Integrins as biomechanical sensors of the microenvironment

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Abstract | Integrins, and integrin-mediated adhesions, have long been recognized to provide the main molecular link attaching cells to the extracellular matrix (ECM) and to serve as bidirectional hubs transmitting signals between cells and their environment. Recent evidence has shown that their combined biochemical and mechanical properties also allow integrins to sense, respond to and interact with ECM of differing properties with exquisite specificity. Here, we review this work first by providing an overview of how integrin function is regulated from both a biochemical and a mechanical perspective, affecting integrin cell-surface availability, binding properties, activation or clustering. Then, we address how this biomechanical regulation allows integrins to respond to different ECM physicochemical properties and signals, such as rigidity, composition and spatial distribution. Finally, we discuss the importance of this sensing for major cell functions by taking cell migration and cancer as examples.

Type I transmembrane proteins
Proteins that span the cell membrane through a single transmembrane α-helix, with the N terminus on the extracellular side of the membrane.

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Integrins cross the plasma membrane and link the extracellular matrix (ECM) to the cell cytoskeleton. This role as ECM–cytoskeletal linkers inspired their name (from their function as 'integrators') and was predicted before the proteins were even identified[1–3]. Since their discovery on a molecular level in the 1980s, integrins have emerged as fundamental cell adhesion receptors that mediate cell and tissue function in a very wide range of scenarios in health and disease[4,5]. It has also been appreciated that integrin function is subject to very tight and complex regulation, from both a biochemical perspective (activation) and a mechanical perspective (mechanotransduction).

Several recent reviews have analysed the details of both biochemical[6,7] and mechanical[8–10] integrin regulation. It is now becoming increasingly possible to elucidate not only how integrins are affected by these biochemical and mechanical signals but also how this multifaceted regulation allows integrins to act as sensors of their environment, the ECM. Accordingly, our understanding of how integrins sense ECM parameters such as its molecular composition and conformation, its physical presentation, its stiffness or forces transmitted through it has greatly expanded in recent years. In this Review, we aim to describe how this sensing occurs. Rather than going into detail about the intricacies of integrin regulation, we lay out its fundamental biochemical and mechanical principles. Then, we discuss how those principles enable integrins to sense ECM properties. Finally, we discuss implications of this sensing in physiological scenarios, focusing on two highly relevant examples: cell migration and the regulation of dormancy and invasion in cancer.

Biochemical regulation of integrins

To act as cell adhesion receptors, integrins need to be transported to the plasma membrane and need to be activated (that is, change their conformation to allow ECM binding). These steps are directly related to integrin structure and also provide means to regulate integrin function.

Integrin structure. Integrins relay signals between the extracellular environment and intracellular pathways, and this communication occurs in both directions[11,12]. Integrins are heterodimeric receptors existing in at least 24 unique combinations of non-covalently interacting α-subunits (18 types) and β-subunits (8 types). This facilitates binding to a wide variety of ECM components but also to counter-receptors on other cell types[2]. Although some subunits appear in only a single heterodimer, 12 integrins contain the β1-subunit and five contain the αv-subunit[13]. Integrin α-subunits and β-subunits are both type I transmembrane proteins composed of a large extracellular domain, a single-pass transmembrane helix and a short cytoplasmic domain (with the exception of the large intracellular domain of the β4-subunit). Newly synthesized integrin α- and β-subunits heterodimerize in the endoplasmic reticulum and are expressed on the cell surface as obligate heterodimers[14] (FIG. 1). Importantly, the β1-subunit is translated in excess, resides in the endoplasmic reticulum as an immature precursor that matures through post-translational modifications, such as glycosylation, and is transported to the plasma membrane only after heterodimerization[7,14,15]. The binding sites for the ECM ligands either comprise epitopes from both subunits

RGD motif
A peptide sequence consisting of arginine, glycine and aspartate. It is found in extracellular matrix molecules such as fibronectin and vitronectin and it serves as a binding site for integrins.

(for example, in the case of α5β1 and αvβ3 integrins, which recognize the RGD motif in proteins such as fibronectin and vitronectin[16–18]) or reside on a specific domain of the α-subunit (as is the case for the collagen-binding integrins α1β1, α2β1, α10β1 and α11β1 (REF.[19])) and the different combinations between various α-subunits and β2 integrins[20] expressed specifically on haematopoietic cells). A characteristic feature of integrins is their ability to bind to several different ECM ligands. ECM ligands, in turn, can also engage different integrin heterodimers[13]. Thus, there is considerable redundancy between specific integrins, which has, for example, complicated the evaluation of the in vivo relevance of these receptors in animal models. However, as discussed in detail below, an emerging theme is that integrins with overlapping ligand specificities possess markedly distinct biomechanical properties. In addition, integrin coupling to other membrane-spanning molecules,

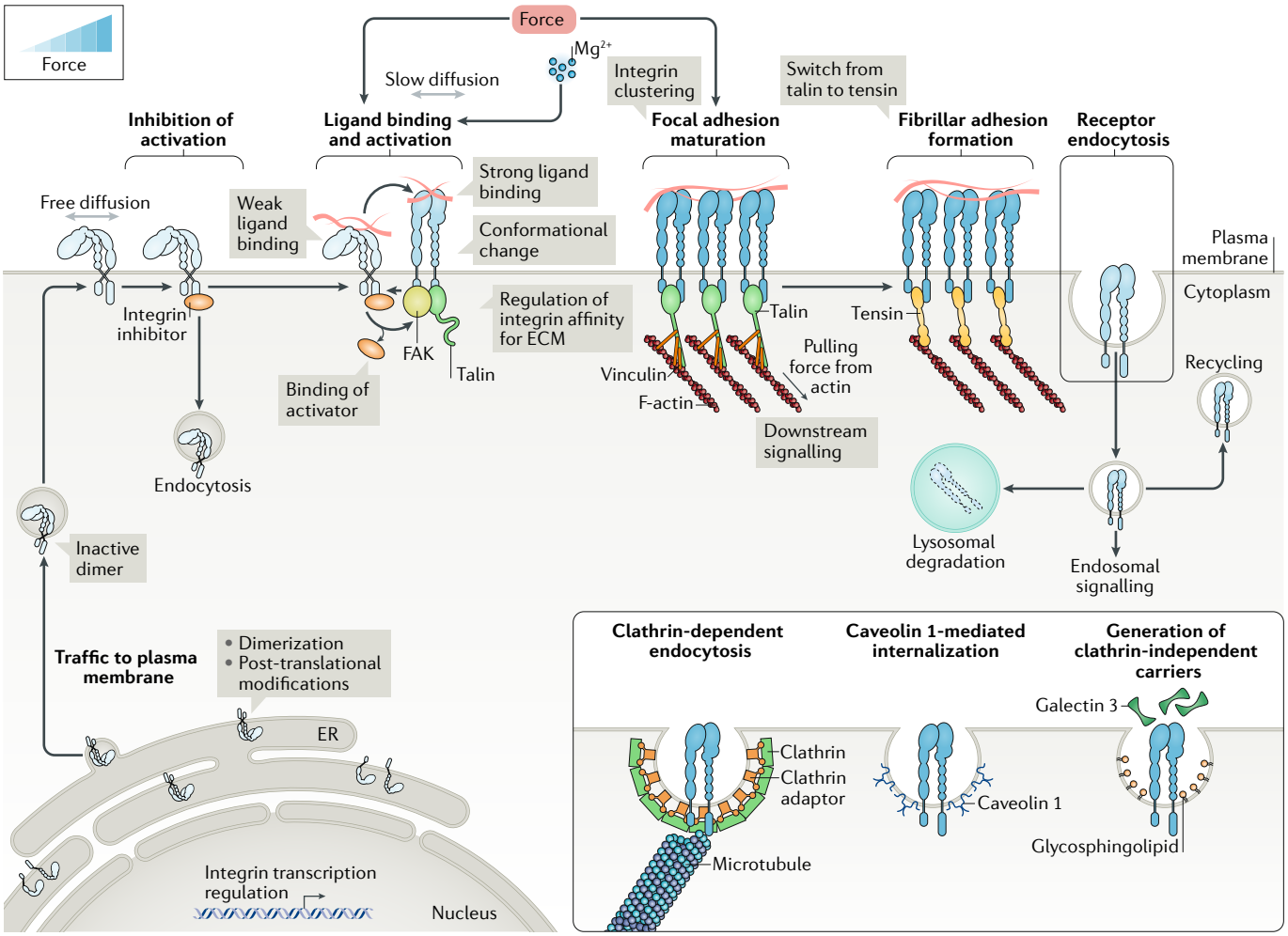


Fig. 1 | **Distinct levels of integrin regulation.** Integrin transcription is regulated by multiple external signals, such as extracellular matrix (ECM) composition or signalling from growth factor receptors. Integrin α-subunit and β-subunit heterodimerize in the endoplasmic reticulum (ER) and Golgi apparatus, mature through post-translational modifications, such as glycosylation, and are transported as an inactive heterodimer (maintained inactive by intraluminal Ca^{2+}) to the plasma membrane[14,52]. On the plasma membrane, inside-out mechanisms[17], in conjugation with extracellular Mg^{2+} (REF.[14]) and the forces from the ECM (see also FIG. 2) allow integrin unbending and the separation of the α integrin and β integrin legs (opening), resulting in activation and increased affinity for ligand binding[14,17]. Inside-out signals regulate displacement of intracellular integrin inhibitors and allow talin binding to integrin β-tails, tightly controlling integrin affinity for ECM ligands[25]. In fibroblasts, recruitment of focal adhesion kinase (FAK) to integrins has been suggested to precede talin recruitment[38]. Integrin activation is also promoted by an outside-in mechanism through ECM binding and force application that slows the diffusion of integrin dimers within the cell membrane. Force application leads to integrin clustering (see also FIG. 2) and the initiation of integrin downstream signalling through the coupling of integrins via talin and vinculin to the actin cytoskeleton (see also FIG. 3). Reciprocally, actin can pull on integrins, further contributing to force generation. In fibroblasts, focal adhesions can mature to fibrillar adhesions where talin is replaced by tensin[30]. Trafficking of integrins regulates their cell-surface availability. Integrins are constantly endocytosed from the plasma membrane. They are then efficiently recycled, with a small subset of the receptors targeted to lysosomal degradation[52]. Integrins may also continue to signal from the endosomal compartment[40,52]. Integrins can be endocytosed via multiple different routes depending on the cell type, adhesion status and cellular signalling pathways that are activated. Integrin cytoplasmic tails contain recognition motifs for clathrin adaptors (DAB2 and ARH bind to β-tail motifs[49], whereas the μ2-subunit of the AP2 clathrin adaptor complex binds to a subset of α-subunit tails[50]), allowing clathrin-mediated endocytosis. Microtubules and clathrin-mediated endocytosis have been implicated in focal adhesion turnover. Clathrin-independent internalization mechanisms include caveolin-dependent pathways and clathrin-independent carriers[51,86,87]. Darker blue shading indicates integrins subjected to higher forces.

such as growth factor receptors[21], proteoglycans[22] and tetraspanins, influences integrin assemblies (see also below) and their cellular functions. In the specific case of the laminin-binding integrins, their high affinity for the tetraspanin CD151 effectively provides them with a 'third subunit' that regulates their ECM-binding properties[23]. Thus, integrins that were previously deemed redundant on the basis of shared ligands or joint downstream signalling functions may still emerge as functionally distinct, owing to other levels of their regulation.

