Localization of an Integrin Binding Site to the C Terminus of Talin*

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Talin, consisting of a 47-kDa N-terminal head domain (residues 1–433) and a 190-kDa C-terminal rod domain (residues 434–2541), links integrins to the actin cytoskeleton. We previously reported that the binding stoichiometry of integrin α1β3-talin is 2:1. More recently, an integrin binding site has been localized to the talin head domain. In the present study, we identified another integrin binding site at the C-terminal region of the talin rod domain. In a solid phase binding assay, RGD affinity-bound human αIbβ3 was bound in a dose-dependent manner to microtiter wells coated with the isolated 190-kDa proteolytic fragment of the talin rod domain. Additionally, α1β3 also bound to the talin rod domain captured by 8d4, an anti-talin monoclonal antibody. Polyclonal antibodies raised against a recombinant protein fragment corresponding to partial sequences of the talin rod domain (anti-talin-R) inhibited α1β3 binding to intact talin by ~50% but completely blocked α1β3 binding to the talin rod domain. To localize the integrin binding site, we examined α1β3 binding to recombinant polypeptide fragments corresponding to partial sequences of the talin rod domain. Whereas α1β3 bound effectively to talin-(1075–2541) and talin-(1984–2541), it failed to bind to talin-(434–1076) and talin-(434–1795). Furthermore, the binding of α1β3 to talin-(1084–2541) was inhibited by anti-talin-R. These results indicate that an integrin binding site is located within residues 1984–2541 of the talin rod domain. Thus, talin contains two integrin binding sites, one in the homologous FERM (band four-point-one, ezrin, radixin, moesin) domain and another near its C terminus. Because talin exists as an anti-parallel homodimer in focal adhesions, the two integrin binding sites in the adjacent talin molecules would be in close proximity with each other.

Integrins are a widely distributed family of αβ heterodimeric transmembrane adhesion receptors involved in myriad biological processes such as hemostasis, immune response, wound healing, angiogenesis, embryogenesis, and tumorigenesis (1). By binding to both extracellular matrix proteins and cytoskeletal elements, integrins transmit signal into cells to regulate cell adhesion, migration, differentiation, proliferation, and survival. On blood platelets, integrin αIIbβ3 is the most prominent adhesion receptor that plays an essential role in platelet aggregation and clot retraction (2, 3). It is well established that αIIbβ3 exists in different conformational states with high and low ligand binding affinity. Recent studies have indicated that interaction of αIIbβ3 with the platelet cytoskeleton may play an important regulatory role in affinity modulation of the receptor (4–6). Furthermore, cytoskeletal attachment of αIIbβ3 has also been shown to be crucial for αIIbβ3-dependent post-ligand occupancy events such as clot retraction, protein tyrosine phosphorylation, receptor clustering, and redistribution (5, 7–9).

Most integrin subunits (except β3) contain short cytoplasmic sequences that are linked to actin filaments via cytoskeletal linkage proteins such as talin (10), α-actinin (11), filamin (12), and skelemin (13). Morphological studies have shown that talin is a prominent component of focal adhesions, suggesting that it plays a pivotal role in integrin-cytoskeleton interaction (14). In functional studies, microinjection of affinity-purified polyclonal anti-talin antibodies or monoclonal antibodies directed against the N- and C-terminal regions of talin into fibroblasts disrupted focal adhesion and actin stress fibers, and inhibited cell migration (15, 16). Furthermore, down-regulation of talin expression using talin antisense RNA showed that talin plays a central role in cell spreading and normal processing of β1 integrins (17). More recently, targeted disruption of the talin gene demonstrated an essential role of talin in focal adhesion assembly and β1 integrin expression in undifferentiated embryonic stem cells (18). As a result, talin−/− embryos are embryonic lethal at the gastrulation stage (19).

