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Mechanisms of talin-dependent integrin signaling and crosstalk

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Abstract

Cells undergo dynamic remodeling of the cytoskeleton during adhesion and migration on various extracellular matrix (ECM) substrates in response to physiological and pathological cues. The major mediators of such cellular responses are the heterodimeric adhesion receptors, the integrins. Extracellular or intracellular signals emanating from different signaling cascades cause inside-out signaling of integrins via talin, a cystokeletal protein that links integrins to the actin cytoskeleton. Various integrin subfamilies communicate with each other and growth factor receptors under diverse cellular contexts to facilitate or inhibit various integrin-mediated functions. Since talin is an essential mediator of integrin activation, much of the integrin crosstalk would therefore be influenced by talin. However, despite the existence of an extensive body of knowledge on the role of talin in integrin activation and as a stabilizer of ECM-actin linkage, information on its role in regulating inter-integrin communication is limited. This review will focus on the structure of talin, its regulation of integrin activation and discuss its potential role in integrin crosstalk.

Introduction

The communication of extracellular matrix (ECM) with intracellular cytoskeleton is crucial for regulating cell adhesion, cell shape change and cell migration. Such communication depends heavily on integrins, a large family of noncovalent heterodimeric (α/β) adhesion receptors [65], [68]. Integrins function by engaging ECM ligands through their large extracellular domains and actin-binding proteins through their short cytoplasmic tails (CT), thereby linking ECM with the cytoskeleton (Fig1). Talin [19], [24], [63], [82], the focus of the article, together with filamin [95] and α-actinin [77], [127], [160], [139], are known to be the key players in this linkage, which can bind directly and simultaneously to both actin and integrin CTs. Numerous other intracellular proteins also connect integrins and the actin cytoskeleton but indirectly via shared binding partners. These extensive integrin-actin networks of protein-protein interactions coalesce to form discrete structures, focal adhesions, podosomes or analogous structures, that constitute dynamic hubs of adhesive and signaling activities [85], [146], [177].

The effects of talin on integrin function are broad. It transduces signals across integrins in both the inside-out and outside-in directions and it also influences the organization of the

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actin network and the composition of focal adhesions [1], [28], [45], [75], [76], [110], [147], [178]. Much of the recent studies on talin have emphasized its unique role in the inside-out signaling of integrins; i.e., their transformation from their basal or “resting” state to a more “active” state in which they can engage their cognate ECM ligands more efficiently (integrin activation). Less emphasized but clearly documented is the influence of talin on integrin outside-in signaling (Fig 1). Much less is known concerning the role of talin in the crosstalk between integrins of the same or different integrin subfamilies or with other signaling pathways. This review will summarize recent advance on talin structure and its control of integrin function and will touch upon its role in integrin crosstalk.

Talin expression, structure and subcellular distribution

Talin was discovered three decades ago as a protein highly enriched at cell adhesion sites [20]. The Tln gene and its orthologs can be traced from vertebrates back to protists [149]. Of the two mammalian isoforms of talin, Tln1 is ubiquitously expressed, being most abundant in the heart and scarce in the brain. Tln2 is enriched in the heart and brain with lower levels detected in the skeletal muscle, liver and lung [115]. Although the Tln1 and Tln2 isoforms in mammals share 74% identity, they do not fully compensate for each other. For example, in skeletal muscle or heart specific knockout models and during epithelial embryogenesis, when Tln1 is inactivated, Tln2 levels do not increase and existing levels do not compensate for Tln1 [29], [101], [108]. Moreover, Tln1 levels remain unelevated and do not compensate functionally for Tln2 in Tln2 deficient mice [29]. Gastrulation defects result in early embryonic lethality of Tln1 global knockout mice while Tln2 knockout mice are viable and fertile although early and severe myopathy in skeletal muscles is observed in the Tln2 knockout which is not prominent in the skeletal muscle specific Tln1 knockout [17], [29]. Platelet or endothelial specific Tln1 deficiency results in severe phenotypes in mouse models [114], [57], [122], [131], although some compensation could be demonstrated by exogenous Tln2 in endothelial cells and upregulated Tln2 expression was observed in undifferentiated embryonic stem cells [83], [179]. The vital role of talin in integrin activation and cell adhesion has been established using multiple model organisms [17], [30], [31], [115] as well as tissue-specific inactivation of the Tln1 gene [108], [114], [122].

