Journal of Thrombosis and Haemostasis, **5**: 1345–1352

REVIEW ARTICLE

Platelet integrin $\alpha_{IIb}\beta_3$: activation mechanisms

Y.-Q. MA, J. QIN and E. F. PLOW

Department of Molecular Cardiology, Joseph J. Jacobs Center for Thrombosis and Vascular Biology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA

To cite this article: Ma Y-Q, Qin J, Plow EF. Platelet integrin $\alpha_{IIb}\beta_3$: activation mechanisms. *J Thromb Haemost* 2007; **5**: 1345–52.

Summary. Integrin $\alpha_{IIb}\beta_3$ plays a critical role in platelet aggregation, a central response in hemostasis and thrombosis. This function of $\alpha_{IIb}\beta_3$ depends upon a transition from a resting to an activated state such that it acquires the capacity to bind soluble ligands. Diverse platelet agonists alter the cytoplasmic domain of $\alpha_{IIb}\beta_3$ and initiate a conformational change that traverses the transmembrane region and ultimately triggers rearrangements in the extracellular domain to permit ligand binding. The membrane-proximal regions of α_{IIb} and β_3 cytoplasmic tails, together with the transmembrane segments of the subunits, contact each other to form a complex which restrains the integrin in the resting state. It is unclasping of this complex that induces integrin activation. This clasping/unclasping process is influenced by multiple cytoplasmic tail binding partners. Among them, talin appears to be a critical trigger of $\alpha_{IIb}\beta_3$ activation, but other binding partners, which function as activators or suppressors, are likely to act as co-regulators of integrin activation.

Keywords: $\alpha_{IIb}\beta_3$, integrins, platelets.

Introduction

Integrins are a large family of adhesion receptors that mediate cell-extracellular matrix (ECM) and cell–cell interactions. Occupancy of integrins by ECM ligands or counter-receptors induces multiple intracellular signaling pathways, which play critical roles in regulating complex cellular responses [1]. $\alpha_{IIb}\beta_3$, the major integrin on platelets, is essential for platelet aggregation, and, thereby, is centrally involved in hemostasis and thrombosis [2,3]. The function of $\alpha_{IIb}\beta_3$ in platelet aggregation depends upon its capacity to undergo activation, a transition from a low to a high affinity state for its ligands. This transformation allows $\alpha_{IIb}\beta_3$ to bind fibrinogen and von Willebrand factor (VWF), ligands that can bridge platelets together, or other ligands, such as vitronectin and fibronectin, which can modulate aggregation. Activation of $\alpha_{IIb}\beta_3$ is tightly regulated through a process termed 'inside-out signaling', in which agonists, such as ADP and thrombin, which engage G-protein coupled receptors, or other adhesive proteins, such as collagen or VWF, which engage GPIb-IX-V or GPVI, initiate intracellular signaling events that ultimately impart ligand competency to the extracellular domain of $\alpha_{IIb}\beta_3$. This review focuses on the inside-out signaling events that render $\alpha_{IIb}\beta_3$ competent to bind ligands. The pathway of 'outside-in' signaling, which is induced by ligand binding to $\alpha_{IIb}\beta_3$, has been examined in other reviews (e.g. [4]).

Correspondence: Edward F. Plow, Department of Molecular Cardiology, Cleveland Clinic, 9500 Euclid Avenue, NB-50, Cleveland, OH 44195, USA.
Tel.: + 1 216 445 8200; fax: + 1 216 445 8204; e-mail: plowe@ccf.org

Received 18 January 2007, accepted 9 March 2007

Structural basis of integrin $\alpha_{IIb}\beta_3$ activation

All integrins, including $\alpha_{IIb}\beta_3$, are α/β heterodimers. Mature αIIb is composed of 1008 amino acids and β_3 of 762 amino acids. Each subunit consists of a large extracellular region, a single transmembrane spanning region and a short cytoplasmic tail. In a landmark publication, the Arnaout group solved crystal structures of the extracellular domain of $\alpha_V\beta_3$, without and with a bound RGD ligand [5,6]. The extracellular domain of the α-subunit is composed of the N-terminal β-propeller domain, the thigh domain and two calf domains (Fig. 1). The extracellular portion of β_3 subunit is composed of an A domain, PSI (plexin/semaphorin/integrin) domain, hybrid domain, four EGF domains and a membrane-proximal βTD domain [5]. The 12 domains of the α and β subunits assemble into an ellipsoid 'head' and two 'tails'. The head is formed by interaction of the seven-bladed β-propeller of the α-subunit and the β_3A domain (Fig. 1).

Changes in the extracellular domain

The crystal structures of $\alpha_V\beta_3$ and subsequent crystal structures of portions of the extracellular domain of $\alpha_{IIb}\beta_3$ [7] document the similarities in organization and ligand binding mechanism of the two β_3 integrins. Bound ligands nestle into the interface between the β-propeller and the A domain [5–7].

While crystal structures provide solutions of integrin structures, the snapshots do not necessarily capture the conformational intermediates or final products, and, hence, the structural mechanism underlying ligand binding remains uncertain. In the presence of Ca^{2+}, the crystal structure of the extracellular domain of $\alpha_V\beta_3$ was severely bent at the 'genu',

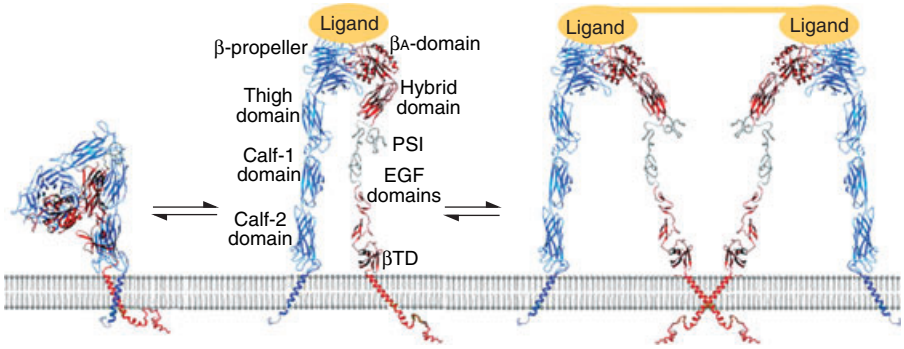

Fig. 1. Structural organization and activation of β_3 integrins. The crystal structure of the extracellular domain, a molecular model of the transmembrane region and NMR structures of the cytoplasmic tails have been adjoined to create a depiction of $\alpha_{IIb}\beta_3$. An equilibrium between a bent and an extended form illustrates the 'switchblade' hypothesis of activation [8,95].

forming a compact 'V' shape. In the presence of Mn^{2+}, a cation that activates integrins, or a ligand peptide, most $\alpha_{IIb}\beta_3$ observed by electron microscopy were in an extended conformation [8]. These observations gave rise to the 'switchblade' hypothesis, in which the extended conformation represents the active state and the bent form is the resting state (Fig. 1), and were supported by disulfide cross-linking experiments showing that stabilization of the bent conformation prevented integrin activation [8]. Nonetheless, a subsequent electron microscopic study using $\alpha v\beta_3$ and a fibronectin fragment indicated that the bent conformation was also capable of binding an activation-dependent ligand [9]. Furthermore, most micrographs of $\alpha_{IIb}\beta_3$ failed to detect a severely bent conformation [10–14].

