Published in final edited form as:

Curr Opin Hematol. 2009 September ; 16(5): 323–328. doi:10.1097/MOH.0b013e32832ea389.

Kindling the flame of integrin activation and function with kindlins

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Abstract

Purpose of review—There are three kindlin family members in vertebrates, which have high-sequence homology and a common organization signature with a C-terminal 4.1, ezrin, radixin, moesin (FERM) domain bisected by a pleckstrin-homology domain. Although the cell and tissue distributions of the three kindlins differ, there is a consistent and close interrelationship between kindlins and integrins, and alterations of kindlin expression affect integrin-dependent functions. However, in-vivo data on the functions of the kindlins and their mechanisms of action have been lacking.

Recent findings—Recent studies have shown that deficiencies of each of the three kindlins result in profound and distinct phenotypes, ranging from skin and intestinal defects (kindlin-1), embryonic lethality due to cardiac developmental problems (kindlin-2), to marked abnormalities in platelet, leukocyte and erythrocyte function (kindlin-3). A human disease characterized by bleeding, frequent infections and osteopetrosis has now been attributed to mutations in the gene encoding for kindlin-3. These defects are consistent with recent data showing that kindlins control integrin activation and function.

Summary—The three members of the kindlin family have now been implicated as essential regulators of integrin function in individual cells and in whole organisms.

Keywords

integrins; kindlin-1; kindlin-2; kindlin-3; talin

Introduction

Whereas scientific investigation usually provides insights that simplify our understanding of biological processes, in the case of integrin activation, the mechanisms have become more complicated by recent discoveries. One such complication comes from recent studies which have unequivocally implicated the three members of the kindlin family of intracellular proteins as key regulators of integrin activation. This review summarizes recent information on the kindlin family and their role in integrin function based upon observations made in cells, animals and humans.

The kindlin family

The kindlins are a family of structurally similar and evolutionarily conserved intracellular proteins. In mammals, there are three kindlin family members, which are designated as

kindlin-1, kindlin-2 and kindlin-3. The kindlin name derives from Theresa Kindler, who described a patient in 1954 with symptoms that included congenital skin blistering, skin fragility and sun sensitivity [1]. This rare autosomal recessive disease, now referred to as Kindler syndrome, is caused by mutations in the gene for kindlin-1 [2,3]. Kindlins are also referred as UNC-related proteins (URPs) based on a *Caenorhabditis elegans* homolog, UNC112 [4]. In some studies, kindlin-2 also has been referred to as Mig2 and kindlin-1 as kindlerin. Genes encoding the kindlins are termed as *KIND* and *FERMT*. Using these various designations in PubMed searches yields about 100 publications on the kindlins, and many are studies on new cases and mutations in Kindler syndrome. However, within the last 18 months, more than 20 publications have appeared. The findings reported in these recent studies have established previously unappreciated functions of the kindlins in mediating cellular responses, in controlling the phenotypes of whole organisms, and in inducing human disease. Among these recent publications is a review of the kindlins and their roles in integrin and matrix biology [5•].

Tissue and cellular distribution of the kindlins

The three kindlins exhibit similar distributions within cells but distinct tissue distributions. These patterns suggest that the kindlins may have overlapping functions but exert their effects in a tissue and cell-specific manner. Kindlin-1 is very abundant in skin and intestines and is localized predominantly to keratinocytes [6]. These localizations are consistent with the skin and intestinal symptoms observed in Kindler syndrome. Kindlin-2 is very broadly distributed with particularly high levels in smooth and skeletal muscle cells. Kindlin-3 appears to be confined to cells of hematopoietic origin [6]. Malignant transformation can alter the expression patterns of kindlins. Kindlin-2 expression is altered in breast and leiomyomas [7,8], and kindlin-1 is expressed highly in colon and lung tumors [9].

The absence of UNC112 in *C. elegans* gives rise to the PAT phenotype, which is also observed when the homologs of the integrin α and β subunits are missing. Indeed, UNC112 colocalizes with PAT-3, the integrin β subunit, and a relationship between kindlin and integrin expression patterns extends to all cells and organisms examined [10]. In mammalian cells, kindlin-1 and kindlin-2 localize to integrin-containing focal adhesion complexes, and kindlin-3 is found in integrin-containing podosomes of hematopoietic cells [6]. As discussed below, this interrelationship depends in part on their direct interaction with the cytoplasmic tails of integrin β subunits and in part on their interaction with other components assembled into these adhesion complexes. Such adhesion complexes change in composition as cells attach, spread and migrate, and the associations of kindlins are likely to change dynamically.

