Integrins, which are heterodimeric transmembrane proteins composed of α and β subunits, are major receptors for cell adhesion (1). Integrin-mediated adhesiveness is determined by the affinity state of each integrin and the number of integrin-ligand bonds formed (valency) (2). The affinity regulation of integrins has been studied extensively, leading to a hypothesis that high affinity states facilitating ligand binding (activation) can be achieved by “inside-out” signals that can break the clasp holding membrane-proximal regions of the α and β tails together and separate the transmembrane domain (TMD)2-tail regions (3, 4). The valency of integrin-ligand binding can be increased by integrin clustering. Several reports suggested that there might be a positive correlation between integrin activation and clustering. For example, Li et al. suggested an insightful idea that integrin activation and clustering can be coupled by homomeric association of separated TMDs of active integrins (5). The idea of homomeric association is supported by several studies showing homomeric association in micelles and membrane bilayers (6–8). However, a study that attempted to identify such a relationship between integrin activation and clustering by inducing αIIb-αIIb clustering through an inducible homodimer system revealed that clustering did not induce integrin activation (9). In the study, integrin activation was measured by binding of PAC1 Fab (10), which can recognize only the active form of integrin regardless of the clustering state of integrins. Similarly, introducing an intermolecular disulfide bond between transmembrane domains of αIIb integrins does not enhance PAC1 Fab binding (11), suggesting that clustering may not be involved in integrin activation. In both of the studies above, clustering of integrins enhanced PAC1 binding, but the enhanced PAC1 binding was attributed to increased valency not to activation of integrins, because PAC1 IgM can form a pentamer with a total 10 available ligand binding sites and thus bind more efficiently to the clustered integrins (10, 11). Therefore, it seems to be accepted that there is no correlation between integrin clustering and activation.

Our previous study showed that overexpression of the integrin αIIb TMD-tail or β3 TMD-tail construct can induce activation of integrin αIIbβ3 (12). The activation was proven to result from heteromeric interactions of the TMD-tail of each construct with native full-length integrins, thus breaking apart the intramolecular α and β TMD-tail interaction within an integrin. This conclusion led us to hypothesize that when α and β TMD-tails of integrins are separated during integrin activation, each TMD-tail may interact heteromerically with the TMD-tail of other integrins, and this may cause activation as well as clustering of integrins in proximity. Therefore, to test our hypotheses on the relationship between integrin clustering and activation, we sought a way to cluster integrins through α-β heteromeric TMD interaction. One such way to achieve this involved the generation of chimeric integrin constructs. These were then used to show the intermolecular TMD-tail interaction between integrins. By using the chimeric integrin, we also showed that integrin activation can be coupled to integrin clus-

**Background:** The relationship between integrin clustering and activation has been controversial.

**Results:** We show that intermolecular transmembrane domain interaction in integrin αIIbβ3 can induce integrin activation.