There is long-standing literature suggesting that disulfide bond rearrangements may be involved in integrin activation, and differences in the free thiol content were found between resting and activated $\alpha_{IIb}\beta_3$ [15]. Protein disulfide isomerase [16] or disulfide isomerase activity intrinsic to $\alpha_{IIb}\beta_3$ [17] have been proposed as mediators of such disulfide rearrangements, but how the generation of free thiols integrates into the structure of integrins and inside-out signaling remains uncertain.

Changes in the transmembrane (TM) domain

The TM of α_{IIb} and β_3 are predicted to form helices, and the two helices are believed to interact with each other in the resting state [14,18], and several mutagenesis studies support this model [19–21]. Recently, it has been shown that peptide mimetics of the α_{IIb}TM induce $\alpha_{IIb}\beta_3$-mediated platelet aggregation, supporting the role of the TM in activation of the integrin [22]. In addition to changes in heterodimerization, the TM of α_{IIb} or β_3 subunit have a propensity to oligomerize [23–25]; multimeric forms of the α_{IIb} and β_3 TM have been detected by ultracentrifugation and gel analyses. Such interactions may contribute to oligomerization of integrin $\alpha_{IIb}\beta_3$ [26], which may in itself induce integrin activation [27].

Changes in the cytoplasmic tail (CT) domain

The structures of the individual CT in aqueous solution and/or membrane mimetic micelles have been examined by various biophysical approaches, including circular dichroism [28,29] and nuclear magnetic resonance (NMR) [30,31]. These studies have detected helices in the membrane-proximal regions of both α_{IIb} and β_3 CT within membrane environments, in aqueous solution with helix promoting sequences, mimicking extension of the TM helices, or in helix promoting agents. Furthermore, in aqueous solution, when the two CT are added together, a complex forms between their membrane-proximal helices [32,33]. This interaction is maintained by multiple hydrophobic and electrostatic interactions [32], including a salt bridge between $\alpha_{IIb}R^{995}$ and β_3D^{723} that was predicted by mutational analyses [34](see Fig. 2A). The 'clasp' between the membrane-proximal α_{IIb} and β_3 CT helices must be weak and was not detected by other groups using similar approaches [35]. However, a series of independent mutational studies support the existence of the clasp between the membrane proximal CT regions, which stabilizes the integrin in a resting state. Selected point mutations that disrupt the hydrophobic or electrostatic bonds between the α_{IIb} and β_3 CT lead to activation of $\alpha_{IIb}\beta_3$ [36–39]. Indeed, disruption of both the electrostatic and hydrophobic bonds is necessary for full activation of $\alpha_{IIb}\beta_3$[39] (see Fig. 2B).As the membrane proximal sequences within the clasp are highly conserved among integrins, it is likely that changes in the association-dissociation equilibrium of the clasp provide a general mechanism for regulating integrin activation.

The membrane distal region of the α_{IIb}CT has two significant structural features, a divalent ion binding site [28] and a turn formed by $NRPP^{999}$ [30]. The significance of both of these structural features remains uncertain. Mutational analyses in which PP^{999} were substituted with AA have led to variable results, ranging from activation [40] to minimal activation [36,39]. Termination of the C-terminus of α_{IIb}CT at residue R^{995} also led to minimal activation [36,39]. The latter results are consistent with NMR studies that showed minimal effects of the PP mutations on the membrane proximal clasp [30]. However, other mutational studies [41], as well as independent approaches, do suggest a role of the C-terminus of α_{IIb}CT in $\alpha_{IIb}\beta_3$ activation [30,42]. Clearly, further investigation is needed.

The membrane-distal region of β_3CT contains two NXXY motifs, sequences that induce a turn structure, are important to

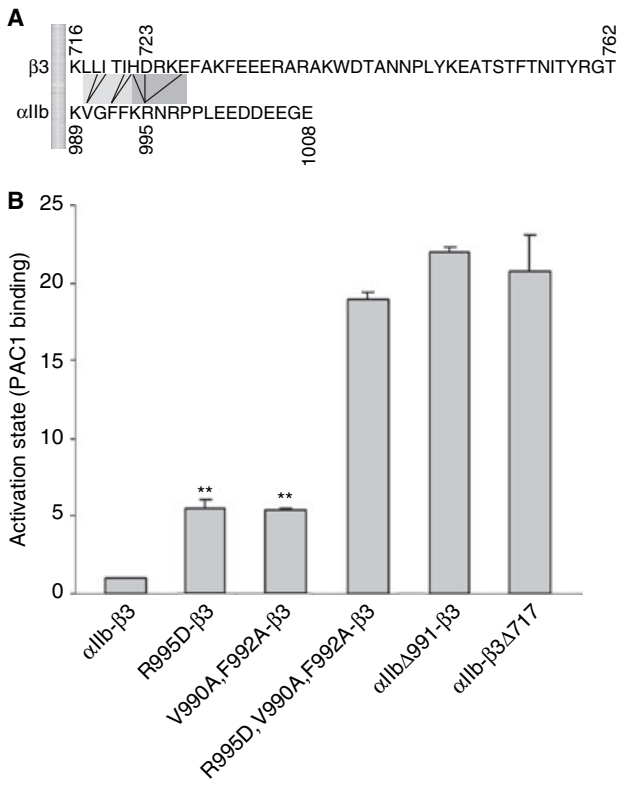

Fig. 2. Unclasping of the $\alpha_{IIb}\beta_3$ membrane-proximal CT complex regulates integrin activation. Nuclear magnetic resonance (NMR) detects both hydrophobic and electrostatic bonds between the α_{IIb} and β_3 cytoplasmic tails (A). Disruption of both classes of non-covalent interactions by mutations in the α_{IIb}CT (electrostatic by $R^{995}D$, hydrophobic by $V^{990}A$, $F^{992}A$, or both by $R^{995}D$, $V^{990}A$, $F^{992}A$) is required for the integrin to reach a fully activated state as defined by the extent of activation attained with deletions (Δ) of the α_{IIb} or β_3 CT (B). Modified from reference 39.

