, suggesting that the inhibitory effect of ICAP1 is not via direct competition. Nonetheless, ICAP1 expression impairs talin-mediated $\beta 1$ activation, and ICAP1 mutants defective in integrin binding do not inhibit this activation. Furthermore, KRIT1 binding to ICAP1 displaces ICAP1 from integrins, facilitating talin-mediated integrin activation⁹⁵.

Intriguingly, a recent study implicates the actin-bundling protein α -actinin in both positive and negative regulation of integrin–talin interactions⁹⁶. The reported α -actinin binding site on integrin β tails⁹⁶ overlaps the talin-binding site (Fig 3), suggesting that α -actinin and talin may compete for binding, and this is now reported for $\beta 3$ integrins⁹⁶. Consistent with this, α -actinin binding suppresses α IIB $\beta 3$ activation in platelets⁹⁷. However, α -actinin apparently enhances talin binding to $\beta 1$ integrins⁹⁶. The data highlight the need to consider α -actinin as a modulator of talin binding and adhesion site maturation, although the different effects on $\beta 1$ and $\beta 3$ integrins remain to be explained.

Integrin–talin interactions may also be inhibited by proteins that bind the talin F3-PTB domain. For example, over-expression of the type 1 PIP-kinase γ -isoform, that binds the talin F3 domain, suppresses integrin activation⁹⁸, even though talin dimers have the potential to engage two different F3-domain ligands simultaneously. In addition, inhibitors of integrin activation that bind the α tail have been identified⁸³, one of which, SHARPIN, is reported to inhibit talin and kindlin binding to the β integrin tail through an unknown mechanism. Thus, a variety of protein–protein interactions can modulate talin–integrin interactions and hence control integrin activation.

CO-OPERATION BETWEEN TALIN AND KINDLIN

Over the past 5 years it has become evident that the kindlins, another family of integrin β -tail-binding, FERM-domain-containing proteins, also play key roles in integrin activation and signalling^{17,19,99,100}. Kindlins directly bind integrin β tails, and kindlin or integrin mutations that inhibit this binding impair talin-mediated integrin activation, strongly suggesting that a direct kindlin–integrin interaction is required for maximal integrin activation. However, in most cell-culture systems, kindlin over-expression does not activate integrins (in some cases it can even suppresses activation¹⁰¹), but when co-expressed with talin head, kindlins can strongly potentiate α IIB $\beta 3$ activation^{101–103}. Surprisingly, even when co-expressed with talin head, over-expressed kindlin does not activate $\alpha 5\beta 1$ integrins¹⁰¹. Indeed, kindlin co-expression can suppress talin head-mediated $\beta 1$ activation. The basis for this suppression, and for the differential behaviour of $\beta 1$ and $\beta 3$ integrins, requires further study. Nonetheless, $\beta 1$ integrin activation is kindlin-dependent as kindlin knockout or knockdown impairs $\beta 1$ activation^{103–105}. Consistent with a role for kindlins as co-activators of integrins, rather than as direct activators, NMR data indicate that kindlin-2, unlike the talin head, is unable to unclasp the inhibitory α IIB- $\beta 3$ tail interaction¹⁰⁶. Furthermore, in engineered cell systems, mutations that block talin binding to β integrin tails block both talin- and kindlin-driven integrin activation, while mutations that inhibit kindlin binding still permit talin-mediated activation, although they block the kindlin enhancement effect¹⁰⁷. Thus, kindlins apparently modulate talin-mediated integrin activation; the major question is how.

Kindlins are structurally related to the talin head

Much less structural information is available for kindlins than for talins, partly because kindlins are difficult to express. Kindlins are highly conserved and revised sequence alignments show that they have a similar domain organisation to the atypical talin FERM domain¹⁰⁸ (FIG 4a). Like talins, kindlins have an F0 domain plus a large unstructured insert in F1. However, unlike talins, kindlin F2 domains contain an inserted PH domain. Structures of kindlin F0^{108,109} and PH domains^{110–112} have been solved and recent SAXS studies indicate that intact kindlin-3 is relatively elongated in solution¹¹³, like the talin head²⁶ (FIG 4b). Consistent with the similarities to the talin FERM domain, pull-down assays demonstrate that kindlins directly bind integrin β -tails and that point mutations in the kindlin F3 domain inhibit this binding^{13,101,102}. Kindlin binds the second NPxY motif in integrin β tails and mutations at this site, or at conserved threonine residues preceding it, inhibit binding. These results have recently been confirmed using various biophysical techniques^{106,113}. In summary, while we still lack high-resolution structures of intact kindlins, the available evidence points to a high degree of similarity to the talin head with the exceptions that kindlins contain an inserted PH domain and bind to the membrane-distal NPxY motif (rather than the membrane-proximal NPxY motif) in integrin β tails (Fig 3).

Kindlins' roles in talin-mediated integrin activation

As described earlier, the molecular basis of talin-mediated integrin activation has been worked out in considerable detail and, in purified systems, talin binding is sufficient to activate membrane-embedded α IIb β 3 integrins¹⁰⁷. However, kindlin knockout, knockdown and over-expression, along with integrin mutants defective in kindlin binding, all implicate kindlins in integrin activation. While we still lack a definitive understanding of how kindlins activate integrins, a number of models have been proposed^{22,100,114} (Fig 4c). *In vitro*, the talin head, kindlin and integrin β tails form a ternary complex^{106,113}, and this may be essential for integrin activation, although the sequential binding of these components, or their binding to adjacent integrins¹¹⁴, have not been excluded. Recent data suggest that the binding of kindlin to integrins neither enhances talin—integrin binding, nor increases talin targeting to the membrane^{106,115}, and no direct talin—kindlin interaction has been reported. This has led to suggestions that kindlin influences events occurring after talin recruitment to the integrin¹¹⁵. Consistent with this, in neutrophils, talin1 is required to generate the intermediate-affinity state of α L β 2 integrin that is responsible for the initial slow rolling of neutrophils, while both talin1 and kindlin-3 are required for high-affinity α L β 2 and neutrophil arrest⁵⁶. Kindlins might enhance talin-mediated integrin tail separation by binding to the second NPxY-like motif in the β integrin tail and anionic membrane phospholipids. Indeed, the kindlin F0 domain, F1 loop and the PH domain can each bind anionic phospholipids (FIG 4a), and such binding is required for kindlin to fully coactivate α IIb β 3 integrin^{109,110,112,116}. This hypothesis could be tested in purified reconstituted systems such as the integrin nanodiscs already used to show talin-mediated integrin activation¹⁰⁷. If kindlin potentiates talin-mediated integrin activation *in vitro*, then it would strongly suggest that the ternary complex is the key to integrin activation. Alternatively, in cells, kindlin binding to the integrin tail may recruit additional activator or signalling proteins, or displace inhibitory proteins that modify talin's ability to activate integrins. In

this regard it is noteworthy that the structurally-defined binding sites for the inhibitors ICAP1⁹⁵ and filamin⁸⁷ in integrin β tails overlap with the kindlin-binding site (FIG 3), suggesting that kindlin binding will displace these inhibitors. Similarly, the binding of 14-3-3 protein to phosphorylated β 2 tails is likely to inhibit kindlin binding⁹³. Kindlins might also influence integrin clustering and so enhance binding of multivalent ligands via increased avidity, rather than through conformational changes that lead to increased affinity for monovalent ligand. Available data suggests kindlins are monomeric so clustering might be mediated by kindlin-binding proteins such as migfilin and ILK, both of which have been implicated in promoting integrin activation^{90,117}. Thus, kindlins' adaptor functions may be critical for enhancing integrin activation and/or clustering.

Integrin and kindlin isoform specificity

All three kindlins bind integrins and impact their activation, but loss of kindlin-1, kindlin-2 or kindlin-3 results in markedly different phenotypes that are only partly due to differential expression^{17,114}. Of the eight different human integrin β subunits, kindlins are currently known to bind β 1, β 2, β 3 and β 6^{99,114,118}. Kindlin-2 preferentially binds β 1 and β 3 integrins, binds more weakly to β 2 and exhibits very little binding to β 6^{106,118}. Kindlin-1 binds strongly to both β 1 and β 6 integrins¹¹⁸. Kindlin-3 binds β 1, β 2 and β 3 integrins and, while differential integrin binding has not been reported for kindlin-3, it has been suggested to preferentially regulate β 1 integrin-dependent processes¹¹⁹. Differential integrin binding may have consequences in cells such as keratinocytes, which express both kindlin-1 and kindlin-2¹¹⁸. In addition to differences in integrin binding, different kindlins also have different subcellular localization and function^{119,120}. Furthermore, despite all three kindlins binding to β 1 and β 3 integrin tails, kindlin-1 and kindlin-2, but not kindlin-3, co-activate α IIb β 3 integrins in CHO cells^{13,101}. Likewise, while loss of kindlin-1 or kindlin-2 impairs β 1 integrin activation, co-expressing talin head and kindlin does not activate β 1 integrins in CHO cells¹⁰¹. The bases for these integrin- and kindlin-specific effects are unknown.

TALIN – BEYOND INTEGRIN ACTIVATION

While talins' roles in integrin activation have been the focus of attention over the last 15 years, talins also play vital roles linking activated integrins to the actin cytoskeleton¹⁰, sensing and reinforcing the response to mechanical force¹²¹ and regulating adhesion formation and turnover.

Role of talin in mechano-transduction

The talin rod is largely made up of 5-helix bundles in which the N-termini and C-termini are positioned at opposite ends; this means that these bundles form a linear chain³⁵ (FIG 1). However, three 4-helix bundles (R2–R4) interrupt the succession of 5-helix bundles, and since their N-termini and C-termini are at the same end of the bundle, they must adopt a more compact structure. Thus, the conformation of the talin rod will change in response to force exerted on integrin–talin–actin complexes, and this will likely impact on its interaction with certain ligands.

The multiple VBSs in the talin rod (FIG 1A) are each defined by hydrophobic residues on one face of an amphipathic helix³¹. These are normally buried within the core of the helical bundles, and vinculin binding therefore requires domain unfolding². Elegant single-molecule experiments show that VBSs can be activated by force¹²², and the recruitment of vinculin to FAs is myosin-II dependent¹²³. Moreover, talin undergoes repeated cycles of actomyosin-dependent stretching in the direction of actin flow¹²⁴. In this context, the R2R3 4-helix bundles contained within the compact N-terminal region of the rod are of particular interest. Unusually, each contains two VBSs (FIG 1A), and while vinculin binding to R2 is inhibited because R2 is stabilised by extensive contacts with R1³⁴, R3 is destabilised by a unique cluster of threonine residues buried within its hydrophobic core³⁵. Therefore, R3 is likely to be amongst the first of the rod domains to bind vinculin.

An additional feature of R2 and R3 is that they each bind the RIAM N-terminal peptide (residues 6–30) albeit with low affinity³⁵. However, it is now apparent that the N-terminal region of RIAM (residues 1–127) contains two talin-binding sites that bind synergistically to R2R3³⁵. Significantly, the talin-binding domain of vinculin, Vd1, displaces RIAM 1–127 from R2R3³⁵ and inhibits binding of the RIAM 6–30 peptide to full-length recombinant talin⁷⁴, suggesting that RIAM and vinculin binding to talin is mutually exclusive. Indeed, RIAM is localised preferentially at the membrane and in nascent adhesions where it supports cell protrusion⁷⁴, while vinculin is abundant in mature FAs^{35,74}. This suggests a model (FIG 5) in which RIAM binding to talin R2R3 initially recruits talin to the plasma membrane in a Rap1-GTP dependent manner. Here, talin activates integrins and triggers the assembly of dynamic nascent adhesions. RIAM also recruits VASP and the actin polymerisation machinery required to drive membrane protrusion. As talin binds F-actin flowing away from the leading edge, force-induced conformational changes in talin R2R3 disrupt high-affinity RIAM binding, exposing the VBSs. Vinculin recruitment stabilises FAs by promoting talin binding to integrins (this is Rap1-GTP independent⁷⁴), therefore maintaining integrins in the activated state^{72,74}. Moreover, vinculin can potentially cross-link talin to actin or acidic membrane phospholipids²⁴ (FIG 5). Vinculin also regulates the recruitment and release of other core FA proteins in a force-dependent manner⁷³.

