Specificities of β1 integrin signaling in the control of cell adhesion and adhesive strength.

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Abstract

Cells exert actomyosin contractility and cytoskeleton-dependent force in response to matrix stiffness cues. Cells dynamically adapt to force by modifying their behavior and remodeling their microenvironment. This adaptation is favored by integrin activation switch and their ability to modulate their clustering and the assembly of an intracellular hub in response to force. Indeed integrins are mechanoreceptors and mediate mechanotransduction by transferring forces to specific adhesion proteins into focal adhesions which are sensitive to tension and activate intracellular signals. $\alpha_5\beta_1$ integrin is considered of major importance for the formation of an elaborate meshwork of fibronectin fibrils and for the extracellular matrix deposition and remodeling. Here we summarize recent progress in the study of mechanisms regulating the activation cycle of β_1 integrin and the specificity of $\alpha_5\beta_1$ integrin in mechanotranduction.

Introduction

Tumors are characterized by extracellular matrix remodeling and stiffening (Cross et al., 2007; Guck et al., 2005) and tissue fibrosis could regulate cancer behavior by influencing the biophysical properties of the microenvironment to alter forces at the cell and/or tissue level (Georges et al., 2007; Levental et al., 2009; Masuzaki et al., 2008). Focal adhesions are the major link connecting the extracellular matrix to actin cytoskeleton through integrin receptors (Geiger et al., 2009). They are promoted by the stiffness of the underlying matrix. They are also linked to matrix assembly since they generate fibrillar adhesions that are required for fibrillogenesis, matrix deposition and remodeling (Mao and Schwarzbauer, 2005; Williams et al., 2008). Integrins are heterodimeric adhesive receptors consisting of an α- and β-subunit, which each have a large ectodomain, a single transmembrane domain and a generally short cytoplasmic tail. Eighteen α subunits and eight β subunits can assemble in 24 different combinations that have overlapping substrate specificity and cell-type-specific expression patterns (Humphries et al., 2006; Hynes, 2002). Most of integrins composed of either β1 or αv subunit bind to the RGD tri-peptide motif that is found in many extracellular matrix components including fibronectin (Danen and Sonnenberg, 2003). α5β1 integrin interaction to fibronectin is potentialized by the nearby synergy site (PHSRN) for maximal binding affinity (Leahy et al., 1996). Alternative splicing of some integrins is a supplementary mechanism to subtly regulate the ligand binding and the downstream signaling activity of integrins. The isoform β1A is the most abundant and ubiquitously expressed subunit associated with a number of α subunits to form distinct heterodimers. The striated muscle specific isoform β1D which supports high tensile forces allowing stability of muscle adhesive structures differs from β1A only on its cytoplasmic domain (Belkin et al., 1997; Belkin et al., 1996). Integrins provide bidirectional signaling by processes known as "outside-in signaling" and "inside-out signaling" which lead to the receptor conformational changes. Indeed integrin ectodomains can exist in bent closed conformation, intermediate extended conformation with a closed head-piece, and extended open conformation (Zhu et al., 2009). These forms could correspond to low affinity, activated and ligand occupied integrin forms, respectively. Outside-in signaling resulting from the binding of integrins to their extracellular matrix ligands controls cell polarity, cytoskeletal architecture, gene expression, cell survival and proliferation. Integrin activation is promoted by the so-called inside-out signaling triggered by the interactions of α and β integrin cytoplasmic domains with each other and with

cytoplasmic proteins leading to the long-range allosteric rearrangements of the integrins that result in increased affinity (O'Toole et al., 1994; Shattil et al., 2010). Inside-out signaling results in increased affinity for extracellular ligands and controls the adhesion strength able to transmit the forces required for cell migration, extracellular remodeling and assembly (Ginsberg et al., 2005). As integrin cytoplasmic tails have no catalytic activity, they must bind accessory molecules that contribute to integrin activation, cytoskeleton organization and downstream signaling pathways involved in cell proliferation and differentiation (Zaidel-Bar et al., 2007). Some of these proteins can be either common or specific between the different β subunits. Finally, integrin cytoplasmic domains are able to organize large complexes built up with highly clustered modules that, through adaptor proteins, initiate signalling cascades, and act as connector modules to strengthen the cytoskeletal link in response to increasing tension. In turn, forcing integrin clustering and focal adhesion formation promotes invasion and inhibiting focal adhesion signaling or tempering tissue stiffening reduces focal adhesions and tumor invasion (Paszek et al., 2005). These observations are consistent with the notion that tension regulates the invasive behavior of tumors by modulating integrin activity, integrin clustering, focal adhesion assembly, and downstream signaling (Paszek et al., 2005). Extracellular matrix stiffness and remodeling could regulate malignancy by enhancing integrin-dependent mechanotransduction (Butcher et al., 2009; Discher et al., 2005). Indeed, different findings have suggested that enhanced integrin signaling rather than just an increase in integrin expression is critical for tumor progression. As $\alpha_5\beta_1$ integrin is considered of major importance for the formation of an elaborate meshwork of fibronectin fibrils and therefore for the extracellular matrix deposition and remodeling, we will detail what we know of the mechanisms regulating the activation cycle of β1 integrin and the specificity of α5β1 integrin in mechanotranduction.

