Talin at a glance

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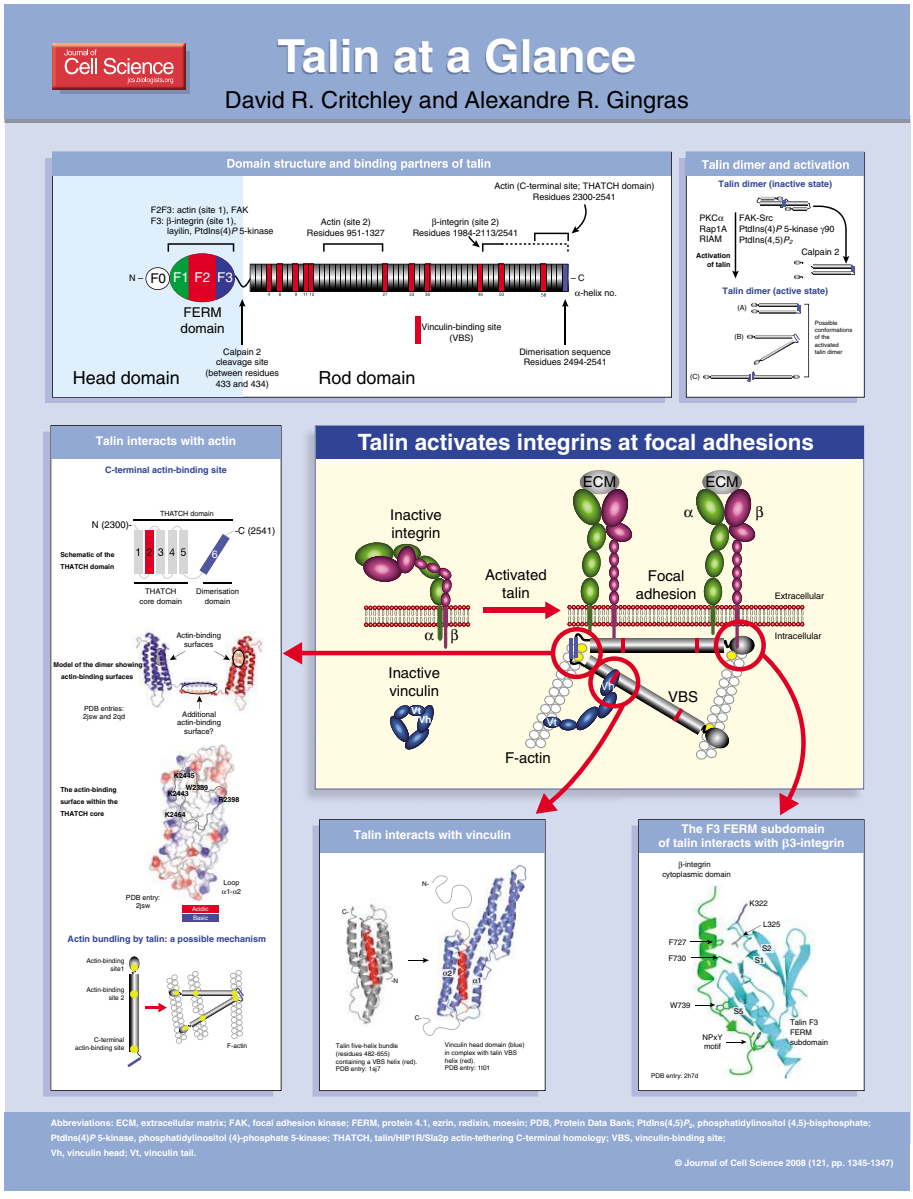
Cell migration, growth and differentiation all require the assembly and disassembly of cellular junctions with the extracellular matrix (ECM). These large multiprotein complexes assemble around the integrin family of cell adhesion molecules (transmembrane αβ heterodimers) that are typically linked to the actin cytoskeleton, with the exception of integrin α6β4, which is coupled to intermediate filaments. Talin is one of several proteins that link the cytoplasmic domains of integrin β subunits to actin filaments (others include α-actinin, filamin, tensin, integrin-linked kinase, melusin and skelemin) (Critchley, 2004; Nayal et al., 2004). Moreover, binding of talin to β-integrin cytoplasmic domains triggers a conformational change in the αβ-integrin extracellular domain that increases its affinity for ECM proteins (Calderwood, 2004) and promotes the assembly of focal adhesions (FAs), cell-ECM junctions that are formed by cells in culture. However, studies in flies expressing mutant integrin alleles show that there is not a simple 1:1 relationship between integrins and talin in cell-ECM junctions, and there must be additional mechanisms that recruit talin to these sites (Devenport et al., 2007).



