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Functional coupling of the *src*-family protein tyrosine kinases p59^{fyn} and p53/56^{lyn} with the interleukin 2 receptor: Implications for redundancy and pleiotropism in cytokine signal transduction

(tyrosine phosphorylation)

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ABSTRACT **The binding of interleukin 2 (IL-2) to the IL-2 receptor (IL-2R) induces a rapid increase in tyrosine phosphorylation of cellular proteins. In a previous study, we have shown that p56^{lck} (lck), a *src*-family protein tyrosine kinase (*src*-PTK), physically and functionally associates with the IL-2R β chain (IL-2Rβ). To further investigate a role of *src*-PTKs in IL-2 signaling, we analyzed a mouse pro-B-cell line, in which *lck* is not expressed detectably. We observed that in this cell line, IL-2 induces activation of at least two *src*-PTKs, p59^{fyn} (*fyn*) and p53/56^{lyn} (*lyn*). Interestingly, stimulation of this cell line with IL-3 also induces activation of *src*-PTKs. The activation of *fyn* or *lyn* seems to be selective for stimulation with IL-2 or IL-3 since stimulation with IL-6 fails to activate them. Furthermore, we provide evidence for the physical association of *fyn* with IL-2Rβ. Taken together with previous results, our current study suggests that different *src*-PTKs, each of which is expressed in a cell-type-specific manner, can participate in the IL-2 signal transduction.**

Interleukin 2 (IL-2) exerts its effects through binding to the functional IL-2 receptor (IL-2R) (1, 2). At least three IL-2R components have been identified: the α chain (IL-2Rα) (3–5), the β chain (IL-2Rβ) (6), and the γ chain (IL-2Rγ) (7). Among them, IL-2Rβ plays a critical role in the intracellular signal transmission of IL-2 (8, 9); however, no kinase activity has been ascribed to IL-2Rβ (6, 10). On the other hand, one of the early biochemical events observed after T-cell stimulation by IL-2 is the increased tyrosine phosphorylation of cellular proteins (11, 12). In this regard, evidence for the physical association of protein tyrosine kinase (PTK) activity with IL-2R has been reported (13, 14), and we have shown that p56^{lck} (*lck*) physically and functionally associates with IL-2Rβ in a natural killer-like cell line. In addition, IL-2 stimulation of peripheral blood lymphocytes results in the activation of *lck in vitro* (15). A similar observation has also been reported in T-cell lines (16).

lck is a member of the *src*-family PTK (*src*-PTK) (17, 18), a family that consists of eight well-described membrane-bound PTKs and is expressed in T cells and natural killer cells predominantly (19, 20). However, IL-2 can also exert biological effects on cells in which *lck* is not expressed detectably. Indeed, B cells and monocytes proliferate and differentiate in response to IL-2 (2). A recent report showed that IL-2-induced cell proliferation is inhibited by specific inhibitors of PTK in an *lck*-negative cell line (21). These observations suggest that a PTK(s) other than *lck* could be involved in the IL-2 signaling pathway.

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Given that the catalytic domain of *lck* is primarily responsible for its interaction with IL-2Rβ (15) and that *src*-PTKs share the highly conserved sequence within this domain (20, 22), we considered the possibility that another *src*-PTK(s) could interact with IL-2Rβ. In this context, we examined a pro-B-cell line in which *lck* was not expressed detectably.

MATERIALS AND METHODS

Cell Culture and DNA Transfection Experiments. The cDNA transfections into COS cells were performed using the calcium phosphate method as described (15). The mouse IL-3-dependent pro-B-cell line BAF-B03 (8) and its derivatives were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and 20% WEHI-3B conditioned medium (as a source of IL-3). BAF-B03 was stably transfected with the expression vector for the human IL-2Rβ, pEF/β (23), along with a neomycin-resistant gene by the electroporation method. After DNA transfer, neomycin-resistant clones were selected as described (9). One of these clones (14B) expresses IL-2Rβ at least 10-fold higher than another BAF-B03-derived transfectant (F7), as judged by flow-cytometric analysis (T.M. and T.T., unpublished data) [F7 expresses about 9000 sites of the high-affinity IL-2R per cell, which is composed of endogenous IL-2Rα and cDNA-directed human IL-2Rβ (8)]. BAFh130 (24) was kindly provided by T. Taga and T. Kishimoto (Osaka University). To induce growth factor starvation, cells were washed three times with RPMI 1640 medium supplemented with 10% fetal calf serum and incubated in medium lacking other added growth factors for 12 hr prior to stimulation.

