1
The SH2 Domain: a Prototype for Protein Interaction Modules

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1.1
The Multidomain Nature of Signaling Proteins and Identification of the SH2 Domain

The discovery of viral oncogenes in the 1970s initiated an intense effort to understand the biochemical and functional properties of their protein products. The striking effects of such transforming proteins on cellular behavior suggested that understanding their modus operandi might reveal new principles underlying the molecular basis for cellular regulation and oncogenesis. Indeed, analysis of the Src, Abl, and Fps/Fes retroviral oncoproteins led to the realization that these proteins have intrinsic protein-tyrosine kinase activity, which correlates with their ability to transform cells from a normal to a cancerous phenotype [1–4]. Furthermore, the normal progenitors of these cytoplasmic proteins, as well as the cell surface receptors for growth factors and insulin, proved to be tyrosine kinases, suggesting that tyrosine phosphorylation is a common physiological mechanism through which cells respond to mitogenic or metabolic hormones [5–8]. Such hormones frequently induce receptor clustering, which stimulates receptor kinase activity, and this effect can be mimicked by oncogenic mutations that constitutively activate the receptor in the absence of an external ligand [9–11].

A logical approach toward understanding the normal and malignant properties of cytoplasmic or receptor tyrosine kinases (RTK) was therefore to pursue the biological consequences of tyrosine phosphorylation. By focusing on the tyrosine kinases themselves, it became apparent that these enzymes are activated by intermolecular autophosphorylation within their kinase domains, leading to a sustained increase in kinase activity [12–14]. Subsequent analysis has shown that such autophosphorylation causes a structural reorganization of the catalytic domain, primarily through movement of the activation loop, which otherwise occludes the kinase active site and interferes with ATP and substrate binding [15, 16]. These observations were consistent with a straightforward model in which tyrosine kinases might regulate the enzymatic activity of exogenous substrates through phosphorylation near their active sites. Curiously, however, receptor tyrosine kinases (RTK) themselves were often found to be the most abundant phosphotyrosine (pTyr)-

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containing proteins in growth factor-stimulated cells, raising the possibility that tyrosine phosphorylation might have unexpected biochemical functions. Furthermore, cytoplasmic protein-tyrosine kinases appeared to have extended polypeptide sequences in addition to the catalytic domain, suggesting that kinases such as Fps, Src, and Abl might have novel, noncatalytic properties. We therefore set out to test the notion that a cytoplasmic tyrosine kinase such as v-Fps might contain multiple functional domains.

Our group had used site-directed mutagenesis, a technique that was then in its infancy, to establish that tyrosine phosphorylation within the C-terminal catalytic domain of the v-Fps cytoplasmic oncoprotein stimulates its kinase activity and transforming potential [13]. We devised a variation of this approach to scan the 130-kDa v-Fps retroviral protein for folded domains that participate in its transforming activity. To this end, we inserted a dipeptide (or oligopeptide) motif at any one of numerous sites throughout the v-Fps protein, with the idea that such an insertion might disrupt the folded structure, and consequently the function, of a globular domain into which it was incorporated, but leave distinct domains intact [17]. At the time we knew that v-Fps had an N-terminal region encoded by the retroviral Gag gene fused to sequences derived from the cellular Fps tyrosine kinase, with the kinase domain located at the extreme C terminus [18]. The insertion mutagenesis approach identified three v-Fps domains that appeared to collaborate to induce cellular transformation. Not surprisingly, insertions in the C-terminal region of v-Fps corresponding to the kinase domain led to a loss of kinase and transforming activities. Insertions in an N-terminal v-Fps sequence caused a significant loss of transforming activity without an obvious effect on catalytic efficiency. This region has more recently been termed the FCH (Fes/Cip4 homology) domain and has been implicated in cytoskeletal localization [19, 20]. Consistent with the rationale for this mutagenesis approach, insertions in the FCH domain destroyed recognition of the v-Fps protein by two anti-Fps monoclonal antibodies that recognize conformation-dependent epitopes [21]. In contrast, insertion in the Gag sequence or in the central region of v-Fps had little effect.

Most interestingly, insertions (and subsequently deletions) in the sequence immediately N-terminal to the kinase domain influenced both kinase activity and substrate recognition and inhibited cellular transformation [17, 22]. Although this region was not necessary for kinase activity per se, it formed a protease-resistant structure that also contained the kinase domain, was important for full kinase activity in vivo, and promoted the tyrosine phosphorylation of selected cellular proteins [22–24]. Inspection of the amino acid sequences of Src and Abl showed that this region of ~100 amino acids N-terminal to the v-Fps kinase domain was relatively conserved in these other cytoplasmic tyrosine kinases and similarly located adjacent to the kinase domain, and we therefore termed it the Src homology 2 (SH2) domain (with the kinase domain implicitly being the SH1 region) [22] (Figure 1.1).

