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Integrin activation by talin, kindlin and mechanical force

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Integrins are the major family of adhesion molecules that mediate cell adhesion to the extracellular matrix. They are essential for embryonic development and influence numerous diseases, including inflammation, cancer cell invasion and metastasis. In this Perspective article, we discuss the current understanding of how talin, kindlin and mechanical forces regulate integrin affinity and avidity and how integrin inactivators function in this framework.

Cell adhesion to extracellular matrix (ECM) proteins and neighbouring cells is essential for multicellular organisms. The ECM is a meshwork of collagens, glycoproteins and proteoglycans, the biochemical and biophysical properties of which are dynamically regulated during development. These properties also vary according to organ type and physiological states, such as ageing and tissue repair, or pathological situations, such as cancer and fibrosis. Cells perceive and respond not only to the biochemical composition of the ECM but also to the biophysical properties of the ECM (for example, stiffness) through mechanotransduction. In this process, forces allosterically modulate the functions of mechanosensitive proteins within adhesion structures to induce biochemical signalling cascades, which trigger both rapid changes in cellular mechanics by affecting cytoskeletal dynamics and long-term changes in cell proliferation and differentiation by modulating gene expression. Integrin-mediated cell adhesion to the ECM mediates the formation of mechanosensitive structures that play key roles in mechanotransduction.

Integrins are heterodimeric receptors composed of α and β subunits that mediate cell adhesion to ECM ligands and cellular counter receptors, such as intercellular adhesion molecule (I-CAM) and vascular cell adhesion protein (V-CAM)[1]. In vertebrates, there are 18 α and 8 β integrin subunits that assemble 24 different heterodimers with distinct ligand-binding specificities and signalling properties[1]. Integrins are composed of a large ectodomain, which mediates ligand binding, a transmembrane domain and a short cytoplasmic tail (except integrin β_4), which indirectly associates with the actomyosin cytoskeleton[2]. Integrins adopt distinct conformations that have different ligand-binding affinities[3] (Fig. 1). In the bent-closed and extended-closed conformations, the lower leg pieces and transmembrane regions of the α and β subunits remain associated and the ligand-binding site is closed[3]. In the extended-open conformation, the swing-away of the hybrid domain from the α subunit and unclasping of the lower leg pieces and transmembrane regions correlate with a rearrangement of the ligand-binding metal-independent adhesion site, a piston-like movement of an α-helix away from the ligand-binding pocket and a complete opening of the ligand-binding site[3]. Whereas the extended-closed conformation may permit low-affinity integrin–ligand interaction, the majority of studies showed that the high-affinity state is primarily associated with the extended-open conformation and thus specifies the on-rate of ligand binding[4–6]. Thus, the shift from the bent-closed/extended-closed conformation to the extended-open conformation is termed 'integrin activation'.

A general hallmark of integrin-mediated adhesion is its mechanosensitivity[7]. Besides the strict requirement for talins and kindlins, mechanical forces are also essential for integrin on-rate regulation. When integrins are activated and bound to their ligands, they cluster and nucleate hundreds of adaptor and signalling molecules at their cytoplasmic tails to assemble a dynamic macromolecular complex, termed the integrin adhesome[8]. The integrin-binding and/or actin-binding protein complexes within the adhesome mechanically couple integrins to the force-generating actomyosin system as a 'gear box', whose dynamic behaviour and regulation can be described as a 'molecular clutch'[9]. The integrin adhesome is organized in a three-dimensional laminated structure with inter-connected modules. Vertically, an integrin activation/signalling module at the plasma membrane is followed by a force transduction module and finally by an actin regulatory module[10]. Laterally, integrins and associated complexes segregate into nanoclusters with distinct integrin activities and mechanical properties[11,12]. Nanoclusters with either strong or weak actomyosin engagement further self-organize into a force-bearing adhesion core and a mechanically relaxed periphery, respectively[13]. Each adhesome module harbours mechanosensitive proteins that can undergo conformational changes or post-translational modifications under tensile force, leading to changes in force transduction and/or induction of biochemical signalling, such as activation of focal adhesion kinase (FAK), Src and Rho family GTPases[7]. For example, actomyosin-mediated stretching of talin leads to the recruitment of vinculin, which strengthens the mechanical linkage between integrin and actomyosin to further increase force transmission[14]. In addition, force can alter the conformation of FAK to increase its catalytic activity and signalling, and of p130Cas (also known as BCAR1) to expose its Src phosphorylation sites[15,16].

Whereas exceedingly high force disrupts protein–protein interactions, exposure of some integrins to an intermediate range of forces (for instance, actomyosin mediated or those produced by blood flow) reduces the off-rates of their bonds with ligand by inducing 'catch' bonds[17]. Together with integrin clustering, which enables dissociated integrin–ligand complexes to rebind, force-induced catch bonds extend the duration of mechanosignalling and the lifetime of integrin adhesion sites[17].

In short, whereas integrin activation culminates with its extended-open conformation and ligand binding, adhesion lifetime is regulated by the formation of catch/strengthened bonds and integrin clustering. As both integrin activation and adhesion lifetime extension are mechanosensitive, here, we discuss the influence of forces in these processes. We evaluate the roles of talin and kindlin and discuss potential functions of the growing number of integrin inactivators, which sequester integrins in their inactive conformation and prevent talin and/or kindlin recruitment.

