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Integrin avidity regulation: are changes in affinity and conformation underemphasized?

Opinion

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Integrins play critical roles in development, wound healing, immunity and cancer. Central to their function is their unique ability to modulate dynamically their adhesiveness through both affinity- and valency-based mechanisms. Recent advances have shed light on the structural basis for affinity regulation and on the signaling mechanisms responsible for both affinity and valency modes of regulation.

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Current Opinion in Cell Biology 2003, **15**:547–556

This review comes from a themed issue on
Cell-to-cell contact and extracellular matrix
Edited by Eric Brown and Elisabetta Dejana

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2003.08.003

Abbreviations

BRET	bioluminescence resonance energy transfer
EM	electron microscopy
FERM	band 4.1, ezrin, radixin, moesin
FRET	fluorescence resonance energy transfer
I	inserted
ICAM	intercellular adhesion molecule
LFA-1	leukocyte-function-associated antigen-1 (integrin αLβ2)
MIDAS	metal-ion-dependent adhesion site
RAPL	regulator for cell adhesion and polarization enriched in lymphoid tissues

Introduction

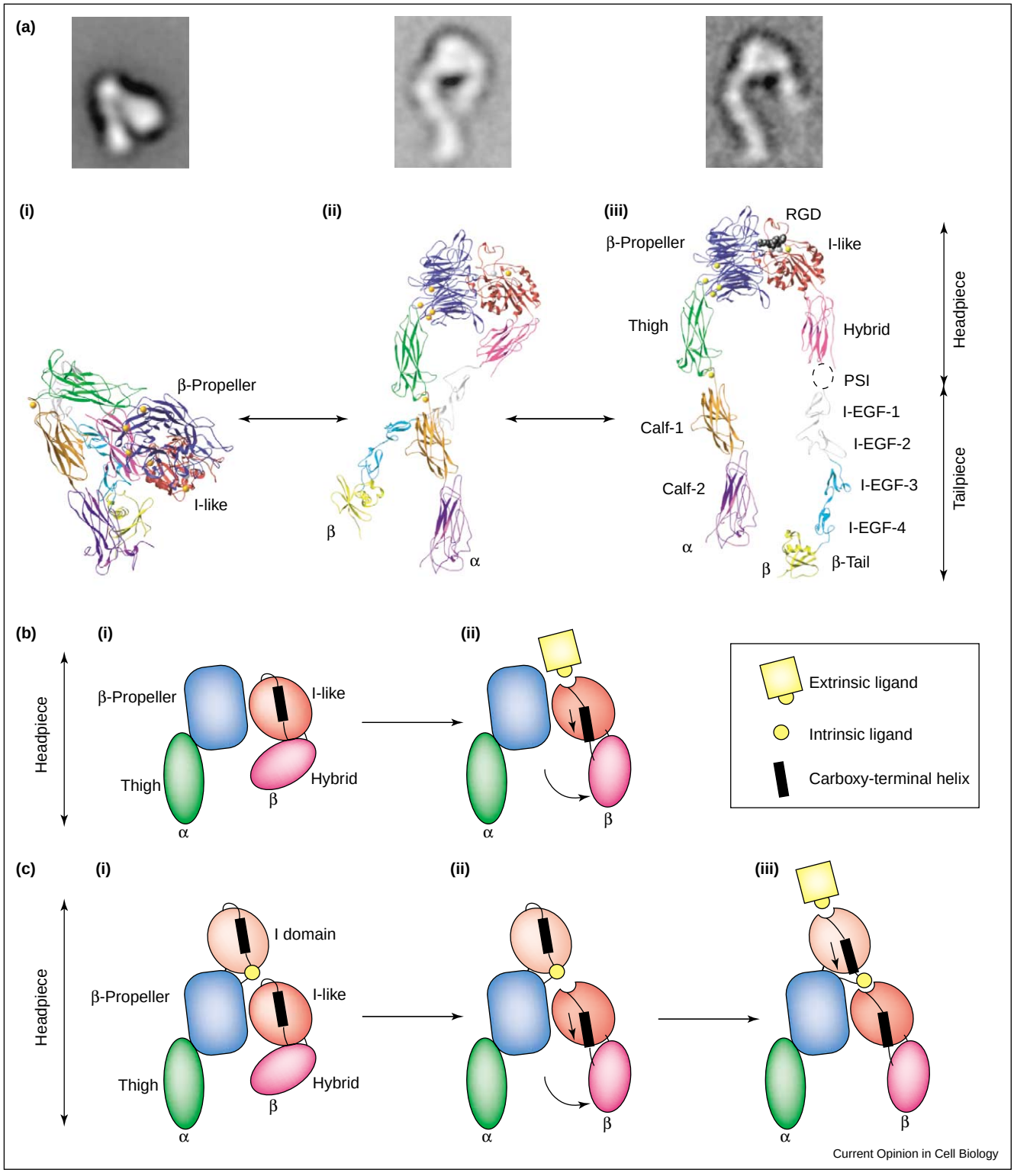
Integrins represent a large family of heterodimeric adhesion receptors composed of α and β subunits that possess the unique ability to regulate dynamically their adhesiveness, through a process termed 'inside–out signaling'. Thus, stimuli received by other cell-surface receptors initiate intracellular signals that impinge on integrin cytoplasmic domains and alter the adhesiveness for extracellular ligand. In addition, ligand binding is transduced from the extracellular domain to the cytoplasm in the classical outside–in direction ('outside–in signaling').

