Src kinase activation by direct interaction with the integrin β cytoplasmic domain

Elena G. Arias-Salgado*, Sergio Lizano*, Sugata Sarkar†, Joan S. Brugge†, Mark H. Ginsberg*, and Sanford J. Shattil*‡§

Departments of *Cell Biology and ‡Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037; and †Department of Cell Biology, Harvard Medical School, Boston, MA 02115

Contributed by Joan S. Brugge, September 24, 2003

Src tyrosine kinases transmit integrin-dependent signals pivotal for cell movement and proliferation. Here, we establish a mechanism for Src activation by integrins. c-Src is shown to bind constitutively and selectively to β3 integrins through an interaction involving the c-Src SH3 domain and the carboxyl-terminal region of the β3 cytoplasmic tail. Clustering of β3 integrins *in vivo* activates c-Src and induces phosphorylation of Tyr-418 in the c-Src activation loop, a reaction essential for adhesion-dependent phosphorylation of Syk, a c-Src substrate. Unlike c-Src, Hck, Lyn, and c-Yes bind more generally to β1A, β2, and β3 cytoplasmic tails. These results invoke a model whereby Src is primed for activation by direct interaction with an integrin β tail, and integrin clustering stabilizes activated Src by inducing intermolecular autophosphorylation. The data provide a paradigm for integrin regulation of Src and a molecular basis for the similar functional defects of osteoclasts or platelets from mice lacking β3 integrins or c-Src.

Integrin adhesion receptors are transmembrane heterodimers. Although integrin α and β cytoplasmic tails are devoid of catalytic activity, engagement of integrins by extracellular matrix ligands triggers "outside-in" signals that collaborate with growth factor-initiated signals to determine cell fate and function (1). A prominent biochemical event required for integrin-dependent functional responses is protein tyrosine phosphorylation due to activation of Src and FAK family protein tyrosine kinases (1–4). In the case of the β3 integrins, αIIbβ3 and αVβ3, a pool of c-Src coimmunoprecipitates with the integrin in nonadherent osteoclasts and platelets and becomes activated upon cell adhesion (5, 6). This process is independent of actin polymerization, focal adhesion assembly, and FAK activation, suggesting a proximal and, perhaps, unique relationship between β3 integrins and c-Src. In this context, mice lacking c-Src or β3 integrins exhibit osteopetrosis/osteosclerosis and defective osteoclast and platelet spreading (6–10), providing genetic evidence for a close functional relationship between these proteins. However, it has not been established whether β3 integrins and c-Src interact directly, nor has it been resolved how c-Src or any other Src family kinase is regulated by integrins. The present studies were carried out to address these issues.

Methods

Cell Lines and Transfections. Chinese hamster ovary (CHO) cell lines that stably express human wild-type or mutant αIIbβ3 have been described (11–13). The amount of β3 integrin expressed on the surface of these cell lines was equivalent, as assessed by flow cytometry by using a β3-specific antibody. Cells were grown in DMEM containing 10% FCS, and transfections were performed by using Lipofectamine reagent (Life Technologies) according to the manufacturer's protocol. To induce αIIbβ3 clustering, CHO cells expressing αIIb(FKBP)β3 were incubated in suspension for 20 min at room temperature with 1 μM AP1510 (Ariad Pharmaceuticals, Cambridge, MA) or an equivalent volume of vehicle (13).

Antibodies and Expression Plasmids. Monoclonal antibody 327 and polyclonal antibody 1671 are specific for the c-Src SH3 domain and carboxyl terminus, respectively. Monoclonal antibody SSA6 and polyclonal antibody 8053 are specific for the integrin β3 subunit (6). Monoclonal antibody 7E2 to the β1 integrin subunit was from R. Juliano (University of North Carolina, Chapel Hill). Polyclonal antibody 828 is specific for the β2 integrin subunit. Antibodies to GST, Syk, c-Fgr, Fyn, and Lyn were from Santa Cruz Biotechnology; antibody to c-Yes and antiphosphotyrosine antibody PY20 were from Transduction Laboratories (Lexington, KY); and phosphospecific antibodies to c-Src Tyr-418 and Tyr-529 were from Biosource International (Camarillo, CA). Horseradish peroxidase-conjugated secondary antibodies were from Bio-Rad.

Vectors pRC-CMV/Src and pLNCX/Src encode wild-type nonneuronal murine c-Src and chicken c-Src, respectively. ECMV/Syk(K402R) encodes kinase-inactive human Syk. Deletion or point mutants of c-Src were generated by using the Exsite site-directed mutagenesis kit (Stratagene), and mutations were confirmed by DNA sequencing. GST expression vectors for the SH3 domains of c-Src, Lyn, and c-Yes have been described (14, 15). For expression in CHO cells, cDNAs encoding GST, GST-c-Src SH3, or GST-c-Src SH3(Δ90–92) were amplified by PCR and cloned into the mammalian expression vector pRC-CMV (Invitrogen). pcDNA3 plasmids encoding αM and β2 integrin subunits were from E. Brown (University of California, San Francisco). Where indicated, c-Src was transcribed and translated *in vitro* by using pRC-CMV/Src and a TNT-coupled rabbit reticulocyte lysate system (Promega) in the presence of T7 RNA polymerase.

