Src kinase regulation by phosphorylation and dephosphorylation

Robert Roskoski Jr.*

Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, 1100 Florida Avenue, New Orleans, LA 70119, USA

Received 2 March 2005
Available online 19 March 2005

Abstract

Src and Src-family protein-tyrosine kinases are regulatory proteins that play key roles in cell differentiation, motility, proliferation, and survival. The initially described phosphorylation sites of Src include an activating phosphotyrosine 416 that results from autophosphorylation, and an inhibiting phosphotyrosine 527 that results from phosphorylation by C-terminal Src kinase (Csk) and Csk homologous kinase. Dephosphorylation of phosphotyrosine 527 increases Src kinase activity. Candidate phosphotyrosine 527 phosphatases include cytoplasmic PTP1B, Shp1 and Shp2, and transmembrane enzymes include CD45, PTPα, PTPε, and PTPκ.

Dephosphorylation of phosphotyrosine 416 decreases Src kinase activity. Thus far PTP-BL, the mouse homologue of human PTP-BAS, has been shown to dephosphorylate phosphotyrosine 416 in a regulatory fashion. The platelet-derived growth factor receptor protein-tyrosine kinase mediates the phosphorylation of Src Tyr138; this phosphorylation has no direct effect on Src kinase activity. The platelet-derived growth factor and the ErbB2/HER2 growth factor receptor protein-tyrosine kinases mediate the phosphorylation of Src Tyr213 and activation of Src kinase activity. Src kinase is also a substrate for protein-serine/threonine kinases including protein kinase C (Ser12), protein kinase A (Ser17), and CDK1/cdc2 (Thr34, Thr46, and Ser72). Of the three protein-serine/threonine kinases, only phosphorylation by CDK1/cdc2 has been demonstrated to increase Src kinase activity. Although considerable information on the phosphoprotein phosphatases that catalyze the hydrolysis of Src phosphotyrosine 527 is at hand, the nature of the phosphatases that mediate the hydrolysis of phosphotyrosine 138 and 213, and phosphoserine and phosphothreonine residues has not been determined.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Cancer; CD45; Epidermal growth factor receptor; ErbB/HER receptors; Integrin receptor; Non-receptor protein-tyrosine kinase; Oncogene; Platelet-derived growth factor receptor; Protein kinase; Protein phosphatase; Proto-oncogene; PTPα; PTPε; PTPκ; PTP-BAS; Shp1; Shp2; Src-family kinases
protein-tyrosine kinases in the human genome of which 58 are transmembrane receptors and 32 are non-receptor proteins.

Phosphoprotein phosphatases reverse the action of protein kinases by catalyzing the following process:

\[
\text{Protein-O}\text{PO}_3^{2-} + \text{H}_2\text{O} \rightarrow \text{protein-OH} + \text{HOPO}_3^{2-}
\]

These enzymes are classified as protein-serine/threonine phosphatases, protein-tyrosine phosphatases, and dual specificity (both protein-phosphoserine/threonine and protein-phosphotyrosine) phosphatases. Alonso et al. [2] report that there are 107 human protein-tyrosine phosphatase genes divided into four classes. The first (class I cysteine-based PTPs) contains 38 classical protein-tyrosine phosphatases and 61 dual specificity phosphatases. The second (class II cysteine-based PTP) contains a single low molecular weight enzyme. The third (class III cysteine-based PTPs) contains three dual specificity enzymes that play a role in cell cycle regulation, and the fourth (class IV aspartate-based PTPs) consists of four dual specificity phosphatases.

**Functions of Src**

Src and Src-family protein kinases play key roles in cell differentiation, motility, proliferation, and survival [3]. From the N- to C-terminus, Src contains an N-terminal 14-carbon myristoyl group, an SH4 domain, a unique segment, an SH3 domain, an SH2 domain, a protein-tyrosine kinase domain, and a C-terminal regulatory tail (Fig. 1). Src-family kinases are controlled by receptor protein-tyrosine kinases, integrin receptors, G-protein coupled receptors, antigen- and Fc-coupled receptors, cytokine receptors, and steroid hormone receptors [3]. Src signals to a variety of downstream effectors including, inter alia, p85 (the regulatory subunit of phosphatidylinositol 3-kinase), RasGAP (Table 1), Shc (Table 1), phospholipase C\(_\gamma\), several integrin signaling proteins (tensin, vinculin, cortactin, talin, and paxillin), and focal adhesion kinase [4]. v-Src (a viral protein) is encoded by the avian cancer-causing oncogene of Rous sarcoma virus, and Src (the cellular homologue in humans, chickens, and other animals) is encoded by a physiological gene, the first of the proto-oncogenes [5].

Src is expressed ubiquitously in vertebrate cells; however, brain, osteoclasts, and platelets express 5- to 200-fold higher levels of this protein than most other cells [4]. In fibroblasts, Src is bound to endosomes, perinuclear membranes, secretory vesicles, and the cytoplasmic face of the plasma membrane where it can interact with a variety of growth factor and integrin receptors [3,4]. The expression of high levels of Src in platelets

![Fig. 1. Protein kinase phosphorylation sites and organization of Src. Except for the aliphatic myristoyl group attached to the SH4 domain, the relative length of the domains is to scale. The chicken numbering system is displayed. S is serine, T is threonine, and Y is tyrosine.](image)

**Table 1**

<table>
<thead>
<tr>
<th>Interacting protein</th>
<th>Function/comments</th>
<th>Swiss-Prot Accession Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>p62Dok</td>
<td>Docking protein downstream of tyrosine kinase; interacts with RasGTPase</td>
<td>Q99704</td>
</tr>
<tr>
<td>Gab1</td>
<td>Grb2-associated binding protein 1</td>
<td>Q13480</td>
</tr>
<tr>
<td>Grb2</td>
<td>Growth factor receptor-bound protein 2</td>
<td>P62993</td>
</tr>
<tr>
<td>PLC(_\gamma)_1</td>
<td>Phospholipase C(_\gamma)_1 catalyzes the hydrolysis of phosphatidylinositol bisphosphate to generate diacylglycerol and inositol 1,4,5-trisphosphate</td>
<td>P19174</td>
</tr>
<tr>
<td>RasGAP</td>
<td>Ras GTPase activating protein; Ras (rat sarcoma) is a signal transduction protein with GTPase activity</td>
<td>P20936</td>
</tr>
<tr>
<td>Rho</td>
<td>A small GTPase that regulates a signal transduction pathway linking plasma membrane receptors to the assembly of focal adhesions and actin stress fibers; rho is a Ras homologue</td>
<td>P61586</td>
</tr>
<tr>
<td>Shc</td>
<td>Src homology 2 domain-containing transforming protein C1; binds Grb2</td>
<td>P29353</td>
</tr>
<tr>
<td>Vav2</td>
<td>A guanine nucleotide exchange factor for Rac, a member of the Rho family of GTPases; vav is the sixth letter of the Hebrew alphabet</td>
<td>P52735</td>
</tr>
</tbody>
</table>

* Human orthologs.
(anucleate cells) and in neurons (which are postmitotic) indicates that Src participates in processes other than cell division.