Talin is a large 235-kDa protein that can be proteolytically cleaved into a 47-kDa N-terminal globular head domain and a 190-kDa C-terminal flexible rod domain (20, 21). Based on the observation that talin caused a leftward shift in the elution profile of the avian integrin (CSAT1 antigen) complex during equilibrium gel filtration, direct interaction of integrins with talin has been suggested (10). Subsequently, biochemical studies have demonstrated that talin binds directly to the cytoplasmic domains of integrin β1A, β1D, β2, and β3 subunits (12, 22–25), and there are two integrin binding sites per talin molecule (24). The talin head domain contains an ~200 amino acid sequence that is homologous to the membrane attachment domains of the band four-point-one, ezrin, radixin, and moesin (FERM) family of membrane-cytoskeleton linkers (21, 26). An integrin binding site has been localized to the FERM domain of talin (22, 25, 27), which also contains binding sites for phosphoinositides (27), actin (28), focal adhesion kinase (29), and layilin (30). Interestingly, interaction of the talin head domain with αIIbβ3 has been shown to lead to receptor activation (22). The talin rod domain contains binding sites for vinculin and actin, thus providing linkage to actin filaments (28, 31). In an earlier study, low affinity interaction of the talin rod domain with the avian CSAT antigen has been demonstrated (10). More recently, the talin rod domain has been shown to bind to

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The abbreviations used are: CSAT, cell-substrate attachment; FERM, band four-point-one, ezrin, radixin, moesin; BSA, bovine serum albumin; PAGE, polyacrylamide gel electrophoresis; kb, kilobase(s).

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Integrin $\alpha_{IIb}\beta_3$ Binds to the Talin Rod Domain

the integrin $\beta_3$ tail, and cooperative binding of the talin head and rod domains to integrins has been suggested (32). Furthermore, post-translational modifications of talin (e.g. calpain cleavage, phosphatidylinositol 4,5-bisphosphate binding) have been shown to regulate its binding affinity to integrins (27, 32).

In the present study, we employed a solid phase binding assay to localize the integrin binding site within the talin rod domain. Results of these studies show that purified $\alpha_{IIb}\beta_3$ binds specifically to a recombinant talin fragment encompassing residues 1984–2541 at the talin C terminus. Talin has been shown to exist as an anti-parallel homodimer in focal adhesions (33, 34). Therefore, the N- and C-terminal integrin binding sites in the adjacent talin subunits may lie in close proximity with each other to interact with different coordinates in the integrin cytoplasmic domains.

MATERIALS AND METHODS

Antibodies and Reagents—The anti-talin monoclonal antibodies, 8d4 (35) and TD7 (16), were from Sigma Chemical Co. and Research Development Corporation. AP-3 (an anti-$\beta_3$, monoclonal antibody (36), was produced as ascites fluid in mice and purified by chromatography on protein A-Sepharose (Amersham Pharmacia Biotech). For binding studies, 8d4 and AP-3 were labeled with carrier-free Na$^{125}$I (Amersham Pharmacia Biotech) using the iodobeads iodination reagent (Pierce) to a specific activity of ~2 $\mu$Ci/ug. The anti-talin-R polyclonal antibody, TD77 (16), was from Sigma Chemical Co. and Research Development Corporation. Material and Methods—Integrin $\alpha_{IIb}$ was isolated from outdated platelets

Fig. 1. Binding of purified $\alpha_{IIb}\beta_3$ to the 190-kDa talin rod domain. A, SDS-PAGE analysis of purified intact talin (lane 1), talin cleaved by calpain (lane 2), and purified talin rod domain (lane 3). Proteins were separated by SDS-PAGE on 7.5% polyacrylamide gels and detected by Coomassie Blue staining. B, microtiter wells were coated with BSA or equal amounts of talin or the talin rod domain. Varying concentrations of $\alpha_{IIb}\beta_3$ were added, and binding proceeded for 3 h at 37 °C. Bound receptor was detected with 50 nm $^{125}$I-AP-3. Background binding (<5% of total) without $\alpha_{IIb}\beta_3$, addition has been subtracted. Data shown are means of triplicate determinations, and error bars represent standard deviations. The data are representative of at least five experiments.

