The Tail of Integrins, Talin, and Kindlins

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Integrins are transmembrane cell–adhesion molecules that carry signals from the outside to the inside of the cell and vice versa. Like other cell surface receptors, integrins signal in response to ligand binding; however, events within the cell can also regulate the affinity of integrins for ligands. This feature is important in physiological situations such as those in blood, in which cells are always in close proximity to their ligands, yet cell-ligand interactions occur only after integrin activation in response to specific external cues. This review focuses on the mechanisms whereby two key proteins, talin and the kindlins, regulate integrin activation by binding the tails of integrin-β subunits.

Integrins are members of a large family of functionally conserved cell-adhesion receptors. They have a critical role in anchoring cells to extracellular matrices and alter cell function by activating intracellular signaling pathways after ligand binding ("outside-in" signaling). Integrins can shift between high- and low-affinity conformations for ligand binding ("inside-out" signaling). This property of integrins is regulated by external cues that are transduced intracellularly and ultimately result in the direct binding of regulatory proteins to the short cytoplasmic domains of integrins. A shift from a low- to a high-affinity state is termed "integrin activation" (*1*, *2*).

Regulation of the affinity with which integrins bind ligands is fundamental for various cellular functions. For example, during development migrating cells require activated integrins at their leading edge to attach newly protruded plasma membrane to the surface on which they are moving and inactivate integrins at their rear. In response to injury, the fibrinogen receptors on platelets, integrin αIIbβ3, are swiftly activated to mediate platelet adhesion and aggregation in order to stop bleeding. Because αIIbβ3 integrins are constantly exposed to fibrinogen, it is vital to keep them inactive so as to prevent pathological platelet aggregation and thrombus formation. Similarly, during inflammation leukocytes require integrin activation in order to adhere to and migrate across the endothelium on their way to affected tissues. Abnormal function of highly modulatable integrins or mutations in integrin-binding proteins required for integrin activation can result in aberrant development or diseases such as bleeding disorders, leukocyte-adhesion deficiencies, and skin blistering. In this review, we discuss recent structural and biochemical studies and data from genetic manipulations in animals that shed new light on how two integrin tail–binding proteins, talin and kindlins, regulate integrin activation.

Integrin Structure

Integrins are formed by noncovalently bound α and β subunits. In mammals, 18 α and 8 β subunits combine in a restricted manner to form 24 specific dimers, which exhibit different ligand-binding properties. Integrin subunits have large extracellular domains (approximately 800 amino acids) that contribute to ligand binding, single transmembrane (TM) domains (approximately 20 amino acids), and short cytoplasmic tails (13 to 70 amino acids, except that of β4). All three domains are required to regulate the affinity of integrins. β2 and β3 integrins can change affinity on a subsecond time scale, and many of the paradigms of integrin structure and function were deduced from studies of these integrins; however, it is not clear whether they can be generalized to all integrins (*1*).

The extracellular domain of the heterodimer consists of a ligand-binding head domain standing on two long legs (Fig. 1A). α integrin subunits contain a seven-bladed β-propeller domain that forms the head, a thigh domain, and calf-1 and calf-2 domains. Half of the α subunits contain an I domain (also referred to as a von Willebrand factor A domain), which when present is nearly always the ligand-binding site. The I domain possesses a conserved metal ion–dependent adhesion site (MIDAS), which binds divalent cations required for ligand binding by integrins. The β subunit is composed of a hybrid domain that connects to the βI domain, which is

