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Molecular Basis for Interaction between Icap1α PTB Domain and β_1 Integrin*

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Icap1α is a 200-amino acid protein that binds to the COOH-terminal 13 amino acids (^{786}AVTTVVNPKYEGK^{798}) of the integrin β_1 subunit. Alanine scanning mutagenesis of this region revealed that Val^{787}, Val^{790}, and ^{792}NPKY^{795} are critical for Icap1α binding. The NP*X*Y motif is a known binding substrate for phosphotyrosine binding (PTB) domain proteins. The sequences of Icap1α, residues 58–200, and the β_1 integrin, residues 786–797, were aligned to the available PTB-peptide structures to generate a high quality structural model. Site-directed mutagenesis showed that Leu^{135}, Ile^{138}, and Ile^{139} of Icap1α, residues predicted by the model to be in close proximity to ^{792}NPKY^{795}, and Leu^{82} and Tyr^{144}, residues expected to form a hydrophobic pocket near Val^{787}, are required for the Icap1α-β_1 integrin interaction. These findings indicate that Icap1α is a PTB domain protein, which recognizes the NP*X*Y motif of β_1 integrin. Furthermore, our date suggest that an interaction between Val^{787} and the hydrophobic pocket created by Leu^{82} and Tyr^{144} of Icap1α forms the basis for the specificity of Icap1α for the β_1 integrin subunit.

Integrins are transmembrane heterodimeric receptors for extracellular matrix and cell surface proteins (1, 2). The binding of integrins to ligands in the extracellular matrix is linked to cell attachment and spreading, which in turn activates various cytosolic signal cascades to promote cell migration, survival, proliferation, and differentiation (3–5). The binding of integrins to their ligands requires integrins to be in an "activated" conformation (6). The regulation of the activation status of integrins and the post-ligand binding activation of various signaling cascades require the integrin cytoplasmic domains. Mutagenesis studies have shown that the cytoplasmic domain of β subunit is important for cell adhesion and migration (7, 8) and for localization of integrins to focal contacts (9, 10).

Integrin β subunit cytoplasmic domain interacts with several cytoskeletal proteins, including α-actinin, talin, paxillin, and filamin (11–16), which may localize integrins to the site of cell-extracellular matrix interaction. Signaling molecules, such as FAK, ILK-1 (17), RACK1 (18), and Shc (19, 20), also bind to the integrin β subunit cytoplasmic domains and may link integrins directly to the cytosolic kinase cascades. In addition to these proteins, which can bind to the cytoplasmic domains of several different β subunits, there are proteins with binding specificity toward a particular β integrin cytoplasmic domain. For example, Cytohesin-1 interacts specifically with the β_2 integrin (21) and regulates the leukocyte-specific $\alpha_L\beta_2$ integrins (22). Similarly, β_3-endonexin binds to the β_3 integrin (23) and may play a role in the affinity regulation of platelet integrin $\alpha_{IIB}\beta_3$ (24). Another of these proteins is Icap1α,[1] which displays a restricted binding toward the β_1 integrins (25).

Although the precise function of Icap1α has not been established, its role in integrin-dependent cell adhesion was suggested from the finding that Icap1α undergoes an adhesion-dependent phosphorylation (25). Furthermore, expression of a mutant Icap1α with a T38D mutation interferes with cell spreading (26). The Icap1α binding site on the β_1 integrin has been mapped to the COOH-terminal region of β_1 integrin (25), which includes one of the two NP*X*Y (or N*X*XY) motifs present in several integrin β subunits. These NP*X*Y motifs are important for the localization of integrins to focal contacts (10), integrin-mediated endocytosis (27), and affinity regulation of integrins (28). The functions of β_1 and β_3 integrins can also be regulated by tyrosine phosphorylation at the NP*X*Y motifs (29–31).

