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REVIEW

Talin and kindlin: the one-two punch in integrin activation

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Abstract **Proper cell-cell and cell-matrix contacts mediated by integrin adhesion receptors are important for development, immune response, hemostasis and wound healing. Integrins pass trans-membrane signals bidirectionally through their regulated affinities for extracellular ligands and intracellular signaling molecules. Such bidirectional signaling by integrins is enabled by the conformational changes that are often linked among extracellular, transmembrane and cytoplasmic domains. Here, we review how talin-integrin and kindlin-integrin interactions, in cooperation with talin-lipid and kindlin-lipid interactions, regulate integrin affinities and how the progress in these areas helps us understand integrin-related diseases.**

Keywords signal transduction; transmembrane domain; nanodisc; integrin; talin; kindling; cell adhesion

Introduction

Evolution from single cells to multicellular organisms necessitates cell-matrix and cell-cell adhesion mechanisms to organize cells into tissue and organs. Integrins, heterodimeric type I transmembrane proteins consisting of α and β subunits, are a major class of adhesion receptors involved in such adhesive events. Each integrin subunit contains a relatively large extracellular domain, a single transmembrane domain (TMD) and a short cytoplasmic tail. 18 α subunits and 8 β subunits dimerize noncovalently to form 24 different integrins [1]. Each integrin exhibits distinct binding affinities to particular ligands. When combined with various integrin expression profiles of different cell types, the ligand specificity results in cells adhering in or migrating toward specific regions where ligands of the particular set of integrins are present [2].

Besides mediating cell adhesions, integrins also transmit signals bidirectionally. In outside-in signaling, they can transmit information on the chemical identity and physical property of their ligands into cells to regulate cell migration, cell survival and growth [3]. Integrins have no enzymatic activities in their cytoplasmic tails, but ligation of integrins with their extracellular ligands can induce conformational changes [4] that result in the separation of the α and β TMD and cytoplasmic tails. These changes may make the cytoplasmic tails of integrins more accessible and favor recruitment of cytoplasmic proteins, such as kinases, phosphotases and scaffold proteins that link integrins to signaling molecules or cytoskeletons [3,5–12]. Furthermore, integrins cluster upon ligation with extracellular matrices which usually present multiple integrin binding sites [13]. At the site of ligand-bound integrins, kinases auto-phosphorylate and phosphorylate other signaling proteins, adaptors and phosphoinositides [3,6,14–25]. This triggers further recruitment of signaling molecules and subsequent signaling events [3,6,14–25]. Integrins can also signal through other transmembrane receptors such as those containing immunoreceptor tyrosine-based activation motifs (ITAMs) [26–28]. Integrin outside-in signaling may also be involved in registering mechanical forces. For example, when talin, one adaptor protein that links integrin to the cytoskeleton, is placed under strain, it undergoes conformational changes that expose binding sites for other adaptors, such as vinculin, to reinforce the integrin-actin linkage [29–32]. Thus, integrin outside-in signaling influences cell behaviors such as adhesion, shape changes and migration following integrin-ECM ligation, and persistent integrin outside-in signaling, specified by different mechanical properties of the ECM, may result in signaling profile and gene expression changes that determine the cell survival and cell fate [33,34].

In integrin inside-out signaling (integrin activation), binding affinity of integrins for specific ligands is swiftly increased in response to intracellular signaling events. Integrin activation, sometimes referred to as integrin affinity modulation, encompasses both affinity increase of individual integrins due to conformational changes and avidity increase due to integrin clustering [6,35,36]. Precise regulation of

