Negative regulators of integrin activity

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Journal of Cell Science 125, 1–10
© 2012. Published by The Company of Biologists Ltd
doi: 10.1242/jcs.093641

Summary
Integrins are heterodimeric transmembrane adhesion receptors composed of α- and β-subunits. They are ubiquitously expressed and have key roles in a number of important biological processes, such as development, maintenance of tissue homeostasis and immunological responses. The activity of integrins, which indicates their affinity towards their ligands, is tightly regulated such that signals inside the cell crucially regulate the switching between active and inactive states. An impaired ability to activate integrins is associated with many human diseases, including bleeding disorders and immune deficiencies, whereas inappropriate integrin activation has been linked to inflammatory disorders and cancer. In recent years, the molecular details of integrin ‘inside-out’ activation have been actively investigated. Binding of cytoplasmic proteins, such as talins and kindlins, to the cytoplasmic tail of β-integrins is widely accepted as being crucial in integrin activation. By contrast, much less is known with regard to the counteracting mechanism involved in switching integrins into an inactive conformation. In this Commentary, we aim to discuss the known mechanisms of integrin inactivation and the molecules involved.

Key words: Activation, Adhesion, Endocytosis, Integrin, SHARPIN, Talin

Introduction
Integrins are heterodimeric transmembrane proteins composed of α- and β-subunits. They are ubiquitously expressed, often in high numbers, and mediate cell–cell adhesion, as well as adhesion of cells to extracellular matrix (ECM) proteins (Hynes, 2002; Legate et al., 2009). The affinity of integrins for their ligands (integrin activation) is allosterically regulated such that the intracellular and extracellular domains of both subunits undergo conformational changes (Moser et al., 2009; Shattil et al., 2010). A controlled regulation of integrin activity is fundamentally important during embryogenesis and is central to many physiological processes in adults. Impaired integrin activation has been linked to diseases, including bleeding disorders, skin blistering and immune deficiencies (Hogg and Bates, 2000; Legate et al., 2009). Conversely, increased integrin activity is associated with chronic inflammation, thrombosis and cancer (Moser et al., 2009; Shattil et al., 2010).

Integrins are unique in their ability to function as bidirectional signalling molecules. Binding of extracellular matrix (ECM) molecules or other ligands to the extracellular domain of integrins transmits a variety of signals into the cell. This ‘outside-in’ activation (Fig. 1) regulates many important cellular processes, including migration, survival, proliferation, gene expression and receptor tyrosine kinase signalling (Ivaska and Heino, 2010; Zaidel-Bar et al., 2007). Conversely, changes in the intracellular environment of the cell can alter the binding of integrins to their ligands through so-called ‘inside-out’ signalling (Fig. 1). Recently, the individual steps involved in integrin inside-out activation have been the focus of intense investigation. Despite some controversy over the details, it is now widely accepted that binding of the cytoplasmic proteins talin-1 and -2 (TLN1, TLN2) and of kindlins (the fermitin family members 1–3, FERMT1–FERMT3; also known as KIND1–KIND3) to the cytoplasmic tail of the integrin β-subunit are crucial for integrin activation (Calderwood et al., 2004; Moser et al., 2009).

Even though factors that keep integrins inactive or that are able to switch activated integrins back into an inactive conformation are likely to be as biologically important as integrin activators for regulating the dynamic nature of integrin function (Fig. 2), they have been studied less than the integrin activating proteins. On the basis of the existing data it appears that integrin activation can be prevented or diminished by at least three mechanisms: (1) interfering with the clasp interactions, (2) competition with talin and kindlin binding, and (3) decreasing the amount of integrins at the plasma membrane by altering integrin trafficking (Fig. 2). In this Commentary, we will describe new and previously described known negative regulators of integrin activity and their relevance to cell biology and human health.