the functions of many receptors, and are highly conserved in integrin β subunits. NXXY motifs serve recognition sites for proteins containing phosphotyrosine binding (PTB) domains, which include many intracellular signaling proteins [43,44]. A major structural feature of the β_3CT structure is a long membrane-proximal helix (K^{716}-A^{735}), which extends beyond the membrane proximal clasping helix. The helix is followed by a flexible loop [31,45], which terminates at the first NXXY motif, $NPLY^{747}$, that forms an inverse turn followed by the second helix region intervening between the first and second NXXY motif, a $NITY^{759}$. The significance of the C-terminal structures has been demonstrated by the effects of $Y^{747}A$ and $S^{752}P$ mutations, which disrupt the C-terminal structures and impair integrin activation [39,46–48]. The structural features of the β_3CT are observed in membrane mimetic micelles and are less apparent in aqueous solution [35]. The fact that a naturally occurring $S^{752}P$ mutation induces Glanzmann thrombasthenia in which $\alpha_{IIb}\beta_3$ does not activate and that this effect can be explained by disruption of the C-terminal helix, supports the validity of the structure observed in micelles. An additional structural feature of the β_3CT observed in micelles is a second membrane contact in addition to its N-terminal insertion. This second contact occurs in the vicinity of the $NPLY^{747}$. The significance of this second insertion is uncertain. However, mutations of the $NPLY^{747}$, which are often interpreted in terms of disruption of its interactions with PTB binding partners, may be due to disruption of this second membrane contact [31].

Post-translational modifications of β_3 cytoplasmic tail

Post-translational modifications occur in the membrane-distal region of β_3CT. The modifications include tyrosine and/or threonine phosphorylations and cleavages by calpain. Functional effects have been ascribed to each of these changes. Phosphorylation of both tyrosine residues, Y^{747} and Y^{759}, in the β_3CT occurs. Platelet aggregation or adhesion to immobilized fibrinogen can trigger phosphorylation of these tyrosines [49,50]. Studies of the DiYF mice in which the two tyrosine residues of β_3CT are replaced by phenylalanines show impaired platelet responses dependent on outside-in signaling across $\alpha_{IIb}\beta_3$ [51]. For the most part, inside-out signaling is unaffected in platelets from these mice [52]. β_3CT undergoes cleavage by activated calpain in aggregated platelets. Four calpain cleavage sites were mapped [53], and tyrosine phosphorylation regulates the susceptibility of these sites to cleavage [54].

Threonine phosphorylation at T^{751} and T^{753} has been detected during platelet activation [55–57]. Currently, the regulatory role of these modifications is not well understood. However, inhibitors of threonine/serine kinases have negative effects on integrin activation, and T^{753} phosphorylation appears to regulate Shc binding [58,59].

Integrin $\alpha_{IIb}\beta_3$ cytoplasmic tail binding partners

With no intrinsic enzymatic activity ascribable to the CT, cytoplasmic proteins that bind to the CT must play a critical role in initiating and propagating the bidirectional signaling events across $\alpha_{IIb}\beta_3$. More than 20 cytoplasmic proteins have been identified as binding partners of the α_{IIb} or β_3 CT (Table 1) [60]. Most of the binding partners have been linked to outside-in signaling. At present, the one interacting molecule that has been most firmly linked to $\alpha_{IIb}\beta_3$ activation is talin. Talin is an abundant cytosolic protein (3% of total platelet protein) capable of linking integrins to the actin cytoskeleton and plays a critical role in integrin-mediated focal adhesion formation [61–63]. Talin's location changes from a uniform distribution to a plasma membrane localization during platelet activation [64]. Talin is composed of a 47 kDa head domain (talin-H) and a 190 kDa rod domain (talin-R). These align in an antiparallel arrangement to form a homodimer. Talin-H is composed of a FERM domain [62], which consists of three subdomains, F_1, F_2 and F_3 [65]. Multiple interactions of talin with the CT of $\alpha_{IIb}\beta_3$ have been demonstrated [66–69]. A crystal structure of the talin F_2-F_3 domain fused to a NPLY peptide has been published [68]. A second interaction of the talin F_3 domain with the membrane proximal region on β_3CT has been detected [32,70,71], and a structure of the F_3 domain in complex with a membrane-proximal β_3CT peptide was

Table 1 Integrin αIIbβ3 associated partners

Proteins/class*	Binding site
Cytoskeletal proteins	
Talin	β3
Myosin	β3-P
Skelemin	β3
Filamin	β3
α-actinin	β3
F-actin	β3
Adaptor and signaling proteins	
Paxillin	β3
Shc	β3-P
Grb2	β3-P
Protein kinases and phosphatase	
Src	β3
Csk	β3
Syk	β3
ILK	β3
FAK	β3
PP1c	αIIb
Others	
BiP	αIIbβ3
Calreticulin	α(IIb)
CIB	αIIb
Icln	αIIb
β3-endonexin	β3
Aup1	αIIb
Protein phosphatase 1	IIb
Transmembrane proteins	
CD9	
CD36	
CD47/IAP	
CD63	
CD98	
CD151	
CD31	

*Because of length constraints, reference citations cannot be given. We apologize to authors for this necessity. The table is adapted from reference 60.

published recently [72]. Talin-PTB appears to recognize the $NPXY^{747}$ motif and is believed to subsequently interact with the membrane-proximal region of β_3CT. The membrane proximal binding site of talin in the β_3CT overlaps extensively with the α_{IIb} binding site and can displace the α_{IIb}CT from the β_3CT, initiating integrin activation [32]. Although the PTB-like domain of talin-H that binds to the $NPXY^{747}$ motif, is homologous to PTB domains in other proteins, such as Dok, Numb or Kindlerin, which also can bind to the $NPLY^{747}$ motif [67,73], these other binding partners lack a structure unique to talin-H that interacts with the membrane-proximal region [72]. The talin story is far from fully unraveled. A binding site for the β_3 CT also is localized in the C-terminal region of rod domain [68,71,74], and cooperative binding of the talin-H and talin-R domains to the β_3CT has been suggested [75]. In addition, it has been suggested that talin also associates with the α_{IIb} CT [70]; however, several other studies have failed to detect such interaction [32,68,71].

