Kindlin-2 (Mig-2): a co-activator of β3 integrins

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Introduction

Integrin activation, the rapid transition from a low to a high affinity state for ligand, regulates the numerous cellular responses consequent to integrin engagement by extracellular matrix proteins or counter-receptors on other cells (Hynes, 2002). This transformation is tightly controlled by the integrin cytoplasmic tails (CTs). It is widely accepted that the head domain of talin (talin-H) can mediate integrin activation by binding to two sites in integrin β3 CT; in integrin β3 this is an NPLY747 motif and the membrane-proximal region. Here, we show that the C-terminal region of integrin β3 CT, composed of a conserved TS752T region and NITY759 motif, supports integrin activation by binding to a cytosolic binding partner, kindlin-2, a widely distributed PTB domain protein. Co-transfection of kindlin-2 with talin-H results in a synergistic enhancement of integrin αIIbβ3 activation. Furthermore, siRNA knockdown of endogenous kindlin-2 impairs talin-induced αIIbβ3 activation in transfected CHO cells and blunts αIIBβ3-mediated adhesion and migration of endothelial cells. Our results thus identify kindlin-2 as a novel regulator of integrin activation; it functions as a coactivator.

Results and discussion

To address the functional significance of the membrane-distal region of the β3 CT, we considered whether it might interact with intracellular regulator(s). A CHO cell line stably expressing αIIbβ3 was transfected with cDNAs encoding for wild-type or mutated β3 CT based on the rationale that these expressed constructs would compete for integrin binding partners. A similar strategy had been used previously to screen the extracellular domain of PSGL-1 so that expression levels of the various β3 CT could be verified. As assessed by flow cytometry (FACS), PSGL-1 expression differed by less than 10%. The membrane-distal region of the β3 CT contains two NXXY turn motifs, NPLY747 and NITY759, which are separated by a short helix containing a T/S cluster, the TS752T region (Fig. 1 A). The head domain of talin (talin-H) docks at the NPLY747 motif through its F3 domain and also interacts with the membrane-proximal region, perturbing the membrane clasp and leading to at least partial integrin activation (Vinogradova et al., 2002; Tadokoro et al., 2003; Wegener et al., 2007). The T/S cluster and the NITY motif are also critical for integrin activation (Chen et al., 1994; O’Toole et al., 1995; Xi et al., 2003; Ma et al., 2006). However, the mechanisms underlying their effects remain unresolved. In this study, we found that kindlin-2, a widely distributed PTB domain protein, interacts with the C terminus of β3 CT at the TS752T and NITY759 motifs and markedly enhances talin-induced integrin activation. Thus, kindlin-2 is identified as a coactivator of integrins.
A reasonable synthesis of the data in Fig. 1 (B and C) is that the membrane-distal region of the \( \beta_3 \) CT regulates integrin activation and does so by interacting with a cytoplasmic binding partner that cooperates with talin but binds to distinct sites. Molecules reported to bind to the membrane-distal conservative regions of \( \beta_3 \) CT include \( \alpha_i \) lamin, which binds to the T/S cluster, and \( \beta_3 \)-endonexin, which binds to the NITY 759 motif. Both have been suggested as regulators of integrin activation (\( \alpha_i \) lamin, a negative regulator, and \( \beta_3 \)-endonexin, a positive regulator) (Eigenthaler et al., 1997; Kiema et al., 2006). To assess their roles in integrin activation, \( \alpha_i \) lamin A Ig-like domain 21 (FLNa21, the \( \beta_3 \) CT binding region) or \( \beta_3 \)-endonexin was transfected or cotransfected together with talin-H into \( \beta_3 \)-CHO cells. Neither modulated talin-induced integrin activation or directly mediated integrin activation (Fig. 2, A and B; and Fig. S1 A, available at http://www.jcb.org/cgi/content/full/jcb.200710196/DC1), thus excluding them as the hypothetical coactivator of integrins. It should be noted that these data are not inconsistent with the proposed role of \( \alpha_i \) lamin A as a negative regulator of integrin activation (Kiema et al., 2006); suppressive effects of FLNa21 may not be evident in the presence of high talin-H levels.