The human talin (Q9Y490, UniProtKB) monomer is a 270 kDa protein composed of 2541 amino acids and consists of an N-terminal head of 433 amino acids (talin-H) followed by a much larger rod domain (talin-R) (Fig 2). Talin-H contains four subdomains, F0, F1, F2 and F3 with F1-F3 being homologous to a typical FERM domain. The F0 and F1 subdomains exhibit ubiquitin-like folds [53] and form a novel linear arrangement with F1-F3 subdomains as opposed to the cloverleaf arrangement typical of most FERM domain proteins [40]. The F1 subdomain also has a 30 amino acid insert that was not visualized in the X-ray structure of talin-H domain [42]. The F3 subdomain is critical to many functions of talin as it binds to many proteins, including integrins [25], phosphatidylinositol-4-phosphate 5-kinase gamma 90 (PIPkinaseγ90) [35], [98] and layilin [15]. Talin F2-F3 has also been implicated in binding to focal adhesion kinase, FAK [26], [90] although the biophysical evidence for such binding is lacking. Using nuclear magnetic resonance (NMR) spectrometry, we failed to detect any significant interaction of ^{15}N-labeled talin F2-F3 with 8-fold excess of a FAK peptide comprising of residues 1011-1042 (Yang et al, unpublished results), a region that was indicated to be the FAK binding site for talin [90]. The F1 and F2 domains anchor to the membrane, which likely position the F3 in an orientation facilitating β integrin engagement [4], [40]. Talin-R consists of a series of helical bundles which contain multiple protein binding sites for vinculin [48], RIAM [93], [55], [91], integrin[163], a THATCH domain and a dimerization sequence at the C-terminus [61]. Notably, talin has been found to contain three actin binding sites: one at talin-H and two at talin-R [32] but the structural details of how these sites mediate actin crosslinking and regulate actin remodeling

remain unknown. Table 1 provides a list of talin binding partners and the specific regions within talin to which they bind.

The overall organization of talin, monomer versus dimer, may be different in specific tissues and/or species. Studies of talin isolated from chicken gizzard revealed that it is monomeric at low protein concentrations at physiological ionic strength [113], [172]. However, studies showed that talin isolated from human platelets exists predominantly as an antiparallel homodimer [50]. Low ionic strength induces a more globular shape to the chicken talin monomer while, at physiological or greater ionic strengths, it assumes an extended and filamentous conformation [113], [172]. Structural studies have revealed that the dimerization domain (2496-2529 residues) is important for the function of its C-terminal actin binding site [45].

In resting state cells, talin distribution is random and diffuse[9] and is autoinhibited from binding to integrins [49], [52], [156] and membrane [5], [156]. Talin-R plays a major role to structurally restrain the talin-H binding to the plasma membrane and integrin [156]. Upon agonist stimulation, talin is rapidly localized to the plasma membrane [10]. Protein kinase C alpha (PKCα), Rap1A, Rap1 effector RIAM, and phosphatadylinositol 4, 5 biphosphate (PIP2) have been shown to play important roles in the talin membrane localization and activation of its integrin binding function [58], [96], [156], [144]. The crystal structure of autoinhibited talin (pdb 4F7G) provides a clear atomic view of the talin-R and F3 interface [136] that is distinct from that proposed in an earlier model [52]. This crystal structure, along with the NMR binding studies suggests a steric and electrostatic mechanism to relieve the autoinhibition of talin. Specifically, the structure reveals that talin F2-F3 in complex with talin-R prevents the membrane association of talin F2-F3. In the autoinhibited talin complex, the negative charges on talin-R are repelled by negative phosphatidylserine on the inner leaflet of the plasma membrane. Higher concentration of negatively charged PIP2 enriched at sites in the membrane is likely to create an attractive force for positive charges on talinF2-F3. Interaction of talin F2-F3 with membrane misaligns the talinR-F3 interface, overcoming the autoinhibition and allowing integrin tail to dock onto talin F3, thus promoting the active conformation of talin via a pull-push mechanism [156]. The most recent model of talin depicts it to be a compact doughnut- shaped dimer with talin-R forming the ring and talin-H occupying the ‘hole’ in the center. This model is based on a combination of electron microscopy, small angle X-ray scattering, NMR and the known X-ray structures of talin fragments [54]. This model implies that for integrin and vinculin to bind talin, large movements of talin domains may be necessary [54]. Actin based mechanical forces are thought to play a role in exposing the vinculin binding sites on talin, but the spatio-temporal and molecular details of how this is accomplished remain to be resolved. The precise mechanisms of how PKC, Rap1A, and RIAM recruit and potentially activate talin are also not clearly understood. Recent studies have shown that multiple RIAM molecules can bind to the sites in talin-R where vinculin also binds, suggesting a turnover mechanism by which RIAM initially recruits talin to the integrin site and is later replaced by vinculin during focal adhesion assembly [55]. Calpain, a calcium regulated protease, can cleave the Q433-Q434 peptide bond in talin [138], thereby liberating talin-H to bind integrin β3CT with a much higher affinity than intact talin [175] and leading to integrin activation. Calpain cleavage of talin at Q433-Q434 as well as K2493-K2494 in talin-R has also been shown to regulate focal adhesion turnover [7], [42]. However, talin activation and consequent integrin activation can occur in the absence of calpain cleavage as ionophore-mediated αLβ2 [38] and RIAM induced β3 integrin activation [58] do not require proteolysis of intact talin by calpain. Thus, there may be several pathways for exposing the integrin binding site in intact talin, and the role of calpain may be geared more to the regulation of focal adhesion turnover.