Here, we examined the structural basis for the interaction between Icap1α and the integrin β_1 cytoplasmic domain. Sequence homology and molecular modeling reveal that Icap1α has a PTB fold. The PTB domains bind their target peptides through extensive hydrogen bonding and packing interactions in which the target peptide is bound to a characteristic grove on the PTB domain. Furthermore, some PTB domains are specific for peptides with sequences and conformation that are similar to canonical NP*X*pY (where pY is phosphotyrosine) peptides but that have Tyr or Phe in place of Tyr(P) (32, 33). All known PTB domain proteins contain additional protein-protein interaction module(s) and are believed to function as adaptor proteins in cell signaling (*e.g.* Shc, IRS-1), lineage determination (*e.g.* Numb), and receptor internalization (*e.g.* X11, Fe65, Disabled) (34).

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The atomic coordinates and structure factors (code 1K11) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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The abbreviations used are: Icap1α, integrin cytoplasmic domain associated protein 1α; PTB, phosphotyrosine binding; IR, insulin receptor; IRS-1, insulin receptor substrate-1; aa, amino acids; GST, glutathione *S*-transferase; βAPP, β-amyloid precursor protein; PDB, Protein Data Bank; mAb, monoclonal antibody.

Sequence of Beta-1 integrin cytoplasmic domain mutants		Growth	β–gal	% β1 E16
KLLMIIHDRREFAKFEKEKMNAKWDTGENPIYKSAVTTVVNPKYEGK	β1 cyto	+++	nd	
GENPIYKSAVTTVVNPKYEGK	β1 E16	+++	48.7 ± 5.5	100
GENPIYKSAATTVVNPKYEGK	β1 V787A	–	<1.0	<1
GENPIYKSAVATVVNPKYEGK	β1 T788A	++	7.3 ± 3.6	15
GENPIYKSAVTAVVNPKYEGK	β1 T789A	++	2.4 ± 0.4	4.9
GENPIYKSAVTTAVNPKYEGK	β1 V790A	–	<1.0	<1
GENPIYKSAVTTVANPKYEGK	β1 V791A	++	9.2 ± 1.3	18.9
GENPIYKSAVTTVVAPKYEGK	β1 N792A	–	nd	
GENPIYKSAVTTVVDPKYEGK	β1 N792D	–	nd	
GENPIYKSAVTTVVNAKYEGK	β1 P793A	–	<1.0	<1
GENPIYKSAVTTVVNPAYEGK	β1 K794A	+++	58.1 ± 9.4	120.5
GENPIYKSAVTTVVNPKAEGK	β1 Y795A	–	<1.0	<1
GENPIYKSAVTTVVNPKFEGK	β1 Y795F	+++	nd	
DNPLFKSATTTVMNPKFAES	β2 E16	–	<1.0	<1
DNPLFKSAVTTVMNPKFAES	β2 T758V	+++	51.2 ± 3.4	105.1
ANNPLYKEATSTFTNITYRGT	β3 cyto	–	nd	
ASNPLYRKPISTHTVDFTFNKSY	β5 cyto	–	nd	
aVttVvNPxYaes	consensus			

FIG. 1. **Characterization of β_1 integrin mutants.** Alanine scanning mutagenesis of the β_1 cytoplasmic domain was carried out to determine the sequence requirement for binding to Icap1α. The interaction of the β_1 cytoplasmic domain mutants and Icap1α PTB domain was characterized by a yeast two-hybrid assay. The strength of interaction was graded based on growth on plates lacking leucine and by β-galactosidase (*β-gal*) activity. *nd*, not determined.

FIG. 2. **Structure-based sequence alignment of PTB domains and bound peptides.** *A*, PTB domains. *B*, bound peptides in the same order *top to bottom* as the PTB domains to which they bind. The X11 protein PTB domain with bound βAPP peptide (PDB structure 1AQC, chains A and C, and PDB structure 1X11, chain D) (40) and the Numb PTB domain with bound Nak peptide (PDB structure 1DDM) (42) were structurally superimposed as described under "Experimental Procedures." Icap1α was aligned by sequence and modeled using these two structures as templates as described under "Experimental Procedures." Residues that are in the α-helix or β-strand in the structures and model are shown in *green* and *red*, respectively. Residues that were disordered and hence not defined in the structures, as well as the aligned residues that were deleted from the model, are in *italics*. The NP*X*Y motifs of the peptides are *underlined*.