The overall strength of cellular adhesiveness (i.e. 'avidity') is governed by the intrinsic affinity of the individual receptor–ligand bonds, and the number of these bonds (valency). Valency is governed by the density of receptor and ligand on the adhesive surfaces, the geometric arrangement of those surfaces, and the ability of the receptor and ligand to move, either passively by diffusion or actively, from other parts of the cell into the zone of cell adhesion. The dynamic regulation of integrin-mediated adhesiveness is thought to involve modulation of all of these parameters. It has previously been questioned whether changes in integrin affinity and conformation were overemphasized [1]; however, recent structural advances reviewed here demonstrate that integrins undergo striking conformational change, and that this dramatically regulates affinity.

Our growing appreciation for the complexity of integrin regulation is confused by inadequate and vague terminology and conceptual plurality, especially for the key concept of avidity. In the first demonstration of regulated adhesiveness through leukocyte-function-associated antigen-1 (LFA-1; integrin αLβ2), it was stated that 'although the mechanism of the regulation of LFA-1 avidity is unclear, a change in the conformation of the ICAM [intercellular adhesion molecule] binding site or redistribution in the membrane seem most likely' [2]. This use of the term 'avidity' was in keeping with prior use in immunochemistry [3] for the total adhesive strength — that is, the multimeric affinity or functional affinity — that results from both the total number of receptor–ligand bonds and the affinity of each of these bonds (monomeric affinity). Thus, avidity can be regulated by either altering valency or affinity. However, the term 'avidity' is used by many workers in the field of integrin biology as encompassing only regulatory mechanisms that do not involve affinity modulation.

In an attempt to clarify the terminology and concepts in the integrin field, we will use the following definitions. Affinity regulation: changes in monomeric affinity that are coupled to alterations in integrin conformation or changes in the equilibrium between different integrin conformational states. Valency regulation: changes in cell surface receptor diffusivity or local density, or in the geometry of the interaction interface, that alter the number of adhesive bonds that can form. Priming (inside–out signalling): regulatory events — either affinity- or valency-based — that precede, or occur independently of, ligand binding, and serve to enhance the propensity to bind ligand efficiently [4]. Adhesion strengthening: ligand-dependent, post-adhesion events that result in enhanced adhesive strength

Figure 1



Global and local integrin conformational changes associated with affinity regulation. **(a)** Switchblade model for global integrin conformation regulation defined by EM [10••] and atomic structures [7,9,85•]. The upper panels show EM averages and the lower panels show ribbon diagrams based on the bent crystal structure or fitting of the latter to the extended EM structures. (i) Bent conformation (low affinity). (ii) Extended conformation with closed headpiece (predicted to be of intermediate affinity). (iii) Extended conformation with open headpiece (high affinity). **(b)** Hybrid domain swing-out and pull spring models for priming of integrins lacking an I domain. The four main domains of the headpiece are drawn based on the

either by accumulation of receptors into the zone of substrate contact, increase in the area of contact or receptor interaction with the cytoskeleton [5]. Ligand-induced activation (outside–in signalling): ligand-induced propagation of intracellular signals, which result from either changes in integrin conformation or cell surface distribution or both [4].

