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RESEARCH HIGHLIGHT

# Pull and push: Talin activation for integrin signaling

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**The inside-out signaling of integrins regulates the ligand-binding affinity of the cell surface receptors in response to changes in the environment for cell survival. The specific binding to the cytoplasmic tail of integrin's β subunit by the intracellular protein talin is the key step of inside-out signaling. A "pull-push" mechanism has been proposed to explain how the PIP2-enriched membrane disrupts the dual auto-inhibition of the N-terminal talin-FERM domain by the C-terminal talin-rod domain such that activated talin-FERM can reach the β-tail for integrin activation.**

A living cell is constantly interacting with its environment to strive, and cell surface receptors play a vital role in mediating these interactions. Integrins are the major metazoan receptors. They are α/β heterodimeric transmembrane molecules. Each of the subunits consists of very large extracellular domains (about 1 000 residues for α and 700 for β subunits, respectively), a single transmembrane segment, and a short cytoplasmic tail. By regulating cell-extracellular matrix contact, cell-cell adhesion and cell-pathogen interaction, integrins take part in a wide-range of biological processes, including development, angiogenesis, immune response, cancer and hemostasis, *etc*. The name of integrin inherently means to integrate the extracellular and intracellular environments. There comes the most important aspect of their function: the bidirectional signaling across the plasma membrane. On one hand, ligand binding to the integrin extracellular domains is transduced to the cytoplasm in the classical "outside-in" direction to regulate intracellular activities. On the other hand, integrins are often expressed on the cell surface in a default low-affinity ligand-binding conformation. When cells become activated, for example by cytokine, integrins are rapidly activated in response to cellular stimulation within the cytoplasm. They undergo large conformational changes, resulting in a dramatic affinity increase for ligand binding. This process is commonly termed "inside-out" signaling. This regulation of integrin activity is essential because inappropriate integrin activation in blood platelets, for instance, results in thrombosis [1-3].

While the α subunit of integrins seems to be more responsible for the extracellular ligand binding specificity in the outside-in event, the short cytoplasmic tail of the β subunit is the region interacting with regulatory proteins in the inside-out process [1]. When integrins are in the resting state, the α and β cytoplasmic tails are in close proximity, which constrains the integrin in a low-affinity conformation. Dissociation of the two tails by signals within the cell subsequently triggers conformational changes, activating integrins [2, 3]. A 2 541-residue long cytoplasmic protein, talin, is the key regulator of integrin affinity. Talins bind to the integrin β-tail, causing the separation of the two tails of integrin subunits, relieving the constraint, and activating integrin (Figure 1A). Talin is composed of an N-terminal globular head, an extended rod of largely helical bundles, and followed by an actin-binding motif. The integrins' β-tail engagement of talin is exerted by its FERM (Four-point-one-protein/Ezrin/Radixin/Moesin) domain within the talin head, whereas the rod domain has multiple binding sites for the F actin-binding protein vinculin. Therefore, talin also plays a key adaptor role by linking the extracellular matrix to the cytoskeleton. The talin-FERM domain (residues 86-400) contains F1, F2 and F3 subdomains [4-6]. The F3 subdomain resembles a phosphotyrosine-binding (PTB) domain and is responsible for specifically recognizing the β-tail. The binding of the F3 domain to the membrane proximal β-tail is fundamental for integrin activation by dissociating the two integrin tails [7-9]. The crystal structure of the F2/F3 domains of talin-FERM in complex with the β-tail of β3 integrin has defined the prototype of how the FERM domain recognizes the NPxY (x represents any amino acid residue) motif conserved on the β-tail across the integrin family, offering solid structural evidence [10]. In

