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Regulation of integrin-mediated adhesions

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Abstract

Integrins are heterodimeric transmembrane adhesion receptors that couple the actin cytoskeleton to the extracellular environment and bidirectionally relay signals across the cell membrane. These processes are critical for cell attachment, migration, differentiation, and survival, and therefore play essential roles in metazoan development, physiology, and pathology. Integrin-mediated adhesions are regulated by diverse factors, including the conformation-specific affinities of integrin receptors for their extracellular ligands, the clustering of integrins and their intracellular binding partners into discrete adhesive structures, mechanical forces exerted on the adhesion, and the intracellular trafficking of integrins themselves. Recent advances shed light onto how the interaction of specific intracellular proteins with the short cytoplasmic tails of integrins controls each of these activities.

Introduction

All animal lineages express integrin adhesion receptors as noncovalently-linked heterodimeric combinations of α and β subunits, each of which is a type I transmembrane glycoprotein with a large extracellular domain and a typically short cytoplasmic tail [1]. Humans have 18 α and 8 β subunits that combine to give 24 different $\alpha\beta$ heterodimers. Integrin ectodomains bind specific extracellular matrix (ECM), cell surface, or soluble protein ligands and their cytoplasmic domains associate with cytoskeletal, signaling, and adaptor proteins [1–3]. Integrins thus permit dynamic bidirectional transmembrane signaling that is essential in cell adhesion, migration, differentiation, and survival.

Extensive studies have led to a good understanding of the structural basis of integrin ligand binding, identification of numerous cytoplasmic tail interacting proteins, appreciation of the diversity of integrin signaling and its impact on the cytoskeleton, and recognition of the important role integrins play in mechanotransduction and motility [1–3, 4•, 5, 6]. Furthermore, knock-out and knock-in studies have uncovered roles of integrins during

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development and, together with human mutations, highlight the importance of integrins in disease [7]. Indeed, integrin inhibitors are used to treat cardiovascular and autoimmune disease and are in trials for additional indications [8].

Given the vital roles of integrins, it is unsurprising that tight control mechanisms have evolved. Here we highlight recent advances in understanding integrin adhesion regulation, focusing primarily on how cytoplasmic integrin tail-binding proteins influence integrin affinity for ligands, integrin clustering, mechanotransduction, and integrin trafficking (Figure 1).

Integrin Conformational Activation

Integrin activation, the process by which integrin heterodimers undergo conformational changes to increase the affinity of the ectodomains for extracellular ligand, provides direct control of integrin-mediated adhesion [1,9]. A detailed discussion of the dynamics of integrin ectodomains is beyond the scope of this mini-review, but X-ray crystallography and electron microscopy studies reveal the molecular basis for integrin-ligand interactions and suggest that activation is coupled to conformational changes associated with ectodomain extension [1,4•]. However, the nature of the ectodomain rearrangements remains controversial and may vary between heterodimers [10]. Nonetheless, most studies agree that activation involves separation of integrin α - and β -subunit cytoplasmic and transmembrane domains (TMDs), which is triggered by talin binding to the β -subunit cytoplasmic tail [2,9,11]. Recent structural and functional studies of integrin α IIB β 3 TMDs [12–15], together with prior studies of the talin-integrin β -tail complex [11,16], have provided a detailed molecular model for integrin activation. Key to this was the understanding that the integrin β -subunit TMD crosses the membrane at an angle and associates with the α -subunit TMD at two sites within the membrane (Figure 2). Glycine packing interactions between the helical TMDs define the “outer membrane clasp,” while the “inner membrane clasp” is made possible because the conserved GFFKR motif at the cytoplasmic end of the α -subunit TMD induces a sharp turn towards the β -subunit, allowing the stacking of hydrophobic residues and facilitating formation of a membrane-proximal α IIB(D723)/ β 3(R995) salt bridge. A key β -subunit lysine residue embedded in the membrane “snorkels” its ϵ -amino group toward the phospholipid head groups of the inner membrane leaflet, contributing to the $\sim 25^\circ$ crossing angle of the longer β -subunit TMD and engaging the inhibitory α - β interactions [15]. Activation occurs when membrane-bound talin binds to a conserved NPLY motif in the integrin β 3-tail using a phosphotyrosine-binding-like domain within talin’s N-terminal atypical FERM domain [11,17,18] (Figure 2). This facilitates additional interactions between the membrane-proximal region of the β -tail and talin, replacing the α IIB(D723)/ β 3(R995) interaction with a new salt bridge between α IIB(D723) and talin-1(K324), which reorients the β -subunit TMD and disengages the inhibitory clasps [11,19,20]. This ultimately results in the transmission of conformational changes to the integrin ectodomains and increased affinity for extracellular ligands (Figure 1).

Although structural, biochemical, and genetic data point to talin as the key activator for most integrins [11,20], additional proteins contribute positively and negatively to integrin activation [3]. Among other activators, the kindlin family of atypical FERM and PH domain

containing proteins (kindlin-1, -2, and -3) has received the most attention [11,20]. Kindlins bind the membrane-distal NPxY motif of integrin β -tails and knockout, knockdown, mutagenesis, and human disease mutations confirm that kindlins perform critical roles in facilitating integrin-mediated adhesion. Biochemical studies indicate that kindlins cooperate with talin [11,20], however their mechanisms of action are less well understood than those of talin and it has been suggested that rather than (or in addition to) directly influencing conformational activation, kindlins promote integrin clustering [21••] or trafficking [22,23].