Mechanism of integrin activation
Recently, the molecules involved in integrin activation have been discussed extensively in many excellent reviews (Margadant et al., 2011; Shattil et al., 2010). Thus, only some details relevant for the discussion are presented here. The conformational changes of integrins are dynamic and we can assume that there is a constant equilibrium between their active and inactive conformations. The known mechanisms for integrin activation include the release of intramembrane interactions (Lau et al., 2009; Partridge and Marcantonio, 2006) and of juxtamembrane cytoplasmic interactions (also termed clasps) (Hughes et al., 1996; Lau et al., 2009) between the α- and β-subunits.
Talin is a large cytoplasmic protein composed of an integrin-binding head domain and a rod domain, which links talin to vinculin (VCL) and the actin cytoskeleton (Critchley, 2009; Grashoff et al., 2010; Humphries et al., 2007). Talin binds to integrin-β tails and induces conformational activation of the integrin by disrupting the integrin clasp (Anthis et al., 2009; Kalli et al., 2011; Wegener et al., 2007). For the platelet integrin αIIbβ3 and for the α-β2 integrin heterodimers, expressed, for example, on leucocytes, there is compelling evidence that a clasp formed by a salt-bridge between the α- and β-tails is crucial for maintaining these receptors in their inactive conformation (Springer and Dustin, 2012; Ye et al., 2012). For the β1 integrins, which predominantly facilitate matrix binding of adherent cell types, the role of the juxtamembrane clasp is not that well established (Czuchra et al., 2006). This might reflect the fact that for most α-β1-integrin-mediated biological processes the switching between integrin activation and inactivation is implicated in adhesion modulation rather than a complete transition between non-adherent and adherent states. Nevertheless, talin binding is also required for β1-integrin activation (Calderwood, 2004). On the basis of in vitro and in vivo data, kindlins are also crucial for integrin activation; they bind to integrin-β subunits and co-activate integrins together with talin (Czuchra et al., 2006; Karaköse et al., 2010; Montanez et al., 2008; Moser et al., 2008). Interestingly, talin is not important for the initial binding of β1 integrins to the matrix, but it is absolutely required for subsequent cell spreading given that fibroblasts lacking both the talin-1 and -2 isoforms fail to spread fully (Zhang et al., 2008). In Drosophila, the talin-null phenotype has defects in the connections between integrin and the cytoskeleton, but the binding of integrin to the extracellular matrix is not abolished (Brown et al., 2002). However, subsequent work has also demonstrated a role for talin binding to the Drosophila integrins in their activation (Tanentzapf and Brown, 2006). These data indicate that integrins can bind the ECM and become activated in the absence of talin, but talin binding to integrin-β tails is crucial for the ability of integrins to fulfil their role as integrators between the ECM and the cytoskeleton.

**Inhibitors interacting with integrin-β tails**

The role of the integrin β-subunit in the regulation of integrin activity has been the main focus in the field (reviewed by Harburger and Calderwood, 2009; Kim and McCulloch, 2011; Shattil et al., 2010) and important inhibitors interacting with the β-tails, such as filamin and integrin cytoplasmic domain-associated protein 1 (ICAP1; encoded by ITGB1BP1) (Liu et al., 2000), have been identified (Fig. 3; Table 1). The main focus of our Commentary is the role of the integrin-α tail in the modulation of integrin activity. However, in the following sections we will also describe the β-tail-binding integrin inhibitors. As we are not aware of clasp-stabilising β-subunit-binding proteins, we will divide the inhibitors in two groups; those that compete with talin and kindlin binding, and those that affect integrin trafficking (as described in the Introduction).

**β-tail-binding inhibitors that compete with the binding of talins and kindlins**

Different integrin β-subunit cytoplasmic domains share two highly conserved NxxY motifs, which are referred to as the
membrane-proximal and membrane-distal motifs. Mutational analyses of these sequences have suggested that they have a crucial role both in integrin ‘inside-out’ and ‘outside-in’ signalling (Liu et al., 2000). Talin binds to the membrane-proximal NPxY motif in the integrin β-subunits through its FERM domain (Goldmann, 2000; Horwitz et al., 1986; Knezevic et al., 1996; Pfaff et al., 1998) and there are at least two classes of proteins that inhibit integrin activity by competing with talin for binding to this motif.

The first class of talin inhibitors are proteins that contain a phosphotyrosine-binding (PTB) domain (Calderwood et al., 2003). The F3 subdomain of the talin FERM domain is similar to PTB domains, and talin interacts with integrins in a similar way to that of PTB domains binding to phosphorylated tyrosine motifs.

### Table 1. Overview of all integrin inhibitors described in this Commentary

<table>
<thead>
<tr>
<th>Integrin inhibitor</th>
<th>α-tail binding</th>
<th>β-tail binding</th>
<th>Actin-binding</th>
<th>Type of inhibition</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAP1</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Competition with talin</td>
<td>(Bouvard et al., 2003)</td>
</tr>
<tr>
<td>Filamin A</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Competition with talin</td>
<td>(Kiema et al., 2006)</td>
</tr>
<tr>
<td>Numb</td>
<td>–</td>
<td>+</td>
<td>?</td>
<td>Competition with talin, endocytosis</td>
<td>(Calderwood et al., 2003; Nishimura and Kaibuchi, 2007)</td>
</tr>
<tr>
<td>DAB2</td>
<td>–</td>
<td>+</td>
<td>?</td>
<td>Endocytosis</td>
<td>(Teckchandani et al., 2009)</td>
</tr>
<tr>
<td>PKCα</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>α-Subunit-specific inhibitor</td>
<td>(Ng et al., 1999; Parsons et al., 2002; Upla et al., 2004)</td>
</tr>
<tr>
<td>Nischarin</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>α-Subunit-specific inhibitor</td>
<td>(Alahari and Nasrallah, 2004)</td>
</tr>
<tr>
<td>CIB1</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>α-Subunit-specific inhibitor</td>
<td>(Gushiken et al., 2008; Naik et al., 1997)</td>
</tr>
<tr>
<td>PP2A</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Other</td>
<td>(Gushiken et al., 2008; Kim et al., 2004)</td>
</tr>
<tr>
<td>SHARPIN</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>Clasp stabiliser or competition with talin and/or kindlin?</td>
<td>(Rantala et al., 2011)</td>
</tr>
<tr>
<td>MDGI</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>Competition with kindlin</td>
<td>(Nevo et al., 2010)</td>
</tr>
<tr>
<td>Rab21</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>Endocytosis</td>
<td>(Pellinen et al., 2006)</td>
</tr>
<tr>
<td>p120RasGAP</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>β1 integrin recycling to the plasma membrane</td>
<td>(Mai et al., 2011)</td>
</tr>
<tr>
<td>ACAP1</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>β1 integrin recycling to the plasma membrane</td>
<td>(Li et al., 2005)</td>
</tr>
<tr>
<td>PKD1</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>β3 integrin recycling to the plasma membrane</td>
<td>(Woods et al., 2004)</td>
</tr>
<tr>
<td>GIPC1</td>
<td>?</td>
<td>?</td>
<td>–</td>
<td>Active α5β1 integrin; endocytosis</td>
<td>(Valdembri et al., 2009)</td>
</tr>
<tr>
<td>MMP8</td>
<td>?</td>
<td>?</td>
<td>–</td>
<td>Inhibits β1 integrin by binding to the extracellular domain</td>
<td>(Pellinen et al., 2012)</td>
</tr>
</tbody>
</table>