Recently, we identified another \( \beta_3 \) CT binding protein, kindlin-2 (Shi et al., 2007), one of a three-member kindlin family that are characterized by bearing a FERM domain (Wick et al., 1994; Siegel et al., 2003; Weinstein et al., 2003; Ussar et al., 2006).
Kindlin-2 contributes to the maturation of focal adhesions during cell shape changes through recruitment of migfilin and filamin (Tu et al., 2003). Targeted disruption of the kindlin-2 gene results in embryonic lethality in mice and causes multiple, severe abnormalities in zebrafish (Dowling et al., 2008). Distinct from talin, its interaction site on β3 CT is not dependent on the NPLY747 motif (Shi et al., 2007). When expressed in αmβ3-CHO cells, kindlin-2 induces statistically significant but very weak integrin activation compared with talin-H (Shi et al., 2007). To consider the role of kindlin-2 as a coactivator with talin-H, both were transfected into αmβ3-CHO cells. As shown in Fig. 2 (C and D), kindlin-2 dramatically enhanced talin-H-mediated αmβ3 activation. This enhancement was not simply additive but represented functional synergism. We assessed the expression levels in different transfectants by Western blots to exclude that coexpression of kindlin-2 enhanced talin-H expression or vice versa; expression of talin-H in single and double transfectants was similar (Fig. 2 E).

To further assess the role of kindlin-2 as a coactivator, GST-fused β3 CT proteins were used to coprecipitate endogenous kindlin-2 in lysates of CHO cells, platelets, and human umbilical vein endothelial cells (HUVECs). As shown in Fig. 3 A, wild-type β3 CT interacts with kindlin-2 but GST alone did not, ascribing specificity to the interactions. The Y747A mutation abrogates talin-H but not kindlin-2 binding to β3 CT. In contrast, the S752P and Y759A mutations still support talin-H binding but dramatically reduce kindlin-2 association (Fig. 3 A). Thus, the binding requirements for talin-H and kindlin-2 on the β3 CT are distinct and both bind to sites known to regulate integrin activation. Consistent with our observations (Fig. 2 A and Fig. S1 A), overexpression of FLNa21 or β3-endonexin, two C-terminal binding proteins of β3 CT, failed to suppress endogenous kindlin-2 binding but dramatically reduce kindlin-2 association (Fig. 3 A). Thus, the binding requirements for talin-H and kindlin-2 on the β3 CT are distinct and both bind to sites known to regulate integrin activation. Consistent with our observations (Fig. 2 A and Fig. S1 A), overexpression of FLNa21 or β3-endonexin, two C-terminal binding proteins of β3 CT, failed to suppress endogenous kindlin-2 binding but dramatically reduce kindlin-2 association (Fig. 3 A).

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Peptides corresponding to Y747-T762 or a variant peptide containing the S752P and Y759A substitutions were synthesized (Fig. 3 B). When added as competitors (200 μM), wild-type Y747-T762 peptide inhibited kindlin-2 coprecipitation with the αmβ3 integrin subunit in both αmβ3-CHO cells and HUVECs (Fig. S2, available at http://www.jcb.org/cgi/content/full/jcb.200710196/DC1).

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kindlin-2 into several fragments, and their β3 CT-binding capacities were evaluated by pull-down assays (Fig. 4 B). Deletion of the N-terminal region of kindlin-2, at N217 or at E345, the border of the PH domain insertion, ablated interaction with the β3 CT. In addition, truncation of kindlin-2 to delete the second part of its F2 and F3 subdomains also disrupted β3 CT interaction (Fig. 4 B). These deletions were more disruptive than the QW615 mutation. However, with deletion of PH domain alone, the mutant GST-β3 CT (Fig. 3 C); the inhibition was ~70% by densitometry. A lower concentration of peptide (100 μM) was still inhibitory but produced only 50% inhibition (unpublished data), suggesting a dose-dependent inhibitory effect. Introduction of S752P and Y759A mutations into the peptide totally abolished its competitive activity (Fig. 3 C). As control, both peptides had no effect on talin-H association with the GST-β3 CT. It is noteworthy that introduction of similar peptides into endothelial cells (Liu et al., 1996) and platelets (Hers et al., 2000) significantly perturbed β3 and IIbβ3 mediated responses, respectively. Thus, our results may provide a molecular explanation for these prior observations.