Immunoprecipitation, Western Blotting, and Src Kinase Assay. Cells were lysed in Nonidet P-40 buffer containing 1% Nonidet P-40, 150 mM NaCl, 50 mM Tris (pH 7.4), 1 mM sodium vanadate, 0.5 mM sodium fluoride, and complete protease inhibitor mixture (Roche Molecular Biochemicals). After clarification, 800 μg of protein were immunoprecipitated with antibodies to the β1, β2, or β3 integrin subunits and protein A-Sepharose. Immunoprecipitates were subjected to Western blotting with the indicated antibodies. For comparison, 20 μg of cell lysate were applied to adjacent lanes. Immunoreactive bands were detected by ECL reagent (Pierce). The activity of Src in β3 integrin immunoprecipitates was determined by using a Src kinase assay kit and a Src-specific peptide substrate (KVEKIGEGTYGVVYK) (Upstate Biotechnology, Lake Placid, NY).

Affinity Chromatography. His_6-recombinant integrin cytoplasmic tail model proteins (Fig. 1*A*) were cloned into pET15b (16) and modified by inserting a sequence encoding the peptide, GLNDIFEAQKIEWHE, at the *Nde*I site immediately downstream of the thrombin cleavage site. This peptide incorporates biotin *in vivo* upon expression in *Escherichia coli* by biotin ligase (Avidity, Denver) (17). Proteins were expressed and purified from *E. coli*

Abbreviation: CHO, Chinese hamster ovary.

§To whom correspondence should be addressed. E-mail: shattil@scripps.edu.

www.pnas.org/cgi/doi/10.1073/pnas.2336149100

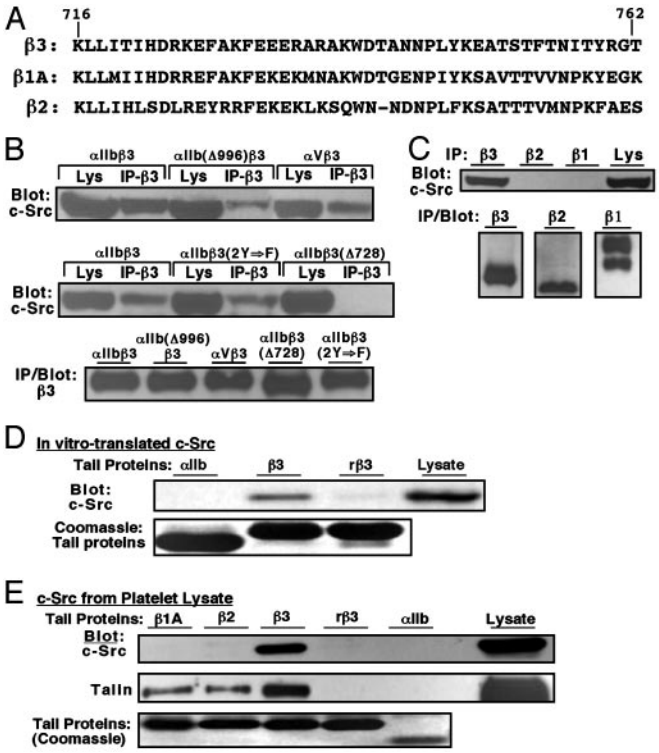

Fig. 1. Interaction of c-Src with the integrin β3 cytoplasmic tail. (*A*) Integrin β tail sequences. Residue numbers shown are for the full-length β3 subunit. (*B*) c-Src was transfected into CHO cells expressing the indicated integrins. β3 immunoprecipitates from resuspended cells were probed on Western blots with antibodies to c-Src or β3. Lys, lysate. (*C*) αIIbβ3 CHO cells were cotransfected with c-Src, αM, and β2. Then β1, β2, and β3 immunoprecipitates were probed as indicated. Immunoprecipitation with preimmune serum yielded no specific bands (data not shown). (*D* and *E*) Neutravidin beads coated with integrin tail model proteins were incubated with *in vitro*-translated c-Src (*D*) or platelet lysate (*E*). Bound c-Src or talin was detected by Western blotting. rβ3 is a random β3 tail sequence. Tail protein loading was assessed by Coomassie staining.

extracts (16). A randomized integrin β3 tail sequence (KLATNEPTTAFIELGKHRTREKITNSYRWFTAEREFK-ILDATNKYA) was constructed by annealing of oligonucleotides followed by PCR and cloned by using flanking restriction sites (*Hin*dIII and *Bam*HI). Affinity chromatography of cell lysates was carried out by using immobilized integrin tail model proteins (16).

GST-SH3 domains of c-Src, Lyn, and c-Yes were expressed in *E. coli* strain BL21 (Novagen). For *in vitro* binding assays, purified GST fusion proteins coupled to glutathione-Sepharose 4B beads (Amersham Pharmacia) were incubated at 4°C with cell lysates in RIPA buffer (50 mM Tris, pH 7.4/75 mM NaCl/1% Nonidet P-40/0.5% deoxycholic acid/0.1% SDS/5 mM EDTA/1 mM sodium vanadate and protease inhibitor mixture). Precipitates were resolved with SDS/PAGE and specific proteins were detected by Western blotting. Competition assays used synthetic peptides (SynPep, Dublin, CA).

