Spatial Coordination of Kindlin-2 with Talin Head Domain in Interaction with Integrin β Cytoplasmic Tails*

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Results: The C-terminal 12 amino acids of β1 and β3 integrins mediate kindlin-2 binding. Kindlin-2 binding to the extreme C terminus allows membrane domains (3, 4). The α/β CT complex restrains the integrin in a resting, low affinity state for extracellular ligands, and a process to unclasp the complex is required to initiate integrin activation (5).

Mechanistically, integrin activation is balanced by multiple CT-associating proteins, including both activators and inhibitors, and the coordination of these positive and negative regulators, together with post-translational modifications of the β CT, defines the clasping state of integrin CT, and thereby integrin activation (6–11). Among many integrin CT binding proteins, the head domain of the cytoskeletal protein talin (talin-H) is a well characterized integrin activator (12, 13). Talin-H resides at the N terminus of talin and resembles a FERM domain, which contains four subdomains (F0, F1, F2, and F3). The integrin binding sites in talin is auto-inhibited but can be exposed by calpain cleavage or binding partners, which allows its F3-phosphotyrosine binding subdomain to directly interact with integrin β CT and unclasp the CT complex to induce integrin activation (14–21). However, quantitative analysis indicated that talin-H alone is insufficient to induce efficient integrin activation, and a co-activation model was proposed (6, 22). Within recent years, it has been found that kindlins could dramatically enhance talin-mediated integrin activation in model cells and were also essential to support efficient integrin activation in vivo, verifying a co-activation mechanism (23–25).

Indeed, deficiencies of specific kindlins are associated with diseases in human, which reflect an inability to effectively activate integrins (26–29). The kindlin family contains three members in mammals, and among them, kindlin-2 is expressed ubiquitously and enriched in the integrin-containing adhesion complexes (30, 31). Interestingly, kindlin family members also contain a FERM-like domain, which is preceded by a distinct F0 subdomain at the N terminus and with a pleckstrin homology domain inserted in the middle of its F2 subdomain, both of which are potential binding sites for membrane phosphoinositides (32–34) While sharing a structurally similar FERM domains, mutations in the integrin β CTs have distinct effects on kindlin and talin-H binding (23–25, 35, 36).

Activation of integrins is a key mechanism to control many cellular adhesive responses including platelet aggregation, leukocyte trafficking, and endothelial cell migration (1, 2). At a molecular level, the integrin activation process is controlled tightly by the heterodimeric α/β cytoplasmic tail (CT) complex formed between the membrane-proximal regions of each integrin subunit, and this complex extends into their transmembrane domains (3, 4). The α/β CT complex restrains the integrin in a resting, low affinity state for extracellular ligands, and a process to unclasp the complex is required to initiate integrin activation (5).

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To further understand the regulatory role of kindlin in integrin activation, we have measured the interaction of kindlin-2 to integrin and detected an unexpected binding preference for different integrin β CTs. Further detailed mapping using synthesized peptides defined a 12-amino acid kindlin-2 binding core in the C terminus of integrin β CT. Residues at the extreme C terminus of this binding core establish the preference of kindlin-2 for different integrin β CTs. Kindlin-2 alone could not unclasp the integrin CT complex, demonstrating that kindlin-2 is dependent upon talin-H to support integrin activation. Finally, despite the relatively large size of kindlin-2 and talin-H, we demonstrate that the short integrin β CTs can accommodate both co-activators simultaneously to achieve integrin co-activation, a transition that governs many cellular responses, including platelet aggregation and tumor metastasis (37–39).

EXPERIMENTAL PROCEDURES

Protein Preparation and Peptide Synthesis—GST-fused integrin β CT, full-length kindlin-2 and His-tagged talin-H (1–429) were expressed, purified, and quantified as described previously (10). Talin-F2,F3(206–405) was prepared as described previously (40). All synthetic peptides were prepared, purified, and authenticated by HPLC and MALDI TOF-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry in the Molecular Biotechnology Core of the Cleveland Clinic.