Intact talin or recombinant talin fragment proteins were then purified by gel filtration on a Sephacryl S-100 column (16 × 600 mm) in a BioLogic HR system (Bio-Rad) and eluted with 20 mM Tris acetate, pH 7.6, 20 mM NaCl, 0.1 mM EDTA, and 0.1% $\beta$-mercaptoethanol. CaCl$_2$ (2 mM) was added and digestion with m-calpain (Sigma) was cleaved between Glu$^{432}$ and Glu$^{434}$ in the endogenous talin sequence (21), thus generating talin-(397–2541), talin-(397–1076), and talin-(397–1975) sequences, the histidine tags were released by digestion with m-calpain (Sigma), which cleaves between Glu$^{432}$ and Glu$^{434}$ in the endogenous talin sequence (21), thus generating talin-(397–2541), talin-(397–1076), and talin-(397–1975) fragments, respectively. For fusion proteins with the histidine tag fused to the N termini of the talin-(1075–2541) and talin-(1984–2541) sequences, the histidine tags were removed by digestion with enterokinase (Novagen). The digested proteins were then purified by gel filtration on a Sephacryl S-100 column (16 × 600 mm) in a BioLogic HR system (Bio-Rad) and eluted with 20 mM Tris acetate, pH 7.6, 20 mM NaCl, 0.1 mM EDTA, 0.1% $\beta$-mercaptoethanol.

Solid-phase Binding Assay—Intact talin or recombinant talin fragment proteins (50–100 $\mu$g/ml, 50 $\mu$g/well) were coated onto microtiter wells (Immumon 4 Removawell strips, Dynex Technologies, Inc.) for 18 h at room temperature. After blocking with 3% BSA, purified $\alpha_{IIb}\beta_3$ was added and the mixture was incubated for 3 h at 37 °C. In inhibition studies, various concentrations of anti-talin-R antibodies or normal rabbit IgG were added and incubated for 1 h at 37 °C prior to the addition of $\alpha_{IIb}\beta_3$. Following extensive washing, bound $\alpha_{IIb}\beta_3$ was detected with $^{125}$I-AP-3 as described previously (24). In some experiments, the amounts of talin, talin-(434–2541), talin-(434–1076), and talin-(434–1975) immobilized onto the wells were quantified by the binding of 50 nm $^{125}$I-IgG.

RESULTS

Binding of $\alpha_{IIb}\beta_3$ to the 190-kDa Talin Rod Domain—Several lines of evidence indicate that the 190-kDa talin rod domain contains an integrin binding site (10, 32). In this study, we isolated the 190-kDa talin rod domain and examined the direct binding of RGD affinity-purified $\alpha_{IIb}\beta_3$ to this talin fragment. Fig. 1A shows that proteolysis of intact talin (lane 1) with m-calpain yielded the 190-kDa head and 75-kDa rod domains (lane 2), which were then purified by gel filtration on a Sephacryl S-300 column. As judged by Coomassie Blue staining, the purified 190-kDa proteolytic fragment was devoid of intact talin, and the 47-kDa fragment (lane 3). The 190-kDa talin rod domain was coated onto microtiter wells, and its ability to support $\alpha_{IIb}\beta_3$ binding was compared with that of...
intact talin. In these experiments, we quantified the immobilization of talin and talin rod domain by the binding of $^{125}$I-8d4, an anti-talin monoclonal antibody whose epitope resides in the talin rod domain. For direct comparison of $\alpha_{IIb}\beta_{3}$ binding, equal amounts of talin (78.3 ± 1.8 fmol/well) and talin rod domain (75.0 ± 4.6 fmol/well) were coated onto the wells. As shown in Fig. 1B, $\alpha_{IIb}\beta_{3}$ bound in a dose-dependent manner to both intact talin and the talin rod domain. However, at all input concentrations of $\alpha_{IIb}\beta_{3}$, the binding of $\alpha_{IIb}\beta_{3}$ to the 190-kDa fragment was approximately half of that to intact talin. Similar results were obtained using a recombinant talin fragment encompassing residues 434–2541 of the talin rod domain (results not shown).

