



# Molecular Mechanisms of SH2- and PTB-Domain-Containing Proteins in Receptor Tyrosine Kinase Signaling

Melany J. Wagner<sup>1,3</sup>, Melissa M. Stacey<sup>1,3</sup>, Bernard A. Liu<sup>1</sup>, and Tony Pawson<sup>1,2</sup>

<sup>1</sup>Lunenfeld Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada

<sup>2</sup>Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Correspondence: pawson@lunenfeld.ca

Intracellular signaling is mediated by reversible posttranslational modifications (PTMs) that include phosphorylation, ubiquitination, and acetylation, among others. In response to extracellular stimuli such as growth factors, receptor tyrosine kinases (RTKs) typically dimerize and initiate signaling through phosphorylation of their cytoplasmic tails and downstream scaffolds. Signaling effectors are recruited to these phosphotyrosine (pTyr) sites primarily through Src homology 2 (SH2) domains and pTyr-binding (PTB) domains. This review describes how these conserved domains specifically recognize pTyr residues and play a major role in mediating precise downstream signaling events.

Receptor tyrosine kinase (RTK) signaling is initiated on binding of soluble growth factors to growth factor receptors such as the insulin receptor (IR) or epidermal growth factor receptor (EGFR), or on binding of membrane-bound ephrins, as is the case for Eph receptors. Intracellular signaling is then propagated through PTMs, which commonly serve to regulate protein function by acting as docking sites for recruitment of modular protein interaction domains. Phosphorylation is the best studied PTM, and is a principle mechanism regulating intracellular signaling.

A common element in RTK signaling involves autophosphorylation of the intracellular

portion of the receptor (Fig. 1). RTKs become activated as a result of ligand-stabilized dimerization or oligomerization. For instance, in the EGFR subfamily (which includes ErbB and EGF receptors), the formation of homo- or heterodimers is initiated by ligand binding and subsequent exposure of a dimerization domain (Hynes and Lane 2005). Dimerization of the RTKs allows autophosphorylation of the RTKs; EGFR is exceptional in that an allosteric interaction between the kinase domains of adjacent monomers is responsible for the receptor activation (Zhang et al. 2006). However, in the majority of cases dimerization enhances RTK catalytic activity through phosphorylation of the

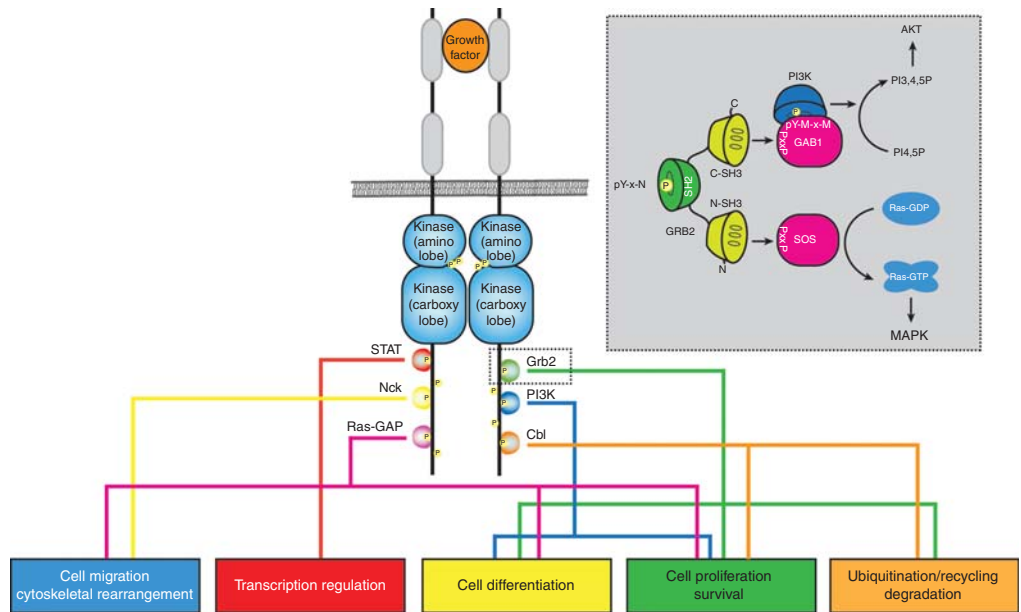
<sup>3</sup>These authors contributed equally to this article.

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**Figure 1.** Receptor tyrosine kinases activate downstream pathways through recruitment of proteins containing pTyr-binding domains. Receptor tyrosine kinases are activated on growth factor binding to the extracellular domain of the receptor, leading to receptor dimerization and tyrosine phosphorylation (yellow circles labeled with a P) of their cytoplasmic tails, which act as docking sites for recruitment of PTB and SH2 domains. Various RTKs can mediate a diverse set of cellular processes (colored boxes) determined by the recruitment of specific SH2- and PTB-domain-containing proteins. The gray box displays how the adaptor Grb2 is recruited to an RTK through recognition of the pY-x-N (pY = pTyr, x = any natural amino acid) and activates cell growth and survival pathways such as MAPK and AKT, respectively, through complex formation via its SH3 domains.

kinase activation loop, and in some instances the juxtamembrane region, and recruits signaling effectors through the creation of pTyr docking sites. The specific interaction of signaling proteins with these pTyr-binding motifs activates signaling pathways, such as canonical signaling through the Ras-mitogen activated protein kinase (MAPK), phosphoinositide-3-kinase (PI3K)-Akt, and phospholipase C-gamma (PLC- $\gamma$ ) pathways. These RTK pathways can result in a variety of cellular processes, including differentiation, proliferation, survival, and migration (Fig. 1). The cellular context of signaling can dictate the biological outcome, and how each RTK initiates a given cellular process remains an area of active research.

Tyrosine phosphorylation mediates RTK signaling through the recruitment and activation of proteins involved in downstream signaling pathways, mediated through pTyr binding

of the SH2 and PTB domains of signaling effectors. SH2 and PTB domains are found in an otherwise diverse set of proteins containing a range of distinct catalytic and interaction domains, and provide a degree of specificity through their recognition of both a pTyr residue and surrounding amino acids. Here we will discuss the properties of proteins that contain SH2 and PTB domains and their roles in signaling downstream of RTKs, as well as the mechanisms by which they regulate the activity of these signaling effectors.

### SH2- AND PTB-DOMAIN-CONTAINING PROTEINS ARE DIVERSE IN NATURE

An SH2 or PTB domain typically recognizes a pTyr residue within the context of a specific amino acid sequence (Songyang et al. 1993). This characteristic allows for the distinct bind-

ing of SH2- and PTB-containing proteins to activated RTKs and to other tyrosine phosphorylated signaling effectors (Sadowski et al. 1986; Anderson et al. 1990; Blaikie et al. 1994; Kavanaugh and Williams 1994). These domains are found in proteins of diverse function, and on RTK activation their recruitment to pTyr sites results in the activation of their host proteins, and the stimulation of downstream signaling pathways. As noted, these proteins usually contain other catalytic and noncatalytic domains that establish their functions, and can be used to categorize them into distinct groups, which are briefly discussed below (Fig. 2).

Adaptor proteins and docking/scaffolding proteins lack intrinsic catalytic activity, but act to assemble signaling complexes capable of selectively stimulating downstream pathways. Adaptors containing both SH2 and SH3 domains use the SH3 domain(s) to aggregate signaling effectors, whereas the SH2 domain mediates recruitment to the active RTK and assembly of the signaling complex. For example, the SH3 domains of Nck bind to effectors involved in the organization of the cytoskeleton (such as N-WASP and PAK) to link the active RTK to the cytoskeleton (Rivero-Lezcano et al. 1995; Zhao et al. 2000). Scaffolding proteins recruit signaling effectors through linear motifs, including pTyr-containing binding sites, and localized assembly of the signaling complex is directed by the scaffold's PTB or SH2 domain. Shc and IRS-1, for instance, localize to pTyr motifs in an active RTK via their PTB domains and bind the Grb2/Sos complex following phosphorylation of internal YXN motifs, allowing for subsequent activation of the Ras-MAPK pathway (Salcini et al. 1994; van der Geer et al. 1996; Kouhara et al. 1997; Saucier et al. 2004).

In combination with a catalytic domain, the SH2 domains of kinases, phosphatases, guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), and the phospholipase PLC- $\gamma$  generally recruit these proteins to their substrates. Such a substrate can be either a tyrosine-phosphorylated protein or a distinct substrate juxtaposed to a tyrosine-phosphorylated protein. Within an enzyme, an SH2 domain can also allow for intramolecular binding

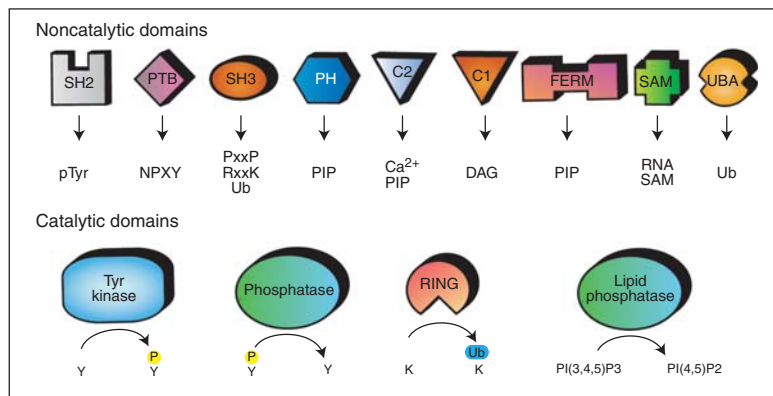
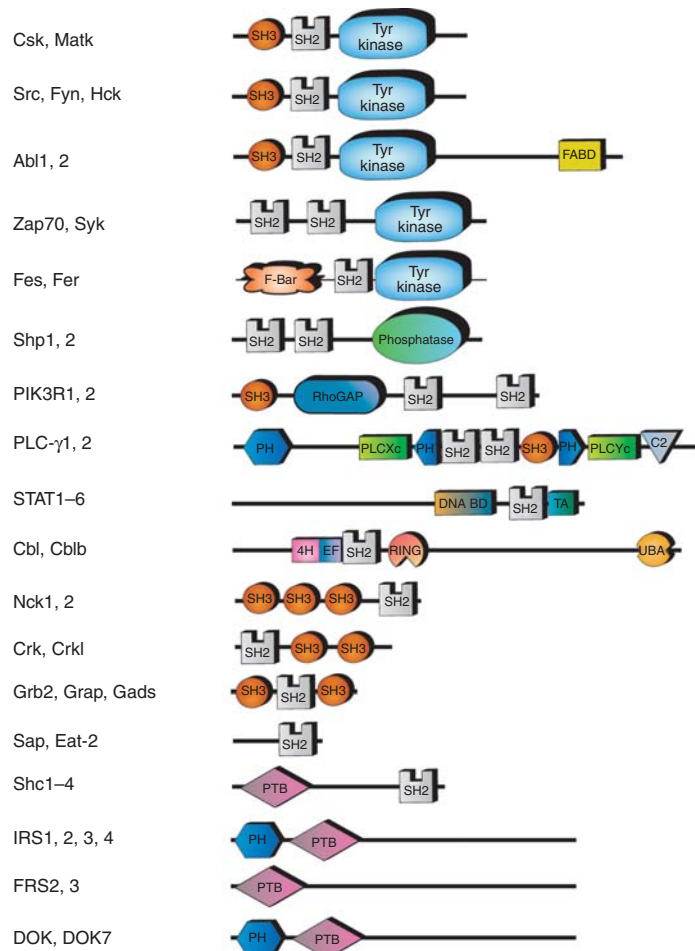
and dynamic regulation of enzymatic activity, and this concept is discussed later in this article. In addition, enzymes containing domains capable of binding secondary messengers (such as PH domains binding phosphatidylinositol 3,4,5-triphosphate (PIP3) or C1 domains binding diacylglycerol (DAG)/phorbol esters) tend to show a higher level of specificity in both localization and activation.

