Integrin and cadherin clusters: A robust way to organize adhesions for cell mechanics

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Recent studies at the nanometer scale have revealed that relatively uniform clusters of adhesion proteins (50–100 nm) constitute the modular units of cell adhesion sites in both cell-matrix and cell-cell adhesions. Super resolution microscopy and membrane protein diffusion studies both suggest that even large focal adhesions are formed of 100 nm clusters that are loosely aggregated. Clusters of 20–50 adhesion molecules (integrins or cadherins) can support large forces through avidity binding interactions but can also be disassembled or endocytosed rapidly. Assembly of the clusters of integrins is force-independent and involves gathering integrins at ligand binding sites where they are stabilized by cytoplasmic adhesion proteins that crosslink the integrin cytoplasmic tails plus connect the clusters to the cell cytoskeleton. Cooperative-signaling events can occur in a single cluster without cascading to other clusters. Thus, the clusters appear to be very important elements in many cellular processes and can be considered as a critical functional module.

Keywords:
- adhesions; cadherin; cell forces; integrin; membrane signaling

Introduction

As cells remodel matrix or shape tissues, they must apply forces to and respond to changes in their physical environment. Aside from general issues of micrometer level shape of the environment, the cell uses contacts with neighboring matrix or other cells to modify tissue morphology. Signaling from externally or internally generated forces commonly occurs at adhesion sites where distinct sets of components that have been described as sets of adhesome proteins (the integrin adhesome and the cadherin adhesome) are assembled into functional modules. In the literature, there are on the order of 200 validated integrin and 125 validated cadherin adhesome proteins [1–3]. Mass spectral analyses of the proteins that differentially bind to integrin adhesions in a force-dependent manner indicate that about 400 proteins are involved [4, 5] (for a list of proteins discussed in this review, see Supplementary Table S1). In any case, adhesions are complex organelles that are easily seen at the microscope level as aggregates of integrins or cadherins that extend for micrometers on matrix-coated surfaces or along cell-cell junctions. With such important functions and a correspondingly large number of components, the adhesion complexes have received considerable attention to understand the basic mechanisms of assembly-disassembly and signaling. Recent findings show that the adhesions are often organized as loose aggregates of tight clusters of integrins or cadherins [6, 7] (Fig. 2), which provides important insights into the functions of adhesions that will hopefully enable us to control them to direct cell functions in wound healing, cancer, cardiovascular disease, and regeneration situations. We will focus first on the integrin adhesions, since more is known about their assembly and dynamics than cadherin adhesions (reviewed in [8]) but we expect that many basic activities will be common to both.

Steps in the formation of integrin-mediated cell-matrix adhesions

In considering adhesion dynamics and function, we describe the early steps of nascent adhesion formation, then the less understood transition to mature adhesions and finally discuss...
how in later steps the clusters might continue to assemble and disassemble during quasi-steady state conditions. The clusters provide important flexibility and robustness for adhesions such that the cells can readily adapt to changes in matrix distribution or matrix mechanics. The lessons from the characterization of the formation of clusters de novo can provide a basis for the analysis of the steady state behaviors.

Formation of nascent adhesions

The first step in integrin adhesion formation is binding of integrin to its ECM ligand and the formation of nascent adhesions that we are describing as clusters (Figs. 1A and 2A). With lipid-linked ligands, the nascent adhesions appear as fluorescent dots and rapidly reach a limiting size as evidenced by either ligand or integrin fluorescence [9]. Advances in super resolution microscopy, stochastic optical reconstruction microscopy (STORM; [10]), or photo-activated localization microscopy (PALM; [11]), reveal that these adhesions are \( \sim 100 \pm 20 \text{ nm} \) under a wide variety of conditions and contain about 50 integrins (Fig. 2A) [7]. This size is maintained constant on substrates of widely different rigidities and over wide ranges of ligand density. Myosin function is not required for the formation of nascent adhesions [7, 12] and they appear immediately after the plasma membrane contacts new matrix sites. An important aspect of integrin adhesions is that monomeric or dimeric ligands must be spaced by less than 60 nm to enable cells to spread on the surface [13, 14] and minimally four ligands are needed in a rectangular cluster of about 100 nm on glass [13]. Alternatively, trimers of the FN peptide (FN7-10) that binds to integrins on single latex beads can link beads to the cytoskeleton through activated integrins that required cytoplasmic talin1 [15]. These studies all say that the formation of the clusters is very robust and raises questions about whether the four ligand bound integrins in a 60 nm rectangle can form a cluster by recruiting unliganded integrins. In addition, there are unanswered questions about what limits the size and density of the clusters. Although many factors such as talin depletion, inhibition of actin filament assembly, and integrin activation by Mn\(^{+}+\) can alter the density of the clusters by altering integrin activation, they do not alter cluster size. Only removal of the integrin-binding domain in the talin rod altered the size of the clusters [7]. It appears that cluster formation depends upon cytoplasmic proteins and we always find talin (1 or 2), FAK, and paxillin in them. Thus, we assume that the nascent adhesions are very robust complexes that can form under a wide variety of conditions. Although it has not been rigorously proven, we hypothesize that integrins will assemble into clusters even in the absence of bound ligands through activation by cytoplasmic proteins or Mn\(^{+}+\).