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integrins' adhesive capacity and ligand specificity is especially important in a cardiovascular system not only for a swift and local response at the site of injury or inflammation but also for the prevention of unwanted consequences such as thrombosis or autoimmunity. Such paradigm of tight affinity control is demonstrated in αIIbβ3 and β2 integrins. αIIbβ3, the most abundant membrane protein on the platelet surface [37], is normally in a resting (inactive) state with low affinity for its physiological ligands, such as fibrinogen. As a result, platelets exhibit low adhesiveness to each other or the blood vessel wall to prevent occlusion of the blood vessel. A range of agonists present at the site of a wound can lead to an increase in αIIbβ3 binding to fibrinogen (i.e., integrin activation), resulting in platelet aggregation, thrombus formation and hemostasis [38]. Similarly, β2 integrins are in an inactive state on circulating leukocytes. At the site of inflammation, agonists induce the activation of β2 integrins, which leads to their binding to intercellular adhesion molecule (ICAM) and/or vascular cell adhesion molecule (VCAM) on the endothelium and thus the arrest of the leukocytes [39]. As molecules important in a number of physiological responses, integrins also contribute to the pathogenesis of many diseases such as thrombosis, cancer, and autoimmunity. Recent years have seen some tremendous progress in understanding the control mechanisms of integrin activation and offer the potential of translating this basic knowledge into therapeutics. In this review, we will summarize the most recent progress in understanding integrin regulation by activators such as talin and kindlins. The readers are referred to an excellent recent review on the negative regulators of integrins [40].

Conformational equilibrium balanced by the phospholipid bilayer and the integrin TMDs

The structural changes in the integrin extracellular domain accompanying its affinity changes have been reviewed in several excellent reviews [35,41–45]. Briefly, the extracellular domain in its inactive form is folded into a V shape with a genu in the middle [46,47]. One view is that upon activation, it adopts an extended conformation with the hybrid domain swung out to form an open head piece [48–50]. However, some groups argued that the bent conformation can be active and fully occupied by physiological ligands [51–53].

Integrin TMDs play an essential role in transmitting signals across the plasma membrane. Truncation of the integrins at the C-termini of extracellular domains results in constitutively active integrins [54], indicating that TMDs and cytoplasmic tails are critical in controlling the activation state of integrins. Furthermore, many activating mutations, from rational mutagenesis studies or genetic screens, map to the α or β TMD [55–58], again showing that the TMDs are critical for integrin regulation. Heterodimeric interactions between α and β TMDs and cytoplasmic tails can be detected in a cellular membrane by co-immunoprecipitations [59], cysteine crosslinking [60,61] and in a reconstituted phospholipid bilayer by NMR [62], but not in detergent micelles [63]. Importantly, mutations in TMDs that activate integrins invariably inhibit α and β TMD-tail interactions [59], and activating integrins alters the relationship of the α and β tails in cellular membranes [7]. Therefore, understanding the structure and interactions of integrin TMDs in a membrane environment is essential for the comprehension of the mechanism of integrin signal transmission.

Integrin TMDs are usually about 20 hydrophobic amino acids in length and proceeded by Trp and charged residues at the C-termini. Two structures of α and β TMD complexes in phospholipid bilayers are available: one determined by NMR with α and β TMDs embedded in a phospholipid bilayer [62] and the other by inter-residue distance restraints inferred from cysteine crosslinking efficiency in a cellular membrane [60]. The two approaches yielded similar structures and revealed two interaction interfaces [60,62]. In both structures, the αIIb TMD helix is short, straight and broken at Gly991, the first residue of the highly-conserved Gly-Phe-Phe-Lys-Arg (GFFKR) motif in the membrane proximal region of the α subunits. The two Phe residues of the αIIb GFFKR motif do not form a continuous helix but instead make a sharp turn toward β3 (Fig. 1). In this way, the hydrophobic side chains of those residues can reside in the hydrophobic core of the lipid bilayer and stack against hydrophobic residues in the β3 TMD, particularly Trp715 and Ile719. The turning of the membrane-proximal region of αIIb also enables the electrostatic interaction between αIIb Arg995 and β3 Asp723 by placing those residues in proximity (Fig. 1). These sets of interactions at the inner membrane interface are termed the inner membrane clasp (IMC). The second interface involves helical packing centered on β3 Gly708 and αIIb G972XXXG976 motif at the outer membrane region and is termed the outer membrane clasp (OMC) (Fig. 1). Integrin β3 TMD makes a long and continuous helix with a 25° tilting angle to enable the multipoint interactions with αIIb and accommodate the extra hydrophobic residues in the β3 TMD.