Several regions of talin bind F-actin², and may therefore play a role in initiating force-induced conformational changes in talin. The best characterised F-actin binding site is in the C-terminal R13 rod domain²⁷, and mutations that compromise this site markedly reduce the ability of talin to rescue cell spreading and FA assembly in talin1 knockdown endothelial cells¹¹. Interestingly, helix 1 in the R13 5-helix bundle negatively regulates actin binding^{27,125}, and mutations that relieve this inhibition increase FA size and stability¹¹. This suggests that force exerted on the R13 bundle may increase its affinity for F-actin, strengthening the talin–actin connection. However, the C-terminal actin-binding site in vinculin is also essential for force transduction, and the repolarisation of cells in response to stretch⁷³. In summary, talin is a mechanosensitive protein that likely changes conformation in response to force; this is predicted to regulate its affinity for RIAM, vinculin and F-actin, and therefore its role during adhesion assembly versus FA maturation.

Role of the integrin-binding site IBS2 in the talin rod

In addition to the well characterized integrin-binding site in the talin F3 FERM domain (IBS1), the talin rod contains an integrin-binding site (IBS2)⁴³ that spans two 5-helix bundles, R11 and R12³⁰. Studies using *D. melanogaster* have established that, despite some redundancy, IBS1 maintains the link between integrins and the ECM, presumably by activating integrins, while IBS2 stabilises the link between integrins and the intracellular adhesion complex that includes paxillin, PINCH and FAK¹²⁶. Interactions between β 1 integrins and an IBS2-containing talin rod polypeptide have been detected by FRET, although surprisingly, no interaction was detected between β 1 integrins and the talin head¹²⁷. This suggests that IBS2 forms a relatively stable association with integrins although it does not activate integrins⁴³; in contrast the IBS1–integrin interaction is more dynamic. Factors that determine which of the two IBSs in talin engage β integrins remain to be defined. Super-resolution fluorescence microscopy of FAs shows that the talin head is close to integrin tails while the C-terminus of the rod co-localises with actin ~40nm from the membrane¹²⁸, an orientation supported by single molecule analysis of talin in cells¹²⁴. This would suggest that IBS2, which is close to the C-terminal end of talin, is not engaged in FAs.

A role for C-terminal talin polypeptides?

Although talin-depleted mammary epithelial cells still spread on ECM proteins, FAK signalling is compromised, p21 levels are elevated and proliferation is inhibited; this phenotype is rescued by expressing membrane-localized FAK, but not a kinase dead mutant⁸². FAK signalling and proliferation was also rescued by expression of just the C-terminal region of talin (residues 1974–2541; FIG 1), suggesting that this part of the talin rod plays a role in assembling FAK signalling complexes, although FAK has not been shown to bind the talin rod. Intriguingly, a similar C-terminal talin polypeptide is generated in cells by calpain2-mediated proteolysis of talin between residues 1902–1903, coupled to arginylation of the liberated 70 kDa talin polypeptide¹²⁹. Moreover, the talin2 gene has at least two internal promoters that encode C-terminal talin2 rod polypeptides¹³⁰. These results raise the possibility that C-terminal polypeptides generated from both talin1 and talin2 may have physiological relevance.

Talin, FA dynamics and turnover

Studies using single protein tracking and super-resolution microscopy have recently begun to provide remarkable new insights into the dynamics of individual molecules within FAs. Integrin immobilisation in FAs requires simultaneous binding to both matrix and talin, while integrins outside FAs are in free diffusion⁸¹. Freely diffusing talin in the cytosol is recruited directly to FAs where it becomes immobilised, implying that talin is activated within FAs, and immediately engages and activates integrins. Intriguingly, integrins within FAs go through periods of immobilisation and slow free diffusion, suggesting that they cycle between active and less active states.

Turnover of FAs is essential for cell migration, and calpain2-mediated cleavage of talin between the head and rod¹³¹ and at a second site that removes the C-terminal dimerisation domain, promotes FA turnover⁴². Interestingly, a FAK mutant (FAK^{E1015A}) that is unable to

bind talin fails to support calpain2-mediated talin cleavage and FA turnover⁴⁰. The possibility that the talin head liberated by calpain2 cleavage is physiologically important is raised by the discovery that its levels appear to be tightly regulated. Thus, the talin head undergoes Smurf1-mediated ubiquitylation, which leads to its degradation, promoting FA turnover. Conversely, Ser425 phosphorylation by cdk5 stabilises the talin head and FAs¹³². Thus, turnover of the liberated talin head might support cycling between active and inactive integrins during FA remodelling, while the liberated talin rod might maintain FAK signalling and therefore cell-cycle progression⁸².

Talin, kindlin and integrin recycling

The Rab GTPases play key roles in integrin recycling¹³³. However, the possibility that talin might also contribute was raised by early observations that talin depletion in Hela cells results in aberrant $\alpha 5\beta 1$ processing¹³⁴, and its delayed export from a secretory compartment¹³⁵. Moreover, talin1 knockout ES cells show a reduced steady state level of $\beta 1$ integrins¹³⁶, and recent studies show that talin1 (but not talin2) protects $\beta 1$ -integrins from proteasomal degradation, and that this is important in epithelial morphogenesis¹³⁷. New evidence points to a role for a talin-PIP-kinase type 1 γ -exocyst complex in promoting trafficking of integrins to the leading edge, the establishment of cell polarity and directional cell migration⁷⁸. Interestingly, talin1 also binds directly to the Rac1-GEF TIAM1⁴¹, which in turn associates with the PAR3 component of the PAR polarity complex. The talin1-TIAM1 interaction is important in transient integrin-mediated Rac1 activation, which occurs via outside-in signalling, and the subsequent lamellipodia protrusion and cell spreading. TIAM1 also co-localises with talin in a sub-population of larger FAs in the front of migrating cells and regulates their turnover. It now emerges that talin also plays a role in $\alpha 5\beta 1$ internalisation, while kindlin-2 binding to the membrane-distal NPxY motif in $\beta 1$ tails appears to prevent lysosomal degradation and promote recycling of internalised, activated $\alpha 5\beta 1$ integrin¹³⁸. However, two recent papers suggest that kindlins dissociate from internalised integrins and that the FERM domain-containing protein sorting nexin-17 interacts with the kindlin binding site on integrin β tails in early endosomes and drives recycling^{139,140}. In conclusion, both talin and kindlins can influence integrin function at several levels.

OUTSTANDING ISSUES

Mechanisms involved in talin-mediated integrin activation are now well understood, but how kindlins and talins cooperate during integrin activation remains a major question. Nonetheless, progress in characterising kindlin structures and interactions, combined with the reconstitution of integrin activation in purified systems¹⁰⁷, advanced imaging techniques such as FRET, super-resolution microscopy and fluorescence correlation spectroscopy^{81,127,128,141}, all hold promise for addressing this question.

A second major question relates to the regulation of talin and kindlin functions. Again, more is known for talin, although the mechanisms remain ill defined. While Rap1A-RIAM recruits talin to the plasma membrane⁴⁴⁻⁴⁶ (FIG 5), it is unclear whether the RIAM binding sites in auto-inhibited talin are exposed and whether RIAM activates talin. Interestingly, the PIP-kinase type 1 γ binding site in auto-inhibited talin is exposed⁶⁵, so perhaps it targets talin

to the plasma membrane (for example, as part of the integrin-containing exocyst complex⁷⁸) (FIG 5), and synthesises the PIP2 that activates talin⁶² (FIG 5). However, if PIP-kinase type I γ occupies talin F3, how does talin bind integrins? In principle, talin dimers can bind both molecules, but could this support integrin clustering? The affinity of talin for integrins is greatly increased by PIP2⁵⁵, so once talin has been delivered to the plasma membrane, PIP2 could shift the binding equilibrium in favour of talin–integrin complexes. Indeed, talin appears to be activated within FAs⁸¹, and PIP2 localised in FAs appear to be important in integrin adhesion and force coupling⁷⁷. The roles of FAK⁴⁰ and vinculin^{70,73} in recruiting talin to FAs, and in the regulation of talin-mediated integrin activation, also require further clarification (FIG 5).

Once integrin–talin complexes engage F-actin, force-induced conformational changes in the talin rod may displace RIAM and promote the vinculin binding required for adhesion maturation^{35,74} (FIG 5). It will be important to establish first, whether the 11 VBSs in the talin rod are differentially activated in response to increasing force, second, whether force relaxation allows the helical bundles in the talin rod to refold displacing vinculin and third, whether vinculin stabilises FAs by cross-linking talin to actin or PIP2 or both? Both *in vitro* molecular tweezer approaches and *in vivo* FRET tension sensors may help to address these questions. In the case of kindlin, similar mechanisms are likely to regulate its activation, namely membrane binding, competition with other partners, phosphorylation and calpain cleavage^{109,110,113,116,142–144}, and possibly even conformational rearrangements¹⁴⁵, but these studies are at an early stage.

Ideas about the regulation and function of talins and kindlins obtained from *in vitro* or cell culture approaches need testing in whole organisms. While progress has been made using talin-knockout in *D. melanogaster*⁸ and conditional talin1 knockout mice (Table 1), studies in vertebrates are complicated by talin2, the function of which remains obscure^{130,146}, although the fact that talin2 expression is tightly regulated by multiple pathways¹⁴⁶ suggests an important function. Likewise, potential redundancy between kindlins can make interpretation of mouse and *D. melanogaster* kindlin knockout phenotypes difficult, and it will be important to assess the isoform-specific interactions and functions of the different kindlins. Although many questions remain unanswered, our understanding of the ‘integrin, talin, kindlin story’ has made remarkable progress in the 30 years since talin was first discovered. It will be fascinating to see how this impacts on our understanding of human diseases, and several recent reports suggest a role for talin-mediated integrin activation in metastasis^{147,148}

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Glossary

Leukocyte adhesion deficiency type III	LAD-III is a rare genetic disease characterized by severe bacterial infections and bleeding disorders. It is caused by mutations in the gene encoding kindlin-3 in hematopoietic cells
FERM domains	found in various cytoskeletal-associated proteins, including band <u>4.1</u> , <u>ezrin</u> , <u>radixin</u> and <u>moesin</u> , the proteins after which this domain was named. One role of these domains is to localize proteins to the plasma membrane. FERM domains typically contain three sub-domains, F1, F2 and F3, normally arranged in a cloverleaf formation
Multiscale molecular dynamics (MD) simulations	these numerically solve Newton's equations for interacting particles and follow their trajectories. MD has become a powerful way of fine-tuning protein structure and dynamics. Major limitations are the relatively small size of the systems and the short timescales of the trajectories that can be studied. Multiscale simulations that combine simplified representations of molecules (coarse grain) with all atom (atomistic) representations circumvent some limitations
Negative headgroup	Lipid headgroups come in various forms with various charged properties. For example, phosphatidyl serine and phosphatidylinositol 4,5-bisphosphate (PIP ₂) have a net negative charge that promotes the binding of some proteins, for example talin, to the membrane
RA domain	The <u>Ras</u> association domain (RA) is found in many effector proteins for the Ras family of small GTPases. The domain adopts a ubiquitin-like fold and supports binding of proteins containing an RA domain to 'active' GTP-loaded Ras family proteins
PH domain	The <u>pleckstrin</u> homology (PH) domain is found in a wide range of intracellular signalling proteins. It often binds to membranes via interactions with PIP ₂
PTB domain	The <u>phosphotyrosine</u> binding (PTB) domain is found in a wide range of intracellular signalling proteins. It commonly binds NPxY motifs; the Y may, or may not, require phosphorylation to support binding
KRIT1	K Ras Interaction Trapped-1, the product of the CCM1 gene, is a multi-domain adaptor protein important for cell–cell and cell matrix adhesion. Loss-of-function mutations in CCM1 cause predisposition to cerebral cavernous malformations, neurovascular anomalies that increase the risk of haemorrhagic stroke
Exocyst complex	An octameric protein complex involved in vesicle trafficking. It targets post-Golgi vesicles to the plasma membrane prior to vesicle fusion
Nanodiscs	A nanodisc is a synthetic model membrane system made from lipids and a scaffold protein. They are useful for the study of incorporated

membrane proteins, such as integrins, because they are relatively small, monodisperse and homogenous, yet provide a native-like environment