In the initial studies of nascent adhesion size, the major surprise was the finding that clusters of the same size with about the same number of integrins formed on RGD-glass as on RGD-lipid in supported bilayers. Thus, the initial clusters on RGD glass must recruit unliganded integrins. To enable cluster growth, interaction between integrins or integrin-adhesion protein would stabilize new molecules in the clusters. In earlier studies, it was found that individual liganded integrins would diffuse in the plasma membrane but would rapidly attach to the cytoskeleton upon clustering [16]. Further, ligand binding was much weaker back from the leading edge, which would enable integrins to diffuse to the early clusters on glass surfaces and to assemble there [17]. Thus, the clusters appear to form as the result of a local activation process with subsequent recruitment and assembly of components. In fully spread cells, there is turnover of integrin molecules in adhesions (t\(_{1/2}\) of \( \sim 3 \) minutes) [18, 19] that is faster than the turnover of the full adhesion but much slower than most of the adhesion proteins (FAK \(<\sim 10\) seconds, paxillin = vinculin \(\sim 20\) seconds, and even talin \(\sim 120\) seconds) [20–23]. This all indicates that adhesions are not fixed structures but are turning over continuously through assembly/disassembly of clusters.

Maturation of nascent adhesions

Once clusters form, subsequent steps depend upon force and other factors to cause adhesion maturation. A very early step after cluster formation involves actin polymerization at the cytoplasmic surface of the cluster by the formin FHOD1 (Fig. 1B) [24, 25]. If bipolar myosin develops force on the actin filaments bound to the clusters, then the cell will spread further (Fig. 1C) [24]. On glass where forces are high, the majority of nascent adhesions disassemble after about 2 minutes and only about 20% of the nascent adhesions transform into mature adhesions. On rigid substrates, \(\alpha\)-actinin associates with sites for the myosinII dependent maturation of the nascent adhesions to focal contacts [12, 26]. Traction forces experienced by nascent adhesions are much lower than the forces experienced by mature adhesions [27] showing that this is a step prior to formation of mature focal adhesions and precedes sensing of substrate rigidity where the forces are much greater per unit area than the forces produced by retrograde flow or even stress fibers (Fig. 1D and E) [28, 29]. In the absence of force, the RGD peptide-induced nascent adhesions will stimulate podosome formation after about 45 minutes and the integrins will be endocytosed on a similar time scale (Fig. 1D1 and E1). All of these cell biological studies emphasize that adhesion formation involves multiple sequential biochemical events and mechanical conditions. Cytoplasmic adhesion proteins change over time as individual clusters grow or shrink but the clusters seem to continue as the basis of the adhesions. An emerging principle is that the clusters provide anchorage and whether forces develop or not causes modification of the cytoplasmic adhesion proteins and cluster behavior.

This concept of micrometer-size adhesions formed of loose aggregates of clusters is replacing the notion that focal adhesions are continuous micrometer-sized structures. Both the structural and force heterogeneity within a single focal adhesion is now being appreciated [30, 31]. The density of integrins within mature adhesions is estimated to be 900 molecules per square micron [32]. However, PALM measurements show a approximately sixfold higher density of integrins within the nascent adhesions [7]. Recent measurements of mobility of integrin receptors in the focal adhesions indicate that mature focal adhesions are also composed of an aggregation of small individual integrin clusters into micron-sized adhesions. In these clusters, about a third of the
integrins are immobile [33, 34] and the rest show confined diffusion between the focal adhesion clusters. These studies report diffusion of $\beta_3$ and $\beta_1$, indicating that integrin clustering may be general and is not restricted to $\beta_3$. Taken together, these results indicate that focal adhesions are generally formed of relatively loose aggregates of integrin clusters. This raises the question of how integrins can diffuse in the presence of an immobile ligand.