Structural organization of kindlins

The three mammalian kindlins have molecular weights of approximately 75 kDa. The human kindlins exhibit extensive amino acid sequence homology, displaying 50–60% sequence identity, and the sequences are highly conserved across species. The amino acids of the kindlins are organized into the same structural domains, and a representative kindlin is shown in Fig. 1. In this presumptive structure, a prominent structural feature is a FERM domain. FERM domains, named for their initial demonstration in 4.1, ezrin, radixin and moesin, link cytosolic proteins to membranes, are prevalent in cytoskeletal related proteins, and are organized into three subdomains: F1, F2, and F3 [11]. The kindlin FERM domains are most similar to that in talin [12]. Both talin and kindlin FERM domains mediate binding to the cytoplasmic tails of integrin β subunits and regulate their function. This interaction depends on the F3 subdomain in both proteins, which has the organization of a phosphotyrosine-like binding (PTB) domain [13,14,15•,16••]. However, there are several features which distinguish the FERM domains within kindlins from that of talin and other

proteins. Whereas the FERM with its PTB subdomain is located in the N-terminal region of talin and most other FERM domain containing proteins, it resides in the C-terminal aspects of the kindlins [5•]. The prototypic kindlin FERM also lacks a F1 subdomain, and its F2 subdomain is bisected by a pleckstrin-homology domain (Fig. 1). Pleckstrin-homology domains mediate binding of phosphatidylinositides, and the expressed pleckstrin-homology domain of kindlin-2 exhibits this function (unpublished).

Integrins, talin and their activation

As a prelude to recent studies of the kindlins, a very brief consideration of integrins and talin is required. As a family, integrins influence numerous physiological and pathological processes, including cell adhesion, migration and survival, and these functions depend upon their ability to bind ligands within their extracellular environment and link them to the cytoskeleton and/or cytoplasmic signaling machinery within the cell [17–19]. Each integrin family member is a heterodimer composed of noncovalently linked α and β subunits. There are 8 integrin β subunits and 18 integrin α subunits. Specific α/β subunit combinations determine which extracellular ligands are recognized. The organizational signature of the α and β subunits is similar. Each contains a large extracellular domain (ECD) containing a large globular headpiece, a single pass transmembrane domain and a short cytoplasmic tail of 15–60 amino acids (except $\beta 4$).

For many integrins, particularly on cells with their integrins exposed to blood, their functions depend on their activation. Integrin activation is defined as the transition from a low (resting state) to a high-affinity state (active state) for their extracellular ligands. This transformation is induced by an inside-out signaling pathway triggered by various cellular agonists. Upon ligand binding and/or clustering of the integrins, outside-in signaling can be initiated to induce events that alter cell shape and function [20,21]. The cytoplasmic tails of integrins act as transmitters and receivers of the bidirectional signaling across integrins. By alignment of all integrin β cytoplasmic tails, several highly conserved regions are evident (Fig. 2) which have been implicated in integrin activation. These regions are: the membrane proximal region; the first NXXY motif, NPLY⁷⁴⁷, in $\beta 3$; a distal region which contains a conserved TxT cluster, in which x is frequently a S or T; and a second NXXY motif, NITY⁷⁵⁹, in $\beta 3$.

As currently envisioned, integrins are maintained in a resting state by transmembrane domain–transmembrane domain and cytoplasmic tail–cytoplasmic tail contacts. Dissociation of these intrasubunit interactions drives global movements of the ECD from a bent to an extended conformation and local rearrangements within the ECD to allow access of ligands to a binding pocket comprised of both subunits [19,22,23,24•]. Nuclear magnetic resonance (NMR) structural studies of a cytoplasmic tail of a prototypical integrin, $\alpha \text{IIb}\beta 3$, suggest that each cytoplasmic tail has a helical structure as it emerges from the membrane into the cytosol [25–27]. Some but not all of these structural studies suggest that these cytoplasmic tail helices interact. Particularly important in maintaining the cytoplasmic tail complex is a salt bridge that forms between residues D⁷²³ in $\beta 3$ and R⁹⁹⁵ in αIIb . Destabilization of this salt bridge by site-directed mutagenesis or naturally occurring mutation leads to at least partial integrin activation [28–30]. Hydrophobic interactions between the cytoplasmic tail helices also have been detected, and mutational data support the role of both electrostatic and hydrophobic bonds in maintaining the cytoplasmic tail complex [25,29]. The clasp formed between the cytoplasmic tail helices maintains the integrin in a resting state, and disruption leads to activation. Only one NMR structure detected the salt bridge, although a large body of mutational and functional data, including analyses of mutations in humans and mice, supports its existence and its importance in regulating the activation of some but not all integrins [30–32]. The α and β transmembrane domain also forms helices that interact

but their interconnection to the cytoplasmic tail clasp remains to be resolved. [33,34]. Unclasping of the cytoplasmic tail is likely to induce separation of the transmembrane domain–transmembrane domain helices to propagate the inside-out signal across the cell membrane to the ECD [35,36].