Like talin-H, kindlin-2 contains a FERM domain; its F2 subdomain is bisected by a PH domain, but its F3 (PTB) subdomain is intact (Fig. 4 A). Our previous experiments had shown that a QW609/AA mutation in F3, a site predicted by molecular modeling to be involved in β CT engagement, did, in fact, disrupt its association with β CT (Shi et al., 2007). We segmented GST-β3 CT (Fig. 3 C); the inhibition was ~70% by densitometry. A lower concentration of peptide (100 μM) was still inhibitory but produced only 50% inhibition (unpublished data), suggesting a dose-dependent inhibitory effect. Introduction of S752P and Y759A mutations into the peptide totally abolished its competitive activity (Fig. 3 C). As control, both peptides had no effect on talin-H association with the GST-β3 CT. It is noteworthy that introduction of similar peptides into endothelial cells (Liu et al., 1996) and platelets (Hers et al., 2000) significantly perturbed αIβ3 and αIIbβ3 mediated responses, respectively. Thus, our results may provide a molecular explanation for these prior observations.

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appears to depend on binding of kindlin-2 through both its N- and C-terminal Fβ subdomains. As to why the C-terminal Fβ of kindlin-2 recognizes the NITY motif rather than the NPLY region of β3 CT will require high resolution structures.

The colocalization of β3 integrin and kindlin-2 was also tested in living cells. We found they dynamically associate with each other in HUVECs during β3 integrin mediated cell spreading on the β3 ligand (Fig. 5 A). At the early stage of spreading (30 min), β3 (green) and kindlin-2 (red) colocalized in the lamellipodia at the edges of spreading cells (Fig. 5 A, top). Over time, both β3 integrin and kindlin-2 moved into focal adhesion sites (Fig. 5 A, bottom, 60 min). The merged images in Fig. 5 A (right) kindlin-2 still retained its capacity to bind the β3 CT. The effects of these mutants on the coactivator activity of kindlin-2 were tested. When cotransfected with talin-H, deletion of either the N- or C-terminal region of kindlin-2 resulted in loss of coactivator activity (Fig. 4 C). The mutant with its PH domain deletion still retained some coactivator activity, although it was less potent than intact kindlin-2. Also, the QW mutant lacked coactivator activity, verifying that this site is involved not only in binding but also in coactivator function. We cannot exclude that some of these mutations may affect global folding of kindlin-2. However, it should be noted that FERM subdomains tend to fold independently into functional units. Thus, coactivator activity
verify the colocalization of kindlin-2 and β3 integrin. β3 integrin and talin also colocalize in spreading HUVECs with a similar pattern (Fig. S3 A, available at http://www.jcb.org/cgi/content/full/jcb.200710196/DC1). These observations place talin and kindlin-2 together, consistent with their cooperativity in function.

To determine if endogenous kindlin-2 supports β3 integrin function, RNA-mediated interference experiments were performed. Small interfering RNAs targeting kindlin-2 (siKind-2) or irrelevant RNAs as control (siControl) were introduced into αmβ3 CHO cells, and kindlin-2 expression levels were analyzed by Western blot. Transfection of siKind-2 but not siControl effectively inhibited the expression of kindlin-2 (Fig. 5 B). The decrease in kindlin-2 protein expression was 70% by densitometry. Neither the siKind-2 nor the siControl changed actin expression, establishing selectivity of the siKind-2 on kindlin-2 expression.

Talin-H can induce αmβ3 activation in transfected αmβ3 CHO cells as shown by others (Tadokoro et al., 2003) and in this study. However, talin-H–mediated integrin activation was significantly blunted when kindlin-2 levels were reduced with siKind-2 but not siControl (Fig. 5 C), indicating that endogenous kindlin-2 supports talin-H–induced αmβ3 activation in these cells.

We also tested the function of kindlin-2 knock-down in cells that express an integrin naturally. HUVECs express and use αvβ3 to mediate cell adhesion and migration on fibrinogen or vitronectin (Plow et al., 2000). Endogenous kindlin-2 could be knocked down in HUVEC using siRNA (Fig. 5 D), and the deficiency of kindlin-2 dramatically suppressed HUVEC adhesion on the β3 integrin ligands, fibrinogen or vitronectin (Fig. 5 E).

