MOLECULAR AND CELLULAR BIOLOGY, Mar. 1995, p. 1431–1438 Vol. 15, No. 3
0270-7306/95/$04.00+0

Activation of Phospholipase Cγ in *Schizosaccharomyces pombe* by Coexpression of Receptor or Nonreceptor Tyrosine Kinases

STEVE ARKINSTALL,* MARK PAYTON, AND KINSEY MAUNDRELL

Glaxo Institute for Molecular Biology, 1228 Plan-les-Ouates, Geneva, Switzerland

Received 12 September 1994/Returned for modification 22 November 1994/Accepted 21 December 1994

The fission yeast *Schizosaccharomyces pombe* has no detectable endogenous receptor tyrosine kinases or associated signalling apparatus, and we have used this cell system to reconstitute mammalian platelet-derived growth factor β (PDGFβ) receptor-linked activation of phospholipase Cγ2 (PLCγ2). The PDGFβ receptor migrates as a glycosylated protein of 165 kDa associated exclusively with membrane fractions. No tyrosine autophosphorylation was detected when PDGFβ was expressed alone. PLCγ2 appears as a 140-kDa protein distributed between particulate and soluble fractions which exhibits characteristic selectivity for phosphatidylinositol 4,5-bisphosphate and is sensitive to powerful activation by Ca^{2+}. When coexpressed, both PDGFβ and PLCγ2 undergo tyrosine phosphorylation, and this is accompanied by a >26-fold increase in [^{3}H]inositol 4,5-biphosphate ([^{3}H]IP_2) and [^{3}H]inositol 1,4,5-triphosphate [^{3}H]IP_3 production. Treatment with the tyrosine phosphatase inhibitor pervanadate further increased PLCγ2 tyrosine phosphorylation as well as [^{3}H]IP_2 and [^{3}H]IP_3 generation. Phosphorylated PLCγ2 was found predominantly in membrane fractions. To test a nonreceptor tyrosine kinase, we then expressed the human proto-oncogene c-*src* together with its negative regulator Csk. These were immunodetectable as bands at 60 kDa (c-Src) and 50 kDa (Csk) and distributed between membrane and cytosolic fractions. When yeast coexpressing c-Src, Csk, and PLCγ2 was incubated with pervanadate, PLCγ2 was tyrosine phosphorylated and [^{3}H]IP_2 and [^{3}H]IP_3 production increased 11.0- and 7.0-fold, respectively. Csk expressed alone with PLCγ2 was ineffective. Similar PLCγ2 activation was observed upon in vitro mixing with extracts expressing either c-Src or the PDGFβ receptor. In summary, this is the first report of a reconstitution of mammalian tyrosine kinase-linked effector activation in yeast cells and also the first demonstration of direct PLCγ2 activation by the proto-oncogene c-*src*. These observations indicate that *S. pombe* provides a powerful cell system in which to study critical molecular interactions and activities underlying receptor and nonreceptor tyrosine kinase-dependent cell signaling.

Activation of many cell surface receptors triggers a rapid increase in phospholipase C (PLC)-dependent hydrolysis of membrane phosphatidylinositol 4,5-bisphosphate (PIP_2). This generates the second messengers inositol 1,4,5-trisphosphate and diacylglycerol which mobilize intracellular Ca^{2+} stores and activate members of the protein kinase C family, respectively (2, 35). Many receptor systems linked to PIP_2 hydrolysis employ tyrosine kinase activation as an immediate and critical step underlying signal transduction (43, 53). They promote the phosphorylation and activation of a subset of PLC subtypes exemplified by isoforms PLCγ1 and PLCγ2 (42), each of which possesses two SH2 domains mediating recognition and high-affinity binding to specific phosphotyrosine residues. Receptors which mediate PLCγ activation include those for epidermal growth factor, platelet-derived growth factor (PDGF), fibroblast growth factor, and nerve growth factor, which have intrinsic tyrosine kinase activity (17, 24, 27, 29, 46), although other receptor systems devoid of intrinsic enzymatic activity are also linked to this effector target. These include receptors for a range of cytokines (3), erythropoietin (41), and thrombin (9), as well as membrane immunoglobulin M and CD40 in B lymphocytes (4, 40), the T-cell antigen receptor (38, 52), the high-affinity immunoglobulin E receptor in basophilic leukemia cells (20, 37), and the immunoglobulin G receptor in monocytic cells (21). Members of the Src and Syk/ZAP70 families are candidate tyrosine kinases mediating this response (22, 42, 53).

Although tyrosine phosphorylation is a critical step mediating PLCγ activation, a precise mechanistic understanding of the molecular and regulatory interactions underlying receptor-linked signalling in an intact cell environment remains elusive. In a few cases purified components have been employed to reconstitute receptor tyrosine kinase-dependent PLCγ activation (34). Under these conditions, however, PLCγ is not catalytically activated by tyrosine phosphorylation per se but rather is relieved from inhibitory constraints mimicked in vitro by substances as diverse as detergents and the actin- and PIP_2-binding protein profilin (6, 34, 51). To overcome difficulties associated with in vitro reconstitution, many studies of PLCγ activation have employed transformed mammalian cell systems in which elements have been recombinantly expressed and/or immunoprecipitated following suitable stimulation (17, 25, 27, 33, 46, 47). These studies have been instrumental in demonstrating basic mechanisms of tyrosine kinase-linked signal transduction processes. Despite this, the presence of endogenous components with tyrosine kinase, SH2 adaptor, and phospholipase activities complicates interpretation of these data and limits our understanding of the precise mechanistic interactions and activities under examination. For instance, association of activated PLCγ with additional phosphoproteins (23, 41, 44, 46), dynamin (8), and cytoskeletal elements (54) has been reported, although their importance to cellular signaling remains unclear.

As an alternative approach to studying the molecular basis for tyrosine kinase-linked signal transduction, we have used a cell system in which there is no detectable endogenous recep-

* Corresponding author. Mailing address: Glaxo Institute for Molecular Biology, 14, chemin des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland. Phone: 41 22 706 96 66. Fax: 41 22 794 69 65. Electronic mail address: SA7182@GGR.CO.UK.

tor tyrosine kinase-linked regulatory processes, namely, the fission yeast *Schizosaccharomyces pombe*. Expression of selected mammalian signalling elements in such a naive system might support productive interactions between heterologous components while eliminating involvement and cross-talk with host cell tyrosine kinase-associated control systems. *S. pombe* has been used by others to study the function and interactions of selected mammalian signalling elements, including protein kinase C isoenzymes (7), Src and Csk (48), Raf-1 and MAPKK (13), and Ras and phosphatidylinositol 3′-kinase (15). As part of an ongoing program to reconstruct mammalian growth factor-linked signalling pathways in the fission yeast, we report here the coexpression of PLCγ2 with either the PDGFβ receptor or the proto-oncogene c-*src*. We show that both tyrosine kinases are able to phosphorylate PLCγ2 and trigger its enzymatic activation to hydrolyze yeast membrane phosphoinositides. This is the first demonstration of Src-dependent activation of a PLCγ isoform and suggests a direct functional interaction. These observations also indicate that heterologous expression of selected signalling elements in *S. pombe* permits the detection and analysis of functional PLCγ activation and paves the way for further systematic and detailed molecular dissection of a range of interactions central to tyrosine kinase-linked transduction processes.

MATERIALS AND METHODS

Strains and media. The host strain for *S. pombe* transformation, Sp31 (*h*$^+$ *leu1-32 ura4-D18 ade6-704 his5-303*) was based on the original wild-type 975h$^+$ strain from the Berne collection and constructed by standard genetic procedures (10). Growth media and the procedure for protoplast transformation are as described elsewhere (31). The bacterial strain for routine work was *Escherichia coli* JM101TR *supE thi srl*::Tn*10 recA* Δ(*lac-proAB*) [F′ *traD36 proAB lacIZ*Δ *M15*], and standard media were used throughout.

