Activation of Syk protein tyrosine kinase through interaction with integrin β cytoplasmic domains

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Syk protein tyrosine kinase is essential for immune system development and function [1] and for the maintenance of vascular integrity [2, 3]. In leukocytes, Syk is activated by binding to diphosphorylated immune receptor tyrosine-based activation motifs (pITAMs) [1]. Syk can also be activated by integrin adhesion receptors [4, 5], but the mechanism of its activation is unknown. Here we report a novel mechanism for Syk's recruitment and activation, which requires that Syk bind to the integrin β3 cytoplasmic tail. We found that both Syk and the related kinase ZAP-70 bound the β3 cytoplasmic tail through their tandem SH2 domains. However, unlike Syk binding to pITAMs, this interaction was independent of tyrosine phosphorylation and of the phosphotyrosine binding function of Syk's tandem SH2 domains. Deletion of the four C-terminal residues of the β3 cytoplasmic tail [β3(759X)] decreased Syk binding and disrupted its physical association with integrin αIIbβ3. Furthermore, cells expressing αIIbβ3(759X) failed to exhibit Syk activation or lamellipodia formation upon cell adhesion to the αIIbβ3 ligand, fibrinogen. In contrast, FAK phosphorylation and focal adhesion formation were unimpaired by this mutation. Thus, the direct binding of Syk kinase to the integrin β3 cytoplasmic tail is a novel and functionally significant mechanism for the regulation of this important non-receptor tyrosine kinase.

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Results and discussion

We examined the possibility of direct interactions between Syk and integrins because Syk activation is an early response to integrin clustering [6] and Syk is found in a protein complex that contains integrins [7, 8]. When platelets adhered to the αIIbβ3 ligand fibrinogen, Syk coprecipitated with integrin αIIbβ3 (Figure 1a). Because integrin-dependent Syk activation [4] depends on integrin cytoplasmic domains [5], we tested for interactions between Syk and recombinant model protein mimics of dimerized integrin cytoplasmic tails. The model proteins are designed with an N-terminal heptad repeat sequence joined to the integrin cytoplasmic domains. The repeats form a coiled-coil homodimer that dimerizes the integrin tails [9]. Syk and Zap-70 from cell lysates bound to a β3 tail model protein (Figure 1b). This association must have been specific because there was no detectable binding to the αIIb tail (Figure 1b) or to a structure-altering [10] β3 tail point mutant β3(Y747A) that perturbs many integrin functions [11] (data not shown). Furthermore, Syk and its paralog, Zap-70, were enriched to a greater extent than Talin (see Figure S1 in the supplementary material available with this article online), a protein known to interact directly with integrin β cytoplasmic tails [9, 12]. To localize β3 integrin binding sites within Syk, we examined the binding of recombinant wild-type and mutant Syk expressed in CHO cells. Wild-type Syk bound to the β3 tail (Figure 1c, left panel). Neither kinase activity nor the kinase domain of Syk was required for binding since both a kinase-inactive Syk(K402R) and a Syk truncation mutant (residues 1–330) lacking the kinase domain bound to the β3 tail (Figure 1c, right panel). Thus, Syk can associate specifically with the β3 integrin cytoplasmic tail, and this interaction involves the N-terminal 1–330 residues of Syk.

To assess whether the association between integrin cytoplasmic tails and Syk family members was direct, we used recombinant fragments of Syk and Zap-70. The Syk family of non-receptor tyrosine kinases consists of N-terminal tandem SH2 domains separated by an intervening sequence termed "interdomain A" [1]. Following the tandem SH2 domains is an "interdomain B" region and a large kinase domain [1]. Fragments containing both SH2 and interdomains A and B [Syk(6–370) and Zap-70 (1–337)] bound to the β3 tail (Figure 2a). No binding was detected with the αIIb or β3 (Y747A) tail. The tandem SH2 domains of Syk recognize phosphorylated tyrosine residues in consensus pITAM motifs [YxxI/L(x)$_{6-8}$YxxI/L]