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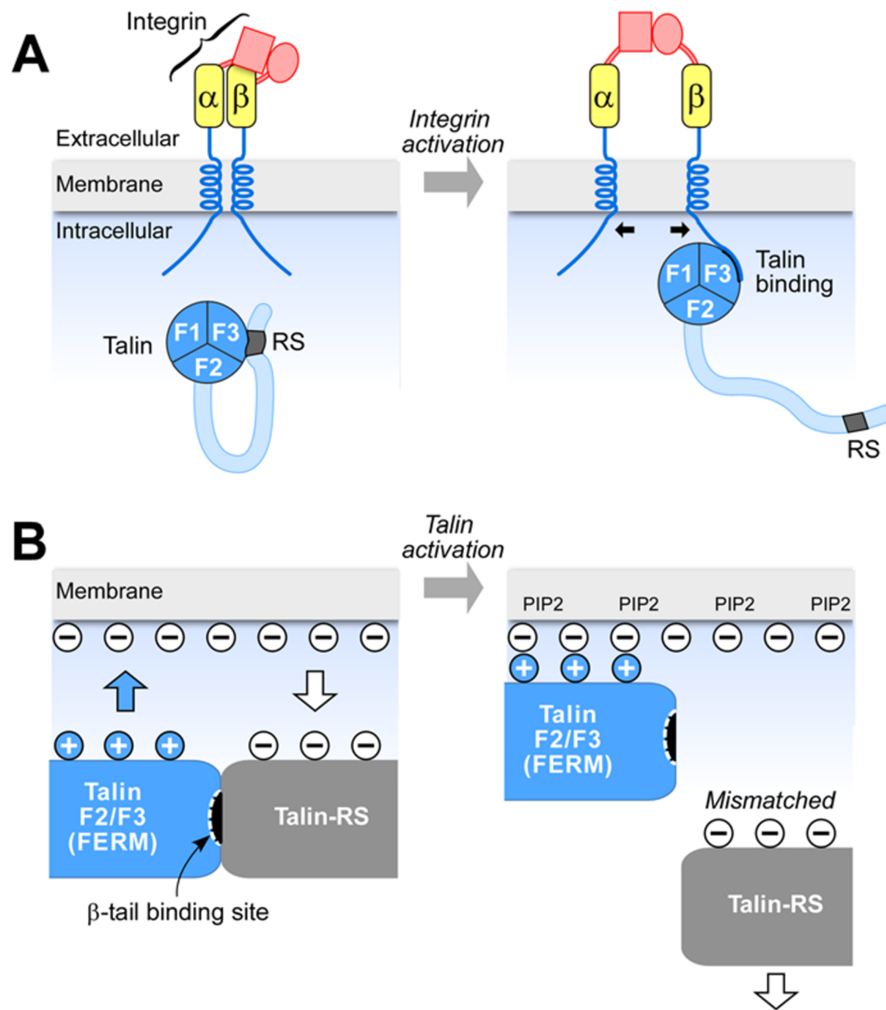


**Figure 1 (A)** Integrin activation. Left panel: both integrin and talin are in an inactivated state. The tails of two integrin subunits are close to each other. The extracellular domains of integrin are in low-affinity conformation for ligand. The talin-RS domain of the inactive talin binds to talin-F3 domain, preventing the F3 domain from accessing the β-tail of integrin. Right panel: The RS domain of talin is relieved from talin-F3 domain. The F3 domain binds to β-tail, causing dissociation of the two tails from each other. This leads to integrin activation. **(B)** Pull-push mechanism of talin activation. Left panel: The talin-RS binds to the talin-F3 domain, blocking the β-tail-binding site of the F3 domain. At the same time, the negatively charged surface of talin-RS repels the talin-F2F3/talin-RS unit away from membrane. This gives a dual inhibition of talin activity. Right panel: PIP2-enriched membrane offers much stronger binding to pull the talin-F2F3 domains to membrane. At the same time, the talin-RS is pushed away, causing the mismatch of talin-RS from talin-F2F3. The β-tail-binding region of F3 becomes available for interaction.

addition, the environment of the phospholipid bilayer membrane modulates the talin-FERM/β-tail interaction. The interaction between the talin-FERM domain and plasma membrane promotes talin-FERM/β-tail binding [11].