Affinity regulation

Global conformation rearrangements

Recently, a striking and unexpected model for integrin global conformational regulation has emerged [6]. Using electron microscopy (EM), it has been known for years that the overall topology of integrins included a globular amino-terminal ligand-binding head domain, containing a critical α and β subunit interface, and two long carboxy-terminal legs or stalks that connect to the transmembrane and cytoplasmic domains of each subunit [7]. The recent X-ray crystal structure of most of the extracellular domain of the integrin αVβ3 provided the surprising finding that the legs were severely bent at the so-called 'genu' or knee, generating a V-shaped topology in which the ligand-binding domains in the headpiece were closely juxtaposed to the membrane-proximal portions of the stalks (i.e. the tailpiece; Figure 1a, first panel) [8••]. Such an orientation appears unfavorable for binding to extracellular matrix or cell-surface ligands. Indeed, nuclear magnetic resonance (NMR), EM, mapping on the structures of epitopes of conformation-sensitive and activating antibodies, and engineering of disulfide bonds across the head–tail interface, have together established that the bent or 'closed' integrin conformation represents the physiological low-affinity state [9,10••,11••]. Moreover, priming and ligand binding are associated with a separation of the α and β tails that is coupled to a large global rearrangement in which the integrin extends with a 'switchblade'-like motion [10••,11••,12•]. Furthermore, the introduction of ectopic glycosylation sites into regions of β1 and β3 that are buried in the closed conformation leads to constitutive adhesiveness of α5β1, αIIbβ3 and αVβ3 [13,14••].

Intradomain conformational regulation

Although the change in orientation and heightened exposure above the cell surface of the integrin headpiece that accompanies global conformational change probably increase integrin access to ligand, extensive evidence also exists for coupled intradomain conformational changes that modulate affinity [11••,12•,15]. Since intradomain conformational change is best understood in integrin α subunit inserted (I) domains, we will review this first and then turn to integrin β subunit I-like domains.

Among the 18 integrin α subunits, half include an I domain between blades 2 and 3 of the β-propeller, which when present represents the major ligand-binding domain [16]. Structures of I domains revealed the existence of two conformations, termed 'closed' and 'open' [16–19]. Compared with the closed conformation, the open conformation exhibits distinct coordination of the metal in the metal-ion-dependent adhesion site (MIDAS), a distinct arrangement of the β6-strand–α7-helix loop, and a 10 Å shift of the carboxy-terminal α7 helix down the side of the I domain [17–19]. Mutations that stabilize the closed or open conformation exhibit constitutively low or high affinity for ligand, respectively [19–22,23•,24–26]. Engineered disulfide bonds that pull the α7 helix downward are sufficient to induce high-affinity ligand binding [19–21,23•,24]. Thus, physiological conformational signals that exert a similar pull might function in priming. The ability to crystallize the mutationally stabilized open conformation in the absence of ligand or a ligand mimetic lattice contact [19], as well as the ability to detect movement of the MIDAS [27] and carboxy-terminal α helix [28] using conformation-sensitive antibodies in intact cells in the absence of ligand demonstrates that the high-affinity conformation can indeed form independently of ligand, and thus that conformational change contributes to integrin priming.

Interdomain communication

In I-domain-containing integrins, the I-like domain is thought to represent a central regulator of the I domain. Allosteric I-like domain antibodies [21], I-like MIDAS mutation [29] and recently identified small-molecule antagonists of the I-like MIDAS [30•] all inhibit ligand binding by the I domain. Mutations in the linker between the I-like domain α7 helix and the β-propeller domain can either activate or inhibit ligand binding, and it was

(Figure 1 Legend continued) orientation, proportions and color scheme depicted in (a). The black cylinder and curved line coming from the 'top' of the I-like domain represent its carboxy-terminal α7 helix and the β6/α7 loop, respectively. The black line coming from the bottom of the α7 helix is one of the two connections to the hybrid domain. (i) Closed headpiece, corresponding to (i) and (ii) in (a). (ii) Open headpiece, corresponding to (iii) in (a). The pivot or 'swing-out' of the hybrid domain by ~80° with respect to the I-like domain is envisioned to pull the I-like α7 helix downward (i.e. as a 'pull spring') and shift the β6–α7 loop and the MIDAS into the open conformation, capable of binding extrinsic ligand with high affinity. **(c)** Hybrid domain swing-out and pull spring models for priming of an I-domain-containing integrin. Compared with (b), an I domain is inserted at the top of the headpiece into the β-propeller at a location that corresponds to the approximate location of the loop connecting blades 2 and 3 in the atomic structure. A black cylinder and curved line coming from the 'top' of the I domain represent its carboxy-terminal α7 helix and the β6–α7 loop, respectively. In addition, the linker connecting the carboxyl terminus of the α7 helix to the β-propeller domain is depicted as a curved line coming from the bottom of I domain α7 helix and connecting to the β-propeller domain. The invariant glutamate (Glu310 in LFA1) that is postulated to serve as an intrinsic ligand for the I-like MIDAS is depicted as a yellow sphere. (i) Closed head piece. (ii) Open headpiece transition. Hybrid domain swing-out and I-like MIDAS conformational change proceed as in (b). (iii) Open headpiece. The open I-like MIDAS binds to the intrinsic ligand in the linker, exerting a pull on the α7 helix that causes it to move down the side of the I domain and the MIDAS to shift into the high-affinity conformation.