**Phosphorylation of Src at tyrosine residues**

Src and Src-family protein kinases are exactlying regulated. One of the important phosphorylation sites of Src is Tyr527, six residues from the C-terminus (Src numbering in this paper refers to the prototypical chicken Src sequence unless otherwise noted). Under basal conditions in vivo, 90–95% of Src is phosphorylated at Tyr527 [6], and phosphotyrosine 527 binds intramolecularly with the Src SH2 domain. This intramolecular association stabilizes a dormant form of the enzyme [7]. The Tyr527Phe Src mutant is more active than the wild-type enzyme and can induce anchorage-independent growth in cell culture and tumors in vivo [8,9]. Src undergoes an intermolecular autophosphorylation at tyrosine 416; this residue is present in the activation loop, and its phosphorylation promotes kinase activity [7].

C-terminal Src kinase (Csk), a cytoplasmic protein-tyrosine kinase, was the first enzyme discovered that catalyzes the phosphorylation of the regulatory C-terminal tail tyrosine of Src. Okada and Nakagawa [10] isolated this enzyme from neonatal rat brain and demonstrated that it catalyzes the phosphorylation of Src at Tyr527. Following phosphorylation, the $K_m$ of Src for ATP and for acid-denatured enolase is unchanged, but the $V_{max}$ is decreased 50%. Using purified Src, the activity of the Tyr527 phosphorylated enzyme in vitro ranges from 0.2% to 20% of the unphosphorylated enzyme depending upon the experimental conditions.

Chk (Csk homology kinase) is a second enzyme that catalyzes the phosphorylation of the inhibitory tyrosine of Src-family kinases [11]. Csk is expressed in all mammalian cells, whereas Chk is limited to breast, hematopoietic cells, neurons, and testes [4]. Csk and Chk consist of an SH3, SH2, and kinase domain; these enzymes lack the N-terminal myristoyl group and the C-terminal regulatory tail phosphorylation site found in Src [7]. Besides inactivating Src by catalytic phosphorylation, Chk forms an inhibitory non-covalent complex with Src. The association of Chk with the activated and autophosphorylated form of Src inhibits Src kinase activity [12]. The action of Chk thereby overrides that of Src. Chk can also bind to unphosphorylated Src and prevent its autophosphorylation.

Besides phosphorylation of Src Tyr527 by Csk and Chk, the platelet-derived growth factor receptor protein-tyrosine kinase mediates the phosphorylation of Tyr138 in the SH2 domain in a recombinant enzyme and in mouse Balb/c cells in culture [13]. Phosphorylation by the PDGF receptor leads to a twofold activation of Src kinase activity. However, if Src is prepared containing pTyr527 after treatment with Csk and ATP, phosphorylation by the PDGF receptor results in 50-fold activation. This is physiologically relevant because 90–95% of Src in cells is phosphorylated at Tyr527 [14]. If Src is first phosphorylated by the PDGF receptor and ATP, and then treated with Csk and ATP, Src inhibition is greatly reduced when compared with the enzyme not treated with PDGF receptor and ATP. Since the amount of PDGF receptor was 1/20th that of Src, activation is related to phosphorylation and not to the formation of a binary enzyme complex. An important conclusion from these experiments is that inhibition by Tyr527 phosphorylation can be overridden by a process other than pTyr527 dephosphorylation, namely Tyr213 phosphorylation [13]. Stover et al. [13] hypothesize that phosphorylation of Tyr213 decreases the ability of the Src SH2 domain to form an intramolecular bond with pTyr527 thereby leading to Src activation.

PDGF treatment of mouse fibroblasts results in phosphorylation of another tyrosine, Tyr138, of Src [15]. Tyr138 of recombinant Src is also phosphorylated by the PDGF receptor in vitro. Src Tyr138 phosphorylation by the PDGF receptor occurs in a Src-kinase dead mutant (Lys297Met), which indicates that this reaction is not related to Src autophosphorylation. Although this phosphorylation requires the association of the PDGF receptor with the Src SH2 domain, phosphorylation occurs at a residue in the Src SH3 domain. As a result, the phosphorylated Src SH3 domain binds less tightly to SH3 ligands. One plausible consequence of Tyr138 phosphorylation is a reduction in Src autoinhibition resulting from the decreased binding of the Src SH3 domain to the SH2-kinase linker [7]. Alternatively, Tyr138 phosphorylation may increase Src activation by extrinsic Src SH3 ligands.

The regulatory role of Src Tyr138 phosphorylation in response to PDGF stimulation is unclear. Tyr138Phe mutants of Src are activated to the same extent as wild-type Src in cells following PDGF stimulation. Thus, Tyr138 phosphorylation is not required for Src activation by the PDGF receptor. The Tyr138Phe mutant does not cause malignant transformation of cells, and this result suggests that this mutant is not constitutively activated. In contrast to the report of Broome and Hunter [15], Stover et al. [13] did not find evidence for the phosphorylation of tyrosine 138 in their experiments, and the reason for this discrepancy is unclear [15].

Vadlamudi et al. [16] examined Src phosphorylation patterns in MCF-7 human breast cancer cells that express ErbB2/HER2 and ErbB3/HER3, which are members of the epidermal growth factor receptor family [17]. These workers found that HER2 signaling increases Src Tyr213 phosphorylation in cells (chicken numbering) and enzyme activity in vitro. Heregulin, a HER3 activator also known as neuregulin [17], also increases
phosphorylation of Tyr861 of focal adhesion kinase. The HER3 receptor responds to heregulin but is kinase dead [17]. HER3 forms a heterodimer with HER2 that results in activation of HER2 protein-tyrosine kinase activity. The heregulin-mediated phosphorylation is downstream of Src as indicated by the lack of Tyr861 phosphorylation of focal adhesion kinase in cells containing a Tyr213Phe Src mutant.