In addition to integrin activators such as talin and kindlin or the recently reported Zasp [24], inhibitors of activation have also been identified [25]. β -tail-binding proteins filamin [26] and ICAP1 [27••,28] compete with and displace activators and are the best-characterized inhibitors. Notably, migfilin competes with integrin for binding to filamin [29] and KRIT1 competes with integrin for binding to ICAP1 [27••], thus migfilin and KRIT1 function as inhibitors-of-inhibitors, restoring integrin activation. Inhibitory proteins may also directly stabilize the inactive clasped integrin conformation. Currently, the α -tail-binding SHARPIN is the only reported example of this mode of inhibition [30] but others may exist. Post-translational modification of integrin tails, or their binding partners, also impacts integrin activation, indeed, tyrosine phosphorylation of the talin-binding NP(I/L)Y motif reduces talin binding and activation [3,11]. Thus, while dramatic progress has been made in understanding integrin activation and inhibition, control of these processes still requires further study.

Adhesion maturation and Integrin Clustering

Whereas conformational integrin activation increases the affinity of individual receptors for ligands, increasing the avidity of the overall interaction by forming a multitude of bonds to multivalent substrates creates stronger cellular adhesions (Figure 1). The relative importance of clustering versus conformational activation has long been debated [31,32] but it is clear that integrins initially form transient (~1 minute lifetime) microclusters in nascent adhesions, which may mature into focal complexes and then into larger focal adhesions (~20 minute half-life), and eventually streaks of fibrillar adhesions [33]. Multivalent ECM ligands certainly drive integrin clustering [34], but the cell glycocalyx was also recently reported to make important contributions [35••]. In the latter case, bulky membrane-bound glycoproteins (which extend up to 80 nm beyond the plasma membrane) impede ECM access to integrins (only 20 nm long) in non-clustered regions, but favor clustering at pre-existing contacts where the membrane and ECM are already in close proximity. This kinetic trap effectively funnels integrins into existing adhesion clusters [35••]. The maturation and growth of integrin adhesions also coincides with the recruitment of a complex platform of scaffolding and signaling proteins that promote integrin clustering [33]. Indeed, seminal studies show that isolated integrin β -tails fused to irrelevant transmembrane and extracellular domains are sufficient for recruitment to existing adhesions [36]. A variety of approaches have revealed the large number of proteins directly or indirectly associated with integrins, the so-called adhesome, and imaging studies have identified constituents preferentially associated with specific adhesion structures [3,33,37,38]. Various integrin-associated cytoplasmic proteins have been implicated in promoting integrin clustering, including talin [39•], kindlin [21••], and α -actinin [40••,41•], but the molecular basis for this

clustering has not been elucidated. Notably, each of these proteins has also been implicated in conformational integrin activation and the two processes are likely linked, making experimental separation of the roles of conformational and micro-clustering in stimulating adhesion challenging. However, soluble monovalent and multivalent integrin ligands in suspended cell binding assays, together with fluorescence resonance energy transfer, high-resolution confocal microscopy, fluorescence fluctuation methods, mathematical models, and molecular dynamics simulations have been used to probe integrin clustering [21••, 34, 40••, 42].

In addition to its well characterized role in conformational activation, overexpression of talin induces $\alpha V\beta 3$ integrin clustering in cultured cells, apparently through a PI(4,5)P₂ lipid-binding interface on the F2 subdomain within the talin FERM domain [43]. Furthermore, studies suggest that for *Drosophila* integrins, the major role of talin is in clustering rather than affinity regulation [39•, 44]. Indeed, in *Drosophila*, a talin mutation that permits integrin β -tail binding but inhibits activation of mammalian integrins does not significantly impinge on talin function during development while another mutation which inhibits both activation of mammalian integrins and clustering of fly integrins produces much more severe phenotypes. The bases for these effects are uncertain but data suggest that defective clustering and defective adhesion reinforcement may be associated with altered intramolecular interactions between the integrin-binding F3 and the lipid-binding F2 subdomains within the talin FERM domain [39•]. How talin drives clustering is not clear but the talin dimer contains four potential integrin β -tail binding sites suggesting that talin may directly link up to four integrins [45••].

Despite its central role in integrin function, a new fluorescence correlation study reports that talin does not begin stably associating with nascent integrin adhesions until after integrin-kindlin interactions have occurred [40••]. Integrin and kindlin apparently associate as they enter the adhesion. Notably, in the absence of α -actinin talin associates with integrin earlier in nascent adhesions, raising the possibility that talin and α -actinin compete for binding to integrin [40••]. Others also suggest competition between talin and α -actinin for binding $\beta 3$ integrins (but not $\beta 1$ integrins) [41•]. However, they suggest talin-integrin interactions form early while force transmitted through α -actinin linkages supports later adhesion maturation. Additional work is required to resolve the different roles of integrin-binding proteins and the timing of their recruitment to adhesions.