+, positive interaction; –, no interaction; ?, not determined
Filamins form the second class of inhibitors of talin binding. The filamin-binding site is located close to the talin-binding site on the β-integrin tails and filamin and talin compete with each other, whereby filamin inhibits integrin activity, as opposed to talin (Kiema et al., 2006). Filamin depletion increases the amount of active integrins on the cell surface, but it is unclear whether any of the developmental defects caused by mutations in filamin genes is the consequence of increased integrin activation (reviewed by Zhou et al., 2010). The genetic analysis of filamin function is complicated by the existence of multiple genes that have partially overlapping expression patterns. In addition to this, migfilin, a filamin-binding protein that is enriched at cell–cell and cell–ECM contact sites, can displace filamin from β1 and β3 integrins and promote integrin activation (Das et al., 2011; Ithychanda et al., 2009; Lad et al., 2008). Therefore, the balance between filamin and migfilin expression will influence the extent to which filamin inhibits integrin activity, and, thus, the filamin–migfilin interaction provides an additional regulatory layer for filamin-induced inhibition of integrins.

**Integrin trafficking regulators that bind the integrin-β tail**

In addition to the regulation of integrin receptor activity on the cell surface by ‘inside-out’ signalling as described above, selective integrin endocytosis can affect the availability of active integrin receptors on the cell surface. Most integrins are constantly endocytosed and recycled back to the plasma membrane in adherent cells and this is regulated in part by proteins interacting with the integrin cytoplasmic tails (Margadant et al., 2011; Pellinen and Ivaska, 2006). However, to date the relationship between integrin activity and integrin endocytic trafficking remains unclear. As mentioned above, many PTB-domain-containing proteins have been shown to interact with integrin-β tails in vitro (Calderwood et al., 2003) and, interestingly, several of these are clathrin adaptor proteins that are involved in endocytosis. According to one report, DAB2 is important for the endocytosis of active integrins from disassembling FAs (Ezratty et al., 2009), whereas another study suggests that there is a role for DAB2 in the endocytosis of

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**Fig. 3. Alignment of activator and inhibitor binding sites on β1 and β3 integrin cytoplasmic regions.** The green box represents the ‘clasp’, which forms between the α- and β-integrin cytoplasmic regions and which is required to keep an integrin in its inactive conformation. The red boxes indicate the membrane-proximal (Prox.) NPxY and membrane-distal (Dist.) NxY motifs. The binding sites for regulators of integrin activity are indicated with lines. Integrin-activating proteins are written in black, proteins that affect integrin trafficking are in green, and proteins that compete with talin or kindlin for binding to the β-integrin cytoplasmic region are in blue (a green rectangle around the name indicates that that protein has also been shown to be involved in integrin trafficking). The β-integrins that the respective integrin activator or inactivator has been shown to bind is given in parentheses. The β-integrin-interacting proteins are talin (Calderwood et al., 1999), kindlins (FERMT1–3) (Moser et al., 2009), filamins (Kiema et al., 2006), tensin (Calderwood et al., 2003; Katz et al., 2007), DAB1, DAB2, numb (Calderwood et al., 2003), DOK1 (Anthis et al., 2009), (Chang et al., 1997; Zhang and Hemler, 1999), PKCz (Parsons et al., 2002) and PKD1 (Woods et al., 2004).
inactive integrins from the dorsal surface of cells (Teckchandani et al., 2009). On the basis of the available structural information, it is conceivable that DAB2 could interact with both active and inactive integrins, as DAB2 binding to the β3-tail involves the membrane distal NPxY motif, which is not bound by talin in active integrins. The PTB-containing clathrin adaptor numb has also been shown to regulate integrin endocytosis and cell migration (Nishimura and Kaibuchi, 2007). Interestingly, the binding sites of talin and numb on the integrin β3 tail overlap (Calderwood et al., 2003), which suggests that numb could specifically regulate endocytosis of inactive integrin receptors by specifically recruiting non-talin-binding inactive receptors for endocytosis. This possibility, however, has not been investigated.

