Mechanisms that regulate adaptor binding to β-integrin cytoplasmic tails

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Summary

Cells recognize and respond to their extracellular environment through transmembrane receptors such as integrins, which physically connect the extracellular matrix to the cytoskeleton. Integrins provide the basis for the assembly of intracellular signaling platforms that link to the cytoskeleton and influence nearly every aspect of cell physiology; however, integrins possess no enzymatic or actin-binding activity of their own and thus rely on adaptor molecules, which bind to the short cytoplasmic tails of integrins, to mediate and regulate these functions. Many adaptors compete for relatively few binding sites on integrin tails, so regulatory mechanisms have evolved to reversibly control the spatial and temporal binding of specific adaptors. This Commentary discusses the adaptor proteins that bind directly to the tails of β integrins and, using talin, tensin, filamin, 14-3-3 and integrin-linked kinase (ILK) as examples, describes the ways in which their binding is regulated.

Key words: Integrins, Protein modification, Signaling, Adaptors

Introduction

Integrins are heterodimeric transmembrane receptors. There are 18 integrin α-subunits and eight β-subunits, which can combine into 24 different heterodimers that recognize overlapping but distinct sets of extracellular ligands (Humphries et al., 2006; Hynes, 2002). The major ligands for integrins are the proteins of the extracellular matrix (ECM), which include fibronectin, collagen, laminins and others. Other ligands include intercellular adhesion molecules (ICAMs) and vascular cell-adhesion molecules (VCAMs), which mediate cell-cell interactions between leukocytes and endothelial cells (Ley et al., 2007; Petri et al., 2008); milk fat globule-EGF factor 8 (MFGE8) and complement factor iC3b, which facilitate phagocytosis of apoptotic cells and pathogens, respectively (Dupuy and Caron, 2008); and the latency-associated peptide of transforming growth factor β (TGFβ), which regulates TGFβ signaling (Sheppard, 2005).

The binding of integrins to the ECM induces them to cluster into focal complexes, which can mature into larger focal adhesions and finally, in the case of the binding of α5β1 integrin to fibronectin, into fibrillar adhesions (Geiger et al., 2001). These adhesions connect the ECM to the actin cytoskeleton to allow the cell to generate force and perform work on its environment (Li et al., 2005a). Additionally, the phosphorylation of signaling proteins such as MAP kinases and Akt [protein kinase B (PKB)] is upregulated following adhesion formation, indicating that integrin-ECM binding has a crucial role in the regulation of signal transduction (Legate et al., 2006; Yee et al., 2008). Notably, the cytoplasmic tails of β integrins are rather short, containing between 40 and 60 amino acids (Fig. 1), and possess no enzymatic or actin-binding activity of their own. Instead, various adaptor proteins bind to specific sites on α- and β-integrin tails to mediate integrin activation and clustering, and to serve as nucleation points for the assembly of larger signaling and structural scaffolds. Although α integrins bind to several well-characterized adaptors [for example, α4 integrin binds to paxillin, and α1 integrin to T-cell protein tyrosine phosphatase (TCPTP) (Liu et al., 1999; Mattila et al., 2005)], the study of α-integrin-tail-binding proteins is only now starting to receive proper attention. The study of β-integrin-tail-binding proteins is significantly more advanced, and will be the focus of this Commentary.

Recently, a search of the literature, in combination with database mining, has defined the integrin 'adhesome' – a list of the proteins that are currently known to assemble into focal adhesions, along with the kinases, phosphatases and proteases that regulate their function (Zaidel-Bar et al., 2007). At the time that the work was published, 90 core components of the adhesome were identified, of which 19 bound directly to β-integrin tails and were therefore potential adaptor proteins. A more focused literature search has identified 42 potential β-integrin-tail-binding adaptor proteins (Table 1), which suggests that the true size of the adhesome was underestimated in the previous survey.

Integrin adaptor proteins fall into three broad categories – adaptors that have a mainly structural function, adaptors that fulfill a scaffolding function by providing binding sites for additional focal-adhesion proteins, and adaptors that have catalytic activity (Table 1). Structural adaptors, including talin, filamin and tensin, bind to F-actin and therefore couple integrins to the cytoskeleton directly. Catalytic adaptors, such as focal adhesion kinase (FAK), integrin-linked kinase (ILK), Src and protein phosphatase 2A (PP2A), facilitate the propagation of signal-transduction pathways from adhesion sites.

By definition, all adaptors bind to other protein partners in addition to integrins. Although there is a clear distinction between the three classes of adaptors, there is significant functional crossover because of the other interactions of these proteins. For example, talin and paxillin can both recruit FAK to focal adhesions (Chen et al., 1995; Hildebrand et al., 1995; Tachibana et al., 1995); FAK can also interface with the actin cytoskeleton through an interaction with the actin-regulatory Arp2-Arp3 (Arp2/3) complex (Serrels et al., 2007). Also, ILK can connect integrins to F-actin by binding to the actin-binding proteins α- and β-parvin (Nikolopoulos and Turner, 2000; Nikolopoulos and Turner, 2001; Yamaji et al., 2004). Furthermore, it was shown in *Drosophila* that adaptor complexes that are centered around talin and ILK can be crosslinked through

Table 1. Adaptor proteins that bind to β-integrin cytoplasmic tails

Adaptor protein	Integrin to which adaptor binds	Reference
Structural adaptors		
α-actinin	β1, β2, β3	(Otey et al., 1993; Pavalko and LaRoche, 1993)
BP180	β4	(Koster et al., 2003; Schaapveld et al., 1998)
Filamin	β1, β2, β3, β7	(Calderwood et al., 2001; Kiema et al., 2006; Loo et al., 1998; Pfaff et al., 1998; Sharma et al., 1995; Travis et al., 2004; Zent et al., 2000)
Myosin	β1, β3, β5	(Jenkins et al., 1998; Sajid et al., 2000; Zhang et al., 2004)
Plectin	β4	(Geerts et al., 1999)
Skelemin	β1, β3	(Reddy et al., 1998)
Talin	β1, β2, β3, β5, β7	(Calderwood et al., 2003; Calderwood et al., 1999; Patil et al., 1999; Pfaff et al., 1998; Sampath et al., 1998)
Tensin	β1, β3, β5, β7	(Calderwood et al., 2003; McCleverty et al., 2007)
Scaffolding adaptors		
14-3-3	β1, β2, β3	(Fagerholm et al., 2005; Han et al., 2001)
β3 endonexin	β3	(Eigenthaler et al., 1997; Shattil et al., 1995)
CD98	β1, β3	(Zent et al., 2000)
Dab1	β1, β2, β3, β5, β7	(Calderwood et al., 2003)
Dab2	β3, β5	(Calderwood et al., 2003)
Dok1	β2, β3, β5, β7	(Calderwood et al., 2003)
Fhl2	β1, β2, β3, β6	(Wixler et al., 2000)
Fhl3	β1	(Samson et al., 2004)
Grb2	β3	(Blystone et al., 1996; Law et al., 1996)
IAP	β3	(Brown et al., 1990)
JAB1	β2	(Bianchi et al., 2000)
Kindlin 2	β1, β3	(Ma et al., 2008; Montanez et al., 2008)
Kindlin 3	β1, β3	(Moser et al., 2008)
Melusin	β1	(Brancaccio et al., 1999)
Numb	β3, β5	(Calderwood et al., 2003)
Paxillin	β1, β3	(Chen et al., 2000; Schaller et al., 1995)
Rack1	β1, β2, β5	(Liliental and Chang, 1998)
Shc	β3, β4	(Dans et al., 2001; Law et al., 1996)
TAP20	β5	(Tang et al., 1999)
WAIT1	β7	(Rietzler et al., 1998)
Catalytic adaptors		
Src	β3	(Arias-Salgado et al., 2003; Arias-Salgado et al., 2005)
Yes	β1, β2, β3	(Arias-Salgado et al., 2005)
Cytohesin 1	β2	(Kolanus et al., 1996)
Eps8	β1, β3, β5	(Calderwood et al., 2003)
ERK2	β6	(Ahmed et al., 2002)
FAK	β1, β2, β3, β5	(Chen et al., 2000; Eliceiri et al., 2002; Schaller et al., 1995)
Fyn	β3	(Arias-Salgado et al., 2005)
ILK	β1, β3	(Hannigan et al., 1996; Pasquet et al., 2002)
Lyn	β1, β2, β3	(Arias-Salgado et al., 2005)
PKD1	β1, β3	(Medeiros et al., 2005; Woods et al., 2004)
PP2A	β1	(Kim et al., 2004)
Shp2	β4	(Bertotti et al., 2006)
Other adaptors		
ICAP1α	β1	(Chang et al., 1997; Zhang and Hemler, 1999)
MIBP	β1	(Li et al., 1999)