Integrin Activation

Talin, a key actor of β1 integrin activation

Talin is a key player in integrin activation, acting as an intracellular ligand: the interaction of talin with integrin cytoplasmic tails causes conformational changes within the extracellular domains, which increase binding affinities for extracellular matrix ligands at the cell surface (Calderwood, 2004b; Tadokoro et al., 2003). Talin consists of a large C-terminal rod and an N-terminal head containing a FERM domain composed of four subdomains from F0 to F3. The F3 subdomain encompasses a PTB-like domain which contains a high affinity binding

site for the membrane proximal NPxY motif on the β integrin tails (Calderwood et al., 2002). Whereas interaction with the F3 domain is required and sufficient for β3 integrin activation (Calderwood et al., 2002), some studies have shown that β1 integrins require larger fragments of talin to generate detectable activation (Bouaouina et al., 2008). This suggests a specific role for the other subdomains of the FERM structure for β_1 integrin activation and the involvement of specific talin-mediated molecular mechanisms occurring for β_1 and not β_3 integrin. Talin binding disrupts a salt bridge between the α and β subunits leading to rearrangement of the integrin transmembrane portion and integrin activation (Luo et al., 2004; Vinogradova et al., 2004) (Fig. 1). Recently, the structure-function analysis of the talin F3 domain with the β3 integrin tail (Wegener et al., 2007) coupled with the structure of the integrin transmembrane portion (Lau et al., 2009; Lau et al., 2008) and the first structure of the complex between β1D integrin tail and talin2 F2-F3 (Anthis et al., 2009) allows a more precise model of integrin activation. The bent and inactive conformational state of integrins is maintained through associations between extracellular, transmembrane and cytoplasmic domains of α and β subunits. The interaction of talin F3 domain with the membrane proximal NPxY motif and its subsequent binding to a membrane-proximal aspartate residue in the β tail creates an ionic interaction at the membrane-proximal helix which disrupts the salt bridge between the α and β subunits cytoplasmic tails known to maintain the integrin inactive state. Such association between talin F3 domain and β_3 subunit tail not only stabilizes the helical structure of the membrane-proximal β tail and the interaction between the F3 domain of talin and β3 tail but also orients a group of lysine residues in F3 towards the negatively charged membrane phospholipids head group. A positively charged patch in talin F2 domain was also reported to establish interactions with cell membrane in case of β1D.

The physiological relevance of talin binding to phospholipids is not only the potential recruitment of talin to the membrane in close proximity to integrin but also the induction of a conformational change or a stabilization of the talin-integrin complex allowing a more efficient association with receptor tails coupled with integrin functionality.

Synergistic effect of kindlin and talin on integrin activation

Even though talin is essential for integrin activation, recent studies have established that kindlins belonging to another family of β-integrin binding proteins might cooperate with talin to activate integrins (Moser et al., 2009a; Moser et al., 2008). Kindlins contain a FERM

domain highly similar to that of talin also composed of three subdomains F1, F2, F3. However the hallmark of kindlins is a PH (pleckstrin homology) domain inserted into and interrupting the F2 subdomains (Kloeker et al., 2004). Kindlin 1 and 2 are able to interact with the cytoplasmic domain of β_1 and β_3 integrin at the membrane distal (T/S)TxxNxxY site through its F3 domain (Harburger et al., 2009). β2 integrin carries a phenylalanine at the position of the distal tyrosine of β_1 and β_3 cytoplasmic tail which allows kindlin-3 binding (Moser et al., 2009a). Kindlin-2 also interacts with two additional proteins called migfilin and ILK also found in focal adhesions (Mackinnon et al., 2002; Montanez et al., 2008; Tu et al., 2003). It has been shown that kindlins and talin are coactivators of integrins and kindlin may exert a synergistic effect on talin activation (Ma et al., 2008). Kindlin proteins have been linked to inherited and acquired human disease including Kindler syndrome, leucocyte adhesion deficiencies and cancer (Meves et al., 2009). Loss of kindlin-2 in mice results in peri-implantation lethality due to the fact that kindlin-2 deficient cells are not able to activate β1 integrins (Montanez et al., 2008) and the loss of kindlin-1 from intestinal epithelial cells or carcinoma colon cells reduced talin-dependent β_1 integrin activation (Ussar et al., 2008). Moreover in CHO cells the activation of the $\alpha_{IIb}\beta_3$ integrin was observed upon the combined overexpression of kindlin-2 and talin head (Harburger et al., 2009; Montanez et al., 2008). However in this biological system, neither kindlin-1 nor kindlin-2 cooperate with the talin head to activate β_1 integrins suggesting that kindlins may exert integrin-specific effects (Harburger et al., 2009) (Fig. 1). So far no evidence was provided showing the sequential or the simultaneous binding of talin and kindlin to an individual β cytoplasmic tail (Meves et al., 2009; Moser et al., 2009b). Kindlin signaling could interfere with suppressor of integrin activation. Indeed, kindlins share the same binding site that ICAP-1 which specifically interacts with the β_1 cytoplasmic tail, competes for talin binding, and blocks integrin activation (Bouvard et al., 2003; Millon-Fremillon et al., 2008). In addition, migfilin, a kindlin-binding protein, binds to filamin A which can also block talin binding to the β cytoplasmic tail (Ithychanda et al., 2009; Lad et al., 2008). Clearly, more work is needed to clarify the role of kindlins in integrin inside-out signaling.