Finally, in the STAT family of transcription factors, SH2 domains enable recruitment to active RTKs; on subsequent RTK-mediated phosphorylation, the SH2 domain mediates STAT dimerization and activation (Stahl et al. 1995; Darnell 1997; Levy and Darnell 2002; Schlesinger and Lemmon 2003). Thus, specific binding of SH2 and PTB domains plays a major role in the localized assembly and activation of signaling effectors. In the sections that follow, we describe in detail how the specific localization of a protein is achieved through the various modes of SH2 binding.

## SH2 DOMAINS

The SH2 domain was initially discovered through the observation that an  $\sim 100$  amino acid sequence in the v-Fps/Fes oncoprotein was necessary for cellular transformation (Sadowski et al. 1986). This domain was named SH2 because of its homology with a corresponding region in Src family and Abl cytoplasmic tyrosine kinases. Since that time, more than 100 human proteins have been found to contain SH2 domains (Sadowski et al. 1986; Liu et al. 2006).

Early on, it was determined that the affinity of an SH2 domain for pTyr depends on the amino acid sequence surrounding the pTyr residue. A given SH2 domain binds to pTyr residues within a preferred peptide with a dissociation constant in the range of 0.2 to 5  $\mu\text{M}$  (Songyang et al. 1993). By comparison, the dissociation constant for an SH2 domain bound to a pTyr-containing motif of random sequence is 4- to 100-fold lower ( $\sim 20 \mu\text{M}$ ) (Songyang et al. 1993). This increase in affinity conveys specificity to each SH2 domain. Thus, domains bind specific pTyr motifs; the Src family kinases (SFKs) bind preferentially to a pYEEI motif,



**Figure 2.** SH2- and PTB-containing proteins are diverse in nature. The modular domain organization of SH2- and PTB-domain proteins displays a diverse set of noncatalytic and catalytic domains for mediating protein–protein interactions and enzyme catalysis, respectively. See the box legend for description of the binding partners and function of these domains. More information on the individual domains portrayed can be found at [www.pawsonlab.mshri.on.ca](http://www.pawsonlab.mshri.on.ca) and [www.smart.embl-heidelberg.de](http://www.smart.embl-heidelberg.de).

whereas the SH2 domains from PI3K or PLC- $\gamma$  bind preferentially to pY $\phi$ X $\phi$  (where  $\phi$  is a residue with a hydrophobic side chain) (Songyang et al. 1993). The specificity of SH2 domains is therefore afforded to some extent by the binding of distinct pTyr-containing motifs, and by other modes of binding described below.

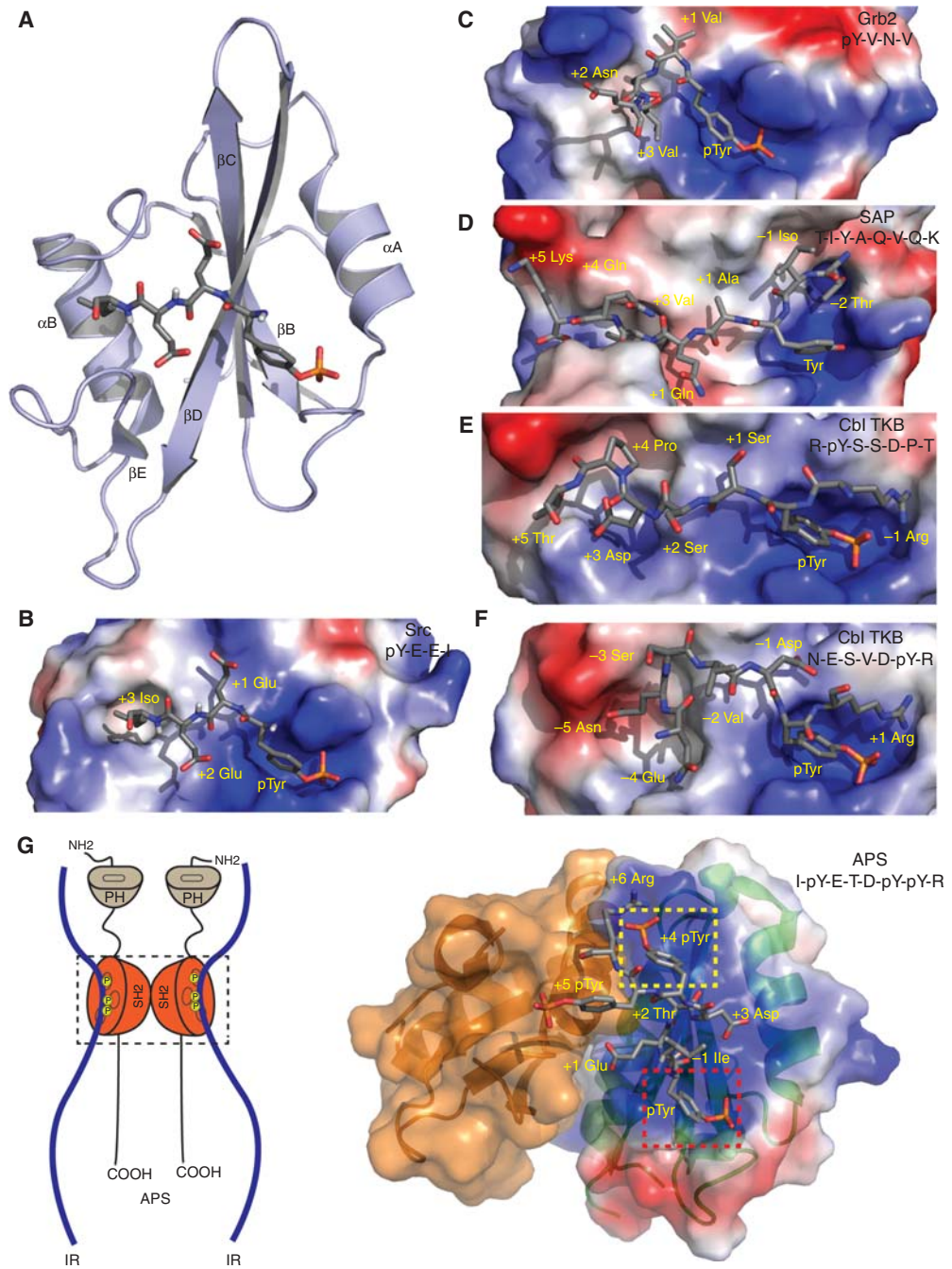
### Canonical SH2 Binding

SH2 domains for which the crystal structures have been solved show a high degree of structural homology (reviewed in Kuriyan and Cowburn 1997 and Schlessinger and Lemmon 2003). The typical SH2 domain fold consists of three or four  $\beta$  strands, which make up an antiparallel  $\beta$  sheet, surrounded by two  $\alpha$  helices. A positively charged binding pocket on the SH2 domain surface uses a critical Arg residue (within a very highly conserved FLVR motif) (Hidaka et al. 1991; Koch et al. 1991) to bind the pTyr of target ligands. In canonical binding, the residues surrounding the critical Arg typically engage amino acids from position +1 to +6 (carboxy terminal of the pTyr) of the ligand, and it is this sequence that dictates the specificity of a given SH2 domain (Songyang et al. 1993; Pawson 1995; Pawson et al. 2001). For example, binding of the preferred pYEEI motif involves regions on either side of the SH2 domain central  $\beta$ -sheet; between the  $\beta$ -sheet and the amino-terminal  $\alpha$ A helix is a deep binding pocket that accommodates the pTyr, whereas on the opposite side of the  $\beta$ -sheet, adjacent to the carboxy-terminal  $\alpha$ B helix, residues form a hydrophobic pocket into which the isoleucine chain extends (Fig. 3A,B) (Waksman et al. 1993). Therefore, the pTyr-binding pocket of an SH2 domain is adjacent to the region that dictates binding specificity and determines whether or not the signaling effector is recruited to a given RTK or scaffold protein. This in turn determines which pathway(s) is subsequently activated downstream of the receptor (reviewed in Songyang et al. 1993; Pawson 1995; Pawson et al. 2001).

The various forms of canonical binding give SH2 domains a complexity that contributes greatly to their selectivity. For example, the SH2 domain of Grb2 preferentially binds pYXNX

(in which X represents any of the natural 20 amino acids) motifs present among its interaction partners including the Shc proteins (Fig. 3C) (Rozakis-Adcock et al. 1993). Grb2's preference for Asn at the +2 position is mediated by a Trp residue (W121) in the EF loop of the SH2 domain whereby mutation of this position (W121T) displays weak binding to pYXNX motifs (Marengere et al. 1994). The SLAM-associated protein (SAP) SH2 domain, which generally requires its target ligands to be phosphorylated, can also bind its target SLAM constitutively and independent of phosphorylation (Fig. 3D) (Sayos et al. 1998; Li et al. 1999; Poy et al. 1999; Ma and Deenick 2011). Tandem SH2 domains in proteins like PI3K, Shp2, and Zap-70 also enhance the specificity of enzyme recruitment to RTKs. Two closely-spaced tyrosine phosphorylated motifs bind to tandem SH2 domains with 20- to 50-fold greater affinity and specificity compared with the binding of a single SH2 domain with a single tyrosine phosphorylated motif (Ottinger et al. 1998). This enhanced selectivity is due in part to the spacing between the two pTyr motifs. In the case of Zap-70, this is known to be caused by the nature of binding; the carboxy-terminal SH2 domain binds one of the pTyr sites in a conventional manner, whereas the second pTyr activation motif is bound by residues from both the amino-terminal and carboxy-terminal SH2 domains (Hatada et al. 1995). To accommodate binding of one pTyr motif by both SH2 domains, a bisphosphorylated peptide would require specific spacing between the pTyr motifs. Similarly, on binding of one of the SH2 domains of Shp2, the SH2 domains maintain a defined relative position that is stabilized at an interface by a disulfide bond and a hydrophobic patch (Eck et al. 1996); this conformational rigidity is most likely responsible for the requirement of defined spacing between pTyr motifs in a Shp2 bisphosphorylated target (Pluskey et al. 1995).