A strong and substantial body of evidence indicates that talin, a 270-kDa cytoskeletal protein, is essential for integrin activation [37–40]. Talin is composed of an N-terminal head (talin-H), which contains a FERM domain, and a long, flexible rod domain (talin-R) [41]. The F3 (PTB) subdomain binds with high affinity to the β cytoplasmic tail and induces integrin activation. The talin F3 subdomain binds to two sites in the β 3 cytoplasmic tail, the NPXY turn and the membrane proximal region. The latter interaction disrupts the clasp between the α and β cytoplasmic tail and initiates activation. This mechanism is supported by structure analyses [25,42] and studies of mice with a point mutation of a talin-binding site [43]. Inherent in this model is the necessity for talin itself to be activated. Such regulation is achieved in two distinct ways, which are identified but not detailed here. First, the cytoskeletal protein filamin acts as a negative regulator [44]. It binds to the β cytoplasmic tail adjacent to the NPXY and impedes access of talin to its binding site. Mechanisms have been identified which displace filamin from its β cytoplasmic tail binding site facilitating integrin activation [45•,46•]. Second, intact talin exists in a conformation in which talin-R interacts with talin-H and precludes its binding to the β cytoplasmic tail [47•, 48•]. The pathway by which this autoinhibition of talin is relieved involves Rap1 and RIAM [49,50].

Enter the kindlins

There is also a substantial evidence from theoretical considerations, mutational data and in-vivo data from humans and mice that regions of the β cytoplasmic tail not directly involved in talin-H binding are critical to integrin activation. As a pivotal example, a naturally occurring mutation of S⁷⁵²P in the β 3 cytoplasmic tail, a mutation that does not affect talin-H binding, gives rise to Glanzmann's thrombasthenia caused by a nonactivatable α IIB β 3 on platelets [51]. Numerous binding partners for the β cytoplasmic tail have been identified which might account for C-terminal region involvement, and kindlin-1 and kindlin-2 were among these. Kindlin-1 had been identified as a transforming growth factor (TGF)- β inducible gene that associated with the β 1 and β 3 cytoplasmic tail and influences cell spreading [12]. Kindlin-2 also associates with the β 1 and β 3 cytoplasmic tail, to strengthen the adhesion of cells and to control focal adhesion formation [52]. Two studies published in 2008 demonstrated that kindlin-2 had minimal activating activity but when coexpressed with talin-H led to synergistic activation of α IIB β 3 [53••,54••]. These studies both showed that this coactivating activity depended on binding of kindlin-2 to the β 3 cytoplasmic tail. This interaction required a functional PTB-like binding domain in kindlin-2 since mutations in this structure QW^{614/615}AA prevented interaction and integrin coactivation. However, rather than binding to the NPLY⁷⁴⁷ turn in the β 3 cytoplasmic tail to which talin binds with high affinity, kindlin-2 binding depended on the membrane distal NITY⁷⁵⁹. Kindlin-2 binding also was abrogated by the S⁷⁵²P mutation, providing a potential explanation for the nonactivatable state of the integrin bearing this mutation. Studies of kindlin-1 [55••] and kindlin-3 [16••] provide evidence for similar structure–function relationships. The C-terminal NXXY motifs in β 1, β 2 and β 3 were shown to be required for interaction with the kindlins, and a single point mutation at Q or W in the PTB domains of kindlins blocked integrin binding and coactivator function [55••]. However, the structure–function relationships in the kindlin/integrin interface are more complex; deletion of the N-terminal region of kindlin-2 or kindlin-3 blocks integrin-activating activity even though its PTB is intact [53••,56••]. Furthermore, kindlin-1 and kindlin-2-bearing mutations that prevent integrin binding exert a negative effect on cell adhesion [55••].