The early association of integrin and kindlin in nascent adhesions is of interest in light of new data implicating kindlin in integrin clustering. How kindlins regulate integrins has been unclear, but using soluble monomeric or multimeric reporters Ye et al [21••] find that kindlins increase integrin $\alpha IIb\beta 3$ avidity for multivalent ligands rather than affinity for monovalent ligands. This requires kindlin-integrin interactions and immunogold electron microscopy supports a role for kindlins in clustering integrins. Kindlin-mediated clustering requires integrin-ECM binding suggesting that kindlins act primarily to cluster ligand-bound $\alpha IIb\beta 3$ integrins [21••], but whether kindlin-mediated clustering extends to other integrins remains to be seen. As kindlins are generally believed to be monomeric, clustering is likely to be driven via kindlin binding partners such as migfilin [46], which can recruit filamin [29], or integrin-linked kinase (ILK) [47•]. Kindlin-ILK interactions are important for

kindlin-mediated $\beta 1$ integrin activation assessed using soluble dimeric reporters [47•], but whether this is due to increased clustering remains to be determined.

Mechanical Forces

Clusters of ligand bound integrins in adhesions are linked to the actin cytoskeleton via integrin-associated actin-binding proteins, allowing bidirectional transmembrane force transmission (Figure 1). The roles of integrins in mechanosensing and mechanotransduction have received considerable interest in recent years and are extensively reviewed elsewhere [5,6,48,49]. In the context of adhesion regulation, forces applied to integrins, either via the ECM or through actomyosin-mediated intracellular tension, are likely to directly influence integrin-ligand interactions and conformational activation [50]. Although experiments using purified integrins embedded in nanodiscs reveal that force is not essential for talin-mediated $\alpha \text{IIb}\beta 3$ ectodomain extension [51], force-mediated remodeling of the $\alpha 5\beta 1$ -fibronectin bond allows formation of additional “synergy site” interactions that support catch-bond behavior, where lifetimes are extended by tension [52,53].

In addition to direct mechanical effects on integrins, pioneering work from the Sheetz lab highlighted the influence of forces on the growth, reinforcement, and actin linkage of adhesions [54,55]. Since then, innovative assays to measure traction forces, the movement of cytoskeletal components and forces across single molecules, and proteomic studies to identify tension regulated adhesome components, have enabled considerable progress in understanding mechanical coupling at adhesions [5,6,37,38,56]. Although many adhesion proteins have been implicated, much of the focus has been on the actin-binding proteins talin and vinculin, and to a lesser extent filamin and α -actinin.

Super-resolution microscopy shows that, in focal adhesions, talin orients with its N-terminal membrane- and integrin-binding FERM domain close to membrane-embedded integrins and its C-terminal actin-binding site deeper in the cell, proximal to F-actin [57]. Talin is thus positioned to transmit force to and from adhesions, although the nature of the talin linkage apparently varies between tissues [45••]. In-cell single-molecule analysis reveals cyclic myosin-mediated stretching of talin [58]. Structural studies of the vinculin-binding domains of talin combined with atomic force microscopy, magnetic tweezers, and molecular dynamics simulations suggest that partial mechanical unfolding of talin helical bundles is needed to permit vinculin binding to otherwise cryptic vinculin-binding sites [11,59,60]. Fluorescence-based, single-molecule force sensors show that vinculin itself is under tension at adhesions but that while vinculin recruitment to adhesions is force dependent, it does not require force transmission across vinculin [56]. Current models propose that force-mediated exposure of the vinculin-binding site on talin recruits vinculin, contributing to vinculin conformational activation facilitating F-actin binding (Figure 1). This allows reinforcement of adhesions and coupling of actin retrograde flow to focal adhesions [61].

Filamin provides another direct mechanically-regulated link between integrins and the actin cytoskeleton [62]. The major integrin-binding site in filamin is occluded by a portion of the adjacent filamin domain [63] and molecular dynamics simulations and single-molecule mechanical measurements [64–66] show that this region of filamin is a mechanosensing

domain and that tension along the filamin molecule can displace the inhibitory strand, increasing integrin binding. Although filamin is not observed in focal adhesions, it is required for breast epithelial cell reorganization of collagen matrices and increasing filamin-integrin interactions permits contraction of stiffer high-density matrices [67]. Thus, while filamin is an integrin-binding mechanosensing molecule its role in mechanical regulation of adhesion is not yet fully understood.

Likewise, little is known about role of α -actinin-integrin interactions in transducing force and influencing adhesion. Despite suggestions that α -actinin resides mostly in the actin zone of adhesions, separated from integrin cytoplasmic tails [57], recent studies demonstrate a role for α -actinin in force-dependent adhesion maturation [41•]. Unlike the situation for talin and filamin, a molecular basis for the response of α -actinin to force has not been determined.

Integrin Trafficking

Integrins are delivered to and from the plasma membrane via vesicular trafficking and it is now appreciated that the localized flux of integrins regulates cell migration, adhesion formation, turnover, and remodeling, as well as cross-talk between integrins and between growth factors and integrins. Due to space limitations, the complexity of integrin endocytic and exocytic recycling pathways, and the many recent detailed reviews of this topic [68–70], we only summarize some general concepts and highlight cross-talk between trafficking and other integrin regulatory mechanisms.