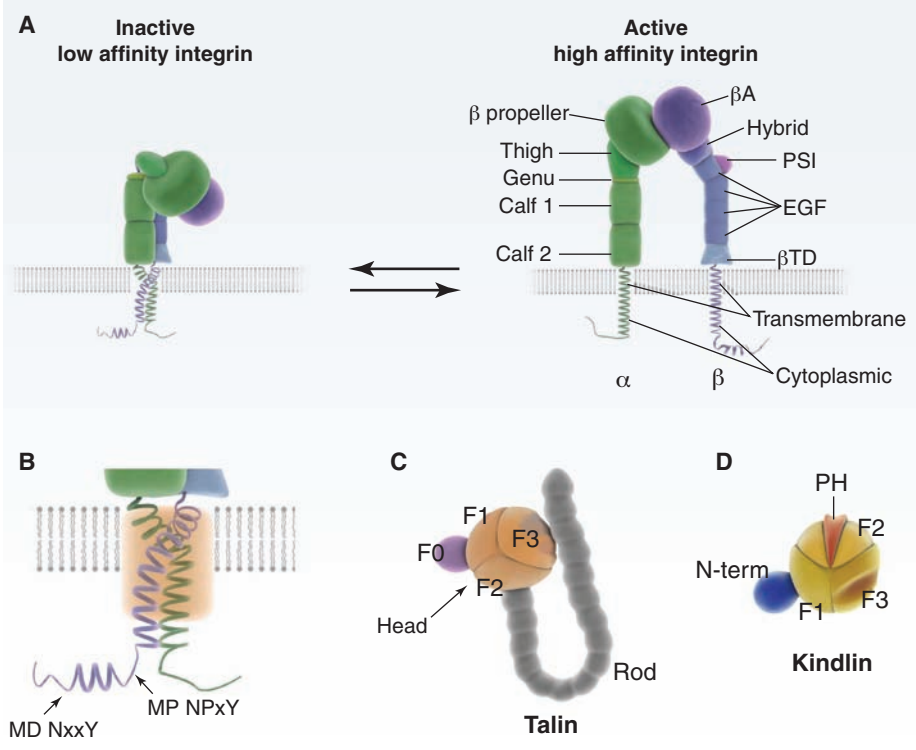

Fig. 1. (**A**) Integrin architecture and schematic representation of integrin activation. Specific contacts between the ectodomains, the TM, and cytoplasmic domains keep the integrin in its bent conformation. Separation of the integrin legs, TM, and cytoplasmic domains occurs during integrin activation, resulting in an extended integrin conformation. The α subunit is shown in green and the β subunit in violet. (**B**) A closer look at the interacting site (orange rectangle) between the TM and membrane proximal cytoplasmic domains of the α and β subunits. The membrane proximal (MP) and distal (MD) NPxY/NxxY motifs within the β tail are indicated. (**C** and **D**) Schematic drawings of the integrin-activating proteins talin (C) and kindlin (D). The FERM domains are depicted as balls subdivided into three subdomains, F1 to F3. Kindlins contain a PH domain inserted into the F2 subdomain. Domain sizes are not to scale, and talin is shown as a monomer for simplicity.

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analogous to the I domain of the α subunit, a PSI (plexin/semaphoring/integrin) domain, four epidermal growth factor (EGF) domains, and a membrane proximal β tail domain (βTD). In integrins without an I domain, ligands bind to a crevice between the αβ subunit interface, where they interact with a metal ion–occupied MIDAS within the β subunit and the propeller domain of the α subunit.

The structure of the short TM domains is poorly defined because of the lack of high-resolution structures of heterodimeric TM domains in their proper context, and only the structures of the β3 and the αIIb subunits are solved in their entirety (*3*–*5*). The β3 TM domain is a 30-residue linear α helix that is longer than the width of a typical lipid bilayer, which implies a pronounced helix tilt within the plasma membrane (*5*). The αIIb TM domain is a 24-residue α helix followed by a backbone reversal and does not exhibit a helix tilt (*4*). This unusual motif is highly conserved in the 18 human integrin α subunits and probably has an important role in the transition from low- to high-affinity states.

A high degree of similarity is found in the short α and β cytoplasmic tails, especially in the membrane proximal region where the GFFKR and HDR(R/K)E sequences are conserved in the α and β subunits, respectively (*6*). Nuclear magnetic resonance (NMR) studies that used integrin-derived αIIβ3 polypeptides proposed that integrins interact with each other through hydrophobic and electrostatic interactions and a salt bridge between the R residue within the GFKKR motif and the D residue within the HDRRE motif (*7*, *8*). However, these interactions were not seen by others, suggesting that tail interactions are very weak at best (*9*). Almost all β tails have two well-defined motifs that are part of a canonical recognition sequence for phosphotyrosine-binding (PTB) domains (*10*), consisting of a membrane proximal NPxY (where x represents any amino acid) motif and a membrane distal NxxY motif (Fig. 1B). These NxxY motifs are binding sites for multiple integrin-binding proteins, including talin and the kindlins.