Canonical SH2 binding may be subject to regulation during cell signaling. A recent publication examining phorbol ester stimulation showed that the PI3K SH2 domains are serine phosphorylated in a manner that occludes pTyr peptide binding (Lee et al. 2011), suggesting



**Figure 3.** Src homology 2 domains recognize specific phosphotyrosine motifs. (A) Ribbon structure of the Src SH2 domain (light blue) bound to a pTyr-Glu-Glu-Ile peptide (PDB: 1SPS) (Waksman et al. 1993). The amino-terminal pTyr of the peptide (gray) occupies the pTyr-binding pocket. The peptide runs over the central  $\beta$  sheet of the SH2 domain, the +1 and +2 glutamates contact the surface of the domain, and the side chain of the +3 Ile (to the left) fits in a hydrophobic pocket. (B) The electrostatic surface of the SH2 domain reveals the positive charged pTyr-binding pocket (blue is positive, red is negative) and the ligand-binding pocket. (C) Grb2 SH2 domain in complex with pYVNV (red) (PDB: 1BMB). (*Legend continues on following page.*)

that this phosphorylation may prevent SH2-mediated recruitment and activation. Further investigation is required to determine whether this represents a broad-spectrum mechanism of negative regulation.

### Alternative Modes of Binding

Some SH2-containing proteins bind their targets in a noncanonical manner. Cbl is an E3 ubiquitin ligase that generally switches “off” protein tyrosine kinase (PTK) targets through ubiquitination and subsequent proteasomal or lysosomal degradation (Sanjay et al. 2001; Ryan et al. 2010). The Cbl SH2 domain has an unusual sequence compared with other SH2 domains (sharing only ~11% sequence homology), and its structure—although similar to other SH2 domains—lacks the conventional BG loop and secondary  $\beta$ -sheet of a typical SH2 domain (Meng et al. 1999). Nevertheless, the Cbl SH2 domain is functionally similar to other SH2 domains and is embedded within the TKB (tyrosine kinase-binding) domain, which includes an EF hand domain and a calcium-binding four-helix bundle (Hu and Hubbard 2005). Extensive interdomain contacts form within the TKB domain, which may contribute to the specificity of the SH2 domain in engaging target phosphopeptides. The extended Cbl SH2 domain recognizes residues both amino and carboxy terminal to the target pTyr, and in the case of binding APS it has been shown that amino acids amino terminal to the pTyr also associate with the four-helix bundle (Hu and Hubbard 2005). Binding of Cbl to proteins including the EGFR, vascular endothelial growth factor receptor, ZAP-70, Src, and Syk involves the canonical (N/D)XpY(S/T)XXP consensus motif

(Fig. 3E) (Lupher et al. 1997; Meng et al. 1999; Schmidt and Dikic 2005), whereas the unrelated DpYR motif in the Met and Plexin families also binds Cbl (Tamagnone et al. 1999; Penengo et al. 2003; Peschard et al. 2004). Surprisingly, the crystal structure of the Cbl TKB domain binding to the pY ligand of Met revealed that Cbl can bind this motif in two orientations: the “canonical” forward orientation and the reverse orientation (amino to carboxyl terminus) (Fig. 3F) (Ng et al. 2008).

In addition, the APS adaptor protein has been shown to dimerize and stabilize the activation of RTKs including the IR through interactions with the activation loop (Ahmed et al. 1999; Moodie et al. 1999; Yokouchi et al. 1999). This dimerization may be important for transphosphorylation—and activation of the kinase domains—of these receptors (Dhe-Paganon et al. 2004; Nishi et al. 2005). The solved structure for the dimerized APS SH2 domains bound to the IR activation loop peptide revealed conventional pTyr binding to Y1158. The dimerization induces a conformational change that creates a second pTyr-binding pocket, and an unusual turn in the peptide ligand running parallel to the  $\beta$ -strands. This new conformation allows for charged interactions between the second pTyr site on IR Y1162 and two Lys residues in the  $\beta$ -D strand (Hu et al. 2003; Dhe-Paganon et al. 2004; Hu and Hubbard 2006).

In some proteins, secondary binding sites in the SH2 domain, distinct from the pTyr motif-binding site, also participate in specific interactions. For example, in the Fes and Abl kinases, an intramolecular interaction between a secondary SH2-binding site and the kinase domain enhances enzymatic activity, as discussed later in this article (Filippakopoulos et al. 2008).

**Figure 3.** (Continued) (D) The SAP SH2 domain can recognize nonphosphorylated SLAM peptide and residues amino terminal to the tyrosine, such as -2 Thr (PDB: 1M27). (E) pTyr-binding pocket of the Cbl SH2 is bound in the canonical fashion with EGFR peptide pYSSDP (gray) with the carboxyl terminus extended across the SH2 surface (PDB: 3BUO). (F) Cbl TKB in complex with the MET peptide is oriented in the reverse direction with the amino acids amino terminal to the pTyr extended across the SH2 domain (PDB: 3BUX). (G) *Left panel:* A graphical representation of the dimerized APS molecules bound to the insulin receptor (IR). *Right panel:* The dimerized SH2 domain of APS bound to the activation loop peptide of the IR with the primary pTyr pocket (red box) and the second pocket (yellow box) indicated.



## PTB DOMAINS

The pTyr-binding (PTB) domain was first identified in the scaffold protein Shc, which has a carboxy-terminal SH2 and amino-terminal PTB domain. This PTB domain was found to bind a pTyr residue in the EGF receptor (Blaikie et al. 1994; Kavanaugh and Williams 1994), and it was originally assumed that a characteristic function of all such domains was recruitment in a phospho-dependent manner.

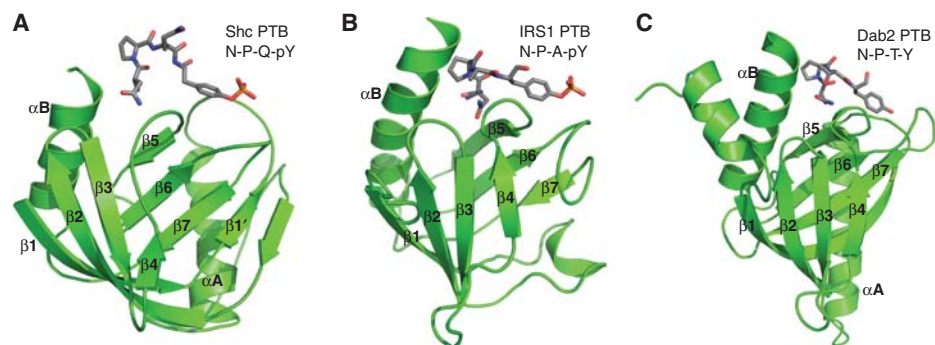
To date, approximately 60 PTB domains have been identified in the human proteome; mutations in six of these have been associated with heritable diseases including Alzheimer's disease, diabetes, and coronary artery disease (reviewed in Uhlik et al. 2005). It turns out that many PTB domains do not in fact rely on ligand tyrosine phosphorylation for binding, but commonly PTB domains do bind phospholipid acidic head groups, which helps to localize them to membrane or juxtamembrane regions where they can easily bind RTKs and mediate downstream signaling. Binding to phospholipid head groups is mediated by a positively charged binding pocket composed of a cluster of basic residues, and is a distinct event from the PTB-domain-containing protein's interactions with pTyr motifs. This section discusses the pTyr-dependent PTB domains.

The 3D structure of the Shc PTB domain was published just 1 year after its identification (Zhou et al. 1995). Surprisingly, its structure re-

sembles that of the pleckstrin homology (PH) domain, despite highly dissimilar sequences (Zhou et al. 1995). To date, all PTB domains for which the crystal structures have been solved appear to share the same folding pattern, including a  $\beta$ -sandwich composed of two virtually orthogonal  $\beta$ -sheets, which is capped by a carboxy-terminal  $\alpha$ -helix (Fig. 4). Together, the  $\beta$ 5 strand and  $\alpha$ -helix make up the ligand-binding pocket (Schlessinger and Lemmon 2003). This binding pocket appears to be highly conserved, not only in its location and spatial arrangement but also in the binding mechanism: a cleft between the  $\beta$ 5 strand and the carboxy-terminal  $\alpha$  helix that accommodates the ligand (Harrison 1996; reviewed in Schlessinger and Lemmon 2003; Uhlik et al. 2005).

## Modes of Peptide Binding

The motif NPXY is common among PTB-domain substrates and is considered the canonical binding motif for PTB-domain-containing proteins. Uhlik and others (Uhlik et al. 2005) conducted an extensive analysis of the PTB-containing proteins; using structural, functional, and evolutionary data, they classified PTB-domain-containing proteins into three groups: pTyr-dependent Shc-like; pTyr-dependent IRS-like; and pTyr-independent Dab-like (Fig. 4). These three classes are distinct in their ability to bind a phosphorylated NPXY motif. The Shc,



**Figure 4.** Structure of phosphotyrosine-binding domains. The ribbon structures shown in green of the PTB domains of Shc (A) (PDB: 1SHC), IRS-1 (B) (PDB: 1IRS), and Dab2 (C) (PTB: 1ME7) bound to their respective N-P-x-(p)Y ligand (gray). x Denotes any natural amino acid; pY represents phosphotyrosine. The mode of ligand binding to the PTB domains shown is similar.



IRS, and Dok scaffolds preferentially bind phosphorylated substrates, whereas the Dab-like PTB-domain-containing proteins bind preferentially to residues that are unphosphorylated or in which the tyrosine is replaced with phenylalanine (Zhang et al. 1997; Dho et al. 1998; Howell et al. 1999; Uhlik et al. 2005).

The three classes of PTB-containing proteins have three distinct modes of peptide binding described below. To accommodate the negatively charged phosphate moiety, the Shc-binding pocket is basic and positively charged, and forms a network of hydrogen bonding with the phosphate. Three key residues are involved in triangulating the oxygen atoms of the phosphate molecule, and these are conserved in all Shc family members: two arginines (Arg67, Arg175 in Shc) and one lysine (Lys169 in Shc) (Uhlik et al. 2005). As with Shc, IRS-like PTB domains have a positively charged pocket, but only two key arginine residues bind the phosphoryl oxygen molecules: these are Arg212 and Arg227 in IRS-1 (Uhlik et al. 2005). The binding of target ligands with Dab-like PTB domains is non-phospho-dependent and the binding pocket is both less basic and more shallow, having a less important role in peptide-binding affinity. Even though the preferred binding motif is unphosphorylated, the tyrosine at position 0 is important; His136 (in Dab1) forms van der Waals contacts with this residue and Gly131 (Dab1) forms a hydrogen bond. A number of other residues in the  $\beta$ 5 strand also form hydrogen bonds with the ligand (Borg et al. 1996; Zhang et al. 1997; Uhlik et al. 2005). A number of hydrophobic contacts and hydrogen bonds mediate extensive contacts between the Dab-like PTB domains and NPXY-containing ligands (Stolt et al. 2003; Yun et al. 2003; Uhlik et al. 2005).

### PTB Domain Function

Proteins containing PTB domains lack inherent catalytic activity. Although a number of these proteins also lack any other distinguishable domains, many contain additional protein–protein interaction modules, such as PH, SH2, SH3, PDZ, and SAM domains (Uhlik et al. 2005). In these proteins, the domains allow

for pTyr-dependent and -independent interactions, whereas the disordered regions can become phosphorylated to recruit additional signaling components.