These landmark structural studies provided good explanations for all the previous mutational studies. The mutations, deletions or truncations to either subunit that result in active integrin in cells interfere with OMC, IMC or both. For example, mutating β3 Gly708, or either Gly in the αIIb G972XXXG976 motif into bulky amino acids disrupts the OMC, resulting in loss of α-β TMD interactions and constitutively active integrins [55–58]. Similarly, mutations in the two Phe residues of αIIb GFFKR motif or in the αIIb Arg995 and β3 Asp723 electrostatic pair destabilize the IMC, also resulting in active integrins [64]. Since the optimal tilting angle of the β subunit is critical to maintain simultaneous OMC and IMC interactions with a short and straight α TMD, one would expect some mechanism to maintain the 25° β3 tilting angle (Figs. 1 and 2). Indeed, C_α of β3 Lys716 resides

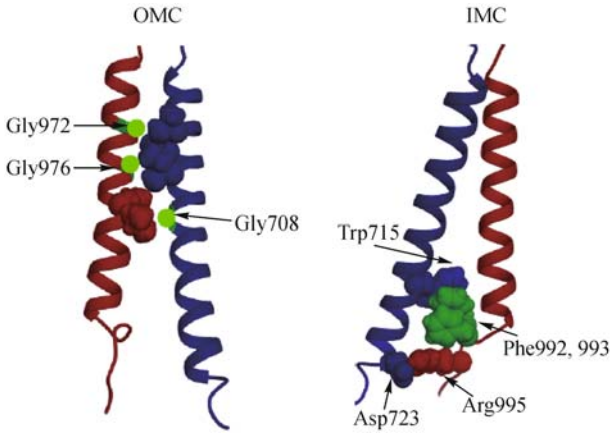

Fig. 1 Structure of integrin αIIbβ3 TMD (ribbon view; αIIb in red and β3 in blue. From PDB 2K9J) showing the two interaction interfaces. Left, outer membrane clasp (OMC). Right, inner membrane clasp (IMC). The important residues for the two interfaces are indicated.

in the hydrophobic region of the lipid bilayer but its positively charged ε-NH_3^+ group snorkels into the negatively charged phosphate head group region (Fig. 2). By doing so, it helps control the tilting angle of β3 TMD [65]. When Lys716 is mutated to negatively charged Glu, it shifts from the hydrophobic core into the aqueous region to avoid the unfavorable placement of negatively charged Glu in negatively charged phosphate head region (Fig. 2). As a result, K716E reduces the embedded length of β3 TMD and the β3 tilting angle, which in turn abolishes α-β TMD interactions and dramatically increases integrin activation (Fig. 2) [65]. Interestingly, integrin activation caused by Lys716 mutation can be reversed by introducing a Pro mutation (A711P) in the middle of β3 TMD. The Pro mutation breaks the continuous β TMD helix into two halves, enables the two helices to adopt different tilting angles to compensate the reduced embedding of β3 TMD, restores simultaneous formation of OMC and IMC and thus reverts integrin activation [65].

Talin "tilts" integrin β TMD and the integrin activation equilibrium

Talin, a cytoplasmic protein, regulates integrin affinity and provides a mechanical link between integrins and the actin cytoskeleton. Talin consists of a 50-kDa N-terminal non-canonical FERM domain (talin head domain or THD) that contains a high-affinity binding site for integrin β subunit and a 220-kDa rod domain that contains multiple binding sites for actin and vinculin [66]. The THD is further divided into F0, F1, F2 and F3 subdomains [66,67]. F3 subdomain, a phosphotyrosine binding domain, binds to the first NPxY motif in integrin β tails [68,69]. The important role of talin in regulating integrin affinity has been well documented in model cells [69–72], transgenic mice [73,74] and reconstituted systems with purified proteins [75]. Overexpression of THD strongly activates αIIbβ3 in nucleated cells [72].