Plasmid constructs. The rat PLCγ2 cDNA (5) was a generous gift from Y. Emori (University of Tokyo). The coding region was excised by digestion with *Kpn*I and *Hin*dIII restriction endonucleases and subcloned into the *Msc*I site of the thiamine-repressible *S. pombe* expression vector pREP3 (26). The cDNA for the wild-type and K602A (K-602 changed to A) point-mutated mouse PDGFβ receptor (55) was kindly provided by L. T. Williams (University of California, San Francisco) and subcloned as a *Bbs*I-*Tth*III1 fragment into the *Msc*I site of pREP4 (26). Human c-Src coding sequence together with 70 bp of upstream untranslated DNA (*Mse*I-*Mse*I fragment) was obtained from T. M. Gilmer (Glaxo Research Institute, Research Triangle Park, N.C.) and expressed from pREP7, a vector based on pREP3 but containing the *S. pombe his5* gene (19a) in place of the *Saccharomyces cerevisiae LEU2* selectable marker. The human Csk cDNA was a kind gift from S. Courtneidge (European Molecular Biology Laboratory, Heidelberg, Germany) and was expressed from the *S. pombe adh* promoter (48) on a plasmid carrying the *S. pombe ade6* selectable marker.

Preparation of yeast homogenates. Suitably transformed *S. pombe* cells were grown overnight to stationary phase in minimal medium containing 4 μM thiamine, after which they were diluted to 5×10^5 cells per ml in fresh minimal medium and cultured with or without exogenously added thiamine for another 18 h until late log phase (1×10^7 to 2×10^7/ml). For homogenization, yeast cells were washed in 0.9% NaCl and broken by vigorous vortexing four times for 1 min each time at 4°C in the presence of 1.5 ml of glass beads (500-μm diameter) and 100 μl of buffer PTP (10 mM Tris-HCl, 5 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 10 μg of leupeptin per ml, 10 μg of chymostatin per ml, 10 μg of aprotonin per ml, 1 mM sodium vanadate, 10 mM sodium PP_i, pH 7.4). A further 400 μl of buffer PTP was then added, and the homogenate was recovered by low-speed centrifugation after the base of the tube was pierced. The homogenate was further centrifuged at $1,000 \times g$ for 5 min to pellet unbroken cells and debris. The supernatant containing both particulate material and soluble proteins was designated crude homogenate. By this protocol, 2×10^9 cells yield routinely crude homogenates with a protein content of 10 to 15 mg/ml.

Measuring PLCγ2 activity with exogenous substrate. Phosphatidyl[*myo*2-^{3}H]-inositol ([^{3}H]PI; 9.2 Ci/mmol) and phosphatidyl[2-^{3}H]inositol 4,5-bisphosphate ([^{3}H]PIP_2; 8.0 Ci/mmol) (New England Nuclear, Regensdorf, Switzerland) were used as exogenous substrates to measure PLCγ2 activity. To prepare substrate, approximately 1 μCi of [^{3}H]PI or [^{3}H]PIP_2 was mixed with 110 μl of phosphatidylethanolamine from bovine brain (10 mg/ml; Sigma Chemie, Buchs, Switzerland) together with (for assays with [^{3}H]PI) 50 μl of phosphatidylinositol from bovine liver (10 mg/ml; Sigma Chemie) in glass tubes. Phospholipids were then dried under vacuum with centrifugation. For assays with [^{3}H]PIP_2, 45 μl of 5 mM PIP_2 from bovine brain (Fluka Chemie AG, Buchs, Switzerland) dissolved in 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) with 100 mM KCl and 10 mM deoxycholate (pH 7.0) was added. Phospholipids were then resuspended in 1.0 ml of 20 mM HEPES (pH 7.2) containing 100 mM NaCl by using a bath sonicator for 5 min and then a probe sonicator at full power four times for 10 s each. To measure PLCγ2 activity, 25 μl of substrate was added to glass tubes together with 25 μl of buffer B (50 mM Tris-HCl, 50 mM maleic acid, 40 mM LiCl, and 20 mM $MgCl_2$ at pH 7.0) and 25 μl of $CaCl_2$ at concentrations calculated to give defined free Ca^{2+} levels between 10^{-8} and 10^{-3} M [based on 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA)]. Reactions were started by adding 25 μl of crude homogenate diluted up to 800-fold in ice-cold buffer C (5 mM Tris-HCl containing 4 mM EGTA with 1 mM dithiothreitol). After incubation at 35°C for 30 min in a shaking water bath, reactions were stopped by adding 1.2 ml of an ice-cold mixture of methanol-chloroform-HCl (200:100:1 [vol/vol]) followed by a further 0.5 ml of chloroform and 0.5 ml of 0.25 M HCl and vortex mixing. Samples were then centrifuged at $1,000 \times g$ for 5 min, and 0.5 ml of the upper aqueous phase was counted by scintillation spectrometry.

For PLCγ2 preactivation, crude homogenates from yeast expressing independently PLCγ2, PDGFβ receptor, or Src with Csk were first diluted 5- to 10-fold in buffer C to give a protein concentration of 2.5 mg/ml. Diluted homogenates containing PLCγ2 (50 μl) were then mixed with 50-μl aliquots of PDGFβ receptor or Src with Csk together with 100 μl of preactivation buffer (50 mM Tris-HCl, 20 mM $MgCl_2$, 10 mM $MnCl_2$, 1 mM dithiothreitol, 2 mM ATP, 5 mM phosphocreatine, 10 U of creatine phosphokinase, pH 7.4) and incubated at 37°C for 15 min. Samples were then diluted a further 20-fold with buffer C and assayed for PLCγ2 activity as described above.

PLCγ2 hydrolysis of yeast phosphoinositides. Following overnight growth into stationary phase, cells were diluted into minimal medium containing 50 μCi of L[*myo*-1,2-^{3}H]inositol (59.6 Ci/mmol; New England Nuclear) and cultured for 18 h as described above. Labelled yeast was used to measure PLCγ2 activity in either membrane fractions or intact cells. To prepare membranes, homogenates prepared as described above were diluted in 30 ml of buffer C, further homogenized for 30 s with an Ultra-Turrax tissue homogenizer (Bender and Hobein, Zurich, Switzerland), and centrifuged at $20,000 \times g$ for 30 min at 4°C. Pellets were then washed by rehomogenization in 30 ml of buffer C, recentrifuged, and resuspended in buffer C to 20% of the original homogenate volume. PLCγ2 activity was measured as described above except that following extraction of the [^{3}H]inositol phosphates, 0.5 ml of the upper phase was diluted with 5.0 ml of water and neutralized with 2% (wt/vol) $NaHCO_3$. Labelled inositol phosphates were then separated by anion-exchange chomatography using minicolumns containing 0.5 ml of washed AG1-X8 resin in the formate form (Bio-Rad Laboratories, Glattbrugg, Switzerland). Following sample loading, columns were washed with 10 ml of water and 10 ml of 60 mM sodium formate in 5 mM sodium tetraborate followed by sequential elution of [^{3}H]IP_1 (inositol monophosphate), [^{3}H]IP_2 (inositol bisphosphate), and [^{3}H]IP_3 (inositol trisphosphate) with 4-ml aliquots of 0.1 M formic acid containing, respectively, 0.15, 0.45, and 1.0 M ammonium formate. Elution profiles were confirmed with authentic labelled [^{3}H]inositol phosphates (New England Nuclear).

For measuring PLCγ2 activity in intact yeast cells, [^{3}H]inositol-labelled cultures were prepared as described above. Where indicated, pervanadate was prepared fresh as a mixture of 300 mM H_2O_2 and 100 mM Na_3VO_4 and 250 μl of this was added to 25-ml cultures which were then incubated under normal growing conditions for a further 1 h. To extract labelled inositol phosphates, 6-ml aliquots of culture (1×10^8 to 2×10^8 cells) were added to 21.6 ml of a mixture of methanol-chloroform-HCl (200:100:2.5 [vol/vol]) and then frozen in a bath of methanol and dry ice. Addition of a further 7.2 ml of chloroform and 7.2 ml of water was followed by vortex mixing. After 30 min on ice to allow for extraction, samples were centrifuged at $1,000 \times g$ for 5 min, and 22 ml of the upper phase was taken for neutralization with 2% (wt/vol) $NaHCO_3$. After centrifugation at $1,000 \times g$ for 5 min, samples were then loaded onto AG1-X8 anion-exchange minicolumns, and [^{3}H]inositol phosphates were isolated as described above.