Phosphorylation of Src at serine and threonine residues

Besides tyrosine phosphorylation, Src is phosphorylated by protein-serine/threonine kinases. Ser12 of mouse Src is a substrate for protein kinase C both in vivo (mouse NIH3T3 cells) and in vitro [14]. Moreover, Ser12 and Ser48 of chicken Src are phosphorylated by PKC in vitro (mammalian Src lacks the residue corresponding to Ser48). Gould and Hunter [14] demonstrated that human, mouse, and rat fibroblasts treated with agents that stimulate phosphatidylinositol turnover lead to phosphorylation of Ser12. Of these agents, PDGF leads to the activation of Src kinase activity measured in immune complexes. The other agents used to stimulate phosphatidylinositol turnover, which included fibroblast growth factor, prostaglandin F2α, serum, vasopressin, phorbol ester, and sodium vanadate, fail to alter Src activity measured in immune complexes. The reason for this discrepancy is unclear. Ser17 is constitutively phosphorylated in fibroblasts [14]. This serine has a protein kinase A consensus sequence (ArgArgArgSerLeu).

A triad of Src serine and threonine residues is phosphorylated by CDK1/cdc2 kinase, an enzyme that plays a key role in transition through the cell cycle. These residues include Thr34, Thr46, and Ser72 (Table 2) [18,19]. The mechanism of activation by the phosphorylation of these residues is unclear. Phosphorylation near the amino terminus (Fig. 1) may promote a conformational change that decreases intramolecular SH2 and SH3 interactions that promote a dormant state [7]. PKA, PKC, and CDK1 are able to catalyze the phosphorylation of residues in pTyr527 and Tyr527 Src in vitro with equal efficiency [19]. In contrast, protein kinase G and ERK are unable to catalyze the phosphorylation of either form of Src.

Src dephosphorylation

An important mechanism for Src activation involves pTyr527 dephosphorylation. Candidate phosphatases include cytoplasmic PTP1B, Shp1 (Src homology 2 domain-containing tyrosine phosphatase 1) and Shp2, and transmembrane enzymes including CD45, PTPα, PTPε, and PTPβ [20]. Thus, far PTP-βL, the mouse homologue of human PTP-BAS, has been shown to dephosphorylate Src phosphotyrosine 416 in a regulatory manner. Although considerable information on Src protein-tyrosine kinases is at hand, the nature of the phosphoprotein phosphatases that mediate the regulatory dephosphorylation of pTyr138, pTyr213, pSer12, pSer17, pThr34, pThr46, and pSer72 in Src has not been determined.

Properties of protein-tyrosine phosphatase 1B (PTP1B), the prototypic protein-tyrosine phosphatase

PTP1B was the first protein-tyrosine phosphatase that was purified, whose cDNA was sequenced, and whose structure was determined by X-ray crystallography [21–24]. PTP1B is a cytosolic protein-tyrosine phosphatase that is expressed in most cells and plays a role in several signal transduction pathways including that of insulin. Moreover, PTP1B has broad substrate specificity when compared with other phosphatases such as PTPα. PTP1B contains eight α-helices and 12 β-strands with a 10-stranded β-sheet that adopts a twisted conformation that spans the length of the molecule [22]. Just as the tertiary structures of protein kinases are similar, the catalytic domains of protein-tyrosine phosphatases resemble that of PTP1B.

Andersen et al. [25] identified 10 conserved signature sequences in the classical PTPs, several of which are described here. The active-site sequence of HCSXGX GR(T/S)G (residues 213–222 in PTP1B) is the defining

<table>
<thead>
<tr>
<th>Residuea</th>
<th>Protein kinase</th>
<th>Citations</th>
<th>Effect on Src activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser12</td>
<td>PKC</td>
<td>[14]</td>
<td>No direct effect</td>
</tr>
<tr>
<td>Ser17</td>
<td>PKA</td>
<td>[14]</td>
<td>Unclear</td>
</tr>
<tr>
<td>Thr34, Thr46, Ser72</td>
<td>CDK1/cdk2</td>
<td>[18,19]</td>
<td>Activation of pTyr527 Src</td>
</tr>
<tr>
<td>Tyr138</td>
<td>PDGF receptor,</td>
<td>[15]</td>
<td>No direct effect</td>
</tr>
<tr>
<td>Tyr213</td>
<td>PDGF receptor, ErbB2/HER2</td>
<td>[13,16]</td>
<td>Activation</td>
</tr>
<tr>
<td>Tyr416</td>
<td>Src autophosphorylation</td>
<td>[7]</td>
<td>Activation</td>
</tr>
<tr>
<td>Tyr527</td>
<td>Csk, Chk</td>
<td>[6–11]</td>
<td>Inhibition</td>
</tr>
</tbody>
</table>

* Chicken Src numbering.
PTP signature motif. This is sometimes abbreviated as CX_5R. This motif forms the phosphate-binding loop that is at the base of the active site cleft, and cysteine and arginine play a key role in mediating hydrolytic removal of phosphate. Cysteine 215 in this segment acts as a nucleophile and accepts phosphate transiently during catalysis (Fig. 2) [26]. Mutation of the catalytic cysteine to serine or alanine abolishes enzyme activity.

The nucleophilicity of the catalytic cysteine (Cys215) results from its close proximity to main-chain amide groups, a hydrogen bond with the side chain of Ser222 of the PTP signature motif, and an invariant arginine (Arg257 in PTP1B) all of which decrease the pK_a of the catalytic cysteine to about 4.6 (the pK_a of the thiol group of free cysteine is about 8). The invariant cationic PTP arginine (Arg221 in PTP1B) binds the anionic phosphotyrosine substrate and stabilizes the phosphoenzyme intermediate [27].

The PTP1B Tyr46 side chain, which forms part of the active site, interacts with the aromatic ring of pTyr in peptide substrates (Fig. 3). The Tyr46Phe mutant protein has reasonable activity toward p-nitrophenylphosphate and a peptide corresponding to the epidermal growth factor receptor (receptor residues 988–996), indicating that the –OH group is dispensable for catalysis [28]. In contrast, a Tyr46Ala mutation greatly diminishes the enzymatic activity toward p-nitrophenylphosphate and the peptide substrate. Based upon steady-state enzyme kinetic studies and X-ray crystallography, the aromatic ring of Tyr46 binds to that of the pTyr substrate.

The side chain of Arg47 interacts with PTP1B substrates in two distinct modes [28]. An acidic or a hydrophobic residue in the pTyr-1 position characterizes PTP1B substrates. The guanidinium group of Arg47 forms a salt bridge with substrates containing an acidic residue at this position. Arg47 adopts a second conformation with the aliphatic portion of its R group interacting with a hydrophobic residue at the pTyr-1 position. The adjacent residue of PTP1B, Asp48, positions peptide substrates in an optimal location for peptide binding and the initial nucleophilic attack by the catalytic cysteine.