Shc is the classical example of a dual SH2 and PTB-domain-containing protein. Shc becomes tyrosine phosphorylated on binding to a receptor, an association that is mediated by its PTB domain (Ravichandran 2001). This pathway is discussed in more detail elsewhere, but the Shc example illustrates how the combination of peptide-binding motifs within a single protein is capable of translating RTK activation into downstream signaling via specific protein–protein interactions.

Similar examples of docking proteins can be seen with IRS-like PTB domains, including IRS-1, FRS2, and Dok-2 (Guy et al. 2002). IRS-like members have been found to bind activated RTKs, including members of the IR, EGF, and RET families (reviewed in Uhlik et al. 2005). Signaling specificity is achieved by variations in binding affinity between recruited proteins (Uhlik et al. 2005). For example, the PTB domain of IRS binds strongly to Tyr960 of the IR juxtamembrane region (Gustafson et al. 1995; Sasaoka and Kobayashi 2000), whereas Shc binds poorly to the same position (van der Geer et al. 1999).

### THE SH2 DOMAIN AS A MOLECULAR SWITCH

RTK signaling is initiated through SH2-mediated binding of active RTKs and phosphorylated scaffolding proteins. In this section, we discuss how this and other SH2-binding events act as “molecular switches” that “turn on” downstream signaling by giving a precise order to the recruitment and activation of signaling effectors.

### SH2-Mediated Recruitment of Signaling Adaptors

Grb2 initiates two signaling pathways following RTK activation and SH2-mediated recruitment: the canonical Ras-MAPK pathway and the canonical PI3K-Akt pathway. Grb2 is recruited to



the cell membrane by binding a pYXNX motif in the RTK itself or in an associated scaffold protein, like Shc or IRS (Fig. 3C). One of the SH3 domains of Grb2 facilitates signaling through the Ras-MAPK pathway by binding the GEF, and activator of Ras, Sos (Fig. 1, inset) (Li et al. 1993; Rozakis-Adcock et al. 1993). Translocation brings Sos proximal to its membrane-bound target Ras. Colocalization seems to be sufficient for Sos to activate Ras, stimulating Ras to exchange GDP for GTP. GTP-bound Ras then activates the serine–threonine kinase Raf, as well as binding to an allosteric regulatory site on Sos to stimulate catalytic activity (Margarit et al. 2003; Sondermann et al. 2004); this ultimately leads to activation of the serine/threonine kinase Erk (extracellular signal-regulated kinase). The outcome of this signaling varies from cell proliferation and survival to differentiation depending on the cellular context, which includes complex mechanisms of Erk regulation and a multiplicity of Erk substrates (Yoon and Seger 2006; Ramos 2008).

A second SH3 domain of Grb2 associates with the scaffold protein Grb2-associated binder 1/2 (Gab) to allow for PI3K signaling. PI3K is activated on recruitment to the cell membrane via binding of a phosphorylated activation motif (pYXXM) in a membrane-proximal protein such as Gab. Once proximal to its lipid substrate, PI3K converts phosphatidylinositol (4,5)-bisphosphate (PIP2) to PIP3. The formation of PIP3 allows for Akt activation because PIP3 specifically binds the PH domains of both Akt and the Akt activator, 3-phosphoinositide-dependent kinase 1 (PDK1) (Newton 2009). Colocalization and a conformational change in Akt (induced by PIP3 binding) allow PDK1 to phosphorylate Akt at threonine 308 (T308) (Stokoe et al. 1997; Stephens et al. 1998). Full Akt activity also requires phosphorylation at serine 473 (S473) by mammalian target of rapamycin complex 2 (mTORC2) (Sarbasov et al. 2005). The final biological outcome of Akt signaling includes promotion of cell growth, proliferation, and survival. Once again, the signaling outcome is thought to be dependent on which substrates are targeted by Akt (Manning and Cantley 2007).

### Controlled Assembly of the Crk-Signaling Complex

The activity of an adaptor can be controlled by mechanisms aside from SH2-mediated recruitment. The Crk adaptor is autoinhibited through intramolecular interactions. Crk consists of an amino-terminal SH2 domain and two SH3 domains separated by a linker region containing a phosphorylation site (Y221). The potential conformations of this molecule include two structures that result in the inhibition of its binding activities. Under basal conditions, Crk is maintained in a compact structure with the inter-SH3 region forming contacts with the SH2 domain and each of its two SH3 domains (Kobashigawa et al. 2007). In this closed conformation, the binding site of the amino-terminal SH3 is occluded by the SH2 domain, leaving it unable to bind to signaling effectors. However, the binding site of the SH2 domain is unobstructed. This suggests that SH2-mediated recruitment to a phosphorylated activation motif may allow for rapid and specific binding of proximal signaling effectors at the amino-terminal SH3 domain. The second inhibitory conformation is thought to be induced as a negative feedback mechanism to limit signaling downstream of RTKs and other receptors. This conformation is induced by active Abl kinase, which phosphorylates Crk at Y221 in the SH3 linker region (Feller et al. 1994). The SH2 domain of Crk then binds to this pTyr and in doing so prevents its binding to intermolecular targets (Feller et al. 1994; Rosen et al. 1995).

### Kinase Inhibition by Intramolecular SH2-Mediated Interactions

The pairing of a kinase domain and a preceding amino-terminal SH2 domain is a configuration that is highly conserved among nonreceptor tyrosine kinases. Whereas this configuration may have initially served to recruit an enzyme to its cognate substrate, it has evolved to play multiple roles in activating and deactivating kinases (Mayer et al. 1995; Li et al. 2008).

SH2 domains stabilize an inactive conformation in nonreceptor tyrosine kinases, including the SFKs and Abl. On a biochemical level,

the activity of these kinases is enhanced as the enzyme switches from a “closed” inactive conformation to an “open” active configuration. For SFKs, this conformational change is regulated by the phosphorylation state of a carboxy-terminal inhibitory tyrosine residue (the equivalent of Y527 in Src). This is regulated by the kinase Csk (c-Src kinase) (Okada and Nakagawa 1989). Crystal structures of inactive SFKs indicate that the closed conformation is characterized by intramolecular interactions between the SH2 domain and the carboxy-terminal inhibitory pTyr motif, and between the SH3 domain and the linker region connecting the SH2 domain and the catalytic domain (Sicheri et al. 1997; Xu et al. 1997). This rigid structure is thought to limit the flexibility of the kinase active site (Young et al. 2001). Inactive Abl mirrors the rigid and closed conformation of inactive Src. However, Abl lacks a regulatory pTyr residue, and an amino-terminal myristoyl group is in part responsible for stabilizing or destabilizing the inhibitory interactions (Hantschel et al. 2003; Nagar et al. 2003).

#### Activation from a State of Autoinhibition

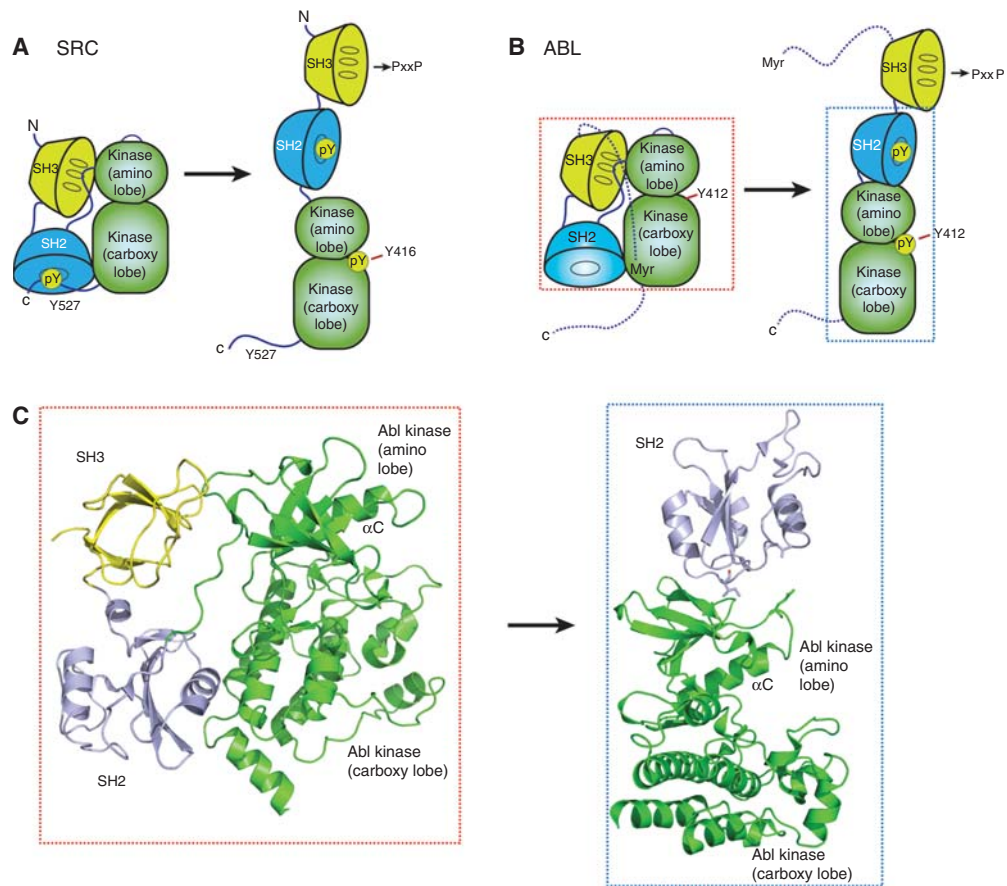
The intramolecular interactions of autoinhibition, such as those of SFKs, Abl, and the adaptor Crk, must be energetically favorable in the absence of a target peptide to prevent constitutive activation. However, they must also be suboptimal for the protein to be labile and able to respond to changes in cell signaling. Structural analysis of the inhibitory conformation of Crk supports this theory because the carboxy-terminal SH3 domain is in a thermodynamically unstable conformation (Cho et al. 2011). Similarly, “strained” conformations can also be observed in SFKs, in which dephosphorylation of the inhibitory tyrosines are sufficient to trigger an open and active conformation (Fig. 5A) (Cooper et al. 1986; Kmiecik and Shalloway 1987; Amrein and Sefton 1988; Xu et al. 1999). In addition, kinase activity can be triggered by the binding of intermolecular target ligands to the SH2 and/or SH3 domains (Liu et al. 1993; Lerner and Smithgall 2002). However, a mutant of the SFK Lck is constitutively inactive and can-

not be activated by T-cell receptor signaling because of a substitution of the native carboxy-terminal inhibitory motif for an alternative optimal SH2-binding sequence: pYEEI (Nika et al. 2007).

#### SH2-Mediated Allosteric Activation of Kinases

The SH2 domains of the nonreceptor tyrosine kinases Csk, Fes, and Abl participate in intramolecular interactions that enhance kinase activity. This is evidenced by the isolated kinase domains from Csk or Fes, which are up to 100-fold less efficient than the full-length kinases (Stone et al. 1984; Sadowski et al. 1986; Filippakopoulos et al. 2008). The isolated Abl kinase domain is also less efficient compared with an SH2-kinase construct of Abl (Fig. 5B) (Filippakopoulos et al. 2008), indicating that the adjacent SH2 domain directly activates the kinase through allosteric effects. In contrast, isolation of the SFK kinase domains (i.e., deletion of the amino-terminal SH2 and SH3 domains) does not hinder activity. Thus, some kinase domains require the SH2 domain for full activity, whereas those of the SFKs do not (Xu and Miller 1996).