Regulation of integrin activation by inside-out signalling. Integrin inside-out signalling — that is, the regulation of integrin activity by molecular interactions with their cytosolic domain — has been intensively studied for more than two decades. Originally the focus was on integrins expressed in cell types that, in their unstimulated state, are non-adherent: αIIβ3 in platelets and β2 integrin heterodimers in white blood cells. In a resting cell, these integrins adopt a bent, inactive conformation, but become activated upon biochemical interactions[24] and force. We first discuss biochemical signalling pathways inducing integrin activation. The role of force in integrin activation is discussed in the section 'The mechanics of integrins'.

Biochemical integrin activation involves the binding of intracellular adaptor protein talin to the cytoplasmic tail of the integrin β-subunit. This binding event induces separation of the cytoplasmic domains of integrin α- and β-subunits (opening) and triggers a global conformational change in the extracellular domain (which unbends and becomes extended, FIG. 1). Whereas there are nuances in different types of integrin conformation depending on the specific case, one can conceptualize activation as the shift in conformation from 'bent closed' (inactive), to 'extended closed', and finally to 'extended open', which has the highest affinity for ligands and initiates firm adhesion[25] (FIG. 1). Talin binding has a key role in the first steps of integrin activation in all cell types, which is then further supported by the binding of additional cytoplasmic effectors, which mediate not only integrin activation but also the clustering of integrins into many different types of adhesive complex. Such complexes range from very early nascent adhesions to mature focal adhesions, and are strongly force sensitive as discussed in more detail below. Apart from talin, a major effector of inside-out integrin signalling is kindlin, which supports integrin-mediated ECM interactions and subsequent cell spreading on the substratum via two mechanisms. First, it supports integrin activation through binding to the integrin β-subunit cytoplasmic tail[26]. Second, kindlin recruits a key focal adhesion component, paxillin, to nascent adhesions to activate the RHO GTPase RAC1, and it directly associates with the actin-polymerizing Arp2/3 complex to induce RAC1-mediated membrane protrusions, which make the spreading possible[27].

On adhesion maturation (which encompasses strengthening and growth of the adhesion by the recruitment of additional integrins and other molecules; see also the section 'Integrins as environmental sensors'), the talin-induced integrin activation can be maintained by binding to the β1-subunit cytoplasmic tail of tensin 1 and tensin 3, which (as talin) couple the integrin to actin[28,29] (FIG. 1). The talin- and tensin-binding sites on the β1-subunit cytoplasmic tail overlap, indicating that integrins switch from talin binding to tensin binding during adhesion maturation. Tyrosine phosphorylation of the cytoplasmic tail has been suggested to favour integrin–tensin interaction and abrogate talin binding[30]. However, the mechanistic details of the talin–tensin switch in maintaining integrin activity and coupling to actin remain unclear. Conversely, integrin inactivation can be supported by cytoplasmic effectors that compete with talin (inhibitors) either directly through binding to overlapping residues on the β-subunit cytoplasmic tail (these include proteins such as ICAP1 (REF.[31]) and filamin) or indirectly by binding to the integrin α-cytoplasmic tail (SHARPIN and MDGI)[11]. Overall, work in adherent cell types such as fibroblasts, epithelial cells and cancer cells has revealed that the balance of integrin-activating and integrin-inactivating proteins, and how this balance is regulated within cells and tissues, modulate cell adhesion, spreading and motility[26].

A key question related to the first steps of integrin activation has centred on how talin is recruited to the plasma membrane. Single-molecule studies indicated that, unlike integrins, which diffuse along the membrane, talin is recruited directly from the cytoplasm[32] but the mechanism of this recruitment has remained controversial. The prevailing view, largely based on studies in platelets, has been that on platelet activation the small GTPase RAP1 recruits the protein RIAM, which then binds and targets talin to the plasma membrane and integrins[33]. However, an alternative mechanism was recently described where direct interaction between RAP1 and the F0 domain of the talin head was suggested to recruit talin to integrins independently of RIAM[34]. This mechanism was previously dismissed owing to the very low affinity of talin–RAP1 interaction in solution. However, in the context of an intact membrane, anchoring of RAP1 at the membrane increases the strength of the interaction, triggering direct membrane targeting of talin by RAP1. Very recently, these data were disputed by a study showing that point mutations of the RAP1-binding site within the talin F0 domain have minimal effect on αIIβ3 integrin activation in vitro and in vivo[35]. Therefore, the exact mechanism of talin recruitment to integrins remains to be clarified. Nevertheless, the specific cellular lipid microenvironment is likely to be an important modulator of talin recruitment to integrins. For example, on proteoliposomes, reconstituted β1 integrin fragments (membrane-embedded transmembrane-cytoplasmic tail domains) synergized with negatively charged membrane phospholipids (phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate) to recruit the talin head FERM domain to the membrane[35]. Taken together, these studies indicate that the strength and specificity of protein–protein interactions involving integrins are strongly influenced by the plasma membrane, and this should be taken into account when evaluating the biological relevance of the

Tetraspanins
Membrane-spanning proteins involved in the formation of specific membrane microdomains that have a role in numerous cellular processes, including cell adhesion, signalling and membrane trafficking. They have four transmembrane α-helices and two extracellular loops with a conserved Cys-Cys-Gly amino acid motif, as well as two other conserved cysteine residues.

Adaptor protein
In the context of integrin adhesions, an adaptor protein is any of the several different types of recruited protein that directly or indirectly link integrins to actin.

Talin
A high molecular weight protein (~270 kDa) that links the integrin β-subunit to actin filaments and promotes the assembly of focal adhesions. It consists of an amino-terminal head region with the F0 and FERM domains, a flexible rod domain and a carboxy-terminal dimerization sequence.

Nascent adhesions
Clusters of activated integrin molecules with sizes smaller than 1 μm. They either undergo fast disassembly or they progress to mature focal adhesions.

Focal adhesions
Nascent adhesions mature to focal adhesions on tension generated by actomyosin contractility or external forces. This leads to protein recruitment, and a change in shape from dotted to larger, elongated structures.

Kindlin
A family of proteins (kindlins 1, 2 and 3) involved in integrin-mediated cell signalling, acting as linkers between the actin cytoskeleton and integrins.

often weak protein–protein interactions of adhesion proteins in solution. Regardless of whether RAP1 recruits talin directly or indirectly via RIAM, this integrin activation step can be inhibited by a family of scaffolding proteins called SHANKs. SHANK1 and SHANK3 share an N-terminal RAS-association domain with high affinity for RAP1–GTP. By this virtue, SHANKs sequester active RAP1 and limit talin recruitment to the plasma membrane, resulting in reduced integrin activity in cancer cells, primary mammary epithelial cells and hippocampal neurons[36]. In turn, this reduced integrin activity decreases cell migration and invasion. At least in the mouse mammary gland, SHANK3 is not expressed in resident fibroblasts, despite the prominent role of fibroblasts in matrix secretion and remodelling, which involve extensive cell–ECM interactions. On the same note, in addition to the key role of RAP1, another mechanism of integrin activation has been described in fibroblasts. Here, the central integrin downstream signalling protein focal adhesion kinase (FAK)[37] can bind talin directly and recruit it to integrins in newly forming nascent adhesions[38]. Altogether, these important observations suggest that cell type-specific and/or tissue-specific mechanisms may exist to fine-tune integrin activity.

Regulation of integrin cell-surface availability. Cell–ECM interactions, mediated by integrins, are regulated on multiple levels. As discussed above, the ligand-binding affinity of integrins is under dynamic regulation by inside-out integrin signalling. Furthermore, integrin engagement with ECM ligands and their subsequent clustering triggers accumulation of complex adaptor and signalling protein hubs[39] regulating integrin downstream signalling pathways — referred to as outside-in integrin signalling — such as activation of the FAK, SRC, AKT and ERK pathways and regulation of small GTPases of the RHO family[5]. These signalling pathways are essential for many integrin-dependent processes such as inhibition of cell death and in consequence cell survival and regulation of cytoskeletal dynamics and cellular structure required for, among other processes, the maintenance of cell polarity and tissue integrity, intracellular transport or migration. However, inhibition of FAK or SRC signalling does not trigger gross changes in the composition of integrin adhesions, indicating that adhesion composition is not a reflection of its ability to relay kinase-dependent integrin outside-in signalling[39].

Both active and inactive integrin heterodimers are constantly endocytosed from the cell surface and active integrins continue signalling from endosomes[40]. Endocytosed integrins are recycled back to the plasma membrane to facilitate the generation of new adhesion sites[41–43] or are trafficked for degradation in lysosomes[44–48] (FIG. 1). Integrins are endocytosed from the cell surface via multiple distinct pathways, including clathrin-dependent and clathrin-independent routes. Clathrin adaptor proteins can mediate integrin uptake and focal adhesion turnover by binding directly to integrin cytoplasmic tails (for example, the clathrin adaptors DAD2 and ARH bind to β-tail motifs[49]; the μ2-subunit of the AP2 clathrin adaptor complex binds to a subset of α-subunit tails[50]). Clathrin-independent integrin uptake is less well understood mechanistically, although it is known that integrin clustering through extracellular lectins induces receptor uptake through the CLIC/GEEC (from 'clathrin-independent carrier/glycosylphosphatidylinositol-enriched endocytic compartments') pathway[51]. The dynamics of integrin traffic have well-established roles in processes such as adhesion turnover and cell migration[43,52].