Integrins Can Exist in Multiple Affinity States for Ligands

Integrins exist in low-, intermediate-, and high-affinity states. On the basis of structural studies, it is thought that integrins are in a low-affinity state when their extracellular domains are bent and in a high-affinity state when those are extended (Fig. 1A). The exact changes that occur in the head domain when integrins move to the high-affinity state are still unclear. Two models have been proposed: The "switchblade" model (*11*) predicts that only extended integrins will bind ligand, and the "deadbolt" model (*12*) suggests that integrin extension occurs only after ligand binding has taken place. In both models, conformational changes within the head domain facilitate ligand binding (*11*, *13*).

The TM domains have a key role in integrin activation. Inactive integrins are proposed to have a coiled-coil interaction between canonical GxxxG dimerization motifs within the TM domains that regulates integrin subunit packing (*14*). Separation of integrin TM domains has been suggested to be a requirement for integrins to adopt the high-affinity state. There are two possible ways by which TM domain interactions can be disrupted. The number of residues of the β integrin TM domain buried within the lipid bilayer may be shortened upon activation, leading to a straightening of the TM domain within the membrane. Alternatively, pistonlike movement of integrin TM domains might cause the disruption of interactions within the membrane by changing the register of TM-domain side chains.

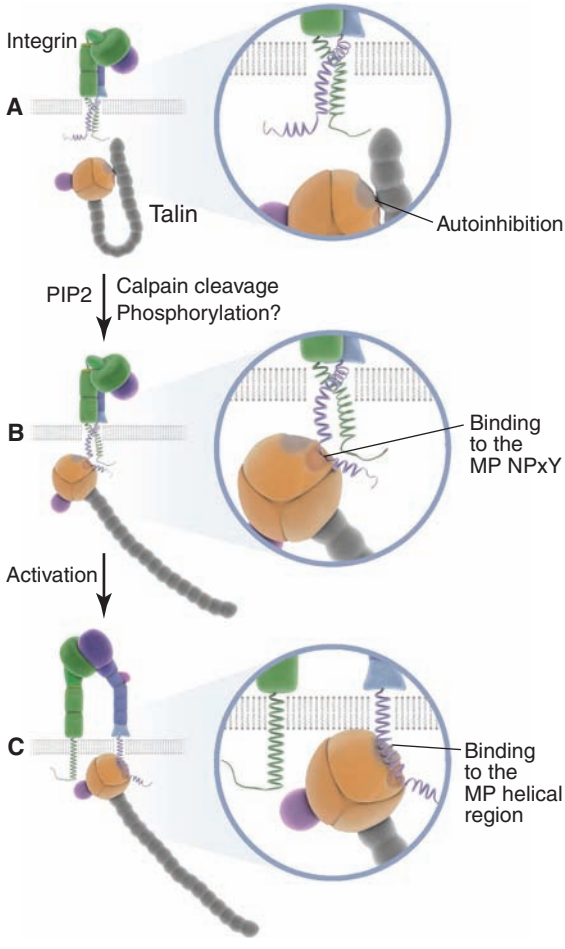

Fig. 2. Integrin activation by talin. (**A**) Cytoplasmic talin becomes activated upon binding phosphatidylinositol 4,5-bisphosphate (PIP_2), which abrogates an autoinhibitory interaction with the rod domain. In addition, calpain cleavage and phosphorylation events may activate talin. (**B**) The talin F3 subdomain engages the membrane proximal NPxY motif in β integrin tails. (**C**) In a second step, a talin-specific loop structure within the F3 subdomain interacts with the membrane-proximal α helix of the β integrin cytoplasmic tail, thereby disrupting the connection between cytoplasmic tails. Pulling forces at the β tail probably reorient the β integrin TM domain, thereby disrupting the packing of the α/β TM domains.

The role of integrin cytoplasmic tails in regulating integrin affinity, especially with respect to the binding of proteins such as talin and kindlins to the highly conserved NxxY motifs, has been extensively examined in the rapidly activated β2 and β3 integrins. Although mutational analysis suggests that the salt bridge is important for maintaining these integrins in a low-affinity state (*15*), this might not be the case for all integrins, especially the β1 integrins (*16*). Despite the controversial role of the salt bridge in maintaining integrins in a low-affinity state, high integrin affinity is thought to be associated with separation of the α and β cytoplasmic tails. Many proteins bind directly to integrin tails, yet only talin and kindlins can regulate integrin affinity. The role of these NxxY motifs–binding proteins in integrin activation and function will now be discussed in detail.