SAXS

small angle X-ray scattering is a technique that gives low-resolution information about the shape of objects. It depends on analysis of the angular intensity distribution of X-rays scattered by molecules in solution

References

- Burridge K, Connell L. A New Protein of Adhesion plaques and ruffling membranes. *J Cell Biol.* 1983; 97:359–367. [PubMed: 6684120]
- Critchley DR. Biochemical and structural properties of the integrin-associated cytoskeletal protein talin. *Annu Rev Biophys.* 2009; 38:235–54. [PubMed: 19416068]
- Calderwood DA, et al. The talin head domain binds to integrin β subunit cytoplasmic tails and regulates integrin activation. *J Biol Chem.* 1999; 274:28071–28704. [PubMed: 10497155]
- Tadokoro S, et al. Talin binding to integrin β tails: a final common step in integrin activation. *Science.* 2003; 302:103–6. [PubMed: 14526080]
- Garcia-Alvarez B, et al. Structural determinants of integrin recognition by talin. *Mol Cell.* 2003; 11:49–58. [PubMed: 12535520]
- Wegener KL, et al. Structural basis of integrin activation by talin. *Cell.* 2007; 128:171–82. [PubMed: 17218263]
- Calderwood DA. Integrin activation. *J Cell Sci.* 2004; 117:657–66. [PubMed: 14754902]
- Brown NH, et al. Talin is essential for integrin function in *Drosophila*. *Dev Cell.* 2002; 3:569–579. [PubMed: 12408808]
- Cram EJ, Clark SG, Schwarzbauer JE. Talin loss-of-function uncovers roles in cell contractility and migration in *C. elegans*. *J Cell Sci.* 2003; 116:3871–8. [PubMed: 12915588]
- Zhang X, et al. Talin depletion reveals independence of initial cell spreading from integrin activation and traction. *Nat Cell Biol.* 2008; 10:1062–8. [PubMed: 19160486]
- Kopp PM, et al. Studies on the morphology and spreading of human endothelial cells define key inter- and intramolecular interactions for talin1. *Eur J Cell Biol.* 2010; 89:661–73. [PubMed: 20605055]
- Monkley SJ, et al. Endothelial cell talin1 is essential for embryonic angiogenesis. *Dev Biol.* 2011; 349:494–502. [PubMed: 21081121]
- Moser M, Nieswandt B, Ussar S, Pozgajova M, Fassler R. Kindlin-3 is essential for integrin activation and platelet aggregation. *Nat Med.* 2008; 14:325–30. First demonstration that loss of kindlin-3 results in severe defects in platelet integrin activation, revealing the importance of kindlins in integrin activation. [PubMed: 18278053]
- Rogalski TM, Mullen GP, Gilbert MM, Williams BD, Moerman DG. The UNC-112 gene in *Caenorhabditis elegans* encodes a novel component of cell-matrix adhesion structures required for integrin localization in the muscle cell membrane. *J Cell Biol.* 2000; 150:253–64. [PubMed: 10893272]
- Siegel DH, et al. 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.* 2003; 73:174–87. [PubMed: 12789646]
- Jobard F, et al. Identification of mutations in a new gene encoding a FERM family protein with a pleckstrin homology domain in Kindler syndrome. *Human molecular genetics.* 2003; 12:925–35. [PubMed: 12668616]
- Karakose E, Schiller HB, Fassler R. The kindlins at a glance. *Journal of cell science.* 2010; 123:2353–6. [PubMed: 20592181]
- Kloeker S, et al. The Kindler syndrome protein is regulated by transforming growth factor- β and involved in integrin-mediated adhesion. *J Biol Chem.* 2004; 279:6824–33. [PubMed: 14634021]

19. Plow EF, Qin J, Byzova T. Kindling the flame of integrin activation and function with kindlins. *Curr Opin Hematol.* 2009; 16:323–8. [PubMed: 19553810]
20. Ye F, Kim C, Ginsberg MH. Molecular mechanism of inside-out integrin regulation. *Journal of thrombosis and haemostasis : JTH.* 2011; 9 (Suppl 1):20–5. [PubMed: 21781238]
21. Anthis NJ, Campbell ID. The tail of integrin activation. *Trends Biochem Sci.* 2011; 36:191–198. [PubMed: 21216149]
22. Shattil SJ, Kim C, Ginsberg MH. The final steps of integrin activation: the end game. *Nat Rev Mol Cell Biol.* 2010; 11:288–300. [PubMed: 20308986]
23. Peng X, Nelson ES, Maiers JL, DeMali KA. New insights into vinculin function and regulation. *Int Rev Cell Mol Biol.* 2011; 287:191–231. [PubMed: 21414589]
24. Ziegler WH, Liddington RC, Critchley DR. The structure and regulation of vinculin. *Trends Cell Biol.* 2006; 16:453–60. [PubMed: 16893648]
25. Winkler J, Lunsdorf H, Jockusch BM. Energy-filtered electron microscopy reveals that talin is a highly flexible protein composed of a series of globular domains. *Eur J Biochem.* 1997; 243:430–436. [PubMed: 9030769]
26. Elliott PR, et al. The Structure of the talin head reveals a novel extended conformation of the FERM domain. *Structure (London, England : 1993).* 2010; 18:1289–99.
27. Gingras AR, et al. The structure of the C-terminal actin-binding domain of talin. *EMBO J.* 2008; 27:458–69. [PubMed: 18157087]
28. Gingras AR, et al. Central region of talin has a unique fold that binds vinculin and actin. *J Biol Chem.* 2010; 285:29577–87. [PubMed: 20610383]
29. Gingras AR, et al. Structural and dynamic characterization of a vinculin binding site in the talin rod. *Biochemistry.* 2006; 45:1805–17. [PubMed: 16460027]
30. Gingras AR, et al. Structural determinants of integrin binding to the talin rod. *J Biol Chem.* 2009; 284:8866–76. [PubMed: 19176533]
31. Gingras AR, et al. Mapping and Consensus Sequence Identification for Multiple Vinculin Binding Sites within the Talin Rod. *J Biol Chem.* 2005; 280:37217–24. [PubMed: 16135522]
32. Goult BT, et al. Structure of a double ubiquitin-like domain in the talin head: a role in integrin activation. *EMBO J.* 2010; 29:1069–80. [PubMed: 20150896]
33. Goult BT, et al. The domain structure of talin: residues 1815–1973 form a five-helix bundle containing a cryptic vinculin-binding site. *FEBS Lett.* 2010; 584:2237–41. [PubMed: 20399778]
34. Papagrigoriou E, et al. Activation of a vinculin-binding site in the talin rod involves rearrangement of a five-helix bundle. *EMBO J.* 2004; 23:2942–2951. [PubMed: 15272303]
35. Goult BT, et al. RIAM and Vinculin Binding to Talin Are Mutually Exclusive and Regulate Adhesion Assembly and Turnover. *J Biol Chem.* 2013; 288:8238–49. Presents a structural model for full-length talin and provides a structural basis for a switch from talin–RIAM to talin–vinculin complexes during adhesion assembly and maturation. [PubMed: 23389036]
36. Anthis NJ, Wegener KL, Critchley DR, Campbell ID. Structural diversity in integrin/talin interactions. *Structure.* 2010; 18:1654–66. [PubMed: 21134644]
37. Anthis NJ, et al. The structure of an integrin/talin complex reveals the basis of inside-out signal transduction. *EMBO J.* 2009; 28:3623–32. The first structure of a full-length β integrin tail bound to the talin F2F3 FERM domains. This, combined with biophysical studies and integrin activation assays, reveals that talin F3 binding to the membrane-proximal helix of the β tail disrupts an inhibitory α tail– β tail interaction, and identifies an important positively charged membrane binding surface on talin F2. [PubMed: 19798053]
38. Wegener KL, et al. Structural Basis for the Interaction between the Cytoplasmic Domain of the Hyaluronate Receptor Layilin and the Talin F3 Subdomain. *J Mol Biol.* 2008; 382:112–126. [PubMed: 18638481]
39. de Pereda JM, et al. Structural basis for phosphatidylinositol phosphate kinase type I γ binding to talin at focal adhesions. *J Biol Chem.* 2005; 280:8381–6. [PubMed: 15623515]
40. Lawson C, et al. FAK promotes recruitment of talin to nascent adhesions to control cell motility. *J Cell Biol.* 2012; 196:223–32. [PubMed: 22270917]

41. Wang S, et al. Tiam1 interaction with the PAR complex promotes talin-mediated Rac1 activation during polarized cell migration. *J Cell Biol.* 2012; 199:331–45. [PubMed: 23071154]
42. Bate N, et al. Talin contains a C-terminal calpain2 cleavage site important in focal adhesion dynamics. *PLoS One.* 2012; 7:e34461. [PubMed: 22496808]
43. Rodius S, et al. The talin rod IBS2 alpha-helix interacts with the beta3 integrin cytoplasmic tail membrane-proximal helix by establishing charge complementary salt bridges. *J Biol Chem.* 2008; 283:24212–23. [PubMed: 18577523]
44. Han J, et al. Reconstructing and Deconstructing Agonist-Induced Activation of Integrin alphaIIb beta3. *Curr Biol.* 2006; 16:1796–806. [PubMed: 16979556]
45. Lee HS, Lim CJ, Puzon-McLaughlin W, Shattil SJ, Ginsberg MH. RIAM activates integrins by linking talin to ras GTPase membrane-targeting sequences. *J Biol Chem.* 2009; 284:5119–27. Provides the first insights into how the Rap1A effector RIAM binds to talin and recruits it to the membrane to activate integrins. [PubMed: 19098287]
46. Watanabe N, et al. Mechanisms and consequences of agonist-induced talin recruitment to platelet integrin alphaIIb beta3. *J Cell Biol.* 2008; 181:1211–22. [PubMed: 18573917]
47. Li G, et al. Full activity of the deleted in liver cancer 1 (DLC1) tumor suppressor depends on an LD-like motif that binds talin and focal adhesion kinase (FAK). *Proc Natl Acad Sci U S A.* 2011; 108:17129–34. [PubMed: 21969587]
48. Sun N, Critchley DR, Paulin D, Li Z, Robson RM. Identification of a repeated domain within mammalian alpha-synemin that interacts directly with talin. *Exp Cell Res.* 2008; 314:1839–1849. [PubMed: 18342854]
49. Petrich BG. Talin-dependent integrin signalling in vivo. *Thromb Haemost.* 2009; 101:1020–4. [PubMed: 19492142]
50. Lau TL, Kim C, Ginsberg MH, Ulmer TS. The structure of the integrin alphaIIb beta3 transmembrane complex explains integrin transmembrane signalling. *EMBO J.* 2009; 28:1351–61. An NMR structure that provides key information about the complex formed by the membrane spanning helices in the inactive integrin state. [PubMed: 19279667]
51. Zhu J, et al. The structure of a receptor with two associating transmembrane domains on the cell surface: integrin alphaIIb beta3. *Mol Cell.* 2009; 34:234–49. A structure of the membrane spanning region of an intact integrin, determined by disulfide crosslinking and molecular modelling. [PubMed: 19394300]
52. Kim C, et al. Basic amino-acid side chains regulate transmembrane integrin signalling. *Nature.* 2011; 481:209–13. [PubMed: 22178926]
53. Kalli AC, Campbell ID, Sansom MSP. Multiscale simulations suggest a mechanism for integrin inside-out activation. *Proc Natl Acad Sci U S A.* 2011; 108:11890–5. [PubMed: 21730166]
54. Kim C, Ye F, Hu X, Ginsberg MH. Talin activates integrins by altering the topology of the beta transmembrane domain. *J Cell Biol.* 2012; 197:605–11. Demonstrates, using environmentally sensitive fluorophores, that talin binding to the integrin β transmembrane domain (TMD) alters the membrane embedding of the β TMD (see also ref 52). [PubMed: 22641344]
55. Moore DT, et al. Affinity of talin-1 for the beta3-integrin cytosolic domain is modulated by its phospholipid bilayer environment. *Proc Natl Acad Sci U S A.* 2012; 109:793–8. [PubMed: 22210111]
56. Lefort CT, et al. Distinct roles for talin-1 and kindlin-3 in LFA-1 extension and affinity regulation. *Blood.* 2012; 119:4275–82. [PubMed: 22431571]
57. Nieswandt B, et al. Loss of talin1 in platelets abrogates integrin activation, platelet aggregation, and thrombus formation in vitro and in vivo. *J Exp Med.* 2007; 204:3113–8. [PubMed: 18086864]
58. Petrich BG, et al. Talin is required for integrin-mediated platelet function in hemostasis and thrombosis. *J Exp Med.* 2007; 204:3103–11. [PubMed: 18086863]
59. Haling JR, Monkley SJ, Critchley DR, Petrich BG. Talin-dependent integrin activation is required for fibrin clot retraction by platelets. *Blood.* 2011; 117:1719–22. [PubMed: 20971947]
60. Kim M, Carman CV, Springer TA. Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. *Science.* 2003; 301:1720–5. [PubMed: 14500982]
61. Bouaouina M, Harburger DS, Calderwood DA. Talin and signaling through integrins. *Methods Mol Biol.* 2012; 757:325–47. [PubMed: 21909921]