Focal adhesion kinase
(FAK). A tyrosine kinase that is involved in the activation and growth of focal adhesions and has a key role in motility and cell survival.

Lectins
Proteins that bind to carbohydrates with high specificity. They are involved in cell adhesion, cell–cell interaction and cell recognition.

The mechanics of integrins

Because they connect the cell cytoskeleton to the microenvironment, integrins are continuously submitted to forces transmitted between cells and the ECM. As such, they are ideally positioned to serve as sensors of mechanical signals (see later). To perform this function, integrins harness the fact that force applied to macromolecules strongly influences protein conformation and function[53]. In general terms, this mechanical regulation can affect three fundamental integrin properties: ligand-binding kinetics; conformation and activation; and clustering and diffusion. The mechanics of the ECM also influence trafficking and subcellular localization of integrins.

Mechanical regulation of integrin–ECM binding kinetics. Perhaps the most obvious effect that force can have at the molecular scale is on the stability of a bond between two molecules. In the simplest case, known as a slip bond, pulling on two molecules that form a bond will tend to dissociate the bond, decreasing its lifetime (or equivalently, increasing its off rate). In physical terms and as first introduced in the 1970s[54], an applied force will tend to reduce the energy barrier between bound and unbound states, thereby promoting unbinding. However, molecular bonds, in general, and integrins, in particular, can also exhibit a more counterintuitive behaviour termed 'catch bond', or more precisely, 'catch–slip bond'. In a catch–slip bond, applied force first strengthens the bond (catch regime), but once the force surpasses a given threshold it starts weakening the bond (slip regime). Different bond configurations can explain this behaviour[55] but the most intuitive example is that provided by the analogy of two attached hooks. If no force is applied, the hooks are loosely bound. As one pulls the hooks apart, the hooks first become locked in place and therefore tightly bound (catch regime). However, if one pulls with sufficient force, the hooks themselves deform and let go (slip regime). Lastly, a final type of bond is that of ideal bonds, in which bond lifetimes do not depend on force. Theoretically, such a regime could exist for integrin–ECM interactions, as integrin–ligand bonds involve a complex interaction surface between the two proteins, which can be affected by force in more than one way. This would lead to opposing effects of force, which could potentially cancel each other out[56].

Despite their less intuitive nature, catch bonds seem to be a common feature of integrin–ligand interactions (FIG. 2a). They occur, for instance, in different integrin bonds involving RGD-containing ligands, such as those between fibronectin and α5β1 (REF.[57]) or αvβ3 (REFS[58,59]). Catch bonds have also been widely reported among

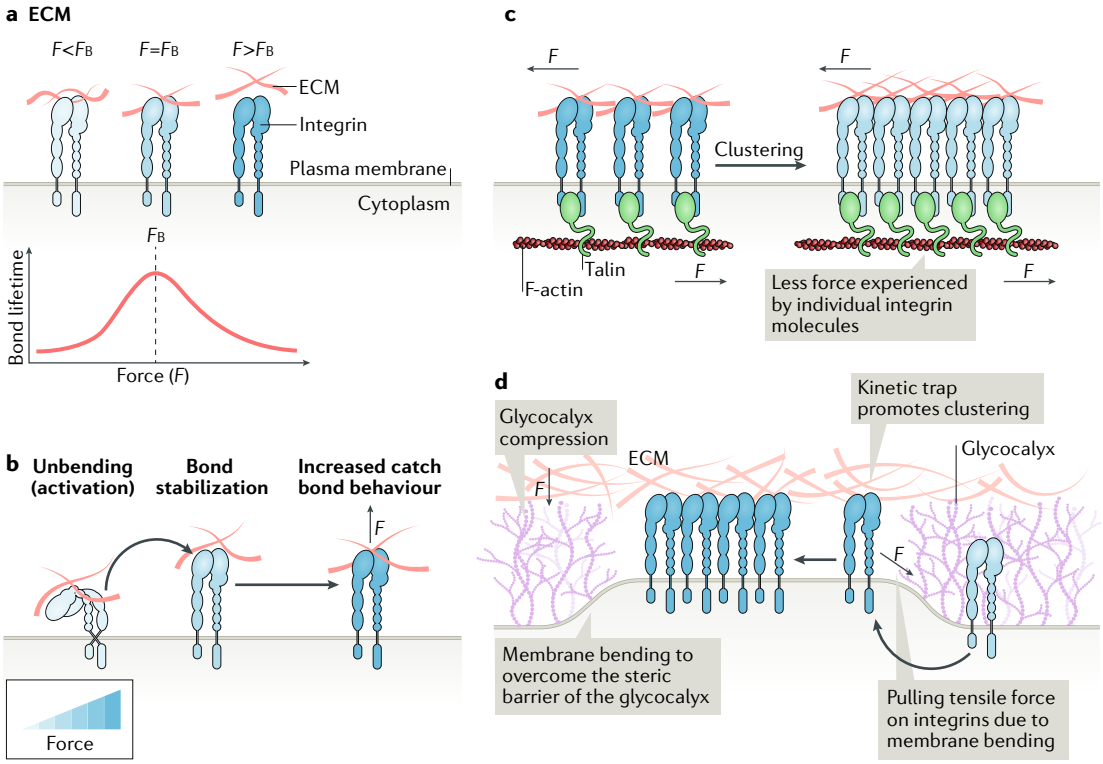


Fig. 2 | **Force regulates integrin properties. a** | Integrin–ligand binding follows a catch bond behaviour[57]. When force (*F*) applied to the ligand-bound integrin is below the optimal bond force (F_B), the strength (lifetime) of the bond increases with force. When *F* exceeds F_B, the bond lifetime decays with force. **b** | Mechanical force (*F*) acting on integrins through their ligands can favour integrin unbending and subsequent activation, thereby triggering outside-in integrin signalling[58]. Activation increases catch bond behaviour, further strengthening the bond. **c** | If a given force (*F*) is applied to an adhesion site, further integrin clustering decreases the force applied to individual integrin dimers. This minimizes elastic energy since it decreases the applied strain, and could thus be promoted. **d** | Because of its bulky nature, the glycocalyx acts as a natural, steric barrier impairing integrin–ligand binding. Binding of the ligand thus necessitates local bending of the plasma membrane. This membrane bending exerts a tensile, pulling force (*F*) on bound integrins resulting from the tendency of the membrane to go back to its non-deformed state and from the local compression of the glycocalyx. This force may modulate integrin properties, such as catch bond dynamics, independently of actomyosin contractility. Finally, the shorter distance between the integrin and the ligand creates a kinetic trap, whereby the close apposition of integrins to their substrate enhances substrate binding and promotes adhesion maturation[83]. Darker blue shading indicates integrins subjected to higher forces. ECM, extracellular matrix.

integrin bonds involved in cell–cell adhesion through surface glycoproteins, such as the bonds between αLβ2 and ICAM1 (REF.[60]), α4β1 and VCAM1 (REF.[61]) and αMβ2 and ICAM1 (REF.[62]). The specific structural mechanisms underlying integrin catch bond behaviour have been challenging to elucidate, largely because the relatively long times associated with integrin bond dissociation (up to the order of seconds) preclude the use of steered molecular dynamics simulations. In the specific case of α5β1 integrin, the presence of a secondary 'synergy' site for integrin binding in fibronectin (other than the main binding site containing the RGD motif) could also influence bond dynamics[63,64]. More generally, structural data have shown that in extended (that is, non-bent) integrins, the progressive change in orientation from the closed to the open conformation increases affinity for RGD ligands by allowing the formation of hydrogen bonds between the integrin and the ligand[65]. Force applied to integrins could reduce fluctuations in integrin conformation, thereby locking them in their open state, increasing affinity for RGD and prolonging bond lifetimes[66,67]. As we discuss in the next section, this mechanism also implies that any change in integrin conformation will also affect bond response to force, potentially even shifting behaviour from that of catch bonds to that of slip bonds. The final type of bond — force-insensitive ideal bonds — has so far been reported for cadherins[68] but not integrins. Finally, it is important to note that whereas the properties under force of integrin–RGD bonds have been intensely studied, there are no reports characterizing force-dependent lifetimes of bonds between integrins and important non-RGD ECM ligands such as collagens or laminins. Thus, whether those highly important physiological interactions behave as catch or slip bonds remains an open question, although indirect data suggest some of them could be catch bonds[69].

Mechanical regulation of integrin activation and conformation. Once an integrin is bound to its ligand and force is applied, this will affect not only ligand-binding kinetics, but also integrin conformation

itself. Since, during their activation, integrins experience major conformational changes that are essential to their properties (FIG. 1), external force is very likely a fundamental means to regulate integrin function. Unlike the biochemical inside-out activation explained earlier, this mechanism would operate from the ECM, being classified thus as outside-in activation. This hypothesis is supported by molecular dynamics simulations, which have shown that forces can trigger several of the steps involved in integrin activation, essentially by pulling integrins open[70–72] (FIG. 2b). As predicted by simulations, experiments pulling on single bonds between fibronectin and integrins αLβ2 (REF.[73]) and αvβ3 (REF.[58]) showed that force induces integrins to transition from their bent configuration to their extended configuration, which strengthens the interaction with the ligand. This leads to a drastic change in bond lifetimes under force: ECM bonds with extended integrins exhibit not only overall longer lifetimes but also a more pronounced catch bond behaviour, which is almost unappreciable for the bent configuration. In T cells, force applied to αLβ2 integrins switches the integrin to an active conformation[74,75]. For α5β1 integrin bonds, force application also promotes integrin activation, strengthening the adhesion (and promoting downstream signalling)[63], even after force is released[76]. This last feature (observed on cyclic force application) is interesting in that the effects of force can persist even after the force is withdrawn, suggesting a possibility of 'memory' of applied force encoded in integrin conformation.