The Rap1 GTPase integrin activation pathway

The small G protein Rap1 (Krev-1), a member of the Ras superfamily, has been brought to the forefront since the discovery of its role in the regulation of diverse cellular processes such as

integrin activation and cell adhesion, cell polarity and cell-cell junction formation. Rap1 stimulates cell adhesion and spreading by activating all integrins that are associated with the actin cytoskeleton, i. e. integrins of the β1, β2, and β3 family. However, the precise role of Rap1 in the signaling pathways that control these processes is not well understood. Importantly, Rap1 promotes talin binding to the cytoplasmic tail of β1 and β3 subunits (Boettner and Van Aelst, 2009; Bos, 2005; Han et al., 2006; Kinbara et al., 2003). Indeed, many cytokines and growth factors promote integrin-dependent cell adhesion through the activation of Rap1. In the last few years, many Rap1 effectors have been identified (Raaijmakers and Bos, 2009). Among these, RIAM (Rap1-GTP-interacting adaptor molecule) is clearly implicated in Rap1-dependent integrin activation (Bergmeier et al., 2007; Bos, 2005; Han et al., 2006; Lafuente et al., 2004; Pasvolsky et al., 2007). Evidence suggests that Rap1A induces formation of an integrin-activation complex containing RIAM and talin, which in turn leads to the unmasking of the integrin-binding site on talin, a critical final step in integrin activation (Han et al., 2006) (Fig. 1). In line with this view, it has been shown that ectopic expression of RIAM can induce cell spreading and formation of lamellipodia as well as activated β1 and β2-containing integrin complexes, whereas RIAM depletion inhibits integrin adhesion on fibronectin and decreases integrin activation induced by the constitutively active Rap1 mutant (Rap V12) (Han et al., 2006; Lafuente et al., 2004). Rap1 dependent cell spreading strictly depends on talin, since this process is blocked by the expression of an integrin mutant (W359A) deficient in talin binding. The model favors a role of Rap1 in the induction of a talin conformational change allowing its membrane translocation and consequently integrin activation. It is noteworthy that RIAM could not only provide a scaffold to bind Rap1 which enables subsequent talin tethering and integrin activation (Watanabe et al., 2008) but also could participate in the local increase in cellular F-actin content through its interaction with ENA, VASP and profilin, proteins known to promote actin polymerization and F-actin formation (Lafuente et al., 2004) .

Code de champ modifié

Negative regulators of β1 integrins

Integrin activation is not sufficient for controlling cell adhesion process. Indeed we have shown that a cycle between the high and the low affinity state of β_1 integrin is required for the proper response of cells to extracellular matrix physicochemical properties (Millon-Fremillon et al., 2008). Indeed depending on the conditions, focal adhesions can assemble, disassemble,

remodel or slide and cellular migration involves cyclic adhesion and detachment between the cell and extracellular matrix suggesting switchable and cyclic affinity regulation. This is achieved by altering the extent of association of proteins with the adhesion complex through competition, phosphorylation and proteolysis. These biochemical mechanisms are also regulated by externally applied or motor driven forces (Zaidel-Bar et al., 2007; Zhang et al., 2008). Several proteins can inhibit integrin activation by competing with talin for binding to the β integrin tail. One regulation loop may be the phosphatidylinositol phosphate kinase type Iγ-90 which is able to compete with β-integrin for talin binding (Calderwood et al., 2004; Ling et al., 2003). In addition, structural analyses have revealed an overlap between talin- and filamin-binding sites on β integrin tails, and this competition for β tail binding can regulate integrin activation (Garcia-Alvarez et al., 2003; Kiema et al., 2006; Nieves et al., 2010). However, this competition model shown for β7 integrin needs to be confirmed for β_1 integrin even though filamin is able to interact with β_1 and β_7 integrin. Migfilin has been shown to competitively inhibit the filamin binding to the β integrin tail and to enhance β_1 and β_3 integrin activation (Ithychanda et al., 2009; Lad et al., 2008). Different integrins also bind specifically to distinct PTB domain-containing proteins described as talin competitors (Calderwood et al., 2003). Dok1 binds specifically β_3 integrins whereas Integrin Cytoplasmic-domain Associated Protein 1 (ICAP1) only binds β_{1A} integrins (Chang et al., 1997; Wegener et al., 2007; Zhang and Hemler, 1999). Focal adhesion disruption and adhesion defect after over-expression of ICAP-1 or a phosphomimetic mutant of ICAP-1 at the CaMKII site respectively (Bouvard and Block, 1998; Bouvard et al., 2003) confirms that ICAP-1 negatively regulates β_1 integrin function. ICAP-1 and β_1 integrin are colocalized at the leading edges of cells during the early stages of spreading (Fournier et al., 2002) but not at mature focal adhesions suggesting a delayed or at least a controlled activation of this integrin during membrane extension. To unravel the role of ICAP-1 in vivo, mice deficient in ICAP-1 expression have been produced and display an osteogenesis defect (Bouvard et al., 2007). *Icap-1* deficient MEF cells show higher β1 integrin affinity for fibronectin and that integrin affinity cycling from low to high affinity is necessary for the proper control of focal adhesion assembly and consequently also for cell spreading, and migration. Modification in the dynamics of β_1 integrin containing focal adhesions in *Icap-1* null cells is also revealed by the faster recruitment of EGFP-talin into focal adhesion observed by FRAP studies (Millon-Fremillon et al., 2008). This observation in living cells fits with talin-ICAP-1 competition for integrin binding in vitro as well as with focal adhesion disruption after ICAP-1