62. Goksoy E, et al. Structural basis for the autoinhibition of talin in regulating integrin activation. *Mol Cell*. 2008; 31:124–33. The first biochemical and structural data to show how binding of the talin head to the rod occludes the integrin binding site in the head, and how talin auto-inhibition might be relieved by PIP2. [PubMed: 18614051]
63. Goult B, et al. Structural studies on full-length talin1 reveal a compact auto-inhibited dimer: Implications for talin activation. *J Struct Biol*. 2013 May 30. pii: S1047-8477(13)00146-9 Epub ahead of print. 10.1016/j.jsb.2013.05.014
64. Goult BT, et al. The structure of an interdomain complex that regulates talin activity. *J Biol Chem*. 2009; 284:15097–106. [PubMed: 19297334]
65. Song X, et al. A novel membrane-dependent on/off switch mechanism of talin FERM domain at sites of cell adhesion. *Cell Res*. 2012; 22:1533–1545. [PubMed: 22710802]
66. Saltel F, et al. New PI(4,5)P₂- and membrane proximal integrin-binding motifs in the talin head control beta3-integrin clustering. *J Cell Biol*. 2009; 187:715–31. [PubMed: 19948488]
67. Helsten TL, et al. Differences in regulation of Drosophila and vertebrate integrin affinity by talin. *Mol Biol Cell*. 2008; 19:3589–98. [PubMed: 18508915]
68. Bunch TA. Integrin alphaIIb beta3 activation in Chinese hamster ovary cells and platelets increases clustering rather than affinity. *J Biol Chem*. 2010; 285:1841–9. [PubMed: 19917607]
69. Molony L, Mccaslin D, Abernethy J, Paschal B, Burrige K. Properties of talin from chicken gizzard smooth-muscle. *J Biol Chem*. 1987; 262:7790–7795. [PubMed: 3108258]
70. Banno A, et al. Subcellular localization of talin is regulated by inter-domain interactions. *J Biol Chem*. 2012; 287:13799–13812. [PubMed: 22351767]
71. Bakolitsa C, et al. Structural basis for vinculin activation at sites of cell adhesion. *Nature*. 2004; 430:583–6. [PubMed: 15195105]
72. Humphries JD, et al. Vinculin controls focal adhesion formation by direct interactions with talin and actin. *J Cell Biol*. 2007; 179:1043–57. [PubMed: 18056416]
73. Carisey A, et al. Vinculin regulates the recruitment and release of core focal adhesion proteins in a force-dependent manner. *Curr Biol*. 2013; 23:271–81. [PubMed: 23375895]
74. Lee HS, Anekal P, Lim CJ, Liu CC, Ginsberg MH. Two Modes of Integrin Activation Form a Binary Molecular Switch in Adhesion Maturation. *Mol Biol Cell*. 2013; 24:1354–1362. Provides evidence that vinculin displaces RIAM from talin, and that this drives the transition from dynamic RIAM-positive nascent adhesions that support membrane protrusion to more stable vinculin-rich force-bearing FAs. [PubMed: 23468527]
75. Colo GP, Lafuente EM, Teixido J. The MRL proteins: Adapting cell adhesion, migration and growth. *Eur J Cell Biol*. 2012; 91:861–8. [PubMed: 22555291]
76. Wynne JP, et al. Rap1-interacting adapter molecule (RIAM) associates with the plasma membrane via a proximity detector. *J Cell Biol*. 2012; 199:317–29. [PubMed: 23045549]
77. Legate KR, et al. Integrin adhesion and force coupling are independently regulated by localized PtdIns(4,5)P₂ synthesis. *EMBO J*. 2011; 30:4539–53. Provides definitive evidence that PIP2 generated by the talin-binding isoform of PIP-kinase type 1 γ in focal adhesions is essential for formation of initial integrin-mediated cell attachment, and for the subsequent linkage of integrins to cytoskeletal actin and the exertion of force on matrix. [PubMed: 21926969]
78. Thapa N, et al. Phosphoinositide signaling regulates the exocyst complex and polarized integrin trafficking in directionally migrating cells. *Developmental cell*. 2012; 22:116–30. [PubMed: 22264730]
79. Li X, et al. Ubiquitination of PIPKI γ 90 by HECTD1 regulates focal adhesion dynamics and cell migration. *J Cell Sci*. 2013
80. Miller NL, Lawson C, Chen XL, Lim ST, Schlaepfer DD. Rgnef (p190RhoGEF) knockout inhibits RhoA activity, focal adhesion establishment, and cell motility downstream of integrins. *PLoS One*. 2012; 7:e37830. [PubMed: 22649559]
81. Rossier O, et al. Integrins beta(1) and beta(3) exhibit distinct dynamic nanoscale organizations inside focal adhesions. *Nat Cell Biol*. 2012; 14:1057–67. [PubMed: 23023225]
82. Wang P, Ballestrem C, Streuli CH. The C-terminus of talin links integrins to cell cycle progression. *J Cell Biol*. 2011; 195:499–513. [PubMed: 22042621]

83. Pouwels J, Nevo J, Pellinen T, Ylanne J, Ivaska J. Negative regulators of integrin activity. *J Cell Sci.* 2012; 125:3271–80. [PubMed: 22822081]
84. Anthis NJ, et al. Beta integrin tyrosine phosphorylation is a conserved mechanism for regulating talin-induced integrin activation. *J Biol Chem.* 2009; 284:36700–10. [PubMed: 19843520]
85. Oxley CL, et al. An integrin phosphorylation switch: the effect of beta3 integrin tail phosphorylation on Dok1 and talin binding. *J Biol Chem.* 2008; 283:5420–6. [PubMed: 18156175]
86. Calderwood DA, et al. Integrin beta cytoplasmic domain interactions with phosphotyrosine-binding domains: a structural prototype for diversity in integrin signaling. *Proc Natl Acad Sci U S A.* 2003; 100:2272–7. [PubMed: 12606711]
87. Kiema T, et al. The molecular basis of filamin binding to integrins and competition with talin. *Mol Cell.* 2006; 21:337–47. [PubMed: 16455489]
88. Ithychanda SS, et al. Migfilin, a molecular switch in regulation of integrin activation. *J Biol Chem.* 2009; 284:4713–22. [PubMed: 19074766]
89. Lad Y, et al. Structural basis of the migfilin-filamin interaction and competition with integrin beta tails. *J Biol Chem.* 2008; 283:35154–63. [PubMed: 18829455]
90. Das M, Ithychanda SS, Qin J, Plow EF. Migfilin and filamin as regulators of integrin activation in endothelial cells and neutrophils. *PLoS one.* 2011; 6:e26355. [PubMed: 22043318]
91. Moik DV, Janbandhu VC, Fassler R. Loss of migfilin expression has no overt consequences on murine development and homeostasis. *J Cell Sci.* 2011; 124:414–21. [PubMed: 21224394]
92. Xiao G, et al. Critical role of filamin-binding LIM protein 1 (FBLP-1)/migfilin in regulation of bone remodeling. *J Biol Chem.* 2012; 287:21450–60. [PubMed: 22556421]
93. Takala H, et al. Beta2 integrin phosphorylation on Thr758 acts as a molecular switch to regulate 14-3-3 and filamin binding. *Blood.* 2008; 112:1853–62. [PubMed: 18550856]
94. Millon-Fremillon A, et al. Cell adaptive response to extracellular matrix density is controlled by ICAP-1-dependent beta1-integrin affinity. *J Cell Biol.* 2008; 180:427–41. [PubMed: 18227284]
95. Liu W, Draheim KM, Zhang R, Calderwood DA, Boggon TJ. Mechanism for KRIT1 Release of ICAP1-Mediated Suppression of Integrin Activation. *Mol Cell.* 2013; 49:719–729. [PubMed: 23317506]
96. Roca-Cusachs P, et al. Integrin-dependent force transmission to the extracellular matrix by alpha-actinin triggers adhesion maturation. *Proc Natl Acad Sci USA.* 2013; 110:E1361–70. [PubMed: 23515331]
97. Tadokoro S, et al. A potential role for alpha-actinin in inside-out alphaIIb beta3 signaling. *Blood.* 2011; 117:250–8. [PubMed: 20940419]
98. Calderwood DA, Tai V, Di Paolo G, De Camilli P, Ginsberg MH. Competition for talin results in trans-dominant inhibition of integrin activation. *J Biol Chem.* 2004; 279:28889–95. [PubMed: 15143061]
99. Bouaouina M, Calderwood DA. Kindlins. *Current biology : CB.* 2011; 21:R99–101. [PubMed: 21300280]
100. Ye F, Petrich BG. Kindlin: helper, co-activator, or booster of talin in integrin activation? *Curr Opin Hematol.* 2011; 18:356–60. [PubMed: 21730832]
101. Harburger DS, Bouaouina M, Calderwood DA. Kindlin-1 and -2 directly bind the C-terminal region of beta integrin cytoplasmic tails and exert integrin-specific activation effects. *J Biol Chem.* 2009; 284:11485–97. [PubMed: 19240021]
102. Ma YQ, Qin J, Wu C, Plow EF. Kindlin-2 (Mig-2): a co-activator of beta3 integrins. *J Cell Biol.* 2008; 181:439–46. Early evidence for cooperativity between the talin head and kindlin-2 in integrin activation. [PubMed: 18458155]
103. Montanez E, et al. Kindlin-2 controls bidirectional signaling of integrins. *Genes Dev.* 2008; 22:1325–30. [PubMed: 18483218]
104. Malinin NL, et al. A point mutation in KINDLIN3 ablates activation of three integrin subfamilies in humans. *Nat Med.* 2009; 15:313–8. [PubMed: 19234460]
105. Qu H, et al. Kindlin-2 regulates podocyte adhesion and fibronectin matrix deposition through interactions with phosphoinositides and integrins. *J Cell Sci.* 2011; 124:879–91. [PubMed: 21325030]