Integrins are internalized via both clathrin-independent (through caveolae and macropinocytosis) and clathrin-dependent mechanisms and are recycled via short or long loop pathways depending on integrin subunit and activation state (Figure 1) [69]. Microtubules can deliver clathrin-associated endocytic adaptors to adhesions where some (such as Numb or Dab2) may interact with NPxY motifs on integrin β -tails, driving endocytosis [3,68]. Consistent with this, loss of the endocytic factor dynamin-2 leads to increased surface integrin levels, but interestingly it also reduces integrin activation and interferes with crosstalk between growth-factors and integrins with severe consequences on angiogenesis [71]. Both active ligand-bound and inactive unbound integrins appear to be endocytosed through clathrin-mediated pathways, although NPxY-binding adaptors presumably displace the integrin activators talin and kindlin, and the large size of ECM components suggests that ECM proteolysis must precede endocytosis of ligand-bound integrins [70]. Following trafficking to early endosomes, sorting nexin-17 or -31 interactions with the β -tail membrane-distal kindlin-binding NPxY motif can drive rapid recycling of inactive integrins back to the plasma membrane [23,72,73]. Active ligand-bound integrins are instead routed to late endosomes and lysosomes but this does not necessarily lead to their degradation, rather the majority of these integrins may be recycled back to the plasma membrane via retrograde transport [74]. Ultimately, these dynamic processes regulate the maturation and architecture of integrin adhesions to control cell motility and migration.

Conclusions

Integrin adhesions are highly organized and dynamically regulated platforms that transduce mechanical and chemical cues between the cell and its environment. Regulation occurs at many levels and their interrelatedness is illustrated by observations that key players influence several different regulatory steps. There have been major recent advances in characterization of the adhesome and in understanding integrin conformational regulation, mechanotransduction, and trafficking, although important details and the bases for heterodimer specificities often remain unclear. Perhaps most elusive are the molecular mechanisms of integrin clustering in adhesions and the chronology of interactions during adhesion maturation. Ongoing model organism studies combined with mathematical modeling and novel cellular biophysical and imaging approaches promise further answers.

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References

1. Campbell ID, Humphries MJ. Integrin structure, activation, and interactions. *Cold Spring Harb Perspect Biol.* 2011; 3
2. Harburger DS, Calderwood DA. Integrin signalling at a glance. *J Cell Sci.* 2009; 122:159–163. [PubMed: 19118207]
3. Morse EM, Brahme NN, Calderwood DA. Integrin cytoplasmic tail interactions. *Biochemistry.* 2014; 53:810–820. [PubMed: 24467163]
4. Zhu J, Zhu J, Springer TA. Complete integrin headpiece opening in eight steps. *J Cell Biol.* 2013; 201:1053–1068. [PubMed: 23798730] By soaking RGD ligands into closed α IIb β 3 headpiece crystals under various conditions the authors reveal 8 distinct conformational steps along the integrin activation and ligand binding pathway.
5. Ross TD, Coon BG, Yun S, Baeyens N, Tanaka K, Ouyang M, Schwartz MA. Integrins in mechanotransduction. *Curr Opin Cell Biol.* 2013; 25:613–618. [PubMed: 23797029]
6. Plotnikov SV, Waterman CM. Guiding cell migration by tugging. *Curr Opin Cell Biol.* 2013; 25:619–626. [PubMed: 23830911]
7. Winograd-Katz SE, Fassler R, Geiger B, Legate KR. The integrin adhesome: from genes and proteins to human disease. *Nat Rev Mol Cell Biol.* 2014; 15:273–288. [PubMed: 24651544]
8. Goodman SL, Picard M. Integrins as therapeutic targets. *Trends Pharmacol Sci.* 2012; 33:405–412. [PubMed: 22633092]
9. Shattil SJ, Kim C, Ginsberg MH. The final steps of integrin activation: the end game. *Nat Rev Mol Cell Biol.* 2010; 11:288–300. [PubMed: 20308986]
10. Xiong JP, Mahalingham B, Alonso JL, Borrelli LA, Rui X, Anand S, Hyman BT, Rysiok T, Muller-Pompalla D, Goodman SL, et al. Crystal structure of the complete integrin α v β 3 ectodomain plus an α / β transmembrane fragment. *J Cell Biol.* 2009; 186:589–600. [PubMed: 19704023]
11. Calderwood DA, Campbell ID, Critchley DR. Talins and kindlins: partners in integrin-mediated adhesion. *Nat Rev Mol Cell Biol.* 2013; 14:503–517. [PubMed: 23860236]
12. Lau TL, Kim C, Ginsberg MH, Ulmer TS. The structure of the integrin α IIb β 3 transmembrane complex explains integrin transmembrane signalling. *EMBO J.* 2009; 28:1351–1361. [PubMed: 19279667]
13. Zhu J, Luo BH, Barth P, Schonbrun J, Baker D, Springer TA. The structure of a receptor with two associating transmembrane domains on the cell surface: integrin α IIb β 3. *Mol Cell.* 2009; 34:234–249. [PubMed: 19394300]