Universally observed nanoscale clusters of adhesion receptors

Receptor clustering is a general concept that is true of other adhesion structures. Indeed, a variety of cell surface receptors cluster. The size of those clusters varies from 50 to 500 nm. In the case of cell-cell adhesions, there is a consistent size to the clusters of cadherin [6, 35]. Importantly, recruitment of...
cadherins to the adhesion appears to be mediated by cis-interactions between cadherins as well as cytoplasmic protein binding. These clusters of about 60 nm form at the plasma membrane (Fig. 2B) even in the absence of ectodomains and truncation of the cytoplasmic tail of cadherins [6]. Small regions of these clusters (~30 nm) reach densities similar to crystal packing of cadherin, indicating these could be stable, in-plane assemblies of cadherin. Initial formation of trans dimers from free monomers was a rare event [36] hence, clusters formed by cis-interactions of cadherins are required for the formation of cell-cell adhesions at physiological time scales.

The universal nature of the clusters formed in cell-cell and cell-matrix adhesions suggests that these are the modular units of adhesion formation and the mature adhesions are a hierarchical organization of the clusters (Fig. 3). This universal phenomenon hence deserves to be considered in more depth. Here we present our hypothesis on how adhesion clusters in the crowded plasma membrane environment could dynamically support cellular mechanical functions as well as signaling to the cell about its mechanical and chemical environment.

**Possible mechanisms of clustering of integrin receptors**

Integrins are transmembrane molecules with large extracellular domains that bind to ECM ligands. Furthermore, they possess small intra-cellular domains [37] capable of interacting with many different proteins that assemble large signaling platforms with over 200 potential binding partners [2, 3]. Lateral interactions between integrin transmembrane domains have been reported as well [38, 39] that can also promote integrin clustering. However, the multiple interactions would predict an ever-increasing nascent adhesion size with continuous ligand-coated surfaces, which contrasts with the many observations of constant nascent adhesion size. Hence, we will explore the role of several physical and chemical factors including lipids, scaffold proteins, and integrin binding proteins in the formation of these adhesion clusters. We draw parallels from extensive studies of other cell surface receptors to understand formation and organization of clusters of cell adhesion receptors.

**Role of lipids and associated proteins in regulating cluster formation and signaling**

When cells contact ECM ligand coated glass, the cells spread. This is accompanied by a decrease in membrane tension. Studies suggest that membrane tension can help promote formation of adhesions [40] but since tension cannot be developed on RGD lipids as cells spread on supported bilayers, membrane tension cannot be a major factor in forming nascent adhesions.

The plasma membrane is a crowded environment with thousands of proteins embedded in a heterogeneous sea of lipids. When proteins aggregate, lipids can aid the aggregation by associating with proteins based upon the length of the hydrophobic domains or other factors. Such lipid (and protein) rafts have been extensively studied and there are situations where proteins can phase separate based on weak structural interactions, into self-limiting rafts of finite sizes [41]. Aspects of the integrin structure, interacting proteins, and lipids such as cholesterol could aid in cluster and adhesion formation. Indeed, nascent adhesion formation has been linked to glycolipid rafts that dissipate upon cholesterol depletion [42]. Rafts are thought to be transient assemblies of few molecules of lipid around the protein hydrophobic domains and they can enhance receptor clustering. In addition, clustering can involve interactions of long saturated acyl chains of GPI anchored proteins on the outer leaflet of the plasma membrane with phosphatidylserine (PS) in the inner leaflet [43–45]. It is unlikely that a dynamic
lipid raft would produce the reliable cluster size that is observed; however, the alteration of lipid composition by sequestering PIP2 with overexpressed binding proteins reduced the density of integrin clusters [46].

Cholesterol-rich domains appear to be peripherally involved in integrin clustering [42]. When cell-matrix adhesions are disrupted in fibroblasts, the raft markers, including GPI-anchored proteins are rapidly internalized [67], which is a phenotype similar to loss of cholesterol in the membrane. Further, manganese activated integrins preferentially localize to lipid rafts [48]. αβ3 integrin is palmitoylated and incorporated into rafts [49, 50] and Ral1 GTPase is identified as an integrin-mediated regulator of raft trafficking [50]. These studies point to a possible role of transient organization of lipids in the vicinity of integrin clusters. Within the clusters, integrins are packed at about a third of their maximal packing density [7] and could include other transmembrane proteins. Interestingly, interactions with several other transmembrane and membrane inserting proteins including uPAR and Glycosylphosphatidylinositol (GPI) anchored PIA-1 have been reported [40, 51]. Thus, the high density of proteins in the clusters could preferentially recruit lipids to the clusters.