Taken together, these data indicated that v-Fps, and likely other cytoplasmic tyrosine kinases, were composed of multiple folded domains. The SH2 sequence was identified as a conserved noncatalytic domain, but one with the potential to

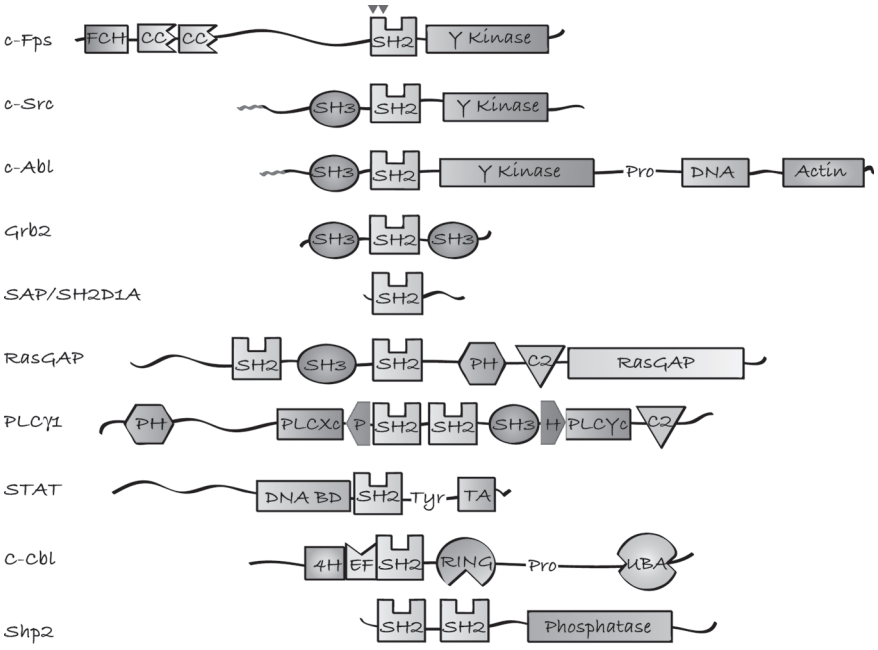


Figure 1.1 The domain organization of several SH2-containing proteins. Arrowheads mark the positions of insertions that led to identification of the SH2 domain in v-Fps.
Y kinase = tyrosine kinase domain; CC = coiled coil; Pro = SH3 binding motifs; BD = binding domain; actin = actin binding domain; PLC X/Yc = split PLC catalytic domain; Tyr = tyrosine phosphorylation site; 4H = 4-helix bundle; EF = EF hard-like domain; TA = transactivation domain; PH = pleckstrin homology; SH = Src homology; DNA = DNA-binding domain; Actin = actin-binding domain; UBA = ubiquitin-associated domain.

regulate both tyrosine kinase activity and substrate recognition and to promote malignant transformation. Such results suggested that the ability of cytoplasmic tyrosine kinases to recognize their substrates is not an exclusive property of the catalytic domain, but also depends on modular noncatalytic domains that target the kinase to specific substrates for phosphorylation.

Support for these ideas came from cloning of the viral Crk oncogene by Hidesaburo Hanafusa [25] and of cDNAs for mammalian phospholipase C (PLC) γ1 and Ras GTPase-activating protein (GAP) [26–28]. Intriguingly, the v-Crk oncoprotein comprised only an SH2 domain and a sequence present in Src, Abl, PLC-γ1, and RasGAP (but absent from Fps), which Hanafusa termed SH3 and which was subsequently shown by the Baltimore lab to bind proline-rich motifs [29]. Despite this lack of intrinsic catalytic activity, the v-Crk oncoprotein stimulated endogenous tyrosine kinase activity in transformed cells and enhanced the phosphorylation of a 130-kDa protein ($p130^{cas}$) at tyrosine, consistent with the view

that the SH2 and SH3 domains might nucleate the formation of multiprotein complexes involved in tyrosine kinase signaling. Sequence analysis indicated that both PLCγ1 and RasGAP contain two SH2 domains and an SH3 domain. Taken with the earlier v-Fps data, these results suggested that SH2 domains are a common element of otherwise disparate enzymes and adaptors that function downstream of tyrosine kinases to control intracellular pathways such as those involved in phospholipid metabolism and Ras GTPase signaling. In support of this idea, activated RTKs were found to induce PLCγ1 and RasGAP phosphorylation at tyrosine, and we also observed that RasGAP associates with two other pTyr-containing proteins in cells with activated or oncogenic tyrosine kinases [30–34]. The variable domain organization of proteins such as Fps, Src, Crk, PLCγ1, and RasGAP indicated that the SH2 and SH3 domains might function in a cassette-like, combinatorial fashion and have broad functions in controlling cellular behavior in response to extracellular signals [35].

The precise means through which SH2 domains might link tyrosine kinases to specific biochemical pathways therefore became a central issue. In this regard, a number of groups found that activated receptor tyrosine kinases (RTK) physically associate with targets such as PLCγ, phosphatidylinositol (PI) 3′ kinase, and RasGAP [30, 36]. Furthermore, autophosphorylation of the PDGF β receptor at a specific tyrosine site (Tyr751) in the noncatalytic kinase insert region proved to specifically recruit PI 3′ kinase to the activated receptor [37]. Extrapolating from these data, we identified SH2 domains as the unifying element in coupling phosphorylated RTKs to their diverse intracellular targets. In these experiments, we found that isolated SH2 domains of different cytoplasmic signaling proteins, including PLCγ1, RasGAP, and Src, share a common ability to bind selectively to activated, autophosphorylated RTKs, as well as to tyrosine-phosphorylated docking proteins (e.g., $p62^{Dok\text{-}1}$) [38, 39]. Of interest, different SH2 domains bound distinct sets of pTyr-containing proteins from growth factor-stimulated cells, suggesting that SH2 domains impose an element of specificity in tyrosine kinase signaling through the selective recognition of tyrosine-phosphorylated proteins. These results indicated that endogenous SH2 domains are independently folding modules that bind selectively to activated RTKs and cytoplasmic proteins in a pTyr-dependent fashion. In a similar vein, recombinant v-Crk protein was found to associate with both tyrosine kinase activity and a series of pTyr-containing proteins from transformed cells [40, 41], consistent with the idea that aberrant binding of SH2 domains to pTyr-containing proteins stimulates malignant transformation. SH2 domain–pTyr interactions were thereby revealed as important in physiological signaling from RTKs and in the induction of malignant transformation by deregulated tyrosine kinase activity.

