The final steps of integrin activation: the end game

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Abstract | Cell-directed changes in the ligand-binding affinity ('activation') of integrins regulate cell adhesion and migration, extracellular matrix assembly and mechanotransduction, thereby contributing to embryonic development and diseases such as atherothrombosis and cancer. Integrin activation comprises triggering events, intermediate signalling events and, finally, the interaction of integrins with cytoplasmic regulators, which changes an integrin's affinity for its ligands. The first two events involve diverse interacting signalling pathways, whereas the final steps are immediately proximal to integrins, thus enabling integrin-focused therapeutic strategies. Recent progress provides insight into the structure of integrin transmembrane domains, and reveals how the final steps of integrin activation are mediated by integrin-binding proteins such as talins and kindlins.

Valency
A term that refers to the number of chemical bonds between two atoms. Here it refers to the number of integrin-binding sites presented by a given adhesive ligand.

Microcluster
A loosely defined term that refers to a non-covalent oligomer of integrins on the cell surface that appears as a point source (that is, with a diameter < 100nm) in fluorescence microscopy.

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Integrins play central roles in the biology of metazoa[1] by controlling cell adhesion to the extracellular matrix (ECM) and cell migration, growth, differentiation and apoptosis. As a result, they contribute to the regulation of development, immunity, inflammation and haemostasis, and to the development of diseases including autoimmunity, atherothrombosis and neoplasia[1]. Integrins are heterodimers of transmembrane α- and β-subunits[1], which each have a large ectodomain, a single transmembrane domain and a generally short cytoplasmic tail (BOX 1). Integrin affinities for their cognate extracellular ligands, such as fibronectin, fibrinogen and collagen, are regulated by cellular signalling, resulting in integrin activation through 'inside–out' signalling[1] (BOX 2). Consequently, integrin activation is important for a wide range of anchorage-dependent cellular events, such as platelet aggregation and leukocyte transmigration[1]. In addition to changes in adhesion, integrin activation can control the polarity of migrating cells and the assembly of the ECM, thereby regulating events such as tumour metastasis[2]. Blockade of integrin activation may therefore be useful in anti-adhesive therapies[3]. The broad biological and potentially therapeutic significance of integrin activation, and interest in this prototype of inside–out signalling, give rise to a fertile field of investigation. Here, we summarize recent progress and controversies in the study of integrin activation, focusing on the terminal events that lead to activation; that is, the 'end game'. We do not consider the ability of integrins to signal into cells ('outside–in' signalling; BOX 2); instead, we emphasize recent advances that identify the cytoplasmic partners that trigger integrin activation, begin to explain how the association of these partners with integrins is regulated by signalling events, explain how these binding interactions activate integrins and identify transmembrane domain structural features that account for the ability of integrins to efficiently transmit signals across cell membranes.

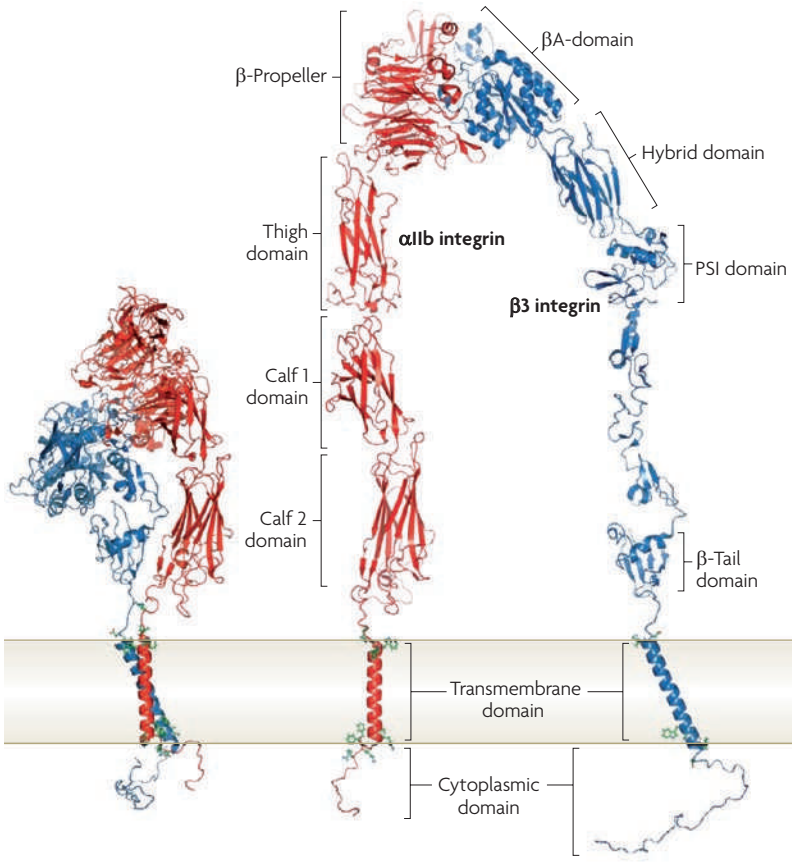
Roles of conformation and clustering

Changes in the conformation of individual integrin heterodimers and clustering of heterodimers into oligomers can influence the binding of ligands[4], the former through changes in single receptor affinity and the latter through increases in receptor valency that accompany integrin clustering[4]. Vigorous debate centres on the relative importance of each mechanism to integrin function[4,5]. Clarification of this debate has been hampered by inexact definitions of clustering and difficulties in quantifying integrin microclusters[4]. Conformational change and clustering are both likely to be important for integrin function, and their relative contributions might vary depending on the integrin, cell type and biological circumstances. For some integrins in circulating blood cells, such as αIIbβ3 integrin in platelets, changes in receptor conformation are the primary means of regulating receptor affinity and ligand binding in response to agonists.

Conformational changes. Much debate surrounds the nature of the extracellular domain conformational changes that underlie integrin activation and several excellent reviews have been devoted to this topic, which is not a main focus of this Review. In brief, integrin ectodomains

Box 1 | **Integrin domain structures**

Integrins are heterodimeric adhesive receptors consisting of an α- and a β-subunit. In mammals, there are 24 canonical integrins formed from combinations of 18 α-subunits and 8 β-subunits. The 'bent conformation' seen in crystal structures (see the figure; left) can be unfolded to facilitate visualization of the domains (see the figure; right). In most integrins the amino-terminal domain in the α- and β-integrin subunits (the β-propeller and the βA domain, respectively), assemble by non-covalent interactions to form a 'head' and provide a ligand binding site. In 8 α-integrin subunits (α1, α2, α10, α11, αL, αM, αX and αD), the αA domain, which is homologous to the βA domain of the β-integrin subunit, is inserted into the β-propeller domain and is the main ligand-binding site in these integrins. In integrins that lack an A domain, such as αIIbβ3 integrin, which is depicted here, the βA domain forms the main ligand-binding site. Note that the plexin, semaphorin and integrin (PSI) domain is at the N terminus of the β-integrin subunit, but is joined by disulphide bonds to more carboxy-terminal residues. The remaining C-terminal extracellular domains of each subunit comprise two long 'legs'. The low affinity state of the integrin for its ligands is maintained by non-covalent interactions between the α- and β-integrin transmembrane and cytoplasmic domains. Figure is modified, with permission, from *EMBO J* REF. 44 © (2009) Macmillan Publishers Ltd. All rights reserved.