The intramolecular mechanisms of activation have been extensively studied for Csk, Fes, and Abl. The crystal structure of full-length Csk shows extensive hydrophobic contacts between a regulatory  $\alpha$ C helix of the kinase domain and the regions surrounding the SH2 domain, the SH3-SH2 linker, and the SH2-kinase domain linker (Ogawa et al. 2002). Similarly, in Fes, acidic residues in the amino-terminal region of the SH2 domain that are distinct from the pTyr-binding pocket form polar interactions with the  $\alpha$ C helix of its kinase domain (Filippakopoulos et al. 2008). In each case, this interaction seems to stabilize the active configuration of the  $\alpha$ C helix and allows for the formation of a lysine-glutamate salt bridge in the active site, and this is critical for orienting the  $\gamma$ -phosphate of ATP. In both structures, the SH2-kinase linker is flexible, suggesting that the SH2 domain can rotate to bind phosphopeptides. Thus, on RTK signaling, SH2-mediated binding of an activation motif may stabilize the active con-



**Figure 5.** SH2 domains regulate tyrosine kinase activation. (A) In the autoinhibited state of Src, the SH2 domain recognizes an intramolecular pTyr site (Y572) and stabilizes the inactive kinase. Additional contacts with the SH3 and the SH2-kinase linker promote stabilization of the inactive conformation of Src. Activation of Src, initiated by dephosphorylation of Y527, frees the SH3 and SH2 to recognize other short linear motifs and further promote kinase activity. (B) The Abl tyrosine kinase remains in an inactive conformation analogous to Src but lacks the intramolecular phosphorylation site. Instead, an intramolecular amino-terminal myristylation stabilizes the inactive conformation of Abl. (C) For Abl kinase to be active, it requires the SH2 domain to stabilize the amino lobe of the kinase domain, thereby allowing it to couple pTyr ligand binding and substrate recognition (PDB:1OPL).

formation. This idea is supported by observations of increased activity of Csk on binding to its phosphorylated target in Cbp (Csk-binding protein) (Takeuchi et al. 2000), and reduced activity in Fes mutants containing a deactivated SH2 pTyr-binding pocket (Filippakopoulos et al. 2008).

Similar to Fes, Abl activity is driven by sites of contact between the SH2 domain (I164) and the amino lobe of the kinase domain (T291/Y331) (Fig. 5C) (Nagar et al. 2006; Filippako-

poulos et al. 2008). This interaction may represent a promising avenue for novel therapeutics because it was shown to be strictly required for the formation of Bcr-Abl-driven leukemia in a mouse model (Grebien et al. 2011).

#### Tandem SH2 Domains Mediate SHP-2 Phosphatase Activity

Although phosphatases are often thought of as negative regulators of signaling, Shp2 is known



to activate the Ras-MAPK pathway downstream of RTKs. In this pathway, the roles of Shp2 as a scaffold for Grb2 and as a phosphatase have yet to be fully elucidated. However, several studies have shown that catalytic activity is necessary for Ras-MAPK activation (Noguchi et al. 1994; Deb et al. 1998; Cunnick et al. 2000; Maroun et al. 2000; Yart et al. 2001).

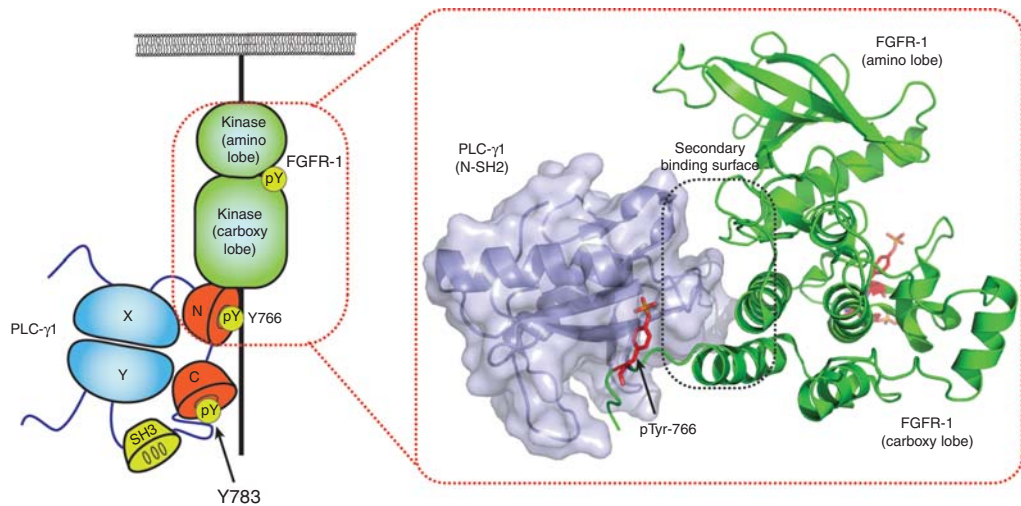
Intramolecular binding of the amino-terminal SH2 domain of Shp2 limits the activity of its PTP (protein tyrosine phosphatase) domain. The crystal structure of Shp2 indicates that the amino-terminal SH2 domain interacts with the PTP domain at residues that are distinct from the pTyr-binding pocket (Hof et al. 1998). Mutation of the amino-terminal SH2 at its PTP interacting residues prevents auto-inhibition, resulting in Shp2 catalytic activity (O'Reilly et al. 2000). Interaction between the SH2 domain and a monophosphorylated activation motif is also sufficient to disrupt the PTP-binding surface and activate the enzyme in vitro (Lechleider et al. 1993; Sugimoto et al. 1994; Hof et al. 1998). Collectively, these data suggest that SH2 target binding simultaneously localizes and disrupts the autoinhibition of Shp2.

Binding of both SH2 domains may further enhance the activity of Shp2. Whereas binding of a monophosphorylated activation motif from IRS-1 is sufficient to activate Shp2, catalytic activity is more potently induced by the corresponding bisphosphorylated motif (Lechleider et al. 1993; Sugimoto et al. 1994; Pluskey et al. 1995). This suggests that Shp2 activity may be enhanced by the engagement of both SH2 domains by IRS-1 in the IR pathway, and potentially by other targets in other RTK pathways. One study suggests that the amino-terminal and carboxy-terminal SH2 domains of Shp2 bind to intramolecular pTyr Y542 and Y580 to enhance activity in vitro (Lu et al. 2001). As well, one of these pTyr is necessary and sufficient to activate the Ras-MAPK pathway in vivo (Lu et al. 2001; Araki et al. 2003). Intramolecular interactions of the SH2 domain may therefore play a role in positively regulating Shp2 phosphatase activity. However, it is of note that some reports suggest that pY542 facilitates binding of

the Grb2 adaptor and that this represents an alternative mechanism of Ras activation (Bennett et al. 1994; Li et al. 1994; Vogel and Ullrich 1996; Araki et al. 2003). The role of these pTyr residues in activating Shp2 and Ras-MAPK signaling warrants further investigation.

### SH2-Mediated Assembly of the PLC- $\gamma$ Catalytic Domain

Active PLC- $\gamma$  cleaves the membrane lipid PIP<sub>2</sub> to give rise to the secondary messengers DAG and IP<sub>3</sub>, and subsequently activates calcium signaling and protein kinase C to induce effects on cell proliferation, cell survival, and cytoskeletal movements. The amino-terminal and carboxy-terminal SH2 domains of PLC- $\gamma$  act in concert to recruit PLC- $\gamma$  to the cell membrane and assemble the two lipase subdomains into an active configuration. PLC- $\gamma$  has an amino-terminal PH domain, a carboxy-terminal C2 domain, as well as a second PH domain and a catalytic domain, each of which is split into two subdomains located on either side of a stretch of amino acids containing two SH2 domains and an SH3 domain. The amino-terminal SH2 domain has been shown to play a dominant role in pTyr-mediated recruitment of PLC- $\gamma$  to the cell membrane downstream of PDGFR (Ji et al. 1999). In the PLC- $\gamma$ -FGFR interaction, the high-affinity binding of the amino-terminal SH2 domain is enhanced by a secondary binding site on the SH2, and this secondary site of contact is required for subsequent enzyme activation (Fig. 6) (Bae et al. 2009). Once bound to an RTK, PLC- $\gamma$  becomes phosphorylated at a critical regulatory tyrosine (Y783) in the linker region between the carboxy-terminal SH2 and the SH3 domains (Kim et al. 1991). The carboxy-terminal SH2 domain binds this pTyr to enhance catalytic activity (Poulin et al. 2005), presumably through a conformational change that unifies the split catalytic domain. Thus the amino-terminal SH2 domain recruits PLC- $\gamma$  for tyrosine phosphorylation and this allows the carboxy-terminal SH2 domain to restructure PLC- $\gamma$  into an active state.



**Figure 6.** Activation of PLC- $\gamma$ . Phospholipase C isoform  $\gamma$  (PLC- $\gamma$ 1) is a tandem SH2 domain-containing protein (see Fig. 2 for a complete view of the domain organization of PLC- $\gamma$ 1) that is recruited to activated FGFR-1. The amino-terminal SH2 domain of PLC- $\gamma$ 1 mediates primary pTyr binding to Y766 on FGFR-1 but also mediates secondary contacts through the B,C loop and D,E loops of the SH2 domain and the  $\alpha$ E, I helix of the carboxy lobe of the kinase domain (PDB: 3GQI). In the activated state of PLC- $\gamma$ 1, the carboxy-terminal SH2 recognizes an intramolecular tyrosine phosphorylation site Y783 bringing together the PLC- $\gamma$ -x (X) and PLC- $\gamma$ -y (Y) lobes, activating the phospholipase enzyme.

### SH2 DOMAINS AND PHOSPHOTYROSINE-MEDIATED SIGNALING IN DISEASE

As reviewed in this article, pTyr binding and other SH2-mediated molecular switches are critical for controlling basal levels of cell signaling and the level of activation following RTK ligation. Loss of these functions is known to cause disease in many instances.

Mutations abrogating pTyr binding of SH2 or PTB domains prevent specific recruitment of the signaling effector following RTK ligation and are sufficient to cause disease. For example, mutation of the c-Cbl SH2-binding site in the RTK c-Met is sufficient to induce malignant transformation of cells *in vitro* and *in vivo* (Park et al. 1986; Abella et al. 2005). Cbl binds RTKs and down-regulates signaling through receptor degradation (Peschard et al. 2001). Exposure to a chemical carcinogen causes loss of the exon that encodes for the juxtamembrane region, including the Cbl SH2-binding site (Park et al. 1986; Vigna et al. 1999; Peschard et al. 2001). This mutation prevents down-reg-

ulation of c-Met signaling and induces cellular transformation. Similarly, mutation of the PTB-binding site of the Dok-7 scaffold is sufficient to cause congenital myasthenic syndrome (CMS). CMS is characterized by malformed muscular junctions and manifests early in life as a muscular weakness that causes hypomobility (Engel 2012). The Dok-7 scaffold is essential for neuromuscular junction formation because it binds the RTK MUSK, and allows for MUSK dimerization and activation in the postsynaptic muscle (Okada et al. 2006; Bergamin et al. 2010). One of the known CMS mutations of Dok7 (Arg 158 to Glu) disrupts a critical salt bridge formed between the pTyr-binding pocket of the PTB domain and the pTyr-binding motif (NPXpY) in MUSK (Bergamin et al. 2010). The resulting disruption of MUSK dimerization and MUSK signaling leads to the malformed muscular junctions of CMS.