Anionic lipids are implicated in integrin-mediated signaling and clustering of other cell surface receptors. In particular, the phosphoinositol bis/tris phosphates (PIP2 and PIP3, respectively) have important signaling roles because of their high density of negative charges [52, 53]. They can bind to the polyanionic sequences present at the juxtamembrane domain of cell surface receptors. Thus, they can help neutralize the charge and stabilize the receptor clusters. Integrins have multiple basic residues at the cytoplasmic membrane surface (e.g. β3 – between 722 and 736; □Iib- 994–997). One of the pathways that is not well understood is the role of PIP kinase binding to talin [54]. Talin binding to integrins could result in PIP kinase activation that would produce a high local concentration of PIP2 at the clusters, which would aid in recruiting PIP2 binding adhesion proteins such as VASP and vinculin [55]. Because PIP2 dynamics are very rapid in cells, rapid dynamics of the adhesions could occur through the localized synthesis and subsequent degradation of PIP2.

**Scaffold proteins can organize receptor clusters**

In general, receptor interactions with scaffold proteins through their cytoplasmic tails play a critical role in formation of receptor clusters. For example, scaffold proteins with multiple binding sites for the same receptor would aid in clustering it. These multiple sites can either bind to the receptor directly or via an intermediate molecule recruited by the active receptor. One example where secondary scaffold proteins are used to cluster receptors is the Lat T cell receptor. It binds to SOS and then to Grb2. Activated Grb2 has dual SOS binding sites. By clustering SOS proteins, Grb2 can mediate clustering of the receptors. In in vitro systems, this behavior leads to gelation at micron scales [56] however, at the plasma membrane ~120 nm clusters of the Lat T receptor are observed. Hence, simple chemical binding of hierarchical molecules leads to receptor clustering and organization at the plasma membrane.

Talin is a scaffolding protein with a FERM domain that dramatically increases clustering of integrins [7, 57, 58]. It is a homodimer of a head domain and a large rod domain that each have one integrin binding site. The FERM domain in the head interacts with PIP2 and helps inside out activation of integrins. In the inactive conformation, integrins form a αβ dimer linked by a salt bridge. Breakage of this salt bridge to separate the α and β dimer tails is a step in integrin activation usually mediated by talin [59, 60]. Cooperative binding of the talin head domain along with PIP2 can mediate integrin activation [61]. This interaction promotes clustering by preventing the β integrin tail from binding its α subunit and by binding PIP2 [62]. Upon separation of the cytoplasmic tail of integrins, several dimeric proteins such as Filamin, α-actinin, and talin bind to it. Talin arrives rapidly at the adhesions whereas integrins accumulate at a relatively slower rate [24, 57]. Stoichiometric measurements suggest that two integrin-kindlin complexes are bound to the heads of one talin dimer, producing a small cluster of activated integrins [57]. Further, the rod domain contains an integrin binding site 2 (IBS2) required for clustering [7], which directly supports lipid bilayers, whereas the rod domain supports polymerization and active dynamics of the clusters on supported lipid bilayers, whereas the rod domain supports aggregation of clusters with the retrograde flow of actin [7]. This could provide a basis for segregation of the different functions of the clusters, which could help assemble a mature adhesion.

![Figure 3. Image of a mouse embryonic fibroblast spread on fibronectin coated substrate for 30 minutes. dSTORM imaging of endogenous Paxillin conjugated (labeled with Paxillin antibody, BD biosciences) to antibody tagged with Alexa 647 (anti mouse secondary antibody conjugated to Alexa 647). Gray scale shows the TIRF image, whereas false color image shows the reconstructed super resolution image. Color code (top left corner, from left to right) indicates increasing intensity of molecules. Encircled in dotted lines from left- to right-cell edge, nascent adhesions, maturing adhesions, mature adhesions. Scale bar 2 μm.](image)
A critical aspect of talin binding to other components is the mechanical unfolding of talin that depends upon the actin cytoskeleton. Hence, next we consider the role of the actin cytoskeleton in organizing the clusters.

