Lyn Associates with the Juxtamembrane Region of c-Kit and Is Activated by Stem Cell Factor in Hematopoietic Cell Lines and Normal Progenitor Cells

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Stem cell factor (SCF) is a cytokine critical for normal hematopoiesis. The receptor for SCF is c-Kit, a receptor tyrosine kinase. Our laboratory is interested in delineating critical components of the SCF signal transduction pathway in hematopoietic tissue. The present study examines activation of Src family members in response to SCF. Stimulation of cell lines as well as normal progenitor cells with SCF rapidly increased tyrosine phosphorylation of the Src family member Lyn. Peak responses were noted 10–20 min after SCF treatment, and phosphorylation of Lyn returned to basal levels 60–90 min after stimulation. SCF also induced increases in Lyn kinase activity in vitro. Lyn coimmunoprecipitated with c-Kit, and studies with GST fusion proteins demonstrated that Lyn readily associated with the juxtamembrane region of c-Kit. Treatment of cells with either Lyn antisense oligonucleotides or PP1, a Src family inhibitor, resulted in dramatic inhibition of SCF-induced proliferation. These data demonstrate that SCF rapidly activates Lyn and suggest that Lyn is critical in SCF-induced proliferation in hematopoietic cells.

Two superfamilies of receptors that interact with hematopoietic growth factors are receptor tyrosine kinases (RTKs) and hematopoietin receptors (1, 2). The hematopoietin receptor superfamily is characterized by an absence of intrinsic tyrosine kinase activity and includes the interleukin-2, interleukin-3, erythropoietin, and granulocyte-macrophage colony-stimulating factor (GM-CSF) receptors, as well as others (1). Recent work has suggested that multiple signal transduction pathways are stimulated by hematopoietin receptor superfamily members, including the Janus kinase-signal transducers and activators of transcription pathway and the Ras-Raf-mitogen-activated protein kinase pathway (3, 4). In addition, activation of Src family members by ligands interacting with hematopoietin receptor superfamily members has been reported (5–12). Src family members are also stimulated by ligands interacting with RTKs (13–18). In fact, recent work by Courtneidge and coworkers (19, 20) has suggested that Src family members are critical in DNA synthesis induced by platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and colony-stimulating factor (CSF-1) in transfected fibroblasts (19, 20).

Our laboratory is interested in delineating signal transduction components that mediate the biological effects of SCF in hematopoietic cells. SCF interacts with the RTK c-Kit and is critical in the development of hematopoietic progenitor and stem cells (21). Interestingly, little is known about the role of Src family members in the signal transduction pathway of SCF. Further, the majority of work examining the role of Src family members in RTK signal transduction has been performed in fibroblasts transfected with the RTK of interest (13–15, 17–20). In this study, we have found that Lyn is the dominant Src family member expressed in SCF-responsive cell lines as well as in normal hematopoietic progenitor cells. Further, we have demonstrated that Lyn associates with the juxtamembrane region of c-Kit (amino acids 544–577, a region just distal of the transmembrane domain) and that SCF induces rapid increases in both tyrosine phosphorylation of Lyn as well as its kinase activity in vitro. Treatment of cells with antisense oligonucleotides specific for Lyn, or a Src family inhibitor, resulted in dramatic inhibition of SCF-induced proliferation. In total, these studies demonstrate that Lyn is activated by SCF and suggest that it plays a critical role in SCF-induced proliferation.