Mutation in the SH2 domain of Shp2 disrupts the inactive conformation of the enzyme and manifests in one of two distinct disease phenotypes: Noonan syndrome or myelomo-



nocytic leukemia. Noonan syndrome is a disease characterized in part by congenital heart defects and reduced postnatal growth. Roughly one-half of Noonan syndrome patients have mutations in Shp2 at or close to the interacting residues of the phosphatase domain and the amino-terminal SH2 domain (Tartaglia et al. 2001). This mutation is predicted to result in a gain of function because this interaction surface is known to prevent Shp2-mediated activation of the Ras-MAPK pathway (Hof et al. 1998). Interestingly, less conservative mutations of the same region can cause juvenile myelomonocytic leukemia (Bentires-Alj et al. 2004), suggesting that autoinhibition of Shp2 plays multiple roles in controlling the development and maintenance of human tissues.

Loss of SH2-mediated autoinhibition leads to cellular transformation by the viral homolog of Src. Rous sarcoma virus contains an Src homolog known as v-Src (viral Src) (Martin 2001). Minor differences in the amino acid sequences differentiate v-Src and cellular Src. This includes the loss of the inhibitory pTyr motif. The result is a constitutively active form of Src that transforms the cell through activation of the Ras-MAPK pathway and the PI3K pathway (Cooper et al. 1986; Penuel and Martin 1999).

### CONCLUDING REMARKS

The discovery of the SH2 domain allowed us to appreciate the importance of protein-interaction domains and pTyr-mediated signaling. We now have a strong understanding of the mechanisms involved in intracellular signaling and how it influences human development and disease. With this knowledge we have developed an array of therapeutics targeting nonreceptor kinases and RTKs in cancer and further advances in the field will hopefully allow us to develop novel therapeutics disrupting the intracellular interactions.

In recent years, there has been a shift from contextual studies of the individual components of signaling pathways to high-throughput methodologies that examine signaling in terms of the genetic requirements along with global changes in tyrosine phosphorylation

and gene expression. Combining this approach with computational analysis of large data sets has the potential to rapidly advance the understanding of RTK signaling and other signaling systems.

### REFERENCES

- Abella JV, Peschard P, Naujokas MA, Lin T, Saucier C, Urbe S, Park M. 2005. Met/hepatocyte growth factor receptor ubiquitination suppresses transformation and is required for Hrs phosphorylation. *Mol Cell Biol* **25**: 9632–9645.
- Ahmed Z, Smith BJ, Kotani K, Wilden P, Pillay TS. 1999. APS, an adapter protein with a PH and SH2 domain, is a substrate for the insulin receptor kinase. *Biochem J* **341**: 665–668.
- Amrein KE, Sefton BM. 1988. Mutation of a site of tyrosine phosphorylation in the lymphocyte-specific tyrosine protein kinase, p56lck, reveals its oncogenic potential in fibroblasts. *Proc Natl Acad Sci* **85**: 4247–4251.
- Anderson D, Koch CA, Grey L, Ellis C, Moran ME, Pawson T. 1990. Binding of SH2 domains of phospholipase C $\gamma$ 1, GAP, and Src to activated growth factor receptors. *Science* **250**: 979–982.
- Araki T, Nawa H, Neel BG. 2003. Tyrosyl phosphorylation of Shp2 is required for normal ERK activation in response to some, but not all, growth factors. *J Biol Chem* **278**: 41677–41684.
- Bae JH, Lew ED, Yuzawa S, Tome F, Lax J, Schlessinger J. 2009. The selectivity of receptor tyrosine kinase signaling is controlled by a secondary SH2 domain binding site. *Cell* **138**: 514–524.
- Bennett AM, Tang TL, Sugimoto S, Walsh CT, Neel BG. 1994. Protein-tyrosine-phosphatase SHPTP2 couples platelet-derived growth factor receptor  $\beta$  to Ras. *Proc Natl Acad Sci* **91**: 7335–7339.
- Bentires-Alj M, Paez JG, David FS, Keilhack H, Halmos B, Naoki K, Maris JM, Richardson A, Bardelli A, Sugarbaker DJ, et al. 2004. Activating mutations of the Noonan syndrome-associated SHP2/PTPN11 gene in human solid tumors and adult acute myelogenous leukemia. *Cancer Res* **64**: 8816–8820.
- Bergamin E, Hallock PT, Burden SJ, Hubbard SR. 2010. The cytoplasmic adaptor protein Dok7 activates the receptor tyrosine kinase MuSK via dimerization. *Mol Cell* **39**: 100–109.
- Blaikie P, Immanuel D, Wu J, Li N, Yajnik V, Margolis B. 1994. A region in Shc distinct from the SH2 domain can bind tyrosine-phosphorylated growth factor receptors. *J Biol Chem* **269**: 32031–32034.
- Borg JP, Ooi J, Levy E, Margolis B. 1996. The phosphotyrosine interaction domains of X11 and FE65 bind to distinct sites on the YENPTY motif of amyloid precursor protein. *Mol Cell Biol* **16**: 6229–6241.
- Cho JH, Muralidharan V, Vila-Perello M, Raleigh DP, Muir TW, Palmer AG 3rd. 2011. Tuning protein autoinhibition by domain destabilization. *Nat Struct Mol Biol* **18**: 550–555.

- Cooper JA, Gould KL, Cartwright CA, Hunter T. 1986. Tyr527 is phosphorylated in pp60c-src: Implications for regulation. *Science* **231**: 1431–1434.
- Cunnick JM, Dorsey JF, Munoz-Antonia T, Mei L, Wu J. 2000. Requirement of SHP2 binding to Grb2-associated binder-1 for mitogen-activated protein kinase activation in response to lysophosphatidic acid and epidermal growth factor. *J Biol Chem* **275**: 13842–13848.
- Darnell JE Jr. 1997. STATs and gene regulation. *Science* **277**: 1630–1635.
- Deb TB, Wong L, Salomon DS, Zhou G, Dixon JE, Gutkind JS, Thompson SA, Johnson GR. 1998. A common requirement for the catalytic activity and both SH2 domains of SHP-2 in mitogen-activated protein (MAP) kinase activation by the ErbB family of receptors. A specific role for SHP-2 in map, but not c-Jun amino-terminal kinase activation. *J Biol Chem* **273**: 16643–16646.
- Dhe-Paganon S, Werner ED, Nishi M, Hansen L, Chi YI, Shoelson SE. 2004. A phenylalanine zipper mediates APS dimerization. *Nat Struct Mol Biol* **11**: 968–974.
- Dho SE, Jacob S, Wolting CD, French MB, Rohrschneider LR, McClade CJ. 1998. The mammalian numb phosphotyrosine-binding domain. Characterization of binding specificity and identification of a novel PDZ domain-containing numb binding protein, LNX. *J Biol Chem* **273**: 9179–9187.
- Eck MJ, Pluskey S, Trub T, Harrison SC, Shoelson SE. 1996. Spatial constraints on the recognition of phosphoproteins by the tandem SH2 domains of the phosphatase SH-PTP2. *Nature* **379**: 277–280.
- Engel AG. 2012. Current status of the congenital myasthenic syndromes. *Neuromuscul Disord* **22**: 99–111.
- Feller SM, Knudsen B, Hanafusa H. 1994. c-Abl kinase regulates the protein binding activity of c-Crk. *EMBO J* **13**: 2341–2351.
- Filippakopoulos P, Kofler M, Hantschel O, Gish GD, Grebien F, Salah E, Neudecker P, Kay LE, Turk BE, Superti-Furga G, et al. 2008. Structural coupling of SH2-kinase domains links Fes and Abl substrate recognition and kinase activation. *Cell* **134**: 793–803.
- Grebien F, Hantschel O, Wojcik J, Kaupé I, Kovacic B, Wyrzucki AM, Gish GD, Cerny-Reiterer S, Koide A, Beug H, et al. 2011. Targeting the SH2-kinase interface in Bcr-Abl inhibits leukemogenesis. *Cell* **147**: 306–319.
- Gustafson TA, He W, Craparo A, Schaub CD, O'Neill TJ. 1995. Phosphotyrosine-dependent interaction of SHC and insulin receptor substrate 1 with the NPEY motif of the insulin receptor via a novel non-SH2 domain. *Mol Cell Biol* **15**: 2500–2508.
- Guy GR, Yusoff P, Bangarusamy D, Fong CW, Wong ES. 2002. Dockers at the crossroads. *Cell Signal* **14**: 11–20.
- Hantschel O, Nagar B, Guettler S, Kretzschmar J, Dorey K, Kuriyan J, Superti-Furga G. 2003. A myristoyl/phosphotyrosine switch regulates c-Abl. *Cell* **112**: 845–857.
- Harrison SC. 1996. Peptide-surface association: The case of PDZ and PTB domains. *Cell* **86**: 341–343.
- Hatada MH, Lu X, Laird ER, Green J, Morgenstern JP, Lou M, Marr CS, Phillips TB, Ram MK, Theriault K, et al. 1995. Molecular basis for interaction of the protein tyrosine kinase ZAP-70 with the T-cell receptor. *Nature* **377**: 32–38.
- Hidaka M, Homma Y, Takenawa T. 1991. Highly conserved eight amino acid sequence in SH2 is important for recognition of phosphotyrosine site. *Biochem Biophys Res Commun* **180**: 1490–1497.
- Hof P, Pluskey S, Dhe-Paganon S, Eck MJ, Shoelson SE. 1998. Crystal structure of the tyrosine phosphatase SHP-2. *Cell* **92**: 441–450.
- Howell BW, Lanier LM, Frank R, Gertler FB, Cooper JA. 1999. The disabled 1 phosphotyrosine-binding domain binds to the internalization signals of transmembrane glycoproteins and to phospholipids. *Mol Cell Biol* **19**: 5179–5188.
- Hu J, Hubbard SR. 2005. Structural characterization of a novel Cbl phosphotyrosine recognition motif in the APS family of adapter proteins. *J Biol Chem* **280**: 18943–18949.
- Hu J, Hubbard SR. 2006. Structural basis for phosphotyrosine recognition by the Src homology-2 domains of the adapter proteins SH2-B and APS. *J Mol Biol* **361**: 69–79.
- Hu J, Liu J, Ghirlando R, Saltiel AR, Hubbard SR. 2003. Structural basis for recruitment of the adaptor protein APS to the activated insulin receptor. *Mol Cell* **12**: 1379–1389.
- Hunter T. 2007. The age of crosstalk: Phosphorylation, ubiquitination, and beyond. *Mol Cell* **28**: 730–738.
- Hynes NE, Lane HA. 2005. ERBB receptors and cancer: The complexity of targeted inhibitors. *Nat Rev Cancer* **5**: 341–354.
- Ji QS, Chattopadhyay A, Vecchi M, Carpenter G. 1999. Physiological requirement for both SH2 domains for phospholipase C- $\gamma$ 1 function and interaction with platelet-derived growth factor receptors. *Mol Cell Biol* **19**: 4961–4970.
- Kavanaugh WM, Williams LT. 1994. An alternative to SH2 domains for binding tyrosine-phosphorylated proteins. *Science* **266**: 1862–1865.
- Kim HK, Kim JW, Zilberstein A, Margolis B, Kim JG, Schlesinger J, Rhee SG. 1991. PDGF stimulation of inositol phospholipid hydrolysis requires PLC- $\gamma$  1 phosphorylation on tyrosine residues 783 and 1254. *Cell* **65**: 435–441.
- Kmieciak TE, Shalloway D. 1987. Activation and suppression of pp60c-src transforming ability by mutation of its primary sites of tyrosine phosphorylation. *Cell* **49**: 65–73.
- Kobashigawa Y, Sakai M, Naito M, Yokochi M, Kumeta H, Makino Y, Ogura K, Tanaka S, Inagaki F. 2007. Structural basis for the transforming activity of human cancer-related signaling adaptor protein CRK. *Nat Struct Mol Biol* **14**: 503–510.
- Koch CA, Anderson D, Moran ME, Ellis C, Pawson T. 1991. SH2 and SH3 domains: Elements that control interactions of cytoplasmic signaling proteins. *Science* **252**: 668–674.
- Kouhara H, Hadari YR, Spivak-Kroizman T, Schilling J, Bar-Sagi D, Lax I, Schlessinger J. 1997. A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway. *Cell* **89**: 693–702.
- Kuriyan J, Cowburn D. 1997. Modular peptide recognition domains in eukaryotic signaling. *Ann Rev Biophys Biomol Struct* **26**: 259–288.
- Lechleider RJ, Sugimoto S, Bennett AM, Kashishian AS, Cooper JA, Shoelson SE, Walsh CT, Neel BG. 1993. Acti-