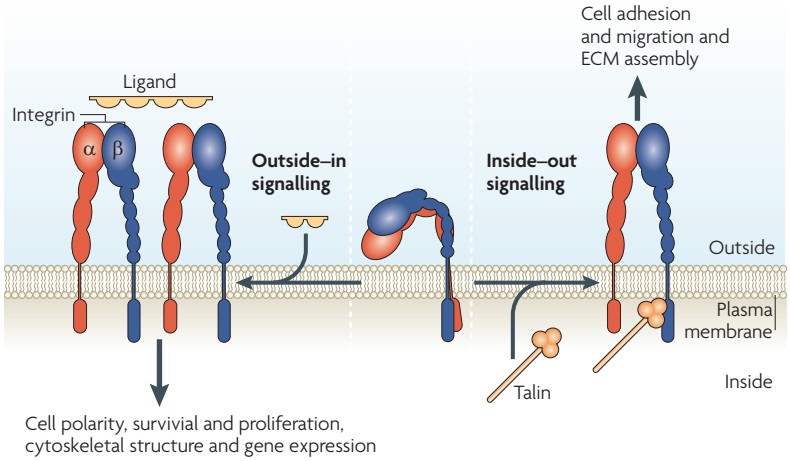
can exist in bent 'closed' conformations, intermediate extended conformations with a closed head-piece, and extended 'open' conformations[6,7]. These may correspond to low affinity, activated, and activated and ligand occupied integrin conformers[8], respectively, on cells[9,10]. The bent form can, in some circumstances, engage ligands such as fibronectin fragments[11]. This result is consistent with the idea that activation involves releasing a 'deadbolt' formed by an interface in the β-integrin subunit between the membrane-proximal β-tail domain and the α7 helix in the ligand-binding βA domain[11,12] (BOX 1). Downwards displacement of this α7 helix leads to conformational activation of the βA domain[13,14]; thus, the deadbolt was proposed to prevent this displacement and block activation[11,12]. However, a structure of the αIIbβ3 integrin ectodomain lacked the deadbolt interface[15]. Furthermore, deletion of the loop connecting helices C and D (CD loop) in the β-tail domain, proposed to form the deadbolt, failed to activate αIIbβ3 integrin or αVβ3 integrin[16], casting further doubt on the deadbolt hypothesis. The conformations of integrins have been studied in isolated ectodomains, or fragments of these, potentially leading to artefacts owing to the release of constraints imposed by interactions of cytoplasmic or transmembrane domains[17]. Electron cryomicroscopy studies of intact integrins in detergent micelles provide insights[18]. However, the structure of the β3 integrin transmembrane domain can differ between detergent micelles and phospholipid bilayers[19]; the latter more accurately mimics biological membranes. Moreover, image selection bias is a potential problem in electron microscopy studies, and has been invoked[11] as an explanation for discrepancies[20]. Electron cryomicroscopy tomography of lipid-embedded αIIbβ3 integrin revealed an average height of 11 nm, much less than the 19 nm height expected of a fully extended integrin[6,21]. Furthermore, addition of Mn^{2+}, which activates integrins directly by interaction with cation coordination sites in the βA domain, did not change the height, indicating a lack of extension[21] — a result in agreement with Förster resonance energy transfer (FRET) studies of αVβ3 integrin in living cells[22]. Steered molecular dynamic modelling[15,23] and experimental studies[24,25] suggest that force can contribute to integrin activation and extension. Furthermore, extension of unoccupied integrins may require either traction forces or collision with other membrane proteins[15]. It seems likely that a resolution of some of these hotly debated issues must take into account the relative strengths of negative stain electron microscopy versus electron cryomicroscopy[26], and awaits structural studies of a lipid-embedded, full-length integrin activated in a physiologically relevant manner.

Clustering. Integrin clustering is defined as the interaction of heterodimers to form hetero-oligomers. It can be caused by inside–out signals that stimulate the recruitment of multivalent protein complexes to integrin cytoplasmic domains[27,28,29], by binding of multivalent extracellular ligands to integrin ectodomains by the homodimerization of integrin transmembrane domains (α-to-α or β-to-β)[30], or by the release of integrins from cytoskeletal constraints that leads to the free diffusion of integrins in the plane of the membrane[31]. Whatever the contribution to the binding of fibronectin or other adhesive ligands, integrin clustering is important for triggering outside–in signalling, integrin recycling[32] and mechanotransduction by adhesion-based intracellular structures that contain integrins and associated molecules[33]. These intracellular structures include focal complexes and focal adhesions[34] in adherent fibroblasts, immunological synapses and kinapses in activated T lymphocytes[35], podosomes in adherent osteoclasts and macrophages, and invadopodia in cancer cells[36]. Technical issues currently limit the separation of integrin clustering from conformational change.

Detergent micelle
A globular aggregate of amphipathic detergent in aqueous solution, with detergent hydrophilic ends facing outside and hydrophobic ends facing inside.

Box 2 | **Bidirectional integrin signalling**

There are two directions of integrin signalling, which have different biological consequences (see the figure). During 'inside–out' signalling, an intracellular activator, such as talin or kindlins, binds to the β-integrin tail, leading to conformational changes that result in increased affinity for extracellular ligands (integrin 'activation'). The relationship between specific conformations and activation remains controversial. Inside–out signalling controls adhesion strength and enables sufficiently strong interactions between integrins and extracellular matrix (ECM) proteins to allow integrins to transmit the forces required for cell migration and ECM remodelling and assembly. Integrins also behave like traditional signalling receptors in transmitting information into cells by 'outside–in' signalling. Binding of integrins to their extracellular ligands changes the conformation of the integrin and, because many of the ligands are multivalent, contributes to integrin clustering. The combination of these two events leads to intracellular signals that control cell polarity, cytoskeletal structure, gene expression and cell survival and proliferation. Although we conceptually separate the two processes, they are often closely linked; for example, integrin activation can increase ligand binding, resulting in outside–in signalling. Conversely, ligand binding can generate signals that cause inside–out signalling.



Förster resonance energy transfer
(FRET). A phenomenon in which one fluorophore (the donor) in its electronic excited state can transfer its energy to another fluorophore (the acceptor) in close proximity, so that excitation of the donor causes the acceptor to emit fluorescence. As the FRET only occurs when the distance between donor and acceptor is less than 10 nm, it is useful for monitoring interactions between two fluorophore-fused molecules.

Focal complex
A relatively small dot-like adhesion (~ 1 μm in width) mainly found in lamellipodia. It is a transient adhesion site during cell migration and can mature into a more stable focal adhesion.

Thus, cell adhesion assays typically reflect the combined effects of integrin conformation and valency regulation on adhesion strength[25,33]. Even the results of soluble ligand binding assays, the classical method to study integrin affinity modulation in non-adherent cells such as leukocytes and platelets, can be subject to ambiguity. For example, since most integrin ligands are multivalent, their binding may be influenced by the cellular regulation of integrin clustering. Furthermore, multimeric ligand binding itself may modify the nature of the bond between integrin and ligand through ligand-induced conformational changes[8], microclustering[37] and outside–in signalling[38]. Finally, because the application of force can prolong the bond lifetimes between integrins and their ligands[25], this so-called 'catch bond' behaviour may erroneously be attributed to integrin clustering.

Advances in the detection of protein–protein interactions in living cells by FRET[39], bioluminescence resonance energy transfer (BRET)[37], image correlation spectroscopy[40] and interferometric photoactivated localization microscopy[41] promise to improve our understanding of integrin clustering at the nanoscale. As certain integrins are expressed at high density (for example, αIIbβ3 integrin molecules are < 200 Å apart in platelets[38]), spontaneous integrin microclusters may be favoured. FRET and BRET also show that $MnCl_2$ activation of leukocyte αLβ2 integrin or platelet αIIbβ3 integrin fails to induce microclustering. Instead, microclustering requires the binding of multivalent ligands to these integrins and is enhanced by cytochalasins, presumably by releasing cytoskeletal constraints[37,42].

Transmembrane domains: signalling conduits

Each α- or β-integrin subunit is a typical type 1 transmembrane protein with the amino terminus outside and a single transmembrane domain that connects to a carboxy-terminal cytoplasmic tail (BOX 1). The transmembrane domain is therefore an essential connection for the transmission of information across the membrane.

The topology of integrin transmembrane domains. Ulmer's laboratory used NMR spectroscopy of the individual αIIb integrin and β3 integrin transmembrane domains and of the heterodimeric complex, to define their structure in phospholipid bicelles and to estimate the extent to which they are embedded in the membrane[19,43,44] (FIG. 1a). Studies of the transmembrane domain of the αIIbβ3 integrin heterodimer subunits show that the β3 integrin transmembrane domain adopts a long helix[19], whereas the αIIb integrin transmembrane domain folds into a shorter helix followed by a backbone reversal that packs Phe992–Phe993 against the transmembrane helix[43](FIG. 1b,c). One important contribution of these studies was clarifying the membrane embedding of the α- and β-integrin transmembrane domains. Prediction methods placed[45] the boundaries between transmembrane and cytoplasmic domains at conserved Lys or Arg residues that precede four to six apolar residues. Armulik and co-workers[46] used enzymatic glycosylation mapping, a method that examines the efficiency of microsomal membrane glycosylation of Asn-X-(Thr/Ser) motifs (where X is any amino acid) placed at varying distances from the presumed transmembrane domain. They predicted that the conserved Lys residues and the C-terminal apolar residues in α1, α2, β1 and β2 integrin subunits are lipid-embedded. Protection from solvent water or paramagnetic relaxation of αIIb and β3 integrin transmembrane domains in bicelles confirmed the predictions of the glycosylation mapping studies[19,43,44] (FIG. 1a). Consequently, for α-integrin subunits, the conserved Gly-Phe-Phe residues C-terminal to Lys-Arg are membrane embedded and terminate in a short transmembrane helix that is perpendicular to the plane of the membrane (FIG. 1b). The β3 integrin transmembrane domain is predicted to be tilted by ~ 25° relative to the plane of the membrane to enable side chains of corresponding hydrophobic residues in the β-subunit[19,43,44] to maintain membrane embedding (FIG. 1b). This β3 integrin transmembrane helical tilt may also be favoured by the propensity of the positively charged side chain of a conserved membrane-embedded Lys-Arg to reside in proximity to the negatively charged phospholipid headgroups[19]. Mutational studies point to a crucial role for these membrane embedded, conserved apolar residues in both subunits in regulating integrin activation[47–49], and the structural basis of the role of the transmembrane domain in activation has now become clear.