Immunodetection of PLCγ2, PDGFβ receptor, c-Src, and Csk. Crude homogenates were prepared as described above. For preparation of membrane fractions for Western blotting (immunoblotting), homogenates were centrifuged at $100,000 \times g$ for 30 min in a TL-100 ultracentrifuge (Beckman Instruments International S.A., Nyon, Switzerland). The supernatant was taken as the cytosolic fraction, while the pellet was washed once in 1.0 ml of 10 mM Tris, pH 7.4, by resuspension using a sonicator probe at full power for 10 s. Following recentrifugation the pellet was suspended in 1 volume of buffer PTP by sonication. For Western blotting, samples were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (7.5 and 10.0% polyacrylamide gels) followed by electrotransfer onto nitrocellulose membranes by standard techniques. Filters were blocked by incubation for 45 min in 20 mM Tris-HCl (pH 7.5) containing 500 mM NaCl and 5% (wt/vol) freeze-dried low-fat milk (TBSM) followed by overnight incubation at 4°C in TBSM containing 0.05% (vol/vol) Tween 20, and antibodies were diluted 500- to 1,000-fold. Antibody binding was detected with horseradish peroxide-conjugated second antibody (Bio-Rad Laboratories) or protein A/G-horseradish peroxidase (Pierce, Zurich, Switzerland) and enhanced chemiluminescence detection (Amersham, Zurich, Switzerland). Antibodies were obtained from the following sources: c-Src (N16; Santa Cruz Biotechnology, Inc., Basel, Switzerland), Csk (Transduction Laboratories, Nottingham, United Kingdom), and phosphotyrosine (4G10; Upstate Biotechnology, Inc., Lake Placid, N.Y.). Antibodies specific for the mouse PDGFβ receptor and

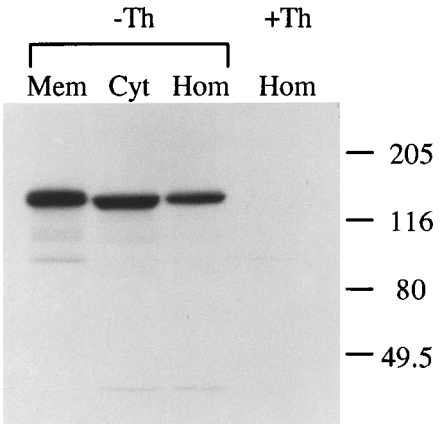

FIG. 1. Rat PLCγ2 expression in *S. pombe*. Western blot showing expression of rat PLCγ2 in a crude homogenate (Hom) and in membrane (Mem) and cytosolic (Cyt) fractions obtained following centrifugation at 100,000 × *g* for 30 min. Yeast cells were grown in either the absence (−Th) or presence (+Th) of thiamine for induction or repression of gene expression, respectively. Sizes are indicated in kilodaltons on the right.

PLCγ2 were kind gifts supplied by L. T. Williams (University of California, San Francisco) and Y. Emori (University of Tokyo), respectively.

PLCγ2 immunprecipitation. Crude homogenates were prepared, and 190-μl aliquots were mixed with 810 μl of ice-cold buffer L (1% Triton X-100, 25 mM Tris-HCl, 150 mM NaCl, 1 mM Na_3VO_4, 10 mM sodium PPi, 10 μg of aprotonin per ml, 10 μg of leupeptin per ml, 10 μg of chymostatin per ml, and 0.5 mM phenylmethylsulfonyl fluoride at pH 7.5), then incubated on ice for 1 h, and centrifuged at 100,000 × *g* for 30 min. For immunprecipitation, 800 μl of cleared lysate was incubated with 25 μl of PLCγ2-specific antibody for 30 min, after which time 100 μl of washed protein A-Sepharose (50%, vol/vol; Pharmacia, Uppsala, Sweden) was added and incubated for a further 2 h on ice. Beads were then sedimented by centrifugation, washed three times in 1.0 ml of buffer L, then resuspended in SDS-PAGE sample buffer, and heated at 95°C for 5 min.

RESULTS

Expression of rat PLCγ2. The rat PLCγ2 cDNA was subcloned into the thiamine-repressible expression vector REP3 and transformed into the fission yeast *S. pombe* (see Materials and Methods). PLCγ2 protein expression was immunodetectable at high levels in crude yeast homogenates after 18 h of growth in the absence of thiamine and, on the basis of separation by SDS-PAGE, migrates as a protein of 140 kDa (Fig. 1). This size is as predicted by the cDNA (5) and identical to that of purified native protein (4, 12). Interestingly, PLCγ2 appears to be localized in both soluble and 100,000 × *g*-centrifuged particulate fractions, with membrane-associated enzyme displaying slightly slower mobility upon SDS-PAGE separation (Fig. 1). To test for enzymatic activity, yeast extracts were incubated with phospholipid vesicles containing phosphatidylethanolamine together with [^{3}H]PI or [^{3}H]PIP_2 in the presence of free Ca^{2+} concentrations between 10^{-8} and 10^{-3} M. While no phosphoinositide hydrolysis could be detected with extracts prepared from control cells, in cells expressing PLCγ2 we measured high levels of activity, with half maximal stimulation of [^{3}H]PI and [^{3}H]PIP_2 hydrolysis occurring at approximately 2 μM free Ca^{2+} (Fig. 2A). Anion-exchange chromatography demonstrated that [^{3}H]IP_1 and [^{3}H]IP_3 were the major products following hydrolysis of [^{3}H]PI and [^{3}H]PIP_2, respectively (data not shown). PLCγ2 exhibits at least 10-fold higher activity with [^{3}H]PIP_2 rather than [^{3}H]PI as a substrate (Fig. 2A), a K_d for PIP_2 of 125 μM, and optimum activity at pH ~7.0 (data not shown). To test whether PLCγ2 could also hydrolyze yeast membrane phosphatidylinositol and PIP_2, we prepared membranes from *S. pombe* grown overnight in the

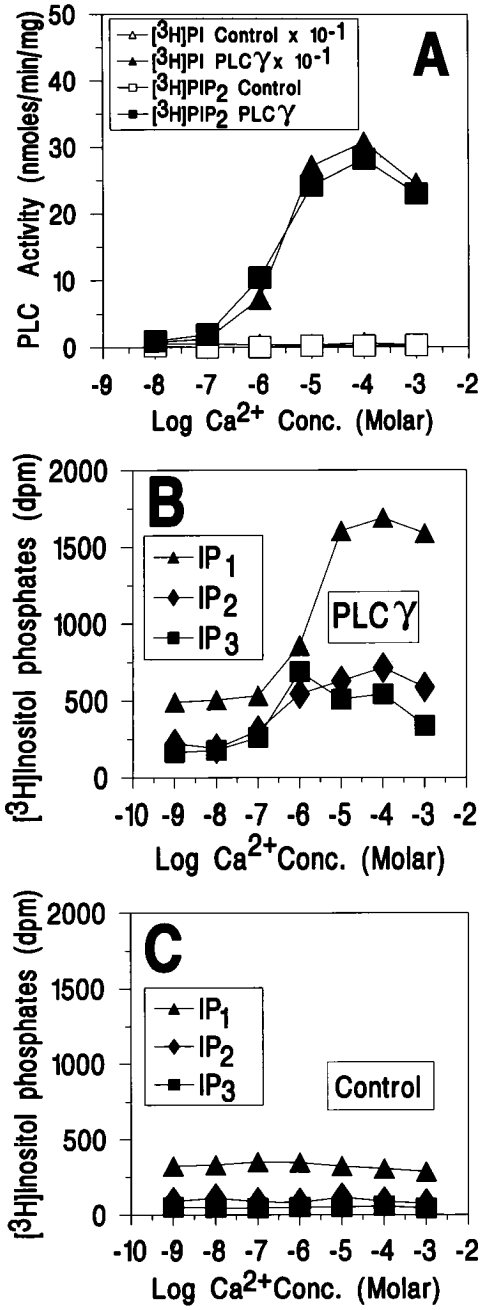

FIG. 2. PLCγ2 enzymatic activity. (A) PLC activity was measured in crude homogenates with either [^{3}H]PI or [^{3}H]PIP_2 as an exogenous substrate. Activities are calculated on the basis of the total protein content of the yeast extract. Homogenates were diluted either 50- or 800-fold for hydrolysis of [^{3}H]PI or [^{3}H]PIP_2, respectively. Solid symbols show extracts from yeast expressing PLCγ2, while open symbols represent control cells. Points are the means from triplicate determinations and typical of more than five independent experiments. Yeast cells expressing PLCγ2 (B) or control cells (C) grown for 18 h in the presence of [^{3}H]inositol to label endogenous phosphoinositides were homogenized, and membranes were incubated in the presence of increasing concentrations of free Ca^{2+}. [^{3}H]inositol phosphates were isolated by anion-exchange chromatography. For clarity, values have been corrected by a factor of 10^{-2} for [^{3}H]IP_1 and a factor of 10^{-1} for [^{3}H]IP_2. Unadjusted values are given for [^{3}H]IP_3. Points are the means from triplicate determinations and representative of three independent experiments.