MATERIALS AND METHODS

Cells, Growth Factors, and Antibodies—Mo7e and MB-02 cells (kindly provided by Doris Morgan, Hahnemann University, Philadelphia, PA) were maintained in RPMI 1640, 10% fetal calf serum (Atlanta Biologics, Norcross, GA), 2 mM l-glutamine, and 1% penicillin-streptomycin (cell culture medium) supplemented with recombinant human GM-CSF (10 ng/ml) and human SCF (20 ng/ml). Human fetal liver was obtained from 13–18-week fetuses after induced abortions (Advanced Bioscience, Palo Alto, CA). All samples were obtained through protocols consistent with IRB guidelines and with maternal consent. Single cell suspensions were made from the tissue and mononuclear cells isolated using lymphocyte separation medium. Human GM-CSF and SCF were purchased from PeproTech (Rocky Hill, NJ). PP1 was purchased from Calbiochem (La Jolla, CA). Polyclonal antibodies used to immunoprecipitate Yes, Fgr, Hck, Blk, Fyn, and Lyn were kindly provided by Dr. Joe Bolen (DNAX, Palo Alto, CA) and have been described previously (22). Monoclonal antibody used to immunoblot Lyn was purchased from Transduction Laboratories (Lexington, KY). Anti-phosphotyrosine immunoblotting was performed with 4G10 (Upstate Biotechnology, Inc., Lake Placid, NY). Immunoblotting of c-Kit was performed with anti-serum obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Detection of Lyn Tyrosine Phosphorylation—Cells were washed twice in RPMI 1640, resuspended at 2 × 10^7 cells/ml in RPMI 1640 with 1% fetal calf serum, stimulated with SCF (100 ng/ml) for the time indicated.
in the text, rapidly pelleted (3000 rpm, 30 s at 4 °C), and lysed in 1 ml of lysis buffer (1% Triton X-100, 50 mM sodium chloride, 10 mM Tris (pH 7.5), 5 mM EDTA, 30 mM sodium pyrophosphate, 5 mM sodium fluoride, 25 mM β-glycerol phosphate, 5 mM sodium orthovanadate, 0.1% p-nitrophenyl phosphate, and 1 mM phenylmethylsulfonyl fluoride). Clarified cell lysates were immunoprecipitated 2 h at 4 °C with polyclonal Lyn antisera and the antigen-antibody complexes captured with protein A-Sepharose. Immunoprecipitates were washed six times with lysis buffer and eluted from the protein A-Sepharose with SDS sample buffer. Samples were resolved with SDS-PAGE, transferred to Immobilon, and immunoblotted with antibody specific for phosphotyrosine (P-Tyr). Phosphotyrosyl proteins were visualized with enhanced chemiluminescence. Blots were then stripped and reprobed with antibody specific for Lyn (lower portion of B and C).

**RESULTS**

To determine whether SCF activated Src family members in hematopoietic cells, we first examined expression of Src family members in the SCF-responsive cell line Mo7e (Fig. 1A). These cells expressed low levels of Fgr, as well as strikingly high levels of both p56<sup>lyn</sup> and p59<sup> lyn</sup>. Lyn was also the dominant Src family member expressed in progenitor cells isolated from human fetal liver and normal human bone marrow (data not shown). The high expression of Lyn in SCF-responsive tissues led us to examine whether SCF induced phosphorylation of Lyn. Addition of SCF to Mo7e cells resulted in a rapid and protracted increase in tyrosine phosphorylation of Lyn (Fig. 1B, upper panel). Similar results were obtained with MB-02 cells (Fig. 1C, upper panel), an erythroidic, SCF-responsive human cell line, as well as with normal progenitor cells isolated from human fetal liver (Fig. 1D). In each of these experiments, as described in the Epicurian Coli TKX instruction manual (Stratagene, La Jolla, CA).

Coprecipitation assays were performed using equivalent amounts of bound fusion proteins incubated with clarified cell lysates for 3 h at 4 °C. Resin was washed three times with lysis buffer. Proteins coprecipitating with the fusion proteins bound to the glutathione-Sepharose were eluted from the resin by boiling in SDS sample buffer. Proteins were resolved by SDS-PAGE, transferred to Immobilon, and immunoblotted for Lyn.