To confirm binding specificity, we produced polyclonal antibodies directed against the talin rod domain (anti-talin-R) and tested for inhibition on $\alpha_{IIb}\beta_{3}$ binding to intact talin and the talin rod domain. On immunoblots, anti-talin-R reacted specifically with intact talin and the 190-kDa talin rod domain, but not with the 47-kDa talin head domain (Fig. 2A). In inhibition studies, microtiter wells coated with intact talin or talin rod domain were pretreated with anti-talin-R prior to the addition of $\alpha_{IIb}\beta_{3}$. Fig. 2 (B and C) shows that anti-talin-R, but not normal rabbit IgG, effectively inhibited the binding of $\alpha_{IIb}\beta_{3}$ to intact talin or to the talin rod domain. However, at high antibody concentrations, anti-talin-R inhibited $\alpha_{IIb}\beta_{3}$ binding to intact talin by ~50% only (Fig. 2B). In contrast, complete inhibition of $\alpha_{IIb}\beta_{3}$ binding to the talin rod domain was observed (Fig. 2C). In control samples, anti-talin-R had no effect on $\alpha_{IIb}\beta_{3}$ binding to the talin head domain (results not shown). Thus, these findings are consistent with the observations that two distinct integrin binding sites are present in talin (24), one in the 47-kDa head domain (22, 25) and another in the 190-kDa rod domain (10, 32).

Because coating of proteins onto microtiter wells would induce conformational changes of the adsorbed proteins, $\alpha_{IIb}\beta_{3}$ may bind to immobilized talin rod domain but not to the native talin fragment. To exclude this possibility, we examined whether $\alpha_{IIb}\beta_{3}$ also binds to the talin rod domain captured by immobilized 8d4, an anti-talin monoclonal antibody. In these experiments, the isolated 190-kDa talin rod domain was allowed to bind to microtiter wells coated with 8d4. After incubation for 2 h at 37°C, unbound protein was removed and the binding of $\alpha_{IIb}\beta_{3}$ to the antibody-captured talin rod domain proceeded as described for receptor binding to the immobilized talin fragment. As shown in Fig. 3, without preincubation of the 190-kDa talin fragment with 8d4-coated wells, negligible binding of $\alpha_{IIb}\beta_{3}$ was detected. However, binding of the talin rod domain to immobilized 8d4 caused a dramatic increase in $\alpha_{IIb}\beta_{3}$ binding. In control samples, parallel binding was performed on microtiter wells coated with normal mouse IgG. As expected, preincubation of the talin rod domain with control IgG-coated wells did not cause any increase in $\alpha_{IIb}\beta_{3}$ binding. Thus, in addition to the immobilized 190-kDa talin fragment, $\alpha_{IIb}\beta_{3}$ also binds to 8d4-captured talin rod domain, which is more likely to assume the conformation of the soluble protein.