Figure 1
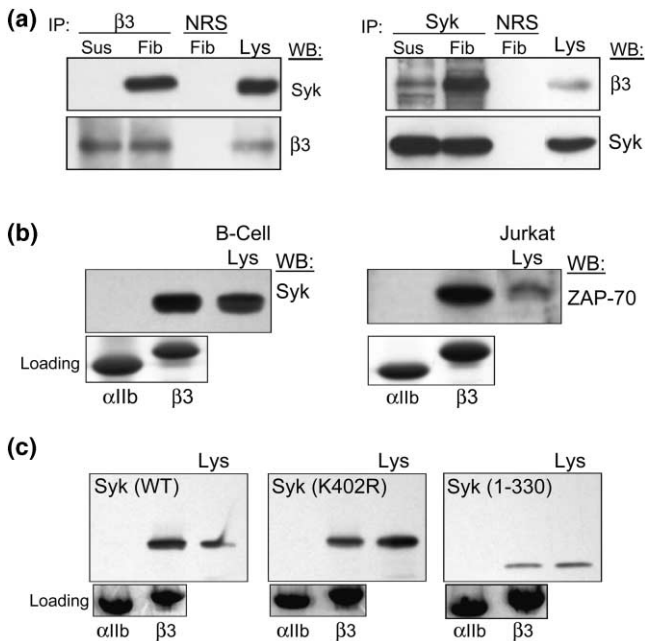
Adhesion-dependent association between Syk and integrin αIIbβ3.
(a) Integrin αIIbβ3 (left panel) or Syk (right panel) was immuno-
precipitated from lysates of fibrinogen (Fib)-adherent or suspended
(Sus) platelets, followed by probing with anti-Syk or anti-β3,
respectively. "NRS" stands for normal rabbit serum. "Lys" stands for
platelet lysate. **(b)** Affinity chromatography of B cell lysates or Jurkat
T cell lysates. Integrin αIIb and β3 cytoplasmic-domain model proteins
were used. **(c)** CHO cells expressing various wild-type and mutant
Syk constructs were lysed, and affinity chromatography was performed
with integrin αIIb and β3 cytoplasmic domains. Coomassie blue
staining of the integrin cytoplasmic-domain model proteins was used
for monitoring the loading of the affinity matrix (Loading). The last
lane of each immunoblot represents 1/20th of the starting cell lysate
(Lys). Depicted is one representative experiment of three performed.

Figure 2

Direct interaction between Syk and the β3 tail involves Syk's tandem
SH2 domain but is independent of phospho-ITAM binding. **(a)** Syk and
Zap-70 direct binding to the integrin β3 cytoplasmic tail. The doublet
(approximately 70 and 62 kDa) of Syk(6–370) seen when one blots
with anti-GST represents intact Syk(6–370), a C-terminal cleavage of
the purified GST-fusion protein. Coomassie blue staining of the
integrin cytoplasmic domain model proteins was used for monitoring
the loading of each construct (Loading). **(b)** Direct binding of β3
tails to purified Syk(6–370) in the presence of excess phospho-ITAM
(pITAM) peptides. Peptides proved to be functional because they
activated Syk kinase in vitro ([14] and data not shown). All experiments
were repeated 3–6 times, and representative results are shown. **(c)**
Elisa analysis of soluble β3 tail binding to immobilized GST-
Syk(6–370) in the presence of FcεRIγ pITAM. The inset image
shows specific inhibition of the binding of the integrin β3 tail to
immobilized GST-Syk. NT represents no treatment. Experiments
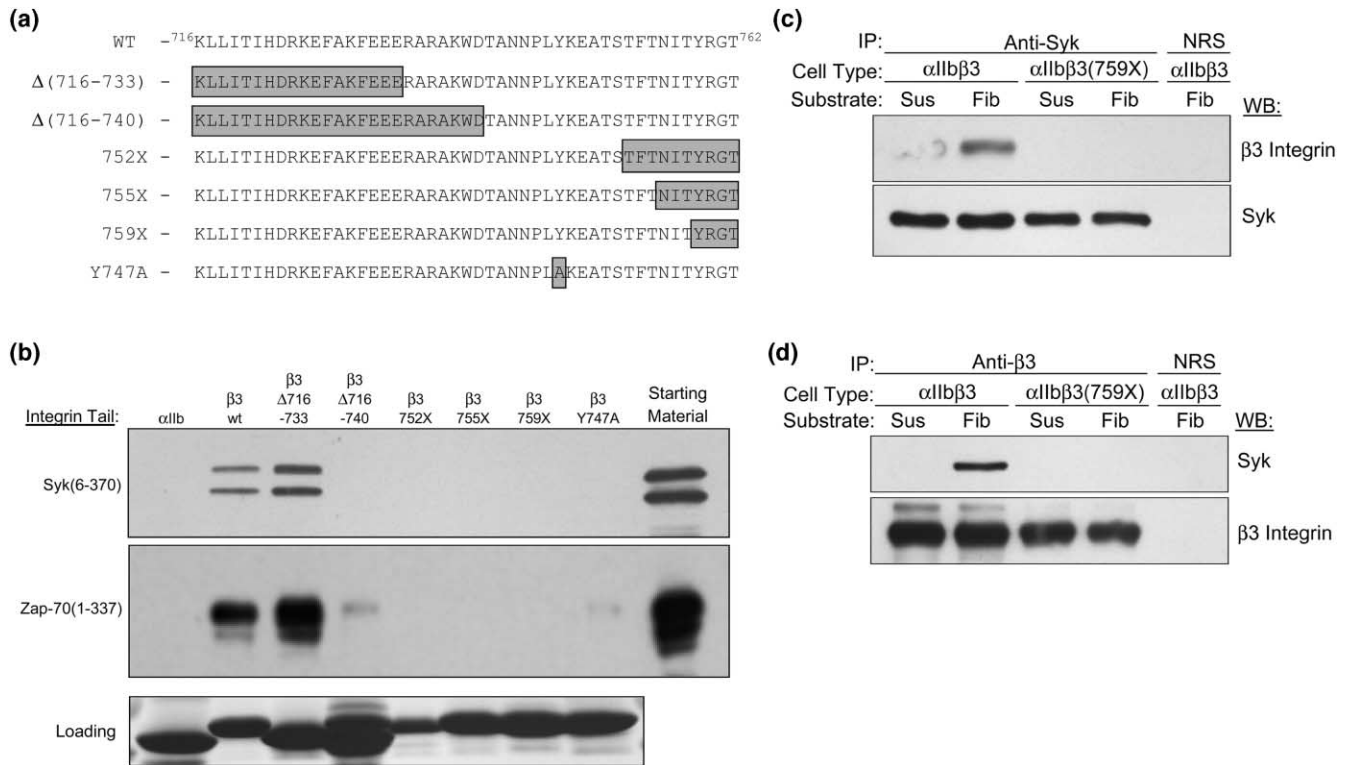were repeated 3–6 times, and representative results are shown.