Antisense Experiments and Proliferation Assays—Mo7e cells were washed twice and resuspended at 10<sup>5</sup>/ml in cell culture medium. Cells were aliquoted into 96-well plates and incubated overnight (37 °C, 5% CO<sub>2</sub>) with or without oligonucleotides at the indicated concentrations. Cells were treated with oligonucleotides for 3 h, and then medium or human SCF (100 ng/ml) was added to triplicate wells. The microtiter plates were incubated 72 h at 37 °C, 5% CO<sub>2</sub>. Each well was pulsed with 1 μCi of [3H]thymidine (6.7 Ci/mM, NEN Life Science Products) for 6 h and then harvested (Skatron semiautomated cell harvester) onto glass fiber filter paper (Filtermat, Skatron, Inc., Sterling, VA). Filter strips were dried and counted in a liquid scintillation counter (model 1216, Pharmacia Biotech Inc.). The sequences for the sense and antisense oligonucleotides for human Lyn have been published previously (26). Unmodified oligomers were synthesized with an automated synthesizer as described previously (27). Proliferation assays with fetal liver cells were performed as described above, except assays were performed in the same buffer (25 mM HEPES, 10 mM MnCl<sub>2</sub>, pH 7.5) containing 100 μCi/ml [3H]thymidine after 72 h of incubation and harvested 12 h later. Proliferation assays with PP1 were performed as described above except that Mo7e cells were treated with either PP1 or medium immediately prior to the addition of human SCF (100 ng/ml) or phosphor myristate acetate (10 ng/ml). All samples were done in triplicate.

**SCF-induced Activation of Lyn in Hematopoietic Cells**

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**Fig. 1. SCF induces tyrosine phosphorylation of Lyn.** A, Lyn is the dominant Src family member expressed in Mo7e cells. Mo7e cells were lysed and immunoprecipitations performed with antisera specific for the Src family members indicated. Immune complex assays were performed as described under “Materials and Methods.” Radiolabeled proteins were visualized with autoradiography. B–D, SCF induces tyrosine phosphorylation of Lyn in SCF-responsive cell lines and in normal progenitor cells isolated from fetal liver. Cells were stimulated the indicated times with SCF, lysed, and immunoprecipitated (IP) with antisera specific for Lyn or control serum (Ct). Samples were resolved using SDS-PAGE, transferred to Immobilon, and immunoblotted (IB) with antibody specific for phosphotyrosine (P-Tyr). Phosphotyrosyl proteins were visualized with enhanced chemiluminescence. Blots were then stripped and reprobed with antibody specific for Lyn (lower portion of B and C).
immunoblotting with antibody specific for Lyn indicated that increases in the levels of Lyn did not account for the increase in tyrosine phosphorylation (Fig. 1, B and C, lower panels). These data demonstrate that SCF induces tyrosine phosphorylation of Lyn in two SCF-responsive cell lines as well as in normal progenitor cells. Interestingly, though p56\textsuperscript{C}lat was the dominant phosphoprotein in each cell line or tissue examined, SCF-induced phosphorylation of p53\textsuperscript{C}lat could also be observed when the experiments shown in Fig. 1 (B–D) were exposed for a longer period of time (data not shown). Thus, SCF induced tyrosine phosphorylation of both Lyn isoforms within minutes of addition to cells.

To determine if SCF activated Lyn kinase activity, we examined the effects of SCF on Lyn autophosphorylation and substrate phosphorylation in \textit{in vitro} immune complex assays. As demonstrated in Fig. 2A, treatment of Mo\textit{7e} cells with SCF induced increases in Lyn autophosphorylation. Although p56\textsuperscript{C}lat was more heavily phosphorylated than p53\textsuperscript{C}lat, darker exposure of Fig. 2A demonstrated that autophosphorylation of p53\textsuperscript{C}lat also increased after SCF treatment (data not shown). In addition to autophosphorylated p56\textsuperscript{C}lat, a 120-kDa phosphoprotein (p120) was detected in immune complex assays performed with Lyn immunoprecipitates but not in control immunoprecipitates. Our preliminary studies suggest that p120 can be reprecipitated with c-cbl antisera and are consistent with a large body of work suggesting that the c-cbl proto-oncogene product associates with Lyn in several hematopoietic lineages (28–30).