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Talin: domains and binding partners

Talin (~270 kDa) comprises a globular N-terminal head region and a flexible rod domain, which can be dissociated by the protease calpain 2. The head contains a FERM (protein 4.1, ezrin, radixin, moesin) domain (subdivided into F1, F2 and F3 subdomains), which has binding sites for the cytoplasmic domains of β-integrins and layilin (a hyaluronan receptor) as well as for filamentous actin (F-actin). The head also binds to two signalling molecules that regulate the dynamics of FA, namely PIPK1γ90 [a splice variant of phosphatidylinositol (4)-phosphate 5-kinase type Iγ] and focal adhesion kinase (FAK), although it is not clear whether binding to FAK is direct. The talin rod contains an additional integrin-binding site, at least two actin-binding sites and several binding sites for vinculin, which itself has multiple partners. Finally, the C-terminal helices of two talin monomers form an anti-parallel dimer, although the relative position of the two subunits within the dimer is uncertain. Yeast two-hybrid and proteomic screens have identified several additional talin-binding proteins (Zaidel-Bar et al., 2007) and numerous phosphorylation sites (Ratnikov et al., 2005).

Structural studies of the integrin-talin interaction

Binding of the talin head to the cytoplasmic domain of integrin β subunits is thought to activate integrins by disrupting a salt bridge between the α- and β-integrin subunits, leading to separation of their cytoplasmic domains (Campbell and Ginsberg, 2004). Expression of the talin head in CHO cells stably expressing the platelet integrin αIIbβ3 resulted in integrin activation, whereas small hairpin RNA (shRNA)-mediated knockdown of talin in these cells compromised activation. Support for the cytoplasmic-domain-separation model comes from fluorescence resonance energy transfer (FRET) studies using the integrin subunits αL and β2 C-terminally tagged with CFP and YFP, respectively, and expressed in K562 cells: the FRET efficiency between the integrin subunits was significantly decreased by the

expression of the talin head (Kim et al., 2003).

The talin F3 FERM subdomain binds to the more N-terminal of the two NPxY motifs found in β-integrin cytoplasmic domains, and the structure of the F3 subdomain bound to residues ^{739}WDTANNPLYDEA^{750} in β3-integrin has been determined (underlined residues indicate key binding determinants). F3 has a phosphotyrosine-binding (PTB) fold comprising seven β-strands (S1-S7) and a C-terminal α-helix. β3-integrin binds predominantly to a hydrophobic surface on strand S5, and mutations in S5 markedly reduce binding. The β3-integrin ^{744}NPLY^{747} sequence adopts a β-turn, and Y747 projects into an acidic pocket in the talin structure, whereas the equivalent region in PTB domains that recognize phosphotyrosine is strongly basic. Phosphorylation of Y747 of β3-integrin could therefore disrupt the talin-integrin interaction, suggesting one way in which the activation of β3-integrin by talin might be regulated. The talin F3 subdomain also contains a hydrophobic pocket, comprising residues R358, A360 and Y377, that is occupied by W739 in the β3-integrin cytoplasmic domain, and alanine substitution of talin R358 markedly reduces integrin binding (Campbell and Ginsberg, 2004). Interestingly, this same surface in talin F3 binds the WVYSPLHY sequence at the C-terminus of PIPK1γ90 and the WVENEIYY sequence in the layilin cytoplasmic domain.

Several other PTB-domain proteins bind to the same NPxY motif in integrins, yet do not activate these proteins, implying that talin F3 must make additional contacts with the integrin cytoplasmic domain (Wegener et al., 2007). The flexible loop between β-strands S1 and S2 in talin F3 forms a second hydrophobic pocket that allows the docking of two residues (F727 and F730) of the membrane-proximal helix of β3-integrin. Mutation of either F727 or F730 in β3-integrin or the interacting residues in the talin F3 subdomain (notably L325) markedly reduces integrin αIIbβ3 activation by a talin F2-F3 fragment. Other PTB-domain proteins lack the flexible loop and so do not activate integrins. This suggests a model of integrin activation in which the talin F3 subdomain initially binds to the β3-integrin NPxY motif and subsequently engages the membrane-proximal helix. In addition, talin K322 in the large loop between β-strands S1 and S2 might make contact with acidic membrane lipids, further stabilizing the integrin-talin complex (Wegener et al., 2007). Interestingly, the talin F3 subdomain is not sufficient to activate β1-integrins and the N-terminal domains F1 and F0 (the domain predicted to precede F1) are also required (Bouaouina et al., 2008).

Pathways that promote integrin activation by talin

The small GTPase Rap1A plays a key role in integrin activation (Bos, 2005). However, the link between Rap1A and talin has only recently been elucidated, by reconstituting platelet integrin-αIIbβ3 activation in CHO cells (Han et al., 2006). PKCα acts upstream and talin acts downstream of Rap1A, as constitutively active Rap1A(G12V) failed to activate αIIbβ3-integrin in cells expressing low levels of talin. Much of talin exists in an inactive cytosolic pool and the Rap1-interacting adaptor molecule RIAM has been implicated in talin activation because it is sufficient to activate αIIbβ3-integrin in the absence of Rap1A(G12V). Other mechanisms for regulating talin and its association with integrins have also been suggested (Critchley, 2004). Integrin signalling via FAK and Src promotes binding of PIPK1γ90 to the talin F3 subdomain, the activation of PIPK1γ90 and translocation of the PIPK1γ90-talin complex to the plasma membrane. This raises the possibility that talin is activated by local synthesis of phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2], and PtdIns(4,5)P_2 has been reported to increase the binding of integrins to talin. Calpain 2 also increases the binding of talin to integrins in vitro, but cleavage of talin appears not to be required for integrin activation in cells (Franco et al., 2004).