- vation of the SH2-containing phosphotyrosine phosphatase SH-PTP2 by its binding site, phosphotyrosine 1009, on the human platelet-derived growth factor receptor. *J Biol Chem* **268**: 21478–21481.
- Lee JY, Chiu YH, Asara J, Cantley LC. 2011. Inhibition of PI3K binding to activators by serine phosphorylation of PI3K regulatory subunit p85 $\alpha$  Src homology-2 domains. *Proc Natl Acad Sci* **108**: 14157–14162.
- Lerner EC, Smithgall TE. 2002. SH3-dependent stimulation of Src-family kinase autophosphorylation without tail release from the SH2 domain in vivo. *Nat Struct Biol* **9**: 365–369.
- Levy DE, Darnell JE Jr. 2002. Stats: Transcriptional control and biological impact. *Nat Rev Mol Cell Biol* **3**: 651–662.
- Li N, Batzer A, Daly R, Yajnik V, Skolnik E, Chardin P, Bar-Sagi D, Margolis B, Schlessinger J. 1993. Guanine-nucleotide-releasing factor hSos1 binds to Grb2 and links receptor tyrosine kinases to Ras signalling. *Nature* **363**: 85–88.
- Li W, Nishimura R, Kashishian A, Batzer AG, Kim WJ, Cooper JA, Schlessinger J. 1994. A new function for a phosphotyrosine phosphatase: Linking GRB2-Sos to a receptor tyrosine kinase. *Mol Cell Biol* **14**: 509–517.
- Li SC, Gish G, Yang D, Coffey AJ, Forman-Kay JD, Ernberg I, Kay LE, Pawson T. 1999. Novel mode of ligand binding by the SH2 domain of the human XLP disease gene product SAP/SH2D1A. *Curr Biol* **9**: 1355–1362.
- Li W, Young SL, King N, Miller WT. 2008. Signaling properties of a non-metazoan Src kinase and the evolutionary history of Src negative regulation. *J Biol Chem* **283**: 15491–15501.
- Liu X, Brodeur SR, Gish G, Songyang Z, Cantley LC, Laudano AP, Pawson T. 1993. Regulation of c-Src tyrosine kinase activity by the Src SH2 domain. *Oncogene* **8**: 1119–1126.
- Liu BA, Jablonowski K, Raina M, Arce M, Pawson T, Nash PD. 2006. The human and mouse complement of SH2 domain proteins—establishing the boundaries of phosphotyrosine signaling. *Mol Cell* **22**: 851–868.
- Lu W, Gong D, Bar-Sagi D, Cole PA. 2001. Site-specific incorporation of a phosphotyrosine mimetic reveals a role for tyrosine phosphorylation of SHP-2 in cell signaling. *Mol Cell* **8**: 759–769.
- Lupher ML Jr, Songyang Z, Shoelson SE, Cantley LC, Band H. 1997. The Cbl phosphotyrosine-binding domain selects a D(N/D)XpY motif and binds to the Tyr<sup>292</sup> negative regulatory phosphorylation site of ZAP-70. *J Biol Chem* **272**: 33140–33144.
- Ma CS, Deenick EK. 2011. The role of SAP and SLAM family molecules in the humoral immune response. *Ann NY Acad Sci* **1217**: 32–44.
- Manning BD, Cantley LC. 2007. AKT/PKB signaling: Navigating downstream. *Cell* **129**: 1261–1274.
- Marengere LE, Songyang Z, Gish GD, Schaller MD, Parsons JT, Stern MJ, Cantley LC, Pawson T. 1994. SH2 domain specificity and activity modified by a single residue. *Nature* **369**: 502–505.
- Margarit SM, Sondermann H, Hall BE, Nagar B, Hoelz A, Pirruccello M, Bar-Sagi D, Kuriyan J. 2003. Structural evidence for feedback activation by Ras.GTP of the Ras-specific nucleotide exchange factor SOS. *Cell* **112**: 685–695.
- Maroun CR, Naujokas MA, Holgado-Madruga M, Wong AJ, Park M. 2000. The tyrosine phosphatase SHP-2 is required for sustained activation of extracellular signal-regulated kinase and epithelial morphogenesis downstream from the met receptor tyrosine kinase. *Mol Cell Biol* **20**: 8513–8525.
- Martin GS. 2001. The hunting of the Src. *Nat Rev Mol Cell Biol* **2**: 467–475.
- Mayer BJ, Hirai H, Sakai R. 1995. Evidence that SH2 domains promote processive phosphorylation by protein-tyrosine kinases. *Curr Biol* **5**: 296–305.
- Meng W, Sawasdikosol S, Burakoff SJ, Eck MJ. 1999. Structure of the amino-terminal domain of Cbl complexed to its binding site on ZAP-70 kinase. *Nature* **398**: 84–90.
- Moodie SA, Alleman-Sposeto J, Gustafson TA. 1999. Identification of the APS protein as a novel insulin receptor substrate. *J Biol Chem* **274**: 11186–11193.
- Nagar B, Hantschel O, Young MA, Scheffzek K, Veach D, Bornmann W, Clarkson B, Superti-Furga G, Kuriyan J. 2003. Structural basis for the autoinhibition of c-Abl tyrosine kinase. *Cell* **112**: 859–871.
- Nagar B, Hantschel O, Seeliger M, Davies JM, Weis WI, Superti-Furga G, Kuriyan J. 2006. Organization of the SH3-SH2 unit in active and inactive forms of the c-Abl tyrosine kinase. *Mol Cell* **21**: 787–798.
- Newton AC. 2009. Lipid activation of protein kinases. *J Lipid Res* **50**: S266–S271.
- Ng C, Jackson RA, Buschdorf JB, Sun Q, Guy GR, Sivaraman J. 2008. Structural basis for a novel intrapeptidyl H-bond and reverse binding of c-Cbl-TKB domain substrates. *EMBO J* **27**: 804–816.
- Nika K, Tautz L, Arimura Y, Vang T, Williams S, Mustelin T. 2007. A weak Lck tail bite is necessary for Lck function in T cell antigen receptor signaling. *J Biol Chem* **282**: 36000–36009.
- Nishi M, Werner ED, Oh BC, Frantz JD, Dhe-Paganon S, Hansen L, Lee J, Shoelson SE. 2005. Kinase activation through dimerization by human SH2-B. *Mol Cell Biol* **25**: 2607–2621.
- Noguchi T, Matozaki T, Horita K, Fujioka Y, Kasuga M. 1994. Role of SH-PTP2, a protein-tyrosine phosphatase with Src homology 2 domains, in insulin-stimulated Ras activation. *Mol Cell Biol* **14**: 6674–6682.
- Ogawa A, Takayama Y, Sakai H, Chong KT, Takeuchi S, Nakagawa A, Nada S, Okada M, Tsukihara T. 2002. Structure of the carboxyl-terminal Src kinase, Csk. *J Biol Chem* **277**: 14351–14354.
- Okada M, Nakagawa H. 1989. A protein tyrosine kinase involved in regulation of pp60c-src function. *J Biol Chem* **264**: 20886–20893.
- Okada K, Inoue A, Okada M, Murata Y, Kakuta S, Jigami T, Kubo S, Shiraishi H, Eguchi K, Motomura M, et al. 2006. The muscle protein Dok-7 is essential for neuromuscular synaptogenesis. *Science* **312**: 1802–1805.
- O'Reilly AM, Pluskey S, Shoelson SE, Neel BG. 2000. Activated mutants of SHP-2 preferentially induce elongation of *Xenopus* animal caps. *Mol Cell Biol* **20**: 299–311.