Deficiencies of kindlins in fish, mice and humans

Loss of kindlin-1 in humans causes Kindler syndrome, with the most common characteristics being congenital skin blistering and poikiloderma, and there is often oral and colonic involvement [2]. Histologically, the blisters show epithelial detachment from the underlying tissue. Deletion of kindlin-1 in mice also gives rise to skin as well as intestinal defects [57••]. The intestinal defect has been attributed to defective integrin activation in epithelial cells. Human deficiencies of kindlin-2 have not been reported and are predicted to be embryonically lethal since knock-out of kindlin-2 in mice and zebrafish results in embryonic lethality [54••,58•]. The phenotypes of the embryos in both species are consistent with defective integrin function, exerting a prominent effect on cardiac development and function. The specific role of kindlin-2 in myogenesis relates to its regulation of myocyte elongation [59••]. Again, phenotypes could be assigned to effects of kindlin-2 on integrin function, and cells derived from the kindlin-2^{-/-} mice or in which kindlin-2 was knocked down by siRNA point to its prominent role in integrin activation [53••,54••]. Knock-out of kindlin-3 is not embryonically lethal but the mice only survive for 1 week [16••]. Initial characterization of the kindlin-3^{-/-} mice was restricted to its role in platelet-dependent responses. Platelets from these mice did not aggregate due to a failure to activate $\alpha\text{IIb}\beta_3$ and consequently the mice failed to form a thrombus in an FeCl_3 model. In a subsequent study, leukocyte transmigration was shown to be blunted in the kindlin-3^{-/-} mice [60••]. The leukocytes were able to roll on endothelium but were unable to adhere firmly, a response requiring β_2 integrins and their activation. Kindlin-3 deficiency in mice was also reported to lead to an abnormal morphology of erythrocytes [61•] and this may reflect integrin-independent functions of this kindlin.

Within the past year, mutations in kindlin-3 have been linked to a human disease [56••,62••, 63••]. The common symptoms in the patients are bleeding and susceptibility to infections, and it is likely that osteopetrosis is common to this disease. Platelets from these patients fail to aggregate, leukocytes fail to spread properly, and mesenchymal cells produce excessive amounts of bone and cartilage. This disease has been referred to as leukocyte adhesion deficiency-III (LADIII) [64,65] or LAD variant (LADIV) [63••] and integrin activation deficiency disease (IADD) [56••]. We coined that latter designation since either LAD designation failed to capture the most troublesome phenotype in our patients, their bleeding symptom, which was related to their aberrant platelet function and required multiple transfusions [56••]. The defect in some patients with these symptoms had been ascribed to mutations in their CALDAG-GEF1 protein, a guanine exchange factor for Rap1; and mice deficient in CALDAG-GEF1 do display certain defects in activation of platelet and leukocyte integrins [66] and were regarded as a model for LADIII [67]. More careful analysis, however, revealed that many of the patients lack mutations in CALDAG-GEF1 but all carry stop-codon mutations in the kindlin-3 gene [63••]. In fact, CALDAG-GEF1 protein and function were normal in many patients and kindlin-3 but not CALDAG-GEF1 rescued the abnormal phenotype of immortalized cells from the patients [56••,62••]. Thus, the defects in the patients were caused by the lack of kindlin-3 and unrelated to CALDAGGEF1 function. The disease symptoms in these patients are consistent with the restricted expression of kindlin-3 to hematopoietic tissues, as is the reversibility of the IADD symptoms by bone marrow transplantation. Also of note, erythrocyte shape abnormalities observed in the kindlin-3^{-/-} mice [61•] were not detected in IADD patients; that is, mice and human may not be identical. However, humans and mice with nonfunctional kindlin-3 did show abnormal leukocyte functions due to dysfunctional β_2 integrins [60••].

Conclusion

Recent publications clearly implicate the kindlins as novel regulators of integrin-mediated responses. One mechanism by which kindlins influence cellular responses is by synergizing with talin to activate integrins, a function that depends on their direct binding to the cytoplasmic tails of integrin β subunits. Kindlins may influence cell adhesive responses in ways that may depend on still other binding partners. The demonstrations that animals with inactivated genes for the kindlins display lethal phenotypes and that human diseases arise from kindlin mutations add legitimacy and importance to analyses of their structure and function.

Acknowledgments

The work was supported by NIH grant HL073311. The authors wish to thank the members of their laboratories with particular appreciation to Yan-Qing Ma and Nikolay Malinin for their efforts in our studies of kindlins. We also thank Dr Cary Wu, University of Pittsburgh, as a collaborator in our studies of kindlins.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 411–412).