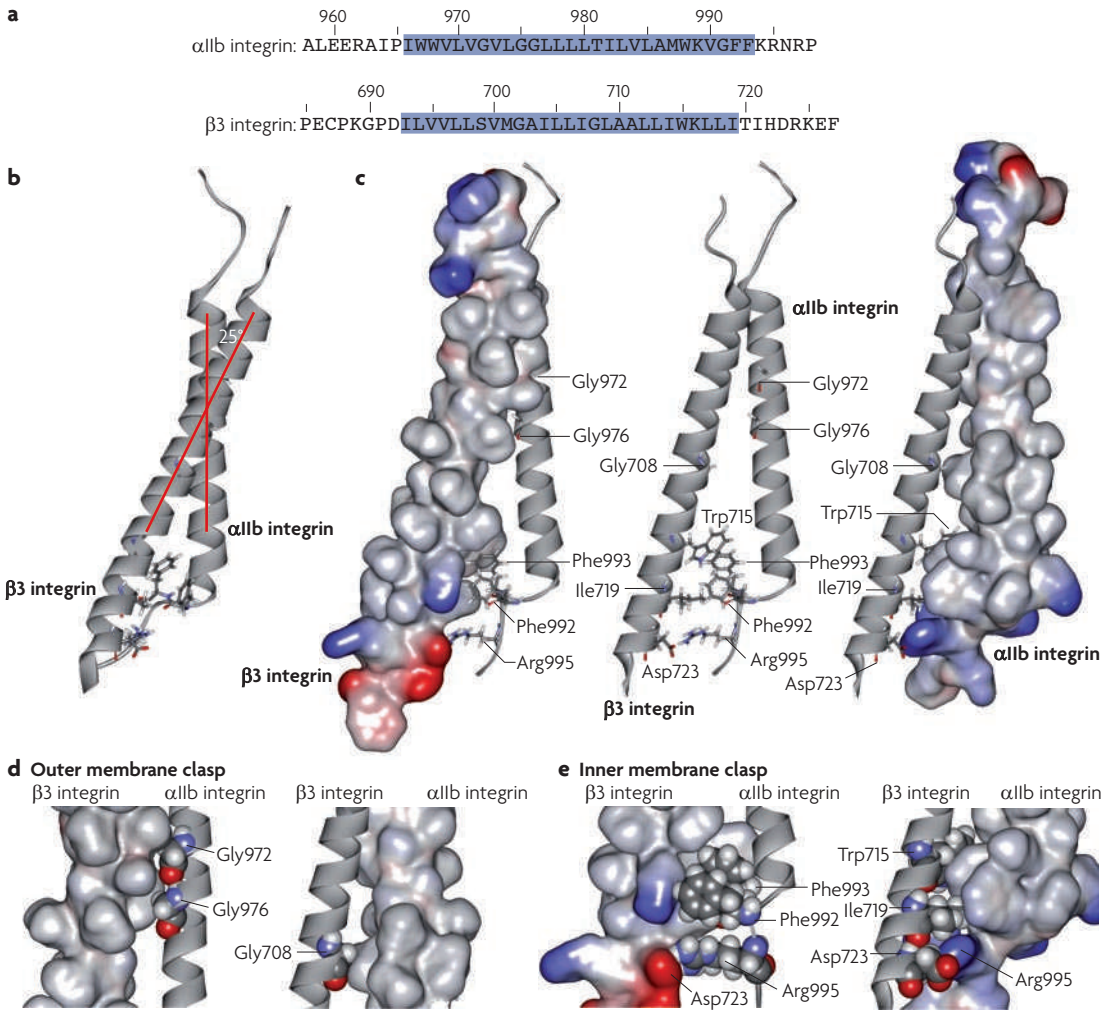


Figure 1 | **The structure of the αIIbβ3 integrin transmembrane complex enables inside–out signal transduction.** The models depicted are based on the average of an ensemble of 20 calculated simulated annealing NMR structures[44] (Protein data bank identifier 2K9J). **a** | Sequences of the αIIb and β3 integrin transmembrane domains. The membrane-embedded segments, as assessed by NMR spectroscopy of integrins in phospholipid bicelles, are highlighted in blue. **b** | Ile966–Arg995 of αIIb integrin and Ile693–Asp723 of β3 integrin adopt well-structured conformations with a predicted crossing angle of 25°. **c** | Rotating the model in part **b** by 90° reveals the two discrete elements that mediate the principal interaction of the transmembrane domains. The β3 (left) or αIIb (right) integrin transmembrane domains are depicted as space-filling models, with the ribbon structure in the middle. Basic residues are blue and acidic residues are red. The association of α- and β-integrin transmembrane domains, through packing of Gly residues in the outer membrane leaflet, forms the outer membrane clasp. The novel assembly in the inner membrane leaflet extending into the membrane–cytosol interface forms the inner membrane clasp. **d** | The outer membrane clasp. Gly972 and Gly976 of αIIb integrin and Gly708 of β3 integrin are shown as atoms that form holes into which side chains from the apposing space filling model of β3 integrin pack (left). Gly708 of β3 integrin forms a hole into which αIIb integrin side chains pack (right). **e** | The αβ-integrin transmembrane interaction is stabilized by interhelical packing mediated by Phe992–Phe993 of αIIb integrin and then the electrostatic interaction of Arg995 of αIIb integrin with Asp723 of β3 integrin to form the inner membrane clasp. The left panel depicts a space-filling model of the β3 integrin transmembrane domain in which Asp723 of β3 integrin is shown in red and space-filling models of the Phe992, Phe993 and Arg995 side chains of αIIb integrin are shown. The right panel depicts a space-filling model of the αIIb integrin transmembrane domain in which Arg995 of αIIb integrin is shown in blue and space-filling models of the Trp715, Ile719 and Asp723 side chains of β3 integrin are shown.

Focal adhesion
A large (2–5 μm in width), elongated, oval-shaped protein complex found on the cell periphery, which connects the actin cytoskeleton (F-actin bundle) to the ECM and provides strong integrin-dependent adhesion.

Immunological synapse
A cell–cell junction between a T lymphocyte and an antigen presenting cell during T lymphocyte activation.

Podosome
A type of ECM contact that is different from focal complexes and focal adhesions. Podosomes have a core actin filament surrounded by a ring structure of integrin adhesive complexes. They are shorter than other ECM contacts in depth (~ 0.2–0.4 μm) and are typically found in monocytic lineages.

Invadopodium
A type of ECM contact that is different from focal complexes and focal adhesions but similar to podosomes. Invadopodia can extend up to ~ 8 μm, associate with ECM-degrading enzymes and are seen in transformed fibroblasts or malignant cells.

Bioluminescence resonance energy transfer
A FRET-like phenomenon in which bioluminescence generated by luciferase (the donor) can excite a nearby fluorophore (the acceptor).

Image correlation spectroscopy
A method used to analyse molecular densities and rates of aggregation and diffusion of fluorescent molecules by autocorrelating the temporal (or spatial) fluctuation of intensities in confocal images.

Interferometric photoactivated localization microscopy
A recently developed fluorescent microscopy that provides 20 nm resolution in three dimensions, thus allowing single-molecule imaging.