an interaction with the NHL-domain protein Wech (Löer et al., 2008). The interconnectedness of protein-protein interactions that are mediated by adaptors is key to the assembly of the complex structural and signaling platform of the focal adhesion.

Fluorescence ratio imaging experiments have shown that adaptor molecules assemble into focal-adhesion sites in a sequential manner (Laukaitis et al., 2001; Zaidel-Bar et al., 2003; Zaidel-Bar et al., 2004; Zamir et al., 1999), so that the maturation of adhesion sites can in part be characterized by the specific composition of adaptor molecules that are incorporated within them. The adaptor integrin-cytoplasmic-domain-associated protein 1α (ICAP1α) localizes with β1 integrins before the formation of focal complexes and regulates adhesion assembly, perhaps by controlling the binding of talin to β1-integrin tails (Bouvard et al., 2003; Fournier et al., 2002; Millon-Frémillon et al., 2008). As focal complexes mature into focal adhesions they lose ICAP1α (Fournier et al., 2002) and the actin-binding protein vinculin (Katz et al., 2000; Zamir et al., 1999), and acquire the actin-regulatory protein zyxin. In some cell types, the integrin adaptor tensin only becomes recruited when focal adhesions progress to fibrillar adhesions (Papp et al., 2007; Zaidel-Bar et al., 2003; Zaidel-Bar et al., 2004; Zamir et al., 1999). The fact that tensin is only found at certain stages of adhesion-structure formation indicates that its binding to integrin tails is tightly regulated.

This Commentary describes how adaptor proteins bind to β-integrin tails, and discusses the strategies by which this binding is regulated. We use specific examples – the binding of talin vs tensin, the binding of 14-3-3 vs filamin, and the co-adaptor-mediated binding of ILK – to demonstrate how the regulated binding of adaptors occurs, and how it can alter the functional properties of adhesion complexes.

Adaptors bind to 'hot spots' on β-integrin tails

Many proteins have been shown to bind to β-integrin cytoplasmic tails (Table 1). The short length of these tails, along with the abundance of β-integrin-binding partners, indicates that there must be a significant overlap between adaptor binding sites. The β3-integrin tail is most often used as the model sequence for mapping precisely the interactions between integrins and adaptors, and the known β3-integrin-binding sites for several adaptors are shown in Fig. 2. Although good structural data exist for the binding interactions between β3-integrin tails and talin (García-Alvarez et al., 2003; Wegener et al., 2007), and nuclear magnetic resonance (NMR) chemical-shift analysis has indicated the sites at which talin and docking protein 1 (Dok1) interact with the β3-integrin tail (Oxley et al., 2008; Ulmer et al., 2003), most binding sites have been deduced from assays using mutated and truncated synthetic integrin-tail peptides. Although the entire length of the tail is used to bind to adaptors, there are three distinct 'hot-spots' that are preferred binding sites for most adaptors and are conserved among β-integrin tails and across species (Fig. 1).

Homo sapiens

β1A	751	WKLLMII **HDRR**EFAKFEKEKMNAKWDTGE**NPIY**KSAVTTVV--------**NPKY**EGK
β1D	751	WKLLMII **HDRR**EFAKFEKEKMNAKWDTGE**NPIY**KSPINNFK--------**NPNY**GRKAGL
β2	723	WKALIHL **SDLR**EYRRFEKEKLKSQWNND-**NPLF**KSATTTVM--------**NPKF**AES
β3	741	WKLLITI **HDRK**EFAKFEEERARAKWDTAN**NPLY**KEATSTFT--------**NITY**RGT
β5	742	WKLLVTI **HDRR**EFAKFQSERSRARYEMAS**NPLY**RKPISTHTVDFTFNKF**NKSY**NGTVD
β6	730	WKLLVSF **HDRK**EVAKFEAERSKAKWQTGT**NPLY**RGSTSTFK--------**NVTY**KHREKQKVDLSTDC
β7	746	YRLSVEI **YDRR**EYSRFEKEQQQLNWKQDS**NPLY**KSAITTTI--------**NPRF**QEADSPTL

Gallus gallus

β1	756	WKLLMII **HDRR**EFAKFEKEKMNAKWDTGE**NPIY**KSAVTTVV--------**NPKY**EGK
β2	725	WRLLTEI **FDRR**EYRRFEKEKSKAKWNEAD**NPLF**KSATTTVM--------**NPRF**DGQ
β3	733	WKLLITI **HDRR**EFARFEEEKARAKWDTGN**NPLY**KEATSTFT--------**NITY**RGNM
β5	755	WKLLVTI **HDRR**EFDRFQSERTRARYEMAS**NPLY**RKPISTHNVEFTFNKL**NKSY**NGTVD

Ictalurus punctatus

β1	760	WKLLMII **HDRR**EFAKFEKEKMNAKWDAGE**NPIY**KSAVTTVV--------**NPKY**EGK
β2	724	IKALFYF **KDLK**EWKKFEKEAQRRQWAKGE**NPLF**QNATTTVA--------**NPAF**TGDS

Danio rerio

β1-1	752	WKLLMII **HDRR**EFAKFEKEKMNAKWDTGE**NPIY**KSAVTTVI--------**NPKY**EGK
β1-2	748	WKLLMII **HDRR**EFAKFEKEKMNAKWDAGE**NPIY**KSAVTTVV--------**NPKY**EGK
β1-3	740	WKLLMVI **HDRR**EFDKFEKEKNNAKWDTGE**NPIY**KSAVTTVV--------**NPKY**EGK
β3.1	742	WKLLITI **HDRR**EFAKFEEERARAKWETGH**NPLY**KGATSTFT--------**NITY**RGKD
β3.2	738	WKLLITI **HDRR**EFAKFEEEKAKAKWEAAN**NPLY**KGATTTFQ--------**NVAY**RGS
β5	768	WKLVITV **HDRR**EFARFQSARSRARYEMAS**NPVY**KRSVPMETDFDMHGIKSLNGGVH