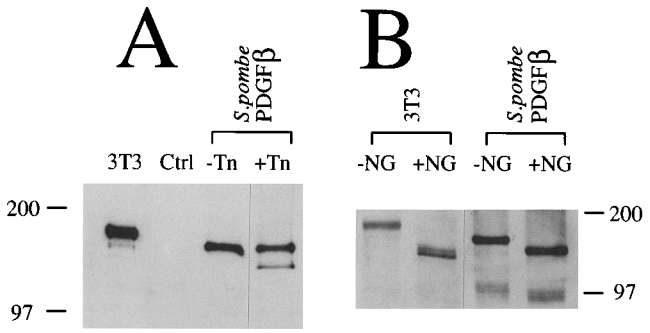

FIG. 3. Mouse PDGFβ receptor expression in *S. pombe*. (A) Yeast samples were crude homogenates of cells grown in the absence of thiamine for 19 h with (+Tn) or without (−Tn) tunicamycin (5 μg/ml) for the final 2 h of culture. The major band migrates as a protein of 165 kDa, although in the presence of tunicamycin an additional band at 135 kDa is detectable. In untreated Swiss 3T3 cell membranes the PDGF receptor is immunodetectable as a major band at 180 kDa. Ctrl, control. (B) Membranes from Swiss 3T3 cells or homogenates from *S. pombe* expressing the mouse PDGFβ receptor were incubated overnight with (+NG) or without (−NG) *N*-glycosidase F (20 U/ml). Enzymatic treatment resulted in a major immunodetectable band at 135 kDa. SDS-PAGE protein separation was performed using 7.5% (A) or 10% (B) polyacrylamide gels. Sizes are indicated in kilodaltons on the left and right.

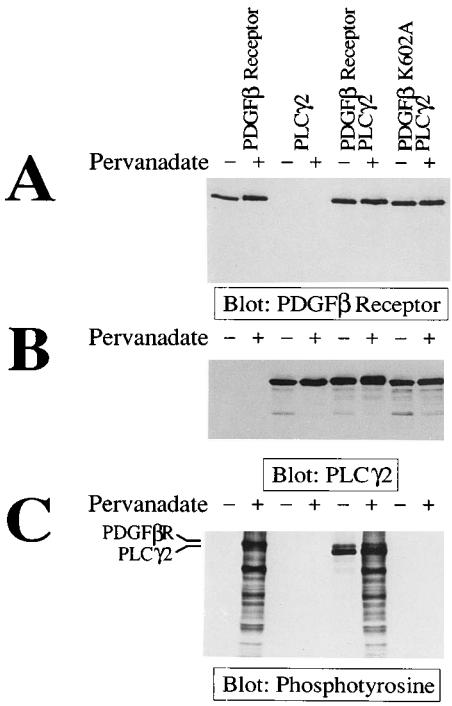

FIG. 4. Pervanadate stimulates tyrosine phosphorylation in *S. pombe* expressing the PDGFβ receptor. Western blot analysis of crude homogenates prepared from cells pretreated with (+) or without (−) pervanadate for 60 min prior to homogenization. Yeast expressed combinations of the PDGFβ receptor and PLCγ2 either alone or together as indicated. The catalytically inactive PDGFβ receptor point mutant K602A was also coexpressed with PLCγ2. Samples were analyzed with antibody specific for the PDGFβ receptor (A), PLCγ2 (B), or phosphotyrosine (C). The positions of phosphorylated PDGFβ and PLCγ2 are indicated (C).

presence of [^{3}H]inositol and isolated [^{3}H]inositol phosphates by anion-exchange chromatography. Similar to observations made with exogenous phospholipids, membrane incubation with free Ca^{2+} levels between 10^{-9} and 10^{-4} M resulted in increased generation of [^{3}H]IP_1, [^{3}H]IP_2, and [^{3}H]IP_3 (Fig. 2B). Increased generation of [^{3}H]inositol phosphates was not detected with prelabelled membranes from control cells (Fig. 2C). It is of note that although PLCγ2 can clearly hydrolyze endogenous yeast phosphoinositides in a manner comparable to that observed with purified [^{3}H]PI and [^{3}H]PIP_2, generation of [^{3}H]IP_3 from labelled membranes is approximately 10-fold more sensitive to free Ca^{2+} than stimulated hydrolysis of exogenous [^{3}H]PIP_2.

Expression of mouse PDGFβ receptor. The mouse PDGFβ receptor expressed in *S. pombe* was immunodetectable as a protein band with an apparent molecular size of 165 kDa by SDS-PAGE (Fig. 3A). This is considerably larger than predicted by the cloned cDNA (~123 kDa), and as indicated in mammalian cells, extensive glycosylation could contribute to this disparity (55). Consistent with such a modification for the PDGFβ receptor expressed in *S. pombe*, yeast cells grown in the presence of tunicamycin for the final 2 h of culture (5.0 μg/ml) gave an immunodetectable band at 135 kDa (Fig. 3A). In addition, treatment of both Swiss 3T3 cell membranes and *S. pombe* extracts with *N*-glycosidase F (overnight; 20 U/ml) yielded PDGFβ receptor migrating identically at 135 kDa (Fig. 3B). Moreover, following membrane solubilization in radioimmunoprecipitation assay buffer, the PDGFβ receptor was immunodetected as a 165-kDa protein following precipitation using either concanavalin A or lectin I bound to agarose beads (data not shown). As expected from a transmembrane receptor, the PDGFβ receptor was detected exclusively in particulate fractions following centrifugation at 100,000 × *g* (see Fig. 7A).

PDGFβ receptor phosphorylates and activates coexpressed PLCγ2. PLCγ2 is a physiological effector target for phosphorylation and activation by the PDGFβ receptor (12). To test whether similar functional interactions can be reconstituted in *S. pombe*, we next coexpressed PLCγ2 with the PDGFβ receptor. Yeast cells expressing PDGFβ and PLCγ2, either alone or together, were analyzed simultaneously by using antibodies detecting receptor, effector, and tyrosine phosphorylation. Upon coexpression (Fig. 4A and B), both the PDGFβ receptor and PLCγ2 become tyrosine phosphorylated (Fig. 4C). This contrasts with a lack of detectable phosphorylation when either PDGFβ or PLCγ2 was expressed alone at similar levels (Fig. 4). To test whether PLCγ2 tyrosine phosphorylation leads to enzymatic activation, *S. pombe* cells were then prelabelled with [^{3}H]inositol and inositol phosphates were isolated by anion-exchange chromatography. In cells coexpressing the mouse PDGFβ receptor and PLCγ2, levels of [^{3}H]inositol phosphates were increased 9.3- ± 3.96-fold (n = 5), 27.4- ± 6.61-fold (n = 5), and 26.0- ± 9.95-fold (n = 5) for [^{3}H]IP_1, [^{3}H]IP_2 and [^{3}H]IP_3, respectively (Fig. 5).

In order to enhance PLCγ2 tyrosine phosphorylation, we exposed cells to the tyrosine phosphatase inhibitor pervanadate formed from a mixture of hydrogen peroxide and Na_3VO_4 (11). Although protein tyrosine phosphorylation remained undetectable in control cells following incubation with pervanadate, in yeast cells expressing the PDGFβ receptor, extensive tyrosine phosphorylation was observed for both PDGFβ and other protein bands ranging in size from >10 to <200 kDa (Fig. 4C). When coexpressed with the PDGFβ receptor, tyrosine phosphorylation of PLCγ2 was increased considerably upon stimulation with pervanadate (Fig. 4C). The kinase-deficient PDGFβ receptor point mutant K602A failed to elicit any phosphorylation under identical conditions (Fig. 4C). The identity of PLCγ2 as the target for tyrosine phosphorylation by the PDGFβ receptor was confirmed by immunoprecipitation using PLCγ2-specific antibody (Fig. 6). As indicated by anal-

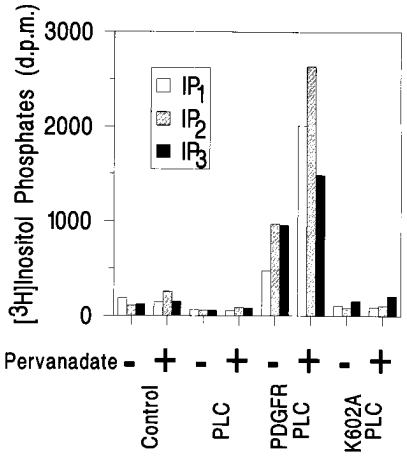

FIG. 5. Tyrosine phosphorylated PLCγ2 is enzymatically active in intact yeast cells. *S. pombe* expressing PLCγ2 (PLC) either alone or together with the PDGFβ receptor (PDGFR) or a catalytically inactive point mutant (K602A) was grown under inducing conditions in the presence of [^{3}H]inositol for 18 h. Cells were stimulated with (+) or without (−) pervanadate for 60 min and extracted with $CHCl_3$-CH_3OH-HCl, and [^{3}H]inositol phosphates were isolated by anion-exchange chromatography. Radioactivity eluting identically with [^{3}H]IP_1, [^{3}H]IP_2, and [^{3}H]IP_3 is indicated as disintegrations per minute. Bars represent the means from duplicate determinations, and data are representative of five separate experiments.