**Actin cytoskeleton provides fences to promote receptor clustering**

The cortical actin cytoskeleton constitutes an actin mesh on the cytoplasmic surface of the plasma membrane. In the lamellipodium, where nascent adhesions assemble, a cross-linked actin network pushes out the membrane in the thin leading edge. A thin portion of the actin meshwork at the surface of the plasma membrane defines barriers for diffusion of proteins in corrals that only weakly inhibit lipid diffusion [65–67]. When measured, integrin receptors exhibit confined diffusion at the micrometer (in milliseconds) scale but free diffusion on the nanometer (in microsecond) scale [68]. Hence, the proposed membrane skeleton fence model [69] defines corrals laid out by the actin mesh where the receptors can freely diffuse. In protruding membranes, receptor proteins collide with the actin mesh and remain confined in the gaps in the mesh, which have an average size of ~200 nm. Hence these receptors (membrane proteins) undergo a short-term free diffusion in the corrals and long-term hop diffusions between compartments [70, 71]. The rapid free diffusion within the mesh would enhance early clustering of receptors and could enable rapid exchange of the receptors at the edge of clusters with a free pool of receptors that is confined by actin filaments. Integrins require actin filaments for clustering into nascent adhesions [7]. Without integrin confinement by actin, the dynamics of cluster formation may be tipped to dissociation thereby accelerating disassembly of clusters once back from the edge (previous studies show that integrins are dissociation thereby accelerating disassembly of clusters once back from the edge [72]). Other more direct roles for actin filaments binding to the nascent adhesions could also account for the decreased density of clusters after actin depolymerization [7].

**Organization of integrin clusters byglycans**

Another mechanism for cluster formation could involve crosslinking of the glycocalyx. Glycoproteins are transmembrane or membrane-anchored proteins covalently linked to sugar moieties that can interact with a secreted family of lectins called galectins. Binding to galectins can cross-link the cell surface receptors creating glycana-based domains [73], thereby compartmentalizing receptors [74, 75] and regulating their diffusion (e.g. EGFR [76]). This can contribute to apparent clustering of receptors [77]. The glycocalyx protein mucin extends ~200 nm outside the plasma membrane. Because of its size, mucin blocks integrins from reaching the ECM (integrins extend only 20 nm outside the plasma membrane) [78]. As a consequence, this favors integrin binding to the ECM in regions where integrin has already bound to the ECM in a type of feed-forward or positive feedback process. This increases the likelihood of interaction of integrins with the ligand only at a limited number of sites since the glycocalyx will be concentrated by its exclusion from those sites, thereby promoting clustering [79]. There is no direct evidence that this mechanism is operative for nascent integrin clusters but there is typically a significant separation between clusters, possibly indicating the depletion of free integrins in that region or alternatively the higher concentration of corralled glycocalyx molecules around the clusters that inhibits nearby cluster formation.

**What are the advantages of the formation of nanometer scale clusters of adhesion receptors?**

Recent advances in super resolution microscopy, have led to immense progress in unraveling the size of diffraction limited receptor clusters. Most cell surface receptors cluster in islands of 50–650 nm with 10–120 molecules per cluster. For example, integrin (~80–120 nm with ~50 molecules [7]), E-Cadherin [6], MHC class 1 (70–600 nm with 25–125 molecules [80]), EGFR [81], lectin (80 nm with ~12 molecules [82]), B cell receptors (80 nm with 20–120 molecules). Nature has used this modular theme extensively and repeatedly. Hence it is likely to have important functional consequences for robust force-bearing and signaling centers.

**Avidity and specificity of ligand receptor interactions**

The obvious advantage of clustering of receptors is the increase in avidity and specificity of the binding to extracellular signals [83–86]. Cell surface receptors that need to respond to low thresholds of ligands and to vastly varying ligand concentrations would benefit if a combination of single receptors in clusters was needed for an active binding event [87]. In the somewhat similar system of T cell activation, the specificity has been explained through models of kinetic proofreading where ligand binding times and kinetics of modifications of the receptors are critical parameters [88]. This is a good way to reduce stochastic noise in the system and increase specificity of the response.