Xenopus laevis

β1	751	WKLLMII **HDRR**EFAKFEKEKMNAKWDTGE**NPIY**KSAVTTVV--------**NPKY**EGK
β3	739	WKLLITI **HDRR**EFAKFEEERAKAKWDTAH**NPLY**KGATSTFT--------**NITY**RGNTE
β5	746	WKLLVTI **HDRR**EFSRFQSDRSRARYEMAS**NPLY**RPAVSTHNVDEMYNMLSKSYNGTT

Drosophila melanogaster

βPS	799	WKLLTTI **HDRR**EFARFEKERMNAKWDTGE**NPIY**KQATSTFK--------**NPMY**AGK
βv	747	WYIRA-- **KDAR**EYAKFEEDQKNSVRQ--E**NPIY**RDPVGRYEVPKALSVKYDENNPFAS

Caenorhabditis elegans

βPat3	760	WKLLTVL **HDRS**EYATFNNERLMAKWDTNE**NPIY**KQATTTFK--------**NPVY**AGKAN

Strongylocentrotus purpuratus

β-C	759	WRLLTYI **HDRR**EFQNFEKERANATWEGGE**NPIY**KPSTSVFK--------**NPTY**QK
β-G	737	WRLLVYV **QDSR**EFASFEKERAGTHWGQNE**NPIY**KPSTSTFK--------**NPTY**QK
β-L	756	WRLYTYV **QDKR**EYAQWENDCKKAQWDQSD**NPIY**KSSTTTFK--------**NPTY**GK

Ophlitaspongia tenuis

βPo1	792	LKGLLLL WDVVEVRKFEREIKNAKYTKNE**NPLY**RSATKDYQ--------**NPLY**GK

Acropora millepora

βCn1	743	IKGLFTM **VDRI**EYKQFERERMHSKWTREK**NPLY**QAAKTTFE--------**NPTY**AGGRQ
β2	724	WKLLATI **QDRR**EFAKFEKDRQNAQWDTGE**NPIF**KQPTTTFQ--------**NPTY**GGK

Fig. 1. Alignment of β-integrin-tail sequences. β-integrin tails from several species were aligned manually. The divergent sequences of human β4 integrin and β8 integrin were not included. Residues that are normally buried in the membrane but that might become available for adaptor binding upon integrin activation are depicted in green. The conserved NxxY motifs and HDR[R/K] motif are highlighted in bold. Residues that might be phosphorylated to regulate adaptor binding are highlighted in red. Residues are numbered according to the National Center for Biotechnology Information (NCBI) sequence.

The HDRK motif

The first hot-spot is a membrane-proximal HDRK motif (HDRR in β1 and β5 integrins) that, in β3 integrin, binds to the Src-family kinase Fyn (Reddy et al., 2008), FAK and paxillin (Schaller et al., 1995), and skelemin (Reddy et al., 1998). Whereas an early report showed that the binding of FAK to β1-integrin-tail peptides in vitro is not dependent on paxillin (Schaller et al., 1995), it is now thought that the association of FAK with integrins in vivo is indirect, and most probably occurs through an interaction with paxillin (Hayashi et al., 2002; Liu et al., 2002). When integrins are inactive, the HDRK sequence binds to α-integrin tails through the formation of a salt bridge between the aspartate (D) residue in HDRK and the arginine (R) residue of the α-integrin-tail GFFKR motif (Hughes et al., 1996; Vinogradova et al., 2002); this interaction is important to stabilize the inactive state of the integrin heterodimer in vitro (Hughes et al., 1996; Sakai et al., 1998). However, the importance of the salt bridge in vivo varies depending on the integrin involved, because mice that express a β1 integrin that lacks an aspartate in the HDRK motif display a normal phenotype (Czuchra et al., 2006), whereas a mutation in the arginine residue within the GFFKR motif of α4 integrin results in aberrant integrin activation (Imai et al., 2008). The close proximity of the β-subunit HDRK motif to the α-subunit in the inactive integrin suggests that this sequence only becomes available for adaptor binding following separation of the two integrin tails, which is a key event in integrin activation. Alternatively, binding of adaptors might promote tail separation – a recent NMR structure of skelemin demonstrates that it can bind to both β3- and αIIb-integrin tails in solution and partially disrupt their interaction, but whether it has any role in integrin activation in vivo is not known (Deshmukh et al., 2007). In vitro peptide-binding studies have demonstrated that skelemin, paxillin and FAK also bind to additional sequences that are N-terminal to the HDRK sequence (Reddy et al., 1998; Schaller et al., 1995); these sequences are normally buried in the membrane, but might become exposed to the cytosol and accessible to adaptors upon integrin activation (Armulik et al., 1999; Li et al., 2002; Stefansson et al., 2004).

The NPxY and NxxY motifs

The second and third β-integrin hot-spots for adaptor binding are a membrane-proximal NPxY motif and a membrane-distal NxxY

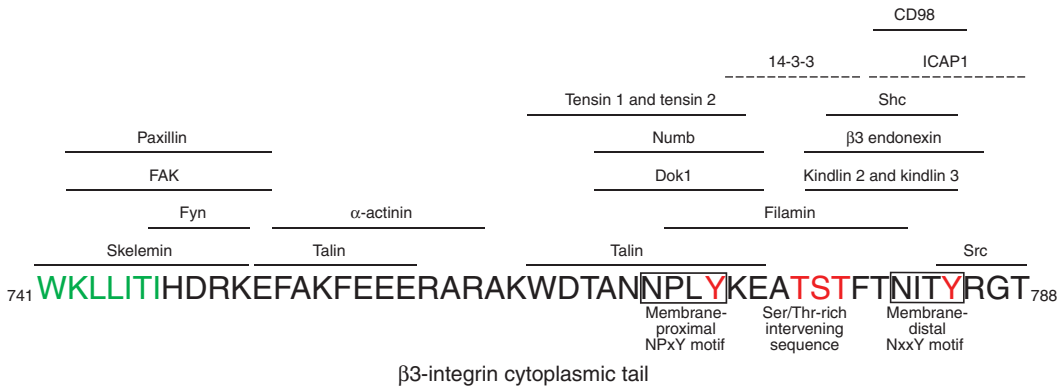

Fig. 2. Adaptor-binding sites along the β3-integrin tail. The mapped positions of adaptors that have been shown to bind to the β3-integrin cytoplasmic tail are indicated by solid lines. Positions of adaptors that have binding sites on other integrins and are discussed in the text, but that have not been shown to bind to β3 integrin, are indicated by broken lines. Residues that are normally buried in the membrane are colored green. Residues that can be phosphorylated by various kinases (see text) are highlighted in red.

motif. Both motifs are recognition sites for phosphotyrosine-binding (PTB) domains (Calderwood et al., 2003), and almost all the adaptors depicted in Fig. 2 that bind to these motifs do so via PTB domains. Numb, Dok1, ICAP1α, tensin and Shc possess isolated PTB domains (Chang et al., 2002; Lo, 2004; Songyang et al., 1995; Verdi et al., 1996; Yamanashi and Baltimore, 1997), whereas the PTB domains of talin, kindlin 1 and kindlin 2 (also known as fermitin family homolog 1 and fermitin family homolog 2, respectively) reside within the larger band 4.1, ezrin, radixin, moesin (FERM) domain (Calderwood et al., 2002; Kloeker et al., 2004; Shi et al., 2007). Only β3 endonexin does not have a described PTB domain.