overexpression (Bouvard and Block, 1998; Bouvard et al., 2003). Our data show that ICAP-1 limits β1 clustering into focal adhesion and slows down focal adhesion assembly by promoting β_1 integrin low affinity state (Fig. 1). ICAP-1 could delay the talin/integrin interaction necessary for integrin activation and clustering (Calderwood, 2004a; Cluzel et al., 2005; Giannone et al., 2003). Some examples indicate that competition between integrin partners can also be modulated by integrin phosphorylation. Indeed different studies not only highlight the role of integrin phosphorylation in the cell but also indicate the targeted nature of this modification, manifested as a switch for integrin activation. For instance, Serine/Threonine phosphorylation of the β-integrin tail (β1 residues 783, 784 or 785 or β2 residue 758), possibly mediated by PKC, inhibits filamin binding without altering talin binding, whereas Src-mediated tyrosine phosphorylation of the conserved integrin NP(I/L)Y motif, inhibits talin binding but enhances the binding of other PTB domain-containing proteins such as filamin, Dok1 or tensin (Kiema et al., 2006; McCleverty et al., 2007; Oxley et al., 2008; Takala et al., 2008). Indeed phosphorylation of Tyr747 switches the binding preference of the β_3 integrin tail from talin to the Dok1 PTB domain. Tyrosine phosphorylation of the β_3 tail thus allows Dok1 to compete strongly with talin, and this would result in down-regulation of integrin activation. These results agree with previous studies that demonstrated a phosphorylation-dependent association of Dok1 with the β_3 integrin tail (Ling et al., 2005) and suggestions that integrin β tail phosphorylation blocks talin binding (Oxley et al., 2008). Although negative regulators of integrins as well as activators have been clearly identified, the actual mechanisms leading to integrin activation cycles remain to be identified.

The specific role of α5β1 integrin in adhesion strength

Integrin-ligand binding provides a transmembrane mechanical link to transmit forces from extracellular contacts to intracellular structures such as actin cytoskeleton that is tensioned by myosin II motors (Riveline et al., 2001). By sensing their extracellular environment, integrins allow cell to adapt its behaviour according to variations in microenvironment chemical composition but also stiffness (Engler et al., 2006; Paszek et al., 2005). This property allows identifying integrins as mechanoreceptors (Wang et al., 1993) and mediates mechanotransduction by transferring forces to specific adhesion proteins into focal adhesions which are sensitive to tension and activate intracellular signals. As it has been largely described above, ligand binding to integrin leads to conformational changes of integrin extracellular domain in “active state” or high affinity state for extracellular matrix and is

necessary for lateral motility and clustering of integrins. This step is important for intracellular signals activation and focal complex or nascent adhesion formation. Then, application of forces on theses complexes induces their growth into larger adhesions called focal adhesions that are connected to actin stress fibers. This is supported by experiments showing that applied tension or rigid substrates induce formation of large focal adhesions more efficiently than flexible substrates of the same chemical composition (Pelham and Wang, 1997; Riveline et al., 2001). The development of internal tension forces applied specifically to the adhesion sites is due to contraction-myosin II machinery and promotes in turn focal adhesion assembly (Galbraith and Sheetz, 1997; Galbraith et al., 2002). Therefore, focal adhesions are individual mechanosensors whose elongation reveals the local balance between the force generated by the cell and extracellular matrix rigidity. As focal adhesion contains the major mechanical elements, they have been associated with the mecano-sensing capabilities of the cell. Focal adhesions grow under tension, generate strong adhesion and downstream signals (Friedland et al., 2009; Gallant et al., 2005; Michael et al., 2009; Wei et al., 2008). Therefore cellular force sensing is thought to be dominated through the regulation of focal adhesion assembly and growth by both intracellular and extracellular forces. This behavior leads to the concept of adhesion strengthening or reinforcement in which adhesion under forces recruits additional proteins and enlarges to keep force per area constant (Balaban et al., 2001; Choquet et al., 1997; Chrzanowska-Wodnicka and Burridge, 1996; Schwartz and DeSimone, 2008) (Fig. 1). Although adhesion maturation depends on intracellular and extracellular tension, the biophysical regulation of force transmission between the actin cytoskeleton and extracellular matrix during this process is largely unknown.

