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Biochemical and Biophysical Research Communications 331 (2005) 1–14
BBRC
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Breakthroughs and Views

Src kinase regulation by phosphorylation and dephosphorylation ☆

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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 PTPλ. 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 receptor 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.

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; PTPλ; PTP-BAS; Shp1; Shp2; Src-family kinases

Protein phosphorylation, which plays a key regulatory role in nearly every aspect of eukaryotic cell biology, is a reversible and dynamic process that is mediated by protein kinases and phosphoprotein phosphatases. Protein kinases catalyze the following reaction:

$$\mathrm{MgATP^{1-} + protein\text{-}OH \rightarrow protein\text{-}OPO_3^{2-} + MgADP + H^+}$$

Based upon the nature of the phosphorylated –OH group, these enzymes are classified as protein-serine/threonine kinases and protein-tyrosine kinases. There is a small group of enzymes, which includes MEK, that catalyzes the phosphorylation of both threonine and tyrosine on target proteins. These enzymes, which closely resemble serine/threonine kinases, are dual specificity kinases. Manning et al. [1] identified 90

☆ *Abbreviations:* CDK1/cdc2, cyclin-dependent kinase 1/cell division cycle 2; Csk, C-terminal Src kinase; Chk, Csk homology kinase; GST, glutathione *S*-transferase; PDGF, platelet-derived growth factor; PKA, protein kinase A; PKC, protein kinase C; PTP, protein-tyrosine phosphatase; pSer, phosphoserine; pThr, phosphothreonine; pTyr, phosphotyrosine; SH2, Src homology 2; SH3, Src homology 3; SH4, Src homology 4.

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0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.03.012

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:

$$\text{Protein-OPO}_3^{2-} + \text{H}_2\text{O} \rightarrow \text{protein-OH} + \text{HOPO}_3^{2-}$$

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 Cγ, 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

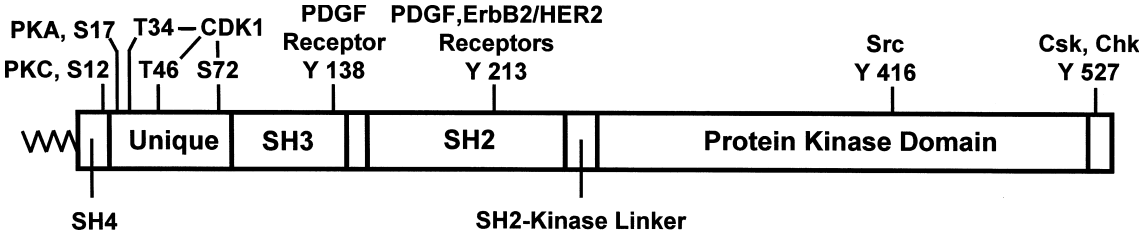

Fig. 1. Protein kinase phosphorylation sites and organization of Src. Except for the aliphatic myristoyl group attached to the SH4 domain, the relative length of the domains is to scale. The chicken numbering system is displayed. S is serine, T is threonine, and Y is tyrosine.

Table 1
Selected proteins interacting with kinases and phosphatases[a]

Interacting protein	Function/comments	Swiss-Prot Accession Nos.
p62Dok	Docking protein downstream of tyrosine kinase; interacts with RasGTPase	Q99704
Gab1	Grb2-associated binding protein 1	Q13480
Grb2	Growth factor receptor-bound protein 2	P62993
PLCγ1	Phospholipase Cγ1 catalyzes the hydrolysis of phosphatidylinositol bisphosphate to generate diacylglycerol and inositol 1,4,5-trisphosphate	P19174
RasGAP	Ras GTPase activating protein; Ras (rat sarcoma) is a signal transduction protein with GTPase activity	P20936
Rho	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	P61586
Shc	Src homology 2 domain-containing transforming protein C1; binds Grb2	P29353
Vav2	A guanine nucleotide exchange factor for Rac, a member of the Rho family of GTPases; vav is the sixth letter of the Hebrew alphabet	P52735

[a] Human orthologs.