αβ-Integrin cytoplasmic domain interactions and signalling. Interactions of integrin cytoplasmic domains with each other or cytoplasmic proteins lead to the long-range allosteric rearrangements of the integrins[48,50] that underlie activation. Recent work provides new insights into how such rearrangements cross the membrane. The association of α- and β-integrin transmembrane and cytoplasmic domains regulates integrin signalling[17,51–54]. Mutational studies suggested that an electrostatic interaction between Asp723 of β3 integrin and Arg995 of αIIb integrin[17] might constrain the C-termini of these integrins to inhibit activation. Subsequent studies indicated that mutations of the corresponding residues in β2 and β1 integrins could activate these integrins[55,56];

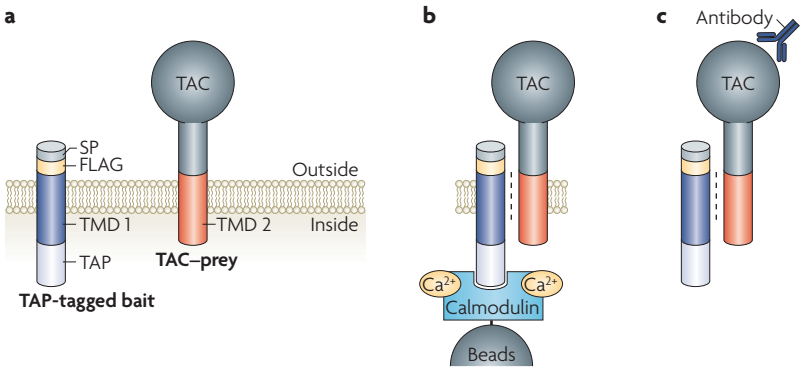


Figure 2 | **An affinity-capture method to study transmembrane domain interactions.** Use of an affinity-capture method reveals the preferential interaction of αIIb and β3 integrin transmembrane domains (TMDs). **a** | The transmembrane domain tail bait and prey constructs are depicted. The bait consists of the transmembrane and cytoplasmic domain fused to a FLAG tag (for detection), with a signal peptide at the amino terminus and a tandem affinity purification (TAP) tag at the carboxy terminus. The transmembrane and cytoplasmic domains of the prey are joined at the N terminus to the extracellular domain of an irrelevant type 1 membrane protein, such as the TAC subunit of the interleukin 2 receptor. **b** | Chinese hamster ovary cells are transiently transfected with baits and preys, cells are lysed and the bait is rapidly and efficiently captured through its TAP tag. Capture of the TAP tag with calmodulin beads is depicted. **c** | Bound preys are detected by western blotting using an anti-TAC antibody. SP, signal peptide.

however, mutation of the Asp residue in β1 integrin produced no evident phenotype in mice[57]. Clasping the cytoplasmic or transmembrane domains together with artificial coiled coils inhibited activation[15,58], as did linking the α- and β-integrin transmembrane domains with disulphides[51]. Nevertheless, efforts to identify interactions of isolated α- and β-integrin tails in aqueous solution by NMR spectroscopy[59] were either unsuccessful, or reported differing structures of the αβ-integrin complex[60,61].

αβ-Integrin transmembrane domains and activation. Mutational studies and molecular modelling suggest that interactions between the transmembrane domain of an α- and a β-integrin subunit are important in maintaining the low affinity inactive state, and that activation requires alteration of these transmembrane interactions[17,51–54,62]. Efforts to identify direct interactions between α- and β-integrin transmembrane domains have yielded differing results[30,63,64]. An affinity capture assay using mini integrins, which have only transmembrane and cytoplasmic domains (FIG. 2), was recently used in conjunction with NMR spectroscopy to reveal preferential heterodimeric association of αIIb integrin transmembrane cytoplasmic tails with those of β3 integrin by specific transmembrane interactions[65]. Furthermore, mutations in αIIb integrin (at Arg995) and β3 integrin (at Asp723) confirmed that an electrostatic interaction stabilizes the association between the αIIb and β3 integrin transmembrane tails. Finally, several transmembrane domain mutations that activate integrins reduce the αβ-integrin association. Thus, this affinity capture assay can be used to study interactions among transmembrane domains, and has documented the importance of αβ-integrin transmembrane interactions in integrin activation.

αβ-Integrin transmembrane domain structure enables signalling. An NMR structure of the αβ-integrin transmembrane complex reveals that the transmembrane domains primarily associate through two structural elements, one in the inner membrane leaflet, which extends into the membrane–cytoplasmic interface, and the other at the outer leaflet of the lipid bilayer[44] (FIG. 1c–e). In this way, perturbations at the cytoplasmic face, or separation of the legs of the ectodomain, can destabilize the αβ-integrin transmembrane dimer. Early models envisaged a coiled-coil-like arrangement for the αβ-integrin transmembrane complex[62]; however, the extended intersubunit interface of such a structure might be too stable to transmit perturbations at the cytoplasmic face to the ectodomain. The αIIbβ3 integrin transmembrane domain structure[44] reveals that the dimer is stabilized by two structural assemblies, termed the inner membrane clasp (IMC) and outer membrane clasp (OMC) (FIG. 1c–e). The OMC is formed by the packing interactions of three Gly residues, Gly972 of αIIb integrin, Gly976 of αIIb integrin and Gly708 of β3 integrin, which cause the α- and β-integrin transmembrane helices to cross within their N-terminal halves at an angle of ~ 25° (FIG. 1d). Because of this crossing angle and their differing lengths, the αIIb and β3 integrin transmembrane helices would dissociate C-terminally to Lys712 of β3 integrin. However, this loss of contact is overcome by the placement of Phe992–Phe993 of αIIb integrin between the transmembrane domains, which brings the aromatic rings of these residues in proximity to the aromatic ring of Trp715 of β3 integrin, and by contacts between Ile719 of β3 integrin and Phe992–Phe993 of αIIb integrin. This structural motif brings Arg995 of αIIb integrin and Asp723 of β3 integrin into sufficient proximity to enable the electrostatic interactions that were predicted nearly 15 years ago[17] to stabilize the IMC (FIG. 1e). Importantly, NMR structures calculated without using this electrostatic interaction as a distance restraint reveal an essentially identical IMC structure[44]. Furthermore, mutations that disrupt this electrostatic interaction lead to destabilization of the αβ-integrin transmembrane dimer, as shown by NMR spectroscopy in bicelles and affinity capture in mammalian cell membranes[65]. Rosetta modelling[66] makes use of computer searches to identify short sequences of known structure for use in the prediction of protein structure from amino acid sequence. Thirty percent of the cluster 1 structures predicted by Rosetta modelling, combined with sparse restraints, indicate that Arg995 of αIIb integrin and Asp723 of β3 integrin are in proximity. Many of the Rosetta structures also indicate that the side chain of Lys716 of β3 integrin forms hydrogen bonds with αIIb integrin backbone carbonyl oxygens to stabilize the α- and β-integrin association. Substitution of this Lys with polar neutral or acidic residues or a bulky hydrophobic residue activated αIIbβ3 integrin, which was interpreted to provide support for this additional αβ-integrin interaction[67]. The IMC structure in phospholipid bicelles differs from that of the same region of the isolated cytoplasmic domains in aqueous solution[60], suggesting that the distinct lipid tail-to-headgroup environment is important in driving IMC assembly. Indeed, the IMC structure formed by the αIIb and β3 integrin transmembrane

Phospholipid bicelle
A planar disc-shaped particle made of a phospholipid mixture. The centre of the bicelle consists of two layers of phospholipids and the edge of the bicelle is covered by phospholipids with shorter lipid chains.

Microsomal membrane
A membrane vesicle that is generated by fragmentation of the endoplasmic reticulum.

Coiled coil
A protein structure generated by dimerization or multimerization of α-helices. These α-helices typically consist of repeats of two hydrophilic residues, followed by a hydrophobic residue that is buried into the binding interface in aqueous solution.

cytoplasmic domains in a 50% acetonitrile/water solution closely resembles that of the isolated cytoplasmic domains in water[68]. Mutations that disrupt either the IMC or OMC destabilize the association of the αIIb and β3 integrin transmembrane domains[44,65], providing experimental validation for the idea that both clasps are required to maintain the transmembrane complex. Thus, the binding of cytoplasmic proteins to the integrin intracellular domains can disrupt the IMC, in a manner that is described below for talin, destabilizing the transmembrane complex and resulting in rearrangements in the ectodomain that lead to integrin activation.