suggested that an invariant glutamate residue in the linker (Glu310 in LFA-1) might function as an intrinsic ligand that, when bound to the I-like domain MIDAS, would exert a downward pull on the I domain α7 helix and induce the high-affinity state (Figure 1c) [6,31,32].

The orientation between the I-like and hybrid domains appears to represent the critical 'translator' for converting global conformational change into local intradomain conformational changes that regulate affinity (Figure 1). High-resolution EM studies demonstrate that the hybrid domain exhibits two distinct orientations with respect to the I-like domain. In the presence of ligand, the hybrid domain swings outward by ~80°, into an 'open orientation' [11••,15]. The I-like domain is inserted into the hybrid domain, to which it is attached by both its amino terminus and its carboxy-terminal α7 helix. The observed direction of pivoting is consistent with downward movement of the α7 helix which is hypothesized to be coupled to a shift of the I-like domain MIDAS to the open conformation (Figure 1) [11••,14••,15,33•]. Thus, I and I-like domains are hypothesized to be activated by similar conformational mechanisms. Addition of ectopic glycosylation sites into β1 and β3, engineered to function as a wedge between the I-like and hybrid domains and enforce the open orientation, leads to constitutive high-affinity ligand binding by both α5β1 and αIIbβ3 [14••]. In addition, hybrid domain epitopes masked by the I-like domain under basal conditions are exposed upon activation [33•]. Furthermore, Leu358→Ala mutation in the I-like domain carboxy-terminal α7 helix of β1 integrin induces both high-affinity ligand binding and expression of I-like domain activation epitopes [33•].

Separation of the integrin α and β subunit transmembrane and cytoplasmic domains has emerged as the critical trigger for initiation of inside–out conformational signaling (i.e. integrin priming; Figure 1). Cytoplasmic-domain mutations are well known activators of integrin adhesiveness, and association between the α and β subunit transmembrane and cytoplasmic domains constrains the inactive state [34]. Recent X-ray crystal [8••], EM [11••] and cryo-EM [35] structures provide direct evidence that the membrane-proximal portions of the extracellular domains, and the transmembrane and cytoplasmic domains of the α and β subunits, are in close juxtaposition in the inactive state. Enforced association of the α and β cytoplasmic domains [9] or of the α and β subunit membrane-proximal stalks [11••,12•] renders integrins inactive, whereas release of these constraints promotes high-affinity ligand binding. NMR structures of the cytoplasmic domains of αIIb and β3 reveal a direct association that is perturbed by both activating mutations and by talin-head-domain binding [36••]. Recent fluorescence resonance energy transfer (FRET)-based studies directly demonstrate that separation of the cytoplasmic domains occurs in living cells during priming induced by chemokine binding to G-protein-coupled receptors, talin-head-domain binding, and as a consequence of activating cytoplasmic domain mutations [37••].

Models for integrin conformation regulation

One of the more prominent models for integrin conformational regulation has been the 'hinge' or 'scissor' hypothesis, in which a fulcrum was suggested to exist within the transmembrane domain, and activation involved separation or dramatic rigid body motion at the α and β subunit interface in the head domain. This model has now been ruled out by the use of engineered intersubunit disulfide bonds in the headpiece [38] and by EM analysis of αVβ3 [11••] and fibronectin-bound α5β1 [15]. Furthermore, results from FRET-based measurements suggest that the large scale of cytoplasmic domain separation that occurs during priming and activation is more consistent with separation of the transmembrane domains than with hinging [37••].