Talin Is an Essential Mediator of Integrin Activation

Talin is a component of adhesion plaques and interacts with integrin cytoplasmic tails (*17*). Its role in altering integrin function was originally demonstrated by its ability to induce a shift in the affinity of a normally inactive integrin expressed in chinese hamster ovary (CHO) cells (*18*, *19*). Knockout and knockdown experiments subsequently reinforced the notion that talin is a key regulator of integrin affinity for ligand, and many mutational and structural studies have described the mechanism by which it accomplishes this task. Talin orthologs have been identified in all multicellular eukaryotes studied; vertebrates encode two talin isoforms, termed talin1 and talin2, whereas lower eukaryotes encode only a single talin isoform corresponding to talin1 (*20*, *21*).

Talins are ~270-kD proteins consisting of an N-terminal 47-kD head domain and a ~220-kD C-terminal flexible rod domain (Fig. 1C). The talin head consists of a FERM (4.1, ezrin, radixin, moesin) domain composed of 3 subdomains (F1, F2, and F3) and an F0 subdomain with no homology to known domains. The F3 subdomain resembles a PTB domain and binds integrin tails, phosphatidylinositol 4-phosphate 5-kinase γ (PIPKIγ), and the hyaluronan receptor layilin (*22*–*25*). The talin rod domain is composed of a series of helical bundles that contain multiple binding sites for the F actin–binding protein vinculin and a second integrin-binding site (*26*). The C

terminus contains a THATCH (talin/HIP1R/Sla2p actin tethering C-terminal homology) domain (also known as an I/LWEQ motif) that mediates dimerization and provides a direct linkage between talin and F actin (*27*, *28*).

The integrin-binding site for the talin head was mapped to the membrane-proximal NPxY motif, a common binding motif for PTB domain–containing proteins (*18*, *19*, *29*). Mutations within the NPxY motif of both β1 (*30*) and β3 (*31*) integrins, as well as mutations in the talin PTB domain (*32*), abolish talin binding and decrease integrin affinity. Insights into how talin increases integrin affinity came from NMR experiments showing that the talin head effectively outcompetes the αIIb tail for binding to the β3 tail (*7*). Fluorescence energy transfer (FRET) experiments in cells confirmed that the talin head induces separation of the integrin tails (in this case αLβ2), which is concomitant with increased basal integrin ligand binding (*33*). Cells depleted of talin1 by small interfering RNA (siRNA) cannot respond to common activation stimuli (*31*). Furthermore, genetic experiments in *Caenorhabditis elegans* (*34*), *Drosophila* (*35*), and mice (*36*–*38*) demonstrated that talin1 ablation universally leads to integrin-adhesion defects. These experiments led to the belief that talin was both necessary and sufficient to activate integrins. However, the claims of sufficiency were later shown to be an oversimplification.

A critical question is why talin can modulate integrin affinity, whereas other PTB domain–containing proteins that bind the same NPxY motif, such as Dok1 (*18*), tensin (*39*), and Numb (*18*), cannot. Mutational and structural studies suggest that this might be because the talin head has an additional binding site on the β integrin tail, in the membrane proximal region where the α and β integrin tails interact (*8*, *40*), whereas Dok1 binds only to the region surrounding the NPxY motif (*41*). Crystallographic data has clarified that talin-dependent integrin activation involves binding of the talin F3 subdomain to the β3 integrin tail at two locations in order to induce the displacement of the α integrin tail and facilitate tail separation (*32*). The talin F3 subdomain contains an extra loop of amino acids that binds to membrane-proximal sequences in the β3 integrin tail. Thus, it was proposed that talin first encounters the β integrin tail by binding the NPxY motif through its PTB domain, and the loop sequence subsequently interacts with membrane proximal sequences within the β tail to displace the α integrin tail and separate the TM domains.

Although the talin head increases integrin affinity, full-length talin is required to cluster integrins into focal adhesions (FA) [reviewed in (*2*)], which are hubs that relay signals from integrins to different cellular compartments. Cells that do not express talin are unable to undergo sustained spreading, which indicates an adhesion defect (*42*). Expressing the talin1 head in these cells partially restored the spreading defect, but FAs were still absent, demonstrating that the clustering of integrins into larger adhesion structures depends on both the head and rod of talin. These studies also showed that talin is essential for coupling the actin cytoskeleton to adhesion structures and established talin as a key adaptor linking the cytoskeleton to the extracellular matrix (*42*). Mutational analysis of talin indicates that a functional dimerization motif is both necessary and sufficient to localize talin to focal adhesions (*28*, *43*). Because talin contains two β integrin–binding sites, one within the FERM and the other within the rod domain, the talin homodimer has up to four integrin-binding sites, which may enable talin to act as an integrin crosslinker in order to promote clustering. Consistent with this hypothesis, cleavage of the talin head from the rod domain by the protease calpain induces focal-adhesion disassembly (*44*).