106. Bledzka K, et al. Spatial coordination of kindlin-2 with talin head domain in interaction with integrin beta cytoplasmic tails. *J Biol Chem.* 2012; 287:24585–94. [PubMed: 22648415]
107. Ye F, et al. Recreation of the terminal events in physiological integrin activation. *J Cell Biol.* 2010; 188:157–73. Establishes that purified talin components, in an *in vitro* nanodisc system, are sufficient to activate isolated membrane-embedded integrins, even in the absence of kindlin. Also provides support for a model where integrins become more ‘upright’ when activated. [PubMed: 20048261]
108. Goult BT, et al. The Structure of the N-Terminus of Kindlin-1: A Domain Important for alphaIIb beta3 Integrin Activation. *J Mol Biol.* 2009; 394:944–56. [PubMed: 19804783]
109. Perera HD, et al. Membrane binding of the N-terminal ubiquitin-like domain of kindlin-2 is crucial for its regulation of integrin activation. *Structure.* 2011; 19:1664–71. [PubMed: 22078565]
110. Liu J, et al. Structural basis of phosphoinositide binding to kindlin-2 protein pleckstrin homology domain in regulating integrin activation. *J Biol Chem.* 2011; 286:43334–42. [PubMed: 22030399]
111. Liu Y, Zhu Y, Ye S, Zhang R. Crystal structure of kindlin-2 PH domain reveals a conformational transition for its membrane anchoring and regulation of integrin activation. *Protein & cell.* 2012; 3:434–40. [PubMed: 22653426]
112. Yates LA, et al. Structural and functional characterisation of the kindlin-1 pleckstrin homology domain. *J Biol Chem.* 2012; 287:43246–61. [PubMed: 23132860]
113. Yates LA, Fuzery AK, Bonet R, Campbell ID, Gilbert RJC. Biophysical Analysis of Kindlin-3 Reveals an Elongated Conformation and Maps Integrin Binding to the Membrane-distal beta-Subunit NPXY Motif. *J Biol Chem.* 2012; 287:37715–31. [PubMed: 22989875]
114. Moser M, Legate KR, Zent R, Fassler R. The tail of integrins, talin, and kindlins. *Science.* 2009; 324:895–9. [PubMed: 19443776]
115. Kahner BN, et al. Kindlins, integrin activation and the regulation of talin recruitment to alphaIIb beta3. *PLoS One.* 2012; 7:e34056. [PubMed: 22457811]
116. Bouaouina M, et al. A conserved lipid-binding loop in the kindlin FERM F1 domain is required for kindlin-mediated alphaIIb beta3 integrin coactivation. *J Biol Chem.* 2012; 287:6979–90. [PubMed: 22235127]
117. Honda S, et al. Integrin-linked kinase associated with integrin activation. *Blood.* 2009; 113:5304–13. [PubMed: 19299337]
118. Bandyopadhyay A, Rothschild G, Kim S, Calderwood DA, Raghavan S. Functional differences between kindlin-1 and kindlin-2 in keratinocytes. *J Cell Sci.* 2012; 125:2172–84. [PubMed: 22328497]
119. Bialkowska K, et al. The integrin co-activator Kindlin-3 is expressed and functional in a non-hematopoietic cell, the endothelial cell. *J Biol Chem.* 2010; 285:18640–9. [PubMed: 20378539]
120. Ussar S, et al. Loss of Kindlin-1 causes skin atrophy and lethal neonatal intestinal epithelial dysfunction. *PLoS Genet.* 2008; 4:e1000289. [PubMed: 19057668]
121. Roca-Cusachs P, Gauthier NC, Del Rio A, Sheetz MP. Clustering of alpha(5)beta(1) integrins determines adhesion strength whereas alpha(v)beta(3) and talin enable mechanotransduction. *Proc Natl Acad Sci USA.* 2009; 106:16245–50. [PubMed: 19805288]
122. del Rio A, et al. Stretching single talin rod molecules activates vinculin binding. *Science.* 2009; 323:638–41. Use of magnetic tweezers, total internal reflection fluorescence, and atomic force microscopy showed that physiologically relevant forces cause stretching of talin and result in exposure of cryptic vinculin-binding sites. [PubMed: 19179532]
123. Pasapera AM, Schneider IC, Rericha E, Schlaepfer DD, Waterman CM. Myosin II activity regulates vinculin recruitment to focal adhesions through FAK-mediated paxillin phosphorylation. *J Cell Biol.* 2010; 188:877–90. [PubMed: 20308429]
124. Margadant F, et al. Mechanotransduction in vivo by repeated talin stretch-relaxation events depends upon vinculin. *PLoS Biol.* 2011; 9:e1001223. [PubMed: 22205879]
125. Smith SJ, McCann RO. A C-Terminal Dimerization Motif Is Required for Focal Adhesion Targeting of Talin1 and the Interaction of the Talin1 ILWEQ Module with F-Actin. *Biochemistry.* 2007; 46:10886–98. [PubMed: 17722883]

126. Ellis SJ, Pines M, Fairchild MJ, Tanentzapf G. In vivo functional analysis reveals specific roles for the integrin-binding sites of talin. *J Cell Sci.* 2011; 124:1844–56. [PubMed: 21558413]
127. Parsons M, Messent AJ, Humphries JD, Deakin NO, Humphries MJ. Quantification of integrin receptor agonism by fluorescence lifetime imaging. *J Cell Sci.* 2008; 121:265–71. [PubMed: 18216331]
128. Kanchanawong P, et al. Nanoscale architecture of integrin-based cell adhesions. *Nature.* 2010; 468:580–4. [PubMed: 21107430]
129. Zhang F, Saha S, Kashina A. Arginylation-dependent regulation of a proteolytic product of talin is essential for cell-cell adhesion. *J Cell Biol.* 2012; 197:819–36. [PubMed: 22665520]
130. Debrand E, et al. Talin 2 is a large and complex gene encoding multiple transcripts and protein isoforms. *FEBS J.* 2009; 276:1610–28. [PubMed: 19220457]
131. Franco SJ, et al. Calpain-mediated proteolysis of talin regulates adhesion dynamics. *Nat Cell Biol.* 2004; 6:977–83. [PubMed: 15448700]
132. Huang C, et al. Talin phosphorylation by Cdk5 regulates Smurf1-mediated talin head ubiquitylation and cell migration. *Nat Cell Biol.* 2009; 11:624–30. [PubMed: 19363486]
133. Bridgewater RE, Norman JC, Caswell PT. Integrin trafficking at a glance. *J Cell Sci.* 2012; 125:3695–701. [PubMed: 23027580]
134. Albiges-Rizo C, Frachet P, Block MR. Down regulation of talin alters cell adhesion and the processing of the $\alpha 5 \beta 1$ integrin. *J Cell Sci.* 1995; 108:3317–3329. [PubMed: 7593292]
135. Martel V, et al. Talin controls the exit of the integrin $\alpha 5 \beta 1$ from an early compartment of the secretory pathway. *J Cell Sci.* 2000; 113:1951–1961. [PubMed: 10806106]
136. Priddle H, et al. Disruption of the talin gene compromises focal adhesion assembly in undifferentiated but not differentiated ES cells. *J Cell Biol.* 1998; 142:1121–1133. [PubMed: 9722622]
137. Liu J, et al. Talin1 regulates integrin turnover to promote embryonic epithelial morphogenesis. *Mol Cell Biol.* 2011; 31:3366–77. [PubMed: 21670148]
138. Margadant C, Kreft M, de Groot DJ, Norman JC, Sonnenberg A. Distinct Roles of Talin and Kindlin in Regulating Integrin $\alpha 5 \beta 1$ Function and Trafficking. *Curr Biol.* 2012; 22:1554–63. [PubMed: 22795696]
139. Bottcher RT, et al. Sorting nexin 17 prevents lysosomal degradation of $\beta 1$ integrins by binding to the $\beta 1$ -integrin tail. *Nat Cell Biol.* 2012; 14:584–92. [PubMed: 22561348]
140. Steinberg F, Heesom KJ, Bass MD, Cullen PJ. SNX17 protects integrins from degradation by sorting between lysosomal and recycling pathways. *J Cell Biol.* 2012; 197:219–30. [PubMed: 22492727]
141. Choi CK, Zareno J, Digman MA, Gratton E, Horwitz AR. Cross-correlated fluctuation analysis reveals phosphorylation-regulated paxillin-FAK complexes in nascent adhesions. *Biophys J.* 2011; 100:583–92. [PubMed: 21281572]
142. Zhao Y, et al. Regulation of cell adhesion and migration by Kindlin-3 cleavage by calpain. *J Biol Chem.* 2012; 287:40012–20. [PubMed: 23012377]
143. Bledzka K, et al. Tyrosine phosphorylation of integrin $\beta 3$ regulates kindlin-2 binding and integrin activation. *J Biol Chem.* 2010; 285:30370–4. [PubMed: 20702409]
144. Liu Y, Zhu Y, Ye S, Zhang R. Crystal structure of kindlin-2 PH domain reveals a conformational transition for its membrane anchoring and regulation of integrin activation. *Protein Cell.* 2012; 3:434–40. [PubMed: 22653426]
145. Qadota H, Moerman DG, Benian GM. A molecular mechanism for the requirement of PAT-4 (integrin-linked kinase (ILK)) for the localization of UNC-112 (Kindlin) to integrin adhesion sites. *J Biol Chem.* 2012; 287:28537–51. [PubMed: 22761445]
146. Debrand E, et al. Mice carrying a complete deletion of the talin2 coding sequence are viable and fertile. *Biochem Biophys Res Commun.* 2012; 426:190–5. [PubMed: 22925892]
147. Desiniotis A, Kyprianou N. Significance of talin in cancer progression and metastasis. *Int Rev Cell Mol Biol.* 2011; 289:117–47. [PubMed: 21749900]
148. Kato H, et al. The primacy of $\beta 1$ integrin activation in the metastatic cascade. *PLoS One.* 2012; 7:e46576. [PubMed: 23056350]

149. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell*. 2002; 110:673–687. [PubMed: 12297042]
150. Dong X, et al. $\alpha(V)\beta(3)$ integrin crystal structures and their functional implications. *Biochemistry*. 2012; 51:8814–28. [PubMed: 23106217]
151. Bouaouina M, et al. Zasp regulates integrin activation. *J Cell Sci*. 2012
152. Monkley SJ, et al. Disruption of the talin gene arrests mouse development at the gastrulation stage. *Dev Dynamics*. 2000; 219:560–574.
153. Wang Y, et al. Loss of PIP5K γ , unlike other PIP5KI isoforms, impairs the integrity of the membrane cytoskeleton in murine megakaryocytes. *J Clin Invest*. 2008; 118:812–9. [PubMed: 18188447]
154. Wernimont SA, et al. Contact-Dependent T Cell Activation and T Cell Stopping Require Talin1. *J Immunol*. 2011; 187:6256–6267. [PubMed: 22075696]
155. Manevich-Mendelson E, et al. Talin1 is required for integrin-dependent B lymphocyte homing to lymph nodes and the bone marrow but not for follicular B-cell maturation in the spleen. *Blood*. 2010; 116:5907–18. [PubMed: 20923969]
156. Lammermann T, et al. Rapid leukocyte migration by integrin-independent flowing and squeezing. *Nature*. 2008; 453:51–5. [PubMed: 18451854]
157. Conti FJ, et al. Progressive myopathy and defects in the maintenance of myotendinous junctions in mice that lack talin 1 in skeletal muscle. *Development*. 2008; 135:2043–53. [PubMed: 18434420]
158. Zou W, et al. Talin1 and Rap1 are Critical for Osteoclast Function. *Mol Cell Biol*. 2012; 33:830–844. [PubMed: 23230271]
159. Manso A, et al. Talin1 has unique expression versus talin 2 in the heart and modifies the hypertrophic response to pressure overload. *J Biol Chem*. 2013; 288:4252–4264. [PubMed: 23266827]
160. Conti FJ, Monkley SJ, Wood MR, Critchley DR, Muller U. Talin 1 and 2 are required for myoblast fusion, sarcomere assembly and the maintenance of myotendinous junctions. *Development*. 2009; 136:3597–606. [PubMed: 19793892]
161. Bouvard D, Pouwels J, De Franceschi N, Ivaska J. Integrin inactivators: balancing cellular functions in vitro and in vivo. *Nature Rev Mol Cell Biol*. 2013; 14:432–44.