**Conclusion:** Integrin clustering can enhance integrin activation.

**Significance:** We provide a new mechanism for integrin activation.
**Experimental Procedures**

Plasmids—To construct the chimeric integrin α5-αIIb, we generated the TMD-tail region of the human integrin αIIb subunit by polymerase chain reaction (PCR) using the forward primer (5′-acaaggtatgcggccttgagctt-3′, HindIII cleavage site underlined) and reverse primer (5′-agctttgagcttccctcttcatc-3′, XbaI cleavage site underlined). The PCR product was digested with HindIII and XbaI and cloned into pcDNA3.1. To construct another chimeric integrin β1-β3, the extracellular domain of the human integrin β3 was first generated by PCR using forward primer (gggcatctgctccgggccttgac-3′, HindIII cleavage site underlined) and overlapping reverse primer (5′-ctctggctcttctaccacatgaaccatgacctcgtt-3′, β1 sequence underlined). The TMD-tail region of the human integrin β3 was also generated by PCR using an overlapping forward primer (5′-acagctgcttctaccagcttacgacgtct-3′, HindIII cleavage site underlined) and reverse primer (5′-actcttgagcttcacccctct-3′, HindIII cleavage site underlined), and the PCR product was cloned into pcDNA3.1 (Invitrogen) containing the αIIb TMD-tail. To construct another chimeric integrin β1-β3, the extracellular domain of the human integrin β1 was first generated by PCR using forward primer (gggcaaggtatgtgatgacgagaagccagag-3′, HindIII cleavage site underlined) and overlapping reverse primer (5′-tgtgatgatgatgacgagaagccagag-3′, HindIII cleavage site underlined), and the PCR product was cloned into pcDNA3.1 (Invitrogen) containing the αIIb TMD-tail. To construct another chimeric integrin β1-β3, the extracellular domain of the human integrin β1 was first generated by PCR using forward primer (gggcaaggtatgtgatgacgagaagccagag-3′, HindIII cleavage site underlined) and overlapping reverse primer (5′-tgtgatgatgatgacgagaagccagag-3′, HindIII cleavage site underlined), and the PCR product was cloned into pcDNA3.1 (Invitrogen) containing the αIIb TMD-tail. To construct another chimeric integrin β1-β3, the extracellular domain of the human integrin β1 was first generated by PCR using forward primer (gggcaaggtatgtgatgacgagaagccagag-3′, HindIII cleavage site underlined) and overlapping reverse primer (5′-tgtgatgatgatgacgagaagccagag-3′, HindIII cleavage site underlined), and the PCR product was cloned into pcDNA3.1 (Invitrogen) containing the αIIb TMD-tail. To construct another chimeric integrin β1-β3, the extracellular domain of the human integrin β1 was first generated by PCR using forward primer (gggcaaggtatgtgatgacgagaagccagag-3′, HindIII cleavage site underlined) and overlapping reverse primer (5′-tgtgatgatgatgacgagaagccagag-3′, HindIII cleavage site underlined), and the PCR product was cloned into pcDNA3.1 (Invitrogen) containing the αIIb TMD-tail.

Cell Lines and Antibodies—Chinese hamster ovary (CHO) and A5 cells were maintained as described previously (12). The CHO-α-VC cell line was kindly provided by Dr. Sanford Shattil (University of California, San Diego) and maintained as CHO cells. For transient transfection, Lipofectamine and Plus reagents (Invitrogen) were used according to the manufacturer’s instructions. An anti-human integrin β1 antibody (MAR4) was purchased from BD Biosciences. An antibody specific to the active form of integrin αIIbβ3, PAC1, has been described previously (10).

Immunoprecipitation and Affinity Capture Assay—A5 cells were transfected with the chimeric integrin constructs α5-αIIb and β1-β3. One day after transfection, cell lysates in lysis buffer I (50 mM Tris-Cl, pH 7.4, 1% Triton X-100, 150 mM NaCl, 0.5 mM MgCl2, and 0.5 mM CaCl2) supplemented with EDTA-free protease inhibitor (Roche Applied Science) were clarified by centrifugation and incubated with the anti-human integrin β1 extracellular domain antibody (P5D2) followed by incubation with protein G-Sepharose (GE Healthcare). The resulting precipitates were analyzed by SDS-PAGE followed by Western blotting using antiserum against integrin αIIb cytoplasmic tail (Rb9449). An affinity capture assay was performed to measuring α and β TMD interaction as described previously (12). Briefly, CHO cells were transfected with αIIb and β3 TMD constructs harboring mutations or not, and lysed with lysis buffer II (20 mM HEPES, 150 mM NaCl, 1% CHAPS, and 2 mM CaCl2) supplemented with EDTA-free protease inhibitor. The lysates were incubated with calmodulin-Sepharose (GE Healthcare), and the bound proteins were analyzed by Western blotting.