in immune response receptor subunits [1]. However, in-
tegrin activation of Syk appears to be ITAM independent
[5]. Indeed, a C-terminal SH2 domain mutant of Syk that
is deficient in binding to pITAMs, but which can be
activated by integrin αIIbβ3 [Syk(R195A)] [5], bound to
the β3 cytoplasmic tail (see Figure S2 in the supplemen-
tary material). Additional mapping studies (our unpub-
lished results) indicated that Syk(6–270) bound to the
integrin β3 tail; however, Syk(163–270), which contains
only the C-terminal SH2 domain, failed to bind. We intro-
duced an R42A mutation (predicted to disrupt the phos-
photyrosine binding pocket in the N-terminal SH2 do-
main) into the Syk(6–270) construct to test its effects on
binding to the β3 tail. This mutation failed to prevent its
binding to the β3 tail in direct binding assays (see Figure
S2 in the supplementary material), suggesting that the
Syk-β3 interaction involved a novel, pITAM-indepen-
dent mechanism. To test this hypothesis, we examined
the effect of an 8000-fold molar excess of pITAM peptides
on the binding of Syk(6–370) to the β3 tail. A dually
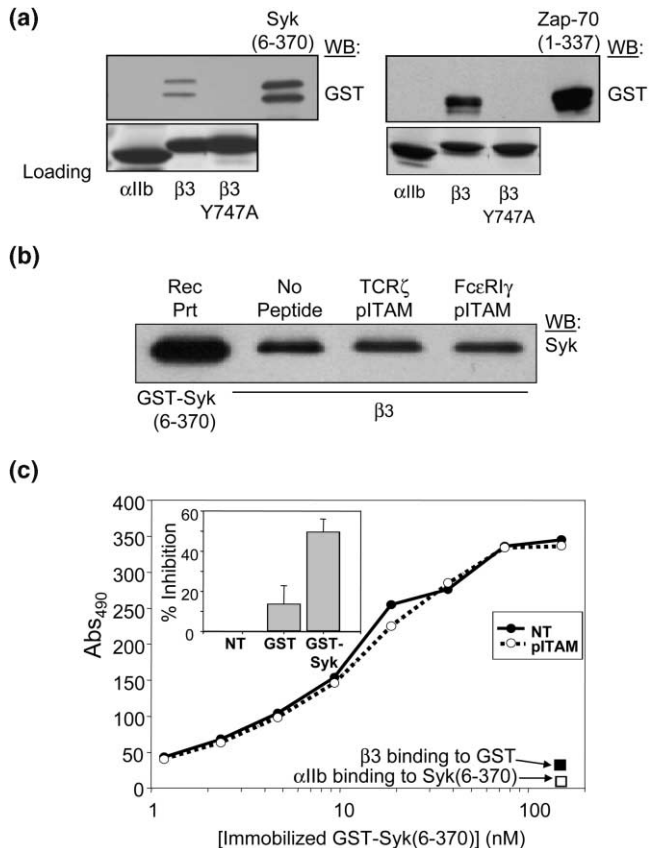
phosphorylated FcεRIγ ITAM peptide [DGVY(PO$_3$)
TGLSTRNQETY(PO$_3$)ETLKTCR] failed to compete
with the β3 integrin cytoplasmic tail for binding to Syk(6–
370) (Figure 2b). pITAM peptide derived from the TCRζ
chain (Figure 2b) also failed to compete. Soluble pITAM
peptide was used at a concentration of 40 μM, well above
the reported 2.6 nM Kd for the interaction between
Syk(6–370) and the FcεRIγ pITAM peptide [13]. In a
quantitative enzyme-linked immunosorbent assay, immo-
bilized Syk(6–370) bound to the soluble β3 tail in the
presence or absence of 10 μM FcεRIγ pITAM peptides