**Localization of the $\alpha_{IIb}\beta_{3}$ Binding Site in the Talin Rod Domain**—To localize the integrin binding site within the 190-kDa talin rod domain, we generated four overlapping talin rod domain fragments and tested their ability to support $\alpha_{IIb}\beta_{3}$ binding (Fig. 4A). These recombinant fragments were produced as histidine-tagged fusion proteins and affinity-purified on Ni$^{2+}$ resin as described under “Materials and Methods.” In preliminary experiments, we found that $\alpha_{IIb}\beta_{3}$ bound nonspecifically to irrelevant histidine-tagged fusion proteins. Therefore, to eliminate nonspecific binding of $\alpha_{IIb}\beta_{3}$, the histidine tags were removed by proteolysis with calpain or enterokinase and the talin fragments were further purified by gel filtration. Fig. 4B shows that the recombinant talin rod fragments migrated at the expected molecular masses on SDS-PAGE. To characterize the talin rod fragments, we performed immunoblotting with 8d4 and TD77 directed against residues 434–1071 and 2269–2541 of the talin rod domain, respectively (16, 22). As expected, both 8d4 and TD77 immunoblotted talin-(434–2541), the entire talin rod domain (Fig. 4, C and D, lane 1). In addition, Fig. 4C shows that 8d4 immunoblotted talin-(434–1076) (lane 2) and talin-(434–1975) (lane 4), but not talin-(1075–2541) (lane 3) and talin-(1984–2541) (lane 5). Conversely, Fig. 4D shows that TD77 immunoblotted talin-(1075–2541) (lane 3) and talin-(1984–2541) (lane 5), but not talin-(434–1076) (lane 2) and talin-(434–1975) (lane 4).

Initially, we examined $\alpha_{IIb}\beta_{3}$ binding to the talin-(434–1076) and talin-(1075–2541) fragments. Fig. 5A shows that $\alpha_{IIb}\beta_{3}$
BOUND dose-dependently to talin-(434–2541), but not to talin-(1075–2541), indicating that the integrin binding site resides within the C-terminal two-thirds of the talin rod domain. To further localize the integrin binding site, we generated two additional recombinant talin fragments corresponding to residues 434–1975 and 1975–2541 and examined their ability to support αIIbβ3 binding. Again, αIIbβ3 bound equally well to talin-(434–2541) and talin-(1075–2541). In addition, αIIbβ3 also bound to talin-(1984–2541), a 60-kDa fragment corresponding to the C-terminal region of the talin rod domain (Fig. 5B). In contrast, negligible αIIbβ3 binding above BSA background was observed with talin-(434–1975) and talin-(434–1975), two N-terminal fragments of the talin rod domain (Fig. 5B). To ascertain that the lack of αIIbβ3 binding to talin-(434–1076) and talin-(434–1975) was not due to the failure of these fragments to be coated onto microtiter wells, we estimated their coating efficiency by the binding of 125I-AP-3. Data shown are means of triplicate determinations, and error bars represent standard deviations. The data are representative of two experiments.

FIG. 3. Binding of αIIbβ3 to the talin rod domain captured by immobilized 8d4. Microtiter wells were coated with normal mouse IgG or 8d4 (50 μg/ml, 100 μl/well). After blocking with BSA, purified recombinant talin-(434–2541) (100 μg/ml) was allowed to bind to the antibody-coated wells for 2 h at 37 °C. Unbound talin-(434–2541) was removed and αIIbβ3 was added. Binding proceeded for 2 h at 37 °C, and bound receptor was detected with 125I-AP-3. Data shown are means of triplicate determinations, and error bars represent standard deviations. The data are representative of two experiments.

FIG. 4. Characterization of recombinant talin rod domain fragments. A, a schematic drawing of the recombinant talin rod domain fragments. The numbers in parentheses represent the residue numbers in the human talin protein sequence. B–D, purified recombinant talin rod fragments were separated on 7.5% polyacrylamide gels. The resolved proteins were analyzed by Coomassie Blue staining (B), or by immunoblotting with 8d4 (C) or TD77 (D).