Thus, there is feedback between mechanical regulation of integrin conformation and binding dynamics: applied forces tend to alter the conformation of integrins by activating them, which in turn increases affinity for ligands and catch bond behaviour. Additionally, any biochemical interaction affecting integrin conformation and activation (as discussed above) will impact how integrins respond to force. For instance, different conformations of integrin α5β1 induced by varying ionic conditions can cause the α5β1–fibronectin bond to behave as either a catch bond or a slip bond[57]. Furthermore, in thymocytes the binding of the cell guidance ligand semaphorin 3E to its receptor plexin D1 drastically reduces the lifetime under force of the α4β1–VCAM1 bond and almost abolishes the catch portion of the catch–slip bond, possibly by disrupting inside-out integrin activation[61]. Similarly, binding of the tight junction protein ZO1 to α5 integrins decreases the lifetime of α5β1–fibronectin bonds under force[77], although the exact effect on integrin conformation remains unknown. Such tight integration between biochemical and mechanical control of integrin function has recently been proposed to be fundamental and required for the process of integrin activation[78]. In this hypothesis, small forces in the low piconewton range would be required to decrease the energy barrier between the bent and extended-open conformations, and allow activation. If one compares force and cytosolic binding partners such as talin as potential activating factors, activation should be more sensitive to force. This is because force would decrease the energy barrier linearly, whereas binding of cytosolic partners would do so only logarithmically (thereby drastically lowering the sensitivity of activation).

Mechanical regulation of integrin clustering. Another fundamental integrin property that can be directly affected by force is the clustering of integrins into adhesion complexes, which can occur through different mechanisms. First, once a given cluster of integrins (crosslinked to each other and to actin through adaptor proteins) is submitted to force, it will be subjected to a given elastic strain. It has been hypothesized that incorporation of an additional integrin into the cluster will be energetically favourable, simply because overall strain will be distributed among more integrins and thereby relaxed[79,80] (FIG. 2c). Second, the ability of ligand-bound integrins to diffuse laterally may be restricted by the underlying mechanical properties of the substrate, affecting their ability to cluster[81]. Finally, the glycocalyx has been shown to mechanically promote integrin clustering[82,83] (FIG. 2d). Because the glycocalyx extends from the membrane well beyond the 20-nm length of a typical integrin[84], it serves as a steric barrier impairing integrin–ligand binding. To bridge this physical glycocalyx barrier and bind their ligand, integrins need to locally bend the membrane towards the ligand. This membrane deformation generates a mechanical resistance, which leads to the application of a pulling tensile force on the bound integrin and a corresponding compressive force on surrounding glycoproteins. Force applied on integrins can then feed back to affect integrin conformation or ligand-binding kinetics. Further, the local membrane deformation induced around the bound integrin acts as a 'kinetic trap' where diffusing integrins are closer to the substrate, and thereby have a higher probability to bind ligands. This then promotes integrin clustering, in a way that is also sensitive to the rigidity of the underlying substrate and ligand density[82]. Other than these three potential mechanisms, mechanical regulation of integrin–ligand binding kinetics and integrin conformation can also feed back to affect clustering, as discussed in the next section.

Mechanical regulation of integrin trafficking. As mentioned above, integrin trafficking importantly influences integrin function, and there is increasing evidence that mechanical cues impact these events. Membrane tension has strong implications in plasma membrane uptake in general. Increased membrane tension, triggered by osmotic shock or in cells subjected to mechanical stretching, inhibits flat-to-curved transition of membranes in clathrin-mediated endocytosis[85] and also attenuates CLIC/GEEC fluid-phase endocytosis[86]. Conversely, reduced cell-surface tension induces clathrin-independent plasma membrane uptake mediated by the BAR protein GRAF1 (REF.[87]). How these relate to regulation of integrin endocytosis or recycling is largely unknown. However, there are some interesting examples of tension-dependent and force-dependent regulation of integrin uptake. A study using mobile RGD ligands on supported lipid membranes (RGD membranes) and rigid RGD ligands on glass (RGD glass)

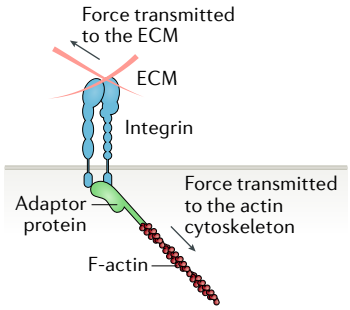
Elastic strain
On application of force to stretch a material, the strain is the change in length divided by the original length. If it is elastic, it will revert to zero when force stops being applied.

Glycocalyx
A meshwork surrounding the cell membrane of many eukaryotic cells and bacteria. It consists of carbohydrates (mostly proteoglycans and glycoproteins) that extend out of the cell membrane.

Fluid-phase endocytosis
Continuous and non-specific uptake of extracellular fluid. This form of endocytosis is not mediated by a specific receptor.

BAR protein
A protein with a BAR (BIN/amphiphysin/RVS) domain. The special banana-shaped conformation of BAR domain dimers creates a pocket of positive charges that could mediate phospholipid binding and curvature sensing or induction.

Box 1 | **Force transmission through integrins**



By providing a link across the plasma membrane, integrins transmit forces between the extracellular matrix (ECM) and the actin cytoskeleton, regardless of where the force originated. This leads to a force balance in which integrins are pulled by the ECM and the cytoskeleton with forces of equal magnitude and opposite sign (see the figure). For instance, any force applied to an ECM fibre will pull on ligand-bound integrins. Integrins do not directly bind actin, but their cytoplasmic tails bind to actin-binding adaptor proteins such as talin, tensin, filamin or α-actinin, which transmit applied forces from integrins to the actin cytoskeleton. Conversely, forces applied to actin (via myosin contraction or actin polymerization, referred to as 'traction forces') are transmitted to the ECM through adaptor proteins and integrins. This general path of force transmission, ECM–integrins–adaptor proteins–actin, is clear, and fluorescence resonance energy transfer measurements confirmed that adaptor proteins such as talin[213,214] and vinculin[215] are indeed under force within integrin adhesion complexes. However, how force is distributed among the many adaptor proteins that directly or indirectly link actin to integrins remains unclear. It is also important to note that the ability of integrins to transmit force is highly regulated by their linkage to both ECM and actin. For instance, if an integrin is pulled through an ECM ligand but the integrin is not linked to actin, the integrin will merely slide along the membrane and not effectively transmit force. The same thing will happen if actomyosin contraction pulls on an integrin that either is not attached to ECM or is attached to a very soft ECM.

demonstrated that cell traction forces inhibit endocytosis of ligand-bound β3 integrins. On RGD glass, force transmitted from the matrix to integrins blocked DAB2 recruitment to activated β3 integrins and supressed clathrin-mediated myosin II-dependent uptake of these receptors. By contrast, on RGD membranes, DAB2 was recruited to integrin adhesions[88], promoting endocytosis. This actomyosin-contractility-dependent clathrin-mediated endocytosis is specific for β3-containing integrins and is not implicated in the endocytosis of RGD-binding α5β1 integrin[88]. This is in line with the notion that β1 integrin turnover from adhesions is insensitive to force[89]. In bone marrow mesenchymal stem cells, endocytosis and subcellular localization of collagen-binding β1 integrins were shown to respond to substrate elasticity: on stiff collagen-coated substrates, β1 integrins were primarily on the plasma membrane, whereas on soft substrates, β1 integrins were primarily endocytosed (via mechanisms involving caveolae and lipid rafts[90]). In addition, a theoretical analysis based on atomic force microscopy data indicated that integrin–ligand complexes are more easily ruptured on soft substrates; this outcome may contribute to the enhancement of integrin internalization on soft substrates[90]. Thus, it seems that the regulated uptake of different β-subunit-containing integrins is sensitive to forces from the ECM but is mediated by alternative endocytic routes (clathrin mediated versus clathrin independent) and may additionally be dictated by other ECM properties, such as ECM composition and biophysical properties. However, these concepts remain to be thoroughly investigated, and deciphering the mechanisms bridging mechanobiological signalling to the availability of integrins on the cell surface will be an exciting area of investigation in the future.

Cell traction forces
Forces that the contractile action of the actomyosin cytoskeleton in cells exert on a substrate measured per unit area.

Caveolae
Small (~50–100 nm) invaginations of the plasma membrane rich in cholesterol. They are shaped by different proteins, of which the caveolin protein family are the principal components.

Lipid rafts
Subdomains of the plasma membrane rich in cholesterol and glycosphingolipids that are resistant to solubilization by non-ionic detergents. They are thought to serve as protein and signalling hubs.

Fibrillar adhesions
Cell–extracellular matrix adhesion sites rich in α5β1 integrin and tensin. They are located towards the cell centre and usually form along extracellular matrix fibrils.

Integrins as environmental sensors

The exquisite sensitivity of integrins to both biochemical and mechanical signals makes these molecules ideal probes of the cell microenvironment. In most cases, combined mechanical and biochemical effects are required to explain cell response to those signals. Here we discuss how the properties of integrins allow them to respond to three fundamental parameters of the microenvironment: force, rigidity and the spatial arrangement of the ECM.

Integrins as force sensors. Tissues in vivo are continuously subjected to mechanical forces generated by cells (largely through the contractile action of the actomyosin cytoskeleton) and by indirect factors such as blood flow in endothelia, air flow in respiratory epithelia or hydrostatic pressure in the mammary gland and bladder[91–96]. Such forces lead to complex tensile and compressive stresses, which cells must sense and respond to so as to maintain homeostasis and which affect processes in development or tumorigenesis[97–99]. Accordingly, and because a large fraction of force is transmitted from the ECM to cells through integrins (see BOX 1), these receptors are considered essential mechanosensors within tissues. Several different single-molecule force sensors have placed the forces experienced by individual integrin molecules within live cells in the wide range 1–100 pN (REFS[100–107]), well within the range where integrin catch bonds (in α5β1 or αvβ3) have their maximum lifetimes (20–30 pN)[57–59]. Once force is applied to integrins, they respond by the processes of reinforcement and adhesion maturation, terms that are often used interchangeably but refer to slightly different concepts. 'Reinforcement' describes the increase in the mechanical resistance of integrin-mediated adhesions on force application. This is usually measured by attaching an ECM-coated probe (such as an atomic force microscope cantilever or a microsphere) to cells through integrins, and checking that once force is applied, it becomes increasingly difficult to either move the probe or detach it from the cell[108–110]. This reinforcement process can happen within 1 s of force application[111], and is likely mediated by the catch bond properties of integrins, without necessarily requiring further protein recruitment.