1. Kindler T. Congenital poikiloderma with traumatic bulla formation and progressive cutaneous atrophy. *Br J Dermatol.* 1954; 66:104–111. [PubMed: 13149722]
2. Ashton GH. Kindler syndrome. *Clin Exp Dermatol.* 2004; 29:116–121. [PubMed: 14987263]
3. Arita K, Wessagowit V, Inamadar AC, et al. Unusual molecular findings in Kindler syndrome. *Br J Dermatol.* 2007; 157:1252–1256. [PubMed: 17854379]
4. Rogalski TM, Mullen GP, Gilbert MM, et al. 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; 150:253–264. [PubMed: 10893272]
- 5•. Larjava H, Plow EF, Wu C. Kindlins: essential regulators of integrin signalling and cell-matrix adhesion. *EMBO Rep.* 2008; 9:1203–1208. This article provides a comprehensive review of kindlins, their structure and function. [PubMed: 18997731]
6. Ussar S, Wang HV, Linder S, et al. The kindlins: subcellular localization and expression during murine development. *Exp Cell Res.* 2006; 312:3142–3151. [PubMed: 16876785]
7. Gozgit JM, Pentecost BT, Marconi SA, et al. Use of an aggressive MCF-7 cell line variant, TMX2-28, to study cell invasion in breast cancer. *Mol Cancer Res.* 2006; 4:905–913. [PubMed: 17189381]
8. Kato K, Shiozawa T, Mitsushita J, et al. Expression of the mitogen-inducible gene-2 (mig-2) is elevated in human uterine leiomyomas but not in leiomyosarcomas. *Hum Pathol.* 2004; 35:55–60. [PubMed: 14745725]
9. Weinstein EJ, Bournier M, Head R, et al. URP1: a member of a novel family of PH and FERM domain-containing membrane-associated proteins is significantly over-expressed in lung and colon carcinomas. *Biochim Biophys Acta.* 2003; 1637:207–216. [PubMed: 12697302]
10. Mackinnon AC, Qadota H, Norman KR, et al. *C. elegans* PAT-4/ILK functions as an adaptor protein within integrin adhesion complexes. *Curr Biol.* 2002; 12:787–797. [PubMed: 12015115]

11. Chishti AH, Kim AC, Marfatia SM, et al. The FERM domain: a unique module involved in the linkage of cytoplasmic proteins to the membrane. *Trends Biochem Sci.* 1998; 23:281–282. [PubMed: 9757824]
12. Kloecker S, Major MB, Calderwood DA, et al. The Kindler syndrome protein is regulated by transforming growth factor-beta and involved in integrin-mediated adhesion. *J Biol Chem.* 2004; 279:6824–6833. [PubMed: 14634021]
13. Calderwood DA, Yan B, de Pereda JM, et al. The phosphotyrosine binding (PTB)-like domain of talin activates integrins. *J Biol Chem.* 2002; 277:21749–21758. [PubMed: 11932255]
14. Tu Y, Wu S, Shi X, et al. Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation. *Cell.* 2003; 113:37–47. [PubMed: 12679033]
15. Shi X, Wu C. A suppressive role of mitogen inducible gene-2 in mesenchymal cancer cell invasion. *Mol Cancer Res.* 2008; 6:715–724. This study demonstrates that kindlin-2 (mig-2) influences the invasive properties of mesenchymal cancer cells. [PubMed: 18505917]
16. Moser M, Nieswandt B, Ussar S, et al. Kindlin-3 is essential for integrin activation and platelet aggregation. *Nat Med.* 2008; 14:325–330. This study describes the hemostatic defects in kindlin-3-deficient mice, showing that platelet aggregation in response to all agonists is absent and thrombus formation is blunted. The hemostatic defect is assigned to an absence of kindlin-3 binding to the cytoplasmic tail of the integrin $\beta 3$ subunit. [PubMed: 18278053]
17. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell.* 2002; 110:673–687. [PubMed: 12297042]
18. Ginsberg MH, Partridge A, Shattil SJ. Integrin regulation. *Curr Opin Cell Biol.* 2005; 17:509–516. [PubMed: 16099636]
19. Luo BH, Carman CV, Springer TA. Structural basis of integrin regulation and signalling. *Annu Rev Immunol.* 2007; 25:619–647. [PubMed: 17201681]
20. Shattil SJ, Newman PJ. Integrins: dynamic scaffolds for adhesion and signaling in platelets. *Blood.* 2004; 104:1606–1615. [PubMed: 15205259]
21. Ma YQ, Qin J, Plow EF. Platelet integrin $\alpha \text{IIb} \beta 3$: activation mechanisms. *J Thromb Haemost.* 2007; 5:1345–1352. [PubMed: 17635696]
22. Arnaout MA. Integrin structure: new twists and turns in dynamic cell adhesion. *Immunol Rev.* 2002; 186:125–146. [PubMed: 12234368]
23. Xiao T, Takagi J, Collier BS, et al. Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. *Nature.* 2004; 432:59–67. [PubMed: 15378069]
24. Zhu J, Luo BH, Xiao T, et al. Structure of a complete integrin ectodomain in a physiologic resting state and activation and deactivation by applied forces. *Mol Cell.* 2008; 32:849–861. This study provides the crystal structure of the ECD of integrin $\alpha \text{IIb} \beta 3$ and shows the transitions that occur during activation of the integrin. [PubMed: 19111664]
25. Vinogradova O, Velyvis A, Velyviene A, et al. A structural mechanism of integrin $\alpha \text{IIb} \beta 3$ ‘inside-out’ activation as regulated by its cytoplasmic face. *Cell.* 2002; 110:587–597. [PubMed: 12230976]
26. Ulmer TS, Yaspan B, Ginsberg MH, Campbell ID. NMR analysis of structure and dynamics of the cytosolic tails of integrin $\alpha \text{IIb} \beta 3$ in aqueous solution. *Biochemistry.* 2001; 40:7498–7508. [PubMed: 11412103]
27. Weljie AM, Hwang PM, Vogel HJ. Solution structures of the cytoplasmic tail complex from platelet integrin αIIb - and $\beta 3$ -subunits. *Proc Natl Acad Sci USA.* 2002; 99:5878–5883. [PubMed: 11983888]
28. Hughes PE, Diaz-Gonzalez F, Leong L, et al. Breaking the integrin hinge. A defined structural constraint regulates integrin signaling. *J Biol Chem.* 1996; 271:6571–6574. [PubMed: 8636068]
29. Ma YQ, Yang J, Pesho MM, et al. Regulation of Integrin $\alpha \text{IIb} \beta 3$ activation by distinct regions of its cytoplasmic tails. *Biochemistry.* 2006; 45:6656–6662. [PubMed: 16716076]
30. Ghevaert C, Salsmann A, Watkins NA, et al. A nonsynonymous SNP in the ITGB3 gene disrupts the conserved membrane-proximal cytoplasmic salt bridge in the $\alpha \text{IIb} \beta 3$ integrin and cosegregates dominantly with abnormal proplatelet formation and macrothrombocytopenia. *Blood.* 2008; 111:3407–3414. [PubMed: 18065693]