FIG. 5. Binding of αIIbβ3 to recombinant talin rod domain fragments. Microtiter wells were coated with purified talin rod domain fragments and blocked with BSA. Purified αIIbβ3, was added, and binding proceeded as described under “Materials and Methods.” A, varying concentrations of αIIbβ3 were added to wells coated with talin-(434–1076) or talin-(1075–2541). B, αIIbβ3 (20 nM) was allowed to bind to wells coated with: 1, talin-(434–2541); 2, talin-(434–1076); 3, talin-(1075–2541); 4, talin-(434–1975); 5, talin-(1984–2541); or BSA as indicated. The amounts of protein fragments coated onto the wells were determined by the binding of 125I-8d4. Data shown are means of triplicate determinations, and error bars represent standard deviations. The data are representative of two experiments.

Located within residues 1984–2541 at the C terminus of the talin rod domain.

To demonstrate the specificity of αIIbβ3 binding to the talin-(1984–2541) fragment, we examined the inhibitory effect of polyclonal anti-talin-R antibodies, which completely inhibited the binding of αIIbβ3 to the talin rod domain (Fig. 2C). As shown in Fig. 6, anti-talin-R also blocked αIIbβ3 binding to the talin-(1984–2541) fragment in a dose-dependent manner, and >75% inhibition was attained at high concentrations of the antibodies.

DISCUSSION

Talin provides linkage between integrins and actin filaments of the cytoskeleton. An integrin binding site has been localized to the N-terminal FERM domain in the talin globular head (22, 25). In the present study, we demonstrated that integrin αIIbβ3 also binds to the talin rod domain in a solid phase binding assay and localized an integrin binding site to residues 1984–2541 at the C-terminal region of talin. These results indicate that talin contains two integrin binding sites, one near each end of the molecule.
Several lines of evidence have indicated that an integrin binding site resides in the talin rod domain. In equilibrium gel filtration, the 190-kDa talin rod domain induces a shift in the elution profile of the avian integrin complex (CSAT antigen) toward a higher molecular weight complex (10). In a solid phase binding assay, the integrin-talin binding stoichiometry was found to be ~2:1 (24), and one of the integrin binding sites has been localized to the talin head domain (22, 25). Recently, a low affinity interaction between the talin rod fragment and an integrin β₃ cytoplasmic domain model protein has been reported (32). Consistent with these studies, we show that RGD affinity-purified α₁β₃β₄ bound specifically to the talin rod domain, and this interaction was completely blocked by polyclonal anti-talin-R antibodies. Because another integrin binding site is present in the talin head domain, anti-talin-R expectedly inhibited α₁β₃ binding to intact talin by ~50%.

Using recombinant polypeptides encompassing partial sequences of the talin rod domain, we further localized the integrin binding site to a 588-amino acid segment at the C terminus of talin. This conclusion is based on results obtained with two sets of overlapping polypeptides. Thus, α₁β₃β₄ bound to talin-(1075–2541) but not to talin-(434–1076). Consistent with these results, α₁β₃β₄ binding was observed with talin-(1984–2541) but not with talin-(434–1984). The inability of α₁β₃β₄ to bind to talin-(434–1076) and talin-(434–1975) was not due to the failure of these protein fragments to be coated onto microtiter wells, because the immobilized fragments were readily detected with the anti-talin monoclonal antibody 8d4 (Fig. 5B). The specificity of α₁β₃β₄ interaction with the talin-(1984–2541) fragment was further substantiated by the observation that anti-talin-R blocked α₁β₃β₄ binding to talin-(1984–2541) but not to the talin head domain. It should be noted that an actin-binding site homologous to the yeast actin binding protein Sla2p is located within the talin-(1984–2541) sequence at residues 2304–2463 (28). A vinculin binding site is also present immediately upstream of this sequence at residues 1944–1969 (31). At present, the relationship between the integrin, vinculin, and actin binding sites within the talin C-terminal end is not known. However, it is interesting to note that vinculin-bound talin exhibits a reduced affinity toward integrin α₁β₃β₄ (38); this may be due to the close proximity between the vinculin and integrin binding sites in the talin C terminus.