Figure 3

(a)

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        WT  -⁷¹⁶KLLITIHDRKEFAKFEEERARAKWDTANNPLYKEATSTFTNITYRGT⁷⁶²

Δ(716-733) -  KLLITIHDRKEFAKFEEE RARAKWDTANNPLYKEATSTFTNITYRGT

Δ(716-740) -  KLLITIHDRKEFAKFEEERARAKWD TANNPLYKEATSTFTNITYRGT

      752X -  KLLITIHDRKEFAKFEEERARAKWDTANNPLYKEATS TFTNITYRGT

      755X -  KLLITIHDRKEFAKFEEERARAKWDTANNPLYKEATSTFT NITYRGT

      759X -  KLLITIHDRKEFAKFEEERARAKWDTANNPLYKEATSTFTNIT YRGT

     Y747A -  KLLITIHDRKEFAKFEEERARAKWDTANNPLA KEATSTFTNITYRGT
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(c)

IP:	Anti-Syk				NRS
Cell Type:	αIIbβ3		αIIbβ3(759X)		αIIbβ3
Substrate:	Sus	Fib	Sus	Fib	Fib

WB: β3 Integrin, Syk

(b)

Integrin Tail: αIIb | β3 wt | β3 Δ716-733 | β3 Δ716-740 | β3 752X | β3 755X | β3 759X | β3 Y747A | Starting Material

Syk(6-370)

Zap-70(1-337)

Loading

(d)

IP:	Anti-β3				NRS
Cell Type:	αIIbβ3		αIIbβ3(759X)		αIIbβ3
Substrate:	Sus	Fib	Sus	Fib	Fib

WB: Syk, β3 Integrin

Removal of the four C-terminal residues of the β3 cytoplasmic tail abrogates Syk binding both in vitro and in vivo. **(a)** Schematic representation of integrin β3 cytoplasmic tail deletion mutants. Shaded boxes indicate deleted residues. **(b)** Direct binding of GST fusions of Syk(6–370) and Zap-70(1–337) to integrin β3 cytoplasmic tail deletion mutants. Bound protein was measured by Western blotting with anti-GST mAb. Bottom panel (Loading) depicts Coomassie staining of cytoplasmic tails. **(c,d)** Lysates were generated from CHO cells stably expressing wild-type integrin αIIbβ3 or integrin αIIbβ3(759X) that were kept in suspension (Sus) or plated on fibrinogen (Fib). Syk or integrin was immunoprecipitated and probed for **(c)** integrin β3 or **(d)** Syk, respectively. Similar amounts of β3 integrin and Syk were expressed in both cell lines (see lower panels of parts [d] and [c], respectively). The figure depicts one representative experiment of three so performed.

(Figure 2c), confirming the lack of competition. The FcεRIγ pITAM peptides were shown to be functional because they activated Syk kinase (EC$_{50}$ 1–2 uM) in vitro [14] (data not shown). Thus, Syk recognition of β3 involves a unique specificity, distinct from Syk's interaction with pITAMs.

To identify regions of the β3 tail involved in Syk binding, we tested deletion mutants represented schematically in Figure 3a. The N-terminal deletion β3(Δ716–733) (which removes residues 716–733 of the integrin β3 cytoplasmic domain; Figure 3a) retained the ability to bind Syk and Zap-70 (Figure 3b). However, deletion of seven more residues [β3(Δ716–740)] prevented binding. Deletion of the last four C-terminal residues (Tyr^{759}-Arg-Gly-Thr^{762}) [β3(759X)] abolished Syk interaction with β3, as did removal of 11 C-terminal residues [residues Thr^{752}–Thr^{762}, (β3752X)] (Figure 3b). Thus, β3 residues Arg^{734}-Thr^{762} are sufficient for its direct interaction with Syk. Furthermore, removal of the four C-terminal residues of integrin β3

abrogates binding in vitro. We assessed the effect of the deletion of these residues on the association between Syk and integrin αIIbβ3. CHO cells expressing αIIbβ3(759X) were generated (see Figure S3 in the supplementary material). These cells expressed similar quantities of αIIbβ3 (Figure S3) and adhered normally to fibrinogen (data not shown), but unlike wild-type αIIbβ3, αIIbβ3(759X) was not coimmunoprecipitated with Syk (Figure 3c,d) from these adherent cells.