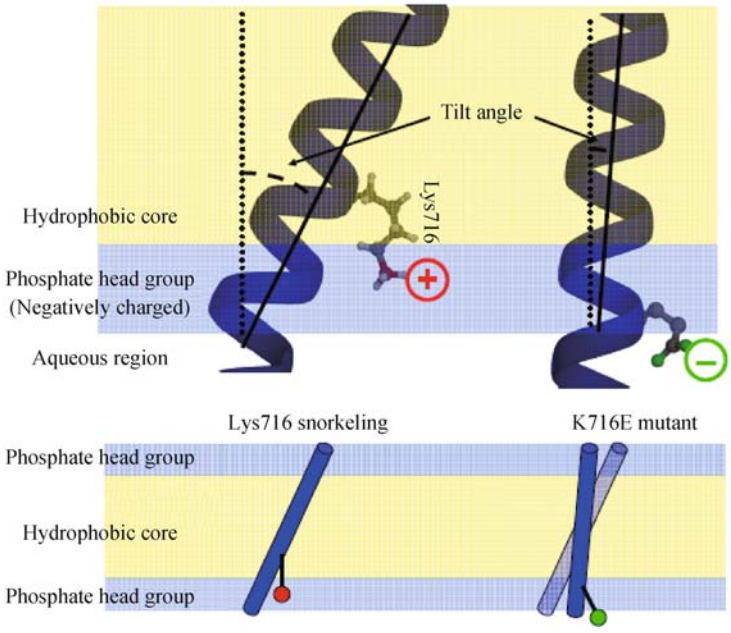

Fig. 2 Snorkeling Lys716 fixes the tilting angle of the β3 TMD. On the left, the C_α of Lys716 resides in the hydrophobic core but its ε-NH_3^+ group snorkels into the negatively charged phosphate head group region. On the right, when Lys716 is mutated to Glu, the residue shifts away from hydrophobic core to place the side chain $-COO^-$ group in the aqueous region. This shift causes reduced embedding of β3 TMD and decreased β3 TMD tilting angle.

Silencing talin in megakaryocytes inhibits agonist-induced integrin activation [76], and disruption of talin-β tail interactions with mutations in either THD or β tail abolishes the capacity of THD to activate integrins [75,77,78]. In *in vitro* systems, recombinant THD alone is sufficient to activate αIIbβ3 reconstituted in both liposomes and phospholipid nanodiscs, and shift the αIIbβ3 toward an extended conformation [75]. Studies in animal models confirmed the role of talin in integrin regulation. Knocking in a mutant talin defective in binding to β tail substantially reduces the ability of talin to strengthen integrin adhesion to the ECM in *Drosophila* [79]. In mouse platelets, genetic ablation of talin severely impairs agonist-induced integrin activation and platelet aggregation [73,74]. Furthermore, a point mutation in β3 integrin (L746A) that selectively disrupts the talin-integrin interaction, or one in talin (L325R) that selectively inhibits the capacity of talin to activate integrins, blocks integrin activation and platelet aggregation [80,81]. Thus, talin binding to the integrin β cytoplasmic tail is a final common step for integrin activation [76].

Recent work from multiple laboratories has elucidated the mechanisms of talin-induced integrin activation at molecular details. Talin binds to two sites on integrin β tails: a strong binding site centered around the first NPxY motif that contributes most of the binding free energy and a weak membrane proximal (MP) binding site that is dependent on the interaction with the NPxY motif [77]. In addition, talin also binds to negatively charged phospholipids through the positively-charged residues on the surface of THD [77,82,83]. The weak interaction at the MP region is a critical differentiating factor for the unique integrin-activating capacity of talin for two reasons: (1) it brings talin Lys324 close to Asp723 of the β tail, thus neutralizing some charge of Asp723, weakening the Arg995-Asp723 electrostatic interactions at IMC and favoring integrin activation [83]; (2) it stabilizes α-helix formation of the β MP region and extends a continuous β TMD-tail helix into the MP region [77,83]. As talin binds to integrin tails and phospholipids, it tilts the rigid continuous β TMD-tail helix further into the membrane, increasing the tilting angle of β TMD (Fig. 3) [84]. Such talin-induced motion was shown by increased fluorescence of solvatochromic dyes attached to the N- or C-terminal of β TMD in the presence of THD [84] and is further supported by molecular dynamics simulations [85]. As described earlier, non-optimal tilting angle destabilizes α-β TMD interactions and shifts the equilibrium toward an activated integrin conformation. The talin-lipid interaction is another critical factor for talin to function as a direct integrin activator, as mutations blocking these interactions or solubilization of integrin in detergent micelle abolishes talin's capacity to activate integrins [75,77,83]. As expected, introducing a flexible proline kink in the middle of the β TMD decouples the TMD C-terminal tilting motion from the N-terminal one, and blocks THD-induced integrin activation [84] (Fig. 3).