31. Imai Y, Park EJ, Peer D, et al. Genetic perturbation of the putative cytoplasmic membrane-proximal salt bridge aberrantly activates $\{\alpha\}$ 4 integrins. *Blood*. 2008; 112:5007–5015. [PubMed: 18809756]
32. Czuchra A, Meyer H, Legate KR, et al. Genetic analysis of beta1 integrin 'activation motifs' in mice. *J Cell Biol*. 2006; 174:889–899. [PubMed: 16954348]
33. Lau TL, Dua V, Ulmer TS. Structure of the integrin alphaIIb transmembrane segment. *J Biol Chem*. 2008; 283:16162–16168. [PubMed: 18417472]
34. Lau TL, Partridge AW, Ginsberg MH, Ulmer TS. Structure of the integrin beta3 transmembrane segment in phospholipid bicelles and detergent micelles. *Biochemistry*. 2008; 47:4008–4016. [PubMed: 18321071]
35. Luo BH, Carman CV, Takagi J, Springer TA. Disrupting integrin transmembrane domain heterodimerization increases ligand binding affinity, not valency or clustering. *Proc Natl Acad Sci U S A*. 2005; 102:3679–3684. [PubMed: 15738420]
36. Kim C, Lau TL, Ulmer TS, Ginsberg MH. Interactions of platelet integrin $\{\alpha\}$ IIb and $\{\beta\}$ 3 transmembrane domains in mammalian cell membranes and their role in integrin activation. *Blood*. 2009; 113:1347–1353.
37. Calderwood DA, Zent R, Grant R, et al. The Talin head domain binds to integrin beta subunit cytoplasmic tails and regulates integrin activation. *J Biol Chem*. 1999; 274:28071–28074. [PubMed: 10497155]
38. Tadokoro S, Shattil SJ, Eto K, et al. Talin binding to integrin β tails: a final common step in integrin activation. *Science*. 2003; 302:103–106. [PubMed: 14526080]
39. Petrich BG, Marchese P, Ruggeri ZM, et al. Talin is required for integrin-mediated platelet function in hemostasis and thrombosis. *J Exp Med*. 2007; 204:3103–3111. [PubMed: 18086863]
40. Nieswandt B, Moser M, Pleines I, et al. Loss of talin1 in platelets abrogates integrin activation, platelet aggregation, and thrombus formation in vitro and in vivo. *J Exp Med*. 2007; 204:3113–3118. [PubMed: 18086864]
41. Critchley DR, Gingras AR. Talin at a glance. *J Cell Sci*. 2008; 121:1345–1347. [PubMed: 18434644]
42. Wegener KL, Partridge AW, Han J, et al. Structural basis of integrin activation by talin. *Cell*. 2007; 128:171–182. [PubMed: 17218263]
43. Petrich BG, Fogelstrand P, Partridge AW, et al. The antithrombotic potential of selective blockade of talin-dependent integrin alpha(IIb)beta(3) (platelet GPIIb-IIIa) activation. *J Clin Invest*. 2007; 117:2250–2259. [PubMed: 17627302]
44. Kiema T, Lad Y, Jiang P, et al. The molecular basis of filamin binding to integrins and competition with talin. *Mol Cell*. 2006; 21:337–347. [PubMed: 16455489]
45. Ithychanda S, Das M, Ma YQ, et al. Migfilin: a molecular switch in regulation of integrin activation. *J Biol Chem*. 2009; 284:4713–4722. This study, together with the next reference, demonstrates the complex molecular interactions that occur to regulate integrin activation. [PubMed: 19074766]
46. Lad Y, Jiang P, Ruskamo S, et al. Structural basis of the migfilin-filamin interaction and competition with integrin beta tails. *J Biol Chem*. 2008; 283:35154–35163. This study, together with the preceding reference, demonstrates the complex molecular interactions that occur to regulate integrin activation. [PubMed: 18829455]
47. Goksoy E, Ma Y-Q, Wang X, et al. Structural basis for the autoinhibition of talin in regulating integrin activation. *Mol Cell*. 2008; 31:124–133. This study, together with the next reference, demonstrates how the intrinsic integrin-activating activity of talin is controlled by its conformation. [PubMed: 18614051]
48. Goult BT, Bate N, Anthis NJ, et al. The structure of an interdomain complex that regulates talin activity. *J Biol Chem*. 2009; 284:15097–15106. This study, together with the preceding reference, demonstrates how the intrinsic integrin-activating activity of talin is controlled by its conformation. [PubMed: 19297334]
49. Han J, Lim CJ, Watanabe N, et al. Reconstructing and deconstructing agonist-induced activation of integrin alphaIIbbeta3. *Curr Biol*. 2006; 16:1796–1806. [PubMed: 16979556]