Subsequent data confirmed that SH2 domains directly recognize short pTyr-containing sequences. The binding sites for SH2 domains on the activated EGF receptor were mapped to the noncatalytic C-terminal tail of the receptor, which contains multiple autophosphorylation sites [42], and SH2 domains were found to bind to denatured pTyr-containing proteins and specific phosphopeptides corresponding to RTK autophosporylation sites [43–46].

1.2
SH2 Domains as a Prototype for Interaction Domains

SH2 domains can be viewed as the forerunner of the large family of interaction domains that control many aspects of signal transduction and cellular regulation, through their ability to bind variously to protein, phospholipid, nucleic acid, or small molecule ligands [47, 48] (Figure 1.2). Such domains have a modular design and mediate simple binary interactions, frequently by binding short peptide motifs. Through their ability to recognize a broad range of post-translational modifications and to discriminate between related binding sequences, interaction domains play a central role in the dynamic and specific cellular responses to extracellular and internal signals. Indeed, the scheme for RTK signaling, in which cytoplasmic sequences of the activated receptor recruit the interaction domains of target proteins, is broadly applicable to a wide range of cell surface receptors and intracellular regulators.

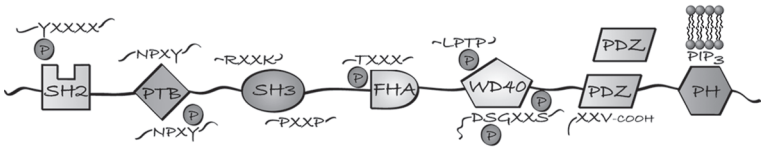


Figure 1.2 Summary of the binding properties of several interaction domains.

Although rather simple at first glance, interaction domains are remarkably versatile. Individual domains can have multiple ligands, and when covalently linked they can function as adaptors to join otherwise distinct polypeptides into a common signaling pathway. The reiterated use of interaction domains can also generate multiprotein assemblies with complex properties. Furthermore, interaction domains can provide allosteric regulation and switch-like behavior to associated catalytic domains and target enzymatic domains to their physiological substrates. In addition, interaction domains can serve as the organizing principle for extended protein networks, which underlie the systems properties of eukaryotic cells [49].

1.3
Structure and Binding Properties of SH2 Domains

The majority of SH2 domains bind short phosphopeptide motifs, which are themselves components of larger polypeptides, such as activated RTKs [50, 51]. A critical feature of phosphopeptide recognition involves association of the SH2 domain with the phosphorylated tyrosine; this interaction alone provides about half the binding energy and therefore results in a ~1000-fold increase in affinity as compared with an unphosphorylated peptide [52–54]. As a consequence, the physiological association of SH2 domains with their binding partners is usually phosphorylation-dependent. An analysis of SH2 domain binding sites on cellular

phosphoproteins, as well as the in-vitro binding preferences of SH2 domains for phosphorylated peptides, indicated that SH2 domains also bind selectively to residues immediately C-terminal to the pTyr, in a fashion that varies from one SH2 domain to another [52, 55]. For example, the SH2 domains of the p85 subunit of PI 3′ kinase typically bind sites on activated RTKs that contain a Met that is three residues C-terminal to the pTyr (the +3 position), and in vitro they bind preferentially to phosphopeptides having the consensus motif pTyr-x-x-Met (where x is any amino acid). The generality of this concept was revealed by using SH2 domains to probe a phosphopeptide library in which a central pTyr is flanked by degenerate positions. This approach showed that different SH2 domains preferentially recognize distinct amino acids at the C-terminal +1 to +3 positions [56, 57]. This technique identified, not only residues that are favored by a particular SH2 domain, but also amino acids that are inhibitory to binding. This is relevant, because the specificity of a particular protein–protein interaction in vivo depends on both permissive forces that direct an interaction domain to its intended target as well as inhibitory effects that block binding to nonphysiological partners.

The dominance of pTyr recognition is necessary for SH2-mediated interactions to be phospho-dependent, but also dictates that the affinity of SH2 domains for the C-terminal specificity residues is not too high, or complex formation could no longer be regulated by phosphorylation. Indeed, the dissociation constants of SH2 domains for their optimal phosphopeptide ligands are typically in the range of 500 nM–1 μM [58]. This relatively modest affinity may have additional biological consequences. Since signaling from cell surface receptors is highly dynamic, the protein–protein interactions that control intracellular pathways must not be too strong, or signaling events would become irreversible. A case in point is provided by the intramolecular interaction in the Src tyrosine kinase between the SH2 domain and a pTyr site (Tyr527) in the C terminus that induces an autoinhibited state (see below). The phosphorylated Tyr527 motif is a poor ligand for the Src SH2 domain, but the interaction proceeds because the two interacting partners are tethered within the same molecule. Replacing the Tyr527 site with an optimal binding motif for the Src SH2 domain yields a Src tyrosine kinase that is locked in the autoinhibited state and cannot be activated, because the inhibitory interaction is too strong [59]. In this example, an SH2-mediated interaction is designed to be weak, so that it can be readily reversed.