Mechanically activated β1 integrin controls its function

Contact with collagen fibrils is not sufficient for integrins activation, however collagen gel rigidity is required for integrins clustering and activation enhancing interaction between β1 integrins, FAK and talin (Wei and Vander Heide, 2008). Until recently models of integrin clustering and activation were viewed as independent of applied tension and were described to be sufficient to form adhesion complexes although insufficient to induce either large adhesion connected with actin cytoskeleton or downstream signaling. However, studies from the Boettiger lab have confirmed the role of the mechanically activated integrin in the control of $\alpha_5\beta_1$ function. The initial $\alpha_5\beta_1$-fibronectin binding or relaxed bond corresponds to the previously described activated-bound state (Takagi et al., 2003) and is independent on

tension. On this conformation, the application of forces switches the relaxed state to a new tensioned state resulting in the increased bond strength through the synergy site on fibronectin (Friedland et al., 2009). This behavior corresponds to the so-called catch bonds. Indeed force can shorten the lifetimes of macromolecular complexes (e.g., integrin-ligand bonds) by accelerating their dissociation. Paradoxically, bond lifetimes can also be prolonged by force. This counterintuitive behavior was named catch bonds, which is in contrast to the ordinary slip bonds that describe the more intuitive behavior of lifetimes being shortened by force (Dembo et al., 1988; Friedland et al., 2009; Kong et al., 2009; Zhu et al., 2005; Zhu and McEver, 2005). That means that a catch bond can function as a molecular clutch that is engaged under tension and will release when tension is released. The identification of molecular interactions that regulate the molecular clutch during focal adhesion assembly is critical to further understanding of cellular mechanotransduction. However clutch regulation can occur intracellularly *via* modulation of focal adhesion proteins that link F-actin to integrins, and/or extracellularly *via* modulation of integrin-extracellular matrix binding (Giannone et al., 2009). This suggests force-dependent molecular switches, cell and substrate tension and reciprocal interactions with the microenvironment.

Adhesion strength at the actin cytoskeleton-integrin interface

The binding between fibronectin and cells occurs partly through the $\alpha 5\beta 1$ and $\alpha v\beta 3$ integrins (Hynes, 2002; Leiss et al., 2008). However the mechano-responsive properties of each integrin are still not very well defined even though it is known that the mechanical properties of the cell's microenvironment are translated into intracellular biochemical pathways. Some intracellular molecules including Src, Cas, talin and vinculin show tension-dependent conformational changes that affect either their localization, kinase activity or phosphorylation levels without any precision on the specificity of integrin type (del Rio et al., 2009; Na et al., 2008; Riveline et al., 2001; Sawada and Sheetz, 2002). The talin1 head domain restored β_1 integrin activation but only full-length talin1 restored the linkage of actin cytoskeleton to extracellular ligand by revealing a binding site of talin rod for vinculin. Then, the contraction of actin filaments now pulls on liganded integrins and causes the assembly of focal adhesion and activation of force-dependent signaling like the phosphorylation of FAK on Tyr 397 (Zhang et al., 2008). Talin may act by increasing the mechanical connection to the cytoskeleton under force through an increase of its binding to vinculin head (del Rio et al., 2009; Galbraith et al., 2002; Zhang et al., 2008) whereas tail domain of vinculin binds to F-

actin and paxillin (Ziegler et al., 2008). More recently, a calibrated biosensor that measures forces across specific proteins in cells with piconewton sensitivity has been developed and applied to measure tension forces at adhesion sites. Insertion of this fluorescent tension-sensor module into vinculin reveals an unexpected regulatory mechanism in which the ability of vinculin to bear force determines whether adhesion assembles or disassembles under tension suggesting that vinculin recruitment to focal adhesion and force transmission across vinculin are regulated separately (Grashoff et al., 2010). As negative regulation of β1 integrin, ICAP-1 slows down focal adhesion assembly by decreasing β_1 integrin affinity. The switch between high and low affinity integrin states through a competition between talin and ICAP-1 is required in order to drive an integrated cell response to the matrix sensing (Millon-Fremillon et al., 2008). Current studies should identify the spatial and temporal regulation of integrin partners contributing to the regulation of the integrin activation cycle.

Adhesion strength at the extracellular matrix-integrin interface

Integrin activation are both enhanced by force (Astrof et al., 2006; Friedland et al., 2009) and depend on its conformation (Shattil et al., 2010). Indeed the structure of integrin is described as a large extracellular head region supported on two legs. This head region is bent under conditions where integrins exhibit low affinity and extended upon activation (Takagi and Springer, 2002; Xiong et al., 2001). As integrin activation is an allosteric process (Luo et al., 2007), it is tempting to think that applied forces by a bound ligand may induce unbending of the extracellular domain of integrin allowing a switch from a low affinity state with short bond lifetimes to a high affinity state with long bond lifetimes (Alon and Dustin, 2007; Chigaev et al., 2003; Luo et al., 2007; McEver and Zhu, 2007). However as $\alpha_5\beta_1$ integrin requires the nearby synergy site (PHSRN) for maximal binding affinity, we can not exclude a peculiar role of the synergic site in $\alpha_5\beta_1$ integrin activation or in elaboration of catch bonds. Recently, using AFM force-clamp experiments on a single purified integrin Kong and co-workers were able to measure the force dependent bond lifetimes of single bond between a fibronectin fragment and an integrin $\alpha_5\beta_1$. Thus, it has been shown that force applied on single fibronectin-$\alpha_5\beta_1$ prolonged bond life times, demonstrating catch bonds between $\alpha_5\beta_1$ and its ligand fibronectin. More accurately, truncating the $\alpha_5\beta_1$ leg regions formed longer-lived catch-bonds that were not affected by cations changes for Mn^{2+}, showing that legs extension is not required for catch bonds. Conversely, inducing the active conformation of the