The accumulating structural and functional data provide strong support for a recently proposed model [11••] for integrin conformational regulation (Figure 1). Priming signals induce binding of proteins, such as talin and possibly RAPL (regulator for cell adhesion and polarization enriched in lymphoid tissues; see below), that destabilize association of — and initiate separation of — the α and β subunit cytoplasmic and transmembrane domains. As a direct consequence, the extracellular interface between the α and β subunits in the tailpiece becomes destabilized, concomitantly perturbing the tailpiece–headpiece interface and facilitating switchblade-like opening [11••]. The hybrid domain is prominent in the tailpiece–headpiece interface, and disruption of this interface appears to be required to enable the hybrid domain to swing out with respect to the I-like domain, facilitating the downward movement of the I-like domain α7 helix that is coupled to MIDAS rearrangement [11••]. For integrins that lack I domains, this represents the final step of priming (Figure 1b). For I-domain-containing integrins, the I-like domain next appears to bind to the intrinsic ligand in the linker between the I domain α7 helix and the β-propeller, thereby exerting a downward pull on the α7 helix of the I domain, leading to affinity modulation of its MIDAS (Figure 1c). The conformational rearrangements that result from modulation of cytoplasmic/transmembrane domain association appear similar to those that result from binding of ligand, except that the conformational signals flow in opposite directions [11••,12•,19,37••].

Linked equilibria, the law of mass action, intermediate affinity states and conformational breathing need to be borne in mind when considering conformational regulation of integrins. EM studies of αVβ3 demonstrate that in many conditions, multiple conformational states co-exist [11••], and physicochemical studies demonstrate that

these states equilibrate on a timescale of less than minutes [11••]. EM studies demonstrate an intermediate global conformation that is extended but has a closed headpiece (Figure 1a, second panel), and is thus expected to have an affinity for ligand intermediate between that of the bent conformation and the extended conformation with the open headpiece. Furthermore, a conformation is seen that resembles the bent conformation, but in which the interface between the headpiece and tailpiece is partially opened [11••]. Moreover, crystal studies of the integrin αL I domain demonstrate an intermediate conformation that appears to be at a low-energy minimum along the pathway between the closed and open conformations, and which has intermediate affinity for ligand [19]. Ligand binding by integrins exhibits multistep kinetics; conversion to a higher-affinity form occurs on a timescale of ~10 s after ligand binding [39–41]. The magnitude of soluble ligand binding induced in cells by physiological adhesion stimulators is intermediate compared with basal and Mn^{2+}-stimulated conditions, which might reflect intermediate affinities as well as fractional priming [42–45]. Thus, regulation of integrin affinity/conformation should be viewed as a shifting of the dynamic equilibrium between closed, intermediate and open conformers, rather than the flipping of a switch. Importantly, ligand might function — just as inside–out signals — to drive the equilibrium toward the open state. Indeed, whereas Mn^{2+} induces a mixture of closed, intermediate and open conformations of αVβ3 (Figure 1a), addition of saturating ligand produces almost exclusively open conformers [11••]. Thus, in the context of cellular adhesion, the combination of inside–out signals and ligand binding should together determine the position of the conformational equilibrium.