Although some adaptors bind specifically to a particular integrin, many adaptors – such as those that bind to the membrane-proximal NPxY motif – are more indiscriminate and can bind to several integrin tails. By contrast, adaptors that bind to the membrane-distal NxxY motif are more restricted with regards to the integrins to which they bind (Calderwood et al., 2003). Binding specificity can result from sequence divergence within the NxxY motif. In β3 integrin, the motif has the sequence NITY and, in β1 integrin, the sequence is NPKY (Fig. 1). The NPKY sequence favors a β-turn structure that is important for the recognition of this motif by many PTB domains. For example, ICAP1α binds specifically to β1 integrin, in part through the membrane-distal NPKY sequence (Chang et al., 2002; Zhang and Hemler, 1999), and introducing an NPKY→NAKY mutation abolishes the binding of ICAP1α to β1-integrin tails in a yeast two-hybrid assay. Structural modeling shows that the bend that is induced by the proline (P) residue is necessary to place the tyrosine (Y) residue in the correct position to make hydrophobic contacts with isoleucine 139 (I139) in ICAP1α. Mutation of either this tyrosine in β1 integrin or I139 in ICAP1α abolishes the interaction of the two proteins (Chang et al., 2002). In contrast to PTB-domain-containing proteins, β3 endonexin binds specifically to the NITY sequence of β3 integrin (Eigenthaler et al., 1997). Mutation of the isoleucine residue to proline abrogated the β3-endonexin–β3-integrin interaction in a yeast two-hybrid assay; conversely, mutation of the proline in β1 integrin to isoleucine imparted β3-endonexin-binding activity to the β1-integrin tail (Eigenthaler et al., 1997).

In summary, many different adaptors have been shown to bind to relatively few sites on β-integrin tails. As the composition of integrin-bound adaptors can affect both the activation status of the integrin and downstream signaling pathways, adaptor binding must be tightly regulated. The following sections discuss how this is achieved.

Regulation of adaptor binding to β-integrin tails

As several PTB-domain-containing adaptor proteins bind to the NPxY and NxxY motifs in integrin tails (Fig. 2), mechanisms to control their binding temporally and spatially must exist. One possible mechanism involves the binding of lipids to the PTB domain of adaptors, whereas another involves the phosphorylation of residues of the β-integrin cytoplasmic tail itself.

Lipid binding by PTB domains

The structure of PTB domains resembles that of phosphoinositide-binding pleckstrin homology (PH) domains, and the PTB domains of talin, Shc, Numb and tensin have been shown to bind to phosphoinositides (Dho et al., 1999; Goksoy et al., 2008; Leone et al., 2008; Zhou et al., 1995). Although the lipid-binding site in PTB domains is distinct from the NPxY/NxxY-binding site, structural studies have shown that phosphoinositide binding can induce long-range conformational changes that increase the affinity for NxxY motifs (Uhlik et al., 2005). Therefore, the local synthesis of appropriate phosphoinositides – for example, by phosphoinositide 3-kinase (PI3K) or phosphatidylinositol 4-phosphate 5-kinase type I γ [PIPKIγ; also known as PtdIns(4)*P*-5-kinase γ], both of which localize to adhesion sites (Chen and Guan, 1994; Di Paolo et al., 2002; Ling et al., 2002) – might increase the affinity of specific PTB-domain-containing adaptors for NxxY motifs. Additionally, Dok1 and the kindlins possess PH domains in addition to PTB domains, so local synthesis of phosphoinositides might direct them to the cell membrane independently of the PTB domain to increase the likelihood that they will encounter integrin ligands.

Phosphorylation of the β-integrin cytoplasmic tail

The adaptor Shc was identified as the founding member of the family of PTB-domain-containing proteins (Blaikie et al., 1994). The Shc PTB domain was so named because it binds specifically to phosphotyrosine-containing NxxY sequences, but a survey of known PTB-domain-containing proteins discovered since then demonstrated that around 75% of PTB domains bind to non-phosphorylated tyrosine residues also in addition to, and sometimes

instead of, phosphotyrosines (Uhlik et al., 2005). Therefore, it seems likely that binding of PTB domains to β-integrin tails can be specified and tuned by regulating phosphorylation of the tyrosine residues in both NxxY motifs. For instance, both of the NPxY tyrosine residues in β1 integrin can be phosphorylated in response to Src transformation (Sakai et al., 2001); mutation of these residues to phenylalanine abolishes phosphorylation of the β1-integrin tail and prevents many of the characteristics of transformation that can be attributed to perturbed integrin function, such as a failure to bind to the ECM and the impairment of directed migration (Sakai et al., 2001). $p60^{src}$ and, to a lesser extent, spleen tyrosine kinase (Syk) phosphorylate both NxxY-motif tyrosine residues in β3-integrin tails in vitro (Law et al., 1996), suggesting that Src-family kinases are the major physiological regulators of integrin NxxY phosphorylation. The phosphorylation of several focal-adhesion proteins, such as paxillin and FAK, is impaired in cells that lack the Src-family kinases Src, Yes and Fyn, but the phosphorylation of integrin tails has not been examined (Cary et al., 2002; Klinghoffer et al., 1999). It is worth noting that the importance of phosphorylation at NxxY sites appears to be integrin-specific. Mice harboring a double Y→F mutation in both NxxY motifs in β1 integrin have no apparent disease phenotype, whereas knock-in mice that express the same mutations in β3 integrin have a bleeding defect, and hematopoietic cells that express the mutant β3-integrin allele show impaired adhesion and clot retraction in vitro (Blystone et al., 1997; Chen et al., 2006; Czuchra et al., 2006; Law et al., 1999).

Integrin tails can also be phosphorylated on serine residues by protein kinase C (PKC). Early work showed that serine phosphorylation of β5 integrin was increased following treatment of human osteosarcoma cells or fibroblasts with phorbol esters, which are potent activators of PKC (Freed et al., 1989). This was confirmed in vitro using purified PKC, but the specific serine that was phosphorylated was not identified (Freed et al., 1989). Phosphorylation of β1 integrin is also increased in vivo upon treatment with phorbol ester; in this case, the affected residue is S785, the only serine residue in the β1-integrin tail, which is not conserved in β5 integrin (Fig. 1) (Hibbs et al., 1991).