14. Yang J, Ma YQ, Page RC, Misra S, Plow EF, Qin J. Structure of an integrin α IIB β 3 transmembrane-cytoplasmic heterocomplex provides insight into integrin activation. *Proc Natl Acad Sci U S A*. 2009; 106:17729–17734. [PubMed: 19805198]
15. Kim C, Schmidt T, Cho EG, Ye F, Ulmer TS, Ginsberg MH. Basic amino-acid side chains regulate transmembrane integrin signalling. *Nature*. 2012; 481:209–213.
16. Anthis NJ, Wegener KL, Ye F, Kim C, Goult BT, Lowe ED, Vakonakis I, Bate N, Critchley DR, Ginsberg MH, et al. The structure of an integrin/talin complex reveals the basis of inside-out signal transduction. *EMBO J*. 2009; 28:3623–3632. [PubMed: 19798053]
17. Goult BT, Bouaouina M, Elliott PR, Bate N, Patel B, Gingras AR, Grossmann JG, Roberts GC, Calderwood DA, Critchley DR, et al. Structure of a double ubiquitin-like domain in the talin head: a role in integrin activation. *EMBO J*. 2010; 29:1069–1080. [PubMed: 20150896]
18. Tadokoro S, Shattil SJ, Eto K, Tai V, Liddington RC, de Pereda JM, Ginsberg MH, Calderwood DA. Talin binding to integrin β tails: a final common step in integrin activation. *Science*. 2003; 302:103–106. [PubMed: 14526080]
19. Kim C, Ye F, Hu X, Ginsberg MH. Talin activates integrins by altering the topology of the β transmembrane domain. *Journal of Cell Biology*. 2012; 197:605–611. [PubMed: 22641344]
20. Ye F, Snider AK, Ginsberg MH. Talin and kindlin: the one-two punch in integrin activation. *Front Med*. 2014; 8:6–16. [PubMed: 24477625]
21. Ye F, Petrich BG, Anekal P, Lefort CT, Kasirer-Friede A, Shattil SJ, Ruppert R, Moser M, Fassler R, Ginsberg MH. The mechanism of kindlin-mediated activation of integrin α IIB β 3. *Curr Biol*. 2013; 23:2288–2295. [PubMed: 24210614] Using single-integrin-containing membrane nanodiscs, flow cytometry with mono- and multi-valent ligands, TIRF, and electron microscopy, this report provides the first detailed analysis of kindlin function showing that rather than mediating conformational activation of α IIB β 3 it instead drives clustering of ligand-bound integrins.
22. Margadant C, Kreft M, de Groot DJ, Norman JC, Sonnenberg A. Distinct Roles of Talin and Kindlin in Regulating Integrin α 5 β 1 Function and Trafficking. *Current Biology*. 2012; 22:1554–1563. [PubMed: 22795696]
23. Bottcher RT, Stremmel C, Meves A, Meyer H, Widmaier M, Tseng HY, Fassler R. Sorting nexin 17 prevents lysosomal degradation of β 1 integrins by binding to the β 1-integrin tail. *Nat Cell Biol*. 2012; 14:584–592. [PubMed: 22561348]
24. Bouaouina M, Jani K, Long JY, Czerniecki S, Morse EM, Ellis SJ, Tanentzapf G, Schock F, Calderwood DA. Zasp regulates integrin activation. *J Cell Sci*. 2012; 125:5647–5657. [PubMed: 22992465]
25. Bouvard D, Pouwels J, De Franceschi N, Ivaska J. Integrin inactivators: balancing cellular functions in vitro and in vivo. *Nat Rev Mol Cell Biol*. 2013; 14:430–442. [PubMed: 23719537]
26. Kiema T, Lad Y, Jiang P, Oxley CL, Baldassarre M, Wegener KL, Campbell ID, Ylanne J, Calderwood DA. The molecular basis of filamin binding to integrins and competition with talin. *Mol Cell*. 2006; 21:337–347. [PubMed: 16455489]
27. Liu W, Draheim KM, Zhang R, Calderwood DA, Boggon TJ. Mechanism for KRIT1 release of ICAP1-mediated suppression of integrin activation. *Mol Cell*. 2013; 49:719–729. [PubMed: 23317506] X-ray crystallographic studies of the ICAP1- β 1 integrin tail complex and of the ICAP-1-KRIT1 complex reveal binding at integrins and KRIT1 bind ICAP1 at overlapping sites. Over-expression, knockdown and structure-guided mutagenesis approaches are used to establish that ICAP1-mediated suppression of β 1 integrin activation can be reversed by KRIT1.
28. Millon-Fremillon A, Bouvard D, Grichine A, Manet-Dupe S, Block MR, Albiges-Rizo C. Cell adaptive response to extracellular matrix density is controlled by ICAP-1-dependent β 1-integrin affinity. *J Cell Biol*. 2008; 180:427–441. [PubMed: 18227284]
29. Lad Y, Jiang P, Ruskamo S, Harburger DS, Ylanne J, Campbell ID, Calderwood DA. Structural basis of the migfilin-filamin interaction and competition with integrin β tails. *J Biol Chem*. 2008; 283:35154–35163. [PubMed: 18829455]
30. Rantala JK, Pouwels J, Pellinen T, Veltel S, Laasola P, Mattila E, Potter CS, Duffy T, Sundberg JP, Kallioniemi O, et al. SHARPIN is an endogenous inhibitor of β 1-integrin activation. *Nat Cell Biol*. 2011; 13:1315–1324. [PubMed: 21947080]