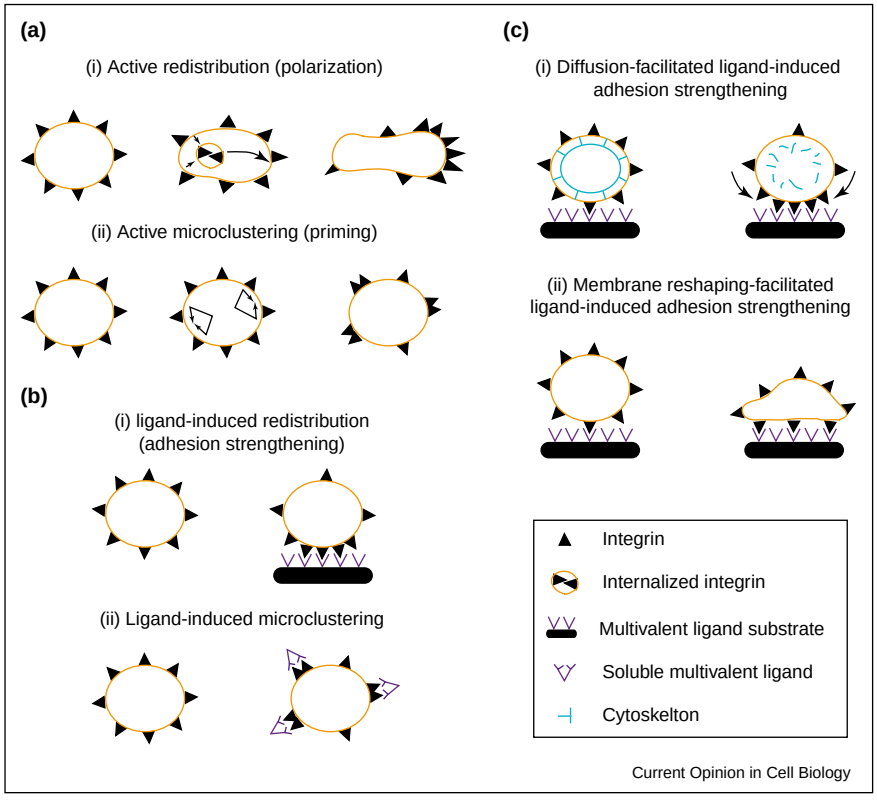
Valency regulation

Elucidation of the precise roles of, and mechanisms for, integrin valency regulation has been clouded by the use of the imprecise term 'avidity regulation' and the frequent use of negative findings as the key justification for concluding that valency regulation is at work. The principle diagnostic used to infer valency-based modes of regulation is the failure to observe high-affinity soluble ligand binding when cells are activated with certain agents that do induce cellular adhesion to ligands on substrates [1,46,47]. However, the failure to detect soluble ligand binding to cells under such conditions could reflect a lack of sensitivity of ligand-binding assays to intermediate levels of affinity, rather than a lack of affinity regulation. For example, the dissociation constant K_d of 200 nM of the high-affinity conformation of the αL I domain [23•] and of wild-type activated αLβ2 [48] is just barely within the range that is detectable by conventional assays for ligand-binding to cells. The K_d of 2 mM for the closed conformation is barely detectable even using surface plasmon resonance. A designed intermediate-affinity αL I domain has a K_d of ~3 μM [19]. Such an intermediate affinity should be sufficient for firm adhesion, but not for detectable binding to soluble ligand. Recent improvements in soluble ligand-binding assays, including careful assessment of kinetics, have clearly demonstrated rapid and transient integrin affinity regulation in response to chemokines [42–45]. Moreover, as mentioned above, sensitive assays often demonstrate that physiological stimuli, as well as phorbol myristate acetate (PMA), induce markedly less soluble ligand binding than Mn^{2+} [49], which is the commonly employed positive control for affinity regulation.

Clustering or patching is often taken as positive evidence for valency regulation; however, at best a correlation rather than a causal relationship with increased adhesiveness can be demonstrated. Furthermore, microscopic definitions of clustering are inconsistent and imprecise, with diverse integrin distribution patterns including punctate, patchy and polarized. Essentially, anything other than an even membrane distribution (as determined using fluorescence microscopy) is termed a 'cluster' and thus ascribed functional relevance. Moreover, inherent to the microscopic approaches used is the arbitrary and flawed assumption that valency regulation should occur on a length scale that is greater than the lower resolution limit of the optical microscope. However, membrane rafts, entities that inherently represent functionally important clusters of lipids and proteins, are generally not visible using such methods and require more sophisticated techniques, such as FRET, for analysis [50]. Finally, experiments designed to assess integrin clustering are often performed under conditions that promote robust homotypic cell aggregation and LFA-1 redistribution to the zone of adhesion [47]. All leukocytes that express integrin αLβ2 also express one or more of its ligands, the ICAMs, facilitating formation of clusters of homotypically adherent cells. αLβ2 redistributed as a consequence of binding to ICAM-1 on adjacent cells would be expected to remain clustered for several minutes after cells are separated by vortexing.

Compounding the experimental issues is confusing terminology. Diverse processes, including diffusion, oligomerization, ligand-dependent redistribution as a consequence of adhesion strengthening, and redistribution as a consequence of polarization and intracellular trafficking, have all been lumped together under the terms 'avidity regulation' and 'clustering' (Figure 2). Thus, we will use more precise terminology, referring to large-scale integrin reorganization as 'redistribution' and referring only to integrins that are close to one another on a molecular length scale of ~100 Å as 'microclusters'. Moreover we differentiate ligand-independent from ligand-dependent redistribution processes as 'active' and 'ligand-induced', respectively, and further differentiate the latter as either 'diffusion-facilitated' or 'membrane-reshaping-facilitated' (Figure 2).