50. Lee HS, Lim CJ, Puzon-McLaughlin W, et al. RIAM activates integrins by linking talin to ras GTPase membrane-targeting sequences. *J Biol Chem.* 2009; 284:5119–5127. [PubMed: 19098287]
51. Chen Y-P, Djaffar I, Pidard D, et al. Ser-752 -> Pro mutation in the cytoplasmic domain of integrin β_3 subunit and defective activation of platelet integrin $\alpha_{IIb}\beta_3$ (GPIIb-IIIa) in a variant of Glanzmann's thrombasthenia. *Proc Natl Acad Sci USA.* 1992; 89:10169–10173. [PubMed: 1438206]
52. Shi X, Ma YQ, Tu Y, et al. The mitogen inducible gene-2 (Mig-2)-integrin interaction strengthens cell-matrix adhesion and modulates cell motility. *J Biol Chem.* 2007; 282:20455–20466. [PubMed: 17513299]
- 53•. Ma YQ, Qin J, Wu C, Plow EF. Kindlin-2 (Mig-2): a co-activator of beta3 integrins. *J Cell Biol.* 2008; 181:439–446. This study, together with the following reference, establishes that kindlin-2 can cooperate with talin to induce integrin activation. This coactivator activity depends on its binding to the β subunit of integrins and involves not only on the FERM domain but also the N-terminal region of kindlin-2. [PubMed: 18458155]
- 54•. Montanez E, Ussar S, Schifferer M, et al. Kindlin-2 controls bidirectional signaling of integrins. *Genes Dev.* 2008; 22:1325–1330. This study establishes that kindlin-2 can cooperate with talin to induce inside-out integrin activation and also influence outside-in signaling across integrins. This coactivator activity of kindlin depends on its binding to the β subunit of integrins. [PubMed: 18483218]
- 55•. 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; 284:11485–11497. This study shows that both kindlin-1 and kindlin-2 bind to the cytoplasmic tails of integrin β subunits. Furthermore, influences of the kindlins on cell spreading independent of integrin binding are demonstrated. [PubMed: 19240021]
- 56•. Malinin NL, Zhang L, Choi J, et al. A point mutation in kindlin-3 ablates activation of three integrin subfamilies in humans. *Nature Med.* 2009; 15:313–318. This manuscript characterizes the symptoms and blood cell responses of two patients with IADD. β_1 , β_2 and β_3 integrins do not activate on the patients' blood cells, leading to bleeding, immune and bone defects. The defect is assigned to a single point mutation in kindlin-3. Re-expression of kindlin-3 restores integrin activation on patients' cells, whereas knockdown of kindlin-3 in normal cells causes integrin activation deficiency. [PubMed: 19234460]
- 57•. Ussar S, Moser M, Widmaier M, et al. Loss of Kindlin-1 causes skin atrophy and lethal neonatal intestinal epithelial dysfunction. *PLoS Genet.* 2008; 4:e1000289. This study describes the phenotype of the kindlin-1-deficient mouse. The symptoms recapitulate those observed frequently in Kindler disease in humans. [PubMed: 19057668]
- 58•. Dowling JJ, Gibbs E, Russell M, et al. Kindlin-2 is an essential component of intercalated discs and is required for vertebrate cardiac structure and function. *Circ Res.* 2008; 102:392–394. This study further investigates the cardiac phenotype in kindlin-2-deficient animals.
- 59•. Dowling JJ, Vreede AP, Kim S, et al. Kindlin-2 is required for myocyte elongation and is essential for myogenesis. *BMC Cell Biol.* 2008; 9:36. This study shows that kindlin-2 deficiency in zebrafish or mice is embryonically lethal. The phenotype implicates kindlin-2 in myogenesis. [PubMed: 18611274]
- 60•. Moser M, Bauer M, Schmid S, et al. Kindlin-3 is required for beta2 integrin-mediated leukocyte adhesion to endothelial cells. *Nat Med.* 2009; 15:300–305. This is a companion paper to [16•] and shows that responses that depend on activation of the β_2 integrins are blunted in the kindlin-3-deficient mice. Leukocytes in these mice and *ex vivo* are unable to firmly adhere and transigrate through endothelium. [PubMed: 19234461]
- 61•. Kruger M, Moser M, Ussar S, et al. SILAC mouse for quantitative proteomics uncovers kindlin-3 as an essential factor for red blood cell function. *Cell.* 2008; 134:353–364. This manuscript shows that the absence of kindlin-3 in mice causes a defect in erythrocyte shape, a function that may be unrelated to its interaction with integrins. [PubMed: 18662549]
- 62•. Svensson L, Howarth K, McDowall A, et al. Leukocyte adhesion deficiency-III is caused by mutations in KINDLIN3 affecting integrin activation. *Nat Med.* 2009; 15:306–312. This manuscript shows that mutations in kindlin-3 give rise to a disease in which integrins on