Talin-integrin interaction

Among the 40 integrin CT binding proteins, talin is unique for its ability to bind and activate integrins. Another family of β integrin CT binding proteins, Kindlins, is also essential for integrin activation [134] and is discussed further below. Interactions of talin-H with the CT of β1, β2, β3, β5 and β7 integrins have been reported [21], [25], [130], [132], [145], [151] but the reported affinity varies widely. Solution NMR was used to estimate the dissociation constants (Kd) of talin F3-β CT to be in the μM range with the highest affinity for β1D, followed by β7, β3 and β1A CT [2], [3], [4]. As a point of reference, the Kd of talin F3 for β3 CT was estimated to be 273μM by NMR. Surface plasmon resonance (SPR) estimated a Kd of talin F2- F3 for the β3 [24] and β2 CT to be 91nM and 12.5 nM [125], respectively. A Kd of talin F2-F3 for β1 was estimated to be 67nM by pulldown assays [16]. The variable affinities probably arise from different technologies, different talin fragments, differences in talin and β CT sequences [3], and sample preparations. Notably, talin binding affinity to β3 CT increases dramatically in the presence of phospholipids [72], [117], suggesting that membrane plays a crucial role in elucidating the physiologically relevant talin-integrin binding. On the other hand, it does appear that the affinity of talin for integrin β CT is of relatively low affinity, which may provide a biological control mechanism so that integrin activation states can be inter-converted readily during cyclic cell adhesion and migration processes. The primary integrins mediating leukocyte (β2 and β7) and platelet (αIIbβ3) functions require rapid transitions between resting and activated states while the ubiquitous β1 integrins are present in a partially active state [59], [68]. Thus, the higher affinity of talin-H for the β2, β3 and β7 integrins may be needed to ensure rapid and efficient integrin activation. The two talin isoforms also exhibit different affinities for the same β CT [2-4]. The Tln2 isoform binds β3 and β1A (non-muscle splice variant) less robustly than Tln1. The tighter association of Tln2 with β1D (muscle specific β1 integrin) may be necessary to withstand high mechanical stress of muscle contraction exerted on myotendinous junctions [3]. Talin has also been reported to bind to the α subunit of the platelet integrin αIIbβ3, but the functional significance of this interaction has not been elucidated [47], [82], [136].

The major integrin binding site of talin is on talin-F3. The F3 domain recognizes two regions in integrin β CT: one on the NPxY/F motif, a conserved sequence in the midsection of most β CTs [21], [43], and a second in the membrane proximal region [165], [168], [171], as represented in Fig 3. The membrane proximal interaction of talin interferes with the integrin β CT interaction with α CT thereby causing the unclasping of the CT complex and initiating integrin activation as initially shown for integrin αIIbβ3 [168]. This mechanism has been also demonstrated with other integrin α/β heterodimers as well [4], [11], [81], [167]. This unclasping process extends into the membrane, where interaction between the α/β transmembrane helices is also disrupted and ultimately induces conformational changes in the extracellular domain of the integrin [79], [89], [176], [180]. Talin binding to the membrane and integrin β3 CT has recently been shown to alter the transmembrane domain topology of the interacting α and β chains which may provide an additional mechanism for transmission of conformational change across the membrane and into the extracellular domain [80]. However, the precise topology of the transmembrane domains of integrin α and β subunits remain controversial [86]. Readers are referred to reviews for further details of the biophysical nature of integrin CT interactions (e.g.[170]).

In addition to the integrin binding site within talin-H, which is known as IBS-1, there is also a second integrin binding site (IBS-2) on talin-R. IBS-2 has not been implicated in regulating integrin activation. An IBS-2 containing talin-R fragment (1974-2293 amino acids) was found to have a binding affinity for β3 and β1 CT similar to that of talin-H [47]. However, this IBS-2-β3 and β1 integrin binding is impeded by residues C-terminal to IBS-2, making the overall affinity of the rod domain weaker than talin-H [47]. An affinity of

34.5nM of talin-R for β3 CT was reported [141], which is higher than published values talin-H [175]. This may suggest that there are two inhibitory mechanisms at play that regulate integrin-talin binding: (i) one involving talin-R(1654-2344 residues) and talin FERM domain that masks the β CT binding site on talin H [49] and (ii) C terminal rod (2300-2541 residues) and IBS-2 (1974-2293 residues) that lowers the strength of the IBS-2-β CT binding. The role of talin-H is to bind and activate integrins, and talin-R may be important in recruitment of adhesion molecules to focal adhesion [169]. It has also been proposed that IBS-1 activates integrins while IBS-2 stabilizes the integrintalin link, freeing talin-H to then interact with other binding partners such as PIPKIγ90 [41].