In addition to these clathrin adaptors, other β-tail-interacting proteins can also trigger integrin endocytosis. For example, protein kinase C alpha (PKCα) binds directly to the integrin β1 tail and its binding sequence spans both NPxY motifs. PKCα induces endocytosis of β1 integrin in migrating cancer cells (Ng et al., 1999; Parsons et al., 2002) and in response to echovirus-1 (EV-1) binding to α2β1 integrin or integrin clustering (Upla et al., 2004). As talin and PKCα probably cannot bind integrin simultaneously due to the overlapping binding sites on the β1-integrin cytoplasmic tail (Fig. 3) (Parsons et al., 2002), it is not surprising that PKCα-dependent EV-1-induced integrin endocytosis is specific for the inactive α2β1 integrin (Jokinen et al., 2010). Recently, it has also been shown that PKCα affects integrin endocytosis through another pathway. Binding of transmembrane proteoglycan syndecan-4 to fibronectin triggers PKCα-dependent RhoG binding to α5β1 integrins (the fibronectin-binding integrin) and subsequent clearance of the integrin from the membrane by accelerated endocytosis (Bass et al., 2011). This transient reduction of integrin levels at the cell surface is important to regulate the adhesive strength of the cells to favour migration (Bass and Humphries, 2002).

The cell surface integrin levels are also influenced by the rate of recycling of the endocytosed receptors to the plasma membrane. For β3 integrins, which predominantly recycle through a Rab4-dependent pathway, the direct binding of protein kinase D1 (PKD1) is required for return of the endocytosed receptor to the plasma membrane (Woods et al., 2004). The recycling of the endocytosed β1 integrins, by contrast, is regulated by binding of ACAP1 (coiled-coil, ANK repeat and PH domain-containing protein 1) to the integrin β1 cytoplasmic domain (Li et al., 2005). Therefore, these proteins contribute to the levels of active integrin present on the cell surface through their effect on receptor recycling.

**Inhibitors interacting with integrin α-tails**

For years, the main focus of the integrin field has been on the role of the β-subunits in regulating integrin activity. As several of the β-subunits (especially β1) can pair with many different α-subunits, proteins that are involved in regulating β-subunits are expected to have important functions in triggering various cellular functions regulated by all β1 integrins. However, in principle, the α-subunits offer two distinct layers of regulating integrin activity. First, they share conserved sequence elements in the membrane-proximal regions of their cytoplasmic tails, which allows for proteins to interact with and regulate all of the different α-subunits at once (Liu et al., 2000; Rantalä et al., 2011). In addition, the poorly conserved specific membrane-distal parts of the α-subunit cytoplasmic tails facilitate protein interactions and could regulate integrin activity in a heterodimer-specific manner. At present, our insight into the molecular mechanisms of α-tail-mediated integrin activity regulation is far from that of the β-tail-binding activators or inactivators. Therefore it is not possible to categorise α-tail-interacting activity regulators into the three mechanistic classes used above to describe different β-tail-binding regulators. Thus, we have opted to group them into common α-tail-binding inhibitors, α-tail-interacting regulators of integrin trafficking and α-subunit-specific inhibitors (Fig. 4; Table 1), as discussed below.

**Integrin inhibitors binding to several or all α-subunits**

**SHARPIN**

Until very recently very little was known about SHARPIN, except that it localises in the postsynaptic density of excitatory synapses in the brain, where it binds Shank proteins (Lim et al., 2001) and that a spontaneous mutation in the Sharpin gene results in eosinophilic proliferative dermatitis and multiorgan inflammation (Li et al., 2011; Seymour et al., 2007). During the last few years, however, new roles of SHARPIN have emerged as it was shown to bind to and inhibit the lipid-phosphatase activity of phosphatase and tensin homolog (PTEN) (He et al., 2010), act as a transcriptional co-activator of eyes absent homolog 1 (EYA1) (Landgraf et al., 2010), and be part of the linear ubiquitin chain assembly complex [LUBAC, composed of heme-oxidised IRP2 ubiquitin ligase 1 (HOIL1)-interacting protein (HOIP), HOIL1 and SHARPIN proteins], regulating nuclear factor kappa B (NFKβ) activity and apoptosis (Gerlach et al., 2011; Ikeda et al., 2011; Tokunaga et al., 2011).

In addition to these functions, a small interfering RNA (siRNA) screen for integrin inhibitors identified SHARPIN as a ubiquitously expressed integrin-inactivating protein (Rantalä et al., 2011). Depletion of SHARPIN increases active β1 integrin activity.