By contrast, 'adhesion maturation' refers to the process by which force application to an integrin–ECM adhesion results in the recruitment of further integrins and adaptor proteins, which link integrins to the cytoskeleton and increase the size of the adhesive complex[112–114]. Of course, this adhesion growth also increases its mechanical resistance to force, thereby increasing reinforcement. The process of adhesion maturation is intricate (see REFS[8,115] for recent reviews) and can lead to many different types of integrin–ECM adhesive complex differing in size, shape and molecular composition, such as nascent adhesions, focal adhesions or fibrillar adhesions[116]. However, the general view is that initial, nascent adhesions (with sizes of the order of 100 nm) form independently of force[117] and then mature in response to force applied either internally by actin structures coupled to integrins at nascent adhesions or by any of the external factors discussed above through

the ECM[59,112,113,118]. Once force is applied, adhesions grow and alter their molecular composition. Concomitantly, both adhesions and associated actin fibres align in the direction of force application[119–121]. As discussed above, integrin clustering can be directly regulated by force. Notably, however, maturation of integrin adhesions also involves mechanosensing events at the level of adaptor proteins within adhesive complexes, such as talin, FAK or SRC[111]. For example, force triggers talin unfolding[122,123], which allows vinculin binding to both talin and actin, consequently leading to the strengthening of the adhesion, and eventually adhesion growth through mechanisms currently unidentified[59,124] (FIG. 3a). In this case, the mechanosensing event does not occur directly in integrins but it is still strongly regulated by the integrin mechanical response. Importantly, forces reach talin through integrins (BOX 1), and the effect of force on the lifetime of the integrin–ECM bond will determine whether talin experiences forces sufficient for its unfolding. Thus, any event regulating activation or bond kinetics of integrin molecules (as described above) will also control force response in integrin-mediated adhesive complexes. Because integrin regulation by forces is highly subtype dependent (as characterized extensively by comparing α5β1 integrin with αvβ3 (REFS[32,89,108,125,126]), α2β1 (REF.[127]) and αvβ6 (REF.[118]) integrins), this specificity is a means to regulate cellular responses to mechanical cues.

In discussion of the role of force in integrin adhesion maturation it is important to consider that the correlation between the level of force that the adhesion complexes experience and their size occurs only for adhesions in early maturation stages and is lost in large and mature adhesions, which do not grow further even under high forces. This suggests saturation of adhesion complex size and stabilization of adhesion complexes with time[128]. A possible explanation for this is that once adhesions surpass a certain size, force no longer reaches all the parts of the adhesion[80], leading to a weaker relationship between adhesion size and force. Further, in certain conditions, forces above a given threshold can also disrupt adhesions[129]. There is also evidence that adhesions can grow through force-independent mechanisms, likely mediated by the actin template provided by stress fibres formed as the adhesion matures[130–132].

In any case, integrin adhesion maturation affects cell downstream responses in different ways. First, it involves the recruitment and activation of signalling proteins such as FAK[133], paxillin[134], SRC[135] or ERK[114]. Mature focal adhesions also lead to enhanced actin polymerization and the formation of actin stress fibres, which produces two types of effect. First, actin polymerization directly affects the nuclear localization and function of mechanosensitive transcription regulators such as MRTFA (also known as MKL1; by releasing it from unpolymerized G-actin[136]) or YAP/TAZ (Yes-associated protein/transcriptional coactivator with PDZ-binding motif) (by releasing it from its binding to the inhibitory SWI/SNF complex[137]). Second, stress fibres mechanically connect the ECM and integrin adhesions to the nucleus via the linker of nucleoskeleton and cytoskeleton (LINC) complex[138], allowing force transmission from the ECM to the nucleus (FIG. 3a). This leads to several outcomes, including changes in nuclear pore conformation promoting YAP/TAZ nuclear import[139]; chromatin remodelling and exposure of specific sites to transcription factors[140]; and changes in the unfolding[141], accessibility[142] orphosphorylation[143] of nuclear proteins, which can further modulate genome organization and expression (see REFS[138,144] for recent reviews).

Integrin responses to ECM rigidity. Another fundamental mechanical property of tissues is their rigidity, and more specifically, that of the ECM. ECM rigidity results from the combined effect of the composition, degree of crosslinking and density of ECM components[145], and it is a major regulator of tissue function. Different tissues and organs within the human body have different levels of rigidity, spanning from very soft tissues in the brain (with a Young's modulus, a measure of stiffness, as low as 10 Pa) to very stiff structures in bone (up to 10^9 Pa)[146]. Modifications in ECM rigidity are associated with, and are known to drive, several processes, both in physiological scenarios (such as embryonic development[147,148]) and in pathological ones (such as cancer[99,149]). Whereas forces are actively transmitted through integrins, rigidity is a passive mechanical parameter, which cannot be directly sensed by cells. To probe rigidity, cells need to actively use their actomyosin cytoskeleton to deform their surrounding ECM through integrin bonds. The resulting forces will depend on ECM rigidity, since a given actomyosin contraction applied by a cell will result in higher or lower forces depending on whether the surrounding matrix is stiff or soft[91] (FIG. 3b). Thus, cells detect rigidity indirectly by measuring forces they apply to the ECM, and understanding this process requires dissecting how rigidity regulates force transmission, and how force transmission (as reviewed in the previous section) in turn triggers mechanotransduction events in integrins and other proteins.

The processes of both force transmission and subsequent mechanotransduction have largely been studied within the framework of the molecular clutch theory (see BOX 2, also recently reviewed in detail[91]). In this regard, the properties of integrin–ligand bonds (and their regulation by integrin activation and conformation) are a key determinant of force transmission. For instance, we have shown that integrin–fibronectin bonds are able to sustain the forces required to unfold talin (5 pN) only on substrates with rigidities above a given threshold, of the order of a few kilopascals[59]. Thereby, integrin mechanical properties (along with other parameters such as myosin contractility and ECM ligand density; see below) control talin mechanosensing and set an ECM rigidity threshold for it. Accordingly, cells attaching to fibronectin through different integrins with different mechanical properties (α5β1 versus αvβ6) exhibit different rigidity thresholds for mechanosensing, with differences in the formation of mature adhesions and the generation of traction forces at increasing rigidities[118]. Different rigidities can also alter the dynamics of force transmission through the actin adaptor protein–integrin–ECM 'clutch', leading to stable versus spatially fluctuating forces, which has an impact on

Stress fibres
Actin bundles rich in non-muscle myosin II and α-actinin. They have an important role in force transmission and cellular contractility in non-muscle cells.

MRTFA
A transcription coactivator whose nuclear translocation is regulated by the balance between F-actin and G-actin in the cell cytoplasm. When not bound to G-actin, it translocates to the nucleus, where it regulates gene expression on association with serum response factor.

YAP/TAZ
Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) are the two mammalian orthologues of *Drosophila melanogaster* Yorkie. Both proteins are regulated by mechanical signals and the Hippo pathway. In response to mechanical stimulation of the cells or on inhibition of the Hippo pathway, YAP and TAZ translocate to the nucleus, where they can regulate gene expression through their binding to transcription factors of the TEAD family.

Linker of nucleoskeleton and cytoskeleton (LINC) complex
A protein complex that links the inner nuclear lamina with the cytoskeleton. It has important roles in cell migration, nuclear mechanosensing and nuclear positioning.