The binding of phosphopeptides to the PTP signature sequence (CX_5R) promotes a major shift in the WPD surface loop (residues 179–185 in PTP1B); the loop moves over the substrate tyrosyl ring and traps the bound phosphotyrosine [29–31]. This loop has a WPDXGXP consensus sequence that contains Asp181 (Fig. 3), a general acid–base catalyst. The movement of the loop shifts the side chain of Phe182 toward the pTyr-binding site, allowing hydrophobic stacking and binding to the phenyl ring of the pTyr residue of the substrate.

The two proline residues and glycine in the bend of the WPDXGXP segment are critical for the dynamics

---

**Fig. 2.** Two-step mechanism of protein phosphotyrosine phosphatases. The reaction proceeds via a covalent thiophosphate intermediate. The numbered residues correspond to human PTP1B.
of the WPD loop motion [29]. Closure of the WPD loop is important for phosphotyrosine hydrolysis because it positions Asp181 close to the scissile oxygen of the tyrosyl substrate allowing Asp181 to donate a proton to the phenolate-leaving group during the first step of hydrolysis [27]. Asp181 functions as a general base in the second step of phosphatase action where it accepts a proton from the attacking hydrolytic water molecule (Fig. 2). The Asp181Ala mutation produces a protein with 1/10^5 the \( k_{\text{cat}} \) of the wild-type enzyme [30]. Asp181Ala mutants of PTP1B have been used to trap substrates non-covalently in vivo and in vitro. This mutation supports the notion that Asp181 is necessary for the first step in the reaction, namely cleavage of the O–P bond of substrate phosphotyrosine by forming an S–P linkage by transphosphorylation [30,31].

Glutamine 262 of the catalytic-water motif (QTXXQYXF, residues 262–269) positions and activates the water molecule involved in the hydrolysis of the phosphocysteine enzyme complex during the second step of catalysis (Fig. 2). This intermediate was visualized by X-ray crystallography in a Gln262Ala mutant [23]. The two glutamine residues in this motif restrict the reaction of the phosphocysteine residue with water and not with other nucleophile acceptors thereby preventing the adventitious transphosphorylation of other proteins from phosphorylated PTP1B.

**PTP1B catalyzed dephosphorylation of Src phosphotyrosine 527**

Src protein-tyrosine kinase activity is elevated in several types of human cancers including those of breast, colon, lung, pancreas, ovary, and stomach [20,32–34]. Activation is attributed to both elevated Src expression and increased specific activity. Src activation by mutation in human cancers is rare [20]. Members of the epidermal growth factor receptor family exhibit increased activity in breast cancer cells [17]; these activated receptors lead to increased Src activity and the production of neoplasms [35].

Bjorge et al. [36] identified an enzyme in MDA-MB-435S cells, a human breast cancer cell line, that dephos-
phosphorylates human Src and a peptide corresponding to the Src carboxyl-terminal negative regulatory tail. Extracts of these cells were chromatographed on a Q-Sepharose column; the fractions that catalyze the hydrolysis of pTyr527 of Src reacted with an anti-PTP1B antibody. In contrast, these fractions failed to react with antibodies against protein-tyrosine phosphatases cdc25b, Shp1, Shp2, PTPx, and CD45 (leukocyte common antigen). The active fractions of cell lysates were subjected to three cycles of immunodepletion with anti-PTP1B, and the dephosphorylation of the Src carboxyl-terminal negative regulatory tail was measured. Immunodepletion decreases the dephosphorylation activity 70–90%. Similar experiments were performed with extracts of BT-483, HS578T, and SK-BR-3 human breast cancer cell lines. The finding that more than 50% of the dephosphorylation activity was immunodepleted by anti-PTP1B indicates that the majority of Src pTyr527 dephosphorylation in these cell lines is due to PTP1B. The least phosphatase activity decrease, which resulted from PTP1B immunodepletion, occurred in a non-tumorigenic human breast cell line (HS-578-Bst). Their findings indicate that PTP1B is capable of dephosphorylating Src pTyr527 in several human breast cancer cell lines.

Bjorge et al. [36] performed a Src inactivation/activation assay in vitro. They first inactivated purified Src by phosphorylation of Tyr527 using recombinant C-terminal Src kinase and ATP. The inactivated enzyme was then immunoprecipitated and incubated with a GST-PTP1B fusion protein for the reactivation step. Reactivation is accompanied by dephosphorylation of pTyr527. This group showed that sodium vanadate, a widely used protein-tyrosine phosphatase inhibitor, blocks the dephosphorylation and reactivation of Src.

PTP1B is ubiquitously expressed and has the potential of playing a role in Src regulation by catalyzing pTyr527 dephosphorylation in a variety of cell types. The studies of Bjorge and co-workers suggest that PTP1B dysregulation (increased expression) may play a role in the pathogenesis of breast cancer. PTP1B has high Src pTyr527 dephosphorylation activity in neoplastic but not in normal breast cell lines. Work needs to be performed to determine whether PTP1B activity is increased in human breast cancers. Depending on the outcome of such studies, PTP1B represents a potential drug target in the treatment of breast cancer.

Src is tethered to perinuclear membranes, endosomes, and secretory vesicles, and the cytoplasmic face of the plasma membrane by an N-terminal myristoyl group [7]. PTP1B is classified as a cytoplasmic enzyme because it lacks a transmembrane segment that occurs in phosphatases such as CD45 and PTPx. However, PTP1B possesses a C-terminus with hydrophobic residues that targets the enzyme to the endoplasmic reticulum [37]. PTP1B can also be targeted to focal adhesions and adherens junctions where Src is also found. These studies suggest that PTP1B can function as a Src pTyr527 phosphatase owing to membrane association and co-localization of these proteins.

**Shp1 catalyzed dephosphorylation of Src phosphotyrosine 527**

Human Shp1 is a cytosolic protein-tyrosine phosphatase containing two tandem SH2 domains (residues 4–100 and 110–213), a phosphatase domain (residues 269–514), and a C-terminal tail (residues 515–595). Shp1 occurs primarily in hematopoietic and epithelial cells. Shp1 is a cytosolic protein-tyrosine phosphatase that behaves as a negative regulator of growth factor signaling in hematopoietic cells and a positive regulator of growth factor signaling in some non-hematopoietic cells. Its N-terminal-SH2 domain blocks the catalytic domain and keeps the enzyme in an inactive conformation [38]. One plausible notion for enzyme regulation involves the recruitment and binding of Shp1 SH2 domains to target phosphotyrosine residues with concomitant phosphatase activation.