Flow Cytometry—A PAC1 binding assay was performed as described previously (13). Briefly, 24 h after transfection of various integrin constructs into A5 or CHO-α-VC cell lines, cells were trypsinized and incubated with PAC1 for 30 min at room temperature. The stained cells were washed twice with Dulbecco’s modified Eagle’s medium (DMEM) and incubated further with anti-IgM conjugated to phycoerythrin. After washing with DMEM, the resulting cells were analyzed using a FACScalibur (BD Biosciences) for PAC1 or MAR4 binding in the FL2 channel and for P5D2 binding or Venus fluorescence in the FL1 channel.

**Results**

TMD-Tail Separation of One Integrin Can Activate Neighboring Integrins—Previously, we showed that overexpression of the integrin αIIb TMD-tail construct results in integrin activation by inducing the interaction between the expressed αIIb TMD-tail and β3 TMD-tail region of intact integrins (Fig. 1A). Thus, we hypothesized that the free tail of integrins may also activate neighboring integrins through the same lateral interaction (Fig. 1B). Similarly, we also hypothesized that clustered integrins may have a greater chance to be activated by this intermolecular TMD-tail interaction than nonclustered integrins, due to the increased chance of the lateral interaction in clustered integrins (Fig. 1C). However, as described above, several trials to induce clustering of integrins failed to produce such activation. According to our hypothesis, the integrin clustering resulting from homomeric TMD-tail interactions would not induce separation of α and β TMD-tails and thus would not have any effect on integrin activation (Fig. 1D). Therefore, to clarify the effect of active integrins on the affinity state of neighboring integrins, we designed a chimeric integrin α5-αIIb β1-β3 that can allow heteromeric α-β TMD-tail interactions between integrins. The chimeric integrin contains the extracellular domains of human α5β1 and the TMD-tail regions of human αIIbβ3. Inclusion of the extracellular domains of integrin α5β1 would ensure specific association between the chimeric integrin subunits, and the TMD-tail regions of integrin αIIbβ3 can interact with wild type integrin αIIbβ3, if such a lateral interaction occurs (Fig. 2A).

Following transfection of the chimeric integrin into CHO cells stably expressing integrin αIIbβ3 (A5 cells), an anti-human integrin β1 extracellular domain-specific antibody that binds to the β1-β3 subunit of the chimeric integrin can successfully precipitate α5-αIIb subunit (Fig. 2B), demonstrating that two chimeric integrin subunits can be associated together as expected. Next, we transfected the chimeric integrin α5-αIIb β1-β3 into A5 cells and tested the effect of its expression on
Intermolecular TMD Interactions Activate Integrins

activate neighboring integrins by inducing intermolecular heteromeric associations of TMD-tails.

Clustering of Integrins Enhances Integrin Activation—Next, we asked whether integrin clustering resulting from an α-β intermolecular TMD-tail interaction among integrins would induce activation of integrins. To visualize and induce the intermolecular heteromeric TMD interaction of integrins, we utilized bimolecular fluorescence complementation using Venus fluorophore (14). For this, we used CHO-α-VC cells that stably express the integrin αIIb subunit fused to the VC as well as integrin β3 subunit as reported previously (15). In addition, we generated a chimeric integrin β1-β3 construct fused to the N-terminal region of Venus (VN) to make β1-β3-VN. The interaction between VN and VC is known to be irreversible; once VN and VC are associated and the fluorescent Venus is assembled, the interaction is stably maintained (16). By using VN-VC dimerization to bring TMD-tails close to each other and thus induce TMD-tail lateral interaction, we asked whether lateral TMD-tail interaction can promote integrin activation (Fig. 3A). However, we note that the VN-VC interaction would hold two integrin together at the end of the cytoplasmic tails through a flexible linker, thus it may not force the intermolecular TMD-tail interaction between the clustered integrins directly but rather increases the probability of such lateral interaction by placing two integrins close together.