We next examined whether SCF induced increases in the capacity of Lyn to phosphorylate a tyrosine kinase substrate \textit{in vitro}. Fig. 2B demonstrates that phosphorylation of enolase was dramatically increased after stimulation of cells with SCF. These data demonstrate that stimulation with SCF induces increases in both Lyn autophosphorylation as well as increases in Lyn kinase activity \textit{in vitro}.

A second protein coimmunoprecipitating with Lyn was a 145-kDa phosphotyrosyl protein readily detectable in antiphosphotyrosine immunoblots of Lyn immunoprecipitates (Fig. 3A). A 145-kDa protein was also observed in Lyn immunoprecipitates from cells metabolically labeled with \[^{35}\text{S}\]methionine (Fig. 3B). Notably, these studies demonstrated that some p145 coimmunoprecipitated with Lyn in resting cells and that the amount of p145 in the immunoprecipitates increased after stimulation with SCF (Fig. 3B). The similarity in the molecular weight of p145 and c-Kit, the SCF receptor, led us to examine whether this protein was c-Kit. Indeed, Fig. 3C demonstrates that c-Kit coimmunoprecipitated with Lyn. We did not assess whether Lyn could be coimmunoprecipitated with antisera specific for c-Kit because both the Mo\textit{7e} and MB-02 cell lines expressed extremely high levels c-Kit as compared with Lyn.

To further examine the interaction of Lyn and c-Kit, we sought to define the region of c-Kit mediating the association. GST fusion proteins encoding specific regions of the intracellular domain of c-Kit were prepared and tested for the capacity to coprecipitate Lyn. We first assessed whether Lyn could be coprecipitated with a GST fusion protein encoding the juxtamembrane region of c-Kit. Competition studies with phosphopeptides have suggested that the juxtamembrane regions of the PDGF and CSF-1 RTKs are involved in interaction with Src family members (16, 31). Because Src family members associate with the PDGF and CSF-1 receptors through SH2 domains, we reasoned that Lyn association with c-Kit was likely to be phosphotyrosine-dependent (14, 15). To generate phosphorylated fusion protein, we expressed the GST-Kit juxtamembrane

![Fig. 2. SCF activates Lyn kinase activity \textit{in vitro}. A. Lyn autophosphorylation is increased after treatment of cells with SCF. Mo\textit{7e} cells were stimulated the indicated times with SCF, lysed, and immunoprecipitated (IP) with antisera specific for Lyn or control serum (Cnt). Immune complex assays were performed, and samples resolved with SDS-PAGE and visualized with autoradiography. B. SCF induces increases in the capacity of Lyn to phosphorylate enolase. Mo\textit{7e} cells were stimulated 10 min with SCF, lysed, and immunoprecipitated with antiserum specific for Lyn or control serum (Cnt). Immune complex assays were performed in the presence of the tyrosine kinase substrate enolase. Samples were resolved with SDS-PAGE and visualized with autoradiography.](image)