The structure of the integrin transmembrane domain described above was obtained with integrin transmembrane peptides in a model membrane, but how does it relate to the structure of an intact integrin in the plasma membrane? Rosetta modelling[66] was combined with a few distance restraints provided by engineered disulphide bonds between introduced Cys mutations in the α- and β-integrin subunits to calculate seven clusters of (collections of similar) low energy models of the structure of the αIIbβ3 integrin transmembrane complex in mammalian cell membranes[67]. The centre structure of the most highly populated cluster is similar to the average structure calculated from NMR restraints obtained with αIIbβ3 integrin transmembrane peptides in bicelles[44]. The calculated NMR structures of the αIIb and β3 integrin transmembrane monomers were available before publication of the Rosetta model; however, the authors emphasized that those structures were not used to inform the Rosetta modelling[67] or the selection of the representative structure. Similarly, models of the αIIbβ3 integrin transmembrane domain, derived by two different methods, both converged on the published NMR structure. These models exhibited close similarities with the averaged NMR structure; the root mean square deviation of α-carbons (or backbone carbons linked to both the amide and carbonyl groups in amino acids) was 1.1 and 1.6 Å from the averaged NMR structure[69]. Consequently, the modelling approaches used complementary methods to independently derive similar overall topographies to the NMR-derived structures of the integrin transmembrane domains in bicelles. Importantly, the sequences that form the IMC are highly conserved between integrins, suggesting that the mechanisms that regulate the IMC to induce integrin activation are likely to be shared. Indeed, the same cytoplasmic proteins (talins and kindlins) are involved in activating multiple classes of integrins (see below). Conversely, the OMC is less conserved in sequence, suggesting that the stability of the OMC might differ between integrins. These sequence variations may account for differences in transmembrane signalling among integrin classes.

How is integrin activation transmitted? After the idea emerged that interactions between integrin cytoplasmic and transmembrane domains might maintain the low affinity state[17], the idea followed that activation involves a rearrangement of these domains. Protein engineering studies established that enforcing the association of the integrin transmembrane or cytoplasmic domains with coiled coils or engineered disulphide bonds prevents integrin activation[15,51,58,70]. More importantly, elegant work showed that mutational activation of a recombinant integrin altered the formation of intersubunit disulphide bonds between Cys mutations in the outer portion of the transmembrane domain, suggesting that a complete separation of the transmembrane domains leads to integrin activation[51]. Oxidation-dependent disulphides formed in the activated integrin at 37°, but not at 0°. The authors emphasized that at 0° the mobility of these transmembrane domains would be greatly reduced, thus the lack of disulphide formation at this temperature indicates a loss of stable transmembrane domain association. In a subsequent paper[67], the same group found that certain activating Cys substitutions did not prevent the formation of engineered disulphides at 0° in the transmembrane or cytoplasmic domains. Inclusion of these disulphides as restraints in the Rosetta calculations did not alter the predicted structures[67]. These results imply that the α- and β-integrin transmembrane domains continue to interact in integrins bearing these activating Cys substitutions. The authors have proposed that this seeming discrepancy may be because "The apparent lack of effect on cross-linking by these activating mutations may result from the use of 0°C and 37°C in cross-linking and activation assays, respectively" (REF. 67). Measures of the interactions of α- and β-integrin transmembrane domains showed that certain activating mutations can weaken but not completely disrupt their association[44,65]. Thus, whereas the idea that integrin activation requires complete disruption of transmembrane assembly is attractive, available evidence does not exclude other plausible rearrangements[45].

Cytoplasmic activators of integrins

The idea that the integrin cytoplasmic domains are the trigger point for conformational changes that result in activation[47,48] led to efforts to find cytoplasmic domain-binding proteins that might mediate this process. Many candidates have been identified[71] and compelling evidence shows that talins and kindlins are major players.

Talins activate integrins. Studies in cultured cells showed that the binding of talin 1 to the cytoplasmic domain of the β-integrin subunit is a common step in β1 and β3 integrin activation *in vitro*[72]. Later studies extended this principle to β2 integrins *in vitro*[73] and to mice, in which deletion of platelet talin 1 blocks activation of platelet β1 and β3 integrins[74,75]. Furthermore, insights from structural studies[76] enabled the creation of mice in which β3 integrin–talin 1 binding was disrupted. These mice were defective in activating αIIbβ3 integrin[3] and protected from pathological thrombosis, without experiencing the severe bleeding associated with complete loss of β3 integrin[3]. Thus, disrupting the β3 integrin–talin 1 interaction may offer an anti-thrombotic benefit by blocking integrin activation. In addition to activating integrins, talin 1 links integrins to filamentous actin (F-actin) and actin-binding proteins (reviewed in REF. 77), thereby linking the actin cytoskeleton to the ECM. More recent analysis of talin 1 structure has identified talin 1 and integrin mutants that have little effect on the binding of talin 1 to the β-integrin tail, or on talin 1 recruitment to integrin in

Pathological thrombosis
The formation of an occlusive mass of fibrin, platelets and leukocytes in a blood vessel, which results in diseases such as myocardial infarction and stroke.

cells, but do block the ability of talin 1 to induce activation[78,79]. These mutants offer the possibility of selectively disrupting the ability of talin 1 to activate integrins, without preventing integrin linkage to the cytoskeleton.

Talin 1 consists of a large C-terminal rod and an N-terminal head domain (THD) containing four subdomains: F0, F1, F2 and F3 (REFS 76,80,81). The F3 subdomain has a PTB domain, which contains a high affinity binding site for β-integrin tails and is sufficient to activate integrins[81]; other portions of the THD enhance activation[82]. A crystal structure of the THD F2 and F3 subdomains in complex with a 12 residue fragment from the mid-portion of the β3 integrin tail reveals that the F3–integrin interaction strongly resembles PTB domain interactions with peptide ligands[76]. Several other PTB domains bind to β3 integrin in a similar manner[83], but talin 1 is uniquely designed to activate integrins because of an additional interaction between it and the membrane-proximal region of the β3 integrin cytoplasmic domain[60,79,84,85]. Mutations in the integrin or talin that block this interaction prevent integrin activation in cells. Thus, talin F3 interacts with β-integrin tails through a PTB-like interaction that is shared with many PTB domain-containing proteins and through a second interaction that is not shared with most of these PTB domains but is required for integrin activation.

How does the interaction of talin 1 with the membrane-proximal portion of the β-integrin tail lead to rearrangement of the integrin transmembranes to cause activation? Talin 1 binding destabilizes the interaction of the αIIb integrin transmembrane tail with the β3 integrin transmembrane tail[60,65]. Structure–function analysis of the talin F3 and β-integrin tail interaction[79], together with the structure of the integrin transmembrane complex[44], provide a compelling model to explain how talin 1 can alter the integrin transmembrane complex. First, binding of talin F3 stabilizes the helical structure of the membrane-proximal β3 integrin tail[79] such that the β3 integrin transmembrane domain forms a continuous helix[19]. Second, the F3–β3 integrin interaction orients a group of Lys residues in F3 towards the negatively charged membrane phospholipid head groups. Mutation of some of these Lys residues disrupts activation[79]. An additional contribution may come from the asymmetric structure of the αIIbβ3 integrin transmembrane at the cytosolic face. In particular, the non-helical Phe992–Phe993 segment of αIIb integrin juxtaposes Arg995 of αIIb integrin and Asp723 of β3 integrin so that an electrostatic interaction can stabilize the transmembrane complex. Arg995 of αIIb integrin and Asp723 of β3 integrin are readily accessible to the THD, which could therefore prevent this electrostatic interaction (FIG. 1). Indeed, a recent structure of the F2–F3 region of a talin 1 paralogue, talin 2, in complex with the β1D integrin cytoplasmic domain revealed that talins can form a salt bridge with the conserved Asp residue of the β-integrin subunit (for example, Asp723 of β3 integrin), thus potentially disrupting its electrostatic interaction with the conserved Arg residue in the α-integrin subunit (for example Arg995 of αIIb integrin)[86]. This structure also identified additional basic residues in F2 that form a 'membrane orientation patch' that can interact with phospholipid head groups to enable talin to alter the tilt angle of the β-integrin transmembrane domain. The predicted capacity of talin to alter this tilt angle explains why talin binding is required for full activation[72,79], even when the interaction of Arg995 of αIIb integrin with Asp723 of β3 integrin is prevented by mutation of Asp723 of β3 integrin. In sum, the THD is exquisitely engineered for activating integrins by binding to β-integrin tails through a PTB-like interaction and by engaging a membrane-proximal β-integrin tail site, which has three important consequences. First, it positions basic patches on talins for an extended electrostatic interaction with the phospholipid head groups of the membrane. Second, it favours the formation of a stable, continuous helix that spans the β-integrin transmembrane and the membrane-proximal portion of the tail, enabling talins to enforce an altered crossing angle on the β-integrin transmembrane domain. Third, talins may directly disrupt the conserved α-integrin Arg and β-integrin Asp interaction by forming a salt bridge with the β-integrin Asp. This unique combination of structural elements in talins, and complementary elements in integrins, explains why they are obligatory partners in the activation process.