31. Carman CV, Springer TA. Integrin avidity regulation: are changes in affinity and conformation underemphasized? *Curr Opin Cell Biol.* 2003; 15:547–556. [PubMed: 14519389]
32. Bazzoni G, Hemler ME. Are changes in integrin affinity and conformation overemphasized? *Trends Biochem Sci.* 1998; 23:30–34. [PubMed: 9478133]
33. Geiger B, Yamada KM. Molecular architecture and function of matrix adhesions. *Cold Spring Harb Perspect Biol.* 2011; 3
34. Kim M, Carman CV, Yang W, Salas A, Springer TA. The primacy of affinity over clustering in regulation of adhesiveness of the integrin α L β 2. *J Cell Biol.* 2004; 167:1241–1253. [PubMed: 15611342]
35. Paszek MJ, DuFort CC, Rossier O, Bainer R, Mouw JK, Godula K, Hudak JE, Lakins JN, Wijekoon AC, Cassereau L, et al. The cancer glycocalyx mechanically primes integrin-mediated growth and survival. *Nature.* 2014; 511:319–325. [PubMed: 25030168] Building on a computational model of glycoprotein and transmembrane receptor organization, the authors show that by separating integrins from ECM ligands, bulky glycocalyx molecules, such as MUC1, can kinetically trap integrins in pre-existing adhesions—favoring integrin clustering.
36. LaFlamme SE, Akiyama SK, Yamada KM. Regulation of fibronectin receptor distribution. *J Cell Biol.* 1992; 117:437–447. [PubMed: 1373145]
37. Kuo JC, Han X, Hsiao CT, Yates JR 3rd, Waterman CM. Analysis of the myosin-II-responsive focal adhesion proteome reveals a role for β -Pix in negative regulation of focal adhesion maturation. *Nat Cell Biol.* 2011; 13:383–393. [PubMed: 21423176]
38. Schiller HB, Friedel CC, Boulegue C, Fassler R. Quantitative proteomics of the integrin adhesome show a myosin II-dependent recruitment of LIM domain proteins. *Embo Reports.* 2011; 12:259–266. [PubMed: 21311561]
39. Ellis SJ, Lostchuck E, Goult BT, Bouaouina M, Fairchild MJ, Lopez-Ceballos P, Calderwood DA, Tanentzapf G. The Talin Head Domain Reinforces Integrin-Mediated Adhesion by Promoting Adhesion Complex Stability and Clustering. *Plos Genetics.* 2014; 10 In this report, the authors demonstrate that in *Drosophila*, talin-integrin interactions are more important for driving integrin clustering than promoting conformational activation through biochemical analyses and developmental studies of a series of targeted talin mutations.
40. Bachir AI, Zareno J, Moissoglu K, Plow EF, Gratton E, Horwitz AR. Integrin-associated complexes form hierarchically with variable stoichiometry in nascent adhesions. *Curr Biol.* 2014; 24:1845–1853. [PubMed: 25088556] In this article, fluorescence fluctuation methods are used to time-resolve and quantify the hierarchical appearance of talin, kindlin, α -actinin, and other integrin adhesion adaptors during the formation and maturation of integrin adhesions.
41. Roca-Cusachs P, del Rio A, Puklin-Faucher E, Gauthier NC, Biais N, Sheetz MP. Integrin-dependent force transmission to the extracellular matrix by α -actinin triggers adhesion maturation. *Proc Natl Acad Sci U S A.* 2013; 110:E1361–E1370. [PubMed: 23515331] This study utilizes TIRF microscopy and magnetic and optical tweezer experiments in cells to investigate cytoskeletal forces on integrin adhesions, reporting that α -actinin is a key linker for mechanotransduction and competes with talin for β 3 integrins in maturing adhesions.
42. Felizzi F, Iber D. Integrin clustering as a result of local membrane deformations and local signaling feedbacks. *Physica a-Statistical Mechanics and Its Applications.* 2014; 408:198–211.
43. Saltel F, Mortier E, Hytonen VP, Jacquier MC, Zimmermann P, Vogel V, Liu W, Wehrle-Haller B. New PI(4,5)P₂- and membrane proximal integrin-binding motifs in the talin head control β 3-integrin clustering. *J Cell Biol.* 2009; 187:715–731. [PubMed: 19948488]
44. Helsten TL, Bunch TA, Kato H, Yamanouchi J, Choi SH, Jannuzzi AL, Feral CC, Ginsberg MH, Brower DL, Shattil SJ. Differences in regulation of *Drosophila* and vertebrate integrin affinity by talin. *Molecular Biology of the Cell.* 2008; 19:3589–3598. [PubMed: 18508915]
45. Klapholz B, Herbert SL, Wellmann J, Johnson R, Parsons M, Brown NH. Alternative mechanisms for talin to mediate integrin function. *Curr Biol.* 2015; 25:847–857. [PubMed: 25754646] Investigating a series of talin mutants in three different *Drosophila* tissues reveals that distinct sets of talin domains are required for different integrin functions during *Drosophila* development and that in some, but not all, cases the partial function of talin mutants relies on vinculin binding. Three different, tissue specific, modes of talin function are proposed.