integrin headpiece with activating monoclonal antibodies, shift catch bonds to lower force range. Thus, the fibronectin-$\alpha_5\beta_1$ catch bond appears to depend on the force-assisted activation of the head-piece but not on integrin extension (Kong et al., 2009). More recently it has been explored how extracellular stiffness impacts focal adhesion assembly and it has been described a tension-dependent clutch at the integrin-extracellular matrix interface on physiologically flexible substrates Cells are spread on fibronectin-coated polyacrylamide gels and form elongated focal adhesion similar in size to those found in cells plated on fibronectin-coated glass meaning that these physiologically flexible substrates gels are stiff enough to enable maturation of focal adhesion. Using myosin-II inhibition as a methodology for studying contractility driven focal adhesion maturation, the authors found that bonds between the integrin and substrat function as an extracellular clutch to modulate the degree of force transmission from the F-actin cytoskeleton to the ECM (Aratyn-Schaus and Gardel, 2010).

As tensioned β1 bonds generated downstream signals, the catch bond mechanism provides a physical basis for force sensing and cell adaptation where different bond lifetimes may correspond to different activation states. Sheetz group explains hint that the mechanotransduction process occurs through a cross-talk between two different integrins coexisting in the same focal adhesion. Fibronectin clustering and integrin $\alpha_5\beta_1$ would determine the adhesion strength while the interactions of extracellular matrix ligands with $\alpha_v\beta_3$ would enable mechanotransduction resulting in the reinforcement of integrin-cytoskeleton linkages through talin-dependent bonds (Roca-Cusachs et al., 2009). The synergy site required for maximal binding affinity in the case of $\alpha_5\beta_1$ integrin may be involved in the adhesion strength. A stable adhesion requires a strong molecular bond to resist high forces provided by clustered $\alpha_5\beta_1$ integrins whereas mechanotransduction might entail force-induced bind/unbind events through fibronectin-$\alpha_v\beta_3$-talin links. The family of transmembrane receptor-like protein tyrosine phosphatases (RPTP-α) has been described as essential for rigidity sensing and as a transducer of mechanical force on $\alpha_v\beta_3$–integrin-cytoskeleton linkages through the activation of Src family kinases (Jiang et al., 2006; von Wichert et al., 2003) which is in correlation with the localization of Src with α_v but not β_1 integrins (Arias-Salgado et al., 2005; Felsenfeld et al., 1999). These results suggest specific mechanical roles and different associated signaling pathways for $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins and could explain their colocalization in adhesion sites at the cell edges and their segregation during the retrograde $\alpha_5\beta_1$ translocation to form fibrillar adhesions (Pankov et al., 2000; Zamir et al., 1999).

The α5β1 integrin mediated cell traction forces direct fibronectin matrix assembly

Fibronectin is a major component of the extracellular matrix. It is a large, dimeric rod-like protein composed of three homologous, repeating modules, types I, II, and III. Fibronectin fibrillogenesis is a cell-mediated, step-wise process that converts soluble fibronectin into an organized matrix of fibronectin fibrils presenting binding sites for multiple extracellular components which are used as growth factor reservoir and to orchestrate the assembly of several other extracellular proteins (Hynes, 2009). Fibronectin fibrillogenesis is an essential biological process mediated by $\alpha_5\beta_1$ integrin and cellular contractile forces (Mao and Schwarzbauer, 2005). Upon binding to fibronectin $\alpha_5\beta_1$ integrins are translocated along actin stress fibers towards the cell center (Clark et al., 2005). This process corresponds to the formation of new adhesion structures called fibrillar adhesions. Fibrillar adhesions are enriched in tensin and phospho-paxillin, in which fibronectin fibrils are aligned with $\alpha_5\beta_1$ integrins and F-actin filaments (Pankov et al., 2000; Zamir et al., 1999). The integrin binding and translocation results in the stretching of the fibronectin molecule to expose hidden multimerization motifs (Mao and Schwarzbauer, 2005; Zhong et al., 1998). Indeed, it has been demonstrated that cell-generated forces are also required to maintain fibronectin in a partially unfolded conformation (Baneyx et al., 2002; Smith et al., 2007) that imparts elasticity to fibronectin fibrils and may modify outside-in mechanotransduction due to the increase in the matrix compliance.