Kindlins cooperate with talins. Talins are essential for integrin activation, but are they sufficient to activate integrins? Recent studies from model organisms and humans have established that another family of β-integrin-binding proteins, the kindlins, are important players in integrin activation. The ~ 76 kDa vertebrate kindlins include kindlin 1 (also known as FERMT1 and URP1), kindlin 2 (also known as FERMT2, MIG2 and URP3) and kindlin 3 (also known as FERMT3 and URP2). Each is structurally related to UNC-112, a *Caenorhabditis elegans* protein implicated in integrin-dependent muscle development[87]. Kindlins and UNC-112 contain a FERM domain near the C-terminus that is similar in sequence to the talin FERM domain, but is unique in that its F2 subdomain is interrupted by a pleckstrin homology domain[88]. The split FERM domain, and its F3 subdomain in particular, mediates the interaction of kindlins with β-integrin cytoplasmic tails. This interaction requires a region of the integrin tails (for example, Asn-X-X-Tyr in β1 and β3 integrins and Asn-X-X-Phe in β2 integrin; where X is any amino acid) that is distal to the talin-binding Asn-Pro-X-Tyr/Phe region, as well as a Ser or Thr in an 8–16 amino acid tract that separates these two regions[89–91]. Kindlin 2 and UNC-112 also interact, through conserved regions N-terminal to their FERM domain, with two additional proteins, migfilin and integrin-linked kinase, which are frequently found in adhesion complexes[87,89,92].

Kindlin 1 deficiency in mice and humans causes Kindler syndrome — epithelial cell dysfunction leading to a skin blistering phenotype and gasterointestinal manifestations[93–95]. Morpholino knockdown of *kindlin 2* in zebrafish embryos causes abnormalities of cardiac muscle development owing to defective cytoskeletal organization at sites of membrane attachment[96], and kindlin 2 deficiency in mice causes peri-implantation lethality owing to detachment of the endoderm and epiblast from basement membranes[89]. Mice deficient in kindlin 3 die

PTB domain
(Phosphotyrosine binding domain). A protein domain that recognizes an Asn-Pro-X-Tyr motif that is found in most β-integrin tails.

FERM domain
(4.1 protein, ezrin, radixin and moesin homology domain). A common domain found in a number of proteins that mediate linkage of the cytoskeleton to the plasma membrane. The FERM domain often interacts with the cytoplasmic tail of transmembrane proteins.

Pleckstrin homology domain
A lipid-binding protein domain originally identified in Pleckstrin.

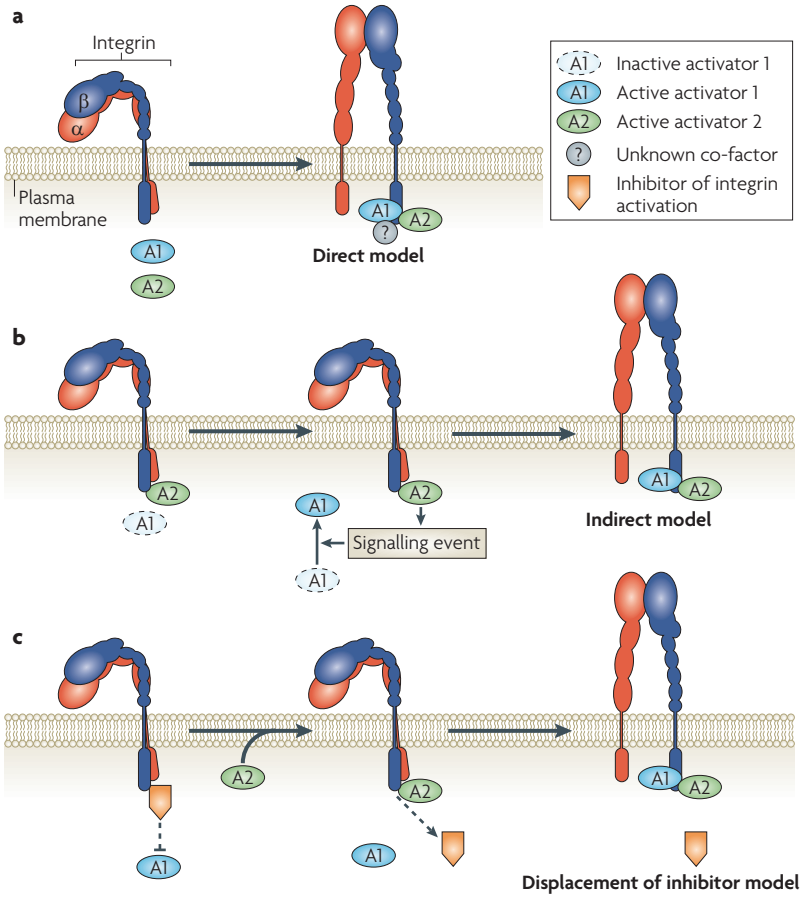


Figure 3 | **Activators, such as talins and kindlins, bind to integrins to cause their activation. a** | In a direct model of integrin activation, both activators (A1 and A2) bind simultaneously to the integrin tail and, together, modify or disrupt the inner membrane clasp. Other proteins might be involved. In the other two general models, A1 is the primary activator and A2 is an 'enabler'. **b** | In an indirect model, A2 regulates a signalling event (for example, synthesis of co-factors) that enables the activator (A1) to bind β-integrin and induce activation. **c** | In a displacement of an inhibitor model, A2–β-integrin binding displaces an inhibitor of A1, enabling A1 to bind and activate the integrin.

with diffuse haemorrhages and osteopetrosis shortly after birth[97]. Given the known integrin and cytoskeletal protein interaction partners of the kindlins, defects in bidirectional integrin signalling probably underlie some of these severe phenotypes.

Studies of cells using knockdown or overexpression strategies indicate that kindlin 1, kindlin 2 and kindlin 3 are capable of regulating the activation of specific integrins, but only in concert with the interaction of talin 1 with the integrin cytoplasmic tail. For example, ligand binding to αIIbβ3 or α5β1 integrins in Chinese hamster ovary (CHO) cells is stimulated by overexpression of the THD. This activation of αIIbβ3 integrin, but not of α5β1 integrin, is increased by co-expression of kindlin 1 or kindlin 2 but not kindlin 3, and is decreased by small interfering RNA knockdown of endogenous kindlin 2. However, neither kindlin 1 nor kindlin 2 are stimulatory in the absence of THD[89–91,93]. In another study, loss of kindlin 1 from intestinal epithelial cells or a colon carcinoma cell line reduced talin-dependent β1 integrin activation and/or β1 integrin-mediated cell adhesion[95]. Thus, kindlins can co-activate integrins and talin 1, but their precise effects may vary with the kindlin, integrin and cell type involved.

Kindlin 3 in leukocyte and platelet integrin activation. Platelets that develop from mice with kindlin 3-deficient haematopoietic precursors exhibit defective activation of the αIIbβ3 integrin fibrinogen receptor and the α2β1 integrin collagen receptor, and impaired aggregation[97]. Kindlin 3-deficient platelets also show reduced adhesion to fibrinogen after direct activation of αIIbβ3 integrin by $MnCl_2$, suggesting an additional defect in outside–in αIIbβ3 integrin signalling. Furthermore, the mice are resistant to mesenteric arteriolar thrombosis following vessel injury by $FeCl_3$. In addition, kindlin 3 deficiency results in defective activation of neutrophil β2 integrins, as evidenced by reduced agonist-dependent binding of intercellular adhesion molecule 1 (ICAM1) and the inactive complement factor 3b fragment (iC3b) *in vitro*, and defective firm adhesion and arrest of neutrophils on activated endothelial cells *in vivo*[98]. These platelet and leukocyte integrin defects in kindlin 3-deficient mice phenocopy blood cell abnormalities in a rare human autosomal recessive disorder called leukocyte adhesion deficiency 1 (LAD1) variant (LAD 1v; also known as LAD3)[99,100]. This disorder is characterized by recurrent bleeding similar to that seen in individuals with Glanzmann thrombasthenia owing to a lack of αIIbβ3 integrin, and by a purulent bacterial infection and leukocytosis similar to that seen in individuals with LAD1 caused by a lack of β2 integrins. In contrast, LAD1v platelets and leukocytes express these integrins but exhibit an impairment of agonist-induced integrin activation.