![Fig. 3. c-Kit coimmunoprecipitates with Lyn. A. coimmunoprecipitation of a 145-kDa phosphotyrosyl protein with Lyn after stimulation of cells with SCF. Mo\textit{7e} cells were treated for the indicated times with SCF, lysed, and immunoprecipitated (IP) with antisera specific for Lyn or control serum (Cnt). Samples were resolved using SDS-PAGE, transferred to Immobilon, and immunoblotted (IB) with antibody specific for phosphotyrosine (P-Tyr). B. Lyn coimmunoprecipitates with p145. Mo\textit{7e} cells were radiolabeled with \[^{35}\text{S}\]methionine, stimulated for the indicated time periods with SCF, lysed, and immunoprecipitated (IP) with antisera specific for Lyn or control serum (Cnt). Samples were resolved using SDS-PAGE, transferred to Immobilon, and immunoblotted (IB) with antisera specific for Lyn.](image)
fusion protein in bacteria that could be induced to express the Elk tyrosine kinase (TKX1). Anti-phosphotyrosine immunoblotting confirmed that the fusion protein produced by the TKX1 bacteria contained significant levels of phosphotyrosine as compared with fusion protein expressed in DH5α bacteria (data not shown). We next examined the ability of phosphorylated and unphosphorylated GST-Kit juxtamembrane fusion protein (isolated from TKX1 or DH5α bacteria, respectively) to coprecipitate Lyn from lysates of Mo7e cells. Fig. 4A demonstrates that Lyn coprecipitated with the tyrosine-phosphorylated GST-Kit juxtamembrane fusion protein (purified from TKX1 bacteria) but not with unphosphorylated fusion protein (purified from DH5α bacteria). In addition, and somewhat surprisingly, tyrosine-phosphorylated GST-Kit juxtamembrane fusion protein coprecipitated Lyn from the lysates of both unstimulated and SCF-stimulated Mo7e cells. These data suggest that activation of Lyn is not necessary for association with phosphorylated c-Kit in vitro. Fig. 4A also demonstrates that the GST control protein did not coprecipitate Lyn. We next examined whether Lyn was capable of associating with other regions in the c-Kit intracellular domain. Fig. 4B demonstrates that Lyn did not associate with GST fusion proteins encoding a significant portion of either catalytic domain (K1 and Y823), the c-Kit kinase insert (KINS), or the carboxyl tail (C-tail) region.

To address the physiological importance of Lyn in the SCF signal transduction pathway, we performed antisense studies with oligonucleotides directed toward the initiation site of Lyn. Mo7e cells were treated overnight with antisense or sense oligonucleotides, treated with additional oligonucleotides the next morning, and then incubated an additional 3 h. Proliferation assays were then performed with either medium or SCF. Shown in Fig. 5A are the effects of sense versus antisense oligonucleotides on SCF-induced proliferation of Mo7e cells. Cells treated with 0.75 μM or more of antisense oligonucleotides demonstrated a 90% inhibition of SCF-induced proliferation as compared with corresponding concentrations of sense oligonucleotides. Further, control experiments with nonsense oligonucleotides demonstrated that the inhibition of SCF-induced proliferation was specific for the antisense sequence (Fig. 5B). The effect of antisense oligonucleotides on expression of both p56Lyn and p53Lyn is demonstrated in Fig. 5C. Although low levels of Lyn protein could still be detected in cells treated with antisense oligonucleotides, expression was decreased significantly as compared with cells treated with sense oligonucleotides in multiple experiments. These data suggest that the decrease in SCF-induced proliferation is related to decreases in expression of Lyn protein. Identical results were obtained with normal progenitor cells isolated from human fetal liver (data not shown). Interestingly, treatment of Mo7e cells with Lyn antisense oligonucleotides also inhibited proliferation induced by

FIG. 4. The juxtamembrane region of c-Kit associates with Lyn. A, tyrosine-phosphorylated GST fusion protein encoding the c-Kit juxtamembrane region coprecipitates Lyn from Mo7e lysates. Mo7e cells (2 × 10⁷) were incubated with either medium or SCF (100 ng/ml) for the indicated times. Cells were lysed and clarified, and coprecipitations were performed for 3 h at 4°C with either GST or the GST-Kit juxtamembrane fusion protein (JXM) isolated from either DH5α or TKX1 bacteria and bound to glutathione-Sepharose. Proteins were resolved by SDS-PAGE, transferred to Immobilon, and immunoblotted with monoclonal antibody specific for Lyn. JXM designates preimmune serum used for control immunoprecipitation. B, Lyn does not associate with the other regions of c-Kit. Clarified lysates from Mo7e cells stimulated with SCF were incubated for 3 h at 4°C with GST or GST fusion proteins (juxtamembrane, designated JXM; kinase region 1, designated K1; kinase insert, designated KINS; the region surrounding tyrosine 823 in kinase region 2, designated Y823; or carboxyl tail, designated C-Tail) bound to glutathione-Sepharose. The GST fusion proteins were isolated from either DH5α (D) or TKX1 (T) bacteria as indicated. Proteins were resolved by SDS-PAGE, transferred to Immobilon and immunoblotted with monoclonal antibody specific for Lyn.