We transfected integrin α5-αIIb and/or β1-β3-VN into CHO-α-VC cells and measured Venus fluorescence and human β1 expression on the cell membrane. As expected, it was possible to observe Venus fluorescence resulting from clustering of those integrins (Fig. 3B). However, transfection of β1-β3-VN alone induced high Venus fluorescence. The Venus fluorescence in this condition looks from the endoplasmic reticulum or Golgi because β1-β3-VN was not exported to the cell surface as determined by surface staining using anti integrin β1 antibody (Fig. 3B, left panel). This intracellular Venus fluorescence is presumably due to nonfunctional complex formation between αIIb-VC and β1-β3-VN mediated by TMD-tail interaction without involvement of head domain interactions. In contrast, when both α5-αIIb and β1-β3-VN constructs were transfected, integrin β1 antibody staining on cell surface was increased (Fig. 3B, middle panel), showing successful and functional assembly of α5-αIIb and β1-β3-VN on the cell surface. These cells also exhibited spontaneous Venus fluorescence resulting from TMD-tail interaction between the chimeric integrin α5-αIIb β1-β3-VN and integrin αIIb-VC β3 (Fig. 3B, middle panel). As a control, we introduced W968C and I693C mutations in α5-αIIb and β1-β3-VN, respectively (Fig. 3C). These mutations are known to block intramolecular TMD-tail separation by producing a spontaneous intersubunit disulfide bond (17), thus blocking the intermolecular lateral interaction. However, despite blocking intramolecular TMD-tail separation by the disulfide bond, we observed Venus fluorescence from integrin αIIbβ3 and the chimeric integrin (Fig. 3B, right panel).

We assume that both integrins fused to VN and VC can be closely localized inside the endoplasmic reticulum or Golgi during biosynthesis. Thus, close proximity during biosynthesis may enable the assembly of the complete Venus protein, presumably due to the intrinsic affinity between VN and VC (16), integrin αIIbβ3 activation using PAC1, which is a ligand mimetic antibody against integrin αIIbβ3 (10). When the wild type chimeric integrin was expressed, there was a little change in the activation status of integrin αIIbβ3 (Fig. 2C, top panel; Fig. 2D, black circles). However, the activation of integrin αIIbβ3 was significantly increased by expression of a chimeric integrin containing a deletion of cytoplasmic tail (Δ717) and a point mutation (G708I) in β3 (Fig. 2C, middle panel; Fig. 2D, red filled circle). These mutations are known to block αIIb-β3 TMD association; this results in the provision of a free αIIb TMD-tail of the chimeric integrin to a neighboring integrin αIIbβ3 (Fig. 2C, diagram of middle panel). To confirm involvement of the chimeric α5-αIIb integrin TMD-tail in the activation of αIIbβ3, we introduced additional mutations (G972L,G976L) into the chimeric integrin that block α-β TMD-tail association (12) (Fig. 2C, bottom panel). As expected, the activating effect of the chimeric integrin is abolished by G972L,G976L mutations (Fig. 2C, bottom panel; Fig. 2D, red empty circle). The effect of chimeric integrins on the affinity state of integrin αIIbβ3 was evident when the expression levels of chimeric integrins were relatively high (Fig. 2, C and D), showing that a high concentration of integrin is required for lateral interaction of integrins. In conclusion, these results suggest that active integrins can...
but not due to intermolecular interaction of those integrins. Alternatively, the intermolecular interactions between the chimeric integrin, integrin αIIbβ3, and Venus protein may be established earlier than the disulfide bond formation within the chimeric integrin. In either case, we were able to induce clustering of integrin αIIbβ3 and the chimeric integrin with the “lock” of TMD separation (Fig. 3C).