Because integrin activation has to be strictly controlled, talin-integrin binding is tightly regulated (Fig. 2). NMR studies revealed an autoinhibitory interaction between the talin C terminus and the PTB domain that blocks the integrin-binding pocket (*45*). Therefore, when talin function is not required it may be maintained in an autoinhibited state. How talin is activated is not clear, but it probably involves binding to the lipid second messenger phosphotidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] because this lipid elicits a conformational change that disrupts the autoinhibitory interaction and enhances integrin-talin binding (*45*, *46*). Although phosphoinositide binding can enhance the affinity of many PTB domains for their substrates (*29*), this does not hold true for the isolated talin head (*46*). Talin binds to PIPKIγ and directs it to focal adhesions (*22*, *24*); thus, a feed-forward loop may exist to enhance talin recruitment to sites of adhesion formation.

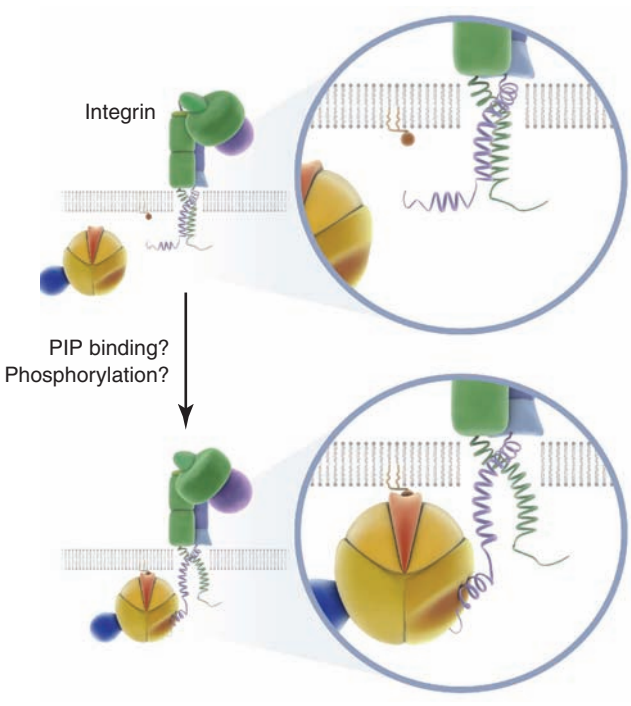

Fig. 3. Hypothetical model of kindlin recruitment and binding to the β integrin cytoplasmic tail. Phosphoinositide binding to the PH domain and/or phosphorylation might activate kindlin proteins and recruit them to the membrane, where they bind via their F3 subdomains to the membrane-distal NxxY motif of β integrin cytoplasmic tails.

In hematopoetic cells, the guanosine triphosphatase (GTPase) Rap1 has been implicated in talin recruitment to integrin tails. Expression of constitutively active Rap1A in T cells increases integrin activation (*47*), and the deletion of Rap1B in platelets decreases αIIbβ3 activation (*48*). Association of the Rap1 effector Rap1-GTP–interacting adaptor molecule (RIAM) resulting in a Rap1-RIAM-talin ternary complex at the integrin tail has been shown to be required for this interaction (*49*, *50*). A similar GTPase-mediated activation mechanism might also occur in nonhematopoetic cells, because a direct interaction between talin and lamellipodin, a member of the MRL (Mig-10/RIAM/Lamellipodin) family of adaptor proteins, also results in integrin activation (*51*).

Talin-integrin interactions are also controlled through phosphorylation of the β integrin tail. The Tyr within the β1 and β3 integrin NPxY motif can be phosphorylated by src family kinases (*52*, *53*), and when mutated to Phe it reverses the integrin-dependent spreading and migration defects in viral-Rous sarcoma oncogene (v-src)–transformed fibroblasts (*53*). The interaction between talin and β integrin tails is regulated by a phosphorylation switch mechanism. Structural analysis showed that the talin PTB–integrin NPxY interaction occurs through acidic and hydrophobic interactions (*23*) and cannot accommodate the introduction of a phosphate group. Accordingly, the affinity of the talin F3 subdomain for a phosphorylated β3 tail peptide is reduced compared with that of the unphosphorylated peptide (*41*). Therefore, phosphorylation could inhibit integrin activation by maintaining an inhibitory complex on inactive integrin tails or by blocking talin binding directly.