Pulldown Assays and Western Blotting—Pulldown assays were performed using GST fusion proteins. Equal amounts of GST-fused integrin β CTs were added together with glutathione-Sepharose 4B (GE Healthcare) to aliquots of the cell lysates from either EGFP-kindlin-2-expressing CHO cells or HUVECs. In peptide inhibition experiments, the indicated peptide was added to the slurries at the selected concentrations. After overnight incubation in a cold room, the precipitates were washed and boiled in Laemmli sample buffer. The eluates were analyzed on gradient acrylamide gels under reducing conditions, and interactions of the integrin β CT with kindlin-2 were determined by Western blotting. The gels were also stained with Coomassie blue to verify that sample loadings were similar.

Surface Plasmon Resonance (SPR)—Real time protein-protein interactions were analyzed using a Biacore3000 instrument (Biacore). Purified proteins were immobilized on the carboxymethyl dextran of CM5 biosensor chip (Biacore, Uppsala, Sweden). Kindlin-2 proteins were dialyzed into 50 mM phosphate buffer (pH 7.0). Talin-H at the concentration of 1.4 mM was injected three times into the solution of kindlin-2 at a 70 μM concentration in a Microcal iTC200 instrument (GE Healthcare). Isothermal titration calorimetry titration curves were obtained by injecting talin-H in 10 3-μl injections with 70 μM kindlin-2 solution in the cell. Titration curves were collected to estimate heat changes associated with the kindlin-2/talin-H interaction.

NMR Spectroscopy—Two-dimensional transferred NOESY experiments were performed with mixing time of 400 ms on Bruker Avance 600 MHz at 25 °C as described (16). The spectra were processed and visualized using nmrPipe (41). Two-dimensional HSQC experiments were used to examine the integrin β CT-target binding were described previously (16).

Cell Spreading—To test the function of the kindlin-2 binding core in a cellular system, a series of chimeric PSGL-1/β3 constructs were expressed in α1β3-CHO cells, and their effects of cell spreading were measured. The chimera consisted of the extracellular and transmembrane domains of PSGL-1 (mPSGL-1) fused to selected segments of the β3CT. A kindlin-2 chimera, described previously (23), allows expression of functional segments or mutants of the β3CT in the cytosol while tethered to PSGL-1, which allows for monitoring of expression levels. The specific chimeric proteins were β3CT/PSGL-1 (β3CT748/PSGL-1 β3(C68–748)), β3CT-CORE/PSGL-1 (β3CTΔ716+749–762), and these were transfected into α1β3-CHO cells. In short, the transiently transfected cells were allowed to adhere and spread on fibrinogen-coated (20 μg/ml) glass coverslips. After incubation at 37 °C for 2 h, the wells were washed three times with PBS, and the adherent cells were fixed with 4% paraformaldehyde and stained with Alexa Fluor 647 phalloidin (Invitrogen). 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 Alexa Fluor 488 (Invitrogen). As controls, nontransfected cells were included in each experiment and always showed no PSGL-1 staining. The positively stained (green) cells were visualized with a ×40 oil immersion objective using a Leica TCS-NT laser scanning confocal microscope (Imaging Core, Cleveland Clinic). Cell area was analyzed with ImageJ software.

Statistical Analysis—Two-tailed Student’s t tests were performed using SigmaPlot (version 11). Differences were considered to be significant with p < 0.05.

RESULTS

Kindlin-2 Exhibits Selective Binding to Integrin β Cytoplasmic Tails—Previous studies have demonstrated that the ability of kindlin-2 to bind and activate β3 and β6 integrins depends on its interaction with the membrane-distal NxxY motif in the β subunit of these integrins (23–25). Because the membrane distal NxxY/F motif is conserved across different integrin β subunits (Fig. 1A), we postulated that kindlin-2 might bind to other integrin β CTs similarly. Surprisingly, we failed to detect interaction of expressed kindlin-2 in cell lysates with GST-fused integrin β3 CT in pulldown assays (Fig. 1B). As expected, kindlin-2 could be precipitated by integrin β1 or β3 CTs under the
same condition. The sequence of all three β CTS, including the β₂ CT, was confirmed, and this preference in kindlin-2 binding was observed with multiple preparations of the β₂ CT. To verify these results, we measured the direct binding of purified kindlin-2 protein to integrin β CTS. Kindlin-2 was expressed as a GST fusion protein in Escherichia coli and purified as described previously (Fig. 1A). Real-time binding of kindlin-2 to full-length integrin β CTS was monitored by SPR in which kindlin-2 protein was injected and allowed to flow over immobilized full-length β₁, β₂, or β₃ CTS. As shown in Fig. 1, D and F, kindlin-2 bound to the β₁ and β₃ CTS. Consistent with the pulldown results, only minimal binding of kindlin-2 to immobilized β₂ CTS was detected (Fig. 1B). The dissociation constants for kindlin-2 with the full-length β₁ and β₃ CTSs were similar at 1.77 × 10⁻⁷ M and 1.36 × 10⁻⁷ M, respectively. Based on the concentrations used, we estimated that the affinity of kindlin-2 for the β₂ CTS was at least 100-fold lower than for the β₁ or β₃ CTS. These results suggest that kindlin-2 can interact selectively with different integrin β CTSs and indicate that the conserved motifs in the integrin β CTSs are insufficient to determine kindlin-2 recognition.