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Thermodynamics of integrin activation

Springer and colleagues have measured the ligand-binding affinities of $\alpha_5\beta_1$ and $\alpha_4\beta_1$ integrins and the free energy required to achieve their different conformations on the cell surface[6,18,19] (Fig. 1). They found that the bent-closed state of $\alpha_5\beta_1$ integrin on K562 cells has the lowest free energy of −3.8 kcal per mol, whereas the extended-closed and extended-open states reach a free energy of 0.4 kcal per mol and 0 kcal per mol, respectively. Hence, the energy landscape favours the bent-closed state of $\alpha_5\beta_1$ integrin, with 99.75% of surface integrins residing in the bent-closed conformation and only 0.1% and 0.15% in the extended-closed and extended-open conformations, respectively[6,20]. Remarkably, the thermodynamically rare extended-open state of $\alpha_5\beta_1$ integrin has a 5,000-fold higher ligand-binding affinity than the bent-closed or extended-closed conformation. Thus, the major energy obstacle for $\alpha_5\beta_1$ integrin activation is to overcome the bent-closed conformation. Once this is achieved, the extended-closed integrin shifts to the high-affinity extended-open conformation because of its slightly lower free energy requirement. Although the $\alpha_4\beta_1$ integrin has a smaller free energy difference between the bent-closed and extended-open states, the ligand-binding affinity of the extended-open state of $\alpha_4\beta_1$ integrin is still 600–800-fold higher than the bent-closed state[19]. As ligand-binding dynamics vary between different integrins and cellular contexts[19], it will be important to test whether other integrins share the same pattern of their energy landscapes.

Talin and kindlin as essential components for integrin activation

Among integrin-binding adaptor proteins, only talin and kindlin are known to be indispensable for integrin activation[21,22]. Mammals express two talin paralogues that share 80% homology. Talin-1 is ubiquitously expressed, whereas talin-2 is enriched in muscle and neuronal tissues[22]. Talins comprise an amino-terminal FERM (protein 4.1, ezrin, radixin and moesin) domain followed by a short, flexible linker and a large carboxy-terminal rod domain. The phosphotyrosine-binding motif in the F3 module of talin's FERM domain binds to the membrane-proximal NPXY motif of β integrin cytoplasmic tails. The talin rod domain harbours multiple actin-binding and vinculin-binding sites, many of which are mechanosensitive and only become exposed under tensile forces. At the end of the rod domain, talin has a dimerization motif of unknown in vivo function. Talin can adopt an auto-inhibitory conformation through an intramolecular interaction between the F3 module of the FERM domain and the R9 module in the rod domain[22]. In leukocytes, chemokine signalling converges on Rap1 GTPases and its effector Rap1-GTP-interacting adapter molecule (RIAM), which binds to and activates talin[23] (Fig. 1). Additional talin activation mechanisms probably exist in other cell types[13]. Although the N-terminal FERM domain of talin is sufficient for integrin binding[24], the actin-binding rod domain is required for cell adhesion and spreading, suggesting that actomyosin-generated forces mediate the latter processes[21,25]. This notion is supported by measurements of tension of around 10 pN across talin molecules in focal adhesion sites[26].

Kindlins are also FERM domain-containing proteins consisting of three members (kindlin-1–3)[27]. Kindlin-2 is expressed ubiquitously outside the haematopoietic system, whereas kindlin-1 is mainly expressed in epithelial cells and kindlin-3 is restricted to the haematopoietic system[27]. Unlike talins, kindlins bind to the membrane distal NxxY motif of integrin tails. Kindlins function as a protein–protein interaction hub by recruiting the integrin-linked protein kinase–PINCH (also known as LIMS1)–parvin complex, paxillin and the Arp2/3 complex to integrins[21,28,29]. This multi-subunit complex probably drives cell spreading by activating FAK, Rac1 and the Arp2/3 complex via the paxillin–β-Pix axis[21,29]. Kindlin may form homodimers, as reported for the *Caenorhabditis elegans* orthologue UNC-112 (ref. [30]). Recombinant mouse kindlin-2 lacking the PH domain can also homodimerize through its F2 module in vitro, but with very slow kinetics[31]. Although a direct interaction between kindlin-2 and F-actin has been observed[32], no intramolecular tension across kindlin has been reported. Thus, talin and probably also kindlin connect integrin with actomyosin and potentially cluster integrins through dimerization. Whereas compelling evidence supports a role of talin in force transmission to integrins, kindlin may instead stabilize cell adhesion through the regulation of actin dynamics.