Mutations and truncations of the β3 integrin tail C terminal to the talin-binding site decrease integrin affinity for ligands (*54*–*57*), which raises the possibility that additional factors also

alter integrin affinity status. Indeed, recent work shows that talin is not the only master regulator of integrin activation and that the kindlin family of proteins, which bind to this region of β1, β2, and β3 integrins, are as important as talin in mediating this function (*58*–*62*).

Kindlins as Regulators of Integrin Activation

Kindlins belong to a family of evolutionarily conserved FERM domain–containing proteins named after the gene mutated in Kindler syndrome, a rare skin blistering disease. There are three kindlin family members in mammals: kindlin-1 [Unc-112 Related Protein 1 (URP1)], kindlin-2 (Mig2), and kindlin-3 (URP2) (*63*). Kindlin-1, which is predominantly expressed in epithelial cells, is found in tissues such as skin, intestine, and kidney; kindlin-2 is expressed in most tissues, with highest amounts in skeletal and smooth muscle cells; and kindlin-3 expression is restricted to cells of hematopoietic origin (*63*–*65*). All three proteins localize to integrin-dependent adhesion sites; kindlin-1 and -2 localize to focal adhesions, and kindlin-3 localizes to podosomes, which are integrin-dependent adhesion sites found in hematopoietic cells.

Kindlins are essential components of the integrin adhesion complex. The *C. elegans* ortholog of kindlin, Unc-112, localizes with integrins in dense bodies and M lines, and loss of its expression results in a muscle detachment phenotype that is similar to that seen in α or β integrin mutants (*66*). Two human diseases caused by kindlin gene mutations have characteristic features of defective integrin function. Kindler syndrome, which is caused by the loss of kindlin-1, is a rare genodermatosis characterized by an epithelial cell-adhesion defect followed by poikiloderma and cutaneous atrophy (*63*, *64*). Mutations in kindlin-3 were implicated in a rare leukocyte-adhesion deficiency (LAD) type III (LAD-III), which results from severe defects in leukocyte and platelet integrin activation (*67*–*70*).

Genetic and siRNA depletion of kindlin-1, -2, and -3 in mice and cells provided definitive experimental proof that kindlins are essential regulators of integrin function because the conformational shift of integrins from the low- to high-affinity state does not occur in the absence of kindlins (*56*, *59*, *61*, *62*). Kindlin-3 deletion causes severe bleeding that is reminiscent of Glanzmann thrombasthenia, a disorder arising from defects in αIIb or β3 integrin subunits. The platelet integrins cannot bind ligands, and platelet aggregation is defective despite normal amounts of talin (*61*). The same phenotype occurs in talin-deficient platelets (*37*, *38*), indicating that both proteins are required to regulate integrin affinity. Furthermore, leukocytes lacking kindlin-3 are unable to transmigrate across the vessel wall into inflamed tissues because of an integrin-mediated adhesion defect (*60*). The phenotypes of these mice resemble LAD-III patients, which led to the identification of mutations in kindlin-3 as a cause of this disease (*67*, *70*). Mice lacking kindlin-1 have a phenotype similar to that of Kindler syndrome patients and exhibit skin atrophy and a detached colon epithelium because of defective integrin function in intestinal epithelial cells (*71*). Kindlin-2 deletion results in death at implantation because of defective integrin function in cells of the endoderm and the epiblast, causing their detachment from the basement membrane (*59*, *72*). This severe phenotype is consistent with the broad expression pattern of kindlin-2.