### Table 1. Integrin activity regulators into the three mechanistic classes used above

<table>
<thead>
<tr>
<th>Mechanistic Class</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clasp</td>
<td>ACAP1, CIB1, MDGI, Rab21, P120RasGAP, SHARPIN</td>
</tr>
<tr>
<td>-tail-binding activators</td>
<td>P120RasGAP, Rab21, MDGI, CIB1, ACAP1</td>
</tr>
<tr>
<td>-tail-binding inactivators</td>
<td>SHARPIN, ACAP1, CIB1, MDGI</td>
</tr>
<tr>
<td>-tail-mediated integrin activity regulation</td>
<td>ACAP1, CIB1, MDGI, SHARPIN</td>
</tr>
</tbody>
</table>

**Fig. 4. Alignment of integrin-inhibitor-binding sites on α-integrin cytoplasmic regions.** As in Fig. 3, the green box represents the ‘clasp’, and binding sites for regulators of integrin activity are indicated with lines. Proteins that affect integrin trafficking are in green and in red are proteins for which the mechanism of integrin inactivation requires further study. The α-integrins that the respective integrin activator or inactivator has been shown to bind in is given parentheses. The α-integrin-interacting proteins are SHARPIN (Rantalä et al., 2011), Rab21 and p120RasGAP (also known as RAS p21 protein activator, RASA1) (May et al., 2011; Pellinen et al., 2006), GIPC1 (Valdembri et al., 2009), MDGI (Nevo et al., 2010), (Barry et al., 2002) and nischarin (Alahari and Nasrallah, 2004).
integrin at the cell surface without affecting the total amount of integrins. SHARPIN binds to the highly conserved WKxGFFKR (Fig. 4) sequence present in the cytoplasmic tail of α-integrins (Rantala et al., 2011) and this interaction is required for SHARPIN-mediated integrin inactivation. The essential amino acids within the integrin cytoplasmic tail are WKxGFF (amino acids 1154–1159 in α2 integrin), whereas the KR residues (the lysine and arginine residues immediately adjacent to the WKxGFF sequence) are not required for SHARPIN binding (Rantala et al., 2011). This observation is compatible with a function of SHARPIN as an integrin inhibitor, as the arginine residue (R) within the α-tail WKxGFFKR sequence has been suggested to form a salt bridge (clasp) with the cytoplasmic tail of the β-integrin subunit, keeping the integrin in an inactive state (O’Toole et al., 1991).

Mechanistically, SHARPIN inhibits the binding of talin and kindlin to the cytoplasmic domain of β1-integrin (Rantala et al., 2011). Thus, on the one hand, the ‘inside-out’ integrin activation is controlled by the balance of counteracting forces that are exerted by SHARPIN (and possibly other integrin inhibitors), and on the other hand and talins and kindlins. It remains to be determined whether this inhibition is direct (through steric hindrance or by stabilising the integrin inactivating clasp) or indirect (for example by recruiting inhibiting kinases). In cells, SHARPIN colocalises with inactive, but not active, integrins in detached ruffles (Rantala et al., 2011), consistent with SHARPIN acting as an integrin-inactivating protein. SHARPIN binding to integrin α-subunits also inhibits α-β1-integrin-dependent functions, such as cell spreading and cell migration.

Epidermal homeostasis is regulated by integrin expression, and downregulation of β1 integrin expression in the suprabasal keratinocytes is a prerequisite for keratinocyte differentiation (Watt, 2002). Interestingly, the proliferative dermatitis phenotype of mice null for SHARPIN resembles that of the transgenic mouse models with forced suprabasal β1 integrin expression under the control of the involucrin promoter (Carroll et al., 1995). Thus, loss of the β1 integrin inactivator SHARPIN and overexpression of β1 integrin results in very similar physiological outcomes in vivo. In addition, the function of SHARPIN as a β1-integrin inhibitor in vivo is supported by the observation that keratin-14-positive keratinocytes from SHARPIN-null mice contain higher active β1-integrin levels than keratinocytes from wild-type mice (Rantala et al., 2011).

Importantly, the integrin inhibitory effect of SHARPIN is independent of the aforementioned other functions of SHARPIN. SHARPIN silencing induces integrin activation in PC3 cells, which are null for PTEN (Gustin et al., 2001) and lack EYA1 expression (Kilpinen et al., 2008). Furthermore, silencing of HOIP (the catalytic subunit of LUBAC) does not affect integrin activity in cells (Rantala et al., 2011). In addition, mice lacking HOIL1 (another member of the LUBAC complex) (i.e. after knockout of the HOIL1-encoding gene Rbck) do not have any obvious integrin-related phenotype (Tokunaga et al., 2009), indicating that SHARPIN has important, LUBAC-independent, functions in vivo.

The identification of SHARPIN opens a new paradigm in integrin regulation as it demonstrates that the dynamic switching between inactive and active integrin conformations is physiologically controlled in vivo by a protein that interacts with the α-subunit cytoplasmic domain. As SHARPIN is expressed in most human tissues and several cancer cell types (Rantala et al., 2011), and it binds several (and potentially all) α-integrins, SHARPIN might represent a general means for cells to suppress integrin activity.