ELISA. Biotinylated recombinant integrin tails (100 ng) were bound at saturating concentrations (20 μg/ml) to wells of neutravidin-coated microtiter plates (Pierce). Purified GST-c-Src SH3, GST-Lyn SH3, or GST-c-Yes SH3 were diluted in PBS/1% BSA, added to wells in triplicate and incubated for 1 h at room temperature. After three washes with PBS/0.2% Tween 20 and subsequent incubations with mouse anti-GST antibody, horseradish peroxidase-conjugated anti-mouse Ig, and *o*-phenylenediamine, bound proteins were quantified in an ELISA reader at 490 nm. Specific binding of GST-c-Src SH3 to the β3 tail was determined by nonlinear regression based on a one-site binding equation with PRISM (GraphPad, San Diego).

Enzyme Activity of Purified c-Src. Purified active human c-Src (10 units; Upstate Biotechnology) was incubated with β3 tail model proteins (20 μg) bound to neutravidin beads (Pierce) in the presence of 150 μM Src-specific peptide substrate. In some samples, 10 μM GST-Src SH3 fusion protein was also present. Reactions were carried out by using a Src kinase assay kit.

Results and Discussion

Selective Interaction of c-Src with β3 Integrins. To explore the molecular basis of β3 integrin signaling, murine c-Src and human β3 integrins were expressed in CHO cells, a model system that recapitulates the features of β3 integrin signaling in platelets (12). c-Src was detected in αIIbβ3 or αVβ3 immunoprecipitates when cells were in suspension (Fig. 1 *A* and *B*), and similar results were obtained when cells were plated on fibrinogen (data not shown). c-Src coprecipitation was slightly reduced if the αIIb tail was deleted [αIIb(Δ996)β3]. However, deletion of the carboxyl-terminal three-quarters of the β3 tail [αIIbβ3(Δ728)] eliminated the c-Src interaction. The interaction was also abolished by conversion of serine 752 to phenylalanine (data not shown), a mutation known to disrupt β3-mediated outside-in signaling in platelets and osteoclasts (9, 18). Substitution of phenylalanines for β3 tail tyrosines 747 and 759 in the β3 tail, substrates for Src kinases [αIIbβ3(2Y→F)], had no effect on the interaction of αIIbβ3 with c-Src. Integrins containing β1A or β2 cytoplasmic tails (α5β1, αMβ2) did not coprecipitate with c-Src, suggesting that the interaction may be specific for β3 integrins (Fig. 1*C*).

To determine whether the β3 tail is sufficient to affinity-isolate c-Src, pull-down assays were performed by using recombinant integrin tail model proteins (16). c-Src expressed by *in vitro* translation bound to the β3 tail but not to a randomized β3 tail sequence or to the αIIb tail (Fig. 1*D*). To further examine integrin interactions with c-Src, β1A, β2, and β3 tail model proteins were used to affinity-isolate c-Src from platelet lysate. c-Src bound selectively to β3 (Fig. 1*E*). The β1A and β2 tails were functional in that they, like β3, bound to talin, a cytoskeletal protein implicated in integrin activation (19). These data establish that c-Src interaction with β3 integrins is selective and is mediated by the β3 tail.

Interaction of the c-Src SH3 Domain with the Integrin β3 Cytoplasmic Tail. c-Src deletion mutants were expressed in CHO cells to identify regions of c-Src necessary for association with αIIbβ3. No association was observed if the amino-terminal myristoylation site, required for membrane association, or the SH3 domain was deleted (Fig. 2). In contrast, deletion of the unique domain or SH2 domain did not affect the association with αIIbβ3. In platelets, Src catalytic activity is not required for c-Src interaction with αIIbβ3 (6). Thus, the SH3 domain and membrane association are required for c-Src interaction with β3 integrins.

To determine whether the c-Src SH3 domain is sufficient for c-Src interaction with β3 integrins, pull-down assays were performed with a GST-c-Src SH3 fusion protein. αIIbβ3 in platelet lysate or purified αIIbβ3 bound specifically to c-Src SH3 but not to c-Src SH3(Δ90–92), which abrogates SH3 interactions with canonical polyproline type II peptides (14) (Fig. 3*A*). αIIbβ3 also bound to Lyn and c-Yes SH3 domains. When GST-c-Src SH3 was expressed with αIIbβ3 in CHO cells, it coimmunoprecipitated with αIIbβ3 in a dose-dependent manner, whereas GST-c-Src SH3(Δ90–92) did not (Fig. 3*B*). Furthermore, GST-c-Src SH3 but not GST-c-Src SH3(Δ90–92) competed with c-Src for asso-

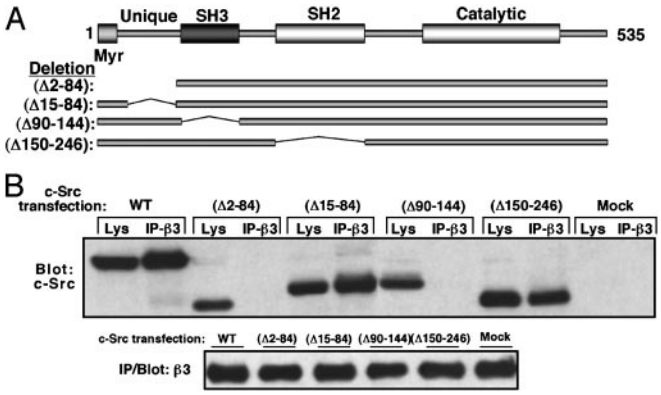

Fig. 2. Regions of c-Src necessary for interaction with αIIbβ3. (*A*) Domain structure of murine c-Src and deletion mutants. (*B*) αIIbβ3 CHO cells were transfected with c-Src or a deletion mutant, and β3 immunoprecipitates were probed as indicated.

ciation with αIIbβ3 (Fig. 3*C*). Thus, the SH3 domain appears necessary and sufficient for c-Src interaction with β3 integrins.