In integrin receptors, the increase in avidity proves especially advantageous to regulate the mechanical forces experienced and exerted by the clusters. Using single molecule studies, the free energy binding of $\alpha_{\beta}$ integrins with the linear GRGDSP peptide was estimated at $-3.1 \text{kcal/mol}$ [89]. Recent evidence shows that when cells attach to RGDbiotin on avidin surfaces, the integrins can rupture avidin-biotin interaction [90]. The biotin-avidin interaction is one of the strongest non-covalent bonds in nature with an absolute free energy of binding of $-18 \text{kcal/mol}$ [91, 92]. Further, the strength of integrin ligand bond would vary depending upon several factors including the loading rate, the integrin, and the ligand but still ranges maximally only between 40 and 90 pN [93–95]. So how can force on the integrin break the biotin-avidin bond? The cooperative strength of a cluster of integrins with increased avidity could explain breakage of the avidin-biotin linkage.
Since cells are subjected to high forces mediated by integrins, the cooperation and organization of several proteins into larger arrays that have greater avidity could reliably link integrins to actin stress fibers and maintain large forces. Clustering could thus, be critical.

Clustering of integrin receptors offers additional advantages in the two force-sensing processes that occur during early cell spreading. Early adhesion formation, unlike adhesion maturation does not require myosin function [12, 57], indicating that it precedes force sensing [7]. Rapidly laying down small adhesions would enable the cell to sense the full area of ligands in its microenvironment and to then mount an appropriate response. The polymerization of actin from the adhesions provides a handle to move the cluster and develop force on it. This is a critical feature of the adhesions in general that must interact with the actin cytoskeleton and there is evidence from several areas that adhesions will act as a site of actin polymerization [96]. Matrix dynamics in the next phase is also aided by the small size of the clusters. For example, the next step on the rigid surfaces is that the majority of the clusters will disassemble; however, some will participate in the formation of contractile sarcomeric units (formed as contractile pairs between these nascent adhesions) to generate traction force that will sense matrix rigidity (Fig. 1D2) [28]. Although we don’t know how adhesions are selected for disassembly or rigidity sensing, it is clear that the limited resources of the cell are focused on a subset of the adhesions through rapid disassembly of most. This not only helps the few adhesions to have the resources to mature into focal adhesions but also allows focus on a few sites for appropriate downstream signaling. The cell should exhibit key differences in downstream signaling depending upon the mechanical characteristics of the integrin ligand. Nascent adhesions aid greatly in the processes involved in cell testing of the matrix, since nascent adhesions can assemble and disassemble rapidly on single matrix fibers while being able to support the large forces involved in rigidity sensing [29]. This enables a complete cell response to the ECM within a few minutes of cell attachment [97]. Because there are 24 different integrins, the steps in matrix-activated cell responses will likely be varied and much more work is needed to understand how cells can use adhesion signals to create proper tissue shapes.

**Clustering of weaker integrin-ligand bonds can regulate adhesion strength and dynamics**

The implications of the clusters for modeling of adhesion phenomena are significant. At a first level, the high local concentration of adhesion receptors will alter the apparent affinity of ligands since the ligands can readily move from one receptor to an adjacent one (an avidity effect). Further, both the dynamic nature of forces in the adhesions and the flow of actin past the adhesions are coupled with enzymatic modifications of adhesions and a variety of cooperative phenomena that are not considered in most physical models of the adhesions. Clutch models (Box 1) of the adhesions often do not consider enzymatic changes but rather assume that the affinities of linker proteins for the receptors and for cytoskeleton are constant [98, 99]. However, for each integrin receptor, the binding to matrix is dependent upon cytoplasmic proteins binding to the cytoplasmic tail (inside out signaling), lateral protein interactions, the phosphorylation state of the cytoplasmic tail, and the rate of force generation on the bond (they often exhibit catch bonds, Box 2) [100, 101]. Phenomena such as dynamic tension wherein the increase in rigidity or force from a substrate will increase the cellular forces on that substrate through an increase in the area of adhesions are well documented and clearly involve the assembly of many more clusters. These changes do not fit with a simple slip bond or even catch bond behavior (Box 2). Further, there is an increasing body of evidence that biochemical changes rapidly follow changes in adhesion patterns [102]. Single molecule measurements of talin length in cells show that talin stretch-relaxation cycles occur on the timescale of 4–15 seconds and typically involve 100–200 nm changes in talin length that correlate with the dynamics of vinculin binding and release [64, 103]. Thus, we favor a multi-step model for the formation of adhesions that has many enzymatic steps as well as decision points that result in dramatic changes in cell state, for example, when rapidly spreading cells start contractions on the substrate to measure substrate rigidity [104]. Even in cells after long times on substrates there are oscillations in the forces on adhesions, new membrane extensions, and actin filaments are continually moving even in stress fibers, indicating that we need more complicated models of the integrin adhesion process. How the disassembly of clusters and ultimately adhesions themselves occurs is poorly understood and this is a critical next step for realistic model building. Tissue formation and repair depend critically upon the development of the proper forces on adhesions in quasi-steady state when adhesions are constantly assembling and disassembling. When either the number of engaged actin binding proteins in the adhesion or the myosin contractility decreases, the force on the cluster decreases and that can activate a disassembly process. A very strong receptor ligand bond is not desired as this would have very long lifetimes of dissociation and thereby hamper the rapid turnover of adhesions. Hence clustering provides an excellent mechanism to use molecules with weaker binding affinities to collectively form stronger adhesions and yet retain the ability to dissociate easily.