In addition, knockdown of kindlin-2 in HUVECs significantly inhibited VEGF-induced cell migration (Fig. 5 F). Under the conditions used, VEGF-induced HUVEC migration on fibrinogen or vitronectin is dependent on αvβ3 activation (Byzova et al., 2000), and there is little cell proliferation (<50% increase) in serum-free medium (unpublished data). Interestingly, we previously found that overexpression of kindlin-2 also inhibited migration for some cancer cells (Shi et al., 2007). These two distinct observations suggest that the supportive role of kindlin-2 in integrin activation might be cell type and/or integrin specific or depends on specific experimental conditions such as ligand concentration (Huttenlocher et al., 1996; Palecek et al., 1997). Furthermore, knocking down kindlin-2 significantly suppressed PMA-induced HUVEC adhesion on fibrinogen (Fig. 5 G), which is also an αvβ3 activation-dependent process. In concert, these results suggest that kindlin-2 plays an important role in supporting β3 integrin functions dependent on activation.

Nonetheless, kindlin-2 is unlikely to be a direct activator of integrin; overexpression of kindlin-2 alone only had a mild effect on integrin activation compared with talin-H (Fig. 2 D). Even though kindlin-2 also bears a FERM-like domain as does talin-H, the binding sites of kindlin-2 on β3 CT are solely localized at its C terminus beyond of the talin-H recognition sites (Fig. 3), which allows kindlin-2 and talin to bind to the β3 CT together. This possibility has been established by the synergistic role of talin-H and kindlin-2 in integrin activation (Fig. 2, C and D) and further verified by the finding that knockdown of endogenous kindlin-2 significantly suppressed talin-H–induced integrin activation (Fig. 5, B and C).

In summary, we found that kindlin-2 is a coactivator of talin in supporting β3 integrin activation. As such, kindlin-2 is the first of the postulated coactivators of integrins (Ma et al., 2007) to be identified. Our data support a model (Fig. 5 H) in which kindlin-2 binds to the C terminus of β3 CT beyond of the talin-binding sites. Functionally, kindlin-2 synergistically enhances talin-induced integrin activation. Kindlin-2 may associate with membrane via its PH domain, an interaction commonly associated with these domains, and interacts with β3 CT via its N terminus and C-terminal PTB domain. Anchoring β3 CT by kindlin-2 could reduce the flexibility of β3 CT in the cytosol, positioning it more favorably for interaction with talin and might also displace other β3 CT binding partners. Due to the variable expression of kindlin-2 in different tissues and cells, e.g., its levels are quite low in human platelets versus HUVECs (Fig. S3 B), one must consider the possibilities that kindlin-2 may exert a “catalytic” effect on integrin activation, where one molecule coactivates multiple integrins, or whether this coactivator activity of kindlin-2 is shared or compensated by other kindlin family members or by other integrin binding partners.

Materials and methods

Plasmid construction and mutagenesis

The cDNA of human αv and β3 subunits were inserted into the mammalian expression vector pcDNA3.1 (Invitrogen). The mouse talin head domain (1–429 amino acids), human kindlin-2, β3-endonexin, and filamin A Ig-like domain 21 (2235–2330 amino acids) were cloned into pEGFP vectors (Clontech Laboratories, Inc.). For the construct of GST-tagged β3 cytoplasmic tail, the fragment of β3 tail (716–762 amino acids) was amplified by PCR and inserted into pGST-parallel-1 vector (Sheffield et al., 1999). The PSGL1/β3 chimera was constructed in pcDNA3.1 vector in which N terminus (1–91 amino acids) of human PSGL1 was fused onto C terminus (468–762 amino acids) of human β3 subunit. All the indicated mutations were introduced into the respective constructs using QuikChange site-directed mutagenesis kit (Stratagene) and confirmed by gene sequencing.

Integrin αvβ3 activation assay

The integrin αvβ3 activation was evaluated with PAC1, a mAb which specifically recognizes active αvβ3. For testing how the membrane-distal regions of β3 CT regulate αvβ3 activation, the β3 subunit bearing different mutations was cotransfected with αvβ3 subunit, with or without R995 D mutation, into CHO-K1 cells using Lipofectamine 2000 (Invitrogen). 24 h after transfection, the cells were collected and PAC1 binding was assessed as described previously (Ma et al., 2006). In brief, PAC1 binding was first normalized by αvβ3 expression level on the cell surfaces measured by mAb 2G12, which is against αvβ3 complex independent of activation status. The values of normalized PAC1 binding on different transfecteds were compared to determine relative integrin activation, defining the basal activation of wild-type αvβ3 as 1.0.