The β_3 CT binding site in talin-H is masked in intact talin, and an exposing event is required for talin-H to become an activator of $\alpha_{IIb}\beta_3$. Calpain cleavage [75], RIAM (Rap 1 interacting adaptor molecule) association [76] or phosphoinositide binding [77] are such exposing events. Calpain itself becomes activated as a consequence of increases in cytosolic Ca^{2+}, which occurs upon agonist stimulation of platelets, and cleavage of talin is detected within activated platelets [78]. Alternatively, activation of Rap1 via the PKC pathway facilitates RIAM, an effector of Rap1, to associate with talin, supporting talin binding to $\alpha_{IIb}\beta_3$.[76] The integrin binding sites in talin can also be exposed by its binding of phosphoinositide $PI4,5P_2$ [77]. These events permit the talin-H domain to bind to the $NPLY^{747}$ motif and the membrane-proximal region of β_3 tail to displace α_{IIb}CT from β_3CT and initiate integrin activation [32,68,71].

Multiple cytoplasmic proteins can associate with β_3CT (see Table 1). Besides talin, several other β_3CT-associated cytoskeletal proteins, α-actinin, myosin and filamin, can interact [50,61,79–82]. As the platelet cytoskeleton plays a role in $\alpha_{IIb}\beta_3$ activation [83], proteins that can link the integrin to the actin cytoskeleton may play a role in inside-out signaling in addition to their more well-defined roles in outside-in signaling. Some of the associations are regulated by post-translational modifications of the β_3CT. For example, GRB2 or myosin binding to β_3CT requires both Y^{747} and Y^{759} phosphorylation [49,50]; SHC association with β_3CT is Y^{759} phosphorylation dependent [49,84] and can be perturbed by T^{753} phosphorylation [59]. Filamin interaction with the threonine-rich region between two NXXY motifs can be inhibited by threonine phosphorylation. Interestingly, c- Src kinase can directly associate with β_3CT in platelets [85], suggesting a potential role in regulating other β_3CT associations.

Several α_{IIb} tail binding proteins have been identified (Table 1). All are present in platelets, appear to bind to the membrane-proximal region of the α_{IIb} CT, and, hence, could play a role in regulating integrin activation. CIB is the most extensively characterized of these but has been reported to be both an inhibitor [86] and an inducer [87] of $\alpha_{IIb}\beta_3$ activation. As it is unlikely that these binding partners could associate simultaneously with this narrow sequence of α_{IIb}, temporal engagement must be envisioned if they are to play a role in activation.

In addition to cytoplasmic proteins, several membrane proteins also associate with $\alpha_{IIb}\beta_3$ (see Table 1). Of these, CD98 is most strongly implicated in integrin activation. This was demonstrated by a genetic screening approach seeking proteins that reversed the inhibitory effect of the β_{1A} CT on $\alpha_{IIb}\beta_3$ activation [88].

Besides these numerous protein partners, the platelet membrane bilayer may regulate integrin activation[31]. The membrane-proximal regions of the α_{IIb} and β_3 CT are hydrophobic and their positioning in the cytosol or within the membrane can vary[89]. Movement of these proximal sequences into or out of the membrane may be part of the activation mechanism. In addition, the β_3 CT region containing the NPLY motif also binds to DPC (dodecylphosphocholine), providing an additional site at which the platelet membrane may regulate integrin activation.

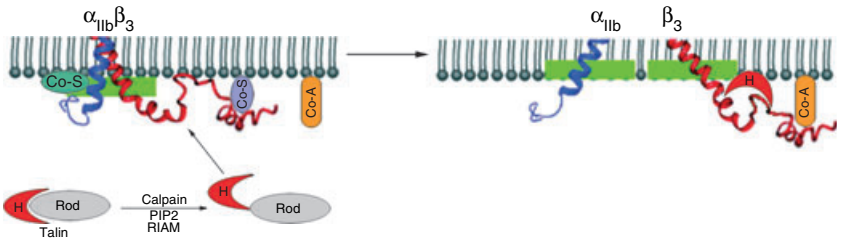

Fig. 3. Model for integrin activation. Activation is initiated by the talin-H, which binds to the NPLY[747] and a site within the membrane proximal region, to unclasp the CT complex. Activation is influenced by co-activators (Co-A), which cooperate with talin-H, or suppressors (Co-S) of activation. Separation of the CT complex may induce membrane insertion and trigger separation of the TM complex, propagating the inside-out signal to the extracellular domain.

In addition to the interest in defining a basic molecular mechanism, the activation of $\alpha_{IIb}\beta_3$ represents a potential therapeutic target that might have a more favorable profile than inhibitors of ligand binding to the receptor. Such considerations have provided and are likely in the future to provide an impetus for proteomic approaches to search for additional binding partners and regulators of $\alpha_{IIb}\beta_3$ activation [90]. A recent application of such a strategy has identified peptides derived from CD226 as inhibitors of inside-out signaling [91].

Model of $\alpha_{IIb}\beta_3$ activation

$\alpha_{IIb}\beta_3$ activation on platelets can be initiated by engagement of various agonist receptors. The multiple signaling cascades that are induced converge at the CT of $\alpha_{IIb}\beta_3$. Based on current data, integrin activation at the CT entails a clasping/unclasping mechanism (see Fig. 3). The clasp is formed between helices on the α_{IIb} and β_3 subunits and is predicted to extend into the TM helices. Unclasping initiated in the CT and extending into the TM provides the driving force to trigger the conformational changes in the extracellular domain. Upon exposure of the talin-H by any one of a series of events (see above), it can interact with the β_3CT and initiate unclasping. Talin-H interacts with the NPLY[747] motif and the membrane-proximal helix of the β_3CT, displacing the α_{IIb}CT from the clasp. It is unclear whether an interaction of the talin-R domain with β_3CT or talin-H with the α_{IIb}CT influences activation. Nonetheless, unclasping appears to be the event that initiates activation.