**Spatial segregation of signals makes the system robust**

With regard to the dynamics of the clusters, there is evidence from receptor diffusion studies indicating that focal adhesions are composed of a conglomeration of individual smaller clusters [33, 34] (see Fig. 3) and that there is rapid exchange of subunits in the clusters with others in the adhesion. This is consistent with the corral model of diffusion since the integrins on the edge of a cluster could be in equilibrium with the free integrins in the corral. In this type of model, disassembly of actin to break the corral and release the free integrins would rapidly cause cluster disassembly. This is consistent with the fact that the dense cortical array of actin in lamellipodia disassembles at the inner edge of the...
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intermediate velocities, persistence, and have both focal expressed together they cooperate and the cells migrate with these pathways have their own advantages and when molecules recruited by the different integrins [106]. Both of to larger areas clearly attributed to different signaling integrins migrate persistently but slowly with few protrusions, whereas to the actin cytoskeleton [26]. Cells expressing adhesion reinforcement in a force-induced manner by binding of time. flexibility in maintaining active adhesions over long periods of time.

Several different integrin receptors are present on the cell surface. Along with different binding affinities for the substrates, each of them has different mechanotransduction and mechanosignaling effects in the cells. For example, in breast epithelium, $\alpha_5\beta_1$ or $\alpha_v\beta_3$ integrins (expressed transiently) adapt to different force regimes. Here, $\alpha_5\beta_1$ has a biphasic response wherein it responds to low and high forces whereas $\alpha_v\beta_3$ responds to intermediate and higher forces [99]. Differential behavior of $\beta_3$ and $\beta_1$ has been shown where $\beta_1$ supports higher traction forces [105]. $\alpha_5\beta_1$ integrins contribute primarily to the strength of the adhesion through their catch-bond binding to the ligand, whereas $\alpha_v\beta_3$ primarily mediates adhesion reinforcement in a force-induced manner by binding to the actin cytoskeleton [26]. Cells expressing $\alpha_v$-class integrins migrate persistently but slowly with few protrusions, large focal adhesions, and lesser spread area whereas cells expressing $\alpha_v\beta_1$ migrate with less persistence but rapidly, have many protrusions as well as focal complexes and spread to larger areas clearly attributed to different signaling molecules recruited by the different integrins [106]. Both of these pathways have their own advantages and when expressed together they cooperate and the cells migrate with intermediate velocities, persistence, and have both focal complexes and focal adhesions [106]. These examples clearly demonstrate that the cells develop motility as a result of a combinatorial response to ligands and force.

The variety of different types of motility needed to shape an organism is significant and the final forms are created with different rigidities and ECM substrates. Small dynamic integrin clusters provide the flexibility and strength to create the proper tissue form and maintain it through the turnover of the membrane proteins. Further, signals generated from the clusters can be combinatorial and add to give an integrated response on the cellular level. This would enable a wide range of responses to physical and biochemical parameters of the ECM with a relatively small number of different biochemical receptors and ligands. This would also allow the cells to sense combinatorial ECM ligands more effectively. Clustering would aid spatial segregation of the receptors within large focal adhesions or other adhesions.

Conclusion

From a number of different studies, small tight modular clusters of adhesion receptors such as integrins or cadherins will assemble into loose aggregates that constitute cell-matrix or cell-cell adhesions (Fig. 2). Because these modular adhesion subunits are dynamic, they can easily form and dissipate in response to microenvironment or cell cytoskeleton changes. They provide digital signals to the cells that depend upon the ligand, rigidity, and force. These adhesions are similar in many respects in that they are assembled from small modular units with a fairly comparable size. The size distribution is remarkably uniform, indicating that there is a favored size that is determined by yet unknown factors, though from talin IBS2 (integrin) and actin rings structures (cadherin) are shown to play an important role.