EXPERIMENTAL PROCEDURES

Molecular Modeling of the Integrin β_1 Peptide Bound to the Icap1α PTB Domain—Structures of PTB domains in the PDB (Protein Data Bank) were identified with SCOP. One undeposited structure was kindly provided by M. Eck (35). The structures were superimposed with MALIGN and then 3DMALIGN of MODELLER (36) using a gap penalty of 4 Å and five iterations of alignment with superposition of Cα, Cβ, Cα, Cβ, and finally Cα atoms. The superimposed structures and the resulting structure-sequence PIR alignment were opened in Look (Molecular Applications Group, Palo Alto, CA). There appeared to be a correlation with structural quality and superposition, with those structures of the best quality superimposing with one another the best. Structures with high quality and similar bound peptides were chosen for a final round of superposition with a gap penalty of 3 Å: PDB code 1AQC, chains A and C; PDB code 1X11, chains B and D; PDB code 1DDM, model 1; and an IRS-1 crystal structure (see Table I).

For the starting sequence alignment between the structures, superposition with a gap penalty of 5 Å was used to obtain a more condensed alignment. Multiple gaps within each loop were condensed into a single gap, leaving insertions/deletions near turns or midpoints of loops. The sequence of human X11 protein, GenBank™ accession number 6226838, was added to reveal the sequence and position of disordered loops that are missing from the X11 structures. The sequence of human Icap1α, residues 58–200, as well as human β_1 residues 786–798, were then aligned. The alignment largely followed the BLAST alignment of human Icap1α to the SMART sequences in the conserved domain data base (37), which include two of the structures, Numb and Shc, and mouse Icap1α. However, human and mouse Icap1α were aligned differently in two different sequence blocks. Furthermore, the Numb and Shc sequence alignment was offset by two residues from the structure-based alignment in a block of 18 residues corresponding to β-strands 2 and 3. A good alignment of Icap1α with Numb but not Shc sequence in this region made alignment clear. Otherwise, only minor alignment adjustments were required. The β_1 peptide could be readily aligned to the peptides in the PDB domain structures using the NP*X*Y motif.

Two modeling programs were used, each of which has proved superior in the past depending on the modeling problem (38–40). Models were made using Segmod (41) with each of the three longer structures as templates (see Fig. 1) and using MODELLER 4 (36) with individual templates and combinations of up to all four templates. Models with the best QUACHK scores and native-like hydrogen bond patterns in the peptide binding cleft were produced by Segmod. The final model utilized as template chains A (X11 protein) and C (β-amyloid precursor protein (βAPP) peptide) of PDB code 1AQC (33) and the first two residues of chain D of PDB code 1X11 (βAPP peptide) (33) because of the longer length of the peptide in this structure. For the last three residues of the β_1 peptide, the Numb-associated kinase (Nak) peptide from PDB code 1DDM (42) was used, which has an extended conformation in this region, instead of the 3–10 helix of the βAPP peptide bound to X11. The PDB code 1DDM peptide was superimposed on that of PDB code 1AQC using the peptide residues MSF and PTY, respectively. QUACHK and NQACHK of WHATIF identified no problematic residues in the peptide or its binding groove on Icap1α. Two long loops were deleted from the model, residues 99–106 and 147–152, because these could not be tem-

plated by the X11 structures, in which the corresponding loops were disordered and therefore missing. These loops are not interacting with the PTB groove. The coordinates have been deposited with the Protein Data Bank (accession number: 1K11).

Mutagenesis of the β_1 Cytoplasmic Domain and Icap1α (aa 54–200)—The LexA fusion protein containing the COOH-terminal 21 aa (^{778}GENPIYKSAVTTVVNPKYEGK^{798}) of the integrin β_1 subunit (pNlex-$\beta_1$cyto) and clone E16–1 containing residues 54–200 of Icap1α in the yeast pJG4–5 vector were described previously (25). Oligonucleotide-directed mutagenesis of the β_1 cytoplasmic domain and Icap1α (aa 54–200) was carried out using standard methods. Each clone used in this study was sequence-verified.