leukocytes and platelets fail to activate. The defect is overcome by expression of kindlin-3 but not by CALDAG-GEF1. [PubMed: 19234463]

- 63•. Kuijpers TW, van de Vijver E, Weterman MA, et al. LAD-1/variant syndrome is caused by mutations in FERMT3. *Blood*. 2009; 113:4740–4746. This study shows the presence of kindlin-3 mutations in LADI variant patients characterized by abnormal integrin activation on blood cells. [PubMed: 19064721]
64. Alon R, Etzioni A. LAD-III, a novel group of leukocyte integrin activation deficiencies. *Trends Immunol*. 2003; 24:561–566. [PubMed: 14552841]
65. McDowall A, Inwald D, Leitinger B, et al. A novel form of integrin dysfunction involving beta1, beta2, and beta3 integrins. *J Clin Invest*. 2003; 111:51–60.
66. Crittenden JR, Bergmeier W, Zhang Y, et al. CalDAG-GEFI integrates signaling for platelet aggregation and thrombus formation. *Nat Med*. 2004; 10:982–986. [PubMed: 15334074]
67. Bergmeier W, Goerge T, Wang HW, et al. Mice lacking the signaling molecule CalDAG-GEFI represent a model for leukocyte adhesion deficiency type III. *J Clin Invest*. 2007; 117:1699–1707. [PubMed: 17492052]

Figure 1.
Domain organization of a prototypic kindlin

Figure 2. Sequence alignment of the cytoplasmic tail of integrin β subunits

Residues in the $\beta 3$ cytoplasmic tail are numbered above and the four regions discussed in the text are numbered below. When conserved, these sequences are underlined in the other integrin β subunits. Regions 1 and 2 are implicated in talin binding, and regions 3 and 4 in kindlin binding.