FIG. 5. Decreased expression of Lyn protein results in a decrease in SCF-induced proliferation. A, SCF-induced proliferation in Mo7e cells is inhibited by Lyn antisense oligonucleotides. Mo7e cells were treated with the indicated concentrations of either sense or antisense oligonucleotide overnight. The following morning cells were treated with oligonucleotides for 3 h and SCF-induced proliferation assessed as described under “Materials and Methods.” Data are presented as the mean of triplicate points. B, a nonsense oligonucleotide sequence does not inhibit SCF-induced proliferation. Mo7e cells were treated with either medium or 0.75 μM antisense, sense, or nonsense oligonucleotides as described in A. Proliferation assays were then performed. Data are presented as the mean of triplicate points. C, treatment with Lyn antisense oligonucleotides results in reduction in expression of Lyn protein. Mo7e cells were treated with 1 μM sense or antisense oligonucleotides as described in A. Cells were lysed and equivalent amounts of protein were resolved using SDS-PAGE and transferred to Immobilon. Immunoblotting (IB) for Lyn protein was performed with antibody specific for Lyn and visualized using enhanced chemiluminescence.
SCF-induced Activation of Lyn in Hematopoietic Cells

Previous work has suggested an important role for Src family members in signal transduction of the RTK ligands EGF, PDGF, and CSF-1 (19, 20). While much is known about activation of Src family members in response to EGF, PDGF, and CSF-1 in fibroblasts transfected with the appropriate RTK, little is known about the role of Src family members in the signal transduction pathway of SCF, a growth factor critical for normal hematopoiesis. The goal of these studies was to determine if SCF activated Src family members in hematopoietic cells and address their possible role in SCF signal transduction. We chose to examine Lyn because it was the most highly expressed Src family member in two SCF-responsive cell lines as well as in normal progenitor cells (Fig. 1A). Our studies demonstrate that SCF induced rapid increases in Lyn tyrosine phosphorylation in both hematopoietic cell lines as well as normal progenitor cells isolated from human fetal liver (Fig. 1). Further, in vitro kinase assays demonstrated that SCF induced increases in Lyn autophosphorylation as well as increases in the capacity of Lyn to phosphophorylate enolase, a tyrosine kinase substrate (Fig. 2). In total, these data demonstrate that treatment of hematopoietic cells with SCF activates the Src family member Lyn.

Previous work has demonstrated that numerous signal transduction components associate either directly or indirectly with RTKs. Specifically, Src and the Src family members Yes and Lyn have been detected in the CSF-1 and PDGF receptor complexes (13–16). Metabolic labeling studies with [35S]methionine demonstrated that a 145-kDa protein coimmunoprecipitated with Lyn in Mo7e cells (Fig. 3B). A 145-kDa phosphotyrosyl protein was also observed in Lyn immunoprecipitates after stimulation with SCF (Fig. 3A). The similarity in size of the 145-kDa protein with the SCF receptor led us to examine whether p145 was c-Kit. Indeed, antisera specific for c-Kit recognized p145 in immunoblotting studies (Fig. 3C). Although our studies indicate that a fraction of the SCF receptor was constitutively associated with Lyn, stimulation with SCF clearly increased the amount of c-Kit that coimmunoprecipitated with Lyn (Fig. 3C). Studies with GST fusion proteins demonstrated that Lyn interacted with tyrosine residues in the c-Kit juxtamembrane region (Fig. 4). In contrast, Lyn did not bind to tyrosine-phosphorylated GST fusion proteins encoding the first catalytic domain, the kinase insert, the majority of the second catalytic domain or the carboxyl tail. Although these data do not exclude the possibility that Lyn is capable of associating with other regions of c-Kit after phosphorylation endogenously, our results demonstrate that Lyn is capable of associating with the tyrosine-phosphorylated c-Kit juxtamembrane region. These findings are consistent with previous work with Src family members and the CSF-1 and PDGF RTKs and suggest that Lyn interacts with the juxtamembrane region of c-Kit through its SH2 domain (14–16, 31). It is likely that coimmunoprecipitation of c-Kit with Lyn in unstimulated cells results from the relatively high basal tyrosine phosphorylation of c-Kit in Mo7e cells (Fig. 3A).