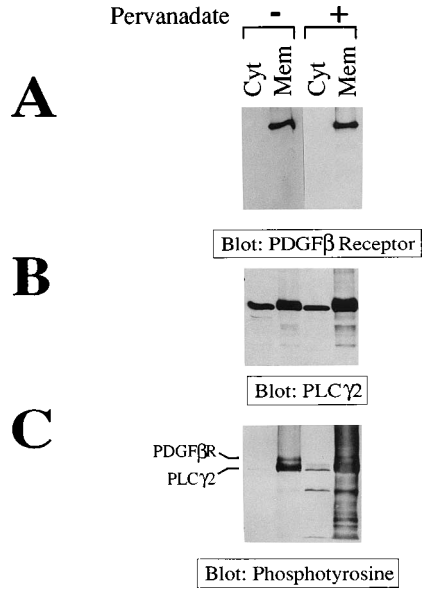

FIG. 7. Distribution of PDGFβ, PLCγ2, and phosphotyrosine in membrane and cytosolic fractions. *S. pombe* cells coexpressing the PDGFβ receptor (PDGFβR) and PLCγ2 were stimulated with (+) or without (−) pervanadate, and membrane (Mem) and cytosolic (Cyt) fractions were prepared from crude homogenates by centrifugation at 100,000 × *g*. Samples were analyzed with antibodies detecting either PDGFβ (A), PLCγ2 (B), or phosphotyrosine (C).

ysis of whole-cell extracts (Fig. 4C), immunprecipitated PLCγ2 is not phosphorylated in the presence of pervanadate when expressed alone (Fig. 6). Interestingly, although PLCγ2 is localized in both particulate and cytosolic fractions (Fig. 7B), tyrosine phosphorylated PLCγ2 is found predominantly in membranes (Fig. 7C). To test whether increased tyrosine phosphorylation of PLCγ2 results in further enzymatic activation,

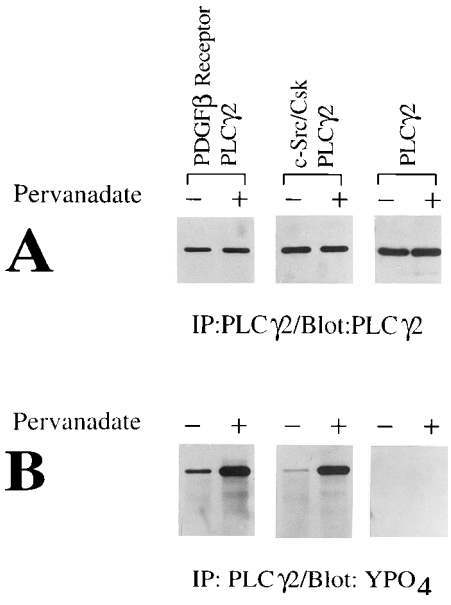

FIG. 6. Tyrosine phosphorylation of immunoprecipitated PLCγ2. Crude homogenates from *S. pombe* expressing PLCγ2 either alone or together with the PDGFβ receptor or c-Src and Csk were stimulated with (+) and without (−) pervanadate and immunoprecipitated with anti-PLCγ2 antibodies and protein A-Sepharose. Samples were analyzed by Western blotting using antibodies detecting either PLCγ2 (A) or phosphotyrosine (B).

cells prelabelled with [^{3}H]inositol were also stimulated with pervanadate. In accordance with the increased phosphorylation state of PLCγ2 (Fig. 6), pervanadate augmented generation of [^{3}H]inositol phosphates 4.0- ± 0.59-fold (n = 6), 3.0- ± 0.24-fold (n = 6), and 2.0- ± 0.4-fold (n = 6) for [^{3}H]IP_1, [^{3}H]IP_2, and [^{3}H]IP_3, respectively (Fig. 5).

Expression of human c-Src with its negative regulator Csk leads to PLCγ2 activation. Functional interaction between PLCγ2 and PDGFβ demonstrates that we can reconstitute known interactions between a receptor tyrosine kinase and effector in *S. pombe*. Members of the Src tyrosine kinase family are additional potential upstream regulators of PLCγ (22, 53), although direct enzymatic activation has not yet been demonstrated. We were therefore curious to extend our studies with fission yeast to test for PLCγ activation by the proto-oncogene c-*src*. Avian Src has been expressed previously in *S. pombe*, where it was shown to be lethal unless coexpressed with the negative regulatory kinase Csk (48). We have confirmed this lethality for human Src (data not shown) and for this reason have also expressed this tyrosine kinase together with Csk. These proteins are immunodetectable as bands at 60 kDa for c-Src and 50 kDa for Csk (Fig. 8A and B), which is as predicted by the expressed cDNAs. Both c-Src and Csk are detected in cytosolic and membrane fractions following centrifugation (Fig. 8A and B). As an initial test of c-*src* activity, we demonstrated phosphorylation of acid-treated enolase using immunoprecipitated Src (not shown). Consistent with this Src kinase activity, upon coexpression of PLCγ2 with c-Src and Csk, low levels of PLCγ2 tyrosine phosphorylation were detected. This can be most clearly seen by analyzing immunoprecipitated PLCγ2 (Fig. 6). This PLCγ2 tyrosine phosphorylation is much lower than is observed upon coexpression with the PDGFβ receptor (Fig. 6), and consistent with a tight relationship between phosphorylation and enzymatic activation, levels of [^{3}H]inositol phosphates generated by prelabelled cells were unaltered compared with control levels (Fig. 9). Despite this,

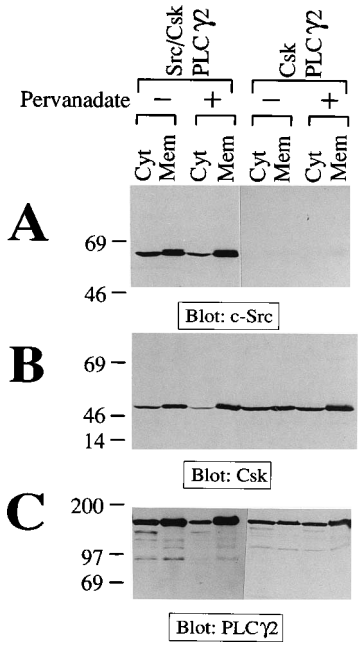

FIG. 8. Human c-Src and Csk expression in *S. pombe*. Yeast expressing PLCγ2 together with c-Src and Csk or Csk alone was grown for 18 h under inducing conditions (no thiamine) and stimulated with (+) or without (−) pervanadate for the final 60 min of culture. Membrane (Mem) and cytosolic (Cyt) fractions were prepared from crude homogenates by centrifugation at 100,000 × *g* and analyzed by Western blotting using antibodies specific for Src (A), Csk (B), or PLCγ2 (C). Sizes are given in kilodaltons on the left.

pervanadate treatment of yeast cells coexpressing Src, Csk, and PLCγ2 augmented PLCγ2 tyrosine phosphorylation considerably (Fig. 6), and this was associated with increased generation of [^{3}H]inositol phosphates (Fig. 9). [^{3}H]IP_1, [^{3}H]IP_2, and [^{3}H]IP_3 generation was elevated 8.8- ± 6.7-fold (n = 4), 11.2- ± 3.01-fold (n = 4), and 6.6- ± 1.45-fold (n = 4), respectively.