Biographies

David Calderwood is an Associate Professor at Yale University School of Medicine, where his group studies integrin activation, signaling and the link to the cytoskeleton. He earned his Ph.D. in Martin Humphries' laboratory at the University of Manchester, U.K., investigating integrin–ligand interactions. In his postdoctoral work with Mark Ginsberg at The Scripps Research Institute, LA Jolla, CA, USA, he identified talin as a key regulator of integrin activation.

Iain Campbell is Emeritus Professor of Structural Biology at the University of Oxford. Trained as a physicist at St Andrews University, he had three main stages in his research career: first developing NMR methods for studying biological systems, second determining the structure of numerous recurring protein module structures, such as Epidermal Growth Factor and, lastly, focusing on structures and interactions associated with focal adhesions.

David Critchley is Emeritus Professor of Biochemistry at the University of Leicester, where he led a multidisciplinary team studying the structure and function of focal adhesion proteins, including talin, vinculin and α -actinin.

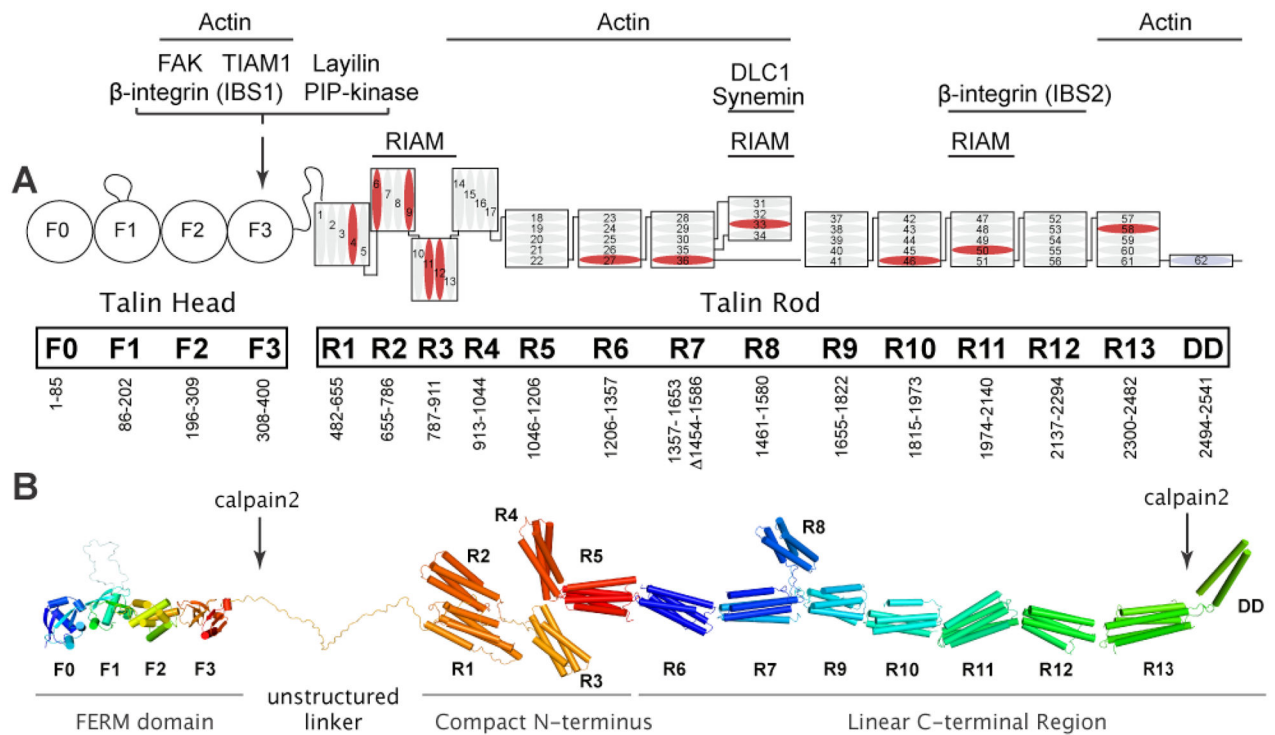


FIG 1. Domain organisation and structural model of full-length talin

A: The domain organisation of talin1³⁵. The N-terminal talin head, which is comprised of an atypical talin FERM domain containing F0, F1, F2, and F3 domains, is joined by an unstructured linker of ~80 residues to the flexible talin rod. The rod is made up of 62 α -helices (numbered blue cylinders) that are organised into thirteen 4- or 5-helix bundles (R1–R13), with a single helical dimerisation domain (DD) at the C-terminus. Domain boundaries and the interaction sites for talin-binding proteins are indicated (IBS; integrin binding site). Helices that bind vinculin are in blue. Talin2 is predicted to have the same domain structure.

B: Structural model of talin assembled from the crystal and NMR structures of the various domains. The position of the calpain2 cleavage sites are indicated. The R1 and R2 domains interact via an extensive hydrophobic interface³⁴, and a long common helix joins R11 and R12³⁰ (not shown). Otherwise, helical bundles are joined by short linkers (not shown since their structures were not determined). Because the N- and C-termini of the three 4-helix bundles (R2R3R4) are positioned at the same end of the bundle, this region will be more compact than the long succession of 5-helix bundles linked via their N- and C-termini.

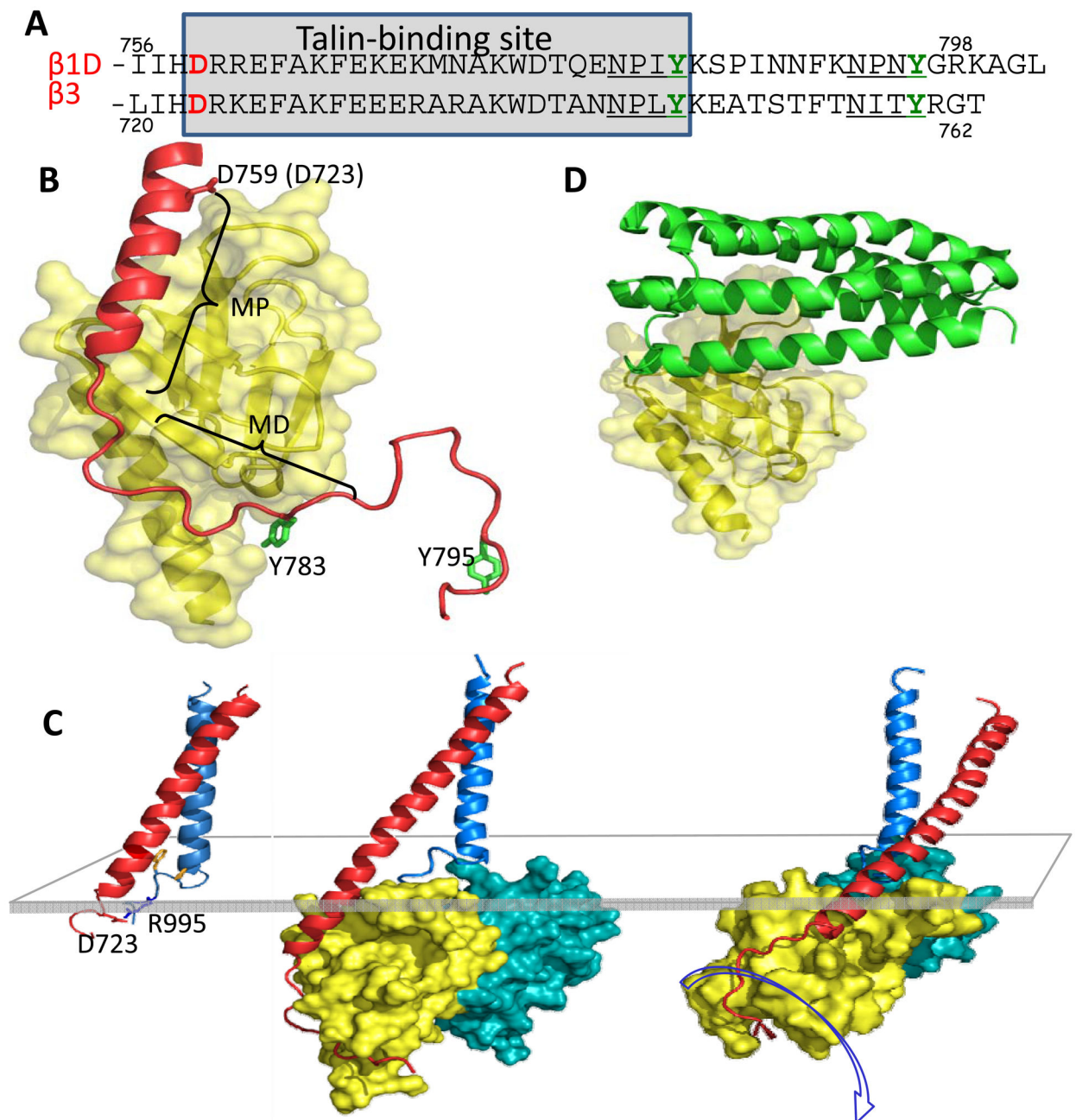


FIG 2. Mechanism of talin-mediated integrin activation

A: The sequences of two representative integrin β -tails, those of integrin $\beta 1D$ and integrin $\beta 3$, are shown. The two NPxY-like motifs are underlined, and the conserved Asp723 (numbered using the $\beta 3$ sequence) that forms a salt bridge with R995 in the αIIb tail, and leads to a low affinity state, is highlighted in red. Talin binds to the indicated region of β -tails via its F3 domain. **B:** The complex between talin2 F3 (yellow) and the $\beta 1D$ tail (red)³⁷ using $\beta 1D$ numbering to indicate key residues. The membrane-proximal (MP) and membrane-distal (MD) regions of the complex are indicated. The first NPxY-like region in the $\beta 1D$ tail is indicated by Y783. The second NPxY-like region in the $\beta 1D$ tail was not seen

in the X-ray structure, but is modelled here (indicated by Y795) to show that it is very exposed, and has the potential to bind kindlins. **C:** The NMR structure of the transmembrane segments of the α IIB β 3 integrin is shown on the left⁵⁰. This NMR structure and the structure of the talin2 F2F3 domains bound to the β 1D-integrin tail³⁷ were used to form the composite structure in the centre. The structure on the right was obtained after 100ns of molecular dynamics simulation in the presence of a membrane bilayer. Formation of favorable electrostatic interactions between talin and the membrane causes rotation of the talin-integrin β tail complex (centre and right structures), increasing the tilt of the β TM helix. This leads to separation of the α and β TM regions⁵³, and hence to integrin activation. The translucent rectangle indicates the position of the cytoplasmic face of the membrane bilayer. **D:** The structure of the autoinhibitory complex between the talin1 F3 domain (yellow) and the R9 domain of the talin1 rod (green)⁶⁵. The talin1 F3 domain is shown in approximately the same orientation as in B. Note how binding of the talin rod to the talin F3 domain occludes the F3 binding site for the membrane-proximal portion of the integrin β tail, effectively preventing integrin binding and activation.