Similarly, although specificity in protein–protein interactions is biologically important, there is often significant flexibility in the binding of interaction domains to their targets. A given SH2 domain may interact physiologically with phosphorylated sites on multiple different proteins, which may allow for cell-specific functions or the formation of more complex signaling networks within a single cell [60]. Conversely, a single phosphorylated motif on an RTK may potentially provide a docking site for several distinct SH2-containing targets [61].

Structural analysis of a number of SH2 domains in association with phosphopeptide ligands has revealed the molecular basis for these biochemical observations (Figure 1.3) [50, 51]. SH2 domains typically have an N-terminal region that forms an α helix (αA) and a central antiparallel β sheet containing strands βA, βB, βC,

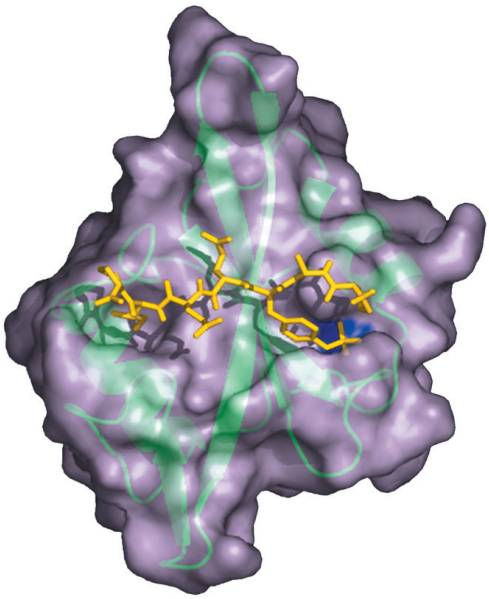


Figure 1.3 Structure of the Src SH2 domain bound to a pTyr-Glu-Glu-Ile peptide (PDB: 1SPS) [67]. The surface of the SH2 domain is in pink, and the secondary structural elements of the SH2 domain in green. The αA helix is to the right and αB helix to the left. The Arg βB5 residue critical for pTyr binding is in blue. The N-terminal pTyr of the peptide (yellow) occupies the pTyr binding pocket. The peptide runs over the central β sheet of the SH2 domain, the +1 and +2 glutamates contact the surface of the domain, and the sidechain of the +3 Ile (to the left) fits in a hydrophobic pocket.

and βD. The C-terminal region has a smaller β sheet (with strands βD′, βE, and βF), followed by another α helix (αB), and a short β strand (βG) that associates with the core β sheet. This creates a bipartite structure with two discrete binding pockets located on either side of the core β sheet [62–64]. Residues in the most highly conserved N-terminal region of the SH2 domain form a positively charged pocket that makes numerous contacts with the pTyr residue. In terms of binding free energy, the most prominent interaction involves a bidentate ionic association between an invariant buried Arg (ArgβB5) in the SH2 domain and two oxygens of the pTyr phosphate (Figure 1.4); substitution of this Arg effectively eliminates phosphopeptide binding [65, 66]. The second binding surface on the SH2 domain is much more variable between different SH2 domains and is responsible for the selective recognition of C-terminal residues. In the Src (or Lck) SH2 domain bound to a pYEEI peptide, the peptide runs orthogonally over the β sheet and adopts an extended conformation so that residues C-terminal to the pTyr contact the more variable specificity pocket. Here, the +1 and +2 glutamates make long-range contacts with the surface of the SH2 domain, while the sidechain of the +3 Ile is buried in a hydrophobic cavity [67, 68] (Figure 1.3).

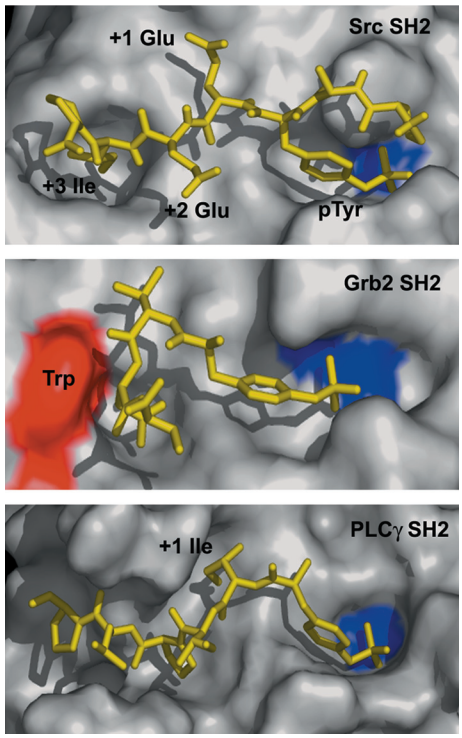


Figure 1.4 Different modes of SH2 domain–phosphopeptide recognition shown for the Src, Grb2, and PLC-γ1 (C terminal) SH2 domains (PDP: 1SPS; PDB: 1TZE; PDB: 2PLD) [67, 70, 72]. In each panel, the surface of the SH2 domain is in gray, and the Arg βB5 required for pTyr recognition in blue. The pTyr binding pocket is to the right, and the more variable surface involved in selective recognition of C-terminal residues is to the left. The phosphopeptides are in yellow, with the N-terminal pTyr to the right and the more C-terminal residues to the left. The +3 Ile of the *po*YEEI peptide occupies a hydrophobic pocket on the Src SH2 domain, as also shown in Figure 1.3. The *po*YVNV peptide bound to the Grb2 SH2 domain is forced into a β turn by the Trp residue at the EF1 position (shown in red). The C-terminal residues of the *po*YIIPLPD peptide bound to the PLC-γ1 SH2 domain occupy an extended hydrophobic cleft.