For determining the regulatory roles of different β3-binding partners in αvβ3 activation, individual EGF/Fused candidate binding partners or combinations of binding partners were transfected into CHO cells stably expressing wild-type αvβ3 (αvβ3-CHO). PAC1 binding to the different transfecteds was analyzed by flow cytometry, gating only on the EGFP-positive cells. Mean fluorescence intensities (MFI) of PAC1 binding were normalized based on the basal level of PAC1 binding to cells transfected with the EGFP vector alone to obtain relative MFI values.

Cell spreading

Monomeric PSGL1 (mPSGL1) or PSGL1/β3 chimera (PSGL1NWednesday) was transfected into αvβ3-CHO cells. The mPSGL1 was obtained by substitution of a single extracellular cysteine at the junction of the transmembrane domain with A to disturb the disulfide bond essential for PSGL1 homodimer formation (McEver and Cummings, 1997). The transiently transfected cells were allowed to adhere and spread on immobilized fibrinogen in Laboratory-Tek II chambers (Nalge Nunc International). After incubation at 37°C for 2 h,
the chambers were washed three times with PBS and the adherent cells were fixed by 4% paraformaldehyde. To identify PSGL-1–expressing cells, the fixed cells were stained by anti-PSGL-1 mAb, KPL-1 (BD Biosciences), followed by goat anti-mouse IgG conjugated with AlexaFluor 488 (Invitrogen). As controls, nontransfected cells with the same treatment were included in each experiment and always showed no PSGL-1 staining. The positively stained (green) cells were observed using a fluorescence microscope (model DMR; Leica) with a 10X objective and recorded with a cooled CCD camera (Retiga Exi; Q-Imaging). Data were analyzed with ImagePro Plus Capture and Analysis software (Media Cybernetics).

GST pull-down assays and Western blotting
Glutathione-Transferase (GST) fusion proteins were expressed in Rosetta2 (DE3) cells, purified by glutathione-affinity chromatography using a GST-PrepFF column (GE Healthcare), and quantified by spectrophotometry based on calculated extinction coefficients and Coomassie blue staining of SDS-PAGE. The cell lysates of transfected CHO cells, out-dated platelets, and HUVECs were prepared in the lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 1% Triton X-100) containing protease inhibitors and centrifuged at 15,000 g for 12 min. For the GST pull-down assays, glutathione-Sepharose 4B (GE Healthcare) and the indicated GST fusion proteins were added to the aliquots of lysate supernatants and incubated at 4°C for 8 h. The antibodies used for Western blotting were anti-kindlin-2 (Tu et al., 2003), anti-GFP (Santa Cruz Biotechnology, Inc.), and anti–human talin (Chemicon International).

Immunofluorescence and confocal microscopy
To observe the distributions of kindlin-2 and β3 integrins, HUVECs were allowed to spread on immobilized fibrinogen at 37°C for 30 min or 60 min. The spread cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and stained with mAb anti-kindlin-2 and polyclonal Ab anti-β3 (Chemicon International) followed by AlexaFluor 568 anti-mouse IgG and AlexaFluor 488 anti-rabbit IgG (Invitrogen). The images were recorded by a confocal microscope with a 63X objective (Leica).

RNA interference
To knock down endogenous kindlin-2, irrelevant control RNAs or designed siRNAs targeting kindlin-2 (from Dharmacon) were transfected to CHO cells using Lipofectamine 2000 (Invitrogen) based on the protocol for siRNA transfection from Invitrogen and HUVECs using targetfect-HUVEC targeting systems according to the manufacturers’ protocols. The extent of suppression and specificity for kindlin-2 were evaluated by Western blotting with anti–kindlin-2 and actin antibodies as controls.

HUVEC adhesion and migration
For cell adhesion assays, the nontransfected or transfected HUVECs, with targeting or control siRNAs, were incubated with the immobilized integrin ligand, fibrinogen (20 μg/ml for coating) or vitronectin (5 μg/ml for coating), for 30 min at 37°C. After washing, the adhered cells were fixed with 4% paraformaldehyde, permeabilized with 1% tadoumine blue, and stained with 1% totalidine blue, and quantified by performing microscopic cell counts.