Shp1 catalyzes the dephosphorylation of Src [39]. Using 32P-labeled recombinant human enzyme, Shp1 (as a GST-Shp1 fusion protein) catalyzes the dephosphorylation in vitro of Src pTyr527 more efficiently than that of pTyr416. An inactive GST-Shp1 fusion protein with a Cys453Ser mutation is without effect. 32P-labeled Src isolated from human platelets and human Jurkat T cells is preferentially dephosphorylated at pTyr527 by recombinant Shp1. Moreover, Src and Shp1 associate physically in interleukin-2 activated human lymphocytes. Anti-Src or anti-Shp1 antibodies alone result in the co-immunoprecipitation of Src and Shp1. Src autophosphorylation in vitro is diminished in extracts prepared from Shp1-deficient mouse thymocytes compared with extracts prepared from wild-type mouse thymocytes thus arguing for Src activation by Shp1-mediated pTyr527 dephosphorylation followed by Tyr416 autophosphorylation. Using antisera directed against C-terminal dephosphorylated and activated Src, immunoprecipititates prepared from Shp1-deficient thymocytes lacked activated Src while those prepared from wild-type thymocytes contained activated Src.

Somani et al. [39] examined Src activation in human HEY ovarian cells transfected with expression constructs encoding wild-type Shp1 or a catalytically incompetent dominant negative construct (Cys453Ser). They found that Src kinase activity is enhanced in cells overexpressing wild-type Shp1 and diminished in cells overexpressing the dominant negative mutant. These findings are consistent with the notion that Shp1 activates Src by catalyzing the dephosphorylation of the inhibitory pTyr527.
Somani et al. [39] tested the idea that the Shp1 SH2 domains play a role in the formation of a physical complex with Src. They prepared GST-fusion proteins containing the N-terminal SH2 domain, the C-terminal SH2 domain, and both domains of Shp1. Their studies showed that phosphorylated Src bound with the fusion protein containing the N-terminal SH2 domain or both SH2 domains. However, phosphorylated Src fails to form a stable complex with the fusion protein containing only the C-terminal SH2 domain of Shp1. These results indicate that the N-terminal SH2 domain, which inhibits Shp1 phosphatase activity, plays a key role in interacting with phosphorylated Src. These workers also showed that recombinant Src catalyzes the phosphorylation of the GST-Shp1 fusion protein in vitro.

Shp1 efficiently catalyzes the dephosphorylation of Src substrates in human embryonic kidney (HEK) cells [40]. Based upon the binding of GST-Shp1 fusion proteins in so-called pull-down assays, interaction of Shp1 with phosphorylated Src substrates is mediated chiefly by the catalytic domain, and not by the SH2 domains, of Shp1. Moreover, a significant correlation exists between the ability of Src to catalyze the phosphorylation of peptides corresponding to bona fide Src protein substrates and the ability of Shp1 to catalyze the dephosphorylation of these peptides. These studies show that peptides that are phosphorylated by Src kinase are good substrates for Shp1 both in vivo and in vitro.

Frank et al. [40] showed that Src catalyzes the activation and phosphorylation of tyrosine residues 538 and 566 in the C-terminal tail of Shp1. Based upon a study of tyrosine to phenylalanine mutants, phosphorylation of the residue corresponding to Tyr538 (not Tyr566) is responsible for Shp1 activation in a recombinant enzyme in vitro, and in human embryonic kidney cells. The Src and Shp1 interaction is an example of a kinase that activates a phosphatase that reverses the effect of the kinase. The net result of regulatory loops of this nature is that Src signaling in the presence of Shp1 should be transient.

In transfected human embryonic kidney (HEK293) cells, Frank et al. [40] showed that Shp1 does not alter the phosphorylation state nor does it activate Src. In contrast, Somani et al. [39] report that Shp1 activates Src in vitro and in human platelets and human Jurkat T cells in vivo. Frank et al. [40] suggest that these disparate findings may reflect a difference in the cell types and protein expression levels. Thus, more work is required to address the issue of the Src kinase regulation by Shp1 and the importance of Shp1 in mediating the dephosphorylation of Src kinase substrates in various cell types. These dissimilar findings indicate that deciphering the mechanism(s) of regulation of Src kinase and its substrates by phosphatases is problematic.

Shp2 catalyzed dephosphorylation of Src phosphotyrosine 527

Human Shp2, like Shp1, is a cytosolic protein-tyrosine phosphatase containing two tandem SH2 domains (residues 6–102 and 112–216), a phosphatase domain (residues 276–517), and a C-terminal tail (residues 518–593). Shp2, in contrast to Shp1, occurs in many types of cells; Shp2 is particularly abundant in heart, brain, and skeletal muscle. The SH2 domains of Shp2 target this enzyme to phosphotyrosine in a variety of growth factor receptors and other signaling molecules [41]. Moreover, its main function is to enhance growth factor and integrin signaling; this strategy involves the augmentation of protein-tyrosine phosphorylation by the tactical application of pTyr dephosphorylation.

Shp2 activates Src in mouse fibroblasts by decreasing Tyr527 phosphorylation and increasing Tyr416 phosphorylation. Zhang et al. [42] studied 3T3 fibroblasts prepared from wild-type and Shp2-deficient mice. They also examined Shp2-deficient mouse fibroblasts that were engineered to express Shp2 (Shp2-rescued mouse cells). They measured the proportion of Src with activation loop phosphorylation (pTyr416) and C-terminal tail inhibitory phosphorylation (pTyr527). Platelet-derived growth factor, epidermal growth factor, and fibroblast growth factor stimulated Src Tyr416 phosphorylation in wild-type and rescued fibroblasts, but not in Shp2-deficient cells.

Basal Tyr527 phosphorylation is increased in Shp2-deficient cells [42]. Moreover, Tyr527 phosphorylation decreases in response to growth factor stimulation in wild-type but not Shp2-deficient cells. Basal and growth factor evoked Tyr416 phosphorylation of Src is markedly decreased in Shp2-deficient cells. When Shp2-deficient cells were detached and replated onto fibronectin coated dishes, Tyr416 phosphorylation increases in rescued but not Shp2-deficient cells. Instead, there is increased Tyr527 phosphorylation in Shp2-deficient cells. Thus, in both receptor protein-tyrosine kinase and integrin signaling, Shp2 reverses or prevents Tyr527 phosphorylation and promotes Tyr416 phosphorylation. Moreover, basal and epidermal growth factor stimulated Src kinase activity is decreased in extracts prepared from Shp−/− cells when compared with wild-type cells.