Box 1

**Clutch model**

The mechanical coupling between the actin and the extracellular matrix is mediated by integrins. This results in a resistance to the myosin II mediated dynamics of actin in the lamellipodia and reduction in the speed of actin retrograde flow. This model assumes a strong binding for the clutch to engage. It predicts distinct regimes on stiff and soft substrate that are not normally observed. On stiff substrates, friction-mediated slippage due to fast retrograde flow and lowtraction would be observed whereas on compliant substrates, the retrograde flow would be slower leading to higher traction forces and result in "load and fail" dynamics. Recently this type of behavior was observed only after talin depletion [116]. This model this helps to explain the mechanosensing of compliant versus rigid substrates under specialized conditions.

**Stick slip model**

This model assumes that actin is present as a dense gel in lamellipodium and it is moving centripetally past lamellipodia where most adhesions disassemble. Force on adhesions preserves them for longer times and those central adhesions may contribute to the nuclear forces that are important for many cell functions including major effects on the transcription profile. Although the overall strength of the multiple adhesion clusters is the same as one large adhesion, there is flexibility in that the individual small clusters have a large surface area to enable exchange of subunits and to allow rapid disassembly of clusters as needed. Thus, the use of multiple strong but small clusters provides important flexibility in maintaining active adhesions over long periods of time.
Box 2 Mechanical bonds in adhesions

The bonds that form between receptors (integrins) and ligands (RGD) or between receptor (cadherins) interactions are non-covalent bonds. These bonds bear mechanical load and will break under very high forces. However, there are different responses of bonds to the rate of force increase that can be generally classified as slip or catch bonds.

**Slip bond**

In this case, non-covalent bond lifetimes become exponentially shorter with increasing tensile forces (see cartoon). This occurs because force moves the components to the edge of the binding range, and then bonds break more easily because the ligand can more easily “slip out” of the binding pocket [111, 112]. As expected, the bond would be strongest at lower forces and as the force increases, the bond will have a higher likelihood of breaking. As the energy applied (force times the length of displacement in the bond) reaches closer to the dissociation constant of the bond, the rate of bond breaking increases exponentially. This is observed in cadherin-cadherin interactions and integrin-fibronectin-cytoskeleton interactions at low forces [15].

**Catch bond**

Alternatively, bond strength can increase as force is rapidly applied to the bond (for example, interlocking hooks or Chinese finger traps have a lower probability of unbinding when force is rapidly applied to them). This counter intuitive behavior is commonly used in nature. Prominent examples include FimH a mannone binding receptor present on *Escherichia coli* that binds to red blood cells; P-selectin – PSGL-1 [113], and the motor protein myosin binding to actin [114]. Several molecular mechanisms can produce this behavior. There is a common allosteric model wherein upon application of tensile force, the receptor molecule undergoes a conformational change that allows for a shift from low to higher binding affinity of the ligand [112]. In addition, there is a deformation model where, like a hook, the bond is stronger upon application of force, but the ligand can easily slide out in low force conditions. Several other models are proposed which rely on the conformational change of the receptor and cofactor binding to the ligand. Catch bond is observed in cadherin mediated and integrin-matrix adhesions at higher forces (>20 pN) [101]. Interestingly, catch bonds between FimH and tri-mannose is much stronger than with the single mannose ligand, suggesting a role of receptor clustering in the function of catch bonds under higher forces [115].

One major function of these adhesions is spatial segregation of signaling information [106, 107]. Several signaling pathways are alternatively activated downstream of both integrin and cadherin mediated adhesions. In case of integrins, to the short cytoplasmic tail a large number (>42) of interacting partners bind [108]. However, spatially, only one to two proteins can bind at any given time. The integrin/cadherin tail by itself lacks any enzymatic or actin binding activity; and hence, it has to rely on binding partners to support mechanical functions. One spatially segregated modular cluster could provide a platform with one type of protein binding to it. These different clusters could exist side by side in a mature adhesion, thereby modulating the numerous cellular functions [109]. Thus, the microenvironment is translated into cellular behavior by the cumulative responses of adhesions.

By extending our knowledge of adhesion behaviors beyond the fibronectin binding integrins (and cadherins), we may be able to predict more complex cell behaviors and the changes that will occur upon perturbation.

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