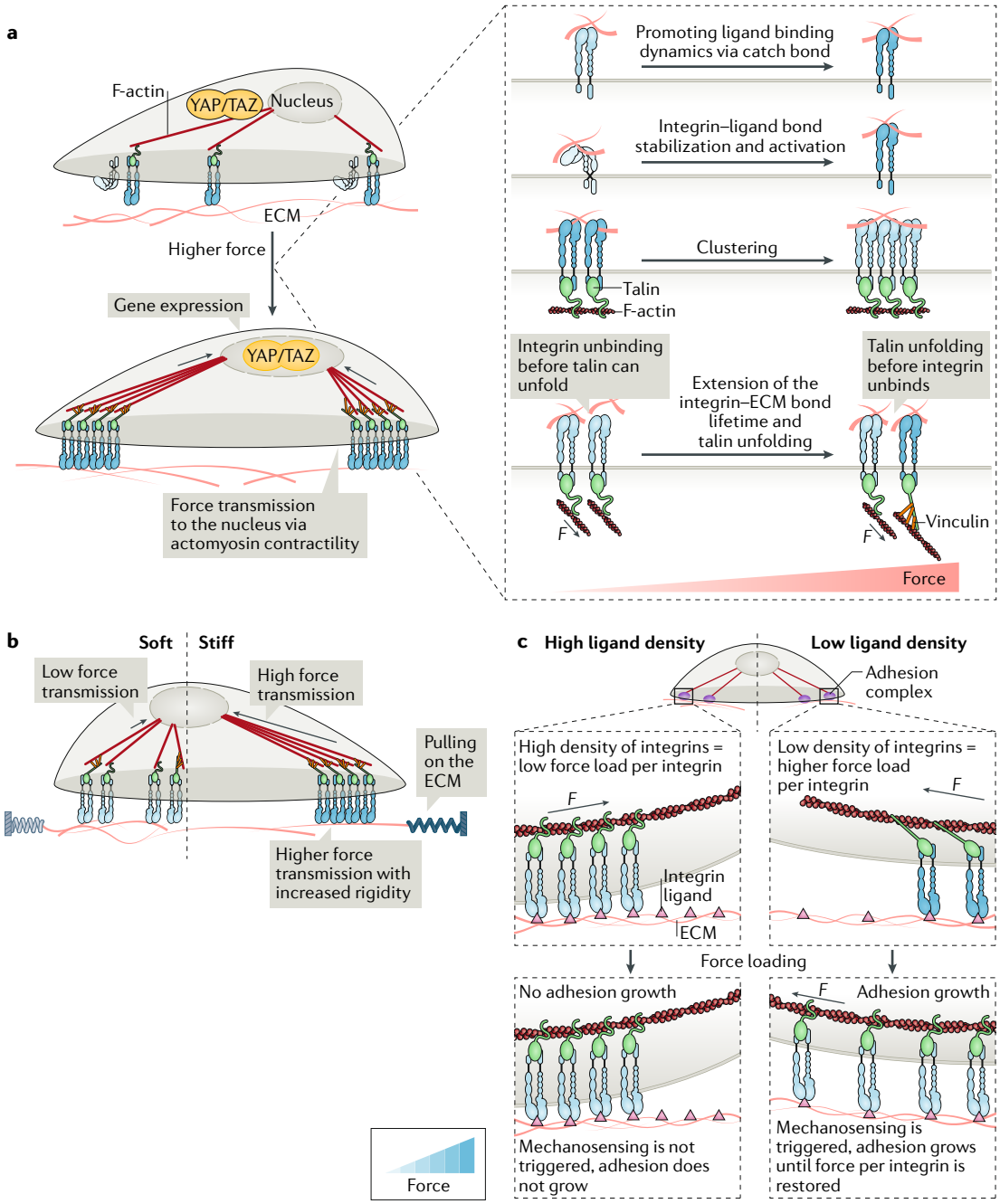


Fig. 3 | **Integrins mediate response to extracellular matrix (ECM) signals such as force, rigidity and ligand distribution. a** | External forces or internal actomyosin contractility affects integrin response via catch bond mechanisms, integrin conformational changes or clustering, as explained in FIG. 2. Increased force can also sufficiently extend integrin bond lifetime, allowing talin unfolding (or other mechanosensitive events), which can then allow the binding of vinculin and the propagation of forces to the actin cytoskeleton[59]. These effects lead to the formation of focal adhesions and stress fibres, allowing forces to reach the nucleus through actomyosin contractility. Forces then influence nuclear shape, affecting nucleocytoplasmic transport and promoting the nuclear translocation of transcription regulators such as YAP/TAZ, ultimately altering gene expression[139]. **b** | Cells continuously pull on the ECM through integrin adhesions and associated actomyosin contractility. High ECM rigidity results in increased force transmission between actin, integrins and the ECM, resulting in the same effects described in part **a**. **c** | Reducing ECM ligand density decreases the number of bound integrins, leading each of them to experience a higher fraction of the force applied by actomyosin contractility[129]. This facilitates the reaching of the threshold of force sensing by integrins, promoting the force-mediated effects described in part **a**. Consequently, integrin adhesions at lower ligand density are more prone to grow and induce downstream signalling in response to force loading. Of note, if ligands are spaced very far apart (and therefore the force per integrin is extremely high), adhesion growth no longer occurs due to an unknown mechanism. Darker blue shading indicates integrins subjected to higher forces.

cell response[134,150]. Such dynamic force transmission through integrins and associated proteins occurs even at the nanoscale, since actomyosin contractile units at the 100-nm scale (about the size of nascent adhesions) bound to the ECM via integrins have been shown to detect and respond to substrate rigidity by triggering the recruitment of the cytoskeletal protein α-actinin[151–153].

Other than bulk rigidity, whether the ECM behaves as a purely elastic material or has viscous and plastic components (as in physiological ECMs) also strongly determines integrin-mediated responses[154,155]. Recent work has shown that cell response to viscous or viscoelastic environments can also be understood through the clutch theory, simply by considering how the change from elastic to viscous properties affects force transmission[156,157].

Sensing of ECM ligand conformation and distribution by integrins. The specificity of integrins towards distinct ECM ligands implies that different integrins will sense the type of ECM protein they are exposed to. Because some ECM binding motifs are only exposed on conformational changes (which can be triggered by force), this also implies that integrins can respond to conformational plasticity of specific ECM proteins, and potentially could 'measure' forces that are associated with these changes. For instance, on matrix metalloproteinase (MMP)-mediated cleavage[158] collagen unwinds and can become denatured at physiological temperatures[159]. Partial denaturing of collagen I leads to the exposure of RGD-containing domains, allowing binding to integrin α5β1 and αv integrins[160]. Similar cryptic integrin-interaction domains exist in fibronectin[161], fibrinogen[162] and potentially other ECM molecules (see REF.[163] for a review).

Box 2 | **Molecular clutch theory**

In essence, the molecular clutch theory considers how the activity of the actomyosin cytoskeleton and its link to the extracellular matrix (ECM) through actin, adaptor proteins and integrins results in force transmission between the different elements (see BOX 1), cytoskeletal dynamics and eventually cell migration. Because all the bonds in the actin–adaptor protein–integrin–ECM link can dynamically form and break, this is referred to as a 'molecular clutch', in analogy to the dynamic connection between shafts in a mechanical engine. Myosin contraction leads to a continuous flow of actin from cell edges towards their centre, known as retrograde flow. Actin polymerization further contributes to this flow by pushing against the plasma membrane[216–219]. Since the bonds linking actin to adaptor proteins, integrins and the ECM continuously break and reform, actomyosin flows and forces are transmitted only partially and slow down progressively from actin to integrins. Adaptor proteins exhibit lower speeds than actin, and in turn integrins exhibit even lower or sometimes even negligible speeds[220]. If one considers how force affects myosin contraction (by slowing it)[118,221] and the binding dynamics between the different molecular players involved (by affecting unbinding rates as a catch or slip bond; see the main text), computational models can be built that predict both the dynamics of cytoskeletal movement and the dynamics of force transmission[222–228]. If the respective speeds of actin retrograde flows and actin polymerization are compared, predictions for cell migration or cell spreading speeds can also be obtained[176,177,222]. Such models can be further refined by also including the force sensitivity of mechanosensitive events in adaptor proteins (such as talin unfolding), leading to predictions of how external factors such as substrate rigidity or ECM ligand distribution regulate whether mechanosensing is triggered[59,118,129]. Model predictions show —and experimental data confirm — that integrin properties under force are crucial determinants of both cell migration and mechanosensing, as discussed in the main text.

Another important mechanical (or at least physical) ECM parameter is the spatial distribution of ligands. Of course, parameters such as ECM density will regulate not only rigidity (see the previous sub-section) but also ligand availability and spatial distribution. Several studies indicate that ECM ligand density and spatial distribution can affect cell responses independently of rigidity. Specifically, it has been shown by use of nanopatterned substrates that integrin clustering and adhesion maturation on very stiff environments occur only when integrin binding sites are placed closer than a given threshold, of the order of a few tens of nanometres[164–167]. This led to the hypothesis that a putative crosslinking molecule of that size crosslinks integrins to each other, thereby serving as a 'molecular ruler' measuring ligand spacing[164,168–170]. However, recent work has shown that on soft substrates, increasing the distances between integrin binding sites above the putative molecular ruler length promoted, rather than inhibited, adhesion growth[129]. This finding is inconsistent with the molecular ruler hypothesis, and could be explained instead by a mechanism regulating force distribution among integrins. It is intuitive to hypothesize that the transmission of actin contractility to the ECM will result in different forces per integrin if the overall distribution and concentration of available binding sites is altered (FIG. 3c). This has indeed been confirmed[171] with use of single-molecule force probes. Consequently, ECM ligand distribution affects adhesion maturation by influencing the force transmitted to individual integrins. Thus, the combined effects of both ligand spacing and substrate rigidity set the force thresholds that will allow mechanotransduction and subsequent adhesion maturation, in a way that can be predicted by the molecular clutch model[129] (BOX 2). Of note, when integrin forces are very high (as in very stiff substrates with highly spaced ligands), mechanotransduction fails due to a yet to be characterized mechanism, leading back to the initial observations that triggered the molecular ruler hypothesis[129].

Roles in physiology and disease

As both a structural scaffold and a conveyor of biochemical and mechanical signals, the ECM regulates a very wide range of processes, from single-cell events driving cell cycle progression[12,13] to complex events in cancer or embryonic development. In such in vivo scenarios, which extend over longer timescales, it is interesting to note that integrins not only sense the ECM but are also able to regulate it, thereby setting up feedback systems. For instance, in the context of fibrosis, force transmitted by fibroblasts through integrins to the ECM can lead to the release of a central mediator of fibrosis, transforming growth factor-β, which is otherwise trapped by latency-associated protein in the ECM. This is mediated by force-dependent unfolding of latency-associated protein[172,173]. In physiological cell migration and cancer cell invasion, such integrin-transmitted forces can also remodel ECM architecture, as we discuss later. Because covering all such relevant physiological scenarios would not be feasible within the scope of this Review, we discuss two cases exemplifying the importance of integrin-mediated ECM interactions: cell migration during

morphogenetic events and the control of cell dormancy and invasion in cancer.

Cell migration and morphogenesis. Cell migration is essential for a wide range of physiological processes, such as embryo morphogenesis, wound healing or regeneration. It is also fundamental in pathological conditions such as cancer, where cells migrate either as individual entities or as highly coordinated collectives in response to molecular and mechanical cues from their environment. Directional cell migration is guided by gradients of different environmental cues, such as diffusible ligands (chemotaxis), substrate-bound ligands in the ECM (haptotaxis) or ECM rigidity (durotaxis)[174,175] (FIG. 4a). Integrins have an essential function in haptotaxis and durotaxis, which naturally derives from their ability to sense ECM rigidity and distribution as described above. Integrin-mediated molecular clutch mechanisms (see BOX 2) have been proposed to explain cell migration speeds in response to ECM rigidity[176] and directional migration in durotaxis in the context of both single-cell migration[134] and collective cell migration[177]. In all cases, the integrin response would be regulated (among other parameters) by the type of integrin–ECM bond being formed, the integrin activation state and myosin contractility levels, providing precise molecular and cell-type specificity to the ECM response.

One prominent in vivo example of directed cell migration is the collective migration of mammary epithelial cells, which in the developing mammary gland migrate collectively into the fat pad to generate ductal branches. This migration process is guided by cell-extrinsic stromal cues, prominently including ECM properties[98] (FIG. 4b). The major structural protein of the mammary gland is fibrillar type I collagen, which underlies the basement membrane[178]. Collagen fibres aligned in the same direction, deposited and remodelled by the mammary gland stromal macrophages[179] and fibroblasts[180], guide the invading epithelial cells, led by the specific tip structures called 'terminal end buds', giving rise to the nascent ducts[181]. Collagen fibre architecture in the mammary gland provides topological cues to guide migration. It also correlates with tissue rigidity during development[180] and during cancer progression[182,183], suggesting that integrin-dependent modulation of rigidity and its sensing could be fundamental to the migration of mammary epithelial cells. Highlighting the involvement of integrins in developmental ductal morphogenesis, stromal deletion of the integrin inhibitor SHARPIN was shown to lead to impaired collagen fibre organization (decreased bundling) as well as decreased collagen deposition and degradation. This impairment of collagen remodelling reduced tissue stiffness in vivo, possibly switching ECM rigidity away from the stiffness optimum of the collectively migrating mammary epithelial cells (FIG. 4b).