Although the $\alpha_5\beta_1$ integrin plays a major role in mediating fibronectin fibrillogenesis and matrix deposition, other integrins such as αv containing integrins can partially compensate for its absence (Wennerberg et al., 1996; Yang and Hynes, 1996). Indeed fibronectin fibrils generated by αv integrins are short and thick, a shape that is reminiscent of those of α_v integrin containing focal adhesions. This observation confirms the importance of the synergy site in matrix assembly (Sechler et al., 1997). It looks like αv integrin are not able to move out from focal adhesions (Wu et al., 1996), limiting the extension of fibronectin fibrils. On the other hand, cell adhesion via either αvβ1 or $\alpha_v\beta_3$ leads to a rapid decrease in Rho activity while, $\alpha_5\beta_1$ integrin but not $\alpha_v\beta_3$ integrin supports sustained high levels of RhoA activity at later stages of cell spreading. RhoA activity is associated with the recruitment of tensin into fibrillar adhesions, integrin translocation and fibronectin fibrillogenesis (Danen et al., 2002). It has now been confirmed that the ability of $\alpha_5\beta_1$ integrin to efficiently bind soluble

fibronectin is coupled with increased RhoA activity which in turn stimulates fibronectin fibrillogenesis (Huveneers et al., 2008). Consistent with these findings, $\alpha_5\beta_1$ integrin has been shown to support ROCK-mediated contractility in fibroblasts (Gaggioli et al., 2007; White et al., 2007). Surprisingly, the use of β_1 and β_3 chimeras has shown that it is the extracellular domain of β1 that controls Rho activity. The role of individual Rho GTPases in fibronectin matrix remodelling has been examined by selectively down regulating their expression in cultured endothelial cells. No significant decrease was detected in the amount of fibronectin deposited by RhoA, RhoB, RhoC, Rac or Cdc42 depleted cells even though pharmacological inhibition of myosin-regulated contractility abrogated matrix assembly (Fernandez-Sauze et al., 2009). It looks like depletion in GTPases lead rather to differences in fiber arrangement. Some new biophysical data indicate that developing fibril orientation is guided by the direction of the traction force applied to that fibril (Lemmon et al., 2009). Furthermore blocking or increasing myosin II activity by treating cells with either blebbistatin or calyculin A abrogates the inward translation of traction forces, the dissipation of compressive strain and fibrillogenesis over time. These results underline the contribution of spatiotemporal changes in traction force and local strain to allow successful matrix assembly. This process likely involves molecular switches such as regulatory GTPases and their specific guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs) and selective interactions with downstream effectors. Recently, it has been show that tensin 2 knockdown significantly reduces the ability of HFF cells to contract 3D collagen gels and is associated with a substantial reduction in Rho A activity without affecting fibrillogenesis (Clark et al., 2010). In that context, inhibition of collagen gel contraction is reversed by depletion of DLC1, a RhoGAP known to bind tensin in focal adhesions (Liao et al., 2007; Qian et al., 2007; Yam et al., 2006). However different isoforms of tensin have been described and their respective functions are poorly understood. Tensin 1 is equally distributed between focal and fibrillar adhesions. Tensin 2 is enriched in focal adhesions at the leading edge whereas tensin 3 translocates rearward and is enriched in fibrillar adhesions (Clark et al., 2010). The ILK/PINCH/PARVIN complex might belong to the signaling pathway controlling maturation of focal adhesions to tensin-rich fibrillar adhesions by down regulating the expression or recruitment of tensin and destabilizing $\alpha_5\beta_1$-integrin-cytoskeleton linkages (Stanchi et al., 2009). The phosphorylation of integrin tails might also act as a switch to drive the disassembly of integrin/talin complex so favoring the formation/stabilization of integrin/tensin complex (McCleverty et al., 2007). The signaling pathways providing a permissive platform

for tensin recruitment are still under debate and need more investigation. It will be important in the future to identify the players such as GEF, GAP and downstream effectors that operate within each spatiotemporal signaling module and identify the cross-talk occurring between these modules.

Conclusion

Numerous different signaling pathways can regulate β_1 integrin activation and talin binding to the β tail is often seen as a final step of the activation process leading to the connection of integrins to actin cytoskeleton. Now, the issue is to understand how these different signaling pathways intersect with talin binding and modulate the physical properties of the extracellular matrix and the behavior of the cell. The challenge is also to decipher the reciprocal cross-talk between cells and extracellular matrix in order to characterize how cells sense and remodel the matrix and how the matrix can trigger through mechanical constraints and chemical outside-in signaling a cellular adaptive response to maintain tissue homeostasis, control morphogenesis or trigger tumor invasion. Unraveling molecular mechanisms able to transmit force is of fundamental importance. The key will be to understand the specific role of β_1 and β_3 integrin localized in a same adhesion site that presents physical restriction of ligand/receptor spatial organization. The addressed question is to know the spatial organization of each integrin activated or not within a same adhesion site and how one type of integrins signals towards another type of integrins either to control initiation of individual adhesion site, to direct collective dynamics or to respond to chemical and physical properties of the extracellular matrix. Future studies should clarify the extent of specific integrin movement into adhesion clusters, the integrin spatial organization within a same cluster, and how these movements and these organizations can correlate with cell behavior.