Mechanosensitive integrin activation with talin, kindlin and low force

The prevailing view of integrin activation is that agonist-induced activation and subsequent binding of talin, and probably kindlin, to the β integrin tail change the tilt angle of the β integrin transmembrane domain and separate the α/β transmembrane and cytoplasmic domains, thereby causing the extension and opening of the ectodomain, and finally ligand binding[33] (Fig. 1a). This model is called integrin inside-out activation to highlight the exclusive and powerful role of talin in inducing conformational changes across the entire integrin molecule from within the cell[34,35]. However, given the thermodynamic landscape of conformations measured for $\alpha_5\beta_1$ and $\alpha_4\beta_1$ integrins, the question is whether talin and kindlin binding to the integrin tail is sufficient to overcome the energy barrier between the bent-closed and the extended-open states. The answer is probably no, as in the absence of the relatively low forces (1–3 pN) that are required to stabilize the rare extended-open conformation[20], non-physiologically high concentrations of talin would be required. Considering that talin is recruited to focal adhesions directly from the cytosol without a discernible enrichment at the plasma membrane[36] and that the affinity of the talin FERM domain for integrin tails is very low[37], it is unlikely that cytosolic talin reaches such high concentrations.

In a new and more plausible model, thermodynamic equilibrium is at the heart of integrin activation. Under this framework, talin and kindlin maintain the rare extended-open integrin states by transducing small mechanical forces to the integrin–ligand complex, rather than by disrupting the transmembrane association of an integrin heterodimer[20]. It is also possible that forces exerted through talin and kindlin may accelerate conformational transitions. Consequently, the mechanical force shifts the thermodynamic equilibrium of integrin conformations in favour of the extended-open state. This mechanosensitive mechanism explains the 'integrin outside-in activation' initiated by the ECM ligand or the integrin counter receptor (Fig. 1b). Besides $\alpha_5\beta_1$ and $\alpha_4\beta_1$ integrins, the large conformational change that correlates with integrin activation and force coupling was also observed for $\alpha_L\beta_2$ integrin in migrating T cells[38,39], suggesting that force-induced activation is used by different integrin classes. However, the dependence of the integrin extended-open conformation on forces may differ between integrins as they presumably display differential talin-binding and/or kindlin-binding affinities and different energy landscapes in the presence of distinct ligands[19,40–42].

As force transduction across integrins requires mechanical resistance from the extracellular side, both the presence of immobilized ECM ligand or the presence of membrane-anchored integrin counter receptors, such as V-CAM1 and I-CAM1/2 are prerequisites to form a stable extended-open conformation. Fibroblast and epithelial cells are constantly exposed to an affluence of immobilized integrin ligands. Moreover, the confinement of the ECM niche and cell–cell interactions permit a sufficiently large time window for integrins to thermodynamically shift their conformations and become actomyosin linked. By contrast, leukocytes in circulation must be rapidly recruited to the inflamed endothelium under high shear stress. Are leukocyte integrins pre-activated by chemokine signalling without ligands? The answer is probably no. Selectins, integrin ligands, such as I-CAMs and V-CAM, and membrane-associated chemokines are

all strongly induced on the endothelial cells by pro-inflammatory cytokines[43]. E-selectins capture circulating leukocytes by binding to selectin receptors, which allows leukocytes to roll on the inner endothelial wall[43]. $\alpha_L\beta_2$ integrins in their extended-open conformation on rolling leukocytes recognize membrane-immobilized I-CAMs and the chemokine receptors bind to endothelial membrane-associated chemokines to trigger talin-1 (and probably kindlin-3) activation. This agonist-induced burst of talin and kindlin activation is a specific property of haematopoietic cells—no agonists are known that activate talin and kindlin in non-haematopoietic cells—to ensure that all extended-open-state integrins on blood flow-exposed leukocytes are instantly linked to actomyosin and stabilized by mechanical forces[43]. Consistently, immobilized chemokines, selectins, an intact actin cytoskeleton and the mechanically resistant I-CAMs are all required for $\alpha_L\beta_2$-mediated leukocyte arrest[44].

The stabilization of the rare extended-open conformation represents the very first step of cell attachment. To ensure stable adhesion, cells strengthen the existing integrin–ligand bond and cluster additional integrins in the growing adhesion site.

Mechanosensitive adhesion strengthening

The catch-bond formation plays a key role in adhesion strengthening. Although relatively low forces (between 1 and 3 pN) are sufficient to stabilize the integrin extended-open state[20], forces transduced across integrins span from 1 to above 10 pN[45,46]. High intramolecular tension across talin and between vinculin and F-actin has also been reported[26,47]. Whereas external forces weaken most protein–protein interactions (the feature of a slip bond), forces between 10 and 30 pN allosterically strengthen bonds between leukocyte function-associated antigen 1 (also known as integrin α_L), Mac-1 (also known as integrin α_M) and I-CAM1, as well as between $\alpha_5\beta_1$ integrin and fibronectin via catch-bond formation[48–50]. In the