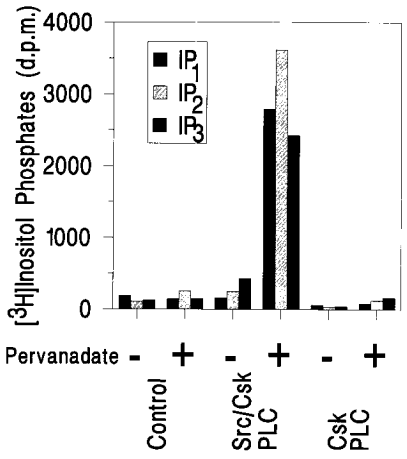

FIG. 9. PLCγ2 tyrosine phosphorylated by c-Src is enzymatically activated in intact yeast cells. *S. pombe* cells expressing PLCγ2 together with either Src and Csk or Csk alone were grown for 18 h under inducing conditions in the presence of [^{3}H]inositol. Cells were stimulated with (+) and without (−) pervanadate for 60 min and [^{3}H]inositol phosphates were isolated by extraction with $CHCl_3$-CH_3OH-HCl followed by anion-exchange chromatography. Radioactivity eluting with [^{3}H]IP_1, [^{3}H]IP_2, and [^{3}H]IP_3 is indicated as disintegrations per minute. Bars represent the means from duplicate determinations, and data are representative of four independent experiments.

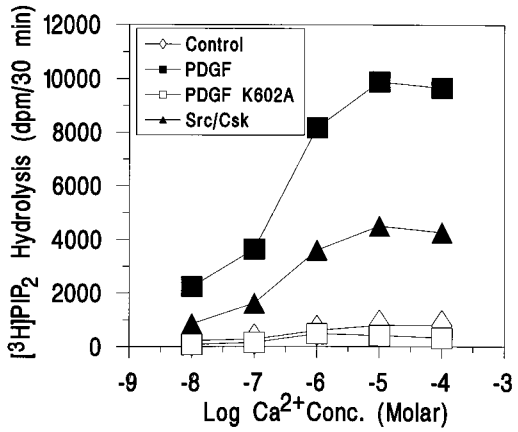

FIG. 10. In vitro PLCγ2 activation by PDGFβ receptor and c-Src. Homogenates from *S. pombe* expressing only PLCγ2 were preincubated with extracts expressing either PDGFβ receptor, the PDGFβ receptor point mutant K602A, or Src with Csk in the presence of ATP (1 mM), $MgCl_2$ (10 mM), and $MnCl_2$ (5 mM). Mixtures were then diluted and incubated with [^{3}H]PIP_2 as exogenous substrate in the presence of increasing concentrations of free Ca^{2+}. Points are the means from triplicate determinations and representative of at least five independent experiments.

It appears likely that Src rather than Csk is responsible for this activity, as in Csk-PLCγ2 transformants no PLCγ2 activation was detected (Fig. 9). Interestingly, pervanadate treatment stimulated a small, but reproducible, decline in c-Src, Csk, and PLCγ2 detectable in cytosolic fractions (Fig. 8), and this may reflect a translocation to particulate fractions.

PLCγ2 in vitro activation by the PDGFβ receptor and by c-Src. In addition to PLCγ2 activation upon stimulation of intact yeast cells with pervanadate, incubating crude homogenates from yeast expressing either the PDGFβ receptor or c-Src (with Csk) with extracts from yeast expressing PLCγ2 leads to enzymatic activation and hydrolysis of exogenous [^{3}H]PIP_2 (Fig. 10). The degree of stimulation is up to 12-fold for PDGFβ and 5-fold for Src-Csk and appears similar at all concentrations of free Ca^{2+} between 10^{-8} and 10^{-4} M. This activation is dependent upon addition of ATP, and consistent with our observations in intact yeast cells, the inactive PDGFβ receptor point mutant K602A (Fig. 10) and Csk expressed alone (data not shown) are totally ineffective in this assay.

DISCUSSION

We have exploited the fission yeast *S. pombe* as cells of host cells in which to coexpress mammalian tyrosine kinases and selected SH2 domain-containing regulators and effector targets. Here we report expression of the PDGFβ receptor or the nonreceptor tyrosine kinase Src (with its negative regulator Csk) together with rat PLCγ2. Both kinases mediate tyrosine phosphorylation and enzymatic activation of PLCγ2, particularly when cells are stimulated with the tyrosine phosphatase inhibitor pervanadate. This functional interaction is consistent with observations in mammalian cells, in which the PDGFβ receptor mediates PLCγ1 tyrosine phosphorylation and catalytic activation (17, 27, 42). PLCγ2 has also been identified as a downstream effector target for the PDGFβ receptor in vascular smooth muscle (12). Our observation that the intracellular environment of *S. pombe* supports PLCγ2 tyrosine phosphorylation and hydrolysis of yeast inositol phospholipids raises an important question fundamental to our understanding of the regulatory interactions necessary for enzymatic ac-

tivation. PLCγ in mammalian cells associates with dynamin (8) as well as several phosphoproteins (23, 41, 44, 46) and cytoskeletal elements (54), and these could play a critical role underlying activation. The need for detergents or the PIP_2-binding protein profilin when reconstituting PLCγ activation (6, 34, 51) may reflect the absence of critical regulatory elements when using purified components. In *S. pombe* we have been unable to detect interactions with additional yeast regulatory elements, as PLCγ2 coimmunoprecipitation revealed no additional unidentified phosphoproteins (Fig. 6). Since *S. pombe* possesses no detectable endogenous receptor-linked tyrosine kinase signal transduction apparatus, the simplest interpretation of our observations is that the mammalian PDGFβ receptor and Src kinases can both interact directly with PLCγ2 to catalyze its tyrosine phosphorylation and enzymatic activation.

Src family tyrosine kinases have been implicated as upstream regulators of PLCγ in a number of mammalian cell systems, although a direct role mediating enzymatic activation has not been demonstrated. For instance, although CD40 and antigen receptors in lymphocytes trigger PLCγ1 and PLCγ2 activation (4, 38, 40, 52), it is unclear whether tyrosine kinases of the Src or Syk/ZAP70 family ultimately mediate effector activation (53). Indeed, although Src family tyrosine kinases can phosphorylate PLCγ1 and PLCγ2 in vitro (22), catalytic activation to hydrolyze PIP_2 has hitherto not been demonstrated. Our observations in fission yeast cells provide evidence that Src catalyzes extensive tyrosine phosphorylation of coexpressed PLCγ2 and that this is accompanied by powerful enzymatic activation to hydrolyze yeast inositol-containing phospholipids. In addition to lymphocyte antigen receptor systems, a role for Src family kinases in activating effector targets appears to have broader significance, as they also appear to relay or amplify signals originating from growth factor tyrosine kinase receptors. This is illustrated by the PDGFβ receptor which interacts with Src through distinct juxtamembrane autophosphorylation sites (32) and which requires Src activation to elicit proliferation (49). Given that PLCγ activation appears crucial for PDGFβ receptor-linked mitogenesis (50), an unresolved question raised by these studies is of the relative contribution of the receptor and Src tyrosine kinase in catalyzing PLCγ phosphorylation and PDGF-stimulated proliferation. Our observations in yeast cells indicate that both receptor and nonreceptor tyrosine kinases play a potentially important role mediating effector activation following growth factor signals at the cell surface.

Coexpression of the PDGFβ receptor and PLCγ2 in *S. pombe* leads to a significant degree of constitutive activity observed both at the level of tyrosine phosphorylation and by increased enzymatic activation leading to generation of [^{3}H]-inositol phosphates. Constitutive activation of the PDGF receptor has been reported previously with high-level expression (14), although in *S. pombe* this appears to be dependent upon coexpression with PLCγ2 (Fig. 4). Indeed, although the PDGFβ receptor is present at similar levels when expressed alone, no receptor autophosphorylation can be detected under these conditions (Fig. 4). PDGFβ is nevertheless uncompromised in its functional potential, as pervanadate elicits significant receptor autophosphorylation and extracts from cells expressing PDGFβ alone mediate powerful in vitro PLCγ2 activation. The mechanism underlying PLCγ2-dependent PDGFβ autophosphorylation is unclear, although there are at least two potential explanations. First, as described for SH2 domains from other components (16, 19), PLCγ2 binding PDGFβ may protect the receptor from dephosphorylation by endogenous tyrosine phosphatase activity. The presence of a yeast phosphatase is clearly indicated by the dramatic increase in cellular tyrosine phosphorylation upon incubation with pervanadate (this study) or phenylarsine (unpublished observations). While at least five tyrosine phosphatases have been identified in *S. pombe* (Cdc25, Pyp1, Pyp2, Pyp3, and Stp1) (1, 30), it is unknown which, if any, of these dephosphorylate heterologously expressed mammalian tyrosine kinases. A second potential explanation for increased PDGFβ activation upon coexpression with PLCγ2 is enhanced receptor dimerization through unconstrained receptor-effector interaction. Indeed, nonhormonal agents causing receptor aggregation, such as cross-linking antibodies, polycations, or protein precipitation, as well as modified receptors with artificial disulfide interactions have been shown to mimic ligand-dependent dimerization and promote receptor activation (28, 45). One potential implication of these observations is that physiologic compartmentalization of signalling elements in mammalian cells, which dictates the concentration of effectors in the immediate proximity of receptor tyrosine kinases, could play a key role controlling receptor activation state and regulating sensitivity to ligand stimulation.