- Ottinger EA, Botfield MC, Shoelson SE. 1998. Tandem SH2 domains confer high specificity in tyrosine kinase signaling. *J Biol Chem* **273**: 729–735.
- Park M, Dean M, Cooper CS, Schmidt M, O'Brien SJ, Blair DG, Vande Woude GF. 1986. Mechanism of met oncogene activation. *Cell* **45**: 895–904.
- Pawson T. 1995. Protein modules and signalling networks. *Nature* **373**: 573–580.
- Pawson T, Gish GD, Nash P. 2001. SH2 domains, interaction modules and cellular wiring. *Trends Cell Biol* **11**: 504–511.
- Penengo L, Rubin C, Yarden Y, Gaudino G. 2003. c-Cbl is a critical modulator of the Ron tyrosine kinase receptor. *Oncogene* **22**: 3669–3679.
- Penuel E, Martin GS. 1999. Transformation by v-Src: Ras-MAPK and PI3K-mTOR mediate parallel pathways. *Mol Biol Cell* **10**: 1693–1703.
- Peschard P, Fournier TM, Lamorte L, Naujokas MA, Band H, Langdon WY, Park M. 2001. Mutation of the c-Cbl TKB domain binding site on the Met receptor tyrosine kinase converts it into a transforming protein. *Mol Cell* **8**: 995–1004.
- Peschard P, Ishiyama N, Lin T, Lipkowitz S, Park M. 2004. A conserved DpYR motif in the juxtamembrane domain of the Met receptor family forms an atypical c-Cbl/Cbl-b tyrosine kinase binding domain binding site required for suppression of oncogenic activation. *J Biol Chem* **279**: 29565–29571.
- Pluskey S, Wandless TJ, Walsh CT, Shoelson SE. 1995. Potent stimulation of SH-PTP2 phosphatase activity by simultaneous occupancy of both SH2 domains. *J Biol Chem* **270**: 2897–2900.
- Poulin B, Sekiya F, Rhee SG. 2005. Intramolecular interaction between phosphorylated tyrosine-783 and the C-terminal Src homology 2 domain activates phospholipase C- $\gamma$ 1. *Proc Natl Acad Sci* **102**: 4276–4281.
- Poy F, Yaffe MB, Sayos J, Saxena K, Morra M, Sumegi J, Cantley LC, Terhorst C, Eck MJ. 1999. Crystal structures of the XLP protein SAP reveal a class of SH2 domains with extended, phosphotyrosine-independent sequence recognition. *Mol Cell* **4**: 555–561.
- Ramos JW. 2008. The regulation of extracellular signal-regulated kinase (ERK) in mammalian cells. *Int J Biochem Cell Biol* **40**: 2707–2719.
- Ravichandran KS. 2001. Signaling via Shc family adapter proteins. *Oncogene* **20**: 6322–6330.
- Rivero-Lezcano OM, Marcilla A, Sameshima JH, Robbins KC. 1995. Wiskott-Aldrich syndrome protein physically associates with Nck through Src homology 3 domains. *Mol Cell Biol* **15**: 5725–5731.
- Rosen MK, Yamazaki T, Gish GD, Kay CM, Pawson T, Kay LE. 1995. Direct demonstration of an intramolecular SH2-phosphotyrosine interaction in the Crk protein. *Nature* **374**: 477–479.
- Rozakis-Adcock M, Fernley R, Wade J, Pawson T, Bowtell D. 1993. The SH2 and SH3 domains of mammalian Grb2 couple the EGF receptor to the Ras activator mSos1. *Nature* **363**: 83–85.
- Ryan PE, Sivadasan-Nair N, Nau MM, Nicholas S, Lipkowitz S. 2010. The N terminus of Cbl-c regulates ubiquitin ligase activity by modulating affinity for the ubiquitin-conjugating enzyme. *J Biol Chem* **285**: 23687–23698.
- Sadowski I, Stone JC, Pawson T. 1986. A noncatalytic domain conserved among cytoplasmic protein-tyrosine kinases modifies the kinase function and transforming activity of Fujinami sarcoma virus P130gag-fps. *Mol Cell Biol* **6**: 4396–4408.
- Salcini AE, McGlade J, Pelicci G, Nicoletti I, Pawson T, Pelicci PG. 1994. Formation of Shc-Grb2 complexes is necessary to induce neoplastic transformation by overexpression of Shc proteins. *Oncogene* **9**: 2827–2836.
- Sanjay A, Horne WC, Baron R. 2001. The Cbl family: Ubiquitin ligases regulating signaling by tyrosine kinases. *Sci STKE* **2001**: e40.
- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. 2005. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* **307**: 1098–1101.
- Sasaoka T, Kobayashi M. 2000. The functional significance of Shc in insulin signaling as a substrate of the insulin receptor. *Endocr J* **47**: 373–381.
- Saucier C, Khoury H, Lai KM, Peschard P, Dankort D, Naujokas MA, Holash J, Yancopoulos GD, Muller WJ, Pawson T, et al. 2004. The Shc adaptor protein is critical for VEGF induction by Met/HGF and ErbB2 receptors and for early onset of tumor angiogenesis. *Proc Natl Acad Sci* **101**: 2345–2350.
- Sayos J, Wu C, Morra M, Wang N, Zhang X, Allen D, van Schaik S, Notarangelo L, Geha R, Roncarolo MG, et al. 1998. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* **395**: 462–469.
- Schlessinger J, Lemmon MA. 2003. SH2 and PTB domains in tyrosine kinase signaling. *Sci STKE* **2003**: RE12.
- Schmidt MH, Dikic I. 2005. The Cbl interactome and its functions. *Nat Rev Mol Cell Biol* **6**: 907–918.
- Sicheri F, Moarefi I, Kuriyan J. 1997. Crystal structure of the Src family tyrosine kinase Hck. *Nature* **385**: 602–609.
- Sondermann H, Soisson SM, Boykevich S, Yang SS, Barsagi D, Kuriyan J. 2004. Structural analysis of autoinhibition in the Ras activator Son of sevenless. *Cell* **119**: 393–405.
- Songyang Z, Shoelson SE, Chaudhuri M, Gish G, Pawson T, Haser WG, King F, Roberts T, Ratnofsky S, Lechleider RJ, et al. 1993. SH2 domains recognize specific phosphopeptide sequences. *Cell* **72**: 767–778.
- Stahl N, Farruggella TJ, Boulton TG, Zhong Z, Darnell JE Jr, Yancopoulos GD. 1995. Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. *Science* **267**: 1349–1353.
- Stephens L, Anderson K, Stokoe D, Erdjument-Bromage H, Painter GF, Holmes AB, Gaffney PR, Reese CB, McCormick F, Tempst P, et al. 1998. Protein kinase B kinases that mediate phosphatidylinositol 3,4,5-trisphosphate-dependent activation of protein kinase B. *Science* **279**: 710–714.
- Stokoe D, Stephens LR, Copeland T, Gaffney PR, Reese CB, Painter GF, Holmes AB, McCormick F, Hawkins PT. 1997. Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B. *Science* **277**: 567–570.
- Stolt PC, Jeon H, Song HK, Herz J, Eck MJ, Blacklow SC. 2003. Origins of peptide selectivity and phosphoinositide



- binding revealed by structures of disabled-1 PTB domain complexes. *Structure* **11**: 569–579.
- Stone JC, Atkinson T, Smith M, Pawson T. 1984. Identification of functional regions in the transforming protein of Fujinami sarcoma virus by in-phase insertion mutagenesis. *Cell* **37**: 549–558.
- Sugimoto S, Wandless TJ, Shoelson SE, Neel BG, Walsh CT. 1994. Activation of the SH2-containing protein tyrosine phosphatase, SH-PTP2, by phosphotyrosine-containing peptides derived from insulin receptor substrate-1. *J Biol Chem* **269**: 13614–13622.
- Takeuchi S, Takayama Y, Ogawa A, Tamura K, Okada M. 2000. Transmembrane phosphoprotein Cbp positively regulates the activity of the carboxyl-terminal Src kinase, Csk. *J Biol Chem* **275**: 29183–29186.
- Tamagnone L, Artigiani S, Chen H, He Z, Ming GI, Song H, Chedotal A, Winberg ML, Goodman CS, Poo M, et al. 1999. Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. *Cell* **99**: 71–80.
- Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, van der Burgt I, Crosby AH, Ion A, Jeffery S, et al. 2001. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. *Nat Genet* **29**: 465–468.
- Uhlík MT, Temple B, Bencharit S, Kimple AJ, Siderovski DP, Johnson GL. 2005. Structural and evolutionary division of phosphotyrosine binding (PTB) domains. *J Mol Biol* **345**: 1–20.
- van der Geer P, Wiley S, Gish GD, Pawson T. 1996. The Shc adaptor protein is highly phosphorylated at conserved, twin tyrosine residues (Y239/240) that mediate protein–protein interactions. *Curr Biol* **6**: 1435–1444.
- van der Geer P, Wiley S, Pawson T. 1999. Re-engineering the target specificity of the insulin receptor by modification of a PTB domain binding site. *Oncogene* **18**: 3071–3075.
- Vigna E, Gramaglia D, Longati P, Bardelli A, Comoglio PM. 1999. Loss of the exon encoding the juxtamembrane domain is essential for the oncogenic activation of TPR-MET. *Oncogene* **18**: 4275–4281.
- Vogel W, Ullrich A. 1996. Multiple in vivo phosphorylated tyrosine phosphatase SHP-2 engages binding to Grb2 via tyrosine 584. *Cell Growth Differ* **7**: 1589–1597.
- Waksman G, Shoelson SE, Pant N, Cowburn D, Kuriyan J. 1993. Binding of a high affinity phosphotyrosyl peptide to the Src SH2 domain: Crystal structures of the complexed and peptide-free forms. *Cell* **72**: 779–790.
- Xu B, Miller WT. 1996. Src homology domains of v-Src stabilize an active conformation of the tyrosine kinase catalytic domain. *Mol Cell Biochem* **158**: 57–63.
- Xu W, Harrison SC, Eck MJ. 1997. Three-dimensional structure of the tyrosine kinase c-Src. *Nature* **385**: 595–602.
- Xu W, Doshi A, Lei M, Eck MJ, Harrison SC. 1999. Crystal structures of c-Src reveal features of its autoinhibitory mechanism. *Mol Cell* **3**: 629–638.
- Yart A, Laffargue M, Mayeux P, Chretien S, Peres C, Tonks N, Roche S, Payrastra B, Chap H, Raynal P. 2001. A critical role for phosphoinositide 3-kinase upstream of Gab1 and SHP2 in the activation of ras and mitogen-activated protein kinases by epidermal growth factor. *J Biol Chem* **276**: 8856–8864.
- Yokouchi M, Wakioka T, Sakamoto H, Yasukawa H, Ohtsuka S, Sasaki A, Ohtsubo M, Valius M, Inoue A, Komiya S, et al. 1999. APS, an adaptor protein containing PH and SH2 domains, is associated with the PDGF receptor and c-Cbl and inhibits PDGF-induced mitogenesis. *Oncogene* **18**: 759–767.
- Yoon S, Seger R. 2006. The extracellular signal-regulated kinase: Multiple substrates regulate diverse cellular functions. *Growth Factors* **24**: 21–44.
- Young MA, Gonfloni S, Superti-Furga G, Roux B, Kuriyan J. 2001. Dynamic coupling between the SH2 and SH3 domains of c-Src and Hck underlies their inactivation by C-terminal tyrosine phosphorylation. *Cell* **105**: 115–126.
- Yun M, Keshvara L, Park CG, Zhang YM, Dickerson JB, Zheng J, Rock CO, Curran T, Park HW. 2003. Crystal structures of the Dab homology domains of mouse disabled 1 and 2. *J Biol Chem* **278**: 36572–36581.
- Zhang Z, Lee CH, Mandiyan V, Borg JP, Margolis B, Schlesinger J, Kuriyan J. 1997. Sequence-specific recognition of the internalization motif of the Alzheimer's amyloid precursor protein by the X11 PTB domain. *EMBO J* **16**: 6141–6150.
- Zhang X, Gureasko J, Shen K, Cole PA, Kuriyan J. 2006. An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. *Cell* **125**: 1137–1149.
- Zhao ZS, Manser E, Lim L. 2000. Interaction between PAK and nck: A template for Nck targets and role of PAK autophosphorylation. *Mol Cell Biol* **20**: 3906–3917.
- Zhou MM, Ravichandran KS, Olejniczak EF, Petros AM, Meadows RP, Sattler M, Harlan JE, Wade WS, Burakoff SJ, Fesik SW. 1995. Structure and ligand recognition of the phosphotyrosine binding domain of Shc. *Nature* **378**: 584–592.