Epidermal growth factor evoked tyrosyl phosphorylation of p62Dok, Vav2, and phospholipase Cγ1 (Table 1) is impaired in Shp2-deficient cells [42]. Tyrosine phosphorylation of the epidermal growth factor receptor, Gab1, and Shc (Table 1) is unaffected, ruling unlikely a global effect of Shp2 deficiency on epidermal growth factor receptor signaling. Focal adhesion kinase tyrosine phosphorylation in response to plating on fibronectin is impaired in Shp2-deficient cells. Focal adhesion kinase is a Src substrate [43] whose phosphorylation is decreased in cells lacking Src family kinases [7] including...
Cd45 catalyzed dephosphorylation of Src phosphotyrosine 527

After the cDNA for PTP1B was sequenced, the data indicated that this protein phosphatase is related to the leukocyte common antigen CD45 [44]. Cd45 is abundantly expressed on all nucleated hematopoietic cells and is critical for classical antigen receptor signaling indicated by the arrested development of B and T cells in mice deficient in CD45. Cd45 catalyzed Src family dephosphorylation is inhibitory. Cd45 catalyzed Src family dephosphorylation may thus play a role in activation of Src phosphatase by Src family kinases by this two-step process. This action explains the role of Shp2 as a positive growth factor signaling molecule.
[6] showed that pTyr789 is required for PTPα neoplastic transformation in mouse NIH3T3 cells. A Tyr789Phe mutation of PTPα blocks pTyr527 dephosphorylation and activation of Src in vivo and in vitro. Wild-type PTPα is able to catalyze the dephosphorylation of Src pTyr527 (the inhibitory site) and pTyr416 (the activation loop site). Wild-type and mutant (Tyr789Phe) PTPα are able to catalyze the dephosphorylation of phosphorylated myelin basic protein (a non-physiological substrate) with equal efficiency. Furthermore, the Tyr789Phe PTPα mutant is able to catalyze the dephosphorylation of pTyr416 but not pTyr527 of Src. The authors conclude that the phosphorylation of Tyr789 does not influence its general phosphatase activity, but it promotes selectively toward pTyr527. Thus, phosphorylation of pTyr416 but not pTyr527 of Src. The phosphorylation of PTPα Tyr789 plays an important role in the regulation of dephosphorylation and activation of Src in NIH3T3 cells.

In the phosphotyrosine displacement model proposed by Zheng et al. [6], pTyr789 of PTPα dislodges Src pTyr527 from the intramolecular Src SH2 domain thereby allowing pTyr527 to interact with the catalytic domain (D1) of PTPα. Phosphotyrosine 789 of PTPα anchors the enzyme to Src permitting it to catalyze the dephosphorylation of Src pTyr527. Zheng and co-workers showed that much more wild-type PTPα than mutant (Cys433Ser) enzyme is co-precipitated with Src in NIH3T3 cell extracts using anti-Src. Moreover, a GST-Src SH2 fusion protein decreases Src association with PTPα and decreases Src activation by PTPα as determined by immunoblotting. Furthermore, these workers showed that the Grb2 (Table 1) SH2 domain is more effective than the Src SH2 domain in blocking Src activation by PTPα. This result is in accord with the greater affinity of the Grb2 SH2 domain than the Src SH2 domain for pTyr589 of PTPα.

PTPα regulates Src kinase activity in human epidermoid A431 cells. These cells express high levels of the epidermal growth factor receptor [53]. Src kinase activity in A431 cell extracts is increased 2- to 3-fold in epidermal growth factor treated cells. Harder et al. [1] found that the phosphotyrosine content of Fyn, Src, and Yes, which are members of the Src kinase family, is diminished in immunoprecipitates prepared from epidermal growth factor-stimulated cells when compared with untreated cells. This association is activated with decreased phosphorylation of the C-terminal regulatory tail Tyr527. There is no change in the amount of the activation loop phosphorylation site (Tyr416). Co-immunoprecipitation studies demonstrated that Fyn, Src, and Yes are associated with PTPα in the A431 cells. These investigators also measured the association of Src kinase with its substrates in these cells. They found an increased association of Src with focal adhesion kinase (one of its substrates) in cells that over-expressed wild-type PTPα but not in cells that overexpressed the inactive mutant Cys433Ala protein. These studies are consistent with the notion that PTPα leads to (1) dephosphorylation of pTyr527, (2) activation of Src, and (3) phosphorylation of Src kinase substrates in A431 cells.

The regulation of PTPα activity is intricate. The SH2 domain of Grb2 binds to pTyr789 of PTPα. Grb2, which has an SH2 domain and two SH3 domains, is an adaptor protein that binds to the phosphorylated epidermal growth factor receptor, the platelet-derived growth factor receptor, and other tyrosine-phosphorylated receptors. Binding of Grb2 to PTPα inhibits PTPα phosphatase activity. When PTPα is released from Grb2, PTPα binds to pTyr527 of Src [53]. Zheng et al. [6] propose that PTPα disrupts the intramolecular Src SH2–pTyr527 link thereby enabling PTPα to dephosphorylate pTyr527.

Brandt et al. [51] showed that treatment of smooth muscle cells with phorbol esters (which activate protein kinase C) promotes reorganization of the actin cytoskeleton and activation of Src. They showed that recombinant PKC isozymes are unable to activate Src directly. They demonstrated that PKCδ phosphorylates Ser180 and 204 in the juxtamembrane region of PTPα resulting in an increase in phosphatase activity [51,52]. Phorbol-ester-stimulated phosphorylation of PTPα by PKC decreases the $K_m$ but does not alter the $V_{max}$ for PTPα substrates [51]. They also showed that cells lacking PKCδ have reduced PTPα and Src activity following phorbol ester treatment. They examined PKC isozymes that are expressed in smooth muscle cells ($\alpha$, $\beta$, $\gamma$, $\delta$, $\epsilon$, and $\eta$), and only PKCδ stimulates PTPα activity. Zheng et al. [52] report that the activity of PTPα increases during mitosis; this increase is postulated to account for the increase in Src activity. PKC is implicated in the control of the G2/M transition [54]. Moreover, PKC activation correlates with the increase in PTPα and Src kinase activity.