Regulation of talin and its interaction with integrins

Autoinhibition provides one mechanism to control the dynamics of talin-mediated integrin activation [49], [156]. Competition with negative regulators filamin [78], [123], ICAP-1 [102], β CT phosphorylation [2], [128] and lipid binding [49], [51], [72] provide additional mechanisms that regulate talin-mediated integrin activation and cytoskeletal-integrin linkage. Talin competes with filamin, a negative regulator of integrin activation [22], [33], [70], [123] or ICAP-1 for overlapping binding sites in integrin β CT. Integrin β CT that have higher affinity for these negative regulators bind talin less well [22], [78], [102]. Phosphorylation of tyrosine residues in the NPxY/F motif on β CT diminishes talin binding and favors binding of other partners, such as Dok1, which binds preferentially to the phosphorylated β CT [2]. Release of inhibitory restraint of talin-R on the talin PTB domain by PIP2 exposes sites involved in integrin activation [49]. This exposure facilitates talin F2-F3 binding to lipids within the plasma membrane thereby orientating talin for more favorable binding to β3CT [72], [117].

Early studies established that talin can be phosphorylated by PKC on serine (S) and threonine residues [99], [100], [8], [8], and subsequent studies indicated that talin was also a substrate for calyculin [121] and CdK5 [64]. Mass spectrometry revealed about 30 phosphorylation sites in talin [137], but only limited data support a functional significance to such post-translational modification. Phosphorylation of S425 within talin-H by Cdk5 prevents binding to and consequently ubiquitination by Smurf1, thereby regulating the intracellular turnover of talin [64]. However, the physiological relevance of phosphorylation of talin in terms of effects on integrin-related activities is less clear. Thrombin stimulated platelets leading to enhanced PKC mediated talin-H phosphorylation is associated with talin redistribution but was independent of its proteolytic cleavage and integrin interaction [10]. In chicken embryo fibroblasts, although stress fiber and focal contact organization was unperturbed by talin phosphorylation, precursors of focal contacts had reduced actin and talin-rich protrusions [8]. It has also been suggested that PKC-mediated talin phosphorylation may be involved in focal adhesion disassembly [164].

Integrin avidity modulation by talin

While the α/β CT unclasping mechanism is believed to be primarily a trigger of affinity modulation, talin also induces clustering of integrins leading to avidity modulation. Avidity modulation results from lateral movement of integrins in the plasma membrane to form integrin-rich microdomains or clusters. Integrin clusters exhibit high avidity for ligands, which are themselves multimeric, allowing for multivalent adhesive linkages. Agonist stimulation of platelets or exogenous talin head expression in CHO cells leads to formation of integrin-enriched clusters. Ligand binding to integrins in these clusters may then also promote conformational change to a high affinity state [18]. This scenario reconciles the data from *Drosophila* integrins where talin seems to be mainly responsible for integrin clustering rather than affinity modulation [17], [60]. In *Drosophila*, talin is not required to stabilize integrins on the cell surface or recruit them to muscle junctions. Instead, the major

role of talin is to connect ECM bound integrins to the cytoskeleton [17], [41], [112], [62], [169]. Formation of microclusters has also been implicated in the function of integrin αLβ2. ICAM-1 binds well to αLβ2 on T-lymphocytes where microclusters can be visualized but not to dendritic cells, which lack such densely packed integrin patches. Talin has been shown to stabilize clusters of αLβ2 in a high affinity state [153]. The post-occupancy signaling from occupied integrins, which, among other events, strengthen their linkages to the actin cytoskeleton, depends upon clustering of integrins even when activated via affinity modulation. This is supported by studies that showed that PIP2 mediated release of talin-H from talin-R is a prerequisite for integrin activation and clustering on immobilized ECM ligands [27], [144]. Receptor clustering was found to precede talin recruitment in *Drosophila* [162]. In skeletal muscle specific $Tln1^{-/-}$ & $Tln2^{-/-}$ mice, talin was shown to be important for clustering of α7, αv and β1 integrins in myotendinous junctions [29]. In other words, while there are theoretical distinctions between affinity and avidity modulation, these processes overlap in responding cells, and talin's role in integrin activation may occur via both affinity and avidity modulation, and specific pathways leading to integrin activation may be more prominent in certain tissues, cells or developmental stages [29], [101].