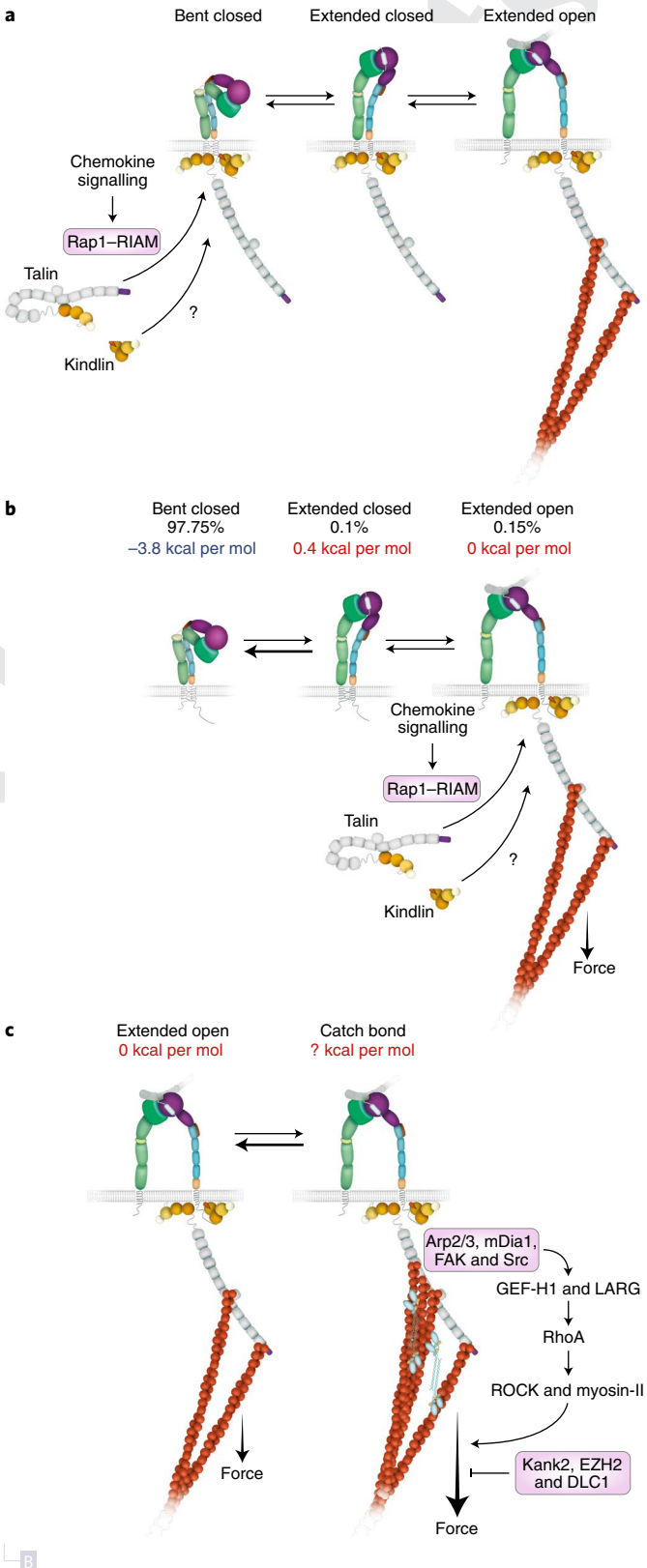


Fig. 1 | a, According to the conventional view of integrin inside-out activation, inactive integrins with a bent-closed conformation have their transmembrane domains closely associated and cytoplasmic tails clasped. Talin recruitment to the cytoplasmic tail of β integrin subunits disrupts the transmembrane association, reorients the transmembrane tilt and therefore induces extension (extended closed) of the integrin ectodomain and finally opening (extended opening) of the integrin ligand-binding pocket enabling ligand binding. Talin in the cytosol is shielded from integrin binding by an autoinhibitory conformation. In haematopoietic cells, agonist-induced (for example, chemokines, ADP and thrombin, among other agonists) signalling induces a burst of talin activation via Rap1–RIAM, which ensures fast and copious binding of talin to β integrin tails. Although it remains unclear whether agonist-induced signalling also regulates kindlin recruitment to β integrin tails (indicated by ?), kindlin was shown to facilitate talin-dependent induction of the integrin extended-open state and integrin clustering. **b**, In the alternative model of mechanosensitive integrin activation (or outside-in activation of integrins), integrins predominantly adopt the thermodynamically stable bent-closed conformation, but can fluctuate to the rare extended-closed and extended-open conformations in a thermodynamic equilibrium. As soon as integrins in the rare extended-open conformation simultaneously bind to immobilized ligand and engage the force-generating actin cytoskeleton via talin and/or kindlin, mechanical force stabilizes the integrin–ligand complex. In blood flow-exposed leukocytes, integrins require immobilized ECM ligands from the extracellular side and surplus amounts of activated talin and kindlin molecules (activated by agonist-induced signalling) to ensure that all extended-open-state integrins are efficiently stabilized by force of the actomyosin system. **c**, ECM-bound integrins can regulate the strength of their bonds with ligand. The ligated integrins nucleate numerous cytoskeletal and signalling regulators, such as vinculin, the Arp2/3 complex, formins, FAK and Src kinases, which in turn reinforces the connection between talin and the actomyosin system, increases force transduction across integrins and eventually induces the formation of catch bonds that strengthen integrin–ligand binding. Increased force transduction also feeds forward to RhoA GTPase signalling via activation of GEF-H1 and LARG, resulting in global elevation of myosin-II activity and overall cell stiffening. DLC1, deleted in liver cancer 1; mDia1, protein diaphanous homologue 1; ROCK, Rho-associated protein kinase.