After we confirmed the surface expression of chimeric integrin α5-αIIB β1-β3-VN and its association with integrin αIIb-VC β3 as shown above, we tested the effect of such clustering on integrin affinity state. CHO-α-VC cells transfected with α5-αIIB and/or β1-β3-VN were detached and stained with PAC1 to measure integrin αIIb-VC β3 activation. Venus fluorescence was also measured to determine the amount of clustered integrins in the cells. In CHO-α-VC cells β1-β3-VN expression alone showed Venus fluorescence due to nonfunctional intracellular TMD-tail interaction as discussed above, and there was of course no increase in PAC1 binding on the cell surface (Fig. 3D, black circle; Fig. 3E, left panel). Interestingly, when both α5-αIIB and β1-β3-VN were expressed, PAC1 binding was significantly increased (Fig. 3D, red filled circle; Fig. 3E, middle panel). However, when the separation of TMD-tail regions was blocked by disulfide bonds in α5-αIIB and β1-β3-VN, there was no increase in PAC1 binding (Fig. 3D, red empty circle; Fig. 3E, right panel), showing that Venus-mediated clustering alone does not contribute to integrin activation. Therefore, we conclude that lateral interaction between integrins (or clustering of integrins) via TMD-tail regions can activate integrins, and the separation of TMD-tails is essential for the lateral interaction-induced integrin activation. We also note that
increased PAC1 binding in the clustered integrins is not due to the increased valency that results from lateral association of the chimeric integrin and \( \alpha 1 \beta 3 \), but is solely due to the activation of \( \alpha 5 \alpha 1 \beta 3 \), because PAC1 cannot recognize the chimeric integrin \( \alpha 5 \alpha 1 \beta 3 \).

To exclude the possibility that the absence of an activating effect of \( \alpha 5 \alpha 1 \beta 3(W968C) \beta 1 \beta 3(I693C)-Vn \) in CHO-\( \alpha 5 \)-VC cells is due to the inability of \( \beta 3(I693C) \) to bind to \( \alpha 1 \beta 3 \), we tested the effect of the I693C mutation in \( \alpha 1 \beta 3 \) TMD-tail interaction. The Tac (interleukin-2 receptor)-fused \( \alpha 1 \beta 3 \) TMD-tail construct (Fig. 4A) bearing the I693C mutation was transfected with the \( \alpha 1 \beta 3 \) TMD-tail construct (Fig. 4A), and their association was measured by a pulldown experiment performed as described previously (12). As shown in Fig. 4B, the degree of \( \alpha 1 \beta 3 \) TMD-tail interaction is similar regardless of whether or...
not the I693C mutation is present. Thus, lack of activation induced by I693C mutation in the β subunit can be attributed to a defect in TMD-tail separation.

**DISCUSSION**

Here, we demonstrated that there may be a lateral interaction between TMD-tails of two integrins and that the lateral interaction among integrins can induce the activation of αIβ3. Based on our previous observation of preferential α-β heterodimeric TMD-tail interaction on CHO cell membrane (12), we favor the interpretation that the activating effect induced by the lateral interaction depends on the heterodimeric TMD-tail interaction between integrins. However, we do not rule out the possibility that homomeric α-α TMD interaction might also contribute to such activating effects, because the G972L,G976L mutations that inhibited the lateral interaction might also contribute to such activating effects, because the G972L,G976L mutations that inhibited the lateral interaction-induced integrin activation in our assay (Fig. 2, C and D) are also known to inhibit the homomeric TMD interaction (7).