Plasmid Construction. The expression vectors for IL-2Rβ (pdKCRβ), for *lck* (pdKCR-lck), and for the chimeric molecule between *lck* and insulin receptor [pdKCR (*lck/insR*)] have been described (15). The plasmids, NT-18 (17), MM23.3 (25), and pmhck, contain the entire coding regions of mouse *lck*, *fyn*, and *hck*, respectively. To construct a vector expressing *lck/hck*, a *Stu* I–*Nco* I fragment from NT-18 and a *Nco* I–*Pvu* II fragment from pmhck were ligated into the *Sma* I-cleaved pdKCRS vector. To construct a vector expressing *lck/fyn*, MM23.3 was digested with *Pma*CI and *Stu* I. This fragment was subcloned into a pUC19 vector, which was cleaved with *Nco* I and *Sma* I, using a synthetic oligonucleotide linker of the sequence:

5′–CATGGGCTAAAGATGCTTGGGAAGTTGCAC–3′
5′–GTGCAACTTCCCAAGCATCTTTAGCC–3′

Abbreviations: IL, interleukin; IL-2R, IL-2 receptor; mAb, monoclonal antibody; PTK, protein tyrosine kinase.

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The resultant recombinant plasmid was digested with *Nco* I and *Hin*cII; the recovered fragment and the *Stu* I–*Nco* I fragment from NT-18 were ligated into the *Sma* I-cleaved pdKCRS vector.

Antibodies and Reagents. The rabbit anti-*lck* antiserum 195.7 has been described (26). Antibodies for each of other *src*-PTKs were kindly provided by the following: rabbit anti-*fyn* antiserum anti-FYN N (27), T. Kawakami (La Jolla Institute for Allergy and Immunology); mouse anti-*lyn* monoclonal antibody (mAb) Lyn 8 (28), Y. Yamanashi and T. Yamamoto (University of Tokyo); mouse anti-*yes* mAb 3H9, T. Akiyama and K. Toyoshima (Osaka University); mouse anti-*fgr* mAb FGA12, Takeda Chemical Industries (Osaka); mouse anti-v-*src* mAb 327, purchased from Oncogene Science (Uniondale, NY). Two mouse mAbs against the human IL-2Rβ, Mik-β1 (29) and Tu-27 (30), were kindly provided by M. Tsudo (Unichika Chuo Hospital, Kyoto) and K. Sugamura (Tohoku University, Sendai, Japan), respectively. The rat mAb M1/9 can recognize T-cell and B-cell forms of mouse CD45. Rabbit polyclonal anti-phosphotyrosine antibody (31) was a generous gift from L. E. Samelson (National Institutes of Health). Recombinant human IL-2, recombinant human IL-6, and the soluble IL-6 receptor (32) were kindly provided by Takeda Chemical Industries, Ajinomoto (Kawasaki, Japan), and Tosoh (Tokyo), respectively.

Immunoprecipitation and Immunoblotting. In transient cDNA expression studies in COS cells, experiments were performed as described (15). BAF-B03 and its derivatives were washed with phosphate-buffered saline and solubilized with lysis buffer [20 mM Tris·HCl (pH 8.0), 1% (vol/vol) Nonidet P-40, 150 mM NaCl, 50 mM NaF, 100 μM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, 10 μg of leupeptin per ml, 10 μg of aprotinin per ml, 1 μg of pepstatin A per ml] for 20 min at 4°C. The samples were immunoprecipitated with indicated antibodies, subjected to SDS/PAGE (10% gel), and electrophoretically transferred to poly(vinylidene difluoride) (PVDF) membrane filters (Immobilon, Millipore). After blocking with TBST milk [10 mM Tris·HCl (pH 8.0), 150 mM NaCl, 0.5% Tween 20, 5% non-fat dry milk], membranes were incubated with anti-FYN N or lyn 8 (1:1000 dilution in TBST milk) overnight at 4°C. Then filters were washed with TBST milk and incubated with alkaline phosphatase-conjugated goat anti-rabbit or anti-mouse immunoglobulin antibody (Tago) (1:1000 dilution in TBST milk) for 2 hr at room temperature. The filters were washed with TBST milk and TBST serially and developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

Factor-starved cells were stimulated with saturating amounts of cytokines, 5 nM human recombinant IL-2, or 20% WEHI-3B conditioned medium for 10 min at 37°C. Cells were centrifuged and solubilized with lysis buffer. After centrifugation, supernatants were mixed with the Laemmli buffer and boiled. Subsequently, samples were subjected to SDS/PAGE (10% gel). Separated proteins were electrophoretically transferred to PVDF membrane filters. The filters were then incubated with anti-phosphotyrosine antibody, washed, and subjected to autoradiography as described (33).

***In Vitro* Kinase Assay.** Cells were stimulated with IL-2 or IL-3 as described above. BAFh130 cells were treated with 30 mg of recombinant human IL-6 per ml in the presence of 10 mg of soluble IL-6 receptor per ml. Preparation of cell lysates and subsequent immunoprecipitation were performed as described above. The immune complexes were washed five times with the lysis buffer and washed once with kinase buffer [25 mM Hepes (pH 7.2), 0.1% (vol/vol) Nonidet P-40, 10 mM $MgCl_2$, 3 mM $MnCl_2$, 30 μM Na_3VO_4] and resuspended in 40 μl of kinase buffer containing 3.75 μM cold ATP and 5 μg of rabbit muscle enolase as an exogenous substrate. After the addition of 10 μCi of [γ-^{32}P]ATP (1 Ci = 37 GBq), the reaction mixture was incubated for 5 min at room temperature and the reaction was terminated by the addition of the Laemmli buffer (during this period of incubation, kinase reactions proceeded linearly; data not shown). Samples were then subjected to SDS/PAGE (10/20% gradient gel) under reducing conditions. The gels were treated with 1 M KOH for 1 hr at 55°C as described (34) and subjected to autoradiography.