In summary, we report the successful functional interaction between mammalian tyrosine kinases and a downstream effector target in a yeast. This system has allowed the first demonstration of PLCγ activation following direct phosphorylation by Src. PLCγ activation is a critical event underlying immunological (42, 53), proliferative (50), chemotactic (18), and differentiative (36, 46) responses to tyrosine kinase stimulation and, in addition, may mediate the transforming actions of some oncogenic tyrosine kinases (39). Heterologous expression in *S. pombe* should therefore provide an excellent system for molecular analysis and dissection of regulatory interactions controlling a signal transduction system critically important under a range of biological and pathophysiological conditions.

ACKNOWLEDGMENTS

We are grateful to S. A. Courtneidge (EMBL, Heidelberg, Germany) for supplying the Csk expression plasmid, T. M. Gilmer (Glaxo Research Institute, Research Triangle Park, N.C.) for cDNA encoding c-Src, Y. Emori (University of Tokyo) for cDNA and antibodies for PLCγ2, and L. T. Williams (University of California, San Francisco) for the PDGFβ receptor cDNA and antibodies. We also thank Corine Gillieron and Elisabeth Schmid for superb technical assistance as well as Jonathan Knowles for his ceaseless encouragement and for reading the manuscript critically.

REFERENCES

1. **Atherton-Fessler, S., G. Hannig, and H. Piwnica-Worms.** 1993. Reversible tyrosine phosphorylation and cell cycle control. Semin. Cell Biol. **4:**433–442.
2. **Berridge, M. J.** 1987. Inositol trisphosphate and diacylglycerol: two interacting second messengers. Annu. Rev. Biochem. **56:**159–193.
3. **Boulton, T. G., N. Stahl, and G. D. Yancopoulous.** 1994. Ciliary neurotrophic factor/leukemia inhibitory factor/interleukin 6/oncostatin M family of cytokines induces tyrosine phosphorylation of a common set of proteins overlapping those induced by other cytokines and growth factors. J. Biol. Chem. **269:**11648–11655.
4. **Coggeshall, K. M., J. C. McHugh, and A. Altman.** 1992. Predominant expression and activation-induced tyrosine phosphorylation of phospholipase C-γ2 in B lymphocytes. Proc. Natl. Acad. Sci. USA **89:**5660–5664.
5. **Emori, Y., Y. Homma, H. Sorimachi, H. Kawasaki, O. Nakanishi, K. Suzuki, and T. Takenawa.** 1989. A second type of rat phosphoinositide-specific phospholipase C containing a Src-related sequence not essential for phosphoinositide-hydrolyzing activity. J. Biol. Chem. **264:**21885–21890.
6. **Goldschmidt-Clermont, P. J., J. W. Kim, L. M. Machesky, S. G. Rhee, and T. D. Pollard.** 1991. Regulation of phospholipase C-γ1 by profilin and tyrosine phosphorylation. Science **251:**1231–1233.
7. **Goode, N. T., and P. J. Parker.** 1994. A phorbol ester-responsive PKC-ζ generated by fusion with the regulatory domain of PKC-δ. FEBS Lett. **340:**145–150.
8. **Gout, I., R. Dhand, I. D. Hiles, M. J. Fry, G. Panayotou, P. Das, O. Truong, N. F. Totty, J. Hsuan, G. W. Booker, I. D. Campbell, and M. D. Waterfield.** 1993. The GTPase dynamin binds to and is activated by a subset of SH3