Integrins exist in an equilibrium between “αo-βn TMD-tail-bound (inactive)” and “αo and βn TMD-tail-separated (active),” where o indicates original integrin pair. Thus, integrin activation can be viewed as the increased probability of the integrin being in the αo and βn TMD-tail-separated state than in the αo-βn TMD-tail-bound state. We reasoned that the separated αo and βn will have some increased likelihood to interact with another αn or βn (where n indicates neighboring integrin pair) of a neighboring integrin in the TMD-tail separated state, if αo or βn concentration is high in the vicinity of the separated αo or βn. This interaction will prolong the αo and βn TMD-tail-separated (active) state and favor the equilibrium toward activated integrin. Because the heteromeric intermolecular interaction between αo and βn (or βo and αo) is identical to the intramolecular TMD-tail interaction between αo and βo, the heteromeric lateral interaction-induced integrin activation, unlike that induced by homomeric TMD interaction proposed by others (5), can be only achieved when there are high concentration of integrins (αo and βn) in the active conformation. Accordingly, the activation achieved by Venus-induced lateral integrin interaction alone is modest (Fig. 3, D and E), presumably due to the competition between intermolecular and intramolecular TMD-tail interactions and to the submaximal clustering induced by VN-VC dimerization. However, when lateral interaction is induced by physiological clustering mechanisms, which may induce higher order clustering (18), and combined with other affinity modulation mechanisms, it may synergize with other mechanisms to dramatically increase integrin activation (9, 18). Furthermore, we speculate that lateral interaction may also contribute to the growth of integrin clusters and integrin adhesion. For example, once an integrin active state is stabilized by binding to ligand, the TMD-tail separation of one integrin would be stabilized (19). Thus, the intermolecular interaction of integrins can be used for a “zipper-like” cell attachment; ligand binding of one integrin recruits another integrin by lateral interaction, and the interaction activates the integrin, thus forming the second adhesion site. Talin is known to bind integrin β tails (20), induce a change in the tilt angle of β TMD (21) that can separate α-β TMD-tails (22), and activate integrins. Because the lateral interaction between integrins is predicted to involve the same α-β binding interface, talin would not only inhibit intramolecular α-β TMD interactions, but also the intermolecular α-β TMD interaction. Therefore, we suggest that lateral interaction-induced integrin activation may be useful for transient cell-substrate interaction where rapid turnover of integrin activity is needed, whereas talin-induced integrin activation may be responsible for more stable cell adhesion.

In conclusion, we suggest here that intermolecular interaction between integrins through heteromeric TMD-tail interactions can activate integrins and that integrin clustering can facilitate the intermolecular interaction by increasing local concentration of integrins. Thus, integrin clustering may be closely related to integrin activation.

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**REFERENCES**

1. Hynes, R. O. (2002) Integrins: bidirectional, allosteric signaling machines. *Cell* **110**, 673–687
2. Carman, C. V., and Springer, T. A. (2003) Integrin avidity regulation: are changes in affinity and conformation underemphasized? *Curr. Opin. Cell Biol.* **15**, 547–556
3. Kim, C., Ye, F., and Ginsberg, M. H. (2011) Regulation of integrin activation. *Annu. Rev. Cell Dev. Biol.* **27**, 321–345
4. Shattil, S. J., Kim, C., and Ginsberg, M. H. (2010) The final steps of integrin

![FIGURE 4. β3(I693C) mutation does not inhibit α-β TMD-tail interaction.](image-url)