1.4 Different Modes of SH2 Domain–Phosphopeptide Recognition

The N-terminal SH2 domain of the Shp-2 tyrosine phosphatase and the C-terminal SH2 domain of phospholipase C-γ1 also bind phosphopeptides, such as those from the platelet-derived growth factor receptor (PDGFR), in an extended conformation [69, 70]. However, in contrast to Src, the specificity region of these SH2 domains

has a long hydrophobic groove that accommodates at least five residues C-terminal to the pTyr, primarily through hydrophobic interactions (Figure 1.4). In PLC-γ1, the +1 Ile of the Tyr1021 motif from the βPDGFR projects into the start of this hydrophobic cleft, facilitated by a cysteine at the βD5 position of the SH2 domain, which provides space for the Ile sidechain. In the Src SH2 domain this space is filled by a tyrosine at βD5, which therefore selects against Ile at the +1 position [71]. The SH2 domain of the Grb2 adaptor has yet another binding mode, conferred by a Trp at the EF1 position (the first residue in the loop between the βE and βF strands) (Figure 1.4). In the Src SH2 domain the corresponding EF1 residue is a Thr that forms part of the +3 hydrophobic binding pocket. In Grb2, the EF1 Trp fills this pocket and projects from the surface of the SH2 domain, forcing the phosphopeptide into a β turn that is accommodated by an Asn at the +2 position of the peptide, which makes hydrogen bonds with backbone atoms of the SH2 domain [72–74]. In the SH2 domain of the Grb7 adaptor, an insertion in the EF loop also occludes the +3-binding pocket and imposes a β-turn conformation on the bound phosphopeptide, which favors a +2 Asn in a similar fashion to Grb2 [75]. These data show how subtle variations in the sequences and structures of SH2 domains can influence their binding preferences. The biological relevance of this selectivity is suggested by the fact that physiological binding sites for SH2-containing proteins often conform to those identified by in-vitro peptide-binding studies and structural analyses.

To test whether the ability of SH2 domains to select specific phosphopeptide motifs is biologically significant, we examined the effects of manipulating their binding selectivity [76]. Examination of multiple SH2 domain sequences revealed the presence of a Trp at the EF1 position that was unique to the Grb2 SH2 domain and which we thought might account for its propensity to bind Asn (as subsequently validated by structural analysis). We found that replacing the EF1 Thr of the Src SH2 domain with Trp resulted in strong selection for peptides having an Asn at the +2 position; structural analysis subsequently revealed that this mutant form of the Src SH2 domain recognizes a physiological Grb2 binding site (pTyr-Val-Asn-Val) in a virtually identical fashion to Grb2 itself [77]. To explore the biological effects of this specificity switch, we made use of the fact that inactivation of the Grb2 gene in *Caenorhabditis elegans* (called *sem-5*) results in multiple defects, including abnormal development of the vulva, which can be rescued by reexpression of wild-type Grb2. We replaced the SH2 domain of Grb2 with that from Src and found that the chimeric Src-Grb2 adaptor, in which the Src SH2 domain is flanked by the Grb2 SH3 domains, was inefficient in rescuing the defect caused by loss of the endogenous gene. However, the same chimeric adaptor, but with Trp replacing Thr at the EF1 position of the Src SH2 domain, restored biological activity to nearly wild-type levels. This experiment showed that SH2 domain selectivity can be attributed to specific residues and demonstrated that the biological function of an SH2 domain correlates with its binding selectivity.

The properties of SH2 domains discussed above are reflected in a number of interaction domains. The ability of post-translational modifications to regulate modular protein–protein interactions is shared by a variety of domains that

selectively recognize peptide motifs after phosphorylation at tyrosine (PTB domain) or serine/threonine (FHA, WD40, MH2, Polo Box, BRCT, FF, and WW domains and 14-3-3 proteins, see Chapter 8) [78, 79], acetylation of lysine residues (bromodomains), arginine methylation (chromodomains) [80], proline hydroxylation (VHL) [81, 82], or monoubiquitination (UIM, CUE, UBA domains) [83]. Many of these domains show a similar structural organization to SH2 domains, in the sense that they have a cassette-like design and typically have distinct binding pockets for the modified amino acid and flanking residues, with the latter providing specificity among different members of the same domain class.