In addition to tyrosine and serine, the threonine residues of β-integrin tails are also phosphorylation targets. In particular, PKC has been shown to phosphorylate β1 integrin on T788 and T789, which fall in the serine/threonine-rich intervening sequence between the membrane-proximal and membrane-distal NxxY motifs. The PKC isoform that is responsible is PKCε, as phosphorylation at this site was not detected in $PKC\varepsilon^{-/-}$ cells (Stawowy et al., 2005). In humans, the threonine residue at position 789 in β1 integrin is conserved across all integrin tails except that of integrin β1D, whereas T788 is replaced with serine in integrins β3, β5 and β6 (Fig. 1). Mutation of this S778 (equivalent to residue 788 in β1 integrin) to alanine in β3 integrin does not affect the activation of the integrin, but does induce morphological changes that are suggestive of a disorganized cytoskeleton (Perrault et al., 1998). Therefore, the 788/789 region is likely to be an important regulatory sequence in all integrins; however, the identity of the kinases involved in its phosphorylation might differ among integrins. T779 in β3 integrin (which is homologous to T789 in β1 integrin) is phosphorylated by Akt and PDK1 in vitro, and activation of Akt in vivo correlates well with β3-integrin phosphorylation at this site (Kirk et al., 2000). Selective-inhibitor studies indicate that T779 might also be a substrate for ERK2 in platelets (Lerea et al., 2007). PKC and Cdc2 also phosphorylate the β3-integrin tail in vitro, but at a site other than T779. The phosphorylation sites for these two kinases have yet to be identified, and whether Cdc2 can phosphorylate β-integrin tails in vivo has not been demonstrated. β2 integrin is phosphorylated at T758, the first threonine in a threonine triplet, in response to phorbol esters and T-cell-receptor stimulation (Hilden et al., 2003; Nurmi et al., 2007; Takala et al., 2008; Valmu and Gahmberg, 1995).

Strategies to regulate adaptor binding – switches and co-adaptor-mediated binding

On the basis of the data presented above, there are three main locations at which β-integrin tails are phosphorylated – the tyrosine residues within the two NxxY motifs, and the serine and threonine residues within the intervening sequence. These three regions provide binding sites for a large number of adaptors (Fig. 2). It is thought that phosphorylation of the NxxY motifs can serve as a switch, displacing PTB domains that do not bind to phosphotyrosine in favor of those that do. This was shown recently for Dok1 and talin – talin binds to a non-phosphorylated β3-integrin tail but, upon phosphorylation of Y773 in the membrane-proximal NPxY motif, Dok1 binds tightly and outcompetes talin for integrin binding (Oxley et al., 2008). This is consistent with the role of Dok1 as a negative regulator of integrin activation (Wegener et al., 2007). Similarly, phosphorylation of the serine/threonine-rich region might serve as a switch to regulate adaptor binding to this region of β-integrin tails, in addition to regulating the binding of PTB domains that have binding sites that overlap into this region. Such a switch has been described for filamin and talin (Kiema et al., 2006). In this model, filamin binds to the unphosphorylated serine/threonine-rich region and prevents binding of talin to the β7-integrin tail. Upon phosphorylation of T783, T784 or T785 in this region, the filamin interaction is disrupted, allowing talin to bind. In the following sections, we introduce two examples of phosphorylation switches that are thought to change the composition of adaptor proteins that are bound to β-integrin tails, and show how this can alter the function of the integrin-ECM linkage. As mutation of phosphorylatable integrin residues results in cellular changes that are consistent with aberrant integrin function (Blystone et al., 1997; Law et al., 1999; Perrault et al., 1998; Sakai et al., 2001), phosphorylation switches are likely to be a general principle of integrin regulation. We also discuss the regulation of integrin binding to ILK, which depends on additional adaptors.

Tensin and talin – a phosphotyrosine switch

Tensin and talin, which are PTB-domain-containing adaptors, both bind to the membrane-proximal NPxY motif of β-integrin tails. The recruitment of talin and tensin to integrins is temporally distinct – talin is recruited to adhesion sites very early, where it has a key role in integrin activation (Tadokoro et al., 2003; Zaidel-Bar et al., 2003), whereas in some cell types tensin is detected only at mature focal adhesions and fibrillar adhesions (Katz et al., 2000; Papp et al., 2007; Zamir et al., 1999). It has been proposed that phosphorylation of β integrins at the NPxY tyrosine residue influences whether tensin or talin is bound at this site, and thereby acts as a regulatory switch (McCleverty et al., 2007; Oxley et al., 2008).

Both tensin and talin have been shown to bind to β1-, β3-, β5- and β7-integrin tails (Calderwood et al., 2003; Calderwood et al., 1999; García-Alvarez et al., 2003; McCleverty et al., 2007), and talin also binds to the β2-integrin tail (Kim et al., 2003; Sampath et al., 1998). Talin binds to β-integrin tails through its FERM domain, which also mediates binding to F-actin, PIPKIγ and its