A, schematic diagram of integrin TMD constructs. αIβ TMD-tail construct (αIβ-TAP) consists of N-terminal signal peptide, FLAG tag, TMD-tail region of integrin αIIb, and tandem affinity purification tag (TAP). β3 TMD-tail construct, Tac-β3 TMD-tail construct transfected with wild type or I693C mutation-bearing β3 TMD-tail construct. Interaction between constructs was measured as described previously (12). Note that the I693C mutation of the integrin β3 TMD-tail construct does not inhibit α-β TMD-tail interaction.
activation: the end game. Nat. Rev. Mol. Cell Biol. 11, 288–300
5. Li, R., Mitra, N., Gratkowski, H., Vilaire, G., Litvinov, R., Nagasami, C., Weisel, J. W., Lear, J. D., DeGrado, W. F., and Bennett, J. S. (2003) Activation of integrin αIIbβ3 by modulation of transmembrane helix associations. Science 300, 795–798
6. Berger, B. W., Kulp, D. W., Span, L. M., DeGrado, J. L., Billings, P. C., Senes, A., Bennett, J. S., and DeGrado, W. F. (2010) Consensus motif for integrin transmembrane helix association. Proc. Natl. Acad. Sci. U.S.A. 107, 703–708
7. Li, W., Metcalf, D. G., Gorelik, R., Li, R., Mitra, N., Nanda, V., Law, P. B., Lear, J. D., Degrado, W. F., and Bennett, J. S. (2005) A push-pull mechanism for regulating integrin function. Proc. Natl. Acad. Sci. U.S.A. 102, 1424–1429
8. Li, R., Babu, C. R., Lear, J. D., Wand, A. J., Bennett, J. S., and DeGrado, W. F. (2001) Oligomerization of the integrin αIIbβ3: roles of the transmembrane and cytoplasmic domains. Proc. Natl. Acad. Sci. U.S.A. 98, 12462–12467
9. Hato, T., Pampori, N., and Shattil, S. J. (1998) Complementary roles for receptor clustering and conformational change in the adhesive and signaling functions of integrin αIIbβ3. J. Cell Biol. 141, 1685–1695
10. Shattil, S. J., Hoxie, J. A., Cunningham, M., and Brass, L. F. (1985) Changes in the platelet membrane glycoprotein IIb-IIIa complex during platelet activation. J. Biol. Chem. 260, 11107–11114
11. Luo, B. H., Carman, C. V., Takagi, J., and Springer, T. A. (2005) Disrupting integrin transmembrane domain heterodimerization increases ligand binding affinity, not valency or clustering. Proc. Natl. Acad. Sci. U.S.A. 102, 3679–3684
12. Kim, C., Lau, T. L., Ulmer, T. S., and Ginsberg, M. H. (2009) Interactions of platelet integrin αIIb and β3 transmembrane domains in mammalian cell membranes and their role in integrin activation. Blood 113, 4747–4753
13. Han, J., Lim, C. J., Watanabe, N., Soriani, A., Ratnikov, B., Calderwood, D. A., Puzon-McLaughlin, W., Lafuente, E. M., Boussiotis, V. A., Shattil, S. J., and Ginsberg, M. H. (2006) Reconstructing and deconstructing agonist-induced activation of integrin αIIbβ3. Curr. Biol. 16, 1796–1806
14. Shyu, Y. J., Liu, H., Deng, X., and Hu, C. D. (2006) Identification of new fluorescent protein fragments for bimolecular fluorescence complementation analysis under physiological conditions. BioTechniques 40, 61–66
15. Watanabe, N., Bodin, L., Pandey, M., Krause, M., Coughlin, S., Boussiotis, V. A., Ginsberg, M. H., and Shattil, S. J. (2008) Mechanisms and consequences of agonist-induced talin recruitment to platelet integrin αIIbβ3. J. Cell Biol. 181, 1211–1222
16. Shyu, Y. J., and Hu, C. D. (2008) Fluorescence complementation: an emerging tool for biological research. Trends Biotechnol. 26, 622–630
17. Luo, B. H., Springer, T. A., and Takagi, J. (2004) A specific interface between integrin transmembrane helices and affinity for ligand. PLoS Biol. 2, e153
18. Ye, F., Petrich, B. G., Anekal, P., Lefort, C. T., Kasirer-Friede, A., Shattil, S. J., Ruppert, R., Moser, M., Fässler, R., and Ginsberg, M. H. (2013) The mechanism of kindlin-mediated activation of integrin αIIbβ3. Curr. Biol. 23, 2288–2295
19. Luo, B. H., Carman, C. V., and Springer, T. A. (2007) Structural basis of integrin regulation and signaling. Annu. Rev. Immunol. 25, 619–647
20. Tadokoro, S., Shattil, S. J., Eto, K., Tai, V., Liddington, R. C., de Pereda, J. M., Ginsberg, M. H., and Calderwood, D. A. (2003) Talin binding to integrin β tails: a final common step in integrin activation. Science 302, 103–106
21. Kim, C., Ye, F., Hu, X., and Ginsberg, M. H. (2012) Talin activates integrins by altering the topology of the β transmembrane domain. J. Cell Biol. 197, 605–611
22. Kim, C., Schmidt, T., Cho, E. G., Ye, F., Ulmer, T. S., and Ginsberg, M. H. (2012) Basic amino-acid side chains regulate transmembrane integrin signalling. Nature 481, 209–213