case of the $\alpha_5\beta_1$ integrin, force induces catch-bond formation by allosterically activating binding to the 'synergy site' located next to the RGD motif in fibronectin[51–53]. The swing away of the hybrid domain from the α integrin subunit is a final step that leads to the high-affinity extended-open conformation, and therefore a prerequisite for the formation of catch bonds[6]. However, the observation that solely stretching the $\alpha_5\beta_1$ integrin head piece is sufficient to induce a catch bond[48] indicates that, differently from the mechanosensitive $\alpha_5\beta_1$ integrin on-rate regulation to the fibronectin RGD site, catch-bond behaviour involves a force-induced conformational change within the integrin head piece that drives the association of the α_5 subunit with the fibronectin synergy site (Fig. 1c). Once formed, the catch bond dramatically prolongs the bond lifetime and provides an explanation for the existence of long-lived integrins (~40 second for β_1 integrin) immobilized within the adhesion sites[54]. Importantly, the synergy site-dependent catch-bond formation of $\alpha_5\beta_1$ integrins greatly differs from those formed by other integrins, including $\alpha_V\beta_3$ and leukocyte function-associated antigen 1, for which a synergy-like binding site is either not required or has not been identified. Direct structural visualization of the integrin–ligand complex under force is needed to elucidate whether a general mechanism of catch-bond formation exists.

Atomic force microscopy-based single-cell force spectroscopy experiments revealed that the adhesion strengthening within the first seconds of integrin–ligand complex formation requires an intact actin cytoskeleton as well as key signalling molecules, such as FAK and Src, but not RhoA GTPases or myosin-II[52]. This mechanism is probably required to initiate the assembly of nascent adhesions[55], whereas adhesion strengthening at later stages is associated with whole-cell stiffening and requires crosstalk between different integrin classes and activation of RhoA- guanine nucleotide exchange factors (GEFs), such as LARG and GEF-H1, and RhoA effectors, such as Rho-associated protein kinase, which controls myosin-II activity[40,56,57] (Fig. 1c).

The single-cell force spectroscopy experiments also demonstrated that talin is essential for catch-bond formation of fibronectin-bound $\alpha_5\beta_1$ integrins, whereas kindlin seems to stabilize $\alpha_5\beta_1$ integrins in their active conformation[52], suggesting that they may contribute to the transmission of different force regimes. Force-induced adhesion strengthening can be attenuated by several mechanisms that converge on talin (Fig. 1c). For instance, Kank2 (KN motif and ankyrin repeat domain-containing protein 2) diminishes the talin–actomyosin linkage and reduces force transmission to integrins behind the cell leading edge[13]. EZH2-mediated methylation of the third actin-binding site in talin may also dampen the force transmission to integrins, facilitating adhesion turnover during extravasation of neutrophils and dendritic cells[58]. Furthermore, deleted in liver cancer 1, a RhoA GTPase-activating protein, is also recruited to the talin rod domain to suppress RhoA signalling-induced actomyosin contractility[59,60]. The resulting decrease in mechanical force shifts integrin–ligand complexes from catch-bond to slip-bond modes, promotes the detachment of integrins from the ligands and thereby weakens cell adhesion.

Mechanosensitive integrin clustering

An additional mechanism to stabilize integrin-mediated adhesions is integrin clustering (avidity), which concentrates multiple integrin–ligand bounds to greatly increase the local adhesion strength. Avidity also allows integrins that are detached from the ligand by high mechanical forces to swiftly rebind to the ligand and significantly extend the adhesion lifetime[7]. However, this property can only operate when the switch from the extended-open state to the thermodynamically favoured bent-closed state proceeds more slowly than the rebinding event. So far, neither the timescales for integrin–ligand rebinding nor the conformational switches have been determined. Finally, integrin clustering stabilizes downstream mechanical linkages by laterally distributing high-tensile loads, thereby contributing to adhesion lifetime extension[17].

The glycocalyx, a layer of glycoprotein–polysaccharide complexes that typically extends>20 nm from the cell surface[61], also facilitates integrin clustering. According to the 'picket fence' model, the exofacial glycocalyx (fence) and transmembrane receptors (pickets) immobilized by cortical actin limit the lateral diffusion of integrins[62] and curtail ligand accessibility for integrins, which have a height of only 11 nm in their bent-closed conformation[18,24,63]. To overcome this mechanical barrier and initiate adhesion, cells mobilize glycocalyx receptors by destabilizing cortical actin structures[64] and compress the glycocalyx by applying protrusive forces through protruding filopodia, lamellipodia[65] and podosomes[66] (Fig. 2a). At its initial phase of assembly, the integrin adhesome is already enriched with talin, kindlin and actin filaments, ensuring that integrins that diffuse into these small, pre-formed adhesions can be captured and mechanically engaged[67]. Compression of the mucin-1 glycocalyx around these pre-formed integrin–ligand complexes increases ECM ligand accessibility to nearby inactive integrins and imposes pulling forces on integrins, thereby promoting adhesion enlargement, strengthening and integrin signalling[68,69] (Fig. 2b). Thus, existing integrin adhesions probably function as a kinetic trap to cluster more integrins and to continuously readjust the thermodynamic equilibrium towards their activation.