1.5 Signaling Pathways and Networks

SH2 domains are embedded in numerous proteins having widely different biochemical functions, which are nonetheless all directly involved in tyrosine kinase signaling owing to the pTyr-binding properties of their SH2 domains. This observation suggests an evolutionary rationale for the pervasive use of interaction domains in cell signaling. According to this scheme, new signaling pathways and cellular functions could be generated through the simple device of inserting a novel interaction domain into an already-existing polypeptide or by organizing interaction domains into new combinations. This would provide the relevant protein with innovative physical connections and would thereby link previously separate cellular processes into a common complex or pathway. This approach has several potential advantages. Incorporating an interaction domain into a specific protein is simpler than evolving a complex mechanism of allosteric regulation. In particular, the same

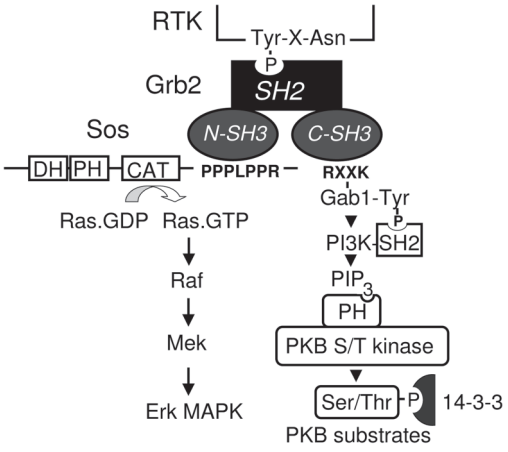


Figure 1.5 A series of modular protein–protein and protein–phospholipid interactions mediate signaling from pTyr-X-Asn motifs on activated RTKs. The N-terminal and C-terminal SH3 domains of Grb2 bind proline-rich and basic motifs, respectively.

interaction domain can be readily coupled to many different types of protein and thereby endow them with a common function, such as pTyr recognition. In addition, interaction domains can themselves provide autoregulatory and switch-like behavior when linked to catalytic domains, through a combination of intra- and intermolecular interactions [84]. In contrast, a regulatory device that depends entirely on conformational change (e.g., induced by phosphorylation) may not be easily transferred from one polypeptide to another.

The various types of SH2 domain proteins can be organized into a number of different classes:

- **Adaptors:** Proteins of the Grb2, Crk, and Nck families are composed exclusively of SH2 and SH3 domains and link specific pTyr motifs recognized by the adaptors' SH2 domains to cellular pathways controlled by proteins that bind selectively to their SH3 domains. Each adaptor recruits a distinct set of SH3-binding proteins and thereby controls a different facet of cellular organization. Grb2, for example, binds to pTyr-X-Asn motifs through its SH2 domain (as discussed above), to the Sos guanine nucleotide exchange factors (GEF) of the Ras GTPase through its N-terminal SH3 domain [85–90], and to the Gab-1 or Gab-2 scaffolding proteins through its C-terminal SH3 domain [91, 92] (Figure 1.5).
  Neither Grb2 nor Sos are notably phosphorylated at tyrosine, and thus activation of Ras by exchange of GDP for GTP may depend primarily on physical recruitment of the Grb2–Sos complex to sites of RTK activation at the plasma membrane, where Ras is also localized. GTP-bound Ras has a number of targets, most significantly the Raf protein-serine/threonine kinase that stimulates the Erk MAP kinase pathway.
  In contrast, Grb2-associated Gab proteins become tyrosine-phosphorylated at sites that bind additional SH2 domain proteins [93], such as PI 3′ kinase, which consequently generates PI-3,4,5-P_3 in the plasma membrane. PIP_3, in turn, is recognized by the PH domain of the protein-serine/threonine kinase PKB/Akt [94], which is activated after recruitment to the membrane and phosphorylates a number of targets, such as Bad, Forkhead transcription factors, and Tuberin involved in apoptosis, cell cycle control, and cell growth [95]. Interestingly, the serine/threonine sites phosphorylated by PKB are often themselves directly recognized by 14-3-3 proteins, which regulate the activity of their associated phosphoproteins [96].
  Because it can bind multiple targets through its SH3 domains, the Grb2 adaptor couples a single pTyr site to a series of interconnected signaling pathways, which act in a cooperative fashion to control the growth, proliferation, survival, and differentiation of growth factor-stimulated cells. These signaling events are assembled from simple, modular protein–protein and protein–phospholipid interactions, which act in concert to form a network with a broad influence on cell behavior.
  The SH2 and SH3 domains of Nck have different binding specificities compared with Grb2. Most strikingly, the Nck SH3 domains bind a series of proteins that regulate the actin cytoskeleton [97], such as N-WASP and the Pak-serine/threonine

kinase [98–100]. This correlates with the ability of Nck to couple tyrosine kinases to cytoskeletal reorganization in cultured cells and intact organisms [101, 102]. Crk, in contrast, links specific pTyr sites to proteins involved in membrane dynamics and cell adhesion, including activators of the Rap and Rac GTPases [103–108]. SH2/SH3 adaptors therefore provide a simple tool through which tyrosine kinases influence a range of cellular activities. Many other types of cell surface receptors, such as TNF receptors, Toll-like receptors, guidance receptors, adhesion molecules and integrins, TGFβ receptors, and Wnt receptors, similarly associate with their cytoplasmic targets through modular interaction domains, often in the form of adaptor proteins [109, 110].

- **Scaffolds:** Scaffold proteins bind multiple signaling components with complementary functions. This applies to SH2 domain proteins such as Blnk (SLP-65) and SLP-76 [111, 112], which act in B cells and T cells as hubs to organize a number of key signaling proteins (including Tec family tyrosine kinases, PLCγ, Nck, and Vav) [113–115] and therefore play a critical role in signaling from antigen receptors [116–118].