Statistical analysis
Quantitative data were compared using a two-tailed t test. P values to determine statistical significance are indicated in the text. For the experiments to observe adherent or migrated cells, 10–20 fields or confocal cell images were randomly taken in at least three independent experiments.

Online supplemental material
Fig. S1 shows that neither FLNa21 nor β3-endonexin has direct effect on integrin αββ3 activation and β3 CT/kindlin-2 association. Fig. S2 shows the interaction of endogenous β3 integrin subunit and kindlin-2. Fig. S3 shows the dynamic colocalization of β3 integrin and talin in HUVECs and kindlin-2 expression in human platelets. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200710196/DC1.

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References
Byzova, T.V., C.K. Goldman, N. Pampori, K.A. Thomas, A. Bett, S.J. Shattil, and E.F. Flow. 2000. A mechanism for modulation of cellular responses to VEGF: activation of the integrins. Mol. Cell. 6:851–860.
Chen, Y.P., I. Djiaffer, D.Pidar, B. Steiner, A.-M. Cituat, J.P. Caen, and J.-P. Rosa. 1992. Ser-752→→Pro mutation in the cytoplasmic domain of integrin β subunit and defective activation of platelet integrin αIIbβ3 in a variant of Glanzmann’s thrombasthenia. Proc. Natl. Acad. Sci. USA. 89:10169–10173.
Chen, Y.P., T.E. O’Toole, J. Ylame, J.P. Rosa, and M.H. Ginsberg. 1994. A point mutation in the integrin beta3 cytoplasmic domain (S752→→P) impairs bidirectional signaling through alpha IIb beta3 (platelet glycoprotein-IIb-IIIa). Blood. 84:1857–1865.
Dowling, J.J., E. Gibbs, M. Russell, D. Goldman, J. Minarcic, J.A. Golden, and E.L. Feldman. 2008. Kindlin-2 is an essential component of intercalated discs and is required for vertebrate cardiac structure and function. Circ. Res. 102:423–431.
Eigenthaler, M., L. Hofferer, S.J. Shattil, and M.H. Ginsberg. 1997. A conserved sequence motif in the integrin beta3 cytoplasmic domain is required for its specific interaction with beta3-endonexin. J. Biol. Chem. 272:7693–7698.
Fenczik, C.A., T. Sethi, J.W. Ramos, P.E. Hughes, and M.H. Ginsberg. 1997. Complementation of dominant suppression implicates CD98 in integrin activation. Nature. 390:81–85.
Hers, I., J. Donath, P.E.M.H. Litjens, G. Van Willigen, and J.W.N. Akkerman. 2000. Inhibition of platelet integrin αIIbβ3 by peptides that interfere with protein kinases and the β, tail. Arterioscler. Thromb. Vasc. Biol. 20:1651–1660.
Hughes, P.E., F. Diaz-Gonzalez, L. Leong, C. Wu, J.A. McDonald, S.J. Shattil, and M.H. Ginsberg. 1996. Breaking the integrin hinge. A defined structural constraint regulates integrin signaling. J. Biol. Chem. 271:6571–6574.
Huttonlocher, A., M.A. Ginsberg, and A.F. Horwitz. 1996. Modulation of cell migration by integrin-mediated cytoskeletal linkages and ligand-binding affinity. J. Cell Biol. 134:1551–1562.
Hynes, R.O. 2002. Integrins: bidirectional, allosteric signaling machines. Cell. 110:673–687.
Kiema, T.Y., L. Lad, P. Jiang, C.L. Oxley, M. Baldassarre, K.L. Wegener, J.D. Campbell, J. Ylame, and D.A. Calderwood. 2006. The molecular basis of flamin binding to integrins and competition with talin. Mol. Cell. 21:337–347.
Kim, M., C.V. Carman, and T.A. Springer. 2003. Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. Science. 301:1720–1725.
Liu, K.Y., S. Timmons, and J. Hawiger. 1996. Identification of a functionally important sequence in the cytoplasmic domain of integrin β by using cell-permeable peptide analogs. Proc. Natl. Acad. Sci. USA. 93:11819–11824.
Ma, Y.Q., J. Yang, M.M. Pesho, O. Vinogradova, J. Qin, and E.F. Flow. 2006. Regulation of integrin alpha(IIb)beta3 activation by distinct regions of 65K/LCY/ICAP-1 subunit cytoplasmic domain. J. Biol. Chem. 281:45659–45666.
Ma, Y.Q., J. Qin, and E.F. Flow. 2007. Platelet integrin alpha(IIb)beta3 activation mechanisms. J. Thromb. Haemost. 5:1345–1352.
McEvoy, R.P., and R.D. Cummings. 1997. Perspectives series: cell adhesion in vascular biology. Role of PSGL-1 binding to selectins in leukocyte recruitment. J. Clin. Invest. 100:485–491.
O’Toole, T.E., J. Ylame, and B.M. Culley. 1995. Regulation of integrin affinity states through an NPYX5 motif in the β subunit cytoplasmic domain. J. Biol. Chem. 270:8553–8558.
Palecek, S.P., J.C. Lotfus, M.H. Ginsberg, D.A. Lauffenburger, and A.F. Horwitz. 1997. Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. Nature. 385:537–540.
Plow, E.F., T.A. Haas, L. Zhang, J. Lotfus, and J.W. Smith. 2000. Ligand binding to integrins. J. Biol. Chem. 275:21785–21788.
Qin, J., O. Vinogradova, and E.F. Flow. 2004. Integrin bidirectional signaling: a molecular view. PLoS Biol. 2:e169.
Sheffield, P., S. Garrard, and Z. Derewenda. 1999. Overcoming expression and purification problems of RhoGDI using a family of “parallel” expression vectors. *Protein Expr. Purif.* 15:34–39.