When integrin αIIbβ3(759X)-expressing CHO cells were plated on fibrinogen, they spread and formed focal adhesions (see Figure S4 in the supplementary material) but did not support adhesion-dependent Syk phosphorylation (Figure 4a, lower panel). In contrast, these cells exhibited adhesion-dependent phosphorylation of another tyrosine kinase, pp125^{FAK} (Figure 4a, upper panel). Thus, the physical association of integrin αIIbβ3 with Syk requires the Syk binding function of the β3 cytoplasmic tail and is required for integrin-dependent activation of Syk. These

Figure 4

The Syk binding site in the β3 tail is required for activation of Syk but not for activation of pp125^{FAK}. **(a)** Plating αIIbβ3(759X) expressing CHO cells on fibrinogen induced the tyrosine phosphorylation of pp125^{FAK} (upper panels) but not Syk (lower panels). **(b)** Phosphotyrosine analysis of pp125^{FAK}, Syk, and Vav1 in fibrinogen-adherent CHO cells upon over-expression of Syk(1–330). CHO cells stably expressing a retrovirally transduced HA-tagged Syk were transfected with vector control/Vav1 or Syk(1–330)/Vav1. Syk, Vav1, or pp125^{FAK} was immunoprecipitated from lysates derived from cells that were kept in suspension (Sus) or plated on fibrinogen (Fib). **(c)** CHO cells stably expressing integrin αIIbβ3 or αIIbβ3(759X) were transfected with either Vav1 or both Vav1 and Syk. Cells were plated onto fibrinogen, fixed, and stained for Vav1 (FITC) and filamentous actin (rhodamine phalloidin). Arrowheads indicate lamellipodia. Quantification of lamellipodia was performed in a blinded fashion, as described [6]. The scale bar represents 10 μm. All experiments were performed at least three times, and representative results are shown.
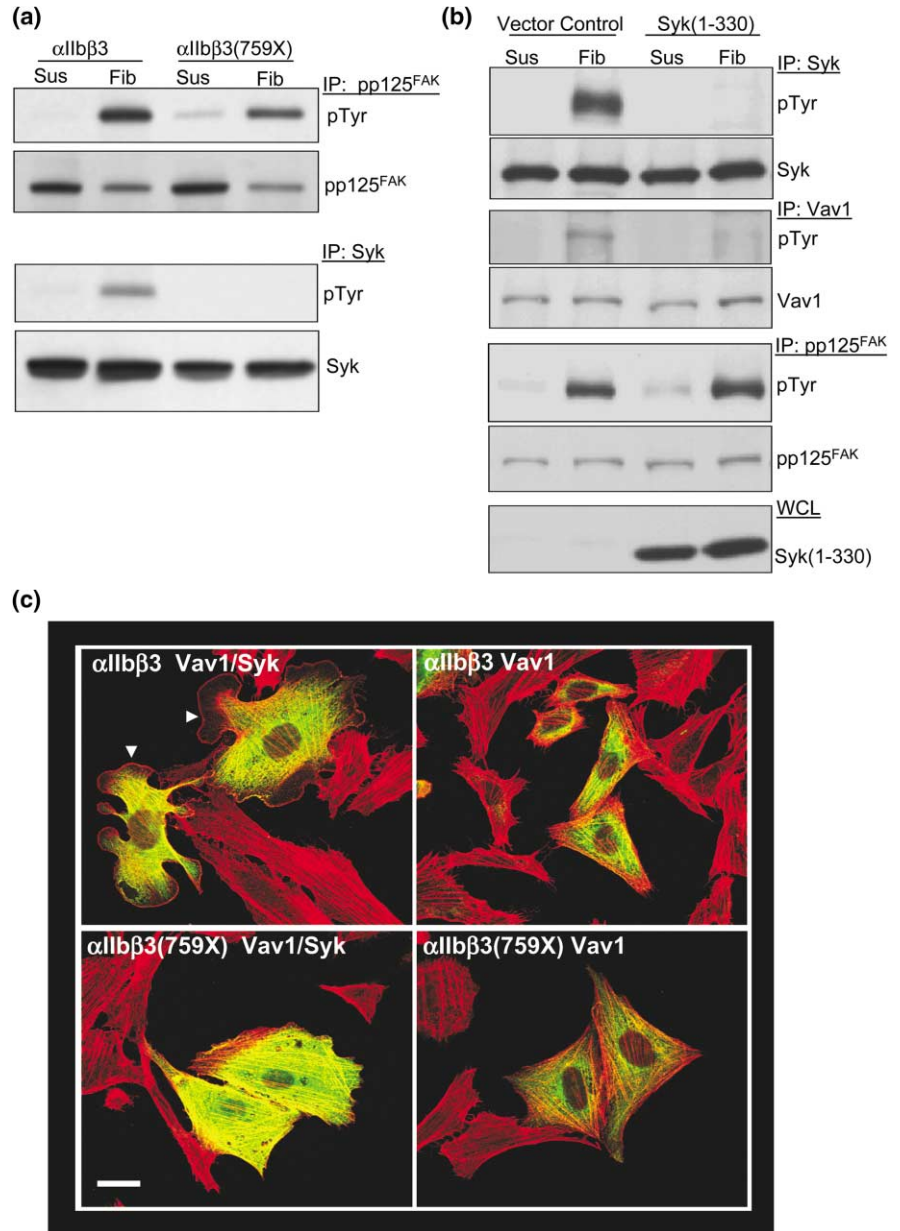
results also suggest that distinct β3 cytoplasmic-domain structural features are responsible for the activation of Syk and pp125^{FAK}.