Talin and integrin outside-in signaling

Integrins are adhesion receptors that are capable of bidirectional signaling. Just as intracellular signals can be transduced across the membrane to activate the ligand binding function of integrins, ligand binding and other external environmental cues can also be transmitted via ligated integrins to the β CT and downstream signaling cascades within the cell that ultimately impact growth, survival, differentiation and proliferation of the cell. These events are referred to as "outside-in signaling" (see Fig. 1). Clustered integrins promote phosphorylation and therefore activation of FAK. Activated FAK can bind Src, a non-receptor tyrosine kinase, or other Src family kinases, (SFK) forming complexes which recruit additional proteins to focal adhesion sites. Signaling via the FAK-Src module affects early stages of adhesion [118], and promotes cell spreading [69], [73], but also can destabilize focal adhesions to allow migration of cells [44]. Thus, many outside-in signaling events from occupied integrins depend on or lead to rearrangements in the actin cytoskeleton. For example, platelet dependent clot retraction depends on occupancy of integrin αIIbβ3 and the associated rearrangements of the actin cytoskeleton [129]. In view of the importance of talin in connecting integrins to the cytoskeleton, it would appear that talin should assist in such outside-in responses, and there are data to support this notion [57]. However, the specific details as to how talin is involved in outside-in signaling remain uncertain. In one scenario, talin-H may dissociate from integrin β CT to facilitate integrin outside-in signaling. This mechanism may very well be involved since only talin-R but not talin-H was detected in focal adhesions in *Drosophila* [162], [161] and $Tln1^{-/-}$ mouse embryonic fibroblasts [112]. This process could be assisted by calpain [42], [7] or PIP2 [144] which separate talin-H from talin-R and is known to play critical role in integrin outside-in signaling [32], [68]. Furthermore, since β CT tyrosine phosphorylation dissociates talin from integrin [2], the notion that there is a rearrangement of talin-mediated integrin-actin connection upon integrin activation and occupancy is supported. Talin-H dissociation would also allow integrin CT to bind other proteins such as kinases and phosphatases known to trigger intracellular signaling pathways, induce cellular responses and cytosleketal rearrangements [68]. However, since most outside-in signaling responses are transduced by clustered integrins, intact talin is needed to bridge integrins and actin at some point during the outside-in signaling response. There is also evidence that not all outside-in responses are talin dependent. In CHO cells, talin recruitment was found to be a post-integrin activation event, promoting cell spreading by inhibiting the integrin β1A-FLNA interactions [123]. GPCR signaling can increase the affinity of α4β1 integrins on monocytes [66], but this increase in affinity appears to be independent of talin, kindlin3 or α-actinin. Rather, these

integrin β binding partners, including talin, seem to stabilize the bonds of α4β1 integrin clusters with the cytoskeleton [67]. Thus, it is likely that the role of talin changes throughout the course of outside-in signaling responses.

Integrins are primary mediators of mechanotransduction. In response to the application of force, talin is recruited to integrin clusters. Thus, under strain, talin's role may not be as an integrin activator, but as a mediator of mechanotransduction signals and strengthener of cytoskeletal connections [140]. This interpretation was supported by studies involving single molecule tracking and super-resolution microscopy in living cells, which suggested that transport of integrins into focal adhesions was talin independent. Integrins diffusing into these focal adhesions were postulated to be subsequently activated by talin recruited from the cytosol. This sequence of events would then lead to fibronectin engagement by the activated integrins in the focal adhesions [143]. Talin may also have functions other than integrin activation. In $Tln1^{-/-}$ embryonic fibroblasts, their adhesive phenotype was promoted only by an intact IBS-2 in talin-R [112]. At focal adhesions, only talin-R and not talin-H was observed [62]. In mammary epithelial cells, talin-R was found to be instrumental in recruiting FAK, vinculin and p21 to regulate cell proliferation [169]. An alternative pathway was demonstrated in which integrin clusters recruited FAK in nascent adhesions without a contribution from talin [90]. In certain tissue- or developmental- specific murine models, integrin activation was unaffected even upon talin knockout [29], [101]. Thus, talin can maintain cytoskeletal integrity [29]) or prevent degradation of β1 integrins besides activating integrins per se [101].

Role of talin in crosstalk involving integrins

The crosstalk between signaling pathways creates complex and interwoven networks of communication, and integrins talk to many different pathways. By virtue of its capacity to control integrin activation and signaling, a role of talin in such integrin-mediated crosstalk can be deduced although direct experimental evidence for talin's involvement is limited. Integrins αVβ3 and α5β1 and their activation [56], [140], [173] are necessary for formation of a fibronectin-rich ECM, but the role of integrins in crosstalk extends beyond communication with the ECM. Signaling by integrins is intricately coordinated with that of various receptor tyrosine kinases (RTKs) and other growth factor receptors. Distinct integrin-growth factor receptor associations may drive different signaling events in response to specific growth factors [142]. These interactions permit bidirectional crosstalk between integrins and RTKs and other growth factor receptors during cell migration, cancer metastasis, angiogenesis and embryonic development (see reviews by [39], [71], [155] and [157] . Since these interactions reciprocally influence signaling via RTK and integrins and is further influenced by the activation states of integrins, this crosstalk will likely be dependent on talin.