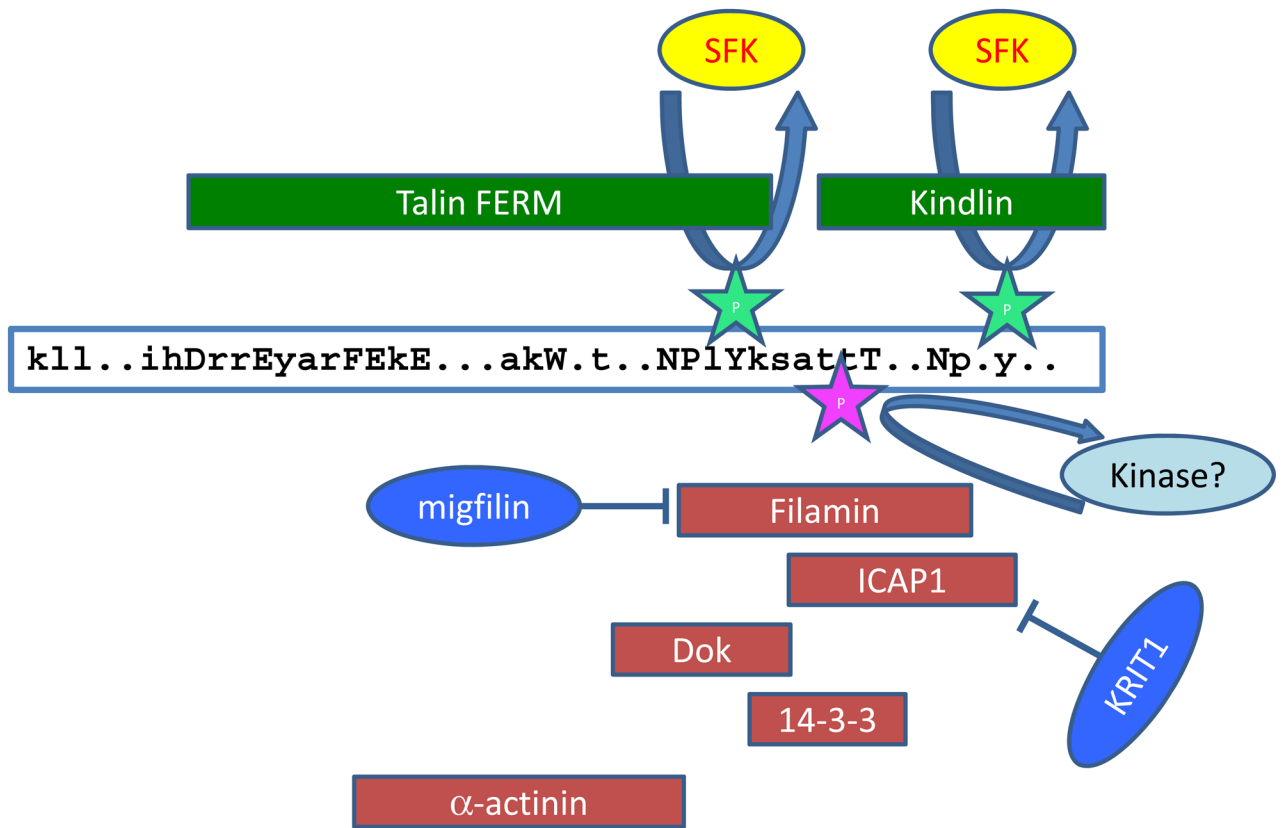


FIG 3. Regulators of talin and kindlin binding to integrins

A schematic of the integrin β tail showing conserved residues: uppercase for near-invariant residues, lowercase for conserved residues with all other residues marked by a dot. Binding sites for integrin activators (green) and inhibitors of integrin activation (red), based on structural and biophysical studies^{87,93,95,96,113}, are indicated by the box that encompasses the protein in question. Talin and kindlin can bind the integrin β tail simultaneously¹⁰⁶, but binding sites for the proteins that inhibit their binding, and thus inhibit integrin activation, overlap, suggesting that only one can bind at a time. Src-family kinase (SFK)-mediated tyrosine phosphorylation of the membrane-proximal or membrane-distal NPxY motif can inhibit talin and kindlin binding, respectively, and enhance binding of the inhibitor Dok^{184,85,143}. Threonine phosphorylation at residues between the NPxY-motifs has the potential to activate or inhibit integrin activation — it suppresses binding of the integrin inhibitor filamin^{87,93} and generates a binding site for 14-3-3 proteins that inhibit integrin activation⁹³. α -actinin can both positively and negatively regulate integrin–talin interactions, depending on the β tail in question⁹⁶. Binding of other proteins, such as migfilin or KRIT1^{89,90,95}, to the integrin inhibitors filamin and ICAP1, respectively, prevents these inhibitors from binding to integrins and hence favours integrin activation.

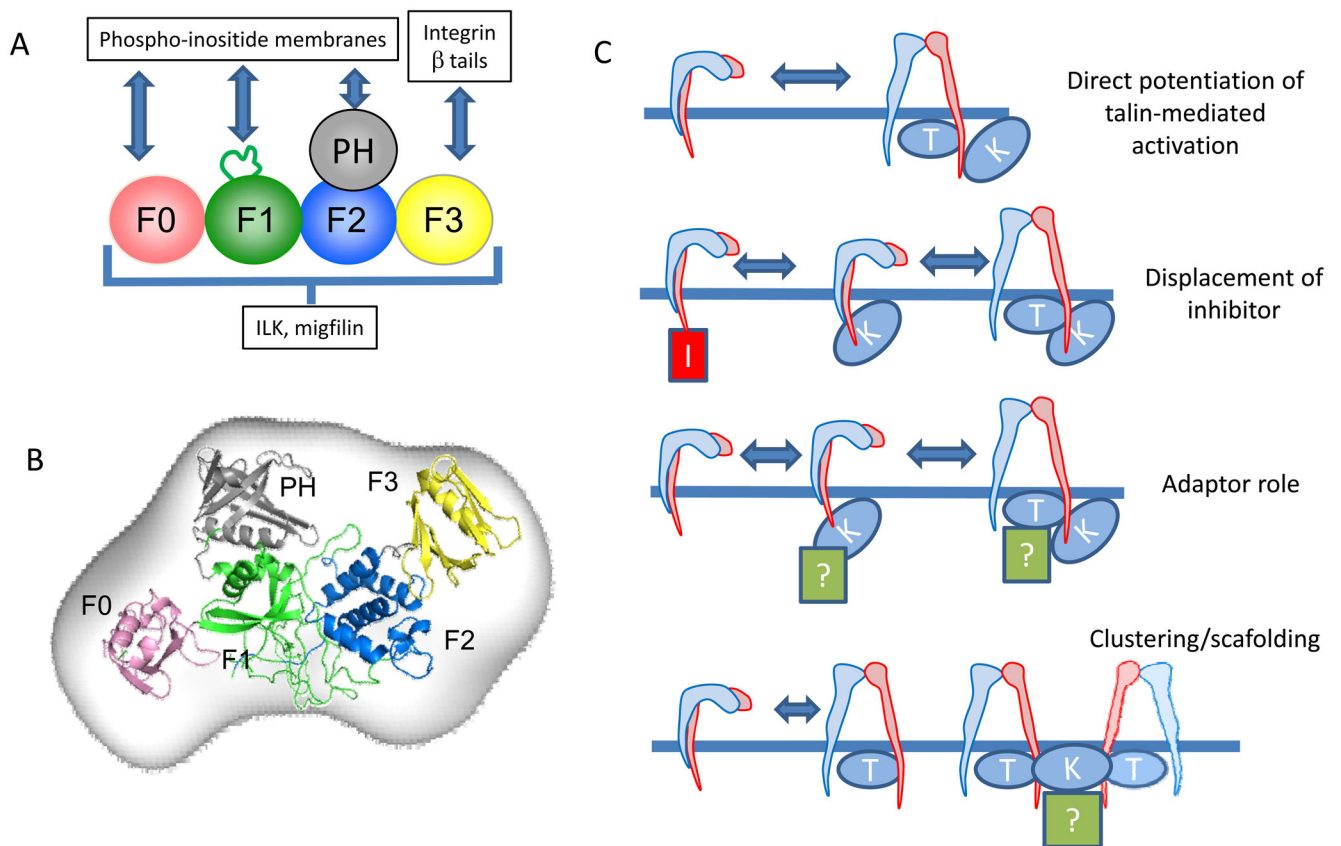


FIG 4. Kindlin: an integrin co-activator

A: A schematic representation of kindlin domain organization, showing where kindlin interaction partners bind. Kindlin is predicted to fold as an atypical FERM domain composed of 5 subdomains (F0–F3 plus a PH domain). Similar to the talin head (Fig 1)²⁶, kindlin is thought to form an extended structure¹¹³. β Integrin tails bind to the F3 subdomain while phospho-inositide membrane-binding sites have been identified in the kindlin F0 and PH domains, and in the large unstructured loop in F1^{109,110,112,116}. Binding sites for ILK and migfilin have yet to be definitively mapped. **B:** Possible orientation of kindlin domains based on x-ray scattering¹¹³, the kindlin-1 PH domain crystal structure¹¹², and NMR structures of the kindlin-1 F0 domain¹⁰⁸ and the talin FERM domain²⁶. Domains are coloured as in part A. **C:** Models for cooperation between talin and kindlin during integrin activation. Binding of kindlin to the β integrin tail may directly potentiate talin-mediated integrin activation (top panel), perhaps by binding both the integrin β tail and the membrane to cause optimal exposure of the talin-binding site in the β tail. Alternatively, kindlin binding to the integrin may displace inhibitors, facilitating talin binding and activation (upper middle panel). In addition, kindlin may recruit other activating or adaptor proteins that cooperate with talin to activate integrins (lower middle panel). Finally, Kindlin may directly or indirectly (via another kindlin binding protein, labelled with a question mark) induce clustering of talin-activated integrins to increase avidity (bottom panel). Note: The Z-band alternatively spliced PDZ-motif containing protein (Zasp) also co-operates with talin to

activate $\alpha 5 \beta 1$ integrin¹⁵¹, although It is unknown whether kindlins have any role in this process.