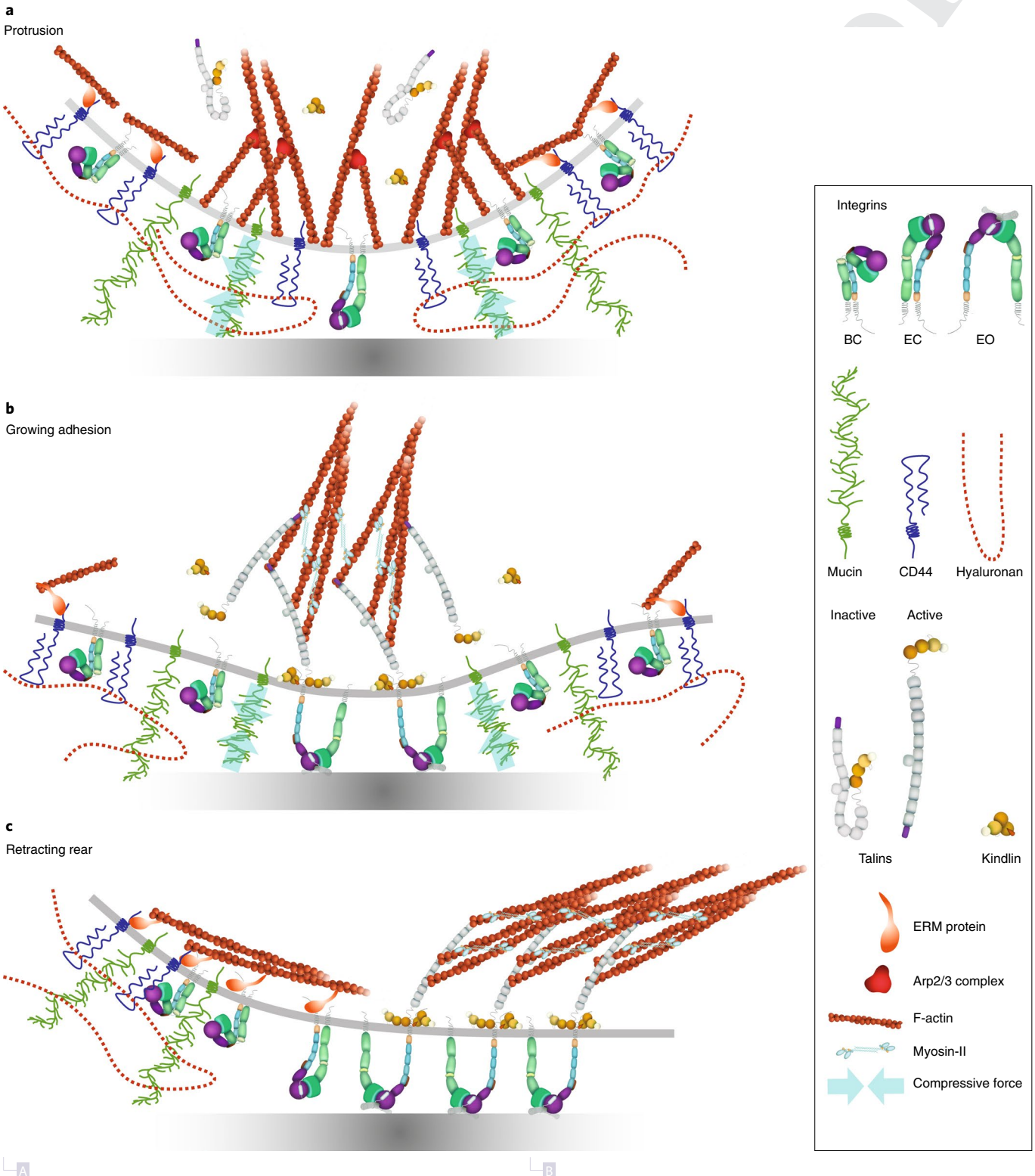
Tensile loads also impose a threshold on integrin clusters, leading to different distances between adjacent integrins and cluster sizes (Fig. 3). High-tensile loads that develop on rigid ECM substrates require that integrins cluster with an interdistance below 60 nm to form a stable adhesion[70], whereas low-tensile loads that develop on soft ECM permit stable adhesion formation with a lower degree of integrin clustering and a minimal interdistance of 200 nm (ref. [71]). The ability of talin, and probably also kindlin, to homodimerize potentially facilitates integrin clustering. Owing to their small size, kindlin dimers may promote high degrees of integrin clustering on rigid ECM[31], whereas the larger talin dimers may mediate low degrees of integrin clustering on soft ECM[72]. The lateral crosslinking of individual adhesion complexes probably also relies on an