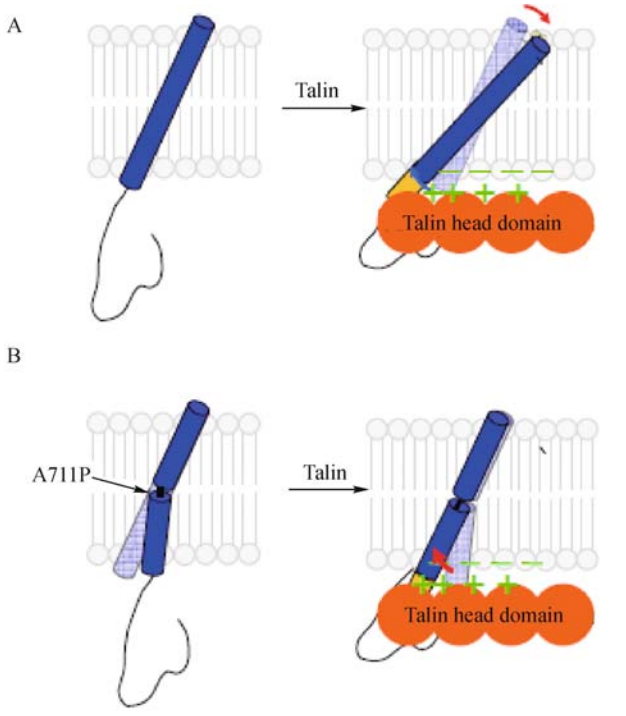

Fig. 3 Talin activates integrin by causing a topology change in β3 TMD. (A) Talin stabilizes the helix in the membrane proximal region of β3 and increases the tilting angle of the continuous β3 TMD. (B) A711P mutation introduces a flexible kink that breaks the continuous β3 TMD, decouples the tilting motion of the two helices, and blocks integrin activation.

Kindlins further tip the balance of integrin activation

Kindlins, a family of cytoplasmic proteins that bind to integrin β tails, are another group of important regulators for integrin activation [36,86]. A kindlin ortholog, UNC-112, co-localizes with integrins and is required for the organization of integrins at muscle body wall junctions in *C. elegans* [87]. There are three mammalian kindlin orthologs: kindlin-1 (also known as URP1 for UNC-112-related protein), kindlin-2 (Mig-2), and kindlin-3 (URP2) [88]. Mutations in and depletion of kindlin-1 result in impaired β1 integrin function and defective epithelial cell attachment to the extracellular matrix [89,90]. Genetic ablation of kindlin-2 in mice inhibits β1 integrin activation and results in embryonic lethality due to severe detachment of the endoderm and epiblast from the basement membrane, phenotypes similar to that of β1 null mice [91]. Paradoxically, overexpression of kindlin-1 or kindlin-2 dramatically inhibits THD-induced β1 integrin activation, and Harburger *et al.* suggested that kindlin might function as a scaffold in β1 regulation [92]. In contrast, overexpressed kindlin-1 and kindlin-2 strongly enhance THD-induced αIIbβ3 activation, although they have little effect by themselves [92,93]. The mechanism for these

integrin specific effects of kindlin is still unknown. Loss of kindlin-3 causes defects in the activation of multiple integrin classes in a number of hematopoietic cell types [94–99]. Thus kindlins regulate integrin activation.