Earlier studies had suggested that LAD1v is due to a splicing defect in the Ca^{2+}- and DAG-regulated guanine nucleotide exchange factor 1 (*CALDAG-GEFI*; also known as *RASGRP1*) gene, resulting in reduced levels of its encoded protein RAP1 guanine nucleotide exchange factor (GEF) in haematopoietic cells[101]. This seemed reasonable because RAP1 is involved in integrin activation[102–104], and *CALDAG-GEFI*-knockout mice[105,106] exhibit defects, albeit partial, in platelet and leukocyte integrin activation. However, recent studies have now shown that *kindlin 3* mutations are a cause of LADIv in several families, including some previously reported to be deficient in CALDAG-GEFI and others with normal levels of CALDAG-GEFI[107–110]. Affected individuals share a common haplotype involving a region on chromosome 11 that harbours mutations in *kindlin 3* that result in a premature stop codon[108–110]. Primary haematopoietic cells or EBV-transformed lymphocytes from affected individuals exhibit reduced levels of *kindlin 3* mRNA[107] or absent kindlin 3 protein[108,109]. Various haematopoietic cells show defective agonist-induced binding of ligands to β1, β2 or β3 integrins[99,107,108]. Importantly, the integrin phenotype in kindlin 3-deficient cells is rescued by expression of recombinant kindlin 3 (REFS 107,108), and RNA interference-mediated knockdown of kindlin 3 in normal haematopoietic cells recapitulates the integrin phenotype[108].

Mesenteric arteriolar thrombosis
Thrombosis that occurs in an arterial vessel of mesentery. Mesentery is the anatomical term indicating the layers of membrane that suspend the small intestine from the back wall of the abdomen.

Glanzmann thrombasthenia
A genetic bleeding disorder caused by a lack of αIIbβ3 integrin or by mutations that inhibit αIIbβ3 integrin function.

Whereas the evidence is clear that kindlins are key regulators of talin-dependent integrin activation by virtue of their association with β-integrin cytoplasmic domains, many questions remain (FIG. 3). Is the kindlin interaction with β-integrin cytoplasmic domains regulated and, if so, how? The kindlin-binding protein, migfilin, binds to filamin A. As filamin A can block talin 1 binding to β-integrin tails, what role, if any, does the shuttling of filamin A on and off integrins have in the ability of kindlins to co-activate integrins[111,112] (for example, see FIG. 3c)? Is talin the direct integrin activator and kindlin the enabler, or is the reverse true (for example, see FIG. 3b)? Do all kindlins activate integrins in the same way? Are there yet unidentified integrin-binding proteins that are required for integrin activation in concert with talins and kindlins (for example, see FIG. 3a)? How do the kindlins participate in outside–in integrin signalling[89]?

RIAM activates talin 1

Agonist stimulation (the triggering event) leads to integrin activation through many signalling intermediaries. If talin 1 binding is a common step in integrin activation, how do these signalling intermediaries regulate the talin–integrin interaction? Recent work has elucidated one such group of signalling intermediaries that are important in this activation — the Ras GTPases[113]. Ras proteins are small monomeric GTPases that cycle between the GTP-bound active form and the GDP-bound inactive form. GEFs promote Ras activity by exchanging bound GDP for GTP, whereas GTPase activating proteins (GAPs) enhance the hydrolysis of Ras-bound GTP to GDP[114]. The Ras subfamily members RAP1A and RAP1B stimulate integrin activation[102–104]. Knockout of RAP1B[115] or its exchange factor *CALDAG-GEFI*[116] in mice, results in the partial impairment of agonist-dependent fibrinogen binding to αIIbβ3 integrin and platelet aggregation. Several RAP1 effectors are implicated in integrin activation[117–119]. RAP1–GTP-interacting adaptor molecule (RIAM; also known as APBB1IP) is a RAP1 effector that is a member of the MIG10, RIAM and lamellipodin (MRL) family of adaptor proteins[118]. RIAM contains Ras association and pleckstrin homology domains and Pro-rich regions. In lymphoid cells, RIAM overexpression induces β1 and β2 integrin-mediated cell adhesion, and RIAM knockdown abolishes RAP1-dependent cell adhesion[118]. RIAM increases cellular F-actin content[118], possibly through its interaction with ENA and VASP — related proteins that can promote actin polymerization to form F-actin. Whereas RIAM is enriched in haematopoietic cells, lamellipodin is a paralogue present in fibroblasts and other cells[120].

Agonists do not efficiently activate recombinant αIIbβ3 integrin expressed in CHO cells; this observation led to a synthetic reconstruction of an integrin activation pathway in CHO cells. Its use in combination with forward and reverse genetics enabled the dissection of a pathway to integrin activation[121]. RAP1 activation induces the association of RAP1, RIAM and talin 1, which leads to αIIbβ3 integrin–talin 1 interactions. More recently, CHO cells transfected with the thrombin receptor proteinase-activated receptor 1 (PAR1; also known as F2R) enabled activation of αIIbβ3 integrin by a natural platelet agonist. Furthermore, bimolecular fluorescence complementation showed that RIAM overexpression stimulates, and RIAM knockdown blocks, talin 1 recruitment to αIIbβ3 integrin in living cells[78]. These studies facilitated the construction of a road map between receptor agonists and integrin activation (FIG.4). Moreover, mapping studies identified short amphipathic helices in RIAM and lamellipodin that bind talin 1; joining these helical peptides to the membrane targeting sequences of RAP1 led to a minimal RAP1–RIAM module that was sufficient to recruit talin 1 to integrins and to activate the integrins[122]. Thus, RIAM functions as a scaffold that connects the membrane-targeting sequences in Ras GTPases to talin 1, thereby recruiting talin 1 to the plasma membrane and activating integrins. An intriguing alternative mechanism was identified in lymphocytes, in which WASP-family verprolin homologue 2 (WAVE2), an actin-nucleating protein, recruited vinculin to the immunological synapse, thereby recruiting talin 1 (REFS 123). Taken together, these studies raise the possibility of a general mechanism for integrin activation: talin-binding proteins that contain membrane-targeting motifs or that associate with proteins that possess such motifs can target talin 1 to integrins and induce activation.

α-Integrin subunit-specific activators

Talin 1 and kindlins bind to β-integrin subunits; however, early experiments pointed to an important role for the cytoplasmic domains of α-integrin subunits in regulating activation[47,124]. Whereas the protein sequences of the membrane proximal α-integrin subunits that form the IMC (for example, Gly-Phe-Phe-Lys-Arg) are well conserved, the sequences of the more distal α-integrin subunits are far more variable. Thus, because there are 18 α-integrin subunits, the complex literature on α-integrin subunit-binding proteins is too large to be thoroughly reviewed here. Nevertheless, a few outstanding examples will be mentioned. Naik, Parise and co-workers identified Ca^{2+}- and integrin-binding protein 1 (CIB1) as an αIIb integrin tail-binding protein[125] and subsequent work reported that it functions to oppose talin 1 binding, thereby serving as an inhibitor of activation[126]. Surprisingly, a lack of platelet CIB1 led to defective thrombosis and no increase in αIIbβ3 integrin activation[127], possibly owing to compensation by CIB1 paralogues[128]. Similarly, Katagiri, Kinashi and co-workers identified regulator for cell adhesion and polarization enriched in lymphoid tissues (RAPL; also known as NORE1 and RASSF5) as a RAP1-binding protein that physically associates with αLβ2 integrin in an αL integrin-specific manner and regulates αLβ2 integrin-mediated adhesiveness[129,130]. More recent elegant *in vivo* studies[131–133] show that RAPL regulates lymphocyte trafficking, in part through MST1 (also known as STK4), a STE20 kinase-like binding partner. In addition, these studies have also clarified the complementary roles of talin 1 and RAPL in the regulation of lymphocyte adhesion by RAP1 (REF. 131). It is clear that the diversity of α-integrin tail sequences and the consequent plethora of α-integrin-binding proteins will continue to be an exciting and fertile area for future investigation.