Yeast Two-hybrid Interaction—Interaction between the β_1 cytoplasmic domain and Icap1α (aa 54–200) was determined in a yeast two-hybrid assay as described previously (25). Semiquantitative measurement of the binding was carried out measuring the β-galactosidase activity of the yeast strain EGY48 (*Mat α his3 trp1 ura3–52 leu2::pLeu2-lexAop6(ΔUAS leu2*) that had been transfected with the JK103 β-galactosidase reporter construct, pNlex-$\beta_1$cyto, and pJG4–5/Icap1α (aa 54–200). Expression of the B42 acidic domain-Icap1α fusion proteins was verified by an immunoblot analysis using the transformed yeast cells cultured in galactose.

Eukaryotic in Vivo GST Pull-down Assay—The binding of β_1 integrins to Icap1α or a mutant Icap1α (I138A) was tested by expressing the PTB domain region of Icap1α (aa 54–200) as a GST fusion protein in 293T cells (25). Cell lysates were prepared 48 h after transfection and incubated with glutathione-Sepharose beads (Sigma) for 16 h at 4 °C. Beads were washed three times, and bound proteins were eluted with SDS sample buffer (non-reducing), run on an SDS-polyacrylamide gel, and immunoblotted with the mAb TS2/16 or anti-GST antibodies as described previously (25).

In Situ Immunofluorescence—The coding sequence of the human integrin β_1 subunit and β_1(V787A) mutant was cloned into the SRα retroviral vector. The virus stock was prepared from the conditioned media of 293T cells that have been transfected with the SRα/β_1 integrin constructs and Ψ-packaging vector. Two ml of the supernatant was used to infect NIH3T3 cells that were seeded the previous night at a density of 250,000 cells/100-mm plate. 48 h later, the infected cells were harvested and analyzed by fluorescence-activated cell sorter using the mAb TS2/16 to determine the surface expression of the human β_1 integrin. The infected cells were then replated on a fibronectin-coated glass coverslip for 4 h and then incubated with the mAb TS2/16 to localize the human β_1 integrins on the cell surface.

RESULTS AND DISCUSSION

The COOH-terminal ^{792}NPKY^{795} Motif and the Val Residues at the −8 and −5 Position from the Tyr^{795} Comprise the Icap1α Binding Site on the β_1 Cytoplasmic Domain—We introduced Ala substitutions at the COOH-terminal 13 amino acids of the β_1 cytoplasmic domain, which comprises the minimal Icap1α binding site (25). An alanine substitution at Asn, Pro, or Tyr of the ^{792}NPKY^{795} motif, Val^{787}, or Val^{790} within this 13-amino acid region effectively abolished the Icap1α binding in a yeast two-hybrid assay (Fig. 1). Tyr^{795}, however, could be changed to Phe without affecting the interaction, indicating that the binding of Icap1α to the peptide target does not require a Tyr(P) residue.

Among different integrin β subunits, only the β_1 cytoplasmic domain fulfilled the requirement of an NPXY motif and Val residues at the −8 and −5 position relative to the Tyr residue (Fig. 1). It is of interest that the β_2 cytoplasmic domain, which has Thr at the −8 position, can be mutated to bind Icap1α by replacing this Thr with Val. Therefore, the Val^{787} residue on the β_1 integrin likely represents the specificity determinant that allows Icap1α to discriminate different β integrin cytoplasmic domains.