Syk activation upon integrin engagement is rapid and, unlike pp125^{FAK} activation, is insensitive to actin-depolymerizing agents [6]. To further examine the dichotomy between integrin-dependent regulation of Syk and pp125^{FAK}, we used the tandem SH2 domains of Syk as a dominant-negative inhibitor. In previous studies, low levels of Syk(1–330) expression did not lead to detectable inhibition of integrin-dependent Syk activation [5]. However, over-expression of Syk(1–330) in CHO cells stably

expressing αIIbβ3 and a single genetic copy of Syk blocked the integrin-dependent phosphorylation of Syk (Figure 4b). Vav1, a Rac guanine nucleotide exchange protein, is phosphorylated and activated upon binding to phosphorylated Tyr^{348} of Syk [1]. Over-expression of Syk(1–330) also inhibited adhesion-dependent phosphorylation of Vav1 (Figure 4b). These results confirm that sequences within the N-terminal half of Syk are involved in its activation via binding to the β3 tail. Over-expression of Syk(1–330) did not, however, inhibit adhesion-induced phosphorylation of pp125^{FAK} (Figure 4b). Thus, Syk activation requires an integrin-dependent signaling pathway that is distinct from that which activates pp125^{FAK}, and

one can selectively block Syk activation and events downstream of Syk without perturbing certain other signaling functions of β3 integrins.

Syk and Vav1 cooperate to remodel the actin cytoskeleton by inducing Rac-dependent lamellipodia formation [6]. To assess the functional effects of the integrin-Syk interaction, we cotransfected CHO cells stably expressing wildtype αIIbβ3 or αIIbβ3(759X) with Vav1, or Syk and Vav1, and assessed integrin-dependent lamellipodia formation. Fibrinogen-adherent cells expressing integrin αIIbβ3(759X) generated at least one actin-rich lamellipodium in 28.3 ± 1.0 percent of cells transfected with Vav1. Cotransfection of Syk and Vav1 did not increase this response (26.3 ± 1.9 percent; Figure 4c). In sharp contrast, transfection of Syk and Vav1 into cells expressing wild-type αIIbβ3 resulted in a dramatic increase in the extent of lamellipodia formation (from 24.0 ± 2.2 in Vav1-transfected cells to 53.3 ± 5.6 percent in Syk/Vav1-transfected cells, Figure 4c). Thus, the interaction between Syk and the β3 integrin cytoplasmic domain initiates Syk-dependent cytoskeletal reorganization.

Our results suggest a novel paradigm for the regulation of Syk kinases. The mechanism of Syk activation by integrins differs from that of immune receptors. pITAMs within immune receptors serve as the binding sites that recruit Syk through its tandem SH2 domains. In contrast, neither the phosphotyrosine binding sites within the Syk SH2 domains nor phosphorylation of tyrosines in the β3 tail is required for Syk interaction with, or activation [5] by, β3 integrins. Thus, integrins and immune receptors have evolved distinct mechanisms for recruiting Syk to transmembrane receptor complexes. Syk is recruited to clustered integrins by its direct interaction with integrin cytoplasmic domains. Src kinases are present in these integrin-dependent protein complexes [15] and are required for maximal integrin-dependent activation of Syk [5]. Thus, the proximity promoted by integrin clustering may promote Syk transphosphorylation by one or more Src family kinase [1] and lead to activation of Syk catalytic activity [16].