Figure 2



Modes of cellular integrin reorganization associated with valency regulation. Integrin reorganization might occur on a relatively large scale and be observable with fluorescence microscopy (redistribution) or on a submicroscopic scale observable using specialized techniques such as BRET or FRET (microclustering). **(a)** Ligand-independent reorganization. (i) Reorganization might occur that requires intrinsic active processes such as vesicular trafficking involved in redistribution associated with cellular polarization, as in the delivery of integrins to the leading edge of a migrating cell [51]. (ii) Alternatively, it is hypothesized that integrins could be actively driven into microclusters either by association with lipid rafts [53••,54•] or by homotypic oligomerization [57,58], events suggested to represent one type of priming mechanism. (Another priming mechanism is affinity regulation.) **(b)** Ligand-dependent reorganization occurs primarily as a consequence of the law of mass action. (i) Gross redistribution of integrin into the zone of contact with ligand during homotypic cell aggregation, heterotypic associations such as the immunological synapse or adhesion to other multivalent substrates such as the extracellular matrix. (ii) Alternatively, microclustering might occur as a consequence of associations of integrins with soluble multivalent ligands such as fibrinogen [62•]. (i) and (ii) might co-exist in adhesion to substrates. **(c)** Adhesion strengthening represents ligand-dependent regulatory events that can occur by several distinct mechanisms, either separately or, more likely, in combination. (i) Evidence suggests that inside–out signals could function to release cytoskeletal restraints and increase diffusivity [59], thereby facilitating ligand-induced redistribution of integrins to the zone of contact with substrate, and enhanced valency of the interaction [41]. (ii) Membrane reshaping (cell spreading) to enhance the complementarity between integrin and ligand-bearing surfaces can also serve to facilitate increased valency of adhesions [86]. Post-ligand association with the cytoskeletal can also contribute to adhesion strengthening, most notably in the context of focal adhesions and focal contacts (not shown).

Active modes of integrin reorganization should occur independently of ligand, implying the existence of intrinsic 'driving forces' for this [1,46]. Vesicular trafficking [51,52] and Rap1-driven polarization [53••,54•] of integrins represent important active modes of integrin reorganization that take place during cell migration, as discussed below. However, mechanistic support for active reorganization of integrins during priming remains tenuous. One hypothesis includes the dynamic recruitment of leukocyte integrins into lipid rafts as a basis for valency priming; however, reports conflict as to whether these integrins are excluded, constitutively associated with, or driven into rafts, and as to whether their adhesive functions are sensitive to raft disruption [55–58]. Another recently suggested mechanism for active integrin microclustering includes formation of homotypic associations between the transmembrane domains of neighboring integrins upon transition to the open conformation [59,60]. However, no direct support for such associations occurring in wild-type integrins in cells yet exists. To the contrary, conditions that promote the open high-affinity conformation in LFA-1 fail to produce either microscopic redistribution or FRET-detectable microclustering [37••].

The best-characterized basis for valency regulation is adhesion strengthening, whereby post-adhesion accumulation of receptor–ligand bonds contributes to overall adhesiveness (Figure 2). An important mechanism for

this is diffusion-facilitated ligand-dependent integrin redistribution, in which signals function to release cytoskeletal constraints and increase diffusion to enable affinity for ligand and mass-action to drive accumulation of integrins into the zone of contact (Figure 2) [42,47,61,62•]. The importance of ligand in driving the reorganization is demonstrated by the finding that PMA, cytochalasin-D and latrunculin, at concentrations that activate adhesion and diffusivity [61] do not promote bioluminescence resonance energy transfer (BRET)-detectable microclustering of αIIbβ3; whereas these agents enhance microclustering in response to soluble multivalent ligands [62•] (Figure 2). Consistently, real-time imaging using green fluorescent protein (GFP)-tagged integrins has shown that visible redistribution occurs long after the initial contacts with the substrate [63,64].

Regulatory signals

Dynamic regulation of integrin-mediated adhesion requires integration of signals initiated by a wide range of stimuli. However, relatively few details are known regarding the integrin-proximal events or their dynamics during cell polarization and migration.

The cytoskeletal protein talin, known for years to associate with integrin cytoplasmic domains, has now clearly been established as an important modulator of affinity for β1, β2 and β3 integrins. Isolated talin-head-domain constructs directly associate with β1, β2 and β3 cytoplasmic domains and, upon overexpression in cells, promote affinity modulation of αIIbβ3 and LFA-1 [37••,65•,66]. Structural studies of αIIb–β3 cytoplasmic domain complexes [36••] and β3–talin FERM (band 4.1, ezrin, radixin, moesin)-domain complexes [67•], together with *in vivo* FRET studies involving the αL and β2 cytoplasmic domains in the presence of talin-head domain [37••], provide strong support for a mechanism whereby the talin head domain promotes an unclasping and separation of the α and β cytoplasmic domains and thus stabilizes the high-affinity conformation. How integrin–talin interactions are regulated remains largely unknown but probably involves unmasking of the talin FERM domain by either calpain cleavage [68] or binding to phosphatidylinositides [69]. Although the talin head domain clearly modulates integrin conformation and affinity, it remains to be determined whether physiologically it functions in initiation of inside–out priming signals, or whether other proteins, such as integrin cytoplasmic domain-associated protein 1 (ICAP-1), function at early stages and are replaced later by talin when focal adhesions are formed [70].