RESULTS

IL-2Rβ Can Physically Associate with the Catalytic Domains of *src*-PTKs. To examine whether homologous catalytic domains of *src*-PTKs can mediate interaction with IL-2Rβ, we generated two cDNAs encoding chimeric kinases called *lck/hck* and *lck/fyn*, containing the modulatory domain of *lck* and the catalytic domain of either *hck* or *fyn* (Fig. 1*A*). These cDNAs were expressed in COS cells with or without IL-2Rβ cDNA. Levels of cell-surface expression of IL-2Rβ in each sample were comparable as assessed by flow-cytometric analysis (data not shown). Immunoblotting of the whole cell lysates with the anti-*lck* antiserum 195.7 revealed that the electrophoretic mobilities of the chimeric proteins were consistent with their predicted sizes [all chimeras contain the epitope (residues 5–148) recognized by 195.7 (26)]. We also occasionally observed bands of slower gel mobilities in addition to those predicted for the wild-type *lck* and chimeras (Fig. 1*B Upper*). The appearance of these slower-mobility bands may be due to variable phosphorylations of serine/threonine residues in the modulatory domain of *lck* (35, 36). From lysates of COS cells, IL-2Rβ was immunoprecipitated with Mik-β1 and immunoprecipitates were analyzed by immunoblotting with 195.7. As shown in Fig. 1*B*, the *lck/hck* and *lck/fyn* as well as the wild-type *lck* were found in Mik-β1 immunoprecipitates (Fig. 1*B Lower*, lanes 7, 9, and 3, respectively). Consistent with our previous observation (15), a chimera containing the catalytic domain of the insulin receptor (*lck/insR*) was not coprecipitated with IL-2Rβ (Fig. 1*B*, lane 5). These results suggest that the catalytic domain of *hck* or *fyn* is also able to mediate interaction with IL-2Rβ (see *Discussion*).

Increased Tyrosine Phosphorylation of Cellular Substrates Following IL-2 or IL-3 Stimulation of a Pro-B-Cell Line. A mouse IL-3-dependent pro-B-cell line, BAF-B03, expresses IL-2Rα and IL-2Rγ, but not IL-2Rβ, endogenously. When the human IL-2Rβ cDNA is expressed in BAF-B03, this cell acquires the ability to proliferate continuously in response to IL-2 (8). 14B is a derivative of BAF-B03, which expresses the human IL-2Rβ at a high level (see *Materials and Methods*). In immunoblotting with anti-phosphotyrosine antibody, IL-2 treatment of 14B induced an apparent increase in the tyrosine phosphorylation of proteins with apparent molecular masses of 85 kDa (pp85) and 52 kDa (pp52) predominantly (Fig. 2). Tyrosine phosphorylation of these proteins increased starting at 5 min after stimulation, reached a peak at 10–20 min, and declined thereafter (data not shown). Interestingly, IL-3 treatment also induced a pattern of tyrosine phosphorylation of cellular proteins similar to that following IL-2 treatment (Fig. 2).

Expression of *src*-PTKs in 14B. The above result suggests that IL-2 or IL-3 stimulation induces activation of PTKs in 14B. RNA blotting analysis of BAF-B03 revealed detectable amounts of *fyn, lyn, src*, and *yes* transcripts but not *lck* transcripts among eight *src*-PTKs assayed (data not shown). To verify the expression of *src*-PTKs in 14B, cell lysates were subjected to immunoprecipitation by a series of antibodies specific for each of the *src*-PTKs. Immunoprecipitated kinases were autophosphorylated by the *in vitro* kinase assay (Fig. 3). An anti-*lyn* antibody immunoprecipitate contained two phosphoproteins at 53 and 56 kDa, corresponding to two alternatively spliced forms of *lyn* (37), following the kinase reaction. Similarly, an anti-*fyn* antibody immunoprecipitate contained a 59-kDa phosphoprotein corresponding to the