Due to the fact that integrin activation has to be strictly controlled, the activity of talin itself is also tightly regulated. In the resting state, a free, intact talin is in an auto-inhibited ‘closed’ conformation [5]. NMR studies have revealed an auto-inhibitory interaction between the FERM domain and the large C-terminal rod domain (residues 482-2 541, termed talin-R). Talin-R specifically masks the β-tail-binding region of talin-FERM (Figure 1A, left panel). This inhibition can be disrupted by talin activator, phosphotidylinositol 4,5-bisphosphate (PIP2) [12]. A paper recently published in *Cell Research* by Song *et al*. [13], employs a combination of X-ray crystallography and NMR to present a compelling molecular mechanism of talin auto-inhibition, and provides insight into a new type of membrane-dependent talin activation .

Song *et al*. [13] determined the crystal structure of a complex between

talin-F2F3 (residues 206-405) and talin-RS (residues 1 654-1 848), a fragment of the C-terminal talin-R. This structure unexpectedly reveals a dual inhibitory topology for talin. First, talin-RS binds to the F3 domain with no apparent contacts with F2. The extensive binding interface on the F3 domain overlaps with the F3's β-tail-binding region, which is a convincing result of the self-masking of the integrin-binding site on the F3 domain by talin-R (Figure 1B, left panel). Moreover, the F2F3/talin-RS complex presents a unique electrostatic surface. In the complex, F2F3 shows a positively charged face that presumably orients toward the negatively charged cell membrane, facilitating β-tail interaction by F3. On the same face, talin-RS exposes a substantially negatively charged surface, which disfavors the complex docking on the membrane (Figure 1B, left panel). Therefore, the auto-inhibitory complex of talin-F2F3/talin-RS sterically masks integrin β-tail binding to F3, and at the same time electrostatically prevents talin from targeting the membrane to access the integrin β-tail.

This crystallographic observation led the authors to explore an extremely intriguing question: how could talin be activated by PIP2 but not other lipids that are also found on the inner surface of the plasma membrane, such as POPS (1-palmitoyl-2-oleoyl-*sn*-glycerol-3-[phospho-L-tidylserine]) and POPC (1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphochoine)? Using NMR, they discovered that PIP2 exhibits the strongest binding capability compared to POPS and POPC. Furthermore, they identified two PIP2-binding sites on talin, first with higher affinity anchoring F2 to PIP2 and then bringing the second site on F3 to PIP2. Based on these experimental data, the authors propose a "pull-push" model to explain that the membrane enriched with PIP2 can activate talin. In accordance with this model, when the membrane is locally enriched with PIP2, the binding affinity of PIP2 to positively charged talin-F2F3 is so strong that the repelling force from negatively charged talin-RS on the same face can no longer prevent the talin-F2F3 from docking on the membrane to access integrin β-tail (the "pull"). Instead, the talin-F2F3/talin-RS interface may be torn to mismatch by the repelling force from the membrane on talin-RS (the "push") (Figure B, right panel). It is noteworthy that according to crystallographic analysis, the talin-F2F3/talin-RS interface is largely hydrophilic, and hence should not be too energetically favorable to be disrupted. The authors performed further meticulous NMR experiments to compare the effects of PIP2 as opposed to POPS and POPC, and of the wild-type talin-F2F3 as opposed to mutant talin-F2F3 that loses PIP2 binding ability to talin-F2. In conclusion, they have combined structural, biochemical and functional evidence to support their "pull-push" talin activation mechanism [13].

The "pull-push" model explains how the PIP2-enriched membrane is capable of attracting talin-F2F3 and relieves the dual inhibition by talin-RS, allowing the activated talin to reach the β-tail for integrin activation. In a broad sense, the authors may provide a new paradigm of a mechanism by which the dynamic surface chemical environment changes of cellular membrane regulate signal transduction through membrane. This investigation bears biological significance beyond the activation of talin/integrin *per se*.

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