Rap1 has emerged as an important signaling effector for chemokine, cytokine, platelet agonist, Fc, T cell and adhesion receptors that regulate β1, β2 and β3 integrin function [71]. Significantly, overexpression of dominant-negative forms of Rap1 or of the Rap1 GTPase-activating protein Spa-1 abrogates chemokine- and T–cell-receptor-induced regulation of LFA-1 [52,53••,54•,72,73]. The recently identified Rap1 effector, RAPL, co-localizes and co-precipitates with αLβ2 in a manner dependent on αL cytoplasmic domain residues Lys1097 and Lys1099 [53••]. Given the proximity of this apparent binding site to the GFFKR sequence (single letter amino acid code) in the αL cytoplasmic domain, it is proposed that RAPL functions analogously to talin by destabilizing the α–β cytoplasmic interface [53••]. Consistently, many studies with a variety of integrins have shown Rap1 and RAPL to promote soluble ligand binding by integrins and expression of activation epitopes [52,53••,72,74–76], although a valency-based mode of regulation has also been suggested [77•].

Cell migration represents a complex process requiring polarization of integrin distribution and function. Several mechanisms for this have recently been identified. During migration of endothelial cells, the leading edge becomes enriched specifically in high-affinity forms of αVβ3 in a Rac-dependent manner [78]. Interestingly, in lymphocytes both talin and RAPL co-localize with LFA-1 at the leading edge and in the immunological synapse, and Rap1/RAPL activity is absolutely required for this polarization [51,52,53••,54•]. Conversely, localized signalling through Rho and Rho-associated kinase (ROCK) is now recognized as an important regulator of de-adhesion in the uropod [79,80•,81,82]. In addition, it has become clear that vesicular trafficking from the uropod to the leading edge is required for migration [51]. Indeed, mutations in the β2 subunit cytoplasmic domain that block internalization cause decreased migration and exaggerated uropods [52].

Finally, both affinity and valency modes of regulation are expected to be uniquely influenced by the many and diverse lateral associations of integrins with proteins such as IAP (integrin-associated protein; CD47), uPAR (urokinase-type plasminogen activator receptor, CD87), tetraspanins and Fcγ receptors [83]. Interestingly, binding of thrombospondin to CD47 modulates the affinity of β3 integrins, through a novel mode of conformational regulation that was likened to modulation by activating antibodies rather than traditional inside–out signaling [84•].

Conclusions

Recent advances in the integrin field have provided a framework for understanding the structural basis of ligand binding, the global conformational rearrangements that constitute priming and ligand-induced activation, and the interdomain linkages that propagate conformational signals. By the application of more quantitative and precise methodologies, such as BRET and FRET, to the problem of integrin distribution dynamics, it appears reasonable to expect the development of a comparable framework for understanding valency regulation. The challenge of this field will be to achieve an integrated understanding of

how all these regulating parameters are spatially and temporally orchestrated in the context of dynamic cell adhesion and migration.

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Nuclear magnetic resonance structures of the epidermal growth factor domains 2 and 3 of the β2 integrin subunit were determined and superpositioned onto the structure of αVβ3 [8••]. Localization of activation epitopes to sterically inaccessible surfaces in the bent integrin suggested that this conformation represents the low-affinity state. A switchblade-like rearrangement was suggested to occur during activation that would make the epitopes accessible for antibody recognition.

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Negative-stain electron micrography with image averaging, coupled to hydrodynamic and surface plasmon resonance ligand-binding studies, were performed on αvβ3 in different activation states. In the resting state in Ca^{2+}/Mg^{2+}, the bent conformer, corresponding exactly to that found in the crystal structure [85•], was observed. Activation by Mn^{2+} induced a switchblade-like extension of the integrin, with a mixture of closed and open headpiece orientations. Cyclic RGD exclusively produced the extended conformer with the open headpiece. Stabilization of the bent conformation on the cell surface by an engineered disulfide bond prevented activation.

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