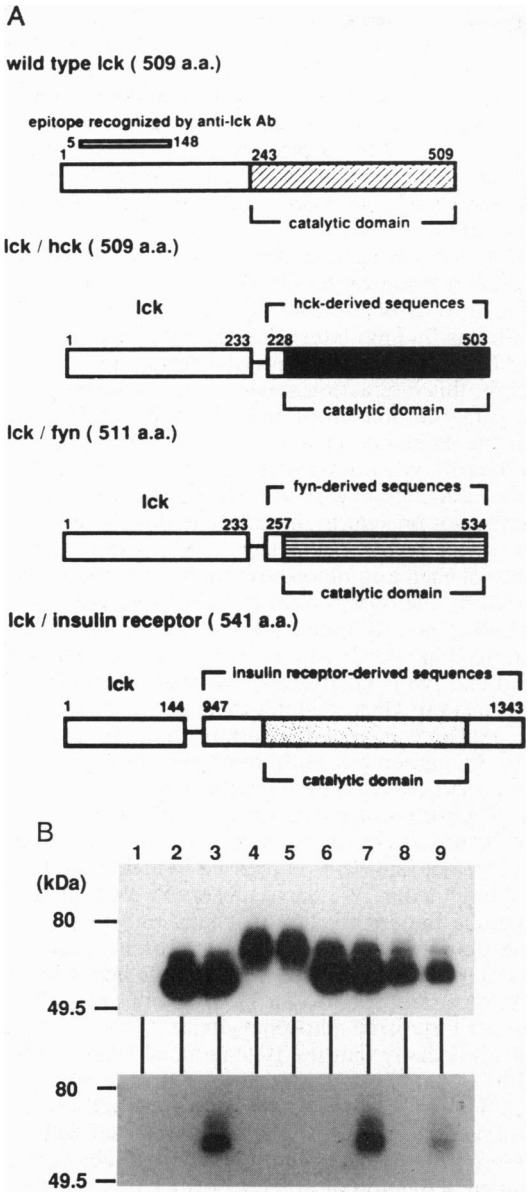

FIG. 1. Association of IL-2Rβ with chimeric *lck* molecules. (*A*) Schematic depiction of chimeric *lck* molecules. Chimeric molecules were constructed as described in the text. In brief, the modulatory domain of *lck* (residues 1–233 or 144) was fused to the catalytic domains of *hck* (residues 228–503), *fyn* (residues 257–534), or the insulin receptor (residues 947–1343), respectively. (*B*) Coprecipitation of the chimeric *lck* molecules with IL-2Rβ. COS cells were transfected with the following cDNAs: IL-2Rβ alone (lane 1), *lck* alone (lane 2), *lck* plus IL-2Rβ (lane 3), *lck/insR* alone (lane 4), *lck/insR* plus IL-2Rβ (lane 5), *lck/hck* alone (lane 6), *lck/hck* plus IL-2Rβ (lane 7), *lck/fyn* alone (lane 8), *lck/fyn* plus IL-2Rβ (lane 9). Associations between the chimeric *lck* molecules and IL-2Rβ were evaluated as described (15). (*Upper*) Anti-*lck* immunoblot of whole cell lysates. (*Lower*) Anti-*lck* immunoblot of Mik-β1 immunoprecipitates.

autophosphorylated form of *fyn*. In contrast, other *src*-PTKs could not be detected.

Activation of *src*-PTKs Following IL-2 or IL-3 Stimulation. We next examined the functional status of these *src*-PTKs following stimulation with IL-2 by the *in vitro* kinase assay using enolase as an exogenous substrate. As shown in Fig. 4*A*, IL-2 treatment induced a 3- to 4-fold increase in the total cellular kinase activities of *lyn* (*Left*) and *fyn* (*Right*), as judged by the extent of phosphorylation of enolase. IL-3 treatment also induced activation of both kinases in a manner

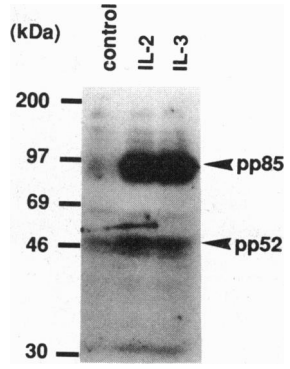

FIG. 2. Increased tyrosine phosphorylation of cellular substrates following IL-2 or IL-3 stimulation of 14B. After stimulation with either IL-2 or IL-3, whole cell lysates of 14B were analyzed by immunoblotting using anti-phosphotyrosine antibody. The arrowheads indicate two major tyrosine-phosphorylated cellular substrates with apparent molecular masses of 52 kDa (pp52) and 85 kDa (pp85).

similar to the IL-2 treatment. This seems to reflect an increase in the specific kinase activity since we observed no detectable change in the amount of *lyn* or *fyn* protein with or without stimulation (Fig. 4*B*). Activation of these kinases begins within 2–5 min after stimulation and peaks at 10 min (data not shown). In BAF-B03 stably transfected with the gp130 cDNA (BAFh130) (24), IL-6 has been shown to induce an increase in the tyrosine phosphorylation of gp130 (38). Therefore we examined whether IL-6 also induces activation of *src*-PTKs (Fig. 4*C*). We found that although IL-3 treatment resulted in the activation of both kinases, stimulation with IL-6 had no effect on the activity of either *lyn* or *fyn*. We confirmed that BAFh130 is capable of responding to IL-6 stimulation on the basis of the apparent increase of [^{3}H]thymidine incorporation (data not shown) as described (38). Therefore these results suggest that the activation of *src*-PTKs in response to IL-2 or IL-3 does not reflect some general event in this cell but rather is selective for activation by these cytokines.