Another example of a rigidity-guided morphogenetic event is the migration of *Xenopus laevis* neural crest cells (FIG. 4c). This embryonic cell population undergoes developmental epithelial–mesenchymal transition (EMT) and becomes migratory[184] — a process with many parallels to invasive migration of human carcinomas[185]. In the developing embryo, rigidity of mesodermal tissue underlying the neural crest increases locally — owing to developmentally programmed convergent extension, which increases cell density — which coincides with the onset of EMT and collective cell migration from the neural crest. Remarkably, this increased rigidity is required for the neural crest cells to respond to chemotactic cues from a critical morphogen, SDF1, and thereby to initiate migration. Furthermore, tissue stiffening above the critical threshold was sufficient to trigger premature migration of non-migratory neural crest cells in vivo[148]. In this setting, the mechanical response was reported to be dependent on β1 integrin–vinculin–talin-mediated mechanosensing and myosin contractility[148], suggesting involvement of the integrin–talin clutch mechanism (BOX 2). Thus, mounting evidence suggests that the mechanobiological principles discovered initially in vitro, with use of model systems, are valid in vivo and have fundamentally important roles in regulating cell migration, with relevance to developmental processes as well as cancer (see also the next sub-section).

Tissue rigidity and ECM remodelling in dormancy and invasion. The ECM in tissue stroma is composed of a complex meshwork of extensively crosslinked proteins. The physical properties and architecture of the ECM are highly tissue specific, ranging from interstitial forms within organs to specialized forms, such as basement membranes underlying epithelia and the vascular endothelium[186]. The chemical composition and the biomechanical properties of the ECM are key to maintenance of tissue homeostasis, and altered ECM properties underpin many human pathological processes, including cancer progression and dissemination[187]. As expected, the fundamental role of integrins as ECM sensors places them as major players in the regulation of both ECM composition and ECM properties, and the cell responses to these parameters.

The most abundant ECM proteins are collagen and fibronectin. During normal development, assembly of a fibronectin network is often the 'seed' and prerequisite for deposition of fibrillary collagen networks[188]. Fibronectin network assembly requires fibronectin-binding integrins, especially α5β1, and mechanical stimulation provided by cellular traction forces. Fibronectin has a high degree of conformational flexibility, and forces transmitted from α5β1 integrins to fibronectin expose cryptic binding sites in fibronectin necessary for its polymerization into fibres[189]. During cancer progression, the tumour stroma is remodelled in multiple ways resulting in increased tissue stiffness, altered biochemical composition and cancer-specific fibre alignment[99,190]. Activated fibroblasts in the cancer stroma, referred to as 'cancer-associated fibroblasts' (CAFs), are the main architects of the cancer stroma through their ability to deposit ECM and to physically remodel the stroma[191] (FIG. 4d). Increased collagen deposition and crosslinking by CAFs facilitate cancer progression by activating integrin downstream signalling pathways such as FAK and YAP/TAZ. These ECM remodelling and subsequent signalling events have been reviewed extensively in several excellent reviews[8,192], and we discuss here only some

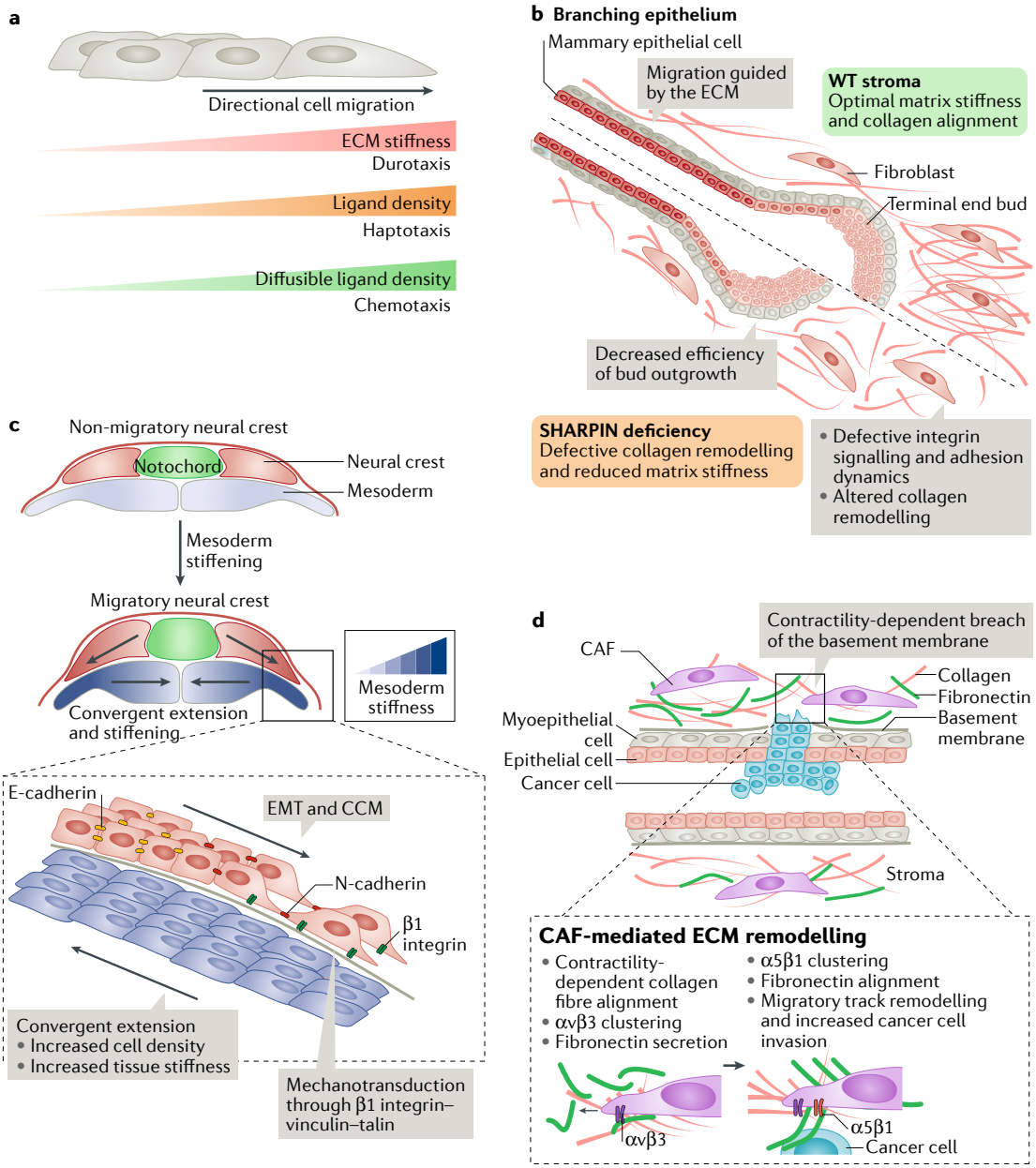
Basement membrane
A sheet-like extracellular matrix structure rich in laminin, collagen IV and nidogen. It acts as a barrier between parenchymal cells and connective tissue.

Neural crest cells
A multipotent group of cells arising at the border between the neural plate and non-neural ectoderm. After gastrulation, they become specified and undergo a process of epithelial–mesenchymal transition during neurulation, migrating to form distinct cell populations in different tissues.

Carcinomas
Cancers that originate in epithelial tissues such as the skin or in tissues that line or cover internal organs. There are different types of carcinoma, including squamous and basal cell carcinomas, adenocarcinomas, melanomas, papillomas and ductal carcinomas.

Convergent extension
A process of collective cell movement during embryonic development by which tissues undergo elongation over one axis and narrowing over the other axis.

Interstitial
Internal to a tissue but not specific to a particular structure.



recent advances in how tumour stroma remodelling and CAFs drive invasion and regulate awakening of dormant disseminated cancer cells, with a focus on the role of integrins in these processes.

Cancer dissemination requires the ability of cancer cells to break through the basement membrane and navigate away from the primary tumour. Imaging of breast cancer tissue has suggested that CAFs contribute to cancer cell invasion by remodelling collagen to establish bundles of straightened and aligned fibres that are oriented perpendicular to the tumour boundary (referred to as a 'tumour-associated collagen signature'), which correlated with poor prognosis and reduced survival in human patients. This remodelled collagen could provide 'migratory tracks' to facilitate invasion following the breach of the basement membrane[183]. In addition to collagen, the tumour stroma is enriched in secreted fibronectin and tenascin C, which favour cell migration and tumour spreading[193,194]. More recently, the role of physical remodelling of fibronectin has been highlighted in cancer invasion (FIG. 4d). In prostate cancer, CAFs were shown to assemble a fibronectin-rich matrix with increased alignment of fibres with respect to each other. In this case, they remodelled fibronectin through actomyosin-contractility-driven traction forces and α5β1 integrin to generate these aligned fibres, which guided cancer cells and promoted directed cell migration[195]. In the model of colon cancer, the role of two fibronectin-binding integrins, αvβ3 and α5β1, was revealed in CAF-mediated fibronectin remodelling, and both integrins were required for cancer cell invasion[196]. In this system, CAFs were seeded in a collagen matrix. They first aligned these collagen fibres in a contractility-dependent manner. Next, downstream of contractility, CAFs secreted fibronectin matrix and aligned the fibronectin fibres. Integrin αvβ3 clustered first, in line