There has been progress in understanding the requirement of kindlin interacting motifs and partners for its integrin-regulating function. Kindlins bind to the second NxxY/F motif on integrin β tails that is distinctive from the talin binding site, and this interaction is required for kindlin to regulate integrin activation and for kindlin localization to focal adhesions [92,93,99,100]. Kindlins are FERM domain proteins based on sequence homology. The kindlin FERM domain is divided into F0, F1, F2, and F3 subdomains; the F2 subdomain is separated into two halves by a pleckstrin homology (PH) domain [36,86]. The PH domain is required for kindlin to promote integrin activation as deletion of PH domain inhibits kindlin-2-induced activation of β1 integrins in podocytes [101] and αIIbβ3 in CHO cells [93,102,103]. Three groups independently reported that the kindlin-2 PH domain preferentially binds to PIP3 and suggested that this PIP3-PH domain interaction is important for proper kindlin function [101,103,104]. Another group reported substantially lower affinities between kindlin PH domains and phosphoinositides and the affinities are further reduced in a phosphate buffer [102]. The authors instead suggested that it is not an inositol phosphate but another phosphorylated species that might be the interaction partner of kindlin-1 PH domain [102]. Kindlin F0 is also required for kindlin to function as an integrin activator, because deletion of this region strongly inhibits the capacity of kindlin-1 to enhance THD-induced αIIbβ3 activation [105]. A more recent report suggested that kindlin F0 domain functions by mediating kindlin binding to PIP2 [106]. In addition, kindlin function depends on a conserved lipid binding loop in kindlin F1 [107]. Thus the emerging picture is that the lipid-kindlin interactions, mediated by multiple kindlin subdomains and specific lipid species, are critical for kindlin function.

In cells, kindlins alone have little effect and can only synergize with talin to activate integrins [92,93]. A number of recent reviews have attempted to explain this phenomenon [36,86,108]. One attractive idea is that kindlin can promote recruitment of talin to integrins. However, recent studies found that kindlin does not increase bi-molecular fluorescence complementation between talin and integrin in CHO cells, nor does it increase binding between talin and β3 tails *in vitro* [109,110]. Furthermore, recombinant kindlin-3 does not alter the β3 TMD tilting angle, the mechanism by which talin activates integrins, nor does it enhance the tilting angle changes induced by talin [84]. Thus kindlins may function by a mechanism distinct from that of talin.

There has been progress in understanding the different regulatory mechanisms by talin and kindlin. Talin is required for integrin-mediated slow rolling of neutrophils on blood vessels, which measures the initial activation of integrins; whereas, kindlin-3 is dispensable for such slow rolling [111]. Both talin and kindlin-3 are required for neutrophil arrest, which correlates with integrins in a high affinity state [111]. Similarly, in T cells, integrin-kindlin-3 interaction is dispensable for initial integrin-ligand binding but is necessary for the strengthening of the integrin-ligand bonds into firm adhesion [112]. Margadant *et al.* reported that the talin-integrin interaction, but not the kindlin-integrin interaction, regulates α5β1 activation; whereas kindlin-integrin interaction plays a separate and distinct role in regulating α5β1 degradation and recycling [113]. A recent work showed that kindlins have little primary effect on affinity of individual αIIbβ3 but increase multivalent ligand binding by promoting the clustering of talin-activated αIIbβ3 (Fig. 4) [114]. Furthermore, kindlin-3 induces integrin αLβ2 clustering in a T cell line [115]. This model, that kindlins promote clustering of talin-activated integrins (Fig. 4) [114], explains why kindlins have little effect in the absence of talin [92,93], why kindlins can synergize with talin to activate integrins [92,93] and why kindlins are required for firm adhesions but not for initial talin-dependent ligand binding [111,112]. It will be interesting to see if this mechanism of kindlin function can be generalized to other integrins.

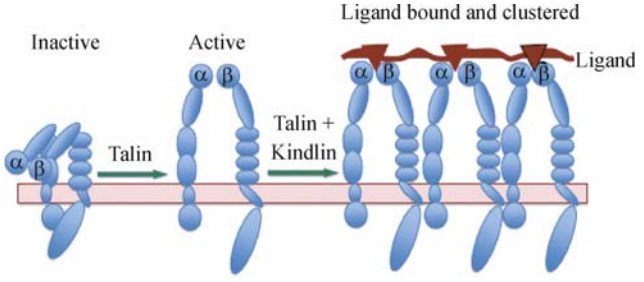

Fig. 4 Model for talin and kindlin function. Talin promotes affinity increase of individual integrin molecules. Kindlins have little primary effect on affinity of individual integrin but increase multivalent ligand binding by promoting the clustering of talin-activated αIIbβ3.