A *src*-PTK, *fyn*, Physically Associates with IL-2Rβ in 14B. We next addressed the issue of whether *lyn* or *fyn* physically interacts with IL-2Rβ. To test this possibility, lysates of 14B were immunoprecipitated either with Mik-β1 or with M1/9 (mAb against CD45) as a control (surface expression of IL-2Rβ and endogenous CD45 were comparable as assessed

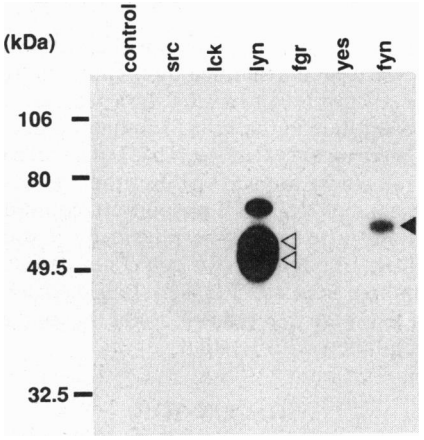

FIG. 3. Panel of *src*-PTKs expressed in 14B. Lysates of 14B were immunoprecipitated with indicated antibodies against each *src*-PTK or rabbit whole IgG (control) (Jackson ImmunoResearch). Individual immunoprecipitates were subjected to *in vitro* kinase assay. Open arrowheads indicate autophosphorylated $p53^{lyn}$ and $p56^{lyn}$; the solid arrowhead indicates autophosphorylated $p59^{fyn}$.

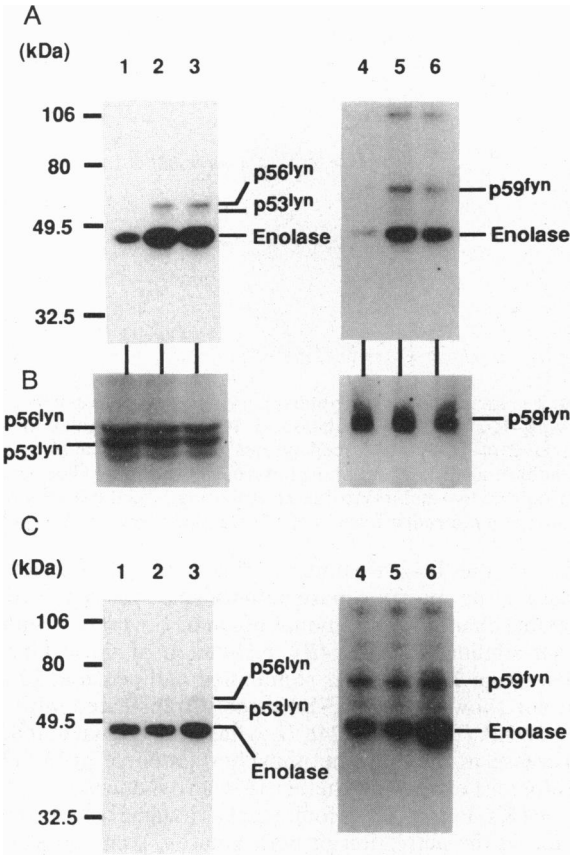

FIG. 4. Activation of *lyn* (*Left*) and *fyn* (*Right*) following stimulation with cytokines. (*A*) *In vitro* kinase assay in 14B. After stimulation with culture medium (control) (lanes 1 and 4), IL-2 (lanes 2 and 5), or IL-3 (lanes 3 and 6), lysates of 14B were immunoprecipitated with specific antibodies for *lyn* or *fyn*. *lyn* or *fyn* kinase was analyzed for *in vitro* kinase activities using enolase as an exogenous substrate. Positions of $p53^{lyn}$, $p56^{lyn}$, $p59^{fyn}$, and enolase are indicated. (*B*) Immunoblots of *lyn* and *fyn* proteins. Whole cell lysates of 14B (5×10^5 cells) were analyzed by immunoblotting using anti-*lyn* antibody (*Left*). The lysates of 14B (1×10^6 cells) were immunoprecipitated with anti-*fyn* antibody and immunoprecipitates were analyzed by immunoblotting using anti-*fyn* antibody (*Right*). Positions of $p53^{lyn}$, $p56^{lyn}$, and $p59^{fyn}$ are indicated. (*C*) *In vitro* kinase assay in BAFh130. BAFh130 was stimulated with culture medium (control) (lanes 1 and 4), IL-6 (lanes 2 and 5), or IL-3 (lanes 3 and 6). Subsequent procedures were performed as described for *A*.

by flow-cytometric analysis; data not shown). The immunoprecipitates were each examined by immunoblotting with an antibody specific for *fyn* or *lyn*. As shown in Fig. 5*A*, *fyn* was specifically identified in the Mik-β1 immunoprecipitate (lane 1). Another mAb against IL-2Rβ, Tu-27, provided essentially identical results (data not shown). For the coprecipitation of *fyn*, the presence of IL-2Rβ is absolutely required (Fig. 5*B*). These results indicate that *fyn* physically associates with IL-2Rβ in 14B. In contrast, we could not detect *lyn* in the Mik-β1 immunoprecipitate (Fig. 5*C*). Based on densitometric analysis, we estimate that $\approx$0.6–1.2% of the total cellular *fyn* were coprecipitated with IL-2Rβ.