- **Enzymes:** A number of RTK targets are enzymes with intrinsic SH2 domains. These include proteins that regulate phospholipid metabolism, such as phospholipase Cγ1 and γ2 and the SHIP 5′-inositol phosphatase [119]. PI 3′ kinase has a noncatalytic p85 adaptor subunit that contains SH2 domains, separated by a coiled-coil region that associates with the p110 catalytic subunit [95, 120]; however, in contrast to other adaptors, p85 is a dedicated component of the PI 3-kinase complex. Other SH2-containing enzymes regulate Ras, Rho, and Rab family GTPases, as well as tyrosine phosphorylation [121–128]. These enzymes are commonly activated by the binding of their SH2 domains to pTyr sites, as discussed in more detail below, and are also frequently substrates for tyrosine phosphorylation.

- **Regulators:** A substantial family of SH2-containing proteins control the duration and localization of tyrosine kinase signals. c-Cbl is an E3 protein–ubiquitin ligase that promotes RTK monoubiquitination and internalization [129–131], and SOCS proteins inhibit cytokine receptor signaling both by directly binding and inhibiting the activity of receptor-associated JAK-tyrosine kinases and by inducing their ubiquitination [132]. The APS protein, in contrast, binds and stabilizes the activated form of the insulin receptor (see below).

- **Transcription factors:** The Stat proteins are SH2-containing transcriptional regulators that provide a direct link between activated cytokine receptors at the plasma membrane, which they bind through their SH2 domain, and gene expression in the nucleus, where they relocalize after phosphorylation and dimerization, as discussed below.

1.6
Plasticity of SH2 Domains

The human genome encodes some 115 SH2 domains. An analysis of existing exon/intron boundaries suggests that these SH2 domains likely evolved from a common ancestor, which may then have been selected for its versatility [133]. Consistent with this possibility, SH2 domains show significant flexibility in their binding properties, beyond their conventional capacity to discriminate between distinct pTyr-containing peptide motifs.

The SAP/SH2D1A protein, for example, is composed almost entirely of a single SH2 domain [134], which binds to a tyrosine-based motif in the T-cell coreceptor SLAM [135] (Figure 1.6). Homotypic engagement of the SLAM extracellular region leads to tyrosine phosphorylation of its cytoplasmic tail, which regulates signaling pathways in stimulated T cells that control the pattern of cytokine production and proliferation of CD8+ T cells. This in turn ensures a limited lymphoid response to viral infection. Mutations in the human SAP gene (see below) have revealed that its product is essential for SLAM signaling, and this has uncovered unusual properties of the SAP SH2 domain. First, it binds preferentially to an extended motif having the consensus sequence Thr-Ile-(p)Tyr-x-x-Val/Ile, which conforms to its physiological binding site on SLAM (Tyr281) [136, 137]. Surprisingly, the SAP SH2 domain binds an unphosphorylated Tyr281 SLAM peptide with a K_d of ~300 nM, and there is only about a 3-fold increase in affinity upon tyrosine phosphorylation [136–138]. As a consequence, SAP can bind to both unphosphorylated and phosphorylated forms of SLAM in T cells. Biochemical and structural analysis has shown that the SAP SH2 domain effectively has three binding pockets, one for the central (p)Tyr, another for the +3 Val, and a third that accommodates the Thr at the –2 position of the peptide ligand [136, 137] (Figure 1.7). It is this unusual interaction with an N-terminal peptide residue that enhances pTyr-independent binding to the SAP SH2 domain. Presumably, the great majority of SH2 domains do not exploit this potential to bind unmodified peptides, because a

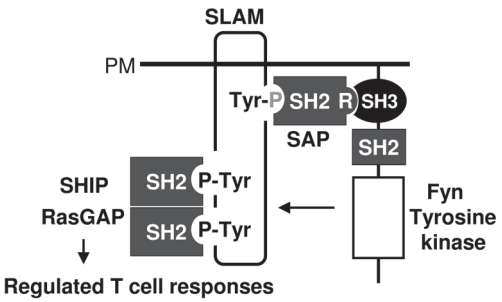


Figure 1.6 The SAP SH2 domain acts as an adaptor to link the SLAM receptor in T cells to the Fyn tyrosine kinase, which consequently phosphorylates SLAM at sites that engage additional SH2-containing proteins. Loss-of-function mutations in the SAP SH2 domain cause X-linked lymphoproliferative syndrome. R = Arg78; PM = plasma membrane.

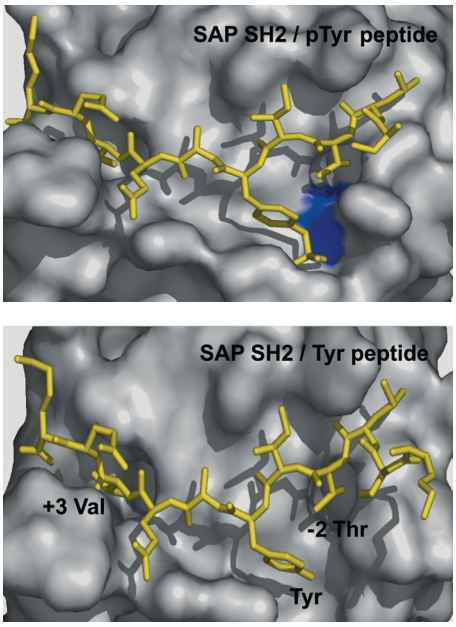


Figure 1.7 Recognition by the SAP SH2 domain of a Tyr-based motif in SLAM in both its unphosphorylated and phosphorylated states. The peptide binding surface of the SAP SH2 domain is in grey, and the Arg βB5 residue in blue. The SH2 domain is complexed with either the phosphorylated SLAM peptide (TIpYAQV) (top) or its unphosphorylated counterpart (bottom). Note the three binding pockets for the –2 Thr, (p)Tyr, and the +3 Val, respectively.

relatively high affinity for the unphosphorylated ligand would nullify the switch-like properties of tyrosine phosphorylation in inducing SH2 domain recognition.