Icap1α Is a PTB Domain Protein—The requirement of the NPXY motif and the hydrophobic Val residues on the β_1 cytoplasmic domain for the Icap1α binding was reminiscent of the interaction between the IR and Shc or IRS-1 in which the NPXY motif in the juxtamembrane region of the IR, upon tyrosine phosphorylation, binds to the PTB domain of Shc or IRS-1 (35, 43–45). BLAST searches with the Icap1α sequence failed to yield any significant homology to the PTB domain of Shc or IRS-1 or any other polypeptides in the NCBI data base. However, a "reverse position-specific iterative BLAST" search

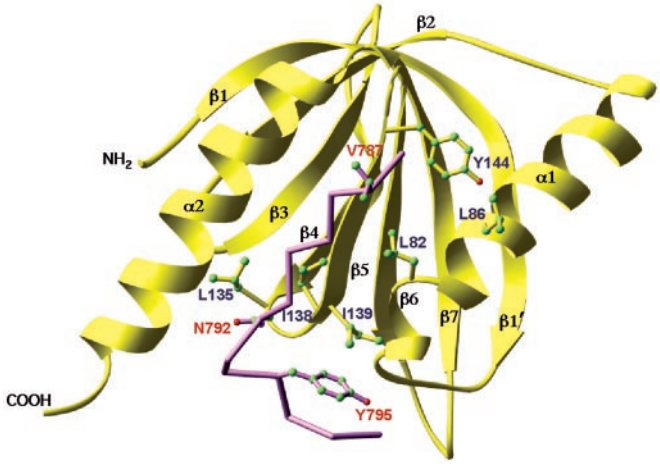

FIG. 3. **Ribbon diagram of the model of the Icap1α PTB domain complexed with β_1 peptide.** The PTB domain is shown as a *yellow* ribbon; the bound β_1 peptide is shown as a *pink* Cα trace. Side chains of key residues are shown with bonds in the same color as the backbone, and atoms are shown in *green* (carbon), and *red* (oxygen). Similar to other PTB domains, the Icap1α PTB domain shares a common pleckstrin homology domain fold with a compact central β sandwich with a COOH-terminal α-helix. The β_1 peptide forms an anti-parallel β-strand with the β_5 strand of Icap1α PTB. Key residues on the β_1 peptide (Val^{787}, Asn^{792}, and Tyr^{795}) and Icap1α (Leu^{82}, Leu^{86}, Leu^{135}, Ile^{138}, Ile^{139}, and Tyr^{144}) are shown with side chains. This figure was prepared with Ribbons (50).

TABLE I
Structural quality of the Icap1α-β_1 peptide model compared with crystal and NMR structures of PTB domain-peptide complexes

Structure or model	Method	PDB code & chains	Residues	QUACHK[a] score	NQACHK[b] Z-score
Icap1α	Model		138	−0.445	−0.71
X11 β-amyloid binding protein	X-ray	1AQC A, C	140	−0.617	+0.03
X11 β-amyloid binding protein	X-ray	1X11 B, D	135	−0.445	+1.01
Drosophila Numb	NMR	1DDM 1	146	−1.991	−5.13
Drosophila Numb	NMR	2NMB 4	154	−2.084	−5.10
Shc	NMR	1SHC	173	−1.980	−5.56
IRS-1	NMR	1IRS	123	−1.292	−2.22
IRS-1[c]	X-ray		113	−0.294	+0.28

[a] Structural average packing environment quality score with the quality check (QUACHK) option of WHATIF (47). Higher (less negative) values are better. Scores receive the following messages: <−2.7, error, certain to be wrong; −2.7 to −2.0, error, quality is very low; −2.0 to −1.4, warning, quality is a bit low, >−1.4, note, quality is within normal ranges.

[b] New or second generation average structural packing environment Z-score with the NQACHK option of WHATIF. Higher (less negative values) are better. The average Z-score for properly refined x-ray structures is 0.0 ± 1.0. Scores receive the following messages: <−5.0, error, the structure is certain to be incorrect; −5.0 to −4.0, error, abnormal score, quality is very low; −4.0 to −3.0, warning, quality is a bit low, the protein is probably threaded correctly; >−3.0, note, quality is within normal ranges.

[c] From Ref. 30, kindly provided by M. Eck, Harvard Medical School, Boston, MA.