Talin exists as an anti-parallel homodimer in focal adhesions (33, 34). The localization of the integrin binding sites to the FERM domain in the talin head (22, 25) and to the C terminus of the talin rod suggest that the two integrin binding sites in the opposing talin molecules may be close to each other (Fig. 7). Similarly, it has been suggested that the two vinculin binding sites at the N-terminal region of the talin rod are closely located with the C-terminal vinculin binding site in the adjacent subunit, thus facilitating high affinity binding of vinculin homotrimers to the talin dimer (31). Therefore, clustering of the integrin binding sites in the talin homodimer may also regulate its affinity for interaction with integrin cytoplasmic domains. In this regard, it has been shown that the talin head domain binds to the integrin β₃ tail with a much higher affinity than the rod domain (32). Nevertheless, in the presence of the talin head domain, cooperative binding of the talin rod domain to the integrin β₃ tail has been observed (32). Furthermore, calpain cleavage of talin or phosphoinositide binding to talin have been demonstrated to induce a conformational change in talin, resulting in increased talin-integrin interaction (27, 32). Based on these observations and our present findings, we propose that both integrin binding sites in the talin head and rod domains are masked by an intermolecular interaction of the talin subunits in the homodimer. Cellular activation induces signal transduction pathways (i.e. calcium mobilization to activate calpain, phosphoinositide metabolism) that lead to post-translational modifications of talin and conformational changes in the talin homodimer, resulting in the exposure of both integrin binding sites. At the same time, ligand occupancy of integrins induces a transmembrane conformational change in the receptors’ cytoplasmic domains possibly to create a high affinity binding site for talin (39). Because the integrin β₃ tail binds cooperatively to the talin head and rod domains to form a ternary complex (32), it is conceivable that an integrin cytoplasmic domain may interact with both integrin binding sites in the opposing talin subunits to modulate the affinity/avidity of integrin-talin interaction.

Because of the lack of homology between the talin head and rod domains (21), as well as the difference in their binding affinities to the integrin β₃ tail (32), it is unlikely that they bind to the same site in the integrin cytoplasmic domain. Previous studies have identified at least two regions in the integrin β tails, namely the membrane proximal helical structure and the NPXY reverse-turn motif, to be involved in talin binding (12, 25, 40, 41). Although both of these two moieties were found to be important for the binding of the talin head domain (22, 25, 42), the site(s) of integrin cytoplasmic tails mediating interaction with the talin rod domain has yet to be defined. In this regard, earlier studies have shown that the distal NPXY motif near the C termini of the β₁, β₂, and β₃ tails also play an important role in focal adhesion formation and cell spreading (43–45). Whether the distal regions of integrin β tails are involved in integrin-talin interaction deserves further investigations.

Microinjection of the entire talin rod domain or its C-terminal fragments into fibroblasts results in a rapid incorporation...
of the injected protein fragments into focal adhesions (28, 46). Although this may be due to the binding of the talin rod fragments to cytoskeletal components in focal adhesions such as vinculin and actin, our present findings suggest that the injected talin fragments may also interact with integrin cytoplasmic domains. Interestingly, microinjection of the talin-(1646–2541) fragment, which contains the C-terminal vinculin and actin binding sites as well as the newly identified integrin binding site, results in the disruption of actin stress fibers and loss of focal adhesions in chicken embryo fibroblasts (28). Similarly, TD77, an anti-talin monoclonal antibody directed against the talin C-terminal region, also causes disruption of stress fibers and focal adhesions, and inhibition of cell motility when microinjected into human fibroblasts (16). Taken together, these functional studies implicate an important role of the talin C-terminal segment in the attachment of integrins to the actin cytoskeleton. In the present study, we have identified an integrin binding site near the C terminus of the talin rod domain and suggested a complex mode of interaction between the integrin cytoplasmic domain and the two cooperative integrin binding sites in the talin homodimer. Detailed mapping of both integrin binding sites in the talin head and rod domains would undoubtedly shed light on the molecular mechanism mediating integrin-talin interaction.

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