Identification of Kindlin-2-binding Site in Integrin β Cytoplasmic Tails—We previously implicated residues in the C-terminal region of the integrin β₁ and β₃ CTSs in kindlin-2 binding (Fig. 2A) (10, 23). To more precisely define the requirements for their interaction, we directly immobilized these β C-terminal peptides conjugated with biotin at their N termini onto SA biosensor chips for SPR analysis. As shown in Fig. 2B, kindlin-2 bound to the immobilized β₁ and β₃ C-terminal peptides, but the association with the β₂ C-terminal peptide was significantly lower, confirming the interactive selectivity of kindlin-2 to integrin β CT. The similar kindlin-2 dissociation constants for the C-terminal peptides and their full-length versions (1.77 × 10⁻⁷ M for full-length β₁ and 1.96 × 10⁻⁷ M for the β₁ C-terminal peptide: 1.36 × 10⁻⁷ M for full-length β₃ CT and 1.81 × 10⁻⁷ M for the β₃ C-terminal peptide) verified that the C termini of the integrin β₁ and β₃ CT served as the main binding region for kindlin-2.

To further define the kindlin-2-binding site, a series of peptides corresponding to the C termini of the β₁ CT with sequential deletion of residues from both the C- and N-terminal sides were synthesized. The β₁ peptides synthesized are shown in Fig. 2C, and they were tested as inhibitors of the β₁-GST/kindlin-2 co-precipitation. As shown in Fig. 2C, the β₁ CTS peptides containing the highlighted residues (AVTTVVKNYEG, designated as the β₁ core) were effective blockers of kindlin-2 binding to the β₃ CT, whereas the β₁ CT peptides lacking these highlighted residues were ineffective inhibitors. The counterpart peptide of β₃ CT (ATSTFTNITYRG, designated as the β₃ core) also effectively blocked kindlin-2 binding to the β₁ or β₃ CT, as did the β₁ core (Fig. 2D and E). Thus, the 12 amino acid residues in the β CTSs identified in Fig. 2A constitute the basic binding core for kindlin-2 recognition with high affinity.

Intriguingly, the kindlin-2 binding core in the integrin β CTSs contains both conserved and variable residues (Figs. 1A and 2A). To further test the contribution of these non-conserved residues in the integrin β CTSs for kindlin-2 interaction, we generated chimeric β peptides in which the last three variable residues in the β₂ and β₃ CTSs were replaced with each other (Fig. 2F). By SPR analysis, we found substitution of the last three residues RGT in the β₁ peptide with the β₂ counterpart residues AES nearly abolished the interaction of β₂ CT peptide with kindlin-2. Surprisingly, replacement of AES in the β₂ CT peptide with RGT significantly enhanced kindlin-2 binding to the β₂ CT peptide (Fig. 2G). These results demonstrate that the variable residues in integrin β CTSs also contribute to kindlin-2
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binding, and they play key roles in determining binding selectivity.

Kindlin-2-binding Core in Integrin β3 Cytoplasmic Tail Inhibits Cell Spreading—The functional significance of kindlin-2 binding core, β3 (Ala475–Gly476), the β3 CT(CORE), was assessed in a cellular system. This segment, as well as control segments of the β3 CT, was expressed as PSGL-1 chimera in CHO cell line stably expressing the binding core, and they play key roles in determining binding selectivity. Kindlin-2 and Talin Head Domain Can Bind Simultaneously to Integrin β3 Cytoplasmic Tail—Because talin-H and kindlin-2 are capable of co-activating integrins by interaction with integrin β CT, it is conceivable that these two proteins might bind to one another or sterically influence the binding of each other to the β CT. To test these possibilities, we performed a series of SPR experiments. First, we considered whether talin-H and kindlin-2 could directly unclasp the integrin CT complex. To test this possibility, we performed a series of functional analyses showing that kindlin-2 enhanced talin-induced integrin activation but alone has only a minimal effect on integrin activation (23, 24).