The association of Lyn and c-Kit also suggests the possibility of transphosphorylation of these two protein-tyrosine kinases. Though phosphorylation of Lyn by c-Kit may mediate some of the increases in Lyn tyrosine phosphorylation observed in situ (Fig. 1, B–D), it is unlikely that this is the sole mechanism mediating SCF-induced increases in Lyn phosphorylation. Indeed, in vitro studies of Lyn autophosphorylation and substrate phosphorylation strongly suggest that SCF induces increases in Lyn kinase activity (Fig. 2). Mapping studies of Lyn phosphorylation sites will ultimately distinguish these possibilities and are presently ongoing.

SCF-induced activation of Lyn and association of Lyn with c-Kit suggested a possible role for Lyn in the SCF signal transduction pathway. Our studies with antisense oligonucleotides demonstrate that reduction of Lyn protein levels resulted in dramatic reduction in SCF-induced proliferation (Fig. 5). Further, treatment of cells with PP1, a Src family inhibitor, also resulted in decreases in SCF-induced proliferative responses (Fig. 6). These findings are consistent with recent studies demonstrating that microinjection of kinase inactive Src family

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**DISCUSSION**

Figure 6. Treatment with PP1 inhibits proliferation induced by SCF but not PMA. A, PP1 inhibition of SCF-induced proliferation is dose-related. Proliferation was assayed using [3H]thymidine incorporation and was performed with Mo7e cells as described under Materials and Methods. B, PP1 does not inhibit PMA-induced proliferation. Mo7e cells were incubated with PMA and SCF (100 ng/ml) in the presence or absence of PP1 (66 μM). Proliferation assays were performed as described under Materials and Methods. Data are presented as the mean of triplicate points.

GM-CSF, a growth factor that interacts with a receptor that is not a RTK (data not shown). We next examined the effects of the Lyn antisense oligonucleotides on PMA-induced proliferation. Unfortunately, the toxicity of these compounds singularly and are presently ongoing.
members or antibodies inhibitory to Src family catalytic activity inhibits EGF-, PDGF-, and CSF-1-mediated proliferation of fibroblasts transfected with the appropriate RTK (19, 20). Indeed, these experimental designs have suggested that Src, Fyn, and Yes are critical in cell cycle progression induced by each of these RTKs. Studies examining the role of Lyn in SCF-induced cell cycle progression of hematopoietic cells are presently in progress in our laboratory.

Activation of Lyn has also been reported in lymphoid and myeloid cells stimulated with ligands interacting with receptors lacking tyrosine kinase activity (GM-CSF, interleukin-3, CD-40 ligand, and anti-immunoglobulin, for example). To address the biological role of Lyn in immune cell function, three groups have generated Lyn-deficient mice (33–35). Although the most dramatic abnormalities reported in these animals have related to B cell function, it will be interesting to determine if, similar to the Stat4- and Stat6-deficient animals, Lyn-deficient mice have other defects in relatively specific aspects of lymphohematopoietic development and activation (36–40).

Our studies represent two important additions to the body of work addressing Src family members and RTK signal transduction: 1) demonstration that SCF activates a Src family member in hematopoietic cells, and 2) demonstration that Lyn coprecipitates with the phosphorylated juxtamembrane region of c-Kit. Finally, studies using both antisense oligonucleotides and PP1 strongly suggest that Lyn is involved in SCF-induced proliferation. Thus, these studies define a novel component of SCF signal transduction in hematopoietic tissue, a critical target population for SCF. Indeed, the cell-specific nature of RTK-mediated signal transduction highlights the importance of examining signal transduction pathways in physiologically appropriate tissues.

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