**PTPα catalyzed dephosphorylation of Src phosphotyrosine 527**

PTPα is related to PTPα and is implicated in the dephosphorylation of Src and Src family kinases including Fyn and Yes [55]. The PTPα subfamily contains four distinct protein species, all products of a single gene. PTPα is an integral membrane protein with an extracellular domain, a transmembrane segment, and intracellular domain that contains two phosphatase components (D1 and D2). Using an alternative promoter, the PTPα gene also encodes a cytosolic protein devoid of a transmembrane segment. p67PTPα, which is produced by internal initiation of translation from PTPα mRNA, and p65PTPα, which is produced by calpain-mediated
proteolytic processing of the larger PTPα forms, are N-terminally truncated forms of PTPε and are cytosolic. The unique N-termini of the four PTPε proteins dictate their distinct subcellular localization patterns and, in turn, their physiological roles [56].

Gil-Henn and Elson [56] studied mice lacking the receptor-like, or transmembrane, PTPε (Ptpre-/- mice). These mice were mated with mouse mammary tumor virus (MMTV)-Neu transgenic mice. Progeny were genotyped and mated among themselves to generate MMTV-Neu mice homozygous for the PTPε-null allele (Ptpre-/- /Neu mice) as well as Ptpre-/- and MMTV-Neu mice. They demonstrated that PTPε is a physiological activator of Src in vivo. The absence of PTPε in Neu-induced mammary tumor cells reduces Src activity and correlates with the phenotype of these cells, whereas expression of exogenous Src, PTPε, or the related PTPα partially reverses the phenotype. Of importance, the effects of PTPε deficiency exist despite expression of PTPα in these tumor cells, suggesting that the closely related PTPα cannot fully compensate for the lack of PTPε.

In agreement, immunoblotting experiments using phospho-specific antibodies reveal that Src autophosphorylation at Tyr416 is reduced by ≈60%, whereas phosphorylation at its C-terminal inhibitory Tyr527 is increased by ≈50% in Ptpre-/- /Neu cells. Both changes in Src phosphorylation are consistent with the observed reduction in Src kinase activity.

A substrate-trapping mutant of PTPε provides further support for the notion that Src is a substrate [56]. Substrate-trapping mutants are inactive, but they typically bind phosphorylated substrates as abortive complexes. Following co-expression of Src with wild-type PTPε in mouse SYF cells, Src was immunoprecipitated and blotted to reveal associated PTPε. Small amounts of wild-type PTPε associate with Src. Replacing wild-type PTPε with the Asp302Ala trapping mutant results in significantly more PTPε being co-precipitated with Src. Increased binding of mutant PTPε to Src is consistent with Src being a substrate of PTPε. The results indicate that PTPε can dephosphorylate and activate Src. Moreover, lack of PTPε is the cause of altered phosphorylation and reduced activity of Src in Ptpre-/- /Neu cells.

Although PTPε and PTPα share an ability to act on Src, decreases in activity and increases in Src phosphorylation in Ptpre-/- /Neu tumor cells occur despite the significant expression of PTPα [56]. Although the functional relationship between PTPα and Src has not been examined in mammary tumors, the data suggest that PTPα (and possibly other phosphatases such as PTP1B) partially activates Src in these cells, but not to the extent of activation mediated by PTPε. It appears that a full complement of Src-activating phosphatases is required for physiological regulation and activation of this kinase.

PTPα, catalyzed dephosphorylation of Src phosphotyrosine 527

Fang et al. [57] studied the ability of chicken PTPα and PTPβ to catalyze the dephosphorylation of chicken Src and v-Src in vitro and in transfected COS cells. These enzymes have an architecture similar to that of CD45 with an extracellular domain, a transmembrane segment, and two intracellular domains (D1 and D2). They found that PTPβ, but not PTPα catalyzes the dephosphorylation of chicken Src whereas each enzyme catalyzes the dephosphorylation of chicken v-Src. v-Src contains the activation loop phosphorylation site (Tyr416) but not the inhibitory Tyr527 site whereas Src contains both phosphorylation sites [7]. In addition to the transmembrane enzymes, these workers generated intracellular enzymes with a myristoylated N-terminus corresponding to chicken PTPα, PTPβ, and human CD45 phosphatase. Myristoylation decreases somewhat the ability of PTPβ to catalyze the dephosphorylation of Src when compared with the transmembrane form. However, all three myristoylated enzymes were able to catalyze the dephosphorylation of Tyr416 in v-Src. When PTPβ was expressed in a non-myristoylated form, it failed to bind to membranes and failed to catalyze the dephosphorylation of Src. The latter experiment indicates that the subcellular localization is important in mediating Src dephosphorylation.

Of concern in these studies [57] is the relatively low pTyr527 dephosphorylating activity of the PTPα chimera tested in these experiments when compared with the work cited above [6,46–53]. The activity of the engineered enzyme may not accurately reflect the wild-type activity because the chimera lacked Ser180 and 204, the activating phosphorylation sites. Moreover, it was not reported whether Tyr789 was phosphorylated (the residue that displaces Src pTyr527 [6]). The ability of each of PTPα, PTPβ, and CD45 to catalyze the dephosphorylation of pTyr416 suggests that this residue exists in an open form that is accessible to many enzymes.

PTPβ expression is low in brain and fibroblasts and is high in spleen, intestine, and pre-B cells, and thus does not match that of Src. This distribution indicates that the physiological role of PTPβ in Src regulation is limited.

Src phosphotyrosine 416 dephosphorylation by PTP-BL, the mouse homologue of human PTP-BAS

Human PTP-BAS is a cytosolic phosphatase that contains a FERM domain (residues 572–872), five PDZ domains (1093–1178, 1368–1452, 1501–1588, 1788–1868, and 1882–1965), and a PTP domain (2237–2485). FERM is the acronym of four point one/ezrin/radixin/moesin. FERM proteins associate with F-actin and with the plasma membrane. PDZ domains are
modular protein interaction domains that bind in a sequence-specific fashion to short sequences that fold as a β-finger. PDZ domains are frequently found in multiple copies. PTP-BAS was derived initially from human white blood cell basophils (BAS refers to basophil) and BL (mouse) refers to basophil like. PTP-BAS co-localizes in membrane fractions with Src family kinases in mouse embryos and cultured central nervous system (cortical) neurons. This enzyme is present in high levels in lung, kidney, and developing brain. PTP-BAS is present in low levels in skeletal muscle and liver.