FIGURE 2. Kindlin-2 interacts with the C termini of integrin β CTs. A, amino acid sequences of the β1, β2, and β3 C-terminal peptides. B, representative binding of one concentration (1 μM) of kindlin-2 to β C-terminal peptides. N-terminally biotinylated peptides were captured on a SA biosensor chip, and kindlin-2 binding signals were recorded after injection (red for β3, green for β3, and blue for β3 peptides) as described in Fig. 1. C, control GST or GST-fused β3 CT was incubated with HUVEC lysate in the presence of the indicated peptide from the β3 CT (final concentration at 200 μM). The endogenous kindlin-2 binding to the β3 CT was precipitated with glutathione-Sepharose and further analyzed by Western blot. GST-fused β3 CT (D) or GST-fused β3 (E) were used to precipitate kindlin-2 protein from HUVEC lysates in the presence of β3 core peptide or β3 core peptide as highlighted in A. F, the chimeric β3 and β3 CT with the last three-amino acid replacement, G, comparison of the binding of one concentration of kindlin-2 to N-terminally biotinylated β peptides (light green for β3-AES, red for β2-RGT, and green for the β3 peptide as shown in A).
titration calorimetry as performed, we could not detect interaction between talin-H and kindlin-2, although interaction of these proteins with \( \beta_3 \) CT indicates the functionality of the protein preparations.

The undetectable interaction between talin-H and kindlin-2 allowed us to test whether they can bind simultaneously to the integrin \( \alpha_{IIb}\beta_3 \) CT by SPR experiments. Full-length \( \beta_3 \) CT was immobilized on the biosensor chip, and talin-H or kindlin-2 was passed over the chip. As shown in Fig. 6A, a typical progress curve was obtained for either kindlin-2 or talin-H. When a mixture of kindlin-2 and talin-H, each at the same concentration, was passed over the immobilized \( \beta_3 \) CT, the response curve was enhanced as compared with that obtained with each individual analyte, indicating that their interaction was not mutually exclusive (Fig. 6A). We further assessed whether occupancy of the immobilized \( \beta_3 \) CT with talin-H would limit kindlin-2 bind-

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**FIGURE 3.** The kindlin-2-binding core in the integrin \( \beta_3 \) CT is sufficient to influence cell spreading. CHO cells stably expressing \( \alpha_{IIb}\beta_3 \) were transiently transfected with plasmids encoding the following \( \beta_3 \) CT-containing chimeras: \( \beta_3 \) CT/PSGL-1, \( \beta_3 \) CT\(\Delta748\)/PSGL-1, and \( \beta_3 \) CT (CORE)/PSGL-1. The cells were allowed to adhere to fibrinogen coated coverslips 12 h after transfection, and cell spreading was measured after an additional 2 h. The adherent cells were fixed and stained with the anti-PSGL-1 mAb, KPL-1, for visualization of the expressing cells by fluorescence microscopy. A displays representative images taken at 40× (bar, 20 \( \mu \)m). B shows effects of the various chimeras on integrin \( \alpha_{IIb}\beta_3 \)-mediated cell spreading. The areas of PSGL-1-positive cells were measured using ImageJ software, and 200 cells were quantified in each experiment. The error bars represent means ± S.D. of three independent experiments. **, \( p < 0.001 \) versus CHO-A5 by Student’s t test, n.s. indicates not significant (\( p = 0.13 \)) versus CHO-A5 by t test.