Crosstalk may also be used to describe how integrin engagement influences the function of other integrin subfamily members. Such integrin mediated crosstalk may enhance or inhibit the function of the targeted integrin. Typically, cell surface levels of the targeted integrin are not reduced but rather the control is exerted via a signaling mechanism [135], [150]. As an example of the positive cooperativity between integrins, αvβ1 integrins were found to work in concert with α5β1 integrin to promote spreading of cells on fibronectin [109]. More extensively studied has been the capacity of one integrin to suppress the activity of another integrin**.** Prerequisites for the suppressive effects of one integrin on another are the ligand-occupied high affinity conformation and an intact β CT [36]. Most emphasis has been given to the communication between β3 and β1 CTs with the β3 CT functioning as the negative regulator [13], [14], [36], [87], [152]; i.e., β3 exerted a trans-dominant effect on β1. These effects have been demonstrated using both adherent, HEK, HUVEC [150], and suspension

K562 cells [13], [14] with inhibition of α5β1 mediated migration on and phagocytosis of fibronectin by ligand-occupied αvβ3. In addition to the requisite role of the β CT, a vital role of the β transmembrane domain in this crosstalk has been demonstrated [150].

Chimeric β integrins have been used to probe endogenous integrin function [87], [152] under the premise that the chimeric receptors could compete with the endogenous integrins for cytoplasmic proteins that are needed for integrin mediated functions. Building upon this hypothesis, Calderwood et al. [23] concluded that it was competition for talin that underlies the trans-dominant inhibition of integrin activation. They showed that mutated β CT defective in talin binding was unable to mediate trans-dominant inhibition and this suppression was reversed by overexpression of integrin activating fragments of talin. Thus, the sequestration of talin by the suppressive species is both necessary and sufficient for trans-dominant inhibition of integrin activation. Talin overexpression was shown to relieve the suppression of specific integrins plated on non-canonical ligands [126]. Ligand occupied β1 integrins have been demonstrated to activate PKA [125] that can suppress flow-induced activation of αvβ3 integrins [126]. PKC is involved in agonist stimulated αIIbβ3 integrin activation on platelets [74]. PKC activated Rap1 [97], [111] can interact with RIAM [88] to promote integrin activation by recruiting talin to the plasma membrane [58]. Thus, the RIAM-talin axis could also be involved in inter-integrin communication. Hence, a model of talin mediated integrin crosstalk can be envisaged whereby stimulating signals unfold the talin molecule to expose talin-H, leading to affinity modulation of integrins via binding of talin-F3 to β integrin CT. Upon ligand engagement and integrin clustering, signaling pathways lead to either inhibition of other integrin species, or amplification of activating signals for talin to further augment integrin activation (Fig 4).

Crosstalk between talin and other integrin binding proteins

Kindlins, a three member family of mammalian proteins, have emerged as important regulators of integrin activation [104]. Talin and kindlins cooperate for optimal integrin activation [12], [103]. Deficiencies/ mutations of kindlins, either in mice [116], [120], [166], or humans [84], [106], [107], [159], can have profound effects on integrin mediated cellular responses. Like talin, kindlins are FERM domain containing proteins. Also like talin, kindlins use their F3 (PTB-like domain) to bind to NPxY/F motifs in integrin β CT. However, talin binds to the membrane proximal of these motifs whereas kindlins engage the membrane distal NPxY/F motif (shown in orange in Fig 3) in β CT. Kindlin-2, talin and β integrin CTs can exist in a ternary complex, showing that talin and kindlin-2 can bind β integrin CT simultaneously and can function as “co-activators” of integrin αIIbβ3 [12]. The mechanism underlying such co-activation is presently unresolved. It has been suggested that talin mediates early stages of integrin activation while kindlin-3 is crucial for induction of the high affinity state of integrins [94]. At this point, it is unclear whether talin binds to the β CT first and facilitates kindlin binding or vice-versa. Based on the displacement model of integrin activation [70], migfilin, which binds to and may be recruited by kindlin to integrin adhesions, could dislodge filamin bound to and make β integrin CT accessible to talin and kindlin binding. Intracellular signaling thus triggered may facilitate further cooperation between talin and kindlin allowing for continuous inter-integrin communication. This will finally result in a robust integrin activation cascade (Fig 5). For more details on kindlins and their roles in integrin activation, readers are referred to recent reviews on this topic [105], [119], [133].

Concluding Remarks

Talin is a key regulator of the communication between the actin cytoskeleton and the ECM. This function depends on the capacity of talin to serve as a binding partner of multiple proteins, including actin and other actin binding proteins and integrins. Integrin engagement

by talin controls their capacity to alter their activation state by affinity and/or avidity modulation. This interaction also assists in mediating the outside-in signaling that occurs through ligated integrins. Integrins speak to one another and to a variety of other membrane receptor systems and the downstream signaling that they orchestrate. Talin, by virtue of its direct association with and regulation of integrins, would appear to be integrally involved in this crosstalk. To comprehend the full role of talin in such crosstalk, mutations of specific functions of talin, some of which have been reported [41] and others that still need to be developed, should be characterized for their effects on the function of other cellular receptor systems with an eye on ultimately testing the consequences of such crosstalk in model cell systems and whole organisms.