In a direct binding assay, GST-c-Src SH3 bound saturably to the β3 tail model protein (EC_{50} ≈10 μM) (Fig. 3*D*), as did the SH3 domains of Lyn and c-Yes. However, GST-c-Src SH3 failed to bind to a random β3 tail sequence, to β3(Δ758), which lacks the four carboxyl-terminal residues, YRGT (Fig. 3*D*), or to β3(S752P) (data not shown). No interaction was observed between GST-c-Src SH3 and integrin β1 or β2 tails (Fig. 3*D*). Interaction between GST-c-Src SH3 and the β3 tail was blocked by a 14-aa peptide from the carboxyl terminus of β3 (β3 748–762) and by optimized polyproline peptides selective for c-Src SH3 (LSSRPLPTLPSP) (20) or Src family SH3 domains (KGGRSLRPLPPLPPPG) (ref. 14; see also Fig. 3*E*). In contrast, no inhibition was observed with an amino-terminal β3 tail peptide (β3 722–741), an αIIb tail peptide (αIIb 989-1008), or a control peptide (KGELRLRNYYYDVV) (14). When added to CHO cell lysates, LSSRPLPTLPSP and KGGRSLRPLPPLPPPG blocked interaction of αIIbβ3 with GST-c-Src SH3 (Fig. 3*F*), but KGELRLRNYYYDVV or a polyproline peptide selective for c-Abl SH3 (APTYPPPLPP) (20) did not. Thus, despite the absence of a polyproline sequence in the β3 tail (Fig. 1*A*), β3 engages in a specific interaction with c-Src SH3 that involves, in part, the polyproline-binding interface of SH3 and the carboxyl-terminal portion of the β3 tail. This may be analogous to certain other atypical Src family SH3 domain interactions (21), but it is unique in mediating binding of c-Src to an adhesion receptor.

Regulation of c-Src by β3 Integrins. Src kinases are maintained in an autoinhibited state by intramolecular interactions of the SH2 and SH3 domains (22–25). In the inhibited configuration, SH3 binding to a polyproline sequence in the linker region between the SH2 and kinase domains regulates the conformation of the catalytic lobes, and disruption of this interaction by other SH3 ligands can increase Src activity (26–28). Because β3 tail binding to c-Src SH3 is inhibited by optimized Src SH3 ligands, β3 binding might disrupt intramolecular, autoinhibitory SH3 interactions. Therefore, we examined the ability of the β3 tail to activate purified c-Src. Incubation of c-Src with beads containing the β3 cytoplasmic tail model protein increased c-Src activity, and this response was blocked by GST-c-Src SH3 (Fig. 4*A*). In contrast, exposure of c-Src to the truncated β3(Δ758) tail or to a random β3 tail sequence had no effect. Thus, the β3 integrin tail can competitively interfere with the natural, intramolecular interactions of c-Src SH3 and promote increased c-Src activity.