Clasping/unclasping is an equilibrium-controlled process, implying reversibility and a potential return to the resting state. Talin-H favors unclasping and its continued association with the CT would impede return to the resting state, thereby pushing the equilibrium toward the activated state. Although talin is clearly implicated in the activation process, several lines of evidence indicate critical roles for other binding partners, both working cooperatively with talin-H as co-regulators and as suppressors of integrin activation. Observations supporting a mechanism more complex than mediated by talin-H alone are: (i) the C-terminus of β_3CT, far beyond the talin-H binding sites, is critical for inside-out as well as outside-in signaling [92]; (ii) some point mutations, such as $S^{752}P$ in the β_3CT, which have no effect on talin-H binding and its unclasping function, still dramatically impair integrin activation [39]; (iii) over-expression of talin-H does not achieve full activation of $\alpha_{IIb}\beta_3$, relative to that induced by deletion of either the α_{IIb} or β_3 CT [39]; (iv) mathematical modeling predicts that talin alone is insufficient to enable ligand-dependent integrin activation [93]and (v) intuitively, other CT binding partners, which may not possess the molecular features needed to initiate unclasping, may still bind and/or displace talin. One such potential co-regulator may be β_3-endonexin, a 111 amino acid protein present in platelets, which binds to the C-terminal NITY[759] motif [94]. On the other hand, filamin A binds to the distal region the β_3CT and appears to function as a suppressor of $\alpha_{IIb}\beta_3$ activation [82]. Thus, roles for both multiple activators and suppressors, collectively co-regulators, all evolving from direct interactions with the CT, must be incorporated into the model of $\alpha_{IIb}\beta_3$ activation (see Fig. 3). In simplistic terms, their functions can be to shift the equilibrium in one direction, the clasped, resting state, or the other, the unclasped, activated state.

How does unclasping lead to $\alpha_{IIb}\beta_3$ activation? One possibility is that the next event triggered by unclasping is insertion of the newly exposed sequences in the α_{IIb} and β_3CT, which contain several hydrophobic residues, into the membrane. NMR experiments in micelles show such an insertion of the α_{IIb} K^{898}-F^{993} and the β_3 K^{716}-I^{121} into the membrane [31]. This insertion would be energetically favorable, burying hydrophobic sequences, and the membrane itself would impede reclasping. The insertion could also provide the impetus to disrupt the associations between the α_{IIb} and β_3 TM helices, thereby propagating the inside-out signal. Interestingly, when modeling the talin-PTB/β_3 CT complex onto the membrane, Wegener *et al.* [72] also found that the membrane-proximal KLLITI should be embedded in the membrane bilayer in the activated state of the integrin. This is consistent with the previous NMR finding and suggestion [31] that membrane insertion might occur during the clasping/unclasping process. Also influencing the equilibrium between states may be the second membrane contact within the β_3CT. This contact may act as a damper of integrin activation by anchoring the β_3CT and suppressing spontaneous separation of the CT clasp. This contact overlaps extensively with the talin binding site at NPLY[747], such that talin could compete with the membrane to bind the β_3CT during activation.

Once traversing the TM, the inside-out signal induces conformational rearrangements that result in activation of ligand binding site in the head domain of $\alpha_{IIb}\beta_3$. The 'switchblade' motion of the head region, translocation of helix-7 within the β_3 A domain and repositioning of the PSI domain, may contribute to the generation of the activated state. As the activated state represents a shift in an equilibrium reaction, in a global sense, events that stabilize the activated state will favor movement of more molecules into the activated state. Hence, events that are usually attributed to outside-in signaling, such as integrin clustering, may also favor the conversion of resting $\alpha_{IIb}\beta_3$ into an activated state.

Acknowledgements

This review was supported by National Institutes of Health Grant PO-1 HL 073 311 and Y.-Q. M. is a recipient of a fellowship from AHA, Ohio Affiliate.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