Shi, X., Y.Q. Ma, Y. Tu, K. Chen, S. Wu, K. Fukuda, J. Qin, E.F. Plow, and C. Wu. 2007. The MIG-2/integrin interaction strengthens cell-matrix adhesion and modulates cell motility. *J. Biol. Chem.* 282:20455–20466.

Siegel, D.H., G.H. Ashton, H.G. Penagos, J.V. Lee, H.S. Feiler, K.C. Wilhelmsen, A.P. South, F.J. Smith, A.R. Prescott, V. Wessagowit, et al. 2003. Loss of kindlin-1, a human homolog of the *Caenorhabditis elegans* actin-extra-cellular-matrix linker protein UNC-112, causes Kindler syndrome. *Am. J. Hum. Genet.* 73:174–187.

Tadokoro, S., S.J. Shattil, K. Eto, V. Tai, R.C. Liddington, J. de Pereda, M.H. Ginsberg, and D.A. Calderwood. 2003. Talin binding to integrin β tails: a final common step in integrin activation. *Science.* 302:103–106.

Tu, Y., S. Wu, X. Shi, K. Chen, and C. Wu. 2003. Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation. *Cell.* 113:37–47.

Ussar, S., H.V. Wang, S. Linder, R. Fassler, and M. Moser. 2006. The Kindlins: subcellular localization and expression during murine development. *Exp. Cell Res.* 312:3142–3151.

Vinogradova, O., A. Velyvis, A. Velyviene, B. Hu, T.A. Haas, E.F. Plow, and J. Qin. 2002. A structural mechanism of integrin αIIbβ3 “inside-out” activation as regulated by its cytoplasmic face. *Cell.* 110:587–597.

Wegener, K.L., A.W. Partridge, J. Han, A.R. Pickford, R.C. Liddington, M.H. Ginsberg, and I.D. Campbell. 2007. Structural basis of integrin activation by talin. *Cell.* 128:171–182.

Weinstein, E.J., M. Bourner, R. Head, H. Zakeri, C. Bauer, and R. Mazzarella. 2003. URP1: a member of a novel family of PH and FERM domain-containing membrane-associated proteins is significantly over-expressed in lung and colon carcinomas. *Biochim. Biophys. Acta.* 1637:207–216.

Wick, M., C. Burger, S. Brusselbach, F.C. Lucibello, and R. Muller. 1994. Identification of serum-inducible genes: different patterns of gene regulation during G0→S and G1→S progression. *J. Cell Sci.* 107(Pt 3):preceeding table of contents.

Xi, X., R.J. Bodnar, Z. Li, S.C. Lam, and X. Du. 2003. 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.* 162:329–339.