This evidence raises the possibility that c-Src bound to β3

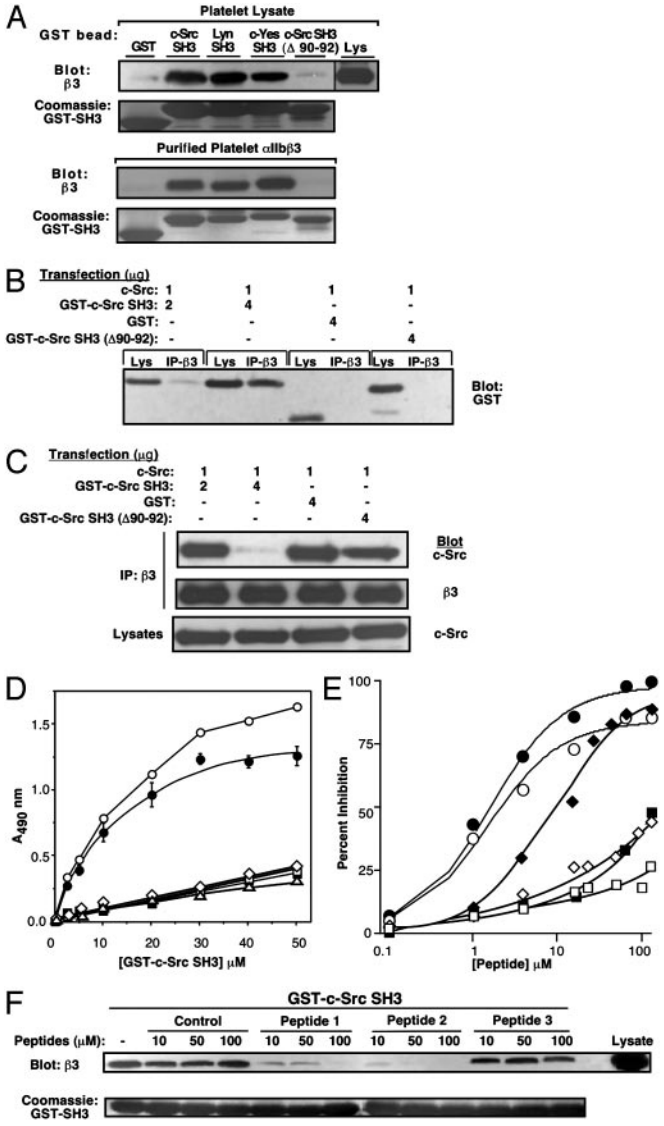

Fig. 3. Interaction of c-Src SH3 with the integrin β3 cytoplasmic tail. (*A*) Platelet lysate or αIIbβ3 purified from platelets was incubated with glutathione-Sepharose beads coated with the indicated GST-SH3 domains. Bound proteins were eluted and probed with antibody to β3. (*B* and *C*) αIIbβ3 CHO cells were cotransfected with c-Src and the indicated GST fusion proteins. β3 immunoprecipitates were probed with antibody to GST (*B*), c-Src, or β3 (*C*). (*D*) Direct binding of purified GST-c-Src SH3 to integrin β cytoplasmic tail proteins, assessed by ELISA: β3 (○), rβ3 (■), β3(Δ758) (□), β1A (◇), and β2 (△). Specific binding of c-Src SH3 to β3 (●). (*E*) Inhibition of the β3 tail/c-Src SH3 interaction by peptides. Immobilized β3 tail and soluble GST-c-Src SH3 (10 μM) were incubated with the following peptides: Src SH3-selective (LSSRPLPTLPSP) (●), Src family SH3-selective (KGGRSLRPLPPLPPPG) (○), β3 tail residues 722–741 (◇), β3 748–762 (◆), αIIb 989-1008 (□), and control (KGELRLRNYYYDVV) (■). Then, binding of GST-c-Src SH3 was detected by ELISA. (*F*) Inhibition of the αIIbβ3/c-Src SH3 interaction by peptides. Lysate from αIIbβ3 CHO cells was incubated with GST-c-Src SH3 coupled to beads in the presence of KGELRLRNYYYDVV (control), KGGRSLRPLPPLPPPG (peptide 1), LSSRPLPTLPSP (peptide 2), or APTYPPPLPP (peptide 3). β3 bound to beads was detected by immunoblotting.

integrins might be activated in quiescent or nonadherent cells. However, c-Src activation in osteoclasts and platelets requires ligand binding to β3 integrins (6, 9). c-Src is positively regulated by trans autophosphorylation on activation loop Tyr-418 (murine; Tyr-416, chicken) (24, 25, 28–31). Therefore, localization of c-Src to unclustered β3 tails in nonadherent cells might spatially restrict trans autophosphorylation but promote it upon integrin