References

1 Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell* 2002; **110**: 673–87.
2 Hodivala-Dilke KM, McHugh KP, Tsakiris DA, Rayburn H, Crowley D, Ullman-Cullere M, Coller BS, Teitelbaum S, Hynes RO. β_3-integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. *J Clin Invest* 1999; **103**: 229–38.
3 French DL, Seligsohn U. Platelet glycoprotein IIb/IIIa receptors and Glanzmann's thrombasthenia. *Arterioscler Thromb Vasc Biol* 2000; **20**: 607–10.
4 Shattil SJ, Newman PJ. Integrins: dynamic scaffolds for adhesion and signaling in platelets. *Blood* 2004; **104**: 1606–15.
5 Xiong JP, Stehle T, Diefenbach B, Zhang R, Dunker R, Scott DL, Joachimiak A, Goodman SL, Arnaout MA. Crystal structure of the extracellular segment of integrin alpha Vbeta3. *Science* 2001; **294**: 339–45.
6 Xiong JP, Stehle T, Zhang R, Joachimiak A, Frech M, Goodman SL, Arnaout MA. Crystal structure of the extracellular segment of integrin alpha Vbeta3 in complex with an Arg-Gly-Asp ligand. *Science* 2002; 296:151–55.
7 Xiao T, Takagi J, Coller BS, Wang JH, Springer TA. Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. *Nature* 2004; **432**: 59–67.
8 Takagi J, Petre BM, Walz T, Springer TA. Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling. *Cell* 2002; **110**: 599–11.
9 Adair BD, Xiong JP, Maddock C, Goodman SL, Arnaout MA, Yeager M. Three-dimensional EM structure of the ectodomain of integrin {alpha}V{beta}3 in a complex with fibronectin. *J Cell Biol* 2005; **168**: 1109–18.
10 Carrell NA, Fitzgerald LA, Steiner B, Erickson HP, Phillips DR. Structure of human platelet membrane glycoproteins IIb and IIIa as determined by electron microscopy. *J Biol Chem* 1985; **260**: 1743–9.
11 Weisel JW, Nagaswami C, Vilaire G, Bennett JS. Examination of the platelet membrane GPIIb-IIIa complex and its interaction with fibrinogen and other ligands by electron microscopy. *J Biol Chem* 1992; **267**: 16637–43.
12 Hantgan RR, Paumi C, Rocco M, Weisel JW. Effects of ligand-mimetic peptides Arg-Gly-Asp-X (X = Phe, Trp, Ser) on alphaIIbbeta3 integrin conformation and oligomerization. *Biochemistry* 1999; **38**: 14461–74.
13 Litvinov RI, Nagaswami C, Vilaire G, Shuman H, Bennett JS, Weisel JW. Functional and structural correlations of individual alphaIIbbeta3 molecules. *Blood* 2004; **104**: 3979–85.
14 Adair BD, Yeager M. Three-dimensional model of the human platelet integrin alpha IIbbeta 3 based on electron cryomicroscopy and x-ray crystallography. *Proc Natl Acad Sci USA* 2002; **99**: 14059–64.
15 Yan BX, Smith JW. A redox site involved in integrin activation. *J Biol Chem* 2000; **275**: 39964–72.
16 Essex DW, Li M. A polyclonal antibody to protein disulfide isomerase induces platelet aggregation and secretion. *Thromb Res* 1999; **96**: 445–50.
17 O'Neill S, Robinson A, Deering A, Ryan M, Fitzgerald DJ, Moran N. The platelet integrin $\alpha_{IIb}\beta_3$ has an endogenous thiol isomerase activity. *J Biol Chem* 2000; **275**: 36984–90.
18 Gottschalk KE. A coiled-coil structure of the alphaIIbbeta3 integrin transmembrane and cytoplasmic domains in its resting state. *Structure* 2005; **13**: 703–12.
19 Luo BH, Springer TA, Takagi J. A specific interface between integrin transmembrane helices and affinity for ligand. *PLoS Biol* 2004; **2**: 776–86.
20 Luo BH, Carman CV, Takagi J, Springer TA. Disrupting integrin transmembrane domain heterodimerization increases ligand binding affinity, not valency or clustering. *Proc Natl Acad Sci USA* 2005; **102**: 3679–84.
21 Partridge AW, Liu S, Kim S, Bowie JU, Ginsberg MH. Transmembrane domain helix packing stabilizes integrin alphaIIbbeta3 in the low affinity state. *J Biol Chem* 2005; **280**: 7294–300.
22 Yin H, Litvinov RI, Vilaire G, Zhu H, Li W, Caputo GA, Moore DT, Lear JD, Weisel JW, DeGrado WF, Bennett JS. Activation of platelet alphaIIbbeta3 by an exogenous peptide corresponding to the transmembrane domain of alphaIIb. *J Biol Chem* 2006; 281:36732–41.
23 Li R, Babu CR, Lear JD, Wand AJ, Bennett JS, DeGrado WF. Oligomerization of the integrin alphaIIbbeta3: roles of the transmembrane and cytoplasmic domains. *Proc Natl Acad Sci USA* 2001; **98**: 12462–7.
24 Li R, Gorelik R, Nanda V, Law PB, Lear JD, DeGrado WF, Bennett JS. Dimerization of the transmembrane domain of integrin alphaIIb subunit in cell membranes. *J Biol Chem* 2004; **279**: 26666–73.
25 Schneider D, Engelman DM. Involvement of transmembrane domain interactions in signal transduction by alpha/beta integrins. *J Biol Chem* 2004; **279**: 9840–6.
26 Fitzgerald LA, Phillips DR. Calcium regulation of the platelet membrane glycoprotein IIb-IIIa complex. *J Biol Chem* 1985; **260**: 11366–74.
27 Buensucesco C, de Virgilio M, Shattil SJ. Detection of Integrin alpha IIbbeta 3 Clustering in Living Cells. *J Biol Chem* 2003; **278**: 15217–24.
28 Haas TA, Plow EF. The cytoplasmic domain of $\alpha_{IIb}\beta_3$: a ternary complex of the integrin α and β subunits and a divalent cation. *J Biol Chem* 1996; **271**: 6017–26.
29 Haas TA, Plow EF. Development of a structural model for the cytoplasmic domain of an integrin. *Protein Eng* 1997; **10**: 1395–405.
30 Vinogradova O, Haas T, Plow EF, Qin J. A structural basis for integrin activation by the cytoplasmic tail of the alpha IIb-subunit. *Proc Natl Acad Sci USA* 2000; **97**: 1450–5.
31 Vinogradova O, Vaynberg J, Kong X, Haas TA, Plow EF, Qin J. Membrane-mediated structural transitions at the cytoplasmic face during integrin activation. *Proc Natl Acad Sci USA* 2004; **101**: 4094–9.
32 Vinogradova O, Velyvis A, Velyviene A, Hu B, Haas TA, Plow EF, Qin J. A structural mechanism of integrin αIIbβ3 "inside-out" activation as regulated by its cytoplasmic face. *Cell* 2002; **110**: 587–97.
33 Weljie AM, Hwang PM, Vogel H. Solution structures of the cytoplasmic tail complex from platelet integrin αIIb- and β3-subunits. *Proc Natl Acad Sci USA* 2002; **99**: 5878–83.

34 Hughes PE, Diaz-Gonzalez F, Leong L, Wu C, McDonald JA, Shattil SJ, Ginsberg MH. Breaking the integrin hinge. A defined structural constraint regulates integrin signaling. *J Biol Chem* 1996; **271**: 6571–4.

35 Ulmer TS, Yaspan B, Ginsberg MH, Campbell ID. NMR analysis of structure and dynamics of the cytosolic tails of integrin alphaIIbbeta3 in aqueous solution. *Biochemistry* 2001; **40**: 7498–508.

36 O'Toole TE, Mandelman D, Forsyth J, Shattil SJ, Plow EF, Ginsberg MH. Modulation of the affinity of integrin $alpha_{IIb}beta_3$ (GPIIb-IIIa) by the cytoplasmic domain of $alpha_{IIb}$. *Science* 1991; **254**: 845–7.

37 O'Toole TE, Katagiri Y, Faull RJ, Peter K, Tamura R, Quaranta V, Loftus JC, Shattil SJ, Ginsberg MH. Integrin cytoplasmic domains mediate inside-out signal transduction. *J Cell Biol* 1994; **124**: 1047–59.

38 Hughes PE, O'Toole TE, Ylanne J, Shattil SJ, Ginsberg MH. The conserved membrane-proximal region of an integrin cytoplasmic domain specifies ligand binding affinity. *J Biol Chem* 1995; **270**: 12411–7.

39 Ma YQ, Yang J, Pesho MM, Vinogradova O, Qin J, Plow EF. Regulation of Integrin alpha(IIb)beta(3) Activation by Distinct Regions of Its Cytoplasmic Tails. *Biochemistry* 2006; **45**: 6656–62.

40 Leisner TM, Wencel-Drake JD, Wang W, Lam SC. Bidirectional transmembrane modulation of integrin $\alpha_{IIb}\beta_3$ conformations. *J Biol Chem* 1999; **274**: 12945–9.

41 Yamanouchi J, Hato T, Tamura T, Fujita S. Suppression of integrin activation by the membrane-distal sequence of the integrin alphaIIb cytoplasmic tail. *Biochem J* 2004; **379**: 317–23.

42 Ginsberg MH, Yaspan B, Forsyth J, Ulmer TS, Campbell ID, Slepak M. A membrane-distal segment of the integrin alpha IIb cytoplasmic domain regulates integrin activation. *J Biol Chem* 2001; **276**: 22514–21.