46. Brahme NN, Harburger DS, Kemp-O'Brien K, Stewart R, Raghavan S, Parsons M, Calderwood DA. Kindlin binds migfilin tandem LIM domains and regulates migfilin focal adhesion localization and recruitment dynamics. *J Biol Chem.* 2013; 288:35604–35616. [PubMed: 24165133]
47. Huet-Calderwood C, Brahme NN, Kumar N, Stiegler AL, Raghavan S, Boggon TJ, Calderwood DA. Differences in binding to the ILK complex determines kindlin isoform adhesion localization and integrin activation. *J Cell Sci.* 2014; 127:4308–4321. [PubMed: 25086068] Kindlin-2 and kindlin-3 differ in their ability to target to focal adhesions and activate $\alpha 5\beta 1$ integrins. Here, the authors use chimeric kindlins to show that this correlates with differences in ILK binding and localise the ILK-binding site in kindlin. They further show that kindlin-ILK interactions are important for kindlin-mediated $\beta 1$ integrin activation.
48. Schiller HB, Fassler R. Mechanosensitivity and compositional dynamics of cell-matrix adhesions. *EMBO Rep.* 2013; 14:509–519. [PubMed: 23681438]
49. Roca-Cusachs P, Iskratsch T, Sheetz MP. Finding the weakest link: exploring integrin-mediated mechanical molecular pathways. *J Cell Sci.* 2012; 125:3025–3038. [PubMed: 22797926]
50. Zhu J, Luo BH, Xiao T, Zhang C, Nishida N, Springer TA. Structure of a complete integrin ectodomain in a physiologic resting state and activation and deactivation by applied forces. *Mol Cell.* 2008; 32:849–861. [PubMed: 19111664]
51. Ye F, Hu G, Taylor D, Ratnikov B, Bobkov AA, McLean MA, Sligar SG, Taylor KA, Ginsberg MH. Recreation of the terminal events in physiological integrin activation. *J Cell Biol.* 2010; 188:157–173. [PubMed: 20048261]
52. Kong F, Garcia AJ, Mould AP, Humphries MJ, Zhu C. Demonstration of catch bonds between an integrin and its ligand. *J Cell Biol.* 2009; 185:1275–1284. [PubMed: 19564406]
53. Friedland JC, Lee MH, Boettiger D. Mechanically Activated Integrin Switch Controls $\alpha 5\beta 1$ Function. *Science.* 2009; 323:642–644. [PubMed: 19179533]
54. Galbraith CG, Yamada KM, Sheetz MP. The relationship between force and focal complex development. *Journal of Cell Biology.* 2002; 159:695–705. [PubMed: 12446745]
55. Choquet D, Felsenfeld DP, Sheetz MP. Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. *Cell.* 1997; 88:39–48. [PubMed: 9019403]
56. Grashoff C, Hoffman BD, Brenner MD, Zhou R, Parsons M, Yang MT, McLean MA, Sligar SG, Chen CS, Ha T, et al. Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. *Nature.* 2010; 466:263–266. [PubMed: 20613844]
57. Kanchanawong P, Shtengel G, Pasapera AM, Ramko EB, Davidson MW, Hess HF, Waterman CM. Nanoscale architecture of integrin-based cell adhesions. *Nature.* 2010; 468:580–584. [PubMed: 21107430]
58. Margadant F, Chew LL, Hu X, Yu H, Bate N, Zhang X, Sheetz M. Mechanotransduction In Vivo by Repeated Talin Stretch-Relaxation Events Depends upon Vinculin. *Plos Biology.* 2011; 9
59. Del Rio A, Perez-Jimenez R, Liu R, Roca-Cusachs P, Fernandez JM, Sheetz M. Stretching Single Talin Rod Molecules Activates Vinculin Binding. *Science.* 2009; 323:638–641. [PubMed: 19179532]
60. Yao MX, Goult BT, Chen H, Cong PW, Sheetz MP, Yan J. Mechanical activation of vinculin binding to talin locks talin in an unfolded conformation. *Scientific Reports.* 2014; 4
61. Thievensen I, Thompson PM, Berlemont S, Plevock KM, Plotnikov SV, Zemljic-Harpf A, Ross RS, Davidson MW, Danuser G, Campbell SL, et al. Vinculin-actin interaction couples actin retrograde flow to focal adhesions, but is dispensable for focal adhesion growth. *J Cell Biol.* 2013; 202:163–177. [PubMed: 23836933]
62. Razinia Z, Makela T, Ylanne J, Calderwood DA. Filamins in mechanosensing and signaling. *Annu Rev Biophys.* 2012; 41:227–246. [PubMed: 22404683]
63. Lad Y, Kiema T, Jiang P, Pentikainen OT, Coles CH, Campbell ID, Calderwood DA, Ylanne J. Structure of three tandem filamin domains reveals auto-inhibition of ligand binding. *EMBO J.* 2007; 26:3993–4004. [PubMed: 17690686]
64. Rognoni L, Stigler J, Pelz B, Ylanne J, Rief M. Dynamic force sensing of filamin revealed in single-molecule experiments. *Proc Natl Acad Sci U S A.* 2012; 109:19679–19684. [PubMed: 23150587]