DISCUSSION

In this study, we demonstrate that a *src*-PTK, *fyn*, physically and functionally associates with IL-2Rβ in an *lck*-negative pro-B-cell line. Taken together with our previous results, it is suggested that several *src*-PTKs can physically and functionally associate with IL-2Rβ. This notion is intriguing since *lck*-negative hematopoietic cells are also known to proliferate or differentiate in response to IL-2 (2). Although it remains unclear how one receptor molecule can associate with different *src*-PTKs, it is possible that a structural similarity conserved within *src*-PTKs is involved in these interactions.

As we reported previously, the N-terminal half of the catalytic domain of *lck* is primarily responsible for its interaction with IL-2Rβ (15). This domain represents the region of highest sequence homology within *src*-PTKs (20, 22). The ability of chimeras, *lck/hck* and *lck/fyn*, to associate with IL-2Rβ appears to reflect their structural similarities. We have found previously that the modulatory domain of *lck* by itself failed to associate with IL-2Rβ. Furthermore, consistent with this finding, internal deletions introduced into this domain did not abrogate the association with IL-2Rβ (15). Therefore, this interaction seems to be mediated principally by the catalytic domain of *hck*, *fyn*, or *lck*. These results suggest the existence of a common structural determinant within the catalytic domains of *src*-PTKs essential for mediating the association with IL-2Rβ. Such a determinant is apparently not present in the catalytic domain of the insulin receptor, which is phylogenically distant from *src*-PTKs. The existence of such a common structural determinant is further supported by the observation that *fyn* physically associates with IL-2Rβ in 14B. Such an association may not be restricted to this pro-B-cell line. In fact, it was recently reported that *fyn* is functionally coupled to IL-2 signaling in a T-cell line (39). Thus, we infer that such functional coupling is also mediated by physical association between *fyn* and IL-2Rβ. In agreement with our results, it was recently reported that activation of *lyn* is induced by IL-2 in F7 (40), another BAF-B03 stably transfected with the IL-2Rβ cDNA (8). We did not detect any coprecipitation of *lyn* with IL-2Rβ, despite the fact that *lyn* is expressed in higher amounts than *fyn* in these cells. We have observed that in BAF-B03 expressing a mutant IL-2Rβ that fails to interact with *lck*, tyrosine phosphorylation of cellular proteins is induced by IL-3 but not by IL-2 (45). This suggests the importance of the physical association between IL-2Rβ and *src*-PTKs in the induction of tyrosine phosphorylation. From our present findings, it is likely that the IL-2-induced PTK activation in BAF-B03 is initially triggered by *fyn*, which interacts with IL-2Rβ. This raises the question as to the activation mechanism of *lyn*, which apparently fails to interact with IL-2Rβ. It is possible that the initial *fyn* activation induces the subsequent activation of other *src*-PTKs that are not physically associated with IL-2Rβ—i.e., activation of *fyn* may be a prerequisite for the activation of *lyn*. Obviously, this issue requires further clarification.

In addition to IL-3, IL-2 (8) or IL-6 (24, 38) also induces proliferative responses in BAF-B03. Accumulating evidence suggests that an increase in tyrosine phosphorylation of cellular proteins is one of the early biochemical events following IL-3 (41) or IL-6 (38) stimulation. Consistent with our current finding, a previous report showed that IL-2 and IL-3 induce similar patterns of tyrosine phosphorylation of cellular proteins in a hematopoietic cell line (42). Furthermore, our results indicate that IL-2 or IL-3, but not IL-6, induces an increase in the *in vitro* kinase activity of *lyn* and *fyn* in BAF-B03. These findings suggest that the signaling pathway of IL-2 or IL-3 is different from that of IL-6, at least in terms of the functional coupling to *fyn* or *lyn*. Recent studies indicate that gp130 is involved in signal transmission of IL-6, leukemia inhibitory factor, and oncostatin M in a redundant manner (43), and, in view of our present findings, it is possible that the signaling pathway(s) for these cytokines is mediated by PTKs other than *src*-PTKs. Interestingly, stimulation with IL-6 provokes ligand-induced DNA synthesis in BAF-B03 but does not support its long-term growth (38). However, it is not clear at present whether this difference is due to utilization of PTKs other than *src*-PTKs.