43 van der Geer P. Pawson T. The PTB domain: a new protein module implicated in signal transduction. *TIBS* 1995; **20**: 277–80.

44 Songyang Z, Gish G, Mbamalu G, Pawson T, Cantley LC. A single point mutation switches the specificity of group III Src homology (SH) 2 domains to that of group I SH2 domains. *J Biol Chem* 1995; **270**: 26029–32.

45 Li R, Babu CR, Valentine K, Wand AJ, Bennett JS, DeGrado WF. Characterization of the monomeric form of the transmembrane and cytoplasmic domains of the integrin beta 3 subunit by NMR spectroscopy. *Biochemistry* 2002; **41**: 15618–24.

46 O'Toole TE, Ylanne J, Culley BM. Regulation of integrin affinity states through an NP*X*Y motif in the β subunit cytoplasmic domain. *J Biol Chem* 1995; **270**: 8553–8.

47 Chen Y-P, Djaffar I, Pidard D, Steiner B, Cieutat A-M, Caen JP, Rosa J-P. Ser-752 - > Pro mutation in the cytoplasmic domain of integrin β_3 subunit and defective activation of platelet integrin $\alpha_{IIb}\beta_3$ (GPIIb-IIIa) in a variant of Glanzmann's thrombasthenia. *Proc Natl Acad Sci USA* 1992; 89:10169–73.

48 Chen YP, O'Toole TE, Ylanne J, Rosa JP, Ginsberg MH. A point mutation in the integrin beta 3 cytoplasmic domain (S752– > P) impairs bidirectional signaling through alpha IIb beta 3 (platelet glycoprotein IIb-IIIa). *Blood* 1994; **84**: 1857–65.

49 Law DA, Nannizzi-Alaimo L, Phillips DR. Outside-in integrin signal transduction. αIIbβ3-(GPIIb-IIIa) tyrosine phosphorylation induced by platelet aggregation. *J Biol Chem* 1996; **271**: 10811–5.

50 Jenkins AL, Nannizzi-Alaimo L, Silver D, Sellers JR, Ginsberg MH, Law DA, Phillips DR. Tyrosine phosphorylation of the β_3 cytoplasmic domain mediates integrin-cytoskeletal interactions. *J Biol Chem* 1998; **273**: 13878–85.

51 Law DA, DeGuzman FR, Heiser P, Ministri-Madrid K, Killeen N, Phillips DR. Integrin cytoplasmic tyrosine motif is required for outside-in alphaIIbbeta3 signalling and platelet function. *Nature* 1999; **401**: 808–11.

52 Prasad KS, Andre P, He M, Bao M, Manganello J, Phillips DR. Soluble CD40 ligand induces beta3 integrin tyrosine phosphorylation and triggers platelet activation by outside-in signaling. *Proc Natl Acad Sci USA* 2003; **100**: 12367–71.

53 Du X, Saido TC, Tsubuki S, Indig FE, Williams MJ, Ginsberg MH. Calpain cleavage of the cytoplasmic domain of the integrin beta 3 subunit. *J Biol Chem* 1995; **270**: 26146–51.

54 Xi X, Flevaris P, Stojanovic A, Chishti A, Phillis DR, Lam SC, Du X. Tyrosine phosphorylation of the integrin beta 3 subunit regulates beta 3 cleavage by calpain. *J Biol Chem* 2006; **281**: 29426–30.

55 Parise LV, Criss AB, Nannizzi L, Wardell MR. Glycoprotein IIIa is phosphorylated in intact human platelets. *Blood* 1990; **75**: 2363–8.

56 Hillery CA, Smyth SS, Parise LV. Phosphorylation of human platelet glycoprotein IIIa (GPIIIa). Dissociation from fibrinogen receptor activation and phosphorylation of GPIIIa in vitro. *J Biol Chem* 1991; **266**: 14663–9.

57 Lerea KM, Cordero KP, Sakariassen KS, Kirk RI, Fried VA. Phosphorylation sites in the integrin β_3 cytoplasmic domain in intact platelets. *J Biol Chem* 1999; **274**: 1914–9.

58 van Willigen G., Hers I, Gorter G, Akkerman JW. Exposure of ligand-binding sites on platelet integrin alpha IIB/beta 3 by phosphorylation of the beta 3 subunit. *Biochem J* 1996; **314**: 769–79.

59 Kirk RI, Sanderson MR, Lerea KM. Threonine phosphorylation of the β_3 integrin cytoplasmic tail, at a site recognized by PDK1 and Akt/PKB *in vitro*, regulates Shc binding. *J Biol Chem* 2000; **275**: 30901–6.

60 Liu SC, Calderwood DA, Ginsberg MH. Integrin cytoplasmic domain-binding proteins. *J Cell Sci* 2000; **113**: 3563–71.

61 Otey CA, Pavalko FM, Burridge K. An interaction between alpha-actinin and the $beta_1$ integrin subunit *in vitro*. *J Cell Biol* 1990; **11**: 721–9.

62 Rees DJ, Ades SE, Singer SJ, Hynes RO. Sequence and domain structure of talin. *Nature* 1990; **347**: 685–9.

63 Burridge K, Chrzanowska-Wodnicka M. Focal adhesions, contractility, and signaling. *Annu Rev Cell Dev Biol* 1996; **12**: 463–518.

64 Beckerle MC, Miller DE, Bertagnolli ME, Locke SJ. Activation-dependent redistribution of the adhesion plaque protein, talin, in intact human platelets. *J Cell Biol* 1989; **109**: 3333–46.

65 Pearson MA, Reczek D, Bretscher A, Karplus PA. Structure of the ERM protein moesin reveals the FERM domain fold masked by an extended actin binding tail domain. *Cell* 2000; **101**: 259–70.

66 Calderwood DA, Zent R, Grant R, Rees DJ, Hynes RO, Ginsberg MH. The Talin head domain binds to integrin beta subunit cytoplasmic tails and regulates integrin activation. *J Biol Chem* 1999; **274**: 28071–4.

67 Calderwood DA, Yan B, de Pereda JM, Garcia-Alvarez B, Fujioka Y, Liddington RC, Ginsberg MH. The phosphotyrosine binding (PTB)-like domain of talin activates integrins. *J Biol Chem* 2002; **277**: 21749–58.

68 Garcia-Alvarez B, de Pereda JM, Calderwood DA, Ulmer TS, Critchley D, Campbell ID Ginsberg MH, Liddington RC. Structural determinants of integrin recognition by talin. *Mol Cell* 2003; **11**: 49–58.