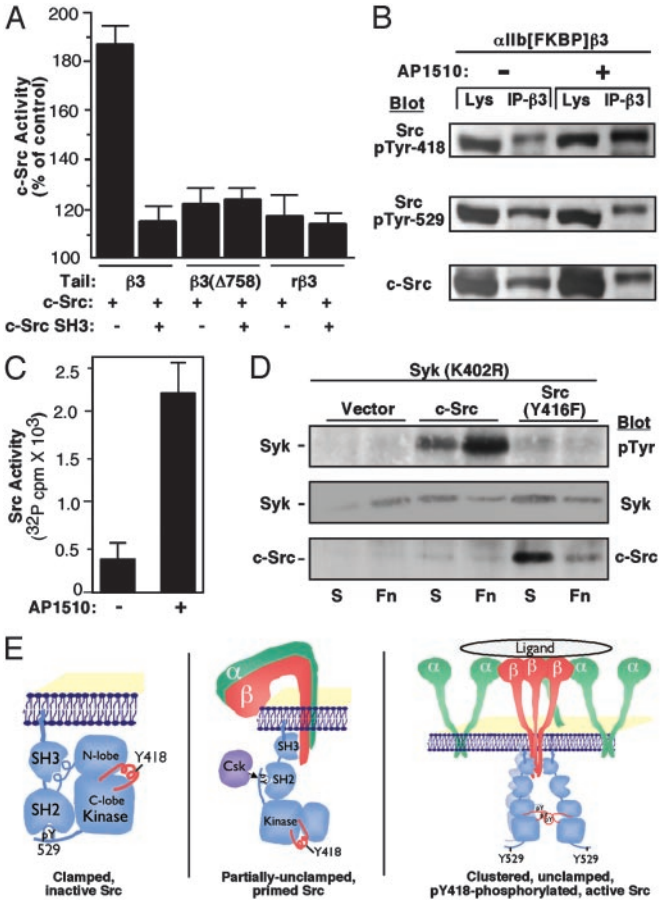

Fig. 4. The integrin β3 cytoplasmic tail modulates c-Src activity. (*A*) Specific binding to the β3 cytoplasmic tail increases the activity of purified c-Src. Activity is expressed relative to a control sample containing c-Src and beads not coated with tail proteins. Data represent means ± SEM of three experiments. (*B* and *C*) Oligomerization of cellular β3 integrins activates c-Src. CHO cells expressing αIIb(FKBP)β3 were transfected with c-Src and incubated in suspension with or without AP1510. Then, β3 immunoprecipitates were subjected to immunoblotting with antibodies specific for pTyr-418 and pTyr-529 (*B*) or *in vitro* Src kinase assay (*C*) (means ± SEM of three experiments). In three immunoblotting experiments and after normalization for gel loading, AP1510 increased Tyr-418 phosphorylation by 210 ± 38% and decreased Tyr-529 phosphorylation by 47 ± 2%. (*D*) c-Src, but not Src(Y416F), can promote adhesion-dependent tyrosine phosphorylation of Syk. c-Src, Src(Y416F), or vector DNA was cotransfected with kinase-inactive Syk(K402R) into CHO cells. Cells were maintained in suspension (S) or plated on fibronectin (Fn) for 30 min, and lysates were subjected to immunoblotting, as indicated. (*E*) Model for Src activation by clustering of β3 integrins, based on current data and recent models for β3 integrins (32, 34, 35) and Src (25).

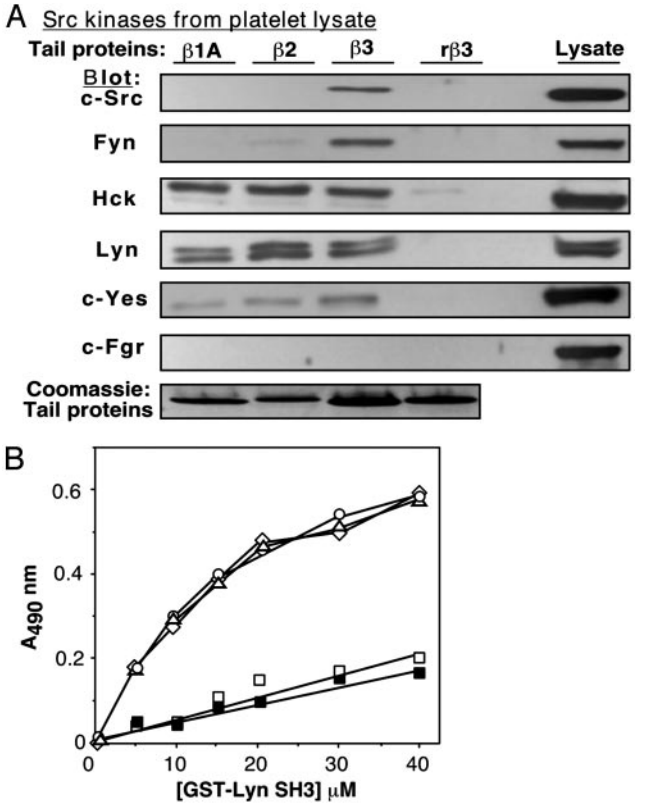

Fig. 5. Interaction of Src family kinases with integrin β cytoplasmic tail proteins. (*A*) Neutravidin beads coated with integrin tail model proteins were incubated with platelet lysate. Bound Src kinases were detected by Western blotting. (*B*) Direct binding of purified GST-Lyn SH3 to integrin β cytoplasmic tail proteins, assessed by ELISA: β1A (◇), β2 (△), β3 (○), rβ1A (□), and rβ3 (■).

clustering (13, 32). To determine whether integrin clustering is sufficient to induce Tyr-418 phosphorylation and c-Src activation *in vivo*, αIIb was fused to an FKBP domain and expressed with β3 in CHO cells. Clustering of αIIbβ3 was achieved by adding AP1510, a bivalent FKBP ligand (13). AP1510 caused an increase in c-Src Tyr-418 phosphorylation (Fig. 4*B*) and c-Src activity (Fig. 4*C*), which is consistent with clustering-induced trans autophosphorylation. Integrin clustering also caused slight but consistent dephosphorylation of c-Src Tyr-529 (Fig. 4*B*), which would further enhance c-Src activity (23).

Mutation of the activation loop tyrosine to phenylalanine reduces c-Src activity *in vitro* (28), but the consequences of this mutation *in vivo* have not been examined. Therefore, we investigated the ability of c-Src to promote adhesion-dependent tyrosine phosphorylation of Syk, a downstream target (6, 12). Whereas expression of chicken c-Src in CHO cells increased basal and adhesion-dependent tyrosine phosphorylation of kinase-inactive Syk(K402R), expression of Src(Y416F) failed to induce Syk phosphorylation (Fig. 4*D*). Thus, c-Src activation loop tyrosine phosphorylation is required for c-Src substrate phosphorylation *in vivo*.

Model for Src Activation by Integrins. These results support the following model for c-Src activation by β3 integrins (Fig. 4*E*). In quiescent cells, intramolecular interactions render the bulk of c-Src clamped and inactive (25). However, a pool of c-Src bound to β3 integrins would be partially unclamped, or primed. This would not lead to full c-Src activation due to absence of activation loop phosphorylation, which is required for realignment of the αC helix and substrate access to the catalytic cleft (22, 31). c-Src activation would also be limited by phosphorylation of Tyr-529 by integrin-associated Csk (6). Clustering of β3 by ligands such as fibrinogen or fibronectin (13), possibly facilitated by homooligomerization of integrin subunits (32), would increase local c-Src concentration, leading to trans autophosphorylation on Tyr-418 and the "switching" of c-Src to a stable activated state (25). Dephosphorylation of Tyr-529, promoted by tyrosine phosphatases (33) and dissociation of Csk from β3 (6), would also contribute to stabilization of the activated state. This model brings together structural, biochemical, and cell biological information on β3 integrins and Src (1, 6, 23, 25, 32, 34, 35) and provides a natural context for understanding how c-Src activation through an adhesion receptor initiates a cascade of tyrosine phosphorylation.