Kindlin-mediated integrin activation requires a direct interaction between kindlin and β integrin tails. The kindlin and talin FERM domains show high levels of sequence similarity (*73*); however, the kindlin FERM domain exhibits the structural hallmark of being split into two halves by a pleckstrin homology (PH)–domain insertion in the F2 subdomain (Fig. 1D). Molecular modeling of the kindlin-2 F3 subdomain that uses the talin F3 subdomain as a structural template suggests that it also resembles a PTB domain capable of recognizing β integrin tails (*74*). Biochemical experiments confirmed the predicted interaction of kindlins with the cytoplasmic tails of β1, β2, and β3 tails (*58*–*61*, *73*, *74*). Kindlin-1 and kindlin-2 PTB-domain mutations abolish their interaction with the β1 integrin tail (*62*, *74*) and impair the ability of kindlin-1 to activate integrins (*61*). Unlike talin, kindlins bind the distal NxxY motif on the β1, β2, and β3 tails (Fig. 3) (*58*–*62*, *74*); additional sequences may also be involved in kindlin binding. Although the intervening sequence between the two NxxY motifs in the β1 and β3 integrin cytoplasmic tails are dispensable for talin binding, mutation of a double Thr or Ser/Thr within this sequence impairs kindlin binding (*61*). Some Glanzmann patients carry a Ser-to-Pro mutation in β3 integrins, and the same mutation abolishes kindlin-3 binding in vitro, which suggests that a lack of kindlin-3 binding might be responsible for the bleeding phenotype (*61*).

Because kindlins and talin bind distinct regions of the β integrin tail, they may cooperate to regulate integrin affinity (*18*, *19*, *58*–*61*). Although kindlins are not sufficient to shift integrins to a high-affinity state, they facilitate talin function. The amount of talin expressed in cells determines the efficacy of kindlins in promoting this function because overexpressing kindlin-2 in cells with relatively little talin (*49*) has little or no effect on integrin affinity modulation and co-expression of the talin-head domain with kindlin-1 or -2 results in a synergistic increase in αIIbβ3 affinity. Conversely, talin depends on kindlins to promote integrin affinity because talin-head overexpression failed to increase αIIbβ3 affinity in CHO cells in which kindlin expression was reduced by siRNA. Thus, kindlins require talin, and talin is not sufficient to increase integrin affinity.

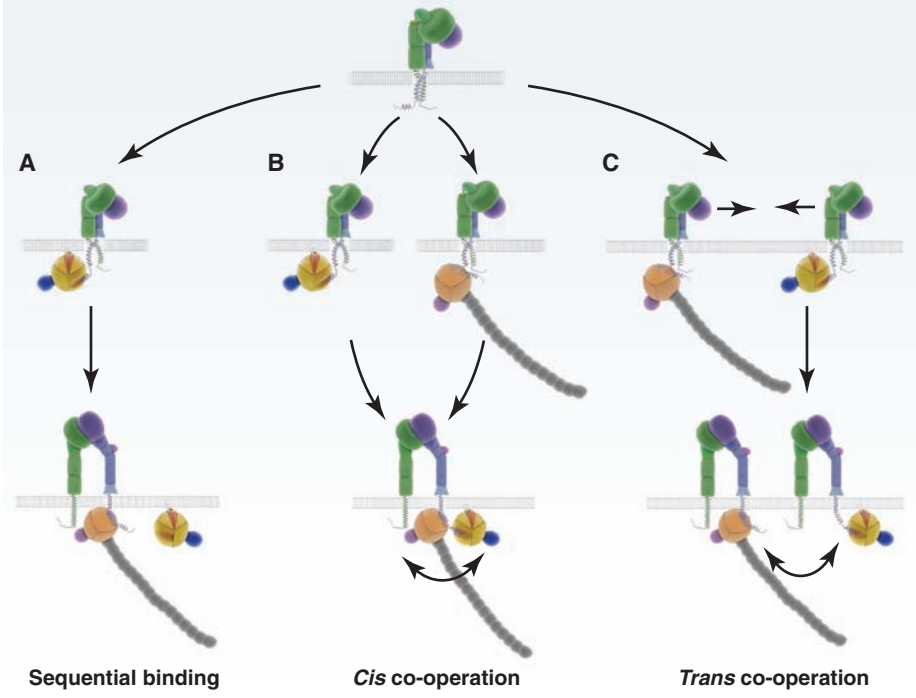

Fig. 4. Putative crosstalk mechanisms between talin and kindlin during integrin activation. (**A**) Model for the sequential binding of kindlin and talin to the integrin tail. Kindlin binding to the MD NxxY motif facilitates talin binding to the MP NPxY motif, which results in the displacement of kindlin from the β tail and final integrin activation. (**B**) Because of the distinct binding sites for talin and kindlin at the β integrin tail, simultaneous binding may be possible. The order in which each protein binds to the integrin tail is not known. (**C**) Communication between talin and kindlin in trans, where each protein is bound to a different integrin tail, can also be envisioned. In this model, talin and kindlin binding to integrin tails results in the formation of integrin nanoclusters and a subsequent talin-kindlin crosstalk (*79*).