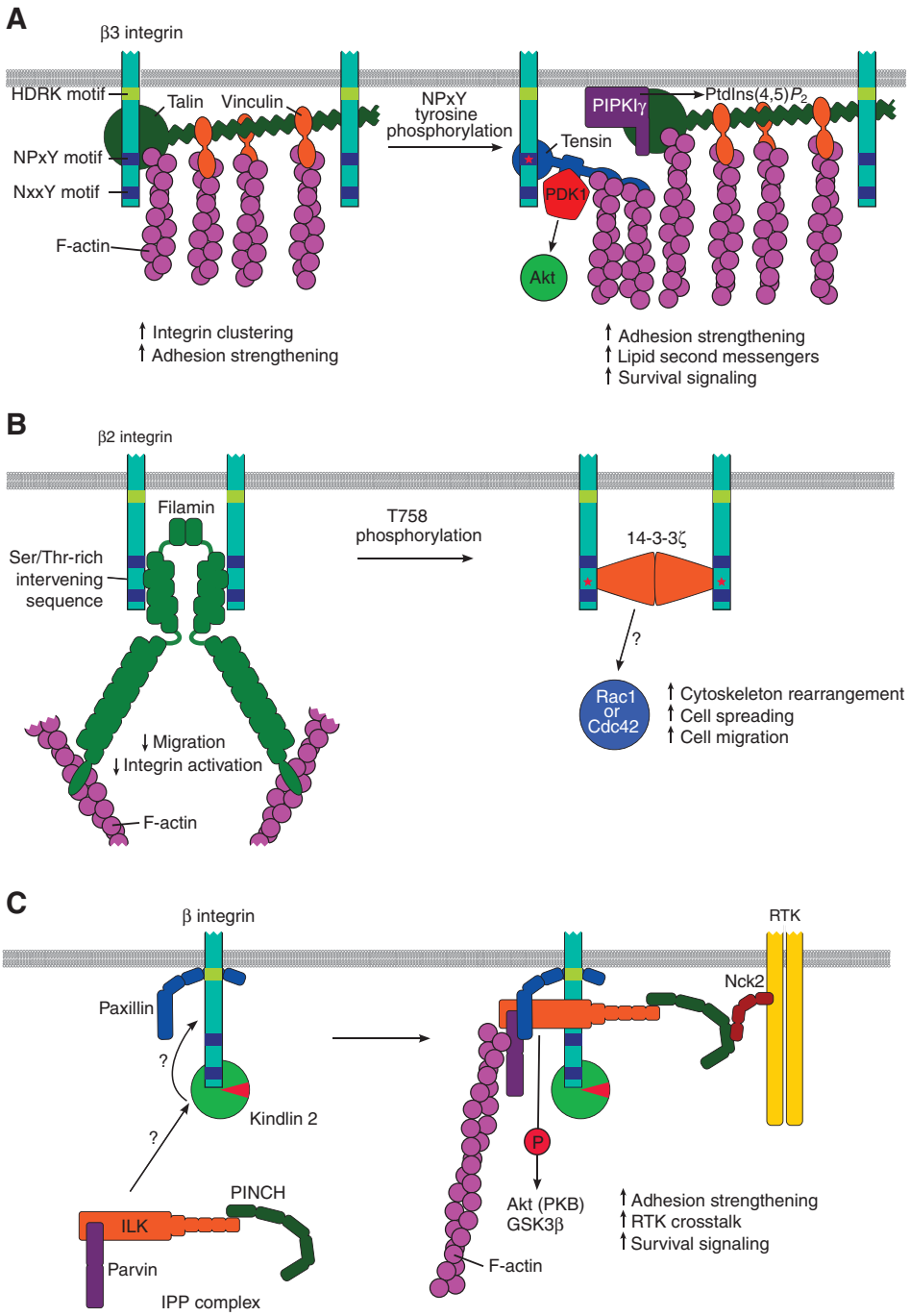

Fig. 3. Proposed mechanisms by which adaptor binding to β integrins is regulated. (A) A phosphotyrosine switch between the binding of talin and tensin to β3 integrin. (Left) A structural scaffold. In the unphosphorylated state, the NPLY motif of β3 integrin binds to the PTB domain of talin. Connections to the actin cytoskeleton occur through direct interactions between talin and actin, as well as through interactions with vinculin. (Right) A signaling scaffold. Phosphorylation (red star) of the NPLY tyrosine residue displaces talin and allows tensin to bind to the β3-integrin tail. Interactions of the adaptor proteins with actin are retained and reinforced, but intracellular signaling can now occur through the production of PtdIns(4,5)P_2 by talin-bound PIPKIγ and by the activation of Akt by tensin-bound PDK1. (B) A phosphothreonine switch between the binding of filamin and 14-3-3ζ to β2 integrin. (Left) Filamin interacts with the serine/threonine-rich intervening sequence when it is unphosphorylated, mostly via hydrophobic interactions. Connections to the actin cytoskeleton occur through the actin-binding domains of the filamin dimer. The filamin-integrin interaction inhibits cell migration and impairs integrin activation in some cell types. (Right) Phosphorylation (red star) of T758 in β2 integrin displaces filamin and allows binding of the adaptor protein 14-3-3ζ, primarily through interactions with the phosphate group of phosphothreonine. The 14-3-3–integrin interaction stimulates cell spreading and migration by promoting actin-cytoskeleton rearrangement in a Rac1- and Cdc42-dependent manner. The steps that lead from 14-3-3 binding to GTPase activation are unknown. Both filamin and 14-3-3 function as dimers, so they might aid integrin clustering by binding to two integrins simultaneously. (C) Co-adaptor-mediated binding of ILK to β integrin. The IPP complex, consisting of ILK, PINCH and parvin, assembles first in the cytosol, and is recruited to integrin tails in a paxillin- and kindlin-2-dependent manner. The details of how the IPP complex is recruited to integrins, and the precise roles of paxillin and kindlin 2 in the process, are unknown. Once the IPP complex is integrated into a focal adhesion, it has both structural and signaling roles. Adhesion strengthening can occur through binding of F-actin to parvin isoforms; crosstalk with receptor tyrosine kinases (RTK) is possible via a PINCH-Nck2 interaction; and ILK participates in signaling by facilitating the phosphorylation (P) of the kinases Akt (PKB) and GSK3β. Molecules are not drawn to scale.

product phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2], the hyaluronate receptor layilin and FAK, although it is not clear whether the talin-FAK interaction is direct (Borowsky and Hynes, 1998; Chen et al., 1995; Di Paolo et al., 2002; Lee et al., 2004; Ling et al., 2002; Martel et al., 2001). When the FERM domain is bound to integrins, PIPKIγ and layilin cannot bind because all three proteins share the same binding site (Barsukov et al., 2003; Wegener et al., 2008). The rod domain of talin contains additional binding sites for β-integrin tails and actin, and at least three binding sites for vinculin (Bass et al., 1999; Gingras et al., 2008; Gingras et al., 2005; Hemmings et al., 1996; Tremuth et al., 2004). Therefore, when talin is bound to β integrins, it functions mainly as a structural adaptor that interacts with the actin cytoskeleton both directly and indirectly (via vinculin). Tensin contains an N-terminal PTEN homology domain and two N-terminal actin-binding domains, which bind to and cap F-actin (Lo et al., 1994), a central Src homology 2 (SH2) domain, and a C-terminal PTB domain that binds to integrin tails (McCleverty et al., 2007). The SH2 domain binds to the Rho GTPase-activating proteins (RhoGAPs) deleted in liver cancer 1 (DLC1) and DLC3, to the kinase PDK1, and to the adaptor Dok2 (Qian et al., 2007; Wavreille and Pei, 2007). It has been reported that PI3K also binds to the SH2 domain (Auger et al., 1996), but this association is likely to be indirect as PI3K does not contain a sequence that matches the binding requirements for the SH2

domain (Wavreille and Pei, 2007). In addition to fulfilling a structural role by binding directly to actin, tensin imparts signaling functions to the focal adhesion by recruiting RhoGAPs, and protein and lipid kinases. Additionally, C-terminal tensin-like protein (CTEN; also known as tensin 4), which lacks actin-binding domains, is a strong promoter of cell migration (Katz et al., 2007).

The binding of tensin or talin is likely to be controlled by phosphorylation of the NPxY tyrosine residue. NMR titration and surface plasmon resonance experiments demonstrate that the affinity of tensin for a β3-derived peptide is insensitive to phosphorylation, whereas binding of talin to the phosphopeptide is reduced (McCleverty et al., 2007; Oxley et al., 2008). A structural comparison of talin and tensin PTB domains that are docked to integrin tails provides a rationale for their differential sensitivity to integrin tyrosine phosphorylation. The PTB domain of talin is an anomalous IRS1-type PTB domain, most of which bind to phosphotyrosine (Uhlik et al., 2005). IRS1-type domains commonly have two arginine residues that bind to the phosphate moiety of phosphotyrosine, but talin contains an aspartate substitution at one of these sites; this creates a steric and electrostatic barrier to phosphotyrosine binding (García-Alvarez et al., 2003). By contrast, the structure of the tensin PTB domain most closely resembles that of the adaptor protein X11α (also known as APBA1), which is a Dab1-type PTB domain (McCleverty et al., 2007); these do not normally bind to phosphotyrosine, but have a rather open binding pocket and minimal contacts with the tyrosine residue, so are often insensitive to phosphorylation (Uhlik et al., 2005). Although tensin contains the basic residues that are used by other PTB domains to bind to phosphotyrosine, this region of the molecule is disordered in the crystal structure, indicating a significant degree of flexibility. Therefore, structural data support the biochemical data demonstrating that NPxY phosphorylation negatively regulates talin binding and thereby favors tensin binding (which is insensitive to NPxY phosphorylation). Cell lines exist that contain tyrosine mutations in the β1- and β3-integrin sequences (Blystone et al., 1997; Chen et al., 2006; Czuchra et al., 2006; Law et al., 1999), so it will only be a matter of time until we see whether these data can be confirmed in vivo.