Interactions of the talin rod with vinculin and actin

The talin rod domain comprises 62 amphipathic α-helices, which form a series of helical bundles. Studies using peptide arrays show that around ten of these helices can bind to a hydrophobic pocket in the vinculin head. Predictably, the key vinculin-binding residues in talin are also hydrophobic, but these are usually buried within the helical bundles (Gingras et al., 2005). Indeed, talin binds to vinculin with low affinity, and it is unclear how vinculin accesses the vinculin-binding sites (VBSs) in talin. It is possible that force exerted on the integrin-talin-actin complex by actomyosin contraction exposes the VBSs in talin and enables vinculin to bind to and stabilize the complex, possibly by crosslinking talin to F-actin (Ziegler et al., 2006). Interestingly, vinculin and not talin has recently been shown to provide the key link between FAs and the actin cytoskeleton (Humphries et al., 2007).

Binding of talin to F-actin is intimately linked to talin dimerisation. The actin-binding site (ABS) in the C-terminal region of the talin rod comprises a five-helix bundle and a C-terminal helix that is required for dimer formation. Together, these constitute a talin/HIP1R/Sla2p actin-tethering C-terminal homology (THATCH) domain (Gingras et al., 2008). The ABS maps to a hydrophobic surface on helices 3 and 4 that is flanked by basic residues. Helix 1, which packs against the opposite side of the bundle, negatively regulates actin binding by an unknown mechanism. Interestingly, actin only binds to the THATCH dimer, and the dimerisation domain itself appears to contribute to binding. Electron microscopy shows that the THATCH dimer binds to three actin monomers along the long pitch of the same actin filament, and does not crosslink F-actin. Presumably, the actin-bundling activity of talin is explained by the presence of at least two other ABSs in talin.

Analysis of talin function in vertebrates

In vertebrates there are two talin genes that encode closely related proteins (74% identity), and recent studies with isoform-specific antibodies indicate that talin 2 is the predominant isoform in brain and striated muscle, whereas talin 1 is the only form that is expressed in platelets (Senetar et al., 2007). Disruption of the *Tln1* gene in mice is embryonic lethal at 8.5-9.5 days post coitum (dpc) owing to arrested gastrulation (Monkley et al., 2000), whereas mice that are homozygous for a *Tln2* gene-trap allele are viable and fertile (Chen and Lo, 2005). More recently, a conditional *Tln1* allele has been used to study the role of talin 1 in platelets. These studies demonstrate for the first time that talin 1 is required for the activation of platelet integrins α2β1 and αIIbβ3 in vivo, and the mice exhibit spontaneous bleeding (Petrich et al., 2007; Nieswandt et al., 2007).

At the cellular level, disruption of both *Tln1* alleles in mouse embryonic stem (ES)

cells confirms previous studies that talin1 is required for FA assembly (Critchley, 2004). Optical trap experiments using *Tln1*$^{-/-}$ fibroblasts and fibronectin-coated (type-III repeats 7-10) silica beads show that talin 1 is required for a characteristic 2pN slip bond or clutch between fibronectin-integrin complexes and the actin cytoskeleton, and it is also required to strengthen the integrin-cytoskeleton linkage (Jiang et al., 2003). In T cells, talin 1 is required for T-cell receptor (TCR)-mediated regulation of the affinity, clustering and polarisation of integrin LFA-1 ($a_L\beta2$), and small interfering RNA (siRNA)-mediated talin 1 knockdown impaired TCR-induced adhesion to and migration on ICAM1, and the conjugation of T cells to antigen-presenting cells (Smith et al., 2005; Simonson et al., 2006). Talin (but not vinculin) is also required for both F-actin and integrin accumulation at the immunological synapse (Nolz et al., 2007), and for the chemokine-induced increase in affinity of integrin VLA-4 ($\alpha4\beta1$) for VCAM1, which is important in lymphocyte-endothelial cell adhesion (Manevich et al., 2007). In macrophages, talin 1 is required for phagocytosis that is mediated by $\alpha M\beta2$ integrin (complement receptor 3) (Lim et al., 2007), whereas a talin-PIPK1γ90 complex has been shown to play a novel role in clathrin-mediated endocytosis in the neuronal synapse (Morgan et al., 2004).

Conclusion

Talin has been shown to play a key role in a wide variety of integrin-mediated events both in vitro and in vivo. However, the signalling pathways that regulate talin activity are only just beginning to emerge, and the structural basis for activation of the many ligand-binding sites in talin is not yet fully understood. In addition, the relative roles of talin 1 and talin 2 remain unexplored, and the availability of mice and cells carrying a conditional *Tln1* allele should help in this regard.

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