domains. Cell **75:**25–36.
9. **Guinebault, C., B. Payrastre, C. Sultan, G. Mauco, M. Breton, S. Levy-Toledano, M. Plantavid, and H. Chap.** 1993. Tyrosine kinases and phosphoinositide metabolism in thrombin-stimulated human platelets. Biochem. J. **292:**851–856.
10. **Gutz, H., H. Heslot, U. Leupold, and N. Loprieno.** 1974. *Schizosaccharomyces pombe*, p. 395–446. *In* R. D. King (ed.), Handbook of genetics, vol. 1. Plenum Press, New York.
11. **Heffetz, D., I. Bushkin, R. Dror, and Y. Zick.** 1990. The insulinomimetic agents H_2O_2 and vanadate stimulated tyrosine phosphorylation in intact cells. J. Biol. Chem. **265:**2896–2902.
12. **Homma, Y., H. Sakamoto, M. Tsunoda, M. Aoki, T. Takenawa, and T. Ooyama.** 1993. Evidence for involvement of phospholipase C-γ2 in signal transduction of platelet-derived growth factor in vascular smooth muscle cells. Biochem. J. **290:**649–653.
13. **Hughes, D. A., A. Ashworth, and C. J. Marshall.** 1993. Complementation of byr1 in fission yeast by mammalian MAP kinase kinase requires coexpression of Raf kinase. Nature (London) **364:**349–352.
14. **Jensen, R. A., J. F. Beeler, M. A. Heidaran, and W. J. LaRochelle.** 1992. Characterization of baculovirus-expressed human α and β platelet-derived growth factor receptors. Biochemistry **31:**10887–10892.
15. **Kodaki, T., R. Woscholski, B. Hallberg, P. Rodriguez-Viciana, J. Downward, and P. J. Parker.** 1994. The activation of phosphatidylinositol 3-kinase by Ras. Curr. Biol. **4:**798–806.
16. **Kuhne, M. R., Z. Zhao, J. Rowles, B. E. Lavan, S.-S. Shen, E. H. Fischer, and G. E. Lienhard.** 1994. Dephosphorylation of insulin receptor substrate 1 by the tyrosine phosphatase PTP2C. J. Biol. Chem. **269:**15833–15837.
17. **Kumjian, D. A., A. Barnstein, S. G. Rhee, and T. O. Daniel.** 1991. Phospholipase C-γ complexes with ligand-activated platelet-derived growth factor receptors. An intermediate implicated in phospholipase activation. J. Biol. Chem. **266:**3973–3980.
18. **Kundra, V., J. A. Escobedo, A. Kazlauskas, H. K. Kim, S. G. Rhee, L. T. Williams, and B. R. Zetter.** 1994. Regulation of chemotaxis by the platelet-derived growth factor receptor-β. Nature (London) **367:**474–476.
19. **Lammers, R., B. Bossenmaier, D. E. Cool, N. K. Tonks, J. Schlessinger, E. H. Fischer, and A. Ullrich.** 1993. Differential activities of protein tyrosine phosphatases in intact cells. J. Biol. Chem. **268:**22456–22462.
19a. **Leberer, E., and K. Maundrell.** Unpublished data.
20. **Li, W., G. G. Deanin, B. Margolis, J. Schlessinger, and J. M. Olivier.** 1992. FcεR1-mediated tyrosine phosphorylation of multiple proteins, including phospholipase Cγ1 and the receptor $\beta\gamma_2$ complex, in RBL-2H3 rat basophilic leukemia cells. Mol. Cell. Biol. **12:**3176–3182.
21. **Liao, F., H. S. Shin, and S. G. Rhee.** 1992. Tyrosine phosphorylation of phospholipase C-γ1 induced by cross-linking of the high-affinity or low-affinity Fc receptor for IgG in U937 cells. Proc. Natl. Acad. Sci. USA **89:**3659–3663.
22. **Liao, F., H. S. Shin, and S. G. Rhee.** 1993. In vitro phosphorylation of PLC-γ1 and PLC-γ2 by src-family protein tyrosine kinases. Biochem. Biophys. Res. Commun. **191:**1028–1033.
23. **Maa, M.-C., T.-H. Leu, B. J. Trandel, J.-H. Chang, and S. J. Parsons.** 1994. A protein that is highly related to GTPase-activating protein-associated p62 complexes with phospholipase Cγ. Mol. Cell. Biol. **14:**5466–5473.
24. **Margolis, B., S. G. Rhee, S. Felder, M. Mervic, R. Lyall, A. Levitzki, A. Ullrich, A. Zilberstein, and J. Schlessinger.** 1989. EGF induces tyrosine phosphorylation of phospholipase C-II: a potential mechanism for EGF receptor signaling. Cell **57:**1101–1107.
25. **Margolis, B., A. Ziberstein, C. Franks, S. Felder, S. Kremer, A. Ullrich, S. G. Rhee, K. Skorecki, and J. Schlessinger.** 1990. Effect of phospholipase C-γ overexpression on PDGF-induced second messengers and mitogenesis. Science **248:**607–610.
26. **Maundrell, K.** 1993. Thiamine-repressible expression vectors pREP and pRIP for fission yeast. Gene **123:**127–130.
27. **Meisenhelder, J., P.-G. Suh, S. G. Rhee, and T. Hunter.** 1989. Phospholipase C-γ is a substrate for the PDGF and EGF receptor protein-tyrosine kinases in vivo and in vitro. Cell **57:**1109–1122.
28. **Mohammadi, M., A. Honegger, A. Sorokin, A. Ullrich, J. Schlessinger, and D. R. Hurwitz.** 1993. Aggregation-induced activation of the epidermal growth factor receptor protein tyrosine kinase. Biochemistry **32:**8742–8748.
29. **Mohammadi, M., A. M. Honegger, D. Rotin, R. Fischer, F. Bellot, W. Li, C. A. Dionne, M. Jaye, M. Rubinstein, and J. Schlessinger.** 1991. A tyrosine-phosphorylated carboxy-terminal peptide of the fibroblast growth factor receptor (Flg) is a binding site for the SH2 domain of phospholipase C-γ1. Mol. Cell. Biol. **11:**5068–5078.
30. **Mondesert, O., S. Moreno, and P. Russell.** 1994. Low molecular weight protein-tyrosine phosphatases are highly conserved between fission yeast and man. J. Biol. Chem. **269:**27996–27999.
31. **Moreno, S., A. Klar, and P. Nurse.** 1991. Molecular genetic analysis of fission yeast, *Schizosaccharomyces pombe*. Methods Enzymol. **194:**795–823.
32. **Mori, S., L. Ronnstrand, K. Yokote, A. Engstrom, S. A. Courtneidge, L. Claesson-Welsh, and C.-H. Heldin.** 1993. Identification of two juxtamembrane autophosphorylation sites in the PDGF β-receptor; involvement in the interaction with Src family tyrosine kinases. EMBO J. **12:**2257–2264.
33. **Morrison, D. K., D. R. Kaplan, S. G. Rhee, and L. T. Williams.** 1990. Platelet-derived growth factor (PDGF)-dependent association of phospholipase C-γ with the PDGF receptor signalling complex. Mol. Cell. Biol. **10:**2359–2366.
34. **Nishibe, S., M. I. Wahl, S. M. T. Hernandez-Sotomayor, N. K. Tonks, S. G. Rhee, and G. Carpenter.** 1990. Increase of the catalytic activity of phospholipase C-γ by tyrosine phosphorylation. Science **250:**1253–1256.
35. **Nishizuka, Y.** 1992. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science **258:**607–614.
36. **Obermeier, A., R. A. Bradshaw, K. Seedorf, A. Choidas, J. Schlessinger, and A. Ullrich.** 1994. Neuronal differentiation signals are controlled by nerve growth factor receptor/Trk binding sites for SHC and PLCγ. EMBO J. **13:**1585–1590.
37. **Park, D. J., H. K. Min, and S. G. Rhee.** 1991. IgE-induced tyrosine phosphorylation of phospholipase C-γ1 in rat basophilic leukemia cells. J. Biol. Chem. **266:**24237–24240.
38. **Park, D. J., H. W. Rho, and S. G. Rhee.** 1991. CD3 stimulation causes phosphorylation of phospholipase C-γ1 on serine and tyrosine residues in a human T-cell line. Proc. Natl. Acad. Sci. USA **88:**5453–5456.
39. **Peles, E., R. B. Levy, E. Or, A. Ullrich, and Y. Yarden.** 1991. Oncogenic forms of the neu/HER2 tyrosine kinase are permanently coupled to phospholipase Cγ. EMBO J. **10:**2077–2086.
40. **Ren, C. L., T. Morio, S. M. Fu, and R. S. Geha.** 1994. Signal transduction via CD40 involves activation of lyn kinase and phosphatidylinositol-3-kinase, and phosphorylation of phospholipase C-γ2. J. Exp. Med. **179:**673–680.
41. **Ren, H.-Y., N. Komatsu, R. Shimizu, K. Okada, and Y. Miura.** 1994. Erythropoietin induces tyrosine phosphorylation and activation of phospholipase C-γ1 in a human erythropoietin-dependent cell line. J. Biol. Chem. **269:**19633–19638.
42. **Rhee, S. G., and K. D. Choi.** 1992. Multiple forms of phospholipase C isozymes and their activation mechanisms. Adv. Second Messenger Phosphoprotein Res. **26:**35–61.
43. **Schlessinger, J., and A. Ullrich.** 1992. Growth factor signaling by receptor tyrosine kinases. Neuron **9:**383–391.
44. **Sieh, M., A. Batzer, J. Schlessinger, and A. Weiss.** 1994. GRB2 and phospholipase C-γ1 associate with a 36- to 38-kilodalton phosphotyrosine protein after T-cell receptor stimulation. Mol. Cell. Biol. **14:**4435–4442.
45. **Sorokin, A., M. A. Lemmon, A. Ullrich, and J. Schlessinger.** 1994. Stabilization of an active dimeric form of the epidermal growth factor receptor by introduction of an inter-receptor disulfide bond. J. Biol. Chem. **269:**9752–9759.
46. **Stephens, R. M., D. M. Loeb, T. D. Copeland, T. Pawson, L. A. Greene, and D. R. Kaplan.** 1994. Trk receptors use redundant signal transduction pathways involving SHC and PLC-γ1 to mediate NGF responses. Neuron **12:**691–705.
47. **Sultzman, L., C. Ellis, L. L. Lin, T. Pawson, and J. Knopf.** 1991. Platelet-derived growth factor increases the in vivo activity of phospholipase C-γ1 and phospholipase C-γ2. Mol. Cell. Biol. **11:**2018–2025.
48. **Superti-Furga, G., S. Fumagalli, M. Koegl, S. A. Courtneidge, and G. Draetta.** 1993. Csk inhibition of c-Src activity requires both the SH2 and SH3 domains of Src. EMBO J. **12:**2625–2634.
49. **Twamley-Stein, G. M., R. Pepperkok, W. Ansorge, and S. A. Courtneidge.** 1993. The Src family tyrosine kinases are required for platelet-derived growth factor-mediated signal transduction in NIH 3T3 cells. Proc. Natl. Acad. Sci. USA **90:**7696–7700.
50. **Valius, M., and A. Kazlauskas.** 1993. Phospholipase C-γ1 and phosphatidylinositol 3 kinase are the downstream mediators of the PDGF receptor's mitogenic signal. Cell **73:**321–334.
51. **Wahl, M. I., G. A. Jones, S. Nishibe, S. G. Rhee, and G. Carpenter.** 1992. Growth factor stimulation of phospholipase C-γ1 activity. Comparative properties of control and activated enzymes. J. Biol. Chem. **267:**10447–10456.
52. **Weiss, A., G. Koretzky, R. C. Schatzman, and T. Kadlecek.** 1991. Functional activation of T-cell antigen receptor induces tyrosine phosphorylation of phospholipase C-γ1. Proc. Natl. Acad. Sci. USA **88:**5484–5488.
53. **Weiss, A., and D. R. Littman.** 1994. Signal transduction by lymphocyte antigen receptors. Cell **76:**263–274.
54. **Yang, L. J., S. G. Rhee, and J. R. Williamson.** 1994. Epidermal growth factor-induced activation and translocation of phospholipase C-γ1 to the cytoskeleton in rat hepatocytes. J. Biol. Chem. **269:**7156–7162.
55. **Yarden, Y., J. A. Escobedo, W.-J. Kuang, T. L. Yang-Feng, T. O. Daniel, P. M. Tremble, E. Y. Chen, M. E. Ando, R. N. Harkins, U. Francke, V. A. Fried, A. Ullrich, and L. T. Williams.** 1986. Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. Nature (London) **323:**226–232.