65. Ehrlicher AJ, Nakamura F, Hartwig JH, Weitz DA, Stossel TP. Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A. *Nature*. 2011; 478:260–263. [PubMed: 21926999]
66. Chen H, Chandrasekar S, Sheetz MP, Stossel TP, Nakamura F, Yan J. Mechanical perturbation of filamin A immunoglobulin repeats 20–21 reveals potential non-equilibrium mechanochemical partner binding function. *Sci Rep*. 2013; 3:1642. [PubMed: 23571456]
67. Gehler S, Baldassarre M, Lad Y, Leight JL, Wozniak MA, Riching KM, Eliceiri KW, Weaver VM, Calderwood DA, Keely PJ. Filamin A- β 1 integrin complex tunes epithelial cell response to matrix tension. *Mol Biol Cell*. 2009; 20:3224–3238. [PubMed: 19458194]
68. De Franceschi N, Hamidi H, Alanko J, Sahgal P, Ivaska J. Integrin traffic - the update. *J Cell Sci*. 2015; 128:839–852. [PubMed: 25663697]
69. Bridgewater RE, Norman JC, Caswell PT. Integrin trafficking at a glance. *J Cell Sci*. 2012; 125:3695–3701. [PubMed: 23027580]
70. Valdembrì D, Serini G. Regulation of adhesion site dynamics by integrin traffic. *Curr Opin Cell Biol*. 2012; 24:582–591. [PubMed: 22981739]
71. Lee MY, Skoura A, Park EJ, Landskroner-Eiger S, Jozsef L, Luciano AK, Murata T, Pasula S, Dong Y, Bouaouina M, et al. Dynamin 2 regulation of integrin endocytosis, but not VEGF signaling, is crucial for developmental angiogenesis. *Development*. 2014; 141:1465–1472. [PubMed: 24598168]
72. Tseng HY, Thoraus N, Ziegler T, Meves A, Fassler R, Bottcher RT. Sorting nexin 31 binds multiple beta integrin cytoplasmic domains and regulates β 1 integrin surface levels and stability. *J Mol Biol*. 2014; 426:3180–3194. [PubMed: 25020227]
73. Steinberg F, Heesom KJ, Bass MD, Cullen PJ. SNX17 protects integrins from degradation by sorting between lysosomal and recycling pathways. *J Cell Biol*. 2012; 197:219–230. [PubMed: 22492727]
74. Dozynkiewicz MA, Jamieson NB, Macpherson I, Grindlay J, van den Berghe PV, von Thun A, Morton JP, Gourley C, Timpson P, Nixon C, et al. Rab25 and CLIC3 collaborate to promote integrin recycling from late endosomes/lysosomes and drive cancer progression. *Dev Cell*. 2012; 22:131–145. [PubMed: 22197222]

Figure 1. Partial summary of the major mechanisms regulating integrin-mediated cell adhesion
 Talin-binding conformationally activates transmembrane integrin adhesion receptors to increase their affinity for extracellular ligand and promotes integrin clustering for high-avidity adhesions to the extracellular matrix. The talin rod domain binds actin filaments to initially enable cytoskeletal force transduction onto integrin adhesions, revealing vinculin-binding sites on talin to promote additional cytoskeletal interactions and reinforcing the adhesion, along with many additional adhesome proteins (grey circles). Both inactive and active ligand-bound integrins may be internalized by endocytic adaptor proteins (triangles) via clathrin-dependent internalization, shuttling through short-loop or long-loop endosomal recycling pathways, although clathrin-independent trafficking also occurs (not depicted).

Figure 2. Conformational Integrin Activation

On the left, the bent and inactive conformation of $\alpha\text{IIb}\beta 3$ integrin is held in place by transmembrane interactions between the α - and β - subunits. The Outer Membrane Clasp (OMC) consists of glycine packing interactions while the Inner Membrane Clasp (IMC) uses hydrophobic stacking residues to promote the formation of an $\alpha\text{IIb(D723)}/\beta 3(\text{R995})$ salt bridge. A snorkeling $\beta 3$ lysine residue produces a 25° helical tilt which embeds this helix at an angle through the membrane. On the right, conformational activation is initiated when the FERM domain of talin binds acidic lipid head groups as well as the $\beta 3$ -tail NPLY motif,

breaking the IMC salt bridge and forming a new salt bridge between the $\beta 3$ tail and talin, increasing the crossing angle of the $\beta 3$ transmembrane helix and slightly extending it into the cytosol. This disengages the OMC, eliciting conformational changes in the integrin ectodomains and promoting high-affinity interactions with extracellular ligands.

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