What might be the function of a talin-tensin phosphotyrosine switch? One possibility is that the regulation of talin and tensin binding to integrins defines a switch from a structural adhesion to an adhesion that participates in signaling (Fig. 3). Early in focal-adhesion formation, talin binds to and activates integrins (Tadokoro et al., 2003). Initial connections between integrins and the cytoskeleton (via talin and vinculin) allow the consolidation of adhesion structures through the generation of force (Fig. 3A) (Humphries et al., 2007; Lee et al., 2007). Once a focal adhesion has formed, however, talin might no longer be required to maintain integrin activation, as adaptors that bind to the membrane-proximal tail sequence might prevent α integrins and β integrins from re-associating with each other, and homomeric associations between integrin transmembrane domains might retain integrins in the active conformation by preventing re-association of the α- and β-integrin tails (Li et al., 2003; Li et al., 2005b). At this point, talin might be displaced in favor of adaptors that have a greater range of functions (Fig. 3B). Src, which localizes to focal adhesions through interactions with FAK and the β-integrin tail itself (Arias-Salgado et al., 2003; Arias-Salgado et al., 2005; Thomas et al., 1998), could phosphorylate the NPxY motif, thus weakening the interaction between talin and β-integrin tails. Talin does not leave the focal adhesion, because it can bind to activated integrins at a membrane-proximal site via its second integrin-binding domain (Rodius et al., 2008); however, the now-unbound talin PTB domain is available for interactions with PIPKIγ or layilin. Tensin might occupy the available phosphorylated NPxY motif and, via its SH2 domain, assemble a signaling complex that includes PDK1, PI3K and RhoGAPs; this complex might regulate downstream signaling pathways through the activation of Akt (via PDK1 and PI3K and reorganization of the actin cytoskeleton (via RhoGAPs).

Filamin and 14-3-3 – a phosphothreonine switch

As has been discussed above, the intervening sequence between the NPxY and NxxY motifs of β-integrin tails can be phosphorylated on serine and threonine residues. Similar to the phosphotyrosine switch described above, it is thought that the phosphorylation of threonine residues in this region acts as a switch to control the binding of adaptor proteins to the cytoplasmic tail. This intervening region is bound by the actin-crosslinking protein filamin and the adaptor protein 14-3-3ζ in β2 integrin (Fagerholm et al., 2005; Sharma et al., 1995; Valmu et al., 1999a; Valmu et al., 1999b). Although the serine and threonine residues in this region are relatively well-conserved across the β-integrin family, the surrounding sequences diverge to a large enough extent that it is risky to assume that these adaptors can bind to all integrins; however, filamin has additionally been shown to bind to β1A-, β3- and β7-integrin tails (Calderwood et al., 2001; Kiema et al., 2006; Loo et al., 1998; Pfaff et al., 1998; Travis et al., 2004; Zent et al., 2000), whereas 14-3-3β binds to the tails of β1- and β3-integrins (Han et al., 2001). Interestingly, deletion of most of the intervening sequence in β1 integrin did not affect its association with 14-3-3β in a yeast two-hybrid assay (Han et al., 2001), so it is possible that 14-3-3β and β1 integrin interact differently than do 14-3-3ζ and β2 integrin. This is supported by evidence that shows that mutating residues outside the usual binding pocket of 14-3-3 abolishes the 14-3-3β–integrin-β1 interaction (Rodriguez and Guan, 2005), so 14-3-3β might not be regulated by the switch mechanism described below.

The binding of filamin to β integrins appears to be negatively regulated by phosphorylation of integrin cytoplasmic tails. Phosphomimicking mutations of T783, T784 and T785 in the β7-integrin tail abolish filamin binding in vitro, with substitution of T784 having the greatest effect (Kiema et al., 2006). Additionally, surface plasmon resonance experiments with β2-integrin tails clearly demonstrate that filamin does not bind to a peptide phosphorylated at T758 (Takala et al., 2008). Although biochemical and structural data strongly suggest that threonine phosphorylation of β2- and β7-integrin tails inhibits filamin binding, this has not yet been tested in vivo. By contrast, 14-3-3 proteins are phosphoserine- and phosphothreonine-binding adaptors, and have been shown to bind to phosphorylated β2 integrin in vitro and in vivo (Fagerholm et al., 2005; Fagerholm et al., 2002). Therefore, phosphorylation of the intervening sequence of β-integrin tails might function as a switch to regulate the binding of filamin or 14-3-3ζ to this region.

A recent structural comparison of β2-integrin-tail peptide bound to either filamin A immunoglobulin-like domain 21 (IgFLNa21) or 14-3-3ζ provides a molecular rationale for how T758 phosphorylation regulates the differential binding of these proteins (Takala et al., 2008). In the IgFLNa21–β2-integrin-tail structure, the integrin tail binds as a β-strand to β-strands C and D of the immunoglobulin fold. The molecular interactions surrounding T758 are largely hydrophobic; the introduction of a phosphate group to

this threonine is expected to be highly unfavorable, and this was confirmed by the surface plasmon resonance experiments mentioned above (Takala et al., 2008). By contrast, the β2-integrin-tail-binding site on 14-3-3ζ lies within a well-characterized basic pocket. Here, electrostatic interactions between the phosphate of phospho-T758 and two arginine residues (R56 and R127) in 14-3-3ζ, as well as a hydrogen bond between the phosphate and Y128, comprise the major binding interactions and are largely responsible for maintaining the complex (Takala et al., 2008). As the phosphate group is the major determinant of the interaction, whereas additional side-chain interactions have at most a minor role, the binding of 14-3-3 adaptors to integrin tails might be more widespread than has been reported.

What might be the effect of a filamin–14-3-3 binding switch? Filamin appears to have an inhibitory function in adhesion and migration, whereas 14-3-3 promotes adhesion, spreading and migration, so the phosphotyrosine switch might regulate these processes. Filamin knockdown in Jurkat cells increases cell adhesion to ICAM1 (Takala et al., 2008). Chinese hamster ovary (CHO) cells expressing chimeric integrins containing mutations that caused an increased affinity for filamin exhibited reduced migration on a fibronectin-coated substrate, owing to decreased polarization and a reduced number of transient membrane protrusions (Calderwood et al., 2001). All cell lines in this study were able to form focal adhesions and reorganize the fibronectin matrix; this suggests that adhesion was not altered in these cells, but that the integrin-filamin interaction, and thus an inhibitory effect of filamin on adhesion, was gradually lost following cell attachment. The potential early effects on adhesion were not studied, but the lack of persistence of membrane protrusions in cells expressing integrins possessing enhanced filamin-binding suggests that early adhesion events are partially inhibited. By contrast, interfering with 14-3-3–integrin binding by introducing a T758A mutation in β2 integrin or expressing a 14-3-3-binding competitor peptide decreases adhesion in COS cells (Fagerholm et al., 2005), and overexpression of 14-3-3β in NIH 3T3 cells results in increased spreading and migration on fibronectin-coated substrates (Han et al., 2001). Both filamin and 14-3-3 can compete with talin for integrin-tail binding in vitro (Kiema et al., 2006; Takala et al., 2008), so it is unlikely that the opposing effects of filamin and 14-3-3 are attributable to changes in talin-mediated integrin activation; rather, the enhancement of spreading and migration upon 14-3-3 binding is probably the result of enhanced Rac1-Cdc42 activity (Nurmi et al., 2007). The intervening steps that might follow 14-3-3 binding to Rac1-Cdc42 are, however, unknown.