Syk is regulated by multiple classes of integrins (β1 [17], β2 [18], and β3 [4]), and Syk binds to multiple classes of integrin tails (β2, β5, β7, and β1A, unpublished results) in addition to β3. Furthermore, β3 tails bind the Syk paralog, Zap-70, which is also subject to integrin-dependent regulation [19]. Thus, the direct interaction between integrin cytoplasmic domains and either Syk or ZAP-70 is likely to represent a general mechanism for the recruitment and activation of these important tyrosine kinases. Given Syk's role in numerous immune receptor-independent processes and its expression in tissues outside of the hematopoietic system [20], the novel mechanism of Syk activation defined here is likely to be of significance in many biological contexts.

Materials and methods

Cells, cDNAs, and peptides
The CHO cell line expressing αIIbβ3(759X) was generated by the transfection of CHO cells with αIIb and β3(759X) cDNA. The CHO A5 cell line stably expressing Syk-HA was created by retroviral transduction with a C-terminal HA-tagged variant of Syk (pLHCX Syk-HA), followed by selection in hygromycin.

Mammalian Syk expression vectors have been described [5], along with the bacterial expression vector GST-Syk(6–370) [21]. A cDNA encoding residues 1–337 of human Zap-70 was cloned into pGEX-2T (Amersham Pharmacia Biotech).

Phospo-ITAMs consisted of the dually phosphorylated FcεRIγ chain ITAM, DGVY(PO₃)TGLSTRNQETY(PO₃)ETLKTCR, and the dually phosphorylated TCRζ chain ITAM, NQLY(PO₃)NELNLGRREEY(PO₃)DVLV [14].

Affinity chromatography/direct binding assays
All model protein mimics of integrin cytoplasmic domains were produced and characterized as previously reported [9, 10]. Ni⁺⁺ beads coated with integrin cytoplasmic tails (5 μl packed) were added to 0.5 mg clarified cell lysate (affinity chromatography) or GST fusion protein (5 nM) in a final volume of 0.5 ml binding buffer (see supplementary material for details). After incubation, beads were washed, and bound protein was analyzed via immunoblotting. For pITAM peptide competition assays, GST-Syk(6–370) was preincubated with 40 μM pITAMs, then direct binding assays were performed.

Elisa competition assay
GST-Syk(6–370) was immobilized onto Immobilon II Elisa plates (Corning) in 0.1 M NaHCO₃ (pH 8.0) for 2 hr at room temperature. The plates were then blocked with heat-inactivated BSA and washed. Phosphopeptide FcεRIγ ITAM (10 μM), followed by the soluble His-tagged integrin cytoplasmic-domain model protein (2.5 μM), were added. After a 1 hr incubation, bound integrin cytoplasmic-domain model proteins were detected with anti-His antibodies and secondary HRP-conjugated antibodies.

Phosphorylation and coprecipitation
Adhesion-dependent phosphorylation assays were carried out as performed previously [5]. For coprecipitation, cells (CHO cells or platelets) on BSA or fibrinogen (1 hr at 37°C) were lysed in 50 mM Tris (pH 7.4) containing 0.5% Nonidet P-40, 50 mM NaCl, and a protease inhibitor cocktail (Boehringer Mannheim, Germany). After clarification at 12,000 rpm for 20 min, 500–750 μg of lysate was incubated with primary antibody (either polyclonal antibody 8053 for β3 integrins or polyclonal antibody 0134 for Syk) overnight at 4°C. Protein A beads (25 μl packed, Amersham Pharmacia Biotech) were then added and rotated for 2 hr at 4°C.

Confocal microscopy
Lamellipodia quantification was carried out as previously described [6].

Supplementary material
For additional methodological details, see the supplementary material available with the electronic version of this article at http://images. cellpress.com/supmat/supmatin.htm.