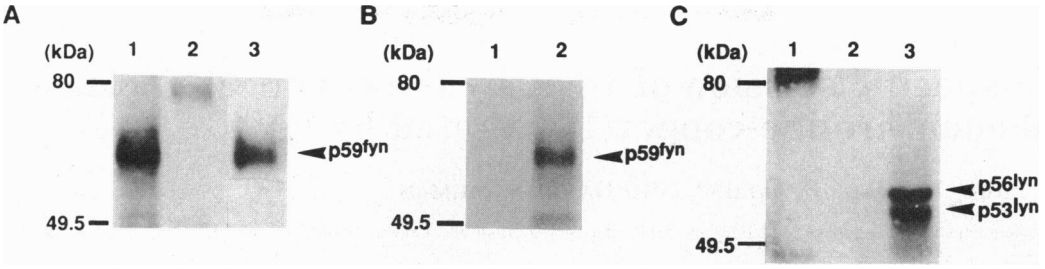

FIG. 5. Coprecipitation of *fyn* with IL-2Rβ in 14B. (*A*) The lysates of 14B (6×10^7 cells) were immunoprecipitated with Mik-β1 (mAb against IL-2Rβ) (lane 1) or M1/9 (mAb against CD45) (lane 2). The lysate of 14B (1×10^6 cells) was immunoprecipitated with anti-*fyn* antibody (lane 3). Immunoprecipitates were subsequently analyzed by immunoblotting using anti-*fyn* antibody. An arrowhead indicates the position of $p59^{fyn}$. (*B*) The lysates of BAF-B03 (lane 1) or 14B (lane 2) (5×10^7 cells, respectively) were immunoprecipitated with Mik-β1. The immunoprecipitates were analyzed by immunoblotting using anti-*fyn* antibody. An arrowhead indicates the coprecipitated $p59^{fyn}$. (*C*) The lysates of 14B (6×10^7 cells) were immunoprecipitated with Mik-β1 (lane 1) or M1/9 (lane 2). Immunoprecipitates were subsequently analyzed by immunoblotting using anti-*lyn* antibody. As a positive control, the whole cell lysate of 14B (5×10^5 cells) was examined (lane 3). Arrowheads indicate positions of $p53^{lyn}$ and $p56^{lyn}$.

In a recent study, it was demonstrated that stimulation of BAF-B03 by IL-2 or IL-3 induces the transcription of a common set of nuclear protooncogenes and that induction of c-*fos* or c-*jun* is apparently mediated by the PTK pathway (44). Our present results suggest that it is most likely that *fyn*, *lyn*, or both, is responsible for the induction of these genes in BAF-B03. The demonstration that signaling by IL-2 or IL-3 induces similar nuclear protooncogenes and involves similar *src*-PTKs implies that they share a common or similar mechanism of signal transmission in this pro-B-cell line.