The phosphothreonine switch between the binding of filamin and 14-3-3 to β2 integrin has been proposed to have an important role in the regulation of T-cell adhesion (Takala et al., 2008). Before T-cell-receptor activation, filamin might bind to β2 integrin, maintaining it in a minimally adhesive state. T758 is phosphorylated in response to T-cell-receptor activation (Hilden et al., 2003; Nurmi et al., 2007), which would facilitate the displacement of filamin and enable 14-3-3ζ to bind to this sequence. Binding of 14-3-3 might ultimately stimulate the activation of Rac1-Cdc42, causing rearrangement of the actin cytoskeleton and thereby strengthening the interaction between T cells and endothelial and antigen-presenting cells. Although this scenario is applicable to β2 integrins and probably β7 integrins, other integrins are less well studied. It is not known whether the interplay between filamin and 14-3-3 has a general role in mediating cell adhesion, migration and polarity, but the finding that filamin is readily detectable in early focal adhesions but nearly absent from mature focal adhesions (Calderwood et al., 2001), combined with the observed threonine phosphorylation of β1- and β3-integrin tails in vivo (Kim et al., 2004; Lerea et al., 1999), suggests that regulation of the filamin-integrin interaction is a general phenomenon.

ILK – co-adaptor-mediated regulation of binding

In addition to β-integrin phosphorylation, binding of adaptors can also be regulated by the binding of additional proteins (co-adaptors). A single example of this regulation has been identified so far, involving the adaptor protein ILK. ILK is a 59-kDa protein that was originally identified in a yeast two-hybrid screen for proteins that bind to the β1-integrin tail (Hannigan et al., 1996); however, the precise ILK-binding site remains unknown. ILK is the central component of the IPP complex, a heterotrimeric complex that also includes one of two PINCH isoforms and one of three parvin isoforms (Legate et al., 2006). The IPP complex is first assembled in the cytosol (Zhang et al., 2002) and is then recruited to adhesion complexes in response to integrin activation and contractile stimuli in tracheal smooth muscle (Sepulveda et al., 2005; Zhang et al., 2007). Although neither PINCH nor parvin bind to integrins directly, truncated versions of either protein that are assembled into the IPP complex fail to target to integrins, as do complexes that contain only two of the three components (Nikolopoulos and Turner, 2001).

The recruitment of ILK to adhesion sites is dependent on its binding to additional adaptors. Paxillin, for instance, binds to both ILK and α-parvin (Nikolopoulos and Turner, 2000; Nikolopoulos and Turner, 2001), and an ILK mutant whose interaction with paxillin is abolished fails to localize to focal adhesions (Nikolopoulos and Turner, 2001; Nikolopoulos and Turner, 2002). The localization of an α-parvin mutant that is defective in paxillin binding is less severely affected, although its recruitment to adhesions is reduced (Nikolopoulos and Turner, 2002). Interestingly, a truncated ILK protein that does not bind to PINCH also fails to bind to paxillin (Nikolopoulos and Turner, 2002). Taken together, these data establish paxillin as an important IPP-complex binding partner that is required to localize ILK to focal adhesions.

In *Caenorhabditis elegans*, UNC-112 was first identified as a binding partner of ILK that was required to direct ILK to muscle-cell attachment sites (Mackinnon et al., 2002). The mammalian UNC-112 homolog kindlin 2 is an integrin adaptor that binds to the membrane-distal NxxY β-integrin motif (Ma et al., 2008; Moser et al., 2008) and to ILK (Montanez et al., 2008). Recent data indicate that kindlin 2 is required to direct ILK to sites of cell adhesion. Cells that are defective in kindlin 2 expression have a severe spreading defect as a consequence of reduced integrin activation (Montanez et al., 2008). Cells that do make focal-adhesion-like structures, however, show localization of paxillin and talin, but not of ILK, to adhesion sites. As paxillin is directed to sites of cell adhesion in the absence of kindlin 2 but ILK is not, the role of paxillin in mediating the localization of ILK is more complicated than a simple shuttling model, in which paxillin directs ILK to focal adhesions on the basis of its own affinity for β-integrin tails. These data suggest that ILK requires both paxillin and kindlin 2 to reach its destination.

Conclusions and perspectives

Many adaptor molecules are now known to bind to the cytoplasmic tails of β integrins, and more are still being discovered. The modes of regulation of adaptor binding are gradually being revealed, but

how the regulators are themselves regulated is still largely unknown. For example, what are the specific steps of focal-adhesion maturation that lead to phosphorylation of the membrane-proximal β-integrin NPxY motif to displace talin and allow the binding of other adaptors? Knowledge of the signaling cascades that lead to integrin-tail phosphorylation, and of the consequences that this has on adaptor binding, might provide insight into how to treat diseases that have an integrin-signaling component.

Dysregulated expression or activity of adaptors such as FAK and ILK have been implicated in pathologies such as cancer, skeletal-muscle dystrophy and heart disease (Hannigan et al., 2007; Hannigan et al., 2005; Mitra and Schlaepfer, 2006; Wang et al., 2008). Accumulation of proteins in focal adhesions can serve to concentrate substrates and additional binding partners, increasing the likelihood of protein-protein interaction; thus, it would be worthwhile to investigate whether the pathogenic effects of ILK and FAK are facilitated by an integrin-binding step, and whether interfering with integrin binding can be a useful therapeutic intervention. First, however, it is important to unravel exactly how both proteins are directed to, and bind to, integrin tails. Detailed information on FAK-integrin and ILK-integrin interactions is currently lacking – the evidence that FAK can bind to integrin tails is over a decade old (Schaller et al., 1995), and subsequent work has not expanded on this early finding, which raises concerns over whether the described interaction is relevant. Given the central role that FAK plays in disease, it is of utmost importance that the molecular interactions that lead to its recruitment to, and retention in, adhesion structures are clearly defined. The in vivo relevance of an ILK-integrin interaction is also open to question. *Drosophila* ILK does not bind to βPS integrin, yet ILK colocalizes with integrins at muscle attachment sites and genetic disruption of *ILK* in *Drosophila* results in defects in actin attachment to the cell membrane, as one would expect from a structural integrin adaptor (Zervas et al., 2001). A molecular analysis of the ILK-integrin interaction in mammals has not been undertaken, and it is not known whether this interaction has a function in mammals that it does not have in the fly. Similar to FAK, ILK appears to be involved in several pathologies, so resolving these open questions is crucial to our understanding of how we can exploit ILK as a therapeutic target.

Finally, it is becoming clear that changes in adaptor composition, as well as those occurring during the maturation of adhesion structures, can also facilitate specific cell behaviors. The phosphothreonine switch between filamin and 14-3-3 (see above), a tensin-CTEN switch that facilitates cell migration (Katz et al., 2007), and the modulation of β1-integrin affinity for the ECM by ICAP1α [which allows the cell to sense and adjust to different ligand concentrations (Millon-Frémillon et al., 2008)] are all recent examples. A challenge for the future will be to identify additional adaptor switches, and define how they are regulated.

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