**MDGI**

Mammary-derived growth inhibitor (MDGI, also known as FABP3) has been named after its origin from lactating bovine mammary glands and its growth inhibitory properties in human mammary carcinoma cell cultures (Böhmer et al., 1987). MDGI is almost ubiquitously expressed, and is particularly abundant in muscle and mammary gland (Haunerland and Spener, 2004). Recently, MDGI has been shown to interact with several different integrin α-subunits (α1, α2, α5, α6, α10 and α11), probably through the highly conserved WKxGFFKR sequence that is present in most, if not all, α-subunits (Nevo et al., 2010). MDGI overexpression reduces adhesion of cells to type I collagen and fibronectin and impairs both migration and invasion specifically in human breast cancer cell lines but not in other cell types (Nevo et al., 2010). These effects are due to an MDGI-induced significant reduction in the active β1integrin conformation on the cell surface as determined with conformation-sensitive antibodies against β1 integrin (Nevo et al., 2010). The exact molecular details of the function of MDGI as a negative regulator of integrin activity remain unsolved, but MDGI overexpression in MDA-MB-231 breast cancer cells reduces the association of kindlin with active β1 integrin, suggesting that MDGI binding to cytoplasmic integrin-α tails could indirectly hamper the binding of integrin agonists to integrin-β tails (Nevo et al., 2010). It remains unclear why these effects of MDGI on integrin activity are specific to breast cancer cells. One possibility is that they are linked to epidermal growth factor receptor (EGFR) overexpression, a common genetic alteration for breast cancer cells. MDGI influences EGFR trafficking so that the receptor is increasingly localised on endosomes (Nevo et al., 2009). As EGFR and β1 integrins participate in extensive crosstalk (Ivaska and Heino, 2011), the altered EGFR dynamics could also influence β1 integrin conformation in breast cancer cells. Regardless of the molecular details, the ability of MDGI to inhibit breast cancer cell invasion in vitro appears to be clinically relevant. Analysis of a tissue microarray of 1331 breast carcinomas revealed that patients with MDGI-positive tumours have a more favourable 10-year disease-free survival prognosis compared with that of patients with MDGI-negative tumours (Nevo et al., 2010).

**Integrin trafficking regulators that bind the integrin α-tail**

As discussed above, the conserved membrane-proximal segment of integrin α-tails is important for SHARPIN and MDGI binding and integrin inactivation. Interestingly, this binding site overlaps with the site on the integrin α-tails, at which the small GTPase Rab21 binds many integrin α–β1 heterodimers and subsequently induces their endocytosis (Pellinen et al., 2006). Furthermore, on endosomes, the GTPase-activating protein (GAP) p120RasGAP (also known as RASA1) competes with Rab21 for integrin binding and regulates the recycling of the receptor to the plasma membrane (Mai et al., 2011). The possible selectivity of Rab21 (and p120RasGAP) for active integrins has not been investigated but, as in the case of the clathrin adaptor Numb, one could envision a scenario, in which integrin activity regulators (for example talin and SHARPIN) compete with endocytosis regulators (Numb and Rab21) for integrin binding. This would provide a mechanism to control the relative amounts of active and inactive integrin receptors on the cell surface.
In endothelial cells, endocytosis of the active α5β1 integrin is also specifically regulated by the α5 tail interacting homomultimeric endocytic adaptor GAIP interacting protein C terminus member 1 (GIPC1). In addition to the α5β1 integrin, GIPC1 also binds to Neuropilin-1 (Nrp-1) and Myosin-6, and formation of this complex triggers endocytosis of the active α5β1-integrin into Rab5-positive endosomes (Valdemori et al., 2009). This results in increased adhesion of endothelial cells to fibronectin, in line with the previously established link between increased integrin traffic and faster cell spreading and adhesion (Pellinen et al., 2006). At present it is not clear how stimulation of endothelial cells with Nrp-1 ligands triggers active-conformer specific endocytosis. However, the selective uptake of the active receptor from the cell surface is absolutely dependent on Neuropilin-1 as endocytosis of the inactive α5β1 is Neuropilin-1 independent. This suggests that in addition to the ability of cells to modulate their adhesion receptor activity on the plasma membrane via ‘inside-out’ signalling, selective endocytosis of a specific integrin conformer may be an important mechanism to regulate the availability of active integrins at the cell surface in a spatially controlled or polarised manner. In addition, very recent data indicate that active and inactive β1-integrins also use distinct recycling pathways to return to the plasma membrane (Arjonen et al., 2012).

Integrin inhibitors binding to specific α-subunits

There are a few proteins that have been described to bind to a specific integrin α-subunit and inhibit integrin function. However, the negative regulatory role of these proteins is controversial, and data demonstrating their actual regulation of integrin activity on the cell surface is lacking. Nevertheless, these examples are discussed here briefly as integrin-heterodimer-specific regulation of integrin activity is an understudied area and at present better examples are lacking.

Nischarin is a ubiquitously expressed cytosolic protein that interacts with the cytoplasmic domain of integrin α5 (Alahari et al., 2000). The interaction involves the α5 membrane proximal sequence IYLILYKLGFFKRSL (residues 1017–1030), for which residues Tyr1018 and Lys1022 are crucial (Alahari and Nasrallah, 2004). As these residues are relatively conserved in other integrin α-subunits it is possible that nischarin could also interact with other integrin-α tails; however, this has not been studied. Overexpression of nischarin inhibits both cell migration and invasion, but not adhesion to fibronectin (Alahari et al., 2000; Alahari, 2003). This is owing to alterations in Rac activation and the organisation of peripheral actin filaments in nischarin-overexpressing cells. Overexpression of the integrin α5 subunit enhances coprecipitation of the serine/threonine-protein kinase PAK1 [p21 protein (Cdc42/Rac)-activated kinase 1] with nischarin, which inhibits PAK1 kinase activity (Alahari and Nasrallah, 2004). This suggests that α5 has a role in the localised control of PAK1 function. Although it appears that nischarin inhibits integrin-dependent processes, data that formally address the effect of nischarin expression on integrin activation are missing. Interestingly, recently nischarin expression has been shown to correlate with low-grade less-aggressive tumours in breast cancer and with reduced tumour growth and lung metastasis in a nude mouse model (Baranwal et al., 2011), which could be linked to reduced integrin activity.