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Highlights

- Talin modulates affinity and avidity of integrins for extracellular matrix ligands
- Role of structural components of talin in modulation of integrin activation
- Integrins communicate with each other and other growth factor receptors
- Signaling via talin-mediated activation can suppress or promote integrin function

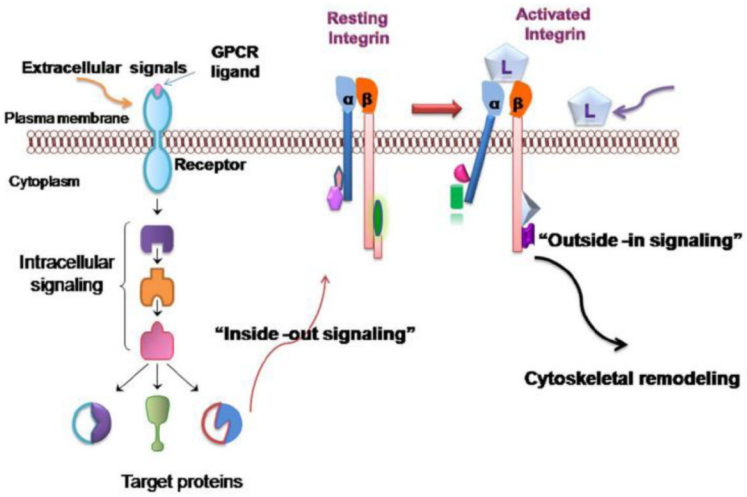

Fig1. Bidirectional signaling across integrins

Agonist stimulation, signaling via G protein coupled receptors (GPCRs) and/or growth factor receptors can lead to "inside-out" signaling of integrins. Extracellular domains of resting integrins open up to bind extracellular matrix ligands (L). This leads to "outside-in" signaling of integrins resulting in cytoskeletal remodeling and downstream signaling cascades.

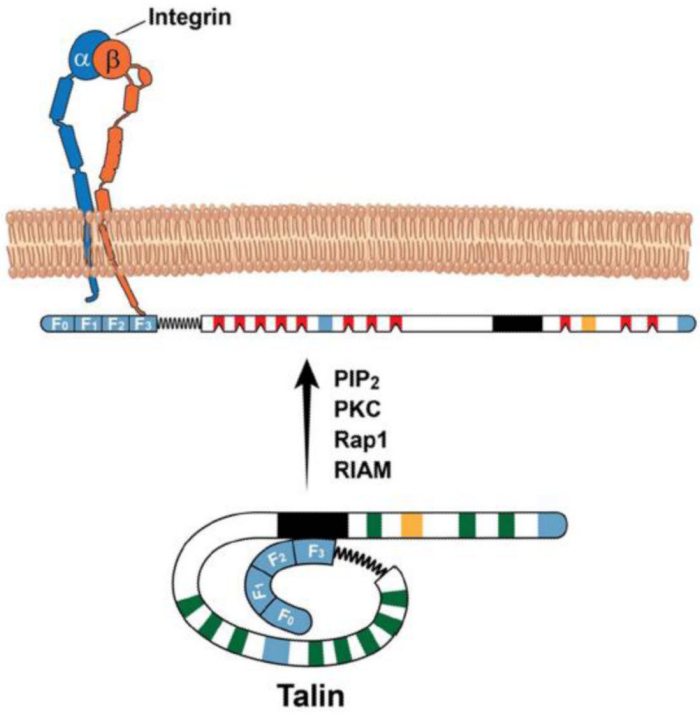

Fig 2. Structure of talin

Talin can be subdivided into head (talin-H) and rod (talin-R) regions. Talin-H is comprised of F0, F1, F2 and F3 domains while the talin-R has ~11 vinculin- and 3 actin-binding sites (in blue). The vinculin binding sites are dormant (in green) and are likely mechanoactivated (in red). PIP2, PKC, Rap1 and or RIAM can relieve the autoinhibitory effect of talin-R (1654-2344aa; black region) on F3 domain, promoting talin binding to β CT. The secondary integrin binding site is in orange.

Fig 3. Regions on different β integrin CTs to which talin has been reported to bind

Talin binds to the conserved membrane proximal region and the first NPxY/F motif (shown in red) on β CTs, as shown by the regions in brackets. However, the talin binding region may well extend to residues beyond these highlighted regions on the β CT. The second NPxY/F motif is shown in orange.