Fig. 5A

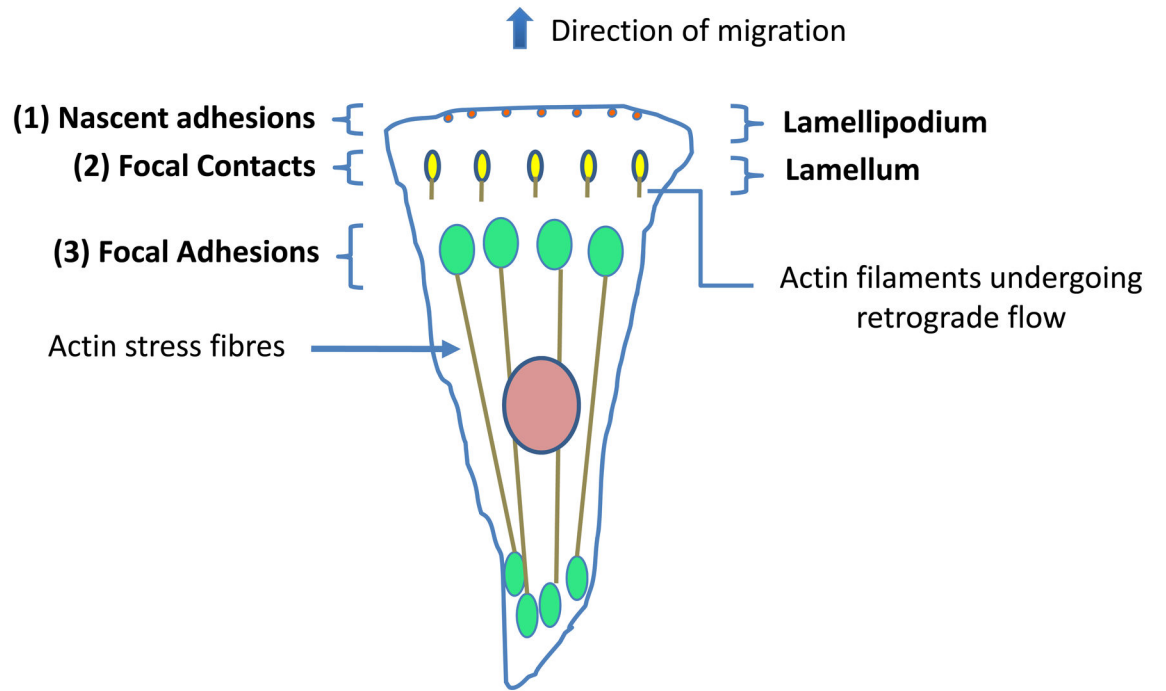


Fig. 5B

(1) Talin Recruitment to lamellipodium and assembly of nascent adhesions

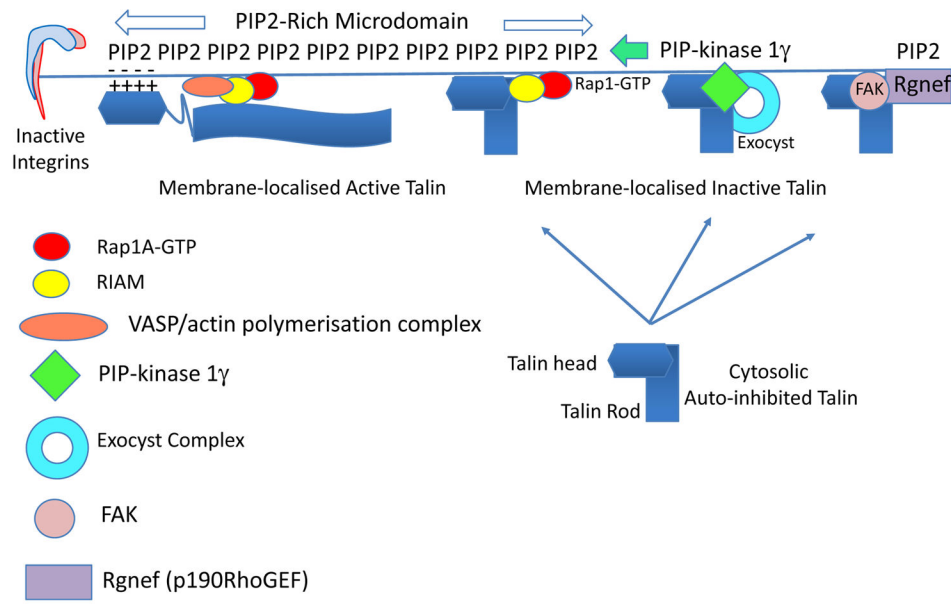


Fig. 5B

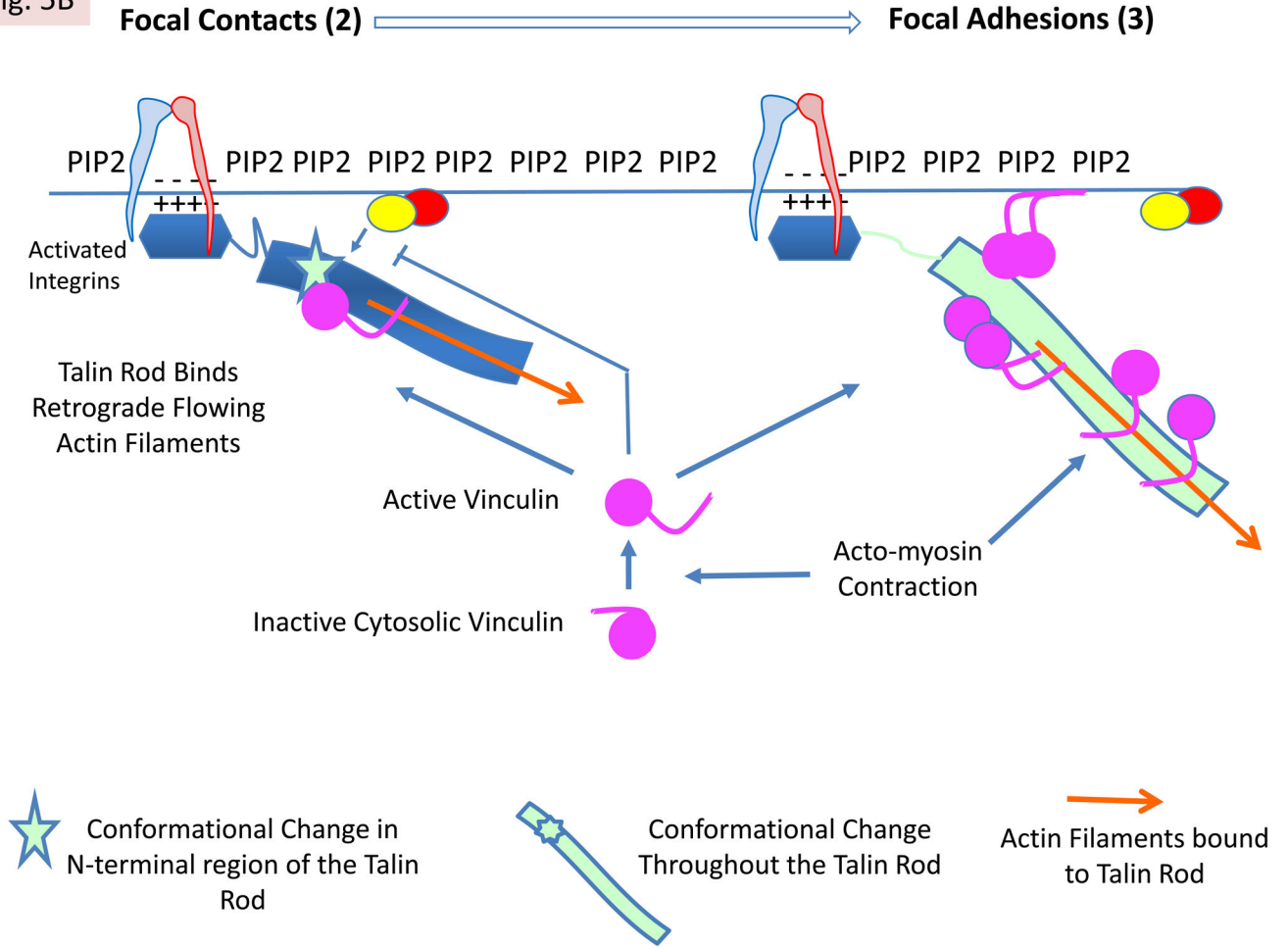


FIG 5. Talin changes partners during adhesion assembly and maturation

A: Mechanisms of talin recruitment to the lamellipodium. The model envisages that auto-inhibited talin in the cytosol can be recruited to PIP2-rich microdomains in the plasma membrane via several mechanisms. First, by binding RIAM, which is complexed to membrane localised Rap1A-GTP^{44,45,74}, via the N-terminal region of the talin rod³⁵. Second, by exocyst complexes⁷⁸ that contain integrins and PIP-kinase type1 γ , which creates the PIP2-rich microdomains. Third, by FAK⁴⁰ complexed to Rgef⁸⁰, a p190RRhoGEF with a PH domain that binds phosphoinositides. Both PIP2 and RIAM binding may contribute to talin activation. Positively charged residues on one surface of the talin head are then envisaged to interact with PIP2²⁶ (see Box 1). Note that only one subunit of talin is shown for simplicity. **B:** Integrin activation following talin activation. **Ba** PIP2 binding to the talin head increases its affinity for β -integrin tails⁵⁵, leading to integrin binding and activation. This triggers the assembly of nascent adhesions. RIAM also drives membrane protrusion⁷⁴, probably by binding VASP, which recruits the actin polymerisation machinery. **Bb** The talin rod then binds F-actin undergoing retrograde flow, and the force exerted on talin¹²⁴ is envisaged to alter the conformation of the N-terminal part of the talin rod. This disrupts the RIAM binding site and increases vinculin binding³⁵, which reinforces the connection of

talin to F-actin and causes dynamic focal contacts to form. The vinculin Vd1 domain binds talin, while its C-terminal tail binds F-actin; it may also cross-link talin to PIP2 in the membrane²⁴. **Bc** Further increases in force exerted on talin by acto-myosin contraction induce more extensive conformational changes in both the talin rod and the unstructured linker between the head and rod. This enhances vinculin binding and promotes the maturation of dynamic focal contacts into more stable focal adhesions that are associated with actin stress fibres. Note that vinculin exists in an auto-inhibited cytoplasmic form and the mechanisms by which it is activated have yet to be defined²⁴, although force exerted by acto-myosin contraction is involved⁷³. A schematic diagram showing the relative positions of nascent adhesions, focal contacts and focal adhesions in a migrating cell, and linking them to the relevant state of integrin activation, is shown.

TABLE 1

Summary of Tln1 and Tln2 gene deletion experiments

Genetic modification	Phenotypic effects	References
<i>Tln1</i>		
Tln1 knockout	Embryonic Lethal E8.5 due to gastrulation defects.	152
Tln1 ^{fl/fl} ; Tie2-Cre (Endothelial cells)	Lethal by E10.5 due to haemorrhage. Defects in angiogenesis and endothelial cell spreading <i>in vivo</i> . Development of heart and other tissues apparently unaffected. Thus, talin1 is not essential for the later stages of development in most tissues.	12
Tln1 ^{fl/fl} ; CreER Tamoxifen injected into pregnant mothers at E8.5, E9.5 or E10.5	Angiogenesis defects and bleeding within 48hrs; death after 72hrs. Development of heart and other tissues apparently unaffected.	12
Tln1 ^{fl/fl} ; PF4-Cre (Megakaryocytes)	Defects in membrane tethering to the cytoskeleton; membrane blebbing. Phenocopied by knockout of the PIP-kinase type1γ isoform.	153
Tln1 ^{fl/fl} ; PF4-Cre (Platelets)	Spontaneous haemorrhaging. Prolonged bleeding times. Defective αIIbβ3 and α2β1 activation and platelet adhesion.	57,58
Tln1 L325R knockin (Platelets)	Defective agonist-induced integrin activation and fibrin clot retraction.	59
Tln1 ^{fl/fl} ; CD4-Cre (T-lymphocytes)	Talin1 is required to stabilise the immune synapse and to support T-cell stopping and contact-dependent cell proliferation.	154
Tln1 ^{fl/fl} ; CD19-Cre (B-lymphocytes)	Talin1 is required for integrin-dependent B-cell homing to lymph nodes, but is not required for follicular B-cell maturation in the spleen.	155
Tln1 ^{fl/fl} ; Mx1-Cre (Leukocytes)	Dendritic cell (DC) migration in 3D is not integrin or talin1 dependent. However, DC extravasation from the blood, which involves adhesion to the endothelium, is integrin and talin dependent.	156
Tln1 ^{fl/fl} ; Human skeletal α-actin-Cre (Skeletal muscle)	Myotendinous junction stability defect. Mild muscular dystrophy and reduced ability of muscle to generate force.	157
Tln1 ^{fl/fl} ; Cathepsin K-Cre (Osteoclasts)	Impaired M-CSF-stimulated integrin activation, reduced adhesion and migration on ECM. Arrested osteoclast maturation into mature resorptive cells leading to increased bone mass.	158
Tln1 ^{fl/fl} ; α-myosin heavy chain-Cre (Heart)	Talin2 is the predominant isoform in cardiomyocytes and talin1 is not required for heart development or basal function. Deletion of talin1 attenuates the hypertrophic response of heart to stress.	159
<i>Tln2</i>		
Tln2 gene traps	No true talin2 null alleles and no phenotype.	130
Tln2 knockout Complete gene deletion	Mice are viable and fertile although they are difficult to breed. Mildly dystrophic phenotype slightly more severe than muscle-specific Tln1 knockout.	146
Tln2 exon1 deletion	Ablates talin2 expression in skeletal muscle but substantial expression of talin2 in other tissues. Myotendinous junction defects. Mildly dystrophic phenotype slightly more severe than muscle-specific Tln1 knockout.	160
<i>Tln1 and Tln2</i>		
Tln1 ^{fl/fl} ; Human skeletal α-actin-Cre; Tln2 exon1 deletion	Deletion of Tln1 and Tln2 from skeletal muscle results in defects in myoblast fusion and sarcomere assembly. Myoblasts still express active β1-integrins, but show defects in coupling integrins to cytoskeletal actin.	160

* The system or cell type in which the gene was modified is shown in brackets.

** Tln1^{fl/fl} indicates that both copies of the Tln1 gene contain a pair of LoxP. Crossing these mice with mice expressing Cre-recombinase from a tissue-specific promoter allows for deletion of the Tln1 gene in selected tissues. Cre-ER is a ubiquitously expressed form of Cre-recombinase that can be activated by injecting tamoxifen into the animal.

*** The phenotypes of mice lacking the various integrin subunits, and integrin activators (including kindlins) and inhibitors, is summarised elsewhere¹⁶¹.