Kindlins also function as cytoskeletal linker molecules in outside-in signaling. Kindlin-1 and -2 bind to integrin-linked kinase (ILK) and the filamin-binding protein migfilin, both of which link kindlins indirectly to the actin cytoskeleton (*59*, *75*, *76*). Both proteins localize to cell-matrix adhesions in a kindlin-dependent manner, demonstrating that kindlins are central linker proteins mediating the assembly of integrin-dependent adhesion complexes (*59*, *76*, *77*). Kindlin-3–deficient platelets exposed to divalent Mn^{2+}, which shifts integrins into the high-affinity state independent of intracellular cues, can adhere to fibrinogen- or collagen-coated surfaces; however, subsequent platelet spreading is impaired, which indicates that integrin-dependent cytoskeletal rearrangements do not occur in the absence of kindlin-3 (*61*). These observations suggest that kindlins remain associated with the adhesion complex and fulfill essential functions as bidirectional signaling molecules.

Perspectives

For years, talin was regarded as the sole regulator of integrin activation, but it now shares the spotlight with kindlins. Whether additional molecules regulate integrin affinity remains to be seen. Many unanswered questions on how kindlins and talin regulate integrin function remain. The binding modes that allow kindlins and talin to cooperatively regulate integrin affinity remain to be determined. Three possible scenarios are presented in Fig. 4. A direct interaction between kindlins and talin has not been detected, but low-affinity interactions cannot be excluded. Structural studies suggest that talin alone mediates the final step in integrin inside-out activation, but perhaps kindlin mediates integrin-talin binding. It is also unclear whether fast- and slow-activating integrins found on different cell types have the same mechanistic requirements for kindlin and talin. Lastly, the fate of kindlins and talin within the adhesion complex has not been determined. Because both talin and kindlins are involved in outside-in signaling, they must remain within the adhesion complex; but does their direct interaction with integrins persist, or does it become indirect through other binding partners? Answers to questions such as these will be essential to gain a full understanding of the mechanism behind inside-out integrin activation.

The activity of kindlin, like that of talin, is probably highly regulated; however, the nature of this control is unknown. Kindlins and talin might respond to the same or different activation signals. Because both kindlins and talin contain lipid-binding domains, they may both be controlled by phosphoinositide signals; however, no specific phosphoinositide is known that binds the kindlin PH domain, and lipid-mediated kindlin regulation has not been reported. Another mode of kindlin regulation may be phosphorylation. Kindlin-1 can be phosphorylated in keratinocytes (*78*), but its role is unidentified, and kindlin-2 and -3 phosphorylation in vivo has not been studied.

There are indications that kindlins have functions other than integrin activation. Kindlin-2 participates in outside-in signaling in FAs by binding to and recruiting ILK and migfilin, thus indirectly linking the integrin tail to the actin cytoskeleton and ILK-mediated signaling pathways. Both kindlin-1 and kindlin-2 also localize to cell-cell adhesion sites, but kindlin-2 cannot compensate for kindlin-1 loss, which suggests that these proteins have separate functions at this location, perhaps by binding distinct interaction partners. Lastly, kindlin-3 is required to maintain the proper architecture of the erythrocyte membrane skeleton (*71*), demonstrating an integrin-independent role for kindlin-3 because erythrocytes do not express integrins. Perhaps kindlins have similar roles in other cell types. Understanding these additional functions of kindlin is required to appreciate the biological importance of this class of proteins.

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The Tail of Integrins, Talin, and Kindlins
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Editor's Summary

Tales of Talin, Kindlin, and Integrin

The integrins are receptors on the surface of animal cells that mediate attachment to the extracellular matrix. Integrins also act as signaling molecules, activating signaling pathways when they bind to their ligands in the matrix. Furthermore, integrins can communicate signals from the inside to the outside of the cell when signals within the cell alter the affinity of integrins for their extracellular ligands. **Moser *et al.*** (p. 895) review recent advances in understanding the roles of the proteins talin and kindlin in such bidirectional signaling and how they influence the function of integrins in health and disease.