(46) with the Icap1α sequence revealed homology to an alignment of PTB domains compiled by SMART (37) with a highly significant expectation value of 2×10^{-10} (data not shown, see Fig. 2). Based on the resulting sequence alignment, amino acids 58–200 of Icap1α can be classified as a single PTB domain. This assignment of the Icap1α PTB domain is in agreement with the minimal region of Icap1α (aa 54–200) required for binding to the β_1 cytoplasmic domain (25). Previously, we reported that Icap1β, an alternatively spliced isoform, does not bind the β_1 cytoplasmic domain (25). The 50 amino acids that are missing in Icap1β correspond to residues 128–177 of Icap1α, which span β-strands 4 to 7, a deletion of which would disrupt the PTB fold.

A High Quality Model of the β_1 Cytoplasmic Domain Bound to Icap1α PTB Domain Can Be Predicted—We constructed a model of Icap1α bound to a β_1 cytoplasmic domain peptide (β_1 peptide: ^{786}AVTTVVNPKYEG^{797}). Seven different PTB domains with bound peptides were compared structurally (see Table I). The two X11 structures, one of the Numb structures, and the two IRS structures bind peptide very similarly; the Numb structure PDB code 2NMB binds to a different peptide that lacks an NPXY consensus. Superposition of the first five structures revealed many structurally equivalent residues in the PTB domains and in the bound peptides. The Icap1α sequence could be readily aligned to the structure-based sequence alignment of these structures (Fig. 2).

The Icap1α-β_1 peptide model (Fig. 3) was found to be of high structural quality using objective tools for structure evaluation that are independent of refinement methods (47, 48). The average structural quality packing environment scores of the model given by QUACHK and NQACHK are within the normal ranges for crystal and NMR structures (Table I and footnote a and b of Table I). Furthermore, the scores for the model are comparable with those for PTB domain crystal structures and comparable with or superior to those for PTB domain NMR structures. The only problematic areas in the structure were in untemplated loops distant from the PTB peptide binding groove, and no structural quality warnings were noted in the PTB domain groove or in the bound peptide. The hydrogen bonding pattern in the model found for the peptide-PTB β-sheet interaction and the internal hydrogen bonds in the β-turn present in the NPXY motif are remarkably well preserved relative to those revealed in the crystal structure templates. The model preserves the hydrogen bond between the backbones of Asn^{792} and Tyr^{795} that form the β-turn in the ^{792}NPKY^{795} motif. Residues 788–791 of β_1 integrin form a β-strand that adds onto the edge of the Icap1α β-sheet, and the hydrogen bond is preserved in the model from the side chain of Asn^{792} to the Icap1α β-sheet that caps the β_1 integrin β-strand.

A Mutational Analysis of Icap1α Supports the Structural Prediction of the Mode of Association between the Icap1α PTB Domain and the β_1 Peptide—Conserved features of PTB domains were noted in the Icap1α-β_1 peptide interaction. For example, a short segment connecting the fourth and fifth β-strands of the Icap1α PTB domain is in close contact with Asn^{792} of the β_1 peptide (Fig. 3). Located within this segment are Leu^{135} and Ile^{138}. Analogous positions in the IRS-1 PTB domain are occupied by Leu and Val, whose carbonyl groups accept hydrogen bonds from the side chain amide group of the Asn of the NPXY motif (35). As predicted, Ala substitution of Leu^{135} or Ile^{138} abolished binding to the β_1 cytoplasmic domain (Fig. 4*A*). This effect was specific because these amino acid substitutions did not alter the expression levels of the mutated Icap1α proteins (Fig. 4*B*). Furthermore, when the Icap1α PTB domain region (aa 54–200) was expressed as a GST fusion protein in 293T cells, the wild type GST-Icap1α PTB, but not the I138A mutant, co-purified with the endogenous β_1 integrins (Fig. 4*C*).

A.