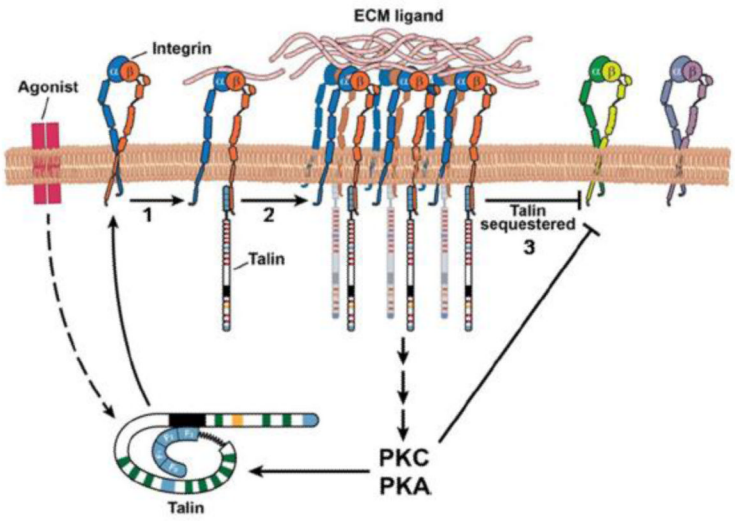

Fig 4. Model of talin mediated integrin crosstalk

(1) Agonists, extracellular or intracellular signals lead to unraveling of the autoinhibited talin by PIP2, RIAM, Rap1 or PKC. (2) Unmasked talin-F3 binds to and activates β integrin CT. (3) Engagement of multivalent ECM ligands via clustered and activated integrins can lead to sequestration of talin, thereby inhibiting other integrin species. Concomitant signaling via PKC or PKA may also contribute to transdominant inhibition of integrins or perpetuation of integrin activation via talin.

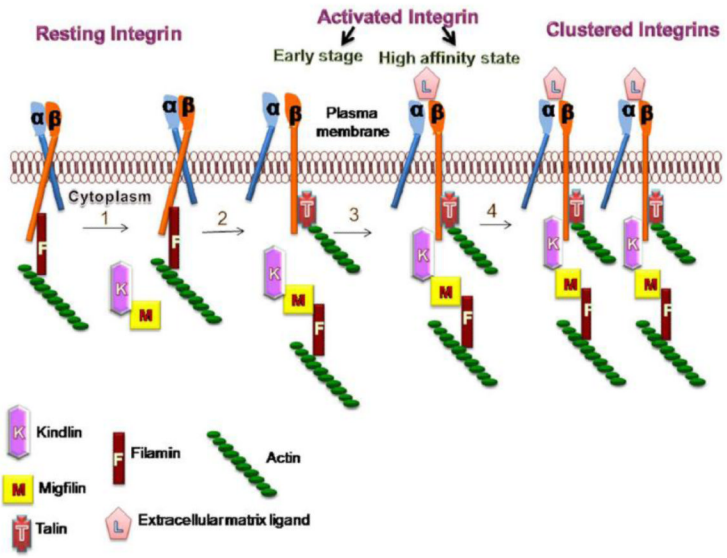

Fig 5. Crosstalk between talin and kindlin in displacement model of integrin activation

(1) In the first step, filamin bound to resting integrins can be displaced from β CT by migfilin, possibly recruited by kindlins to the vicinity of integrin tails. (2) This makes integrin β CT available for binding by talin at the proximal NPxY/F motif, which unclasps the integrins. 3) Kindlin binding converts the early stage of activated integrin to a high affinity state, allowing ligand binding. 4) Cooperative talin and kindlin binding to β CT may lead to crosstalk between integrins leading to clustered integrins and outside-in-signaling. It is still not clear if migfilin remains associated with kindlin after kindlin-β CT engagement and what are the events that trigger dissociation of migfilin-filamin contacts during this process.

Table 1

Binding partners of talin (talin H is 1-433 aa; talin-R is 482-2541 aa; FERM domain is 86-400aa, comprising of F1, F2 and F3 domains; IBS-Integrin Binding site; ABSActin Binding site; DD-Dimerization Domain; aa-amino acids)

Interacting protein	Binding region on talin (amino acid residues)	References
Actin		
	ABS-1 (102-435)	[92]
	ABS-2 (951-1327)	[61]
	ABS-3 (2300-2541)	[45], [61], [110], [148], [154]
β-integrin		
	IBS-1 (206-435)	[24]
	IBS-2 (1974-2293)	[47], [112], [141], [163], [174]
Talin (full length)	DD (2496-2529)	[45]
Focal adhesion kinase	225-357	[26], [90]
Layilin	280-435	[15], [170]
PIPkinaseɣ 90	308-400	[6], [34], [35]
PIPkinaseɣ 661	150-480	[98]
Phospholipids (acidic)	385-406	[37], [51], [124]
RIAM	655-786 (R2)	[55]
	787-911 (R3)	
	1461-1580 (R8)	
	1974-2140 (R11)	
Smurf1	393-433	[64]
Synemin	1327-1948	[158]
	1359-1659	[46]
Talin F3	1655-1822	[49]
Vinculin	Helices 4,8,9,11,12,27,33,36 ,46,50, 58	[48], [55]