69 Tadokoro S, Shattil SJ, Eto K, Tai V, Liddington RC, de Pereda JM, Ginsberg MH, Calderwood DA. Talin binding to integrin β tails: a final common step in integrin activation. *Science* 2003; **302**: 103–6.

70 Knezevic I, Leisner TM, Lam SCT. Direct binding of the platelet integrin $\alpha_{IIb}\beta_3$ (GPIIb- IIIa) to talin – evidence that interaction is mediated through the cytoplasmic domains of both α_{IIb} and β_3. *J Biol Chem* 1996; **271**: 16416–21.

71 Ulmer TS, Calderwood DA, Ginsberg MH, Campbell ID. Domain-specific interactions of talin with the membrane-proximal region of the integrin beta3 subunit. *Biochemistry* 2003; **42**: 8307–12.

72 Wegener KL, Partridge AW, Han J, Pickford AR, Liddington RC, Ginsberg MH, Campbell ID. Structural basis of integrin activation by talin. *Cell* 2007; **128**: 171–82.

73 Kloeker S, Major MB, Calderwood DA, Ginsberg MH, Jones DA, Beckerle MC. The Kindler syndrome protein is regulated by transforming growth factor-beta and involved in integrin-mediated adhesion. *J Biol Chem* 2004; **279**: 6824–33.

74 Xing B, Jedsadayanmata A, Lam SC. Localization of an integrin binding site to the C terminus of talin. *J Biol Chem* 2001; **276**: 44373–8.

75 Yan B, Calderwood DA, Yaspan B, Ginsberg MH. Calpain cleavage promotes talin binding to the beta 3 integrin cytoplasmic domain. *J Biol Chem* 2001; **276**: 28164–70.
76 Han J, Lim CJ, Watanabe N, Soriani A, Ratnikov B, Calderwood DA, Puzon-McLaughlin W, Lafuente EM, Boussiotis VA, Shattil SJ, Ginsberg MH. Reconstructing and Deconstructing Agonist-induced Activation of Integrin alphaIIbbeta3. *Curr Biol* 2006; **16**: 1796–806.
77 Martel V, Racaud-Sultan C, Dupe S, Marie C, Paulhe F, Galmiche A, Block MR, Albiges-Rizo C. Conformation, localization, and integrin binding of talin depend on its interaction with phosphoinositides. *J Biol Chem* 2001; **276**: 21217–27.
78 Hayashi M, Suzuki H, Kawashima S, Saido TC, Inomata M. The behavior of calpain-generated N- and C-terminal fragments of talin in integrin-mediated signaling pathways. *Arch Biochem Biophys* 1999; **371**: 133–41.
79 Schoenwaelder SM, Burridge K. Bidirectional signaling between the cytoskeleton and integrins. *Curr Opin Cell Biol* 1999; **11**: 274–86.
80 Kelly DF, Taylor DW, Bakolitsa C, Bobkov AA, Bankston L, Liddington RC, Taylor KA. Structure of the alpha-actinin-vinculin head domain complex determined by cryo-electron microscopy. *J Mol Biol* 2006; **357**: 562–73.
81 Goldmann WH. Kinetic determination of focal adhesion protein formation. *Biochem Biophys Res Commun* 2000; **271**: 553–7.
82 Kiema T, Lad Y, Jiang P, Oxley CL, Baldassarre M, Wegener KL, Campbell ID, Ylanne J, Calderwood DA. The molecular basis of filamin binding to integrins and competition with talin. *Mol Cell* 2006; **21**: 337–47.
83 Bennett JS, Zigmond S, Vilaire G, Cunningham ME, Bednar B. The platelet cytoskeleton regulates the affinity of the integrin $\alpha_{IIb}\beta_3$ for fibrinogen. *J Biol Chem* 1999; **274**: 25301–7.
84 Cowan KJ, Law DA, Phillips DR. Identification of Shc as the primary protein binding to the tyrosine-phosphorylated β_3 subunit of $\alpha_{IIb}\beta_3$ during outside-in integrin platelet signaling. *J Biol Chem* 2000; **275**: 36423–9.
85 Kralisz U, Cierniewski CS. Association of pp60c-src with alpha IIb beta 3 in resting platelets. *Biochem Mol Biol Int* 1998; **45**: 735–43.
86 Yuan W, Leisner TM, McFadden AW, Wang Z, Larson MK, Clark S, Boudignon-Proudhon C, Lam SC, Parise LV. CIB1 is an endogenous inhibitor of agonist-induced integrin alphaIIbbeta3 activation. *J Cell Biol* 2006; **172**: 169–75.
87 Tsuboi S. Calcium integrin-binding protein activates platelet integrin alpha IIbbeta 3. *J Biol Chem* 2002; **277**: 1919–23.
88 Fenczik CA, Sethi T, Ramos JW, Hughes PE, Ginsberg MH. Complementation of dominant suppression implicates CD98 in integrin activation. *Nature* 1997; **390**: 81–5.
89 Armulik A, Nilsson I, von Heijne G, Johansson S. Determination of the border between the transmembrane and cytoplasmic domains of human integrin subunits. *J Biol Chem* 1999; **274**: 37030–4.
90 Maguire PB, Moran N, Cagney G, Fitzgerald DJ. Application of proteomics to the study of platelet regulatory mechanisms. *Trends Cardiovasc Med* 2004; **14**: 207–20.
91 Edwards RJ, Moran N, Devocelle M, Kiernan A, Meade G, Signac W, Foy M, Park SD, Dunne E, Kenny D, Shields DC. Bioinformatic discovery of novel bioactive peptides. *Nat Chem Biol* 2007; **3**: 108–12.
92 Xi X, Bodnar RJ, Li Z, Lam SC, Du X. Critical roles for the COOH-terminal NITY and RGT sequences of the integrin beta3 cytoplasmic domain in inside-out and outside-in signaling. *J Cell Biol* 2003; **162**: 329–39.
93 Iber D, Campbell ID. Integrin activation – the importance of a positive feedback. *Bull Math Biol* 2006; **68**: 945–56.
94 Eigenthaler M, Hofferer L, Shattil SJ, Ginsberg MH. A conserved sequence motif in the integrin beta3 cytoplasmic domain is required for its specific interaction with beta3-endonexin. *J Biol Chem* 1997; **272**: 7693–8.
95 Shimaoka M, Takagi J, Springer TA. Conformational regulation of integrin structure and function. *Annu Rev Biophys Biomol Struct* 2002; **31**: 485–516.