Icap1 mutation	Growth	β-gal (% w.t.)
w.t (Icap 54-200)	+++	100.0 %
L82A	++	28.2 %
L82Q	+++	101.5 %
L86Q	+++	103.4 %
L135A	-	3.2 %
I138A	-	4.1 %
I139A	+	4.4 %
Y144T	+	5.1 %
L82A/Y144T	+	1.9 %
L86Q/Y144T	+	2.1 %

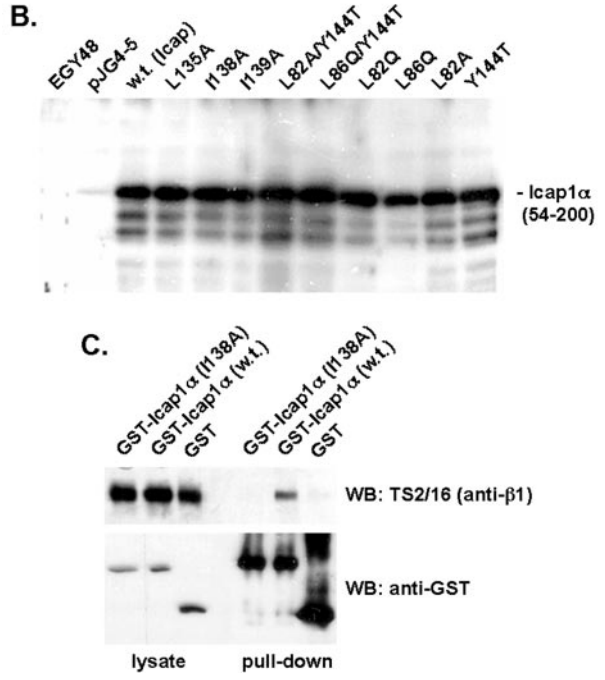

FIG. 4. **Characterization of Icap1α PTB domain mutants.** The interaction between β_1 cytoplasmic domain and Icap1α PTB domain mutants was characterized by a yeast two-hybrid assay (*A*). The strength of interaction was graded based on growth on plates lacking leucine and by β-galactosidase (β-*gal*) activity. An immunoblot of yeast cell lysates with anti-Icap1 antisera (25) demonstrates equivalent expression of each Icap1α PTB domain mutant (*B*). EGY48 and pJG4–5 represent parental yeast strain and EGY48 transformed with empty vector, respectively. The Icap1α PTB domain (aa 54–200) was expressed as a GST fusion protein in 293T cells to assay its binding to the endogenous β_1 integrins (*C*). An immunoblot of glutathione-Sepharose bound proteins revealed a specific interaction of β_1 integrins with the wild type (*w. t.*) Icap1α PTB domain but not with the Icap1α PTB (I138A) mutant. Equivalent loading of GST fusion proteins was verified by an anti-GST immunoblot.

Our model also revealed interactions that would determine the specificity of Icap1α for the β_1 peptide, including those that are unique for Icap1α compared with other PTB domains (Fig. 2). The Tyr^{795} residue of the ^{792}NPKY^{795} motif buries Ile^{139} of Icap1α in an extensive hydrophobic contact. In the structure of IRS-1 bound to the IR peptide, the side chain of Tyr(P)^{1009} of the IR buries the aliphatic portion of the structurally homologous Arg^{212} side chain and hydrogen bonds to its guanido group (35). By contrast, the PTB domains of X11, Numb, and Shc contain a Ser at the homologous position (33, 49), which is a more typical residue in PTB domains. In agreement with the prediction of its functional importance, mutation of Ile^{139} of Icap1α to alanine abolished the Icap1α-β_1 peptide interaction (Fig. 4*A*).

The binding of IRS-1 PTB to the IR peptide is also favored by

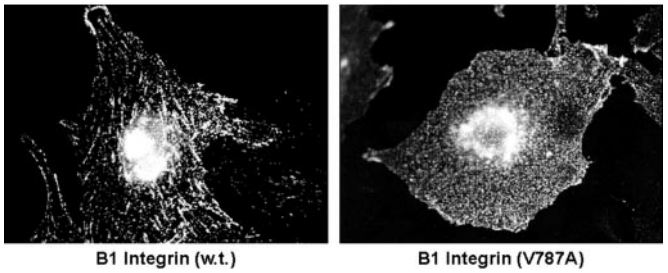

FIG. 5. **Subcellular localization of β_1 integrin with V787A mutation.** NIH3T3 cells induced with human β_1 integrin (wild type, *w. t.*) or human β_1 integrin with alanine substitution at the Val^{787} position (V787A) were plated on fibronectin-coated glass coverslips and stained with the TS2/16 mAb.