Fig. 2 | a, Integrins on the cell surface are overlaid by a glycocalyx (fence) containing long mucins and hyaluronan and transmembrane receptors (pickets), such as CD44, that are immobilized by the cortical actin structure via ERM proteins. The glycocalyx not only imposes an exofacial mechanical barrier but also restricts lateral diffusion of integrins. Cells deploy two strategies to overcome this barrier. First, they produce protrusive force by actin polymerization to push the plasma membrane forward and locally compress the glycocalyx (indicated by the arrows), which shortens the distance between integrins and the ECM and promotes ligand binding. Such forces are produced by protruding filopodia (up to 3 pN), lamellipodia (up to 20 pN)[65] and the core of podosomes (up to 100 pN)[66]. Second, cells mobilize glycocalyx receptors by destabilizing or disrupting their connection with the cortical actin, which leads to local clearing of the glycocalyx in the cell protrusion where integrins can enter and access ECM proteins. **b**, The initial foothold of integrin adhesion serves as a kinetic trap to attract additional integrins for adhesion growth. The local enrichment of talins, the shorter distance between the plasma membrane and ECM proteins and the absence of immobilized glycocalyx receptors facilitate integrin recruitment and activation. Furthermore, compressed large mucin molecules around the preformed adhesion sites reciprocally exert tensile force on ligated integrins, which promotes catch-bond formation and integrin signalling. **c**, At the rear of migrating cells, ERM family proteins, such as moesin, compete with talin for integrin binding and sequester inactive integrins by associating with CD44 within the glycocalyx, thereby curbing integrin clustering and inducing adhesion disassembly. BC, bent closed; EC, extended closed; EO, extended open.

intact F-actin cytoskeleton and actin-crosslinking proteins that are recruited to talin and kindlin.

Roles of integrin inactivators

The essential requirement of actomyosin pulling forces transduced across talin, and possibly kindlin, to stabilize the integrin–ligand bond raises the question of whether integrin inactivators[73] are required to destabilize the integrin complex and reverse integrin–ligand binding. Among identified integrin inactivators, integrin cytoplasmic domain-associated protein 1, moesin and filamin A bind to the β tails and potentially prevent the recruitment of talins and/or kindlins[73]. SHANK-associated RH domain-interacting protein (SHARPIN) and mammary-derived growth inhibitor associates with α integrin tails to stabilize integrins in their low-affinity conformation[74]. SHANK, a SHARPIN-interacting protein, binds to Rap1 and inhibits RIAM binding[75]. Given the energy landscape of integrin conformations,