Palmer et al. [58] found that the bacterially expressed mouse PTP-BL phosphatase domain, but not a catalytically inactive mutant, dephosphorylates mouse Src specifically on pTyr416. In contrast, this enzyme does not dephosphorylate pTyr527. These workers found that ephrinB2, an important regulator of morphogenesis, leads to the activation of Src kinase in mouse NIH3T3 cells. Activation is apparent at 10 min and is absent at 30 min. The deactivation is associated with the recruitment of PTP-BL and dephosphorylation of Src pTyr416. As noted above, pTyr416 occurs in the activation loop and is associated with increased Src activity. This activation results from intermolecular auto-phosphorylation mediated by another Src kinase molecule. It is likely that other phosphatases are involved in regulatory pTyr416 dephosphorylation. It is noteworthy that the ephrin receptor family of protein-tyrosine kinases is the largest family of protein kinases in the human genome with 14 members [1].

Epilogue

The state of protein phosphorylation is determined by the relative activities of protein kinases and phosphatases. Owing to the participation of protein phosphorylation in nearly all aspects of cell biology, it is imperative that the extent of phosphorylation be exactingly controlled. Otherwise, cellular regulation would be severely compromised. The ratio of protein serine/threonine/tyrosine phosphate in normal animal cells is about 3000/300/1 [59]. Despite the relative paucity of protein phosphotyrosine in cells, this modification is of critical importance. Protein-tyrosine phosphorylation is an initial step in signal transduction mediated by insulin, growth factors, cytokines, and a variety of other regulatory stimuli. Our knowledge base of protein-tyrosine kinases is much greater than that of phosphatases. One explanation for this state of affairs is that protein kinases were purified and sequenced a decade before work commenced on phosphoprotein phosphatases [2]. However, the discovery of the enzymatic mechanism of phosphotyrosine phosphatases involving a thiophosphate covalent intermediate by Guan and Dixon [26] is particularly noteworthy.

While the protein-serine/threonine and protein-tyrosine kinases are evolutionarily and structurally related, the protein-tyrosine and protein-serine/threonine phosphatases are in different gene families [23]. The dual specificity protein kinases in humans are related to protein-serine/threonine kinases [1]. These dual specificity protein-kinases, which are related to MEK, include seven members (MEK1–7). These enzymes catalyze the phosphorylation and activation of threonine and tyrosine (Thr-Xxx-Tyr) in the activation loop [7] of substrates including ERK (extracellular signal regulated kinases), JNK (Jun N-terminal kinases), and p38 protein kinases. In contrast to the apparently limited number of dual specificity protein kinases, the number of dual specificity phosphatases (68) included in the PTP family of the human genome is considerably larger [2].

The classical protein-tyrosine phosphatases have a catalytic cleft of about 9 Å that accommodates phosphotyrosine but not phosphoserine or phosphothreonine. In contrast, the catalytic cleft of the dual specificity phosphatases is about 6 Å in depth and can accommodate all three phosphorylated residues [24]. Although classified as dual specificity phosphatases based upon their activity in vitro, these enzymes might function as phosphoserine/phosphothreonine or phosphotyrosine phosphatases in vivo. Just as the structures of protein kinases are similar, the catalytic domains of protein-tyrosine phosphatases resemble that of PTP1B, a classical protein-tyrosine phosphatase.

Phosphorylation by protein kinases can stimulate or inhibit the activity of substrate proteins or provide binding sites for downstream effectors depending upon the substrate and pathway. Phosphoprotein phosphatases, which counteract the action of kinases, can reverse the activity of substrate proteins or eliminate binding sites for downstream effectors. C-terminal Src kinase, a protein-tyrosine kinase, mediates Src Tyr527 phosphorylation and inhibits the signaling of Src, also a protein-tyrosine kinase. In contrast, PTPz, a phosphoprotein phosphatase, mediates pTyr527 dephosphorylation and stimulates Src kinase signaling. If the phosphotyrosine phosphatase is not targeted to the correct cellular location, substrate dephosphorylation is impaired [57].

The phosphate on Tyr527, which forms an intramolecular complex with the Src SH2 domain, is buried. Shalloway and co-workers [6] have provided evidence for a two-step process for Src dephosphorylation involving (1) the displacement of pTyr527 from the Src SH2 domain by PTPz pTyr586 and (2) the catalytic removal of the phosphate. Shp1 and PTPz may employ a similar phosphotyrosine displacement mechanism [6]. Although the phosphorylation state of proteins is regulated, the turnover number of phosphotyrosine protein phosphatases is 10–1000 that of protein-tyrosine kinases in vitro [21].
Of the 107 protein-tyrosine phosphatase genes in the human genome, a total of 81 are thought to be catalytically active toward protein-tyrosine phosphate and 26 are not. Of the 26, two dephosphorylate mRNA, 13 dephosphorylate inositol phospholipids, and eleven are not. Of the 90 protein-tyrosine kinase gene products, a total of 85 are catalytically active [1]. Thus, the number of protein-tyrosine kinase and protein-tyrosine phosphatase genes is nearly the same.

The number of genes encoding human protein kinases and phosphatases is large (>650) [1,2]. This is in line with the notion that a third of all human proteins can serve as physiological substrates for protein kinases. In some cases, a given serine, threonine, or tyrosine is a substrate for more than one kinase. Furthermore, a given protein commonly contains sites for multiple protein kinases. This situation more than likely applies to phosphoprotein phosphatases. When multiple phosphatases can catalyze dephosphorylation of a specific protein residue and the phosphatases are expressed in the same cell, a deficiency of one of them still can result in dysregulation [56]. Thus, a full complement of phosphatases is required for regulation under physiological conditions. This suggests that there may be a role for cytoplasmic PTPβB, Shp1, Shp2, and transmembrane CD45, PTPζ, PTPε, and PTPδ in the regulatory dephosphorylation of Src pTyr527 depending upon their cellular expression. It is likely that a role for other phosphatases in Src dephosphorylation will also be found.

The elucidation of protein phosphorylation signaling pathways is an important undertaking. Besides establishing the identity of protein substrates and the specific residues that are phosphorylated, the determination of which kinases and phosphatases catalyze these reactions in vitro and, more importantly, in vivo represents a major challenge. Moreover, determining the rates of phosphorylation and dephosphorylation compounds the problem. Adding to this, when a phosphatase such as PTPβ catalyzes the dephosphorylation of an inhibiting site (Src pTyr527) and an activating site (Src pTyr416) of the same protein at different rates, determining the net kinase activity is even more difficult. The many challenges in phosphorylation signal transduction include the determination of the combinatorial action of kinases and phosphatases, the rates of phosphorylation and dephosphorylation, and the actual activity of the substrate protein.

References