Fine-tuned platelet aggregation is responsible for physiological homeostasis and aberrant platelet function can lead to pathological thrombosis. The ubiquitously expressed calcium- and integrin-binding protein 1 (CIB1) has a key role in the regulation of the platelet integrin αIIbβ3. CIB1 interacts directly with the cytoplasmic domain of αIIb (Naik et al., 1997), of which the αIIb membrane-proximal sequence LVLAMWKFGFFKRN (residues 983 to 997) forms a minimal-binding domain for CIB1 (Barry et al., 2002). Residues Leu983, Trp988, Phe992 and Ca2+-binding are crucial for the complex formation (Barry et al., 2002; Shock et al., 1999). CIB1 has been reported to function as an endogenous inhibitor of agonist-induced αIIbβ3 integrin activation (Yuan et al., 2006). However, CIB1 has several other binding partners apart from integrins and they might explain the variable roles that have been described for the CIB1–αIIb complex. For example, in contrast to the proposed role as an integrin inactivator, CIB1 has been shown to activate αIIbβ3 integrin ‘inside-out’ signalling by converting the integrin from the resting state into its active conformation (Tsuboi, 2002) and to have an important role in platelet spreading on immobilised fibrinogen (Naik and Naik, 2003). Finally, the physiological consequences of loss of CIB1 (in knockout mice) are impaired thrombosis and increased mouse tail-bleeding time (Naik et al., 2009), suggesting that in vivo CIB1 functions as a positive regulator of αIIbβ3.

Serine-threonine phosphatase 2A (PP2A) has been shown to interact directly with the membrane-proximal KVGFFKR sequence of αIIb integrin (Gushiken et al., 2008). In human embryonal kidney (HEK) 293 cells, PP2A overexpression decreases αIIbβ3-mediated adhesion to immobilised fibrinogen (Gushiken et al., 2008). By contrast, PP2A association with α1 integrin followed by dephosphorylation of crucial residues within the β1 integrin cytoplasmic tail is required for integrin localisation to focal contacts and subsequent activation of downstream signalling (Kim et al., 2004; Mulrooney et al., 2000).

Taken together, at present there are no clear examples of proteins binding to specific α-subunits as bona fide integrin inhibitors. For all the examples discussed above there are data indicating context dependent roles for these proteins in both integrin inactivation and activation.

Matrix metalloproteinase 8 (MMP8)

As described here, integrin activity can also be regulated by extracellular proteins. Recently, we reported an siRNA screen that revealed new genes that were involved in regulating β1 integrin activity (Pellinen et al., 2012). One particularly interesting finding is that the secreted collagenase MMP8 is a negative regulator of β1 integrin activity in several prostate cancer and one breast cancer cell line. MMP8 has been previously associated with tumour suppression owing to its anti-inflammatory or anti-metastatic role in several cancers (Dejonckheere et al., 2011). The increase in integrin activity caused by MMP8 silencing is counteracted by the addition of recombinant MMP8 to the growth medium and correlates with increased in vitro invasion through Matrigel (Pellinen et al., 2012). In a mouse metastasis assay, MMP8-silenced cancer cells extravasate to the lungs to a much greater extent than do control cells. In addition, MMP8 co-precipitates with β1 integrin, implying that binding of MMP8 to the extracellular part of the β1 integrin could modulate integrin activity, although we cannot rule out that MMP8 binds to integrin indirectly through its interactions with collagen. It is also possible that the collagenase activity of MMP8 modifies the integrin ligand collagen in a way...
that abrogates integrin binding. In the future, it will be interesting to study the exact mechanism underlying the effect of MMP8 on integrin activity and how it might be related to the anti-inflammatory role of MMP8.