Amphipathic helix
An α-helix that contains hydrophilic amino acids on one side and hydrophobic amino acids on the other.

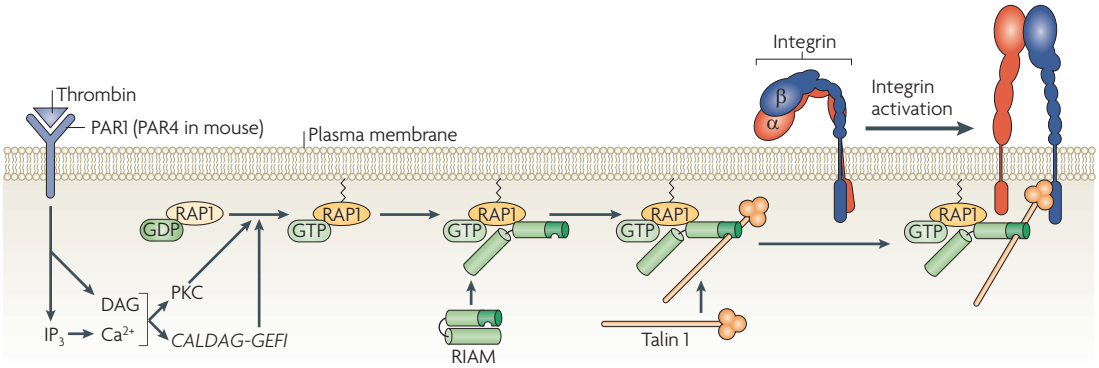


Figure 4 | **A road map from thrombin receptors to αIIbβ3 integrin activation.** The schematic represents the minimal elements of one pathway of αIIbβ3 integrin activation by thrombin receptors, which were identified through the synthetic reconstruction of pathway components in Chinese hamster ovary cells and studies of gene-targeted platelets. Thrombin cleavage or ligand occupancy of the thrombin receptor proteinase-activated receptor 1 (PAR1; also known as F2R) in human platelets, or PAR4 receptors in mouse platelets, stimulates phospholipid hydrolysis, which results in the generation of inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 stimulates an increase in cytosolic free Ca^{2+}, activating Ca^{2+}- and DAG-regulated (*CALDAG-GEFI*; also known as *RASGRP1*), which in turn converts its encoded protein, RAP1, from a GDP-bound to an active GTP-bound form. Ca^{2+} and DAG also activate certain protein kinase C (PKC) isoforms, including PKCα, which among other actions may facilitate the activation of *CALDAG-GEFI*. Activation of RAP1 leads to recruitment of its effector, RAP1–GTP-interacting adaptor molecule (RIAM; also known as APBB1IP), and its binding partner, talin 1, to the plasma membrane. This enables talin binding to the β3 integrin tail and talin-induced activation of αIIbβ3 integrin. Kindlin 3 plays a crucial role in this process, but because its mechanistic role is uncertain, it is not depicted here.

Endogenous suppressors of integrins

Negative regulators of integrin activation might be as important as positive regulators. In principle, negative regulation might occur at any step in the process of inside–out signalling. This is exemplified in platelets by the blockade of specific agonist pathways to αIIbβ3 integrin activation by aspirin, which inhibits cyclooxygenase, thus blocking synthesis of thromboxane, or by clopidogrel, which blocks P2Y12 ADP receptors[134]. Similarly, enforced activation of extracellular signal-regulated kinase 1 (ERK1; also known as MAPK3) and ERK2 (also known as MAPK1) by activated HRas suppresses integrin activation in many cell types[135], an effect that may be pertinent to the changes in adhesion and ECM assembly of transformed cells[136]. ERK1 and ERK2 kinase activity is required for this suppression, and they exert their effects at the plasma membrane[137]. However, the relevant ERK1 and ERK2 substrate or substrates have not been identified.

Most pertinent to this review is the potential for the regulation of integrin activation at its final steps — through the interactions of talin 1 or kindlins with integrin tails. One example of this type of regulation may be the expression and localization to adhesion sites of phosphatidylinositol phosphate kinase type Iγ-90, a protein that competes with β-integrin tails for binding to talin 1 (REFS 138,139). Another example is the Tyr phosphorylation of β-integrin tails that is triggered by ligand binding to integrins, and mediated by Src family kinases[140]. Tyr in the membrane-proximal Asn-Pro-X-Tyr motif of β-integrin tails may exert multiple effects on cell adhesion through phosphorylation-dependent and phosphorylation-independent mechanisms[141–144]. Phosphorylation of this Tyr may serve as an integrin activation 'off switch' by interfering with required acidic and hydrophobic interactions between this region of the β-integrin tail and talin 1, thereby reducing the affinity of the interaction. Moreover, Tyr phosphorylation promotes the interaction of the β-integrin tail with competing PTB domain-containing proteins, such as docking protein 1 (DOK1), which, unlike talin 1, do not activate integrins[83,145]. Furthermore, integrin cytoplasmic domain-associated protein 1 (ICAP1; also known as ITGB1BP1) can bind to the β1A integrin tail and compete for talin 1 binding, thus blocking activation[146]. Another potential negative regulator of integrin activation is filamin A, the blockade of talin 1 binding to β-integrin tails by which may be regulated by kindlin through their mutual binding partner, migfilin[111,147]. To date, studies of negative regulation of integrin activation have been conducted largely with purified proteins or cell lines. Determining their biological significance will require further work in model organisms and humans.

Activation of integrins from the outside

Although the focus of this review is on the inside–out activation of integrins, integrins can be activated directly by extracellular factors, including ECM ligands, and ligand binding to integrins triggers outside–in signalling[148]. Non-physiological reducing agents such as dithiothreitol[149] have been used experimentally for years to activate purified integrins and integrins in cells. For example, reducing agents activate αIIbβ3 integrin in platelets, a response attributed to disulphide exchange between selected Cys residues in the Cys-rich extracellular epidermal growth factor (EGF)-like domains of β3 integrin[150]. Disulphide exchange involving αIIbβ3 or αVβ3 integrins may occur during agonist-induced integrin activation and require thiol isomerases, such

as protein disulphide isomerase or endoplasmic reticulum protein 5, or thiol isomerase activity intrinsic to β3 integrin[149,151,152]. However, the role of disulphide exchange in integrin activation in cells, and how it relates to talin-dependent activation, will require more study.

Integrin affinity can also be modulated extrinsically by the binding of ligands. Even the binding of monovalent ligands, such as short Arg-Gly-Asp peptides, can induce conformational changes in integrin ectodomains[153], as reported by ligand-induced binding site antibodies (anti-LIBS)[154]. FRET studies indicate that these conformational changes can be propagated across the plasma membrane, leading to alteration of the α- and β-integrin tails[155]. Consequently, inside–out and outside–in signalling responses are coupled by dynamic interactions of the integrins with proteins on both sides of the plasma membrane, and they are further modified by forces applied to integrins in adherent cells by virtue of integrin linkages with the ECM and the cytoskeleton.

Perspectives

Integrin activation has been studied for over two decades by a range of experimental techniques. Progress in this area has accelerated in recent years owing to studies using forward and reverse genetics, biochemistry and cell and structural biology. In addition, studies of integrin activation are a prime example of successful bidirectional information transfer between basic scientists and inquisitive clinicians making careful observations on patients with perplexing abnormalities of cell adhesion. Consequently, this field has come closer to a molecular understanding of the 'end game', the final cell signalling events that regulate activation at the level of integrin transmembrane and cytoplasmic domains. Predictably, new discoveries have led to new questions, such as the precise relationships between talins, kindlins and other regulatory proteins during integrin activation, and the structural basis of integrin activation in the context of intact integrin heterodimers in their native membrane environments. Thus, for integrinologists the end game is not the end of the game.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene: www.ncbi.nlm.nih.gov/gene
CALDAG-GEFI
OMIM: www.ncbi.nlm.nih.gov/omim
Kindler syndrome | LAD1 | LAD1v
UniProtKB: www.uniprot.org
α1 | α2 | αIIbβ3 | α5 | αLβ2 | αV | β1 | CIB1 | ERK1 | ERK2 | ICAM1 | ICAP1 | kindlin 1 | kindlin 2 | kindlin 3 | MST1 | RAP1A | RAP1B | RAPL | RIAM | PAR1 | UNC-112 | WAVE2

FURTHER INFORMATION
Mark H. Ginsberg's homepage:
http://pstp.ucsd.edu/ginsberg

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