Another unexplained but interesting phenomenon is the integrin- and cell- specific effects of kindlins. Loss of kindlin-3 in LAD-III eliminates LFA-1 but not VLA-4 adhesiveness developed under shear flow conditions [116]. Kindlin-1 or kindlin-2 synergize with THD in promoting αIIbβ3 activation but dramatically inhibit THD-induced activation of α5β1 in CHO cells [92]. Kindlin-3 neither localizes to nor activates αIIbβ3 in CHO cells, but activates α5β1 in a macrophage cell line [99]. In β1 null keratinocytes, kindlin-1, but not kindlin-2, localizes to the integrin-β6-rich adhesions, and kindlin-1 cannot compensate the defects resulted from kindlin-2 depletion, despite the high homology of the two kindlin isoforms [100]. What cause the kindlins to associate differently with integrins in the same cell is still unknown.

Kindlins have functions other than regulating integrin

activation. For example, kindlins also affect integrin surface expression. Overexpression of kindlin-1 or kindlin-2 increases αIIbβ3 expression levels in CHO cells [92]. Genetic ablation of kindlin-3 reduces surface αIIbβ3 in platelets by 25% [99]. This may be due to altered integrin mRNA levels as recently shown by Bottcher *et al.* in the kindlin-2 null cell [117]. Kindlin and sorting nexin 17 (SNX17), an integrin binding protein that controls the recycling of integrins, have overlapping binding sites on integrin β1 tail [113,117]. Thus mutations to the kindlin binding sites also affect integrin-SNX17 interactions and therefore interfere with the integrin recycling pathway [113,117]. Furthermore, kindlin binds integrin linked kinase (ILK), an important adaptor protein in integrin outside-in signaling [91,118,119]. In kindlin-2 null cells, ILK is not targeted properly to focal adhesions, suggesting loss of kindlin may also affect integrin outside-in signaling through ILK [91]. A recent study in *C. elegans* suggests that PAT-4 (ILK ortholog) enables UNC-112 (kindlin-3 ortholog) binding to PAT-3 (β integrin ortholog) by changing the conformation of UNC-112 [120]. Thus the functions of ILK and kindlins may be mutually dependent. Migfilin, a kindlin binding protein that also binds to filamin, was initially proposed as a likely switch for kindlin function [121]. The hypothesis was that kindlins recruit migfilin to the integrin β cytoplasmic tail, where migfilin displaces the integrin inhibitor, filamin. However, migfilin null mice do not show similar phenotypes to kindlin deficient mice, disfavoring the hypothesis [122].

Integrin activation is a dynamic equilibrium

It is worth emphasizing here that the inactive and active integrins exist in a shifting equilibrium. The measured average integrin affinity reflects the net effects of all the relevant factors on the activation equilibrium. For example, integrins with weakened α-β TMD interactions can be further activated by THD [83] and can also be reverted by silencing endogenous talin or by mutations blocking talin-integrin or kindlin-integrin interactions [76,93]. β3 integrin activation induced by a K716E mutation, which alters β TMD tilting angle, can also be partially reverted by mutations blocking talin-integrin interactions [65]. Integrin clustering and conformational changes can synergistically enhance multivalent ligand binding to cellular integrins [123]. Therefore, THD, which increases the affinity of individual integrins, and kindlins, which promote integrin clustering, can synergize with each other in activating integrins [92,93,114]. Moreover, agents that act via the extracellular domain can synergize with ones acting via the cytoplasmic domain in shifting the conformational equilibrium toward the high affinity state [114,124,125]. On the other hand, cytoplasmic integrin activators can be antagonized by negative cellular regulators [40]. Thus, integrin activation is a dynamic process in which factors with opposing effects can cancel each other and factors with the same effects can add to or synergize with each other (Fig. 5).

Integrins in disease

Given the important roles of integrins in multiple physiological processes, it is not surprising to find diseases involving genetic mutations in integrins or integrin regulators. There are well known diseases due to integrin mutations, Glanzmann thrombasthenia (GT) and leukocyte adhesion deficiency I (LAD I), and to kindlin mutations, LAD III (kindlin-3) and Kindler syndrome (kindlin-1).