**In vivo roles of integrin activators and inactivators**

Above we have discussed several integrin activity inhibitors which all have the ability to induce the inactive integrin conformation on the cell surface, albeit the mechanisms involved differ between the proteins. In cells, these inactivators are counteracting the integrin-activating talin and kindlin proteins such that switching of the integrins between active and inactive states is inhibited. However, a more complex picture emerges when the phenotypes of mice lacking these activators or inactivators are compared (Table 2). In general, mice lacking the integrin activators (especially talins) appear to have a much stronger phenotype than mice lacking integrin inactivators. This might be explained by the relatively large number of integrin activators compared with that of integrin inactivators. In addition, this could indicate that integrin inactivators have more redundant roles than activators in vivo. Furthermore, differences in the expression profiles and tissue distribution could account for some of the relatively mild phenotypes, such as for example in *ecap1*-knockout mice, in which it is mostly bone formation that appears to be affected (Bouvard et al., 2007), or in the kindlin-3 (*Fermi3*)-knockout mouse, in which only leukocytes are affected (Moser et al., 2008). Another complication is that several integrin inhibitors also have integrin-independent functions, such as SHARPIN (Gerlach et al., 2011; He et al., 2010; Ikeda et al., 2011; Landgraf et al., 2010; Tokunaga et al., 2011) and filamins (for a review, see Zhou et al., 2011), which will also contribute to the overall phenotype observed. Finally, mice lacking one integrin inactivator might compensate by increasing the expression or activity of other activators, albeit examples of this remain to be identified. Clearly, unravelling the in vivo roles of integrin inhibitors is an important challenge for the future.

**Concluding remarks**

In this Commentary, we have aimed to present the known mechanisms of integrin activation and inactivation with a main focus on the less-well understood role of integrin inactivating proteins. Many of the molecular details regarding the mechanism of integrin inactivation remain to be investigated for proteins such as SHARPIN, MDGI and others. However, one feature appears to be shared by many of these inactivating proteins. Unlike the best-studied integrin activator talin, which provides the integrins with a link to the actin cytoskeleton, integrin inactivators appear to uncouple integrin receptors from the cytoskeleton. Rapid actin-based movement has been shown to transport active but unengaged integrins (i.e. integrins binding talin but not ECM ligands) along the leading edge to newly formed protrusions (Galbraith et al., 2007), suggesting that the final targeting of the active integrin receptor to adhesion sites is driven by actin. In cells, inactive integrins are often found located in detached ruffles (Rantalai et al., 2011) or dorsally (Teckchandani et al., 2009), and uncoupling from the actin cytoskeleton would probably allow for a more rapid and free diffusion of integrins along the membrane to areas in which activation of integrins and cell adhesion are triggered. Surprisingly little is known about the switch between active and inactive integrin conformations, and examples of heterodimer-specific regulation of integrin activity are particularly sparse. Specific integrin heterodimers have very different biological functions in vitro and in vivo (Hynes, 2002), with the consequence that animals lacking a specific integrin display diverse phenotypes that span from early embryonic lethal to very mild defects. On the basis of this finding, it is plausible to assume that mechanisms that specifically inactivate a particular x-β-heterodimer exist. The identification of these mechanisms, or of regulators of specific integrins, will in the future allow the elucidation of additional layers of complexity to the fine-tuned regulation of integrin function in man.

### Table 2. Overview of some of the phenotypes observed in knockout mice for integrin activators and inactivators

<table>
<thead>
<tr>
<th>Protein</th>
<th>Phenotype</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAP1</td>
<td>Reduced osteoblast proliferation and delayed bone mineralisation</td>
<td>(Bouvard et al., 2007)</td>
</tr>
<tr>
<td>Filamin A</td>
<td>Incomplete septation of the heart during gestation</td>
<td>(Feng et al., 2006; Hart et al., 2006)</td>
</tr>
<tr>
<td>Filamin B</td>
<td>Boomerang Dysplasia; lethal early embryonic or postnatal</td>
<td>(Bicknell et al., 2007)</td>
</tr>
<tr>
<td>Filamin C</td>
<td>Reduced birth weight and respiratory failure shortly after birth</td>
<td>(Dalkilic et al., 2006)</td>
</tr>
<tr>
<td>MDGI</td>
<td>No overt phenotype in the mammary gland (only tissue examined in knockout or overexpressing mice)</td>
<td>(Binas et al., 1995; Clark et al., 2000)</td>
</tr>
<tr>
<td>Talin1</td>
<td>Arrest mouse development at gastrulation stage</td>
<td>(Monkley et al., 2000)</td>
</tr>
<tr>
<td>Talin2</td>
<td>Myopathy with centrally nucleated fibres</td>
<td>(Conti et al., 2009)</td>
</tr>
<tr>
<td>Kindlin1</td>
<td>Skin atrophy and lethal neonatal intestinal epithelial dysfunction</td>
<td>(Usur et al., 2008)</td>
</tr>
<tr>
<td>Kindlin2</td>
<td>Impaired angiogenesis</td>
<td>(Pluskota et al., 2011)</td>
</tr>
<tr>
<td>Kindlin3</td>
<td>Impaired leukocyte adhesion to endothelial cells</td>
<td>(Moser et al., 2008)</td>
</tr>
<tr>
<td>SHARPIN</td>
<td>Multiorgan inflammation, eosinophilic proliferative dermatitis, epidermal hyperplasia, dermal granulocytic infiltration, reduced osteoblast proliferation</td>
<td>(Li et al., 2011; Seymour et al., 2007; Xia et al., 2011)</td>
</tr>
</tbody>
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**Funding**

This study has been supported by Academy of Finland, ERC Starting Grant, Sigrid Juselius Foundation and Finnish Cancer Organizations. J.P is a recipient of the Finnish Academy postdoc and Cancer Institute postdoctoral grants.

**References**


Integrin inactivators