These results raise the question of whether other Src family

members bind to $\beta 3$ or other integrin β subunits. To address this question, we examined the ability of $\beta 1$A, $\beta 2$, and $\beta 3$ tail model proteins to affinity-isolate Src kinases from platelet lysates. As with c-Src, Fyn bound selectively to the $\beta 3$ tail (Fig. 5*A*). In contrast, Hck, Lyn, and c-Yes bound to $\beta 1$A, $\beta 2$, and $\beta 3$ tails, whereas c-Fgr bound to none of these. Furthermore, GST-Lyn SH3 bound to the $\beta 1$A, $\beta 2$, and $\beta 3$ tails (Fig. 5*B*), and binding was blocked by the polyproline peptide selective for Src family SH3 domains (KGGRSLRPLPPLPPPG). Similar results were obtained with GST-c-Yes SH3. In addition, endogenous c-Yes could be coimmunoprecipitated with $\beta 1$ integrin from CHO cells (data not shown). These data indicate that Fyn interaction with $\beta 3$ integrins is selective, whereas other Src family kinases can interact with multiple β cytoplasmic tails in a manner that depends on the SH3 domain.

In solution, the membrane-proximal region of integrin β tails is α-helical, highly conserved, and interacts with talin to regulate integrin affinity. The carboxyl-terminal region of the $\beta 3$ cytoplasmic tail, shown here to be necessary for c-Src interaction, is disordered, and its sequence is divergent from other β tails (19, 34). When the $\beta 3$ transmembrane and cytoplasmic domains together are analyzed in membrane-mimicking detergents, the membrane-distal portion of the $\beta 3$ tail assumes a turn-helix configuration, similar to that of an immunoreceptor-based activation motif (36). However, without knowing the structure of the $\beta 3$ tail in a native membrane, it is difficult to predict *a priori* how it interacts with the c-Src SH3 domain or whether the tail-binding sequences can assume a type II polyproline-type helix, as observed for the intramolecular SH3 binding sequence of c-Src, which lacks a PXXP motif. It is noteworthy that the intramolecular SH3 ligand in Src kinases is not conserved and was not predicted to be a ligand before derivation of the crystal structure. Likewise, it is difficult to explain the β tail specificities of distinct SH3 domains observed in this report. Structurally similar Src family SH3 domains can exhibit differences in ligand-recognition specificities, especially in regions flanking the core PXXP motifs (15, 37, 38). However, further structural information will be required to fully understand the basis for specificity of the c-Src SH3/$\beta 3$ complex and the manner in which other Src family kinases interact with $\beta 1$A and $\beta 2$ integrins. Nonetheless, these results provide a conceptual basis for integrin regulation of Src, for the similar functional defects of osteoclasts or platelets lacking c-Src or $\beta 3$ integrins (6, 7, 9), for the deficient lamellipodia formation in αIIb$\beta 3(\Delta 758)$ CHO cells plated on fibrinogen (39), and for the selective modulation of $\beta 3$ integrin traction forces by c-Src (40). Because $\beta 3$ integrins promote vascular responses to injury and tumor metastasis, disruption of the Src/$\beta 3$ integrin complex may represent a useful therapeutic strategy.

We thank Eric Brown, Rudolph Juliano, and V. Rivera (Ariad Pharmaceuticals) for reagents and Steve Harrison for helpful discussions. These studies were supported by research grants from the National Institutes of Health and by a postdoctoral fellowship (to E.G.A.-S.) from Secretaria de Estado de Educacion y Universidades (Spain) and Fondo Social Europeo.