◀ Fig. 4 | **Integrin-mediated regulation of cell migration. a** | Directional cell migration of individual cells and especially of cell clusters can be regulated by different gradients present in the extracellular matrix (ECM): migration towards increased ECM rigidity (durotaxis), increased concentrations of ECM ligands (haptotaxis) or increased concentrations of soluble cues such as growth factors or chemokines (chemotaxis)[174,175]. **b** | During puberty, the ductal outgrowth of the mammary gland involves a process of collective migration, generating the ductal tree that fills the mammary fat pad. The ductal invasion is an integrin-dependent migration process of mammary epithelial cells led by a tip structure called the terminal end bud. The process is regulated by the stroma, and principally by collagen fibre bundling and consequent increase in stromal rigidity — features that are controlled by mammary gland stromal fibroblasts with an important role of integrin signalling. Specifically, it has been shown that integrin activation status is involved in fibroblast-mediated mammary stroma remodelling — interference with the integrin inhibitor SHARPIN gives rise to impaired collagen fibre organization (decreased bundling) and inhibits collagen deposition and degradation. This reduces tissue stiffness and impairs migration of mammary epithelial cells, likely owing to a change in ECM rigidity away from the optimum[98,180]. **c** | During development, mechanical cues regulate the collective cell migration (CCM) of the *Xenopus laevis* neural crest cells. As a result of developmentally programmed convergent extension, the mesoderm underlying the neural crest stiffens, which occurs just before the onset of neural crest collective migration. This generates a mechanoresponsive signal through an β1 integrin–vinculin–talin axis, triggering epithelial–mesenchymal transition (EMT) of neural crest cells and their subsequent CCM[148]. **d** | In carcinomas, fibroblasts in the tumour microenvironment become activated to give rise to cancer-associated fibroblasts (CAFs). These contribute mechanically to cancer cell migration and invasion via numerous distinct mechanisms. The more recently described mechanisms include stromal ECM remodelling, involving contractility-dependent collagen fibre alignment and subsequent deposition of fibronectin and its remodelling into aligned fibres, which is mediated by αvβ3 integrin and subsequently α5β1 integrin and is required for cancer cell migration[195–198]. CAF contractility was also demonstrated to be crucial to breach the basement membrane, allowing pore formation and thereby making it permissive for cancer cell invasion[195–198]. WT, wild type.

with its role in force-dependent focal adhesion maturation[109,117] and was required for the initial stages of fibronectin network assembly. Integrin α5β1 was recruited later to more mature, fibrillar adhesions and was critical only in later stages of fibronectin assembly and generation of myosin contractility at these sites and in fibre alignment[196]. Although not directly addressed in these studies, it is interesting to speculate that the differences in force applied by αvβ3 or α5β1 integrins on the ECM ligand could explain their different roles, because these integrins could activate downstream signals at different force thresholds. These studies also highlight a fundamental 'seeding' role for fibronectin in coordinating stromal architecture and suggest that it may be a key ECM mechanoeffector in the stroma owing to the effect of force on its conformation[189].

CAFs are additionally implicated in the switch from carcinoma in situ to an invasive carcinoma, which involves cancer cells breaking through the basement membrane to the underlying stroma and is generally considered to be regulated by cancer cell-secreted MMPs such as the collagenase MT1-MMP[197]. However, CAFs may contribute to breaching the basement membrane through their unique biophysical properties. A recent study, using decellularized mouse mesenteric basement membrane, demonstrated that CAFs interact with the basement membrane in a contractility-dependent manner (presumably through integrins) and pull and stretch the matrix, triggering softening and eventually the formation of gaps that are permissive for cancer cell invasion[198]. Another barrier for tumour dissemination may lie within the mechanical properties of the polarized glandular architecture of the mammary gland epithelium. It is composed of basal myoepithelial cells adhering to the basement membrane and apically positioned luminal cells[199]. Several transcription factors involved in EMT, such as TWIST1, are linked to poor prognosis and metastasis in breast cancer[200]. β1 Integrin is upregulated by TWIST1 in breast cancer and contributes to increased invasion[201,202], suggesting that integrin-linked EMT-type changes contribute to breast cancer metastasis. Accordingly, expression of TWIST1 in mammary gland myoepithelial cells increases cancer cell dissemination into the surrounding tissue[203]. However, invasion induced by luminal-specific activation of TWIST1 was mechanically blocked by the underlying genetically normal myoepithelial cells, which were capable of capturing the invading luminal cells, forcing them back into the epithelial structure[203]. Although the exact role of integrins was not studied here, at least two potential biomechanical mechanisms related to integrins could be at play here: first, activation of TWIST1 and subsequently EMT induction downstream of integrin-mediated mechanosensing[204]; and second, changes in mechanosensing induced by a switch in integrins — from integrin types dominant before EMT to upregulation of β1 integrin — which show different properties under force. In any case, these data indicated that the biomechanics of the tissue have the capacity to act against EMT-driven integrin-linked invasion.

Disseminated cancer cells can remain dormant in tissue over extended periods, and the cues triggering their awakening are still largely unknown. However, integrin-mediated adhesion is key to several of the mechanisms involved in regulating cancer cell dormancy described thus far. First, integrin-rich filopodia-type adhesions driven by formin-mediated actin polymerization have been linked to overcoming dormancy[205]. Furthermore, association of α5β1 integrin with urokinase plasminogen activator receptor can trigger FAK-dependent and ERK-dependent escape from dormancy[206]. It was also shown that contact of dormant cancer cells with fibrotic and hence stiffer collagen-rich ECM triggers awakening. This ECM stiffness-induced cancer cell activation was shown to depend on the interaction of collagen with β1 integrin and integrin-mediated activation of SRC and FAK, leading to induced actomyosin contractility, actin stress fibre formation and consequent strong adherence to the ECM that favours proliferation[207]. The transition from quiescence to proliferation was also shown to be supported by fibronectin secretion into the ECM, which could be overcome by inhibition of β1 integrin or cellular contractility[208]. Overall, these studies suggest that integrin-mediated signalling regulates the transition from a quiescent state to a proliferative state of cancer cells in vitro, which depends on the integrin-mediated increase in contractility and cell spreading. β1 integrin-mediated cell adhesion to another ECM protein, laminin, can suppress proliferation and malignant features of breast cancer cells[209]. However, during sustained lung inflammation, neutrophil-released proteases process laminin via two-step processing, giving rise to laminin fragments that activate α3β1 integrin and its downstream signalling to induced proliferation

Mesenteric basement membrane
A set of connective tissues that attaches the intestine to the abdominal wall. It contains blood vessels, nerves, lymph nodes and fat.

Urokinase plasminogen activator receptor
(uPAR). A glycosylphosphatidylinositol-anchored cell membrane receptor that acts as a receptor for urokinase plasminogen activator. The binding of urokinase plasminogen activator to its receptor is instrumental for the activation of plasminogen to plasmin — an important blood protease implicated in blood clot resolution, which has also been shown to degrade extracellular matrix and has been linked to cancer progression.

Box 3 | **Anti-integrin drugs**

The broad biological significance of different integrins in human pathological conditions such as inflammation, fibrosis, angiogenesis and cancer has resulted in a drive towards developing integrin antagonists. However, despite numerous clinical trials, therapeutic targeting of integrins with FDA-approved antagonists is currently limited to only three of the 24 known human integrins (for further details, see REF.[229]) and more interestingly to integrins expressed on blood cells. For example, abciximab (monoclonal antibody) blocks fibrinogen binding to the platelet integrin αIIbβ3, an essential event for platelet aggregation, and is administered after percutaneous coronary intervention to prevent thrombotic complications. In leukocytes, disruption of integrin-mediated cell–cell and cell–extracellular matrix adhesions appears to be a central therapeutic nexus in several diseases. Vedolizumab (monoclonal antibody), clinically approved for the treatment of inflammatory bowel diseases such as ulcerative colitis and Crohn's disease, interferes with the leukocyte α4β7 integrin binding to mucosal addressin cell adhesion molecule 1 (MadCAM1). Consequently, vedolizumab disrupts leukocyte interaction with the affected epithelial tissue and prevents extravasation at these sites. In addition to vedolizumab, new antibodies targeting either the β7 integrin subunit or MadCAM1 are currently undergoing clinical trials for these inflammatory bowel diseases. A monoclonal antibody targeting the leukocyte integrin αLβ2 (cognate ligand intercellular adhesion molecule 1) was previously on the market as a drug for treatment of psoriasis but was withdrawn in 2009 due to a rare but severe side effect, progressive multifocal leukoencephalopathy. Integrin α4β1 is also expressed on leukocytes, in addition to other cell types, where it mediates adhesion to fibronectin and VCAM1. Natalizumab is an important anti-α4β1 integrin antibody approved for the treatment of multiple sclerosis. This antibody reduces immune cell migration across the blood–brain barrier and thereby decreases infiltration into the central nervous system. Although this therapy is also linked to a risk of developing progressive multifocal leukoencephalopathy, its substantial benefits to patients with multiple sclerosis are considered to far outweigh the risks. Notably, all of the approved integrin antagonists disrupt integrin–ligand interactions predominantly in non-adherent blood cells, where the role of integrin-mediated mechanotransduction may be less important. Nevertheless, the targeting of integrins in their capacity as mechanotransducers may have broad applications for the treatment of many human diseases, but this potential remains to be tested.

of dormant breast cancer cells in vitro and in vivo[210]. This was linked to increased cell tension after binding to processed laminin, suggesting that integrin-mediated mechanics are key regulators of disseminated cancer cells in the lung[210]. This is concordant with the notion that increased ECM stiffness in lung fibrosis models induces cancer cell awakening from dormancy[207]. The tissue-specific distribution of different ECM components and local differences in mechanical ECM properties together with differences in expression of integrin types on cancer cells may be fundamental drivers of the specificity and context dependence of cancer metastasis. In this regard, cancer cell dormancy and possible awakening could be strongly influenced by the different behaviour of each integrin–ECM pair under context-dependent mechanical cues.

Conclusions and perspectives

By combining sensitivity to both biochemical regulators and mechanical forces, integrins are poised to exert a fundamental regulatory role virtually in any scenario that involves sensing and remodelling of the cellular microenvironment. In this regard, a fundamental challenge is to link specific sensing mechanisms (precisely dissected in simplified single-molecule or cell culture systems[59,123]) to observed effects of biochemical or mechanical integrin regulation in vivo. As exemplified in the last section of this Review, this link is often unclear, and unravelling it will require the development of experimental setups with the use of tissue engineering to combine sufficient levels of complexity with the ability to perform precise mechanical and biochemical measurements. This is critical not only to unravel the fundamental principles involved in integrin-mediated processes but also to design potentially more precise integrin-based therapies in ECM-related diseases such as cancer and fibrosis (BOX 3). Attempts to target integrins in the treatment of solid tumours have often failed in clinical trials, potentially owing to their very general, structural function of integrin adhesion in adherent cells and their inherent functional redundancy. Additional complications may have arisen from possible side effects such as promoting angiogenesis[211] or metastasis by aiding in cancer cell detachment from primary tumours[212]. A precise understanding of the combined biochemical and biophysical mechanisms involved in integrin sensing could lead to more-focused strategies to target integrins for therapeutic benefit. For instance, therapies specifically designed not to block adhesion but to inhibit or modulate integrin-mediated mechanosensing have not been attempted so far and could open an entirely new approach to the treatment of diseases such as cancer.

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