Arg^{227} of the IRS-1 PTB, whose side chain extends from the loop connecting the β6 and β7 strands of the IRS-1 PTB to donate hydrogen bonds to the phosphate of $Tyr(P)^{1009}$ of the IR (35). In Icap1α PTB, the equivalent position is occupied by Thr^{160}, whose hydroxyl group, together with the hydroxyl group of Tyr^{167}, creates a small pocket to accommodate the hydroxyl group of Tyr^{795} of the β_1 peptide (Fig. 2). In our model, the binding of Icap1α PTB to tyrosine-phosphorylated β_1 peptide is disfavored because Thr^{160} and Tyr^{167} fill the space that would be occupied by a phosphate and because Ile^{139} endows a hydrophobic character to the pocket.

The Val^{787} residue of the β_1 cytoplasmic domain is a critical determinant of the Icap1α binding specificity (Fig. 1). Only the approximate position of Val^{787} could be deduced from the model because it is the first templated residue in the peptide, and random perturbation of initial conditions for modeling resulted in different orientations of the Val^{787} side chain in five different models. However, the model predicted three residues of Icap1α, Leu^{82}, Leu^{86}, and Tyr^{144} to form a hydrophobic pocket near Val^{787} (Fig. 2). Leu^{82} and Leu^{86} are both in the first α-helix of the Icap1α PTB domain. When Leu^{82} was changed to Ala, a residue with a propensity for the α-helix, the binding to the β_1 peptide was severely diminished (Fig. 4*A*). Introduction of a structurally neutral Gln at either Leu^{82} or Leu^{86} did not affect binding. Tyr^{144} is predicted to be near the end of the fifth β-sheet of Icap1α PTB. Changing Tyr^{144} to Thr completely abolished the β_1 peptide binding. Thr has a propensity for the β-sheet, and therefore, the loss of the side chain interaction between Val^{787} of the β_1 integrin and Tyr^{144} of Icap1α, rather than the disruption of the Icap1α PTB domain, is likely to account for the loss of interaction.

The Icap1α Binding Site on the Integrin β_1 Cytoplasmic Domain Is Functionally Important—The ^{792}NPKY^{795} motif on the β_1 cytoplasmic domain is required for the β_1 integrins to be localized to the focal contacts (9, 10). To assess the significance of the Val^{787} of the β_1 cytoplasmic domain, we introduced a V787A substitution to the β_1 integrin and expressed either the wild type human β_1 integrin or this β_1(V787A) mutant in NIH3T3 cells. Fluorescence-activated cell sorter analysis using mAb TS2/16 confirmed equivalent surface expression of the wild type β_1 and β_1(V787A) mutant in the transduced cells (data not shown). *In situ* immunofluorescence revealed that the wild type β_1 integrins were distributed in streaks, in a pattern typical for the distribution of focal contacts in NIH3T3 cells (Fig. 5). The V787A mutant, however, was distributed diffusely, suggesting that Val^{787} of the β_1 integrin cytoplasmic domain is involved in both Icap1α binding and the localization of the β_1 integrins to the focal contacts.

Conclusion—We have shown that Icap1α has a PTB domain, which recognizes the conserved ^{792}NPKY^{795} motif on the β_1 cytoplasmic domain. Icap1α is the only known example of a protein that essentially consists of a single PTB domain. The binding of Icap1α to the β_1 peptide can occur with unphosphorylated Tyr^{795} or when Tyr^{795} is mutated to Phe. In fact, our model strongly disfavors the binding of Icap1α to the β_1 peptide with $Tyr(P)^{795}$, raising the possibility that Icap1α may dissociate from the β_1 integrins when the β_1 cytoplasmic domain is phosphorylated at the ^{792}NPKY^{795} motif.

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