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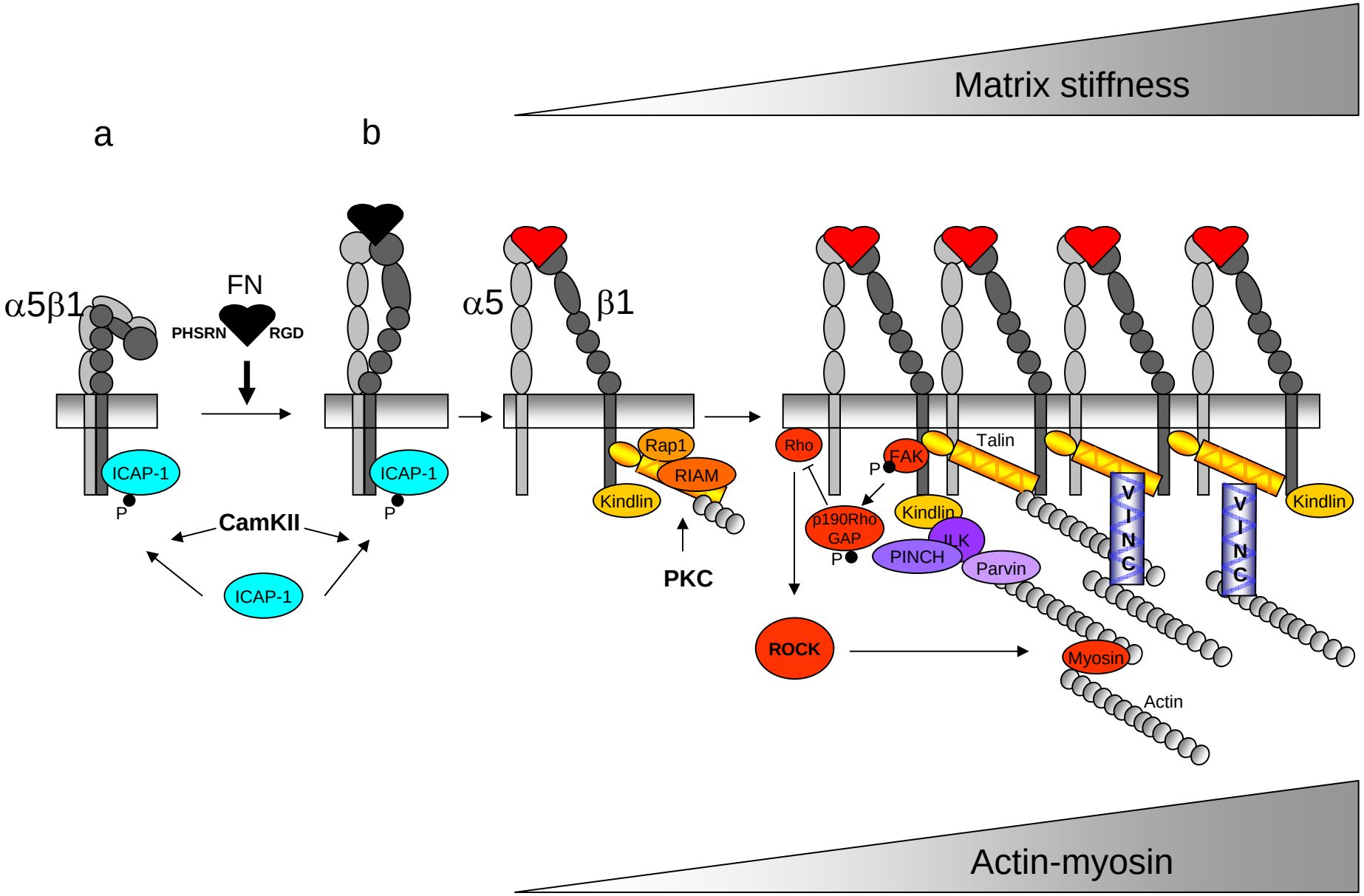
Legends:

Figure 1: Cells tune their contractility in response to matrix stiffness

Cell adhesion to fibronectin depends on the RGD motif on fibronectin. A feature of α5β1 integrin is its requirement for an additional binding site to RGD on fibronectin called the PHSRN synergy site for obtaining maximum binding affinity (fibronectin in red). Integrins can exist in an inactive state which can be activated by inside-out or outside-in cues. The low affinity state of the integrin is maintained by non-covalent interactions between the α and β integrin transmembrane and cytoplasmic domains. The phosphorylation of ICAP-1 on CamKII consensus site is likely to enhance binding to β1 integrin tail and might account for inhibition of α5β1 activation. ICAP-1 binding on β1 integrin could either be associated with the bent conformation (a) or the low affinity extended conformation (b). Following recruitment of an activation complex, ICAP-1 is released, and integrin extension is permitted favoring extracellular ligand binding. Integrin is linked to actin network and force could induce the catch bound process resulting in an increase affinity for fibronectin. Integrin extension is triggered by transmembrane domain separation followed by headpiece transition from the closed to open conformation. The current model is that the integrin cytoplasmic domains are the trigger point for conformational changes that results in integrin activation. Talin and kindlin are likely the major players. Increase in matrix stiffness favors integrin activation, clustering and protein stretching. Cells exert actomyosin contractility and cytoskeleton-dependent force through Rho signaling and feedback loop in response to matrix stiffness cues.

Note that the interaction of ICAP-1 with a define conformation of integrin is still unclear but this interaction is associated with low affinity state of integrin.


Matrix stiffness
a
b
α5β1
FN
PHSRN
RGD
ICAP-1
P
CamKII
ICAP-1
ICAP-1
P
α5
β1
Rap1
RIAM
Kindlin
PKC
Rho
FAK
P
Talin
p190Rho
GAP
P
Kindlin
ILK
PINCH
Parvin
V
I
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C
Kindlin
ROCK
Myosin
Actin
Actin-myosin