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1. Taniguchi, T., Matsui, H., Fujita, T., Hatakeyama, M., Kashima, N., Fuse, A., Hamuro, J., Nishi-Takaoka, C. & Yamada, G. (1986) *Immunol. Rev.* **92**, 121–133.
2. Waldmann, T. A. (1989) *Annu. Rev. Biochem.* **58**, 875–911.
3. Leonard, W. J., Depper, J. M., Crabtree, G. R., Rudikoff, S., Pumphrey, J., Robb, R. J., Kronke, M., Svetlik, P. B., Peffer, N. J., Waldmann, T. A. & Greene, W. C. (1984) *Nature (London)* **311**, 626–631.
4. Nikaido, T., Shimizu, A., Ishida, N., Sabe, H., Teshigawara, K., Maeda, M., Uchiyama, T., Yodoi, J. & Honjo, T. (1984) *Nature (London)* **311**, 631–635.
5. Cosman, D., Cerretti, D. P., Larsen, A., Park, L., March, C., Dower, S., Gillis, S. & Urdal, D. (1984) *Nature (London)* **312**, 768–771.
6. Hatakeyama, M., Tsudo, M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyasaka, M. & Taniguchi, T. (1989) *Science* **244**, 551–556.
7. Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M. & Sugamura, K. (1992) *Science* **257**, 379–382.
8. Hatakeyama, M., Mori, H., Doi, T. & Taniguchi, T. (1989) *Cell* **59**, 837–845.
9. Doi, T., Hatakeyama, M., Minamoto, S., Kono, T., Mori, H. & Taniguchi, T. (1989) *Eur. J. Immunol.* **19**, 2375–2378.
10. Kono, T., Doi, T., Yamada, G., Hatakeyama, M., Minamoto, S., Tsudo, M., Miyasaka, M., Miyata, T. & Taniguchi, T. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1806–1810.
11. Saltzman, E. M., Thom, R. R. & Casnellie, J. E. (1988) *J. Biol. Chem.* **263**, 6956–6959.
12. Ferris, D. K., Willette-Brown, J., Ortaldo, J. R. & Farrar, W. L. (1989) *J. Immunol.* **143**, 870–876.
13. Merida, I. & Gaulton, G. N. (1990) *J. Biol. Chem.* **265**, 5690–5694.
14. Fung, M. R., Scearce, R. M., Hoffman, J. A., Peffer, N. J., Hammes, S. R., Hosking, J. B., Schmandt, R., Kuziel, W. A., Haynes, B. F., Mills, G. B. & Greene, W. C. (1991) *J. Immunol.* **147**, 1253–1260.
15. Hatakeyama, M., Kono, T., Kobayashi, N., Kawahara, A., Levin, S. D., Perlmutter, R. M. & Taniguchi, T. (1991) *Science* **252**, 1523–1528.
16. Horak, I. D., Gress, R. E., Lucas, P. J., Horak, E. M., Waldmann, T. A. & Bolen, J. B. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1996–2000.
17. Marth, J. D., Peet, R., Krebs, E. G. & Perlmutter, R. M. (1985) *Cell* **43**, 393–404.
18. Voronova, A. F. & Sefton, B. M. (1986) *Nature (London)* **319**, 682–685.
19. Perlmutter, R. M., Marth, J. D., Ziegler, S. F., Garvin, A. M., Pawar, S., Cooke, M. P. & Abraham, K. M. (1988) *Biochim. Biophys. Acta* **948**, 245–262.
20. Dymecki, S. M., Niederhuber, J. E. & Desiderio, S. V. (1990) *Science* **247**, 332–336.
21. Otani, H., Siegel, J. P., Erdos, M., Gnarra, J. R., Toledano, M. B., Sharon, M., Mostowski, H., Feinberg, M. B., Pierce, J. H. & Leonard, W. J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2789–2793.
22. Hanks, S. K., Quinn, A. M. & Hunter, T. (1988) *Science* **241**, 42–52.
23. Okamoto, Y., Minamoto, S., Shimizu, K., Mogami, H. & Taniguchi, T. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6584–6588.
24. Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T. & Kishimoto, T. (1990) *Cell* **63**, 1149–1157.
25. Cooke, M. P. & Perlmutter, R. M. (1989) *New Biol.* **1**, 66–74.
26. Louie, R. R., King, C. S., MacAuley, A., Marth, J. D., Perlmutter, R. M., Eckhart, W. & Cooper, J. A. (1988) *J. Virol.* **62**, 4673–4679.
27. Kawakami, T., Kawakami, Y., Aaronson, S. A. & Robbins, K. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3870–3874.
28. Yamanashi, Y., Fukui, Y., Wongsasant, B., Kinoshita, Y., Ichimori, Y., Toyoshima, K. & Yamamoto, T. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1118–1122.
29. Tsudo, M., Kitamura, F. & Miyasaka, M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1982–1986.
30. Takeshita, T., Goto, Y., Tada, K., Nagata, K., Asao, H. & Sugamura, K. (1989) *J. Exp. Med.* **169**, 1323–1332.
31. Wang, J. Y. J. (1985) *Mol. Cell. Biol.* **5**, 3640–3643.
32. Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirano, T. & Kishimoto, T. (1989) *Cell* **58**, 573–581.
33. Hsi, E. D., Siegel, J. N., Minami, Y., Luong, E. T., Klausner, R. D. & Samelson, L. E. (1989) *J. Biol. Chem.* **264**, 10836–10842.
34. Cooper, J. A., Sefton, B. M. & Hunter, T. (1983) *Methods Enzymol.* **99**, 387–402.
35. Veillette, A., Horak, I. D., Horak, E. M., Bookman, M. A. & Bolen, J. B. (1988) *Mol. Cell. Biol.* **8**, 4353–4361.
36. Veillette, A., Horak, I. D. & Bolen, J. B. (1988) *Oncogene Res.* **2**, 385–401.
37. Yi, T., Bolen, J. B. & Ihle, J. N. (1991) *Mol. Cell. Biol.* **11**, 2391–2398.
38. Murakami, M., Narazaki, M., Hibi, M., Yawata, H., Yasukawa, K., Hamaguchi, M., Taga, T. & Kishimoto, T. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11349–11353.
39. Augustine, J. A., Sutor, S. L. & Abraham, R. T. (1991) *Mol. Cell. Biol.* **11**, 4431–4440.
40. Torigoe, T., Saragovi, H. U. & Reed, J. C. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2674–2678.
41. Koyasu, S., Tojo, A., Miyajima, A., Akiyama, T., Kasuga, M., Urabe, A., Schreurs, J., Arai, K., Takaku, F. & Yahara, I. (1987) *EMBO J.* **6**, 3979–3984.
42. Sabe, H., Kuno, J., Koromilas, A., Saito, Y., Kinashi, T., Ueda, M., Takamatsu, T., Hamaguchi, M., Kawakami, T. & Honjo, T. (1991) *Int. Immunol.* **3**, 1137–1148.
43. Gearing, D. P., Comeau, M. R., Friend, D. J., Gimpel, S. D., Thut, C. J., McGourty, J., Brasher, K. K., King, J. A., Gillis, S., Mosley, B., Ziegler, S. F. & Cosman, D. (1992) *Science* **255**, 1434–1437.
44. Shibuya, H., Yoneyama, M., Ninomiya-Tsuji, J., Matsumoto, K. & Taniguchi, T. (1992) *Cell* **70**, 57–67.
45. Minami, Y., Kono, T., Yamada, K., Kobayashi, N., Kawahara, A., Perlmutter, R. M. & Taniguchi, T. (1993) *EMBO J.* **12**, 759–768.