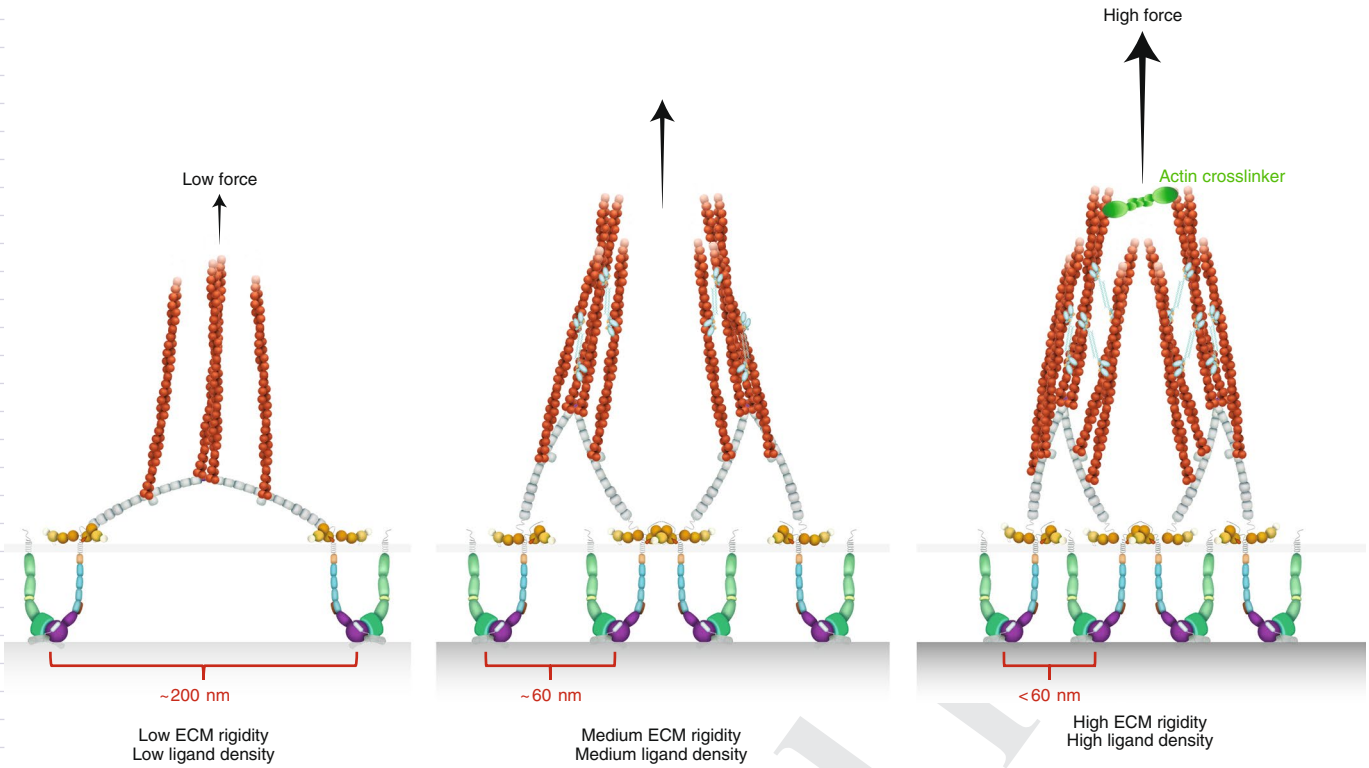


Fig. 3 | A model of how force controls modalities of integrin clustering. Tensile loads can be redistributed laterally between adjacent force-transducing linkages within integrin clusters. Hence, the degree of integrin clustering and ligand spacing correlates with the tensile loads and adhesion lifetime. On soft ECM (~1.5 kPa), integrins assemble into clusters with a large interdistance of ~200 nm between adjacent integrin–ligand complexes, which may be accomplished by talin dimerization. On stiffer ECM (~150 kPa), integrins form dense clusters with an interdistance of ~60 nm to contribute to withstanding high-tensile loads, which may be accomplished by kindlin dimerization. F-actin crosslinkers may further assist a higher degree of integrin clustering.

there is, in principle, no necessity for integrin inactivators to turn off activated integrins. All that is needed is to release the force. However, there are two scenarios in which integrin inactivators could have a function.

First, inactivators may spatially impose a higher-energy barrier for integrin activation. In migrating T cells, SHARPIN localizes in their trailing edge where it may promote rear retraction by preventing excessive integrin activation[76]. As a consequence, SHARPIN-deficient lymphocytes exhibit slower cell migration speed on I-CAM1 and reduced transendothelial migration[76]. SHARPIN-deficient mice exhibit chronic proliferative dermatitis (cpdm), multiple organ inflammation and disrupted lymphoid architecture[77–79]. The pleiotropic phenotypes of *cpdm* mice have been attributed to defective tumour necrosis factor-α-induced nuclear factor-κB (NF-κB) signalling, leading to apoptotic and necroptotic cell death followed by systemic inflammation[80,81]. SHARPIN is part of the linear ubiquitin chain assembly complex, which generates linear ubiquitin chains on proteins such as NF-κB essential modulator[77,78,82]. It is not clear whether the linear ubiquitin chain assembly complex-independent SHARPIN-induced integrin inactivation or the recently reported caspase 1 inhibition is involved in the *cpdm* phenotype[83]. Genetic ablation of the gene encoding the tumour necrosis factor-α receptor in *cpdm* mice rescues the inflammatory phenotype[80,81] and reduces β_1 integrin activity[84], arguing for an involvement of NF-κB-induced inflammatory signalling in integrin regulation.

Second, integrin inactivators may sequester integrins from reactivation or ECM rebinding within integrin clusters, thereby accelerating adhesion disassembly. Such functions can be assigned to the ezrin, radixin and moesin (ERM) family proteins, which are characterized by an N-terminal FERM domain that binds to the hyaluronan receptor CD44, followed by an α-helical linker domain and a C-terminal F-actin-binding tail[85]. ERM proteins adopt an autoinhibited conformation in the cytosol due to an intramolecular interaction between the FERM domain and the C-terminal tail. Once activated, they attach a 'picket fence' of hyaluronan and CD44 to the cortical F-actin network preferentially at the retracting rear of polarized cells[64,86]. In addition, the FERM domain of moesin can bind to β_1 integrin tails by outcompeting talin[87] (Fig. 2c). Thus, moesin-deficient endothelial cells fail to disassemble β_1 integrin-mediated adhesion structures and to retract cell protrusions and instead accumulate large numbers of β_1 integrin-mediated adhesion structures and cannot sprout in three-dimensional environments[87]. Moesin's binding sites for CD44 and integrin do not overlap[88,89], suggesting that even certain glycocalyx-binding receptors may indirectly regulate integrin activity.

Conclusions and outlook

The key role of mechanical force in integrin regulation has revolutionized our understanding of integrin activation, clustering and adhesion strengthening. Under this framework, integrins thermodynamically fluctuate between different conformations, with the rare high-affinity conformation captured by ECM ligands and stabilized by mechanical forces transduced via talin and kindlin. Subsequently, additional adaptor proteins are recruited to strengthen the integrin and actomyosin linkage, and adhesion is further stabilized by the formation of catch bonds and integrin clusters. Mechanosensitive signalling, such as that mediated by Rap1 GTPases, Rho family GTPases, FAK and Rho-associated protein kinases, control integrin-mediated adhesion by regulating talin activity and actomyosin dynamics. However, several questions remain to be investigated. For example, the precise role

of kindlins in the mechanosensitive regulation of integrins remains to be fully delineated. In addition, how talin and kindlin cooperate in the initial integrin activation, clustering and adhesion strengthening remains elusive. Furthermore, controversies regarding the biological importance of putative integrin inactivators abound. Future work will clarify these points and expand our understanding of integrin-mediated adhesion, for which mechanosensitivity has become the leitmotif.

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Competing interests

The authors declare no competing interests. Q15

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