Glanzmann thrombasthenia is a hereditary hemorrhagic disorder caused by loss of αIIbβ3 expression or function. There are three subsets of genotypes for this disease. The first subset completely loses either αIIb or β3 expression due to nonsense genetic mutations [126,127], or has much reduced αIIbβ3 surface expression due to mutations disrupting folding, post-translational processing, or transportation of either αIIb or β3 [128]. The second subset of GT patients has normal αIIbβ3 surface expression but carries mutations in the αIIbβ3 extracellular ligand binding pockets or in the β3 cytoplasmic domain. The former mutations, such as R214W or D119Y, directly block ligand binding [129,130] and the latter ones, such as S752P or R724Ter [131,132], block integrin activation by preventing the binding of integrin regulators such as talin and kindlin. The third subset of patients carries mutations that lock αIIbβ3 in an activated conformation, including C560R and C598Y in β3 cysteine rich domains [133,134]. The activated platelet αIIbβ3 is

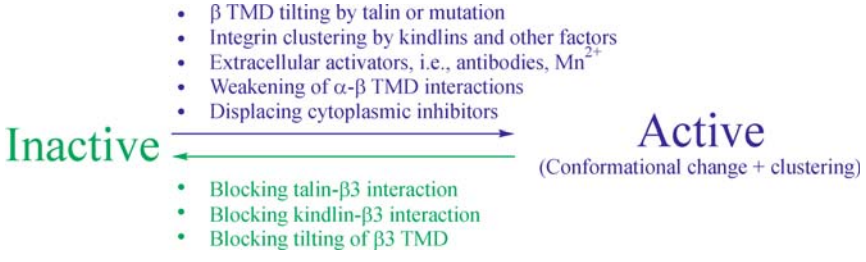

Fig. 5 The dynamic equilibrium of integrin activation functions as signal integrator. The factors that shift the equilibrium to the same direction can add to or synergize with each other. Opposing factors can cancel each other.

constitutively occupied by ligand and thus not available to bridge αIIbβ3 receptors from other platelets at the site of a wound. Therefore, these platelets with constitutively active αIIbβ3 fail to aggregate, resulting in prolonged bleeding.

Leukocyte adhesion deficiency I and III are hereditary immune deficiencies characterized by leukocytosis and repeated infections (LAD II is caused by loss of selectin ligands and thus not integrin-related). LAD I is caused by mutations that cause loss of integrin β2 expression or function. Consequently, leukocytes from LAD I patients fail to firmly adhere to the endothelium near the inflammation site or interact with antigen presenting cells, both of which are critical for mounting an effective immune response [135]. LAD III patients carry mutations in kindlin-3, resulting in non-functional kindlin-3 fragment or absence of kindlin-3 [95,97,98]. In the immune cells of LAD III patients, integrin activation in response to agonist stimulation is defective. Consequently, patients' leukocytes fail to arrest on the vascular endothelium and are incapable of extravasation to the site of infection. Since kindlin-3 also plays a role in regulating αIIbβ3 activation, LAD III patients exhibit GT-like symptoms [135].

Kindler syndrome is a hereditary skin disease caused by nonsense mutations in kindlin-1. The disease is characterized by skin blistering, increased skin sensitivity to light, patchy discoloration of the skin and widespread skin breakdown [136,137]. Knockout of kindlin-1 in mice caused skin atrophy that resembles human Kindler syndrome [89]. Reduced β1 integrin activation has been suggested to play a role in this disease because of defective β1-mediated keratinocytes adhesion to laminin, collagen or fibronectin matrix [89]. Recent work suggested that kindlin-1-deficient keratinocytes respond to cell stress by upregulating the expression of cytokines, which activate fibroblasts and induce their differentiation into myofibroblasts, leading to matrix protein deposition and mucocutaneous fibrosis in patients [138]. Thus, there may also be integrin-independent mechanisms contributing to this disease.

Concluding remarks

The regulation of integrins is important for development and many physiological and pathological events. Thus, this continues to be an area of intense interest. Exciting progress has been made in identifying the key players and understanding their mechanisms of action. Nevertheless, important unanswered questions, such as the mechanism of integrin- and cell-specific effects of kindlins, remain. The remarkable progress in the basic understanding of integrin activation may enable the development of new therapies in the future.

Compliance with ethics guidelines

Feng Ye, Adam Snider, and Mark Ginsberg declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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