References

1. Chu DH, Morita CT, Weiss A: **The Syk family of protein tyrosine kinases in T-cell activation and development.** *Immunol Rev* 1998, **165:**167-180.
2. Cheng AM, Rowley B, Pao W, Hayday A, Bolen JB, Pawson T: **Syk tyrosine kinase required for mouse viability and B-cell development.** *Nature* 1995, **378:**303-306.
3. Turner M, Mee PJ, Costello PS, Williams O, Price AA, Duddy LP, *et al.*: **Perinatal lethality and blocked B-cell development in mice lacking the tyrosine kinase Syk.** *Nature* 1995, **378:**298-302.
4. Clark EA, Shattil SJ, Ginsberg MH, Bolen J, Brugge JS: **Regulation of the protein tyrosine kinase pp72SYK by platelet agonists and the integrin αIIbβ3.** *J Biol Chem* 1994, **269:**28859-28864.
5. Gao J, Zoller KE, Ginsberg MH, Brugge JS, Shattil SJ: **Regulation of the pp72syk protein tyrosine kinase by platelet integrin alpha IIb beta 3.** *EMBO J* 1997, **16:**6414-6425.
6. Miranti CK, Leng L, Maschberger P, Brugge JS, Shattil SJ: **Identification of a novel integrin signaling pathway involving the kinase Syk and the guanine nucleotide exchange factor Vav1.** *Curr Biol* 1998, **8:**1289-1299.
7. Sarkar S, Rooney MM, Lord ST: **Activation of integrin-beta3-associated syk in platelets.** *Biochem J* 1999, **338:**677-680.
8. Saci A, Rendu F, Bachelot-Loza C: **Platelet alpha IIb-beta 3 integrin engagement induces the tyrosine phosphorylation of Cbl and its association with phosphoinositide 3-kinase and Syk.** *Biochem J* 2000, **351:**669-676.
9. Pfaff M, Liu S, Erle DJ, Ginsberg MH: **Integrin β cytoplasmic domains differentially bind to cytoskeletal proteins.** *J Biol Chem* 1998, **273:**6104-6109.
10. Ulmer TS, Yaspan B, Ginsberg MH, Campbell ID: **NMR analysis of structure and dynamics of the cytosolic tails of integrin alphaIIbbeta3 in aqueous solution.** *Biochemistry* 2001, **40:**7498-7508.
11. Ylanne J, Huuskonen J, O'Toole TE, Ginsberg MH, Virtanen I, Gahmberg CG: **Mutation of the cytoplasmic domain of the integrin β3 subunit.** *J Biol Chem* 1995, **270:**9550-9557.
12. Knezevic I, Leisner T, Lam S: **Direct binding of the platelet integrin αIIbβ3 (GPIIb-IIIa) to talin.** *J Biol Chem* 1996, **271:**16416-16421.
13. Ottinger EA, Botfield MC, Shoelson SE: **Tandem SH2 domains confer high specificity in tyrosine kinase signaling.** *J Biol Chem* 1998, **273:**729-735.
14. Shiue L, Zoller MJ, Brugge JS: **Syk is activated by phosphotyrosine-containing peptides representing the tyrosine-based activation motifs of the high affinity receptor for IgE.** *J Biol Chem* 1995, **270:**10498-10502.
15. Hruska KA, Rolnick F, Huskey M, Alvarez U, Cheresh D: **Engagement of the osteoclast integrin alpha v beta 3 by osteopontin stimulates phosphatidylinositol 3-hydroxyl kinase activity.** *Endocrinology* 1995, **136:**2984-2992.
16. El Hillal O, Kurosaki T, Yamamura H, Kinet JP, Scharenberg AM: **syk kinase activation by a src kinase-initiated activation loop phosphorylation chain reaction.** *Proc Natl Acad Sci USA* 1997, **94:**1919-1924.
17. Lin TH, Rosales C, Mondal K, Bolen JB, Haskill S, Juliano RL: **Integrin-mediated tyrosine phosphorylation and cytokine message induction in monocytic cells. A possible signaling role for the Syk tyrosine kinase.** *J Biol Chem* 1995, **270:**16189-16197.
18. Yan SR, Huang M, Berton G: **Signaling by adhesion in human neutrophils: activation of the p72syk tyrosine kinase and formation of protein complexes containing p72syk and Src family kinases in neutrophils spreading over fibrinogen.** *J Immunol* 1997, **158:**1902-1910.
19. Soede RD, Wijnands YM, Kouteren-Cobzaru I, Roos E: **ZAP-70 tyrosine kinase is required for LFA-1-dependent T cell migration.** *J Cell Biol* 1998, **142:**1371-1379.
20. Sada K, Takano T, Yanagi S, Yamamura H: **Structure and function of syk protein-tyrosine kinase.** *J Biochem* 2001, **130:**177-186.
21. Shiue L, Green J, Green OM, Karas JL, Morgenstern JP, Ram MK, *et al.*: **Interaction of p72syk with the gamma and beta subunits of the high-affinity receptor for immunoglobulin E, Fc epsilon RI.** *Mol Cell Biol* 1995, **15:**272-281.