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**FIGURE 4.** Kindlin-2 fails to unclasp the integrin \( \alpha_{IIb}\beta_3 \) CT complex. A, overlay of the selective region of transferred NOESY spectra of 2 mM \( \alpha_{IIb}\beta_3 \) in the presence of 0.1 mM MBP-\( \beta_3 \) (black) versus 2 mM \( \alpha_{IIb}\beta_3 \) in the presence of 0.1 mM MBP-\( \beta_3 \) and 0.2 mM kindlin-2 (red), showing that kindlin-2 does not disrupt the integrin \( \alpha_{IIb}\beta_3 \) CT complex formation. B, overlay of the selective region of transferred NOESY spectra of 2 mM \( \alpha_{IIb}\beta_3 \) in the presence of 0.1 mM MBP-\( \beta_3 \) (black) versus 2 mM \( \alpha_{IIb}\beta_3 \) in the presence of 0.1 mM MBP-\( \beta_3 \) and 0.05 mM talin-H (red), showing that talin-H disrupts the integrin \( \alpha_{IIb}\beta_3 \) CT complex.
Conclusion that talin-H and kindlin-2 can interact simultaneously with integrin β-CTs and exert minimal effects on the binding of one another to the β CT.

To provide independent evidence for the ability of talin-H, kindlin-2, and β3 CT to form a ternary complex, we again used SPR imaging. As shown in Fig. 6B, after the β3 CT-coated chip was saturated with talin-H, the binding signal induced by kindlin-2 reached the level observed in the absence of talin-H. Furthermore, we measured the binding pattern of talin-H to immobilized β3 CT by sequential injections (500-s intervals) in the presence or absence of kindlin-2. The binding signals obtained with multiple injections of talin-H onto immobilized β3 CT was unaffected by kindlin-2 in the running buffer (Fig. 6, C and D). The same results were obtained when we used β1 CT instead of β3 CT in these experiments. Together, these data support the DISCUSSION

Kindlin family members were recently identified as integrin co-activators that play indispensable roles in integrin activation (43). Our current study reports several novel findings to further interpret the mechanism by which kindlin-2 facilitates integrin activation. First, kindlin-2 shows selectivity in binding to integrin CT; it binds with substantially lower affinity to β3 than to β1 and β3 CT. β2-integrins also require kindlins for activation, and this need may be met by kindlin-3 in myeloid cells (27–29). Second, the kindlin-2 binding core of 12 amino acids has been identified in integrin β-CTs, which includes the conserved membrane-distal NxxY region and S/T cluster as well as the variable residues flanking these two motifs. The functional importance of this kindlin-2 binding core in intact cells was supported by cell spreading assays. Expression of the C-terminal kindlin-2 binding region alone was sufficient to suppress integrin-mediated cell spreading. The identification of the kindlin-2 binding core provides a context in which to interpret the effects of previously reported mutations on integrin activation. Thus, the effects of β3Δ755, as well single point mutations at S752P or Y759A in the β3 CT on integrin activation (44–49) can be attributed to inhibition of kindlin-2 binding. Also, the inhibition of integrin α10β3 activation in platelets by importable peptides corresponding to β3 (743–750) and β3 (749–756), which overlap the kindlin-2 binding core (50, 51), can now be attributed to effects on kindlin binding. Expression of the β3.CTΔ748 and β3 CT (CORE) as a PSGL chimeric protein also suppressed integrin activation, suggesting that the membrane proximal and membrane distal regions of the β3 CT contribute independently to integrin-mediated responses, observations consistent with the cooperativity between talin and kindlin-2 in integrin function. We cannot exclude that the effects of these two regions may also reflect their interaction with other integrin binding partners. For example, Integrin Linked Kinase (52) and Src
have been shown to bind to the C-terminal region of the \( \beta_3 \) CT as well. However, in the \( \alpha_{IIb}\beta_3 \)-CHO cell system, these specific binding partners do not influence \( \alpha_{IIb}\beta_3 \) activation or co-activation with talin-H (54)\(^4\). Instead of NxxY in \( \beta_1 \) and \( \beta_3 \) tails, the counterpart motif in the \( \beta_2 \) CT is NxxF. However, substitution of Phe with Tyr in \( \beta_2 \) CT did not improve the binding affinity (data not shown), suggesting that the non-conserved residues in the integrin \( \beta \) core region might be also critical for kindlin-2 recognition. This proposition was supported experimentally. A \( \beta_2 \) CT peptide with its C-terminal AES replaced by the \( \beta_3 \) RGT showed higher kindlin-2 binding, and conversely, a \( \beta_3 \) CT peptide in which it C-terminal RGT was replaced with the \( \beta_2 \) AES sequence had blunted kindlin-2 binding (Fig. 2). Even though this binding core is sufficient for kindlin-2 binding to \( \beta_1 \) and \( \beta_3 \) CT, we do not exclude the possibility that more membrane proximal residues in the CT might help to stabilize the interaction and support integrin activation. More detailed structural studies are still required to further understand the binding specificity of the kindlin/\( \beta \)CT interaction. Third, kindlin-2 is unable to directly unclasp the integrin CT complex, which provides a mechanistic explanation for the very limited ability of kindlin-2 alone on integrin activation; for efficient activation, talin-H also has to be present (23, 24). One possibility for the talin/kindlin-2 co-activating requirement is that talin-H might directly interact with kindlin-2. However, we failed to detect their direct interaction experimentally. Indeed, talin-H and kindlin-2 can bind simultaneously to integrin \( \beta \) CT with no evidence of mutual perturbation. The capacity of kindlin-2 and talin-H to co-assemble on the \( \beta_3 \) CT to form a ternary complex was indicated by our SPR data, where the resonance unit signal, a reflection of mass of the bound constituents (55, 56) increased upon binding of both talin-H and kindlin-2 to the immobilized \( \beta_3 \) CT. Independent evidence of formation of a ternary complex also was provided by NMR experiments showing that the chemical shifts induced by talin-H or kindlin-2 binding to the \( \beta_3 \) CT were further shifted by the presence of the two constituents; such changes are also indicatve of simultaneous interaction of the three constituents, i.e. formation of a ternary complex. Of note, the ability to detect chemical shift changes in the \( \beta_3 \) CT upon addition of kindlin-2 suggests that it may be feasible to locate the interactive residues in \( \beta_3 \) CT engaged in kindlin-2 binding. In view of the relatively low affinity of kindlin-2 for the \( \beta_3 \) CT, which we estimated from our SPR experiments to be \( 1.36 \times 10^{-7} \) M, such NMR mapping may be more