1. Hynes, R. (2002) *Cell* **110,** 673–687.
2. Lowell, C. A., Fumagalli, L. & Berton, G. (1996) *J. Cell Biol.* **133,** 895–910.
3. Klinghoffer, R. A., Sachsenmaier, C., Cooper, J. A. & Soriano, P. (1999) *EMBO J.* **18,** 2459–2471.
4. Parsons, J. T. (2003) *J. Cell Sci.* **116,** 1409–1416.
5. Hruska, K. A., Rolnick, F., Huskey, M., Alvarez, U. & Cheresh, D. (1995) *Endocrinology* **136,** 2984–2992.
6. Obergfell, A., Eto, K., Mocsai, A., Buensuceso, C., Moores, S. L., Brugge, J. S., Lowell, C. A. & Shattil, S. J. (2002) *J. Cell Biol.* **157,** 265–275.
7. Soriano, P., Montgomery, C., Geske, R. & Bradley, A. (1991) *Cell* **64,** 693–702.
8. McHugh, K. P., Hodivala-Dilke, K., Zheng, M. H., Namba, N., Lam, J., Novack, D., Feng, X., Ross, F. P., Hynes, R. O. & Teitelbaum, S. L. (2000) *J. Clin. Invest.* **105,** 433–440.
9. Feng, X., Novack, D. V., Faccio, R., Ory, D. S., Aya, K., Boyer, M. I., McHugh, K. P., Ross, F. P. & Teitelbaum, S. L. (2001) *J. Clin. Invest.* **107,** 1137–1144.
10. Lakkakorpi, P. T., Nakamura, I., Young, M., Lipfert, L., Rodan, G. A. & Duong, L. T. (2001) *J. Cell Sci.* **114,** 149–160.
11. Gao, J., Zoller, K., Ginsberg, M. H., Brugge, J. S. & Shattil, S. J. (1997) *EMBO J.* **16,** 6414–6425.
12. Miranti, C., Leng, L., Maschberger, P., Brugge, J. S. & Shattil, S. J. (1998) *Curr. Biol.* **8,** 1289–1299.
13. Buensuceso, C., De Virgilio, M. & Shattil, S. J. (2003) *J. Biol. Chem.* **278,** 15217–15224.
14. Weng, Z., Thomas, S. M., Rickles, R. J., Taylor, J. A., Brauer, A. W., Seidel-Dugan, C., Michael, W. M., Dreyfuss, G. & Brugge, J. S. (1994) *Mol. Cell. Biol.* **14,** 4509–4521.
15. Rickles, R. J., Botfield, M. C., Zhou, X. M., Henry, P. A., Brugge, J. S. & Zoller, M. J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 10909–10913.
16. Pfaff, M., Liu, S., Erle, D. J. & Ginsberg, M. H. (1998) *J. Biol. Chem.* **273,** 6104–6109.
17. Schatz, P. J. (1993) *Biotechnology* **11,** 1138–1143.
18. Chen, Y.-P., O'Toole, T. E., Ylänne, J., Rosa, J.-P. & Ginsberg, M. H. (1994) *Blood* **84,** 1857–1865.
19. Calderwood, D. A., Zent, R., Grant, R., Rees, D. J. G., Hynes, R. O. & Ginsberg, M. H. (1999) *J. Biol. Chem.* **274,** 28071–28074.
20. Panni, S., Dente, L. & Cesareni, G. (2002) *J. Biol. Chem.* **277,** 21666–21674.
21. Chan, B., Lanyi, A., Song, H. K., Griesbach, J., Simarro-Grande, M., Poy, F., Howie, D., Sumegi, J., Terhorst, C. & Eck, M. J. (2003) *Nat. Cell Biol.* **5,** 155–160.
22. Sicheri, F., Moarefi, I. & Kuriyan, J. (1997) *Nature* **385,** 602–609.
23. Sicheri, F. & Kuriyan, J. (1997) *Curr. Opin. Struct. Biol.* **7,** 777–785.
24. Young, M. A., Gonfloni, S., Superti-Furga, G., Roux, B. & Kuriyan, J. (2001) *Cell* **105,** 115–126.
25. Harrison, S. C. (2003) *Cell* **112,** 737–740.
26. Moarefi, I., LaFevre-Bernt, M., Sicheri, F., Huse, M., Lee, C. H., Kuriyan, J. & Miller, W. T. (1997) *Nature* **385,** 650–653.
27. Luttrell, L. M., Ferguson, S. S. G., Daaka, Y., Miller, W. E., Maudsley, S., Della Rocca, G. J., Lin, F. T., Kawakatsu, H., Owada, K., Luttrell, D. K., *et al.* (1999) *Science* **283,** 655–661.
28. Sun, G., Ramdas, L., Wang, W., Vinci, J., McMurray, J. & Budde, R. J. (2002) *Arch. Biochem. Biophys.* **397,** 11–17.
29. Kmiecik, T. E. & Shalloway, D. (1987) *Cell* **49,** 65–73.
30. Cooper, J. A. & MacAuley, A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4232–4236.
31. Yamaguchi, H. & Hendrickson, W. A. (1996) *Nature* **384,** 484–489.
32. Li, R., Mitra, N., Gratkowski, H., Vilaire, G., Litvinov, R., Nagasami, C., Weisel, J. W., Lear, J. D., DeGrado, W. F. & Bennett, J. S. (2003) *Science* **300,** 795–798.
33. Su, J., Muranjan, M. & Sap, J. (1999) *Curr. Biol.* **9,** 505–511.
34. Vinogradova, O., Velyvis, A., Velyviene, A., Hu, B., Haas, T., Plow, E. & Qin, J. (2002) *Cell* **110,** 587–597.
35. Takagi, J., Petre, B., Walz, T. & Springer, T. (2002) *Cell* **110,** 599–611.
36. Li, R., Babu, C. R., Valentine, K., Lear, J. D., Wand, A. J., Bennett, J. S. & DeGrado, W. F. (2002) *Biochemistry* **41,** 15618–15624.
37. Rickles, R. J., Botfield, M. C., Weng, Z., Taylor, J. A., Green, O. M., Brugge, J. S. & Zoller, M. J. (1994) *EMBO J.* **13,** 5598–5604.
38. Sparks, A. B., Rider, J. E., Hoffman, N. G., Fowlkes, D. M., Quillam, L. A. & Kay, B. K. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 1540–1544.
39. Woodside, D., Obergfell, A., Leng, L., Wilsbacher, J. L., Miranti, C. K., Brugge, J. S., Shattil, S. J. & Ginsberg, M. H. (2001) *Curr. Biol.* **11,** 1799–1804.
40. Felsenfeld, D. P., Schwartzberg, P. L., Venegas, A., Tse, R. & Sheetz, M. P. (1999) *Nat. Cell Biol.* **1,** 200–206.