\(^4\) K. Bledzka, J. Liu, Z. Xu, H. D. Perera, S. P. Yadav, K. Bialkowska, J. Qin, Y.-Q. Ma, and E. F. Plow, unpublished observations.
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FIGURE 7. A, demonstration of a ternary complex between kindlin-2, β3CT, and talin by NMR. 15N-labeled HSQC of β3 CT (60 μM) in the absence (black) and presence (red) of unlabeled talin-F2F3 (100 μM), pH 6.1. Significant line-broadening and some chemical shift changes occur upon addition of talin-F2F3 to the 15N-labeled β3 CT (B). 15N-labeled HSQC of β3 CT (60 μM) in the absence (black) and presence (green) of unlabeled kindlin-2 (100 μM), pH 6.1. The pattern of line-broadening and chemical shift changes differ from that of A by talin-F2F3, indicating a different binding mode. C, 15N-labeled HSQC of β3CT (60 μM) in the absence (black) and presence (cyan) of talin-F2F3 (100 μM) and kindlin-2 (100 μM), pH 6.1. More line broadening and chemical shift changes occur with some new peaks, which differ from A and B, indicating formation of a ternary complex.

feasible for detecting and dissecting the ternary complex than other approaches that are difficult to apply for lower affinity interactions. However, because of the relatively large molecular weight involving kindlin-2 (~70 kDa) and talin-H (~50 kDa), future investigation will employ deuteration and transverse relaxation optimized spectroscopy-type NMR experiments to reduce the line-broadening effect. Although we did not detect any synergistic binding effect of kindlin-2 and talin-H on integrin βCT, their simultaneous binding might be the first key step to attain synergy in integrin activation through alternative pathways: i) kindlin-2 and talin-H could jointly displace negative regulator(s) such as filamin from the integrin CTs. The site that we have mapped for kindlin-2 binding on integrin CT clearly overlaps with that identified for filamin (7); ii) kindlin-2 and talin might be linked by an as yet identified bridging molecule(s), thereby enhancing their interaction with integrin; iii) more directly, kindlin-2 could attach onto the plasma membrane and place β CT in a specific orientation advantageous for talin-H recognition. Although these possible regulatory mechanisms remain to be vigorously dissected, our studies herein have provided significant insights into our understanding of how kindlin-2 and talin may be simultaneously situated on integrin β CT and synergistically promote integrin activation. The capacity of talin and kindlins to associate simultaneously provides an example as to how large molecules such as talin and kindlins can associate in close proximity on a small segment of receptor, the β CTs, to orchestrate a physiologically relevant cellular response, integrin activation.

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