The SAP SH2 domain also has an adaptor function, due to a second binding surface that associates with the SH3 domain of a Src-like cytoplasmic tyrosine kinase, Fyn [139]. Although SH3 domains usually recognize proline-rich motifs, some bind to basic sites [140]; in the Fyn–SAP complex, the Fyn SH3 domain recognizes such a basic surface on the SAP SH2 domain, focused on Arg78 [141, 142]. This surface of the SAP SH2 domain does not overlap the conventional peptide binding site, and the SAP SH2 domain can therefore bind simultaneously to SLAM and the Fyn tyrosine kinase. The SAP-binding site on the Fyn SH3 domain, however, overlaps the regions involved in PxxP recognition and the autoinhibitory intramolecular interaction that represses Fyn activity. SAP therefore bridges SLAM to active Fyn, which consequently phosphorylates SLAM at additional tyrosine sites for the recruitment of SH2-containing effectors such as the SHIP 5′-inositol phosphatase and RasGAP (Figure 1.6).

A number of more general points can be taken from this scheme. First, the reiterated use of rather simple binary interactions can be employed to assemble more sophisticated multiprotein complexes. Second, interaction domains such as

SH2 and SH3 can potentially employ their primary binding surface to engage distinct types of peptide ligand (i.e., phosphorylated or unphosphorylated peptides for SH2 domains, proline-rich or basic motifs for SH3 domains). Third, a single domain can use distinct surfaces to bind distinct types of ligand, as shown by the ability of the SAP SH2 domain to engage both SLAM and Fyn. These are themes that are frequently encountered in the analysis of interaction domains. For example, PTB domains can bind both tyrosine phosphorylated and unphosphorylated peptide ligands [143–145], as well as phospholipids [146]; indeed, a number of interaction domains with functions ranging from phosphoinositide recognition to the binding of proline-rich sequences (such as PH and EVH1 domains, see Chapters 4 and 17) have the same fold as PTB domains [147].

1.7 SH2 Domain Dimerization

The versatility with which SH2 domains bind pTyr-containing sequences is exemplified by the SH2 domain of the APS adaptor, which recognizes the activation loop of the insulin receptor kinase (IRK) catalytic domain. Upon insulin stimulation, the activation loop of IRK is autophosphorylated on three tyrosine residues and is consequently displaced from an inhibitory conformation that occludes the active site. One of these phosphorylated tyrosines (pTyr1163) interacts with residues in the kinase domain to lock the activation segment in place, but the other two (pTyr1158 and pTyr1162) are exposed [148]. The APS SH2 domain binds to these two pTyr residues in an unusual fashion [149]. The C-terminal region of the APS SH2 domain forms an unusually long αB helix, and this allows two SH2 domain monomers to dimerize through an extensive hydrophobic interface. pTyr1158 of IRK occupies the normal pTyr-binding pocket of the SH2 domain, but the subsequent residues of the IRK activation loop are sterically blocked from following a typical path perpendicular to the central β sheet, due to the C-terminal helix of the other monomer in the APS SH2 dimer. Rather, the activation loop binds in the plane of the β sheet, facilitated by numerous specific interactions with the IRK kinase domain. Notably, there is a second pTyr-binding site in the APS SH2 domain, which is occupied by pTyr1162 of the IRK activation loop. Thus, two IRK chains are bridged by two (dimeric) APS SH2 domains. APS may therefore enhance and prolong the activated state of IRK by protecting the phosphorylated activation loop from phosphatases and stabilizing the active form of the kinase domain (Figure 1.8).

These results emphasize several points made above (i.e., the ability of SH2 domains to bind multiple ligands – here, the phosphorylated IRK and another APS SH2 domain – and the plasticity of SH2 domain pTyr-binding sites), but also demonstrate that SH2 domains can form homodimers. The Grb10 SH2 domain also forms a dimer through an interface that involves the C-terminal α helix (αB) [150]. In addition the SH2 domains of the STAT transcription factors dimerize in response to cytokine stimulation (see below) [151]. In this case, however, dimerization is induced by phosphorylation of a tyrosine just C-terminal to the SH2 domain,

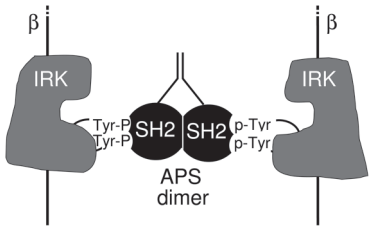


Figure 1.8 The APS SH2 domain homodimerizes and binds the phosphorylated activation loop of the insulin receptor kinase (IRK). IRK is itself a heterotetramer; only the cytoplasmic regions of its β subunits are shown.

which leads to reciprocal intermolecular SH2 domain–pTyr interactions between two STAT proteins. Oligomerization is a common theme among interaction domains, which frequently self-associate into homo- or heterodimers.

1.8 Tandem SH2 Domains

A number of proteins, including the Syk and ZAP-70 cytoplasmic tyrosine kinases, PLC-γ, RasGAP, and the Shp-1 and Shp-2 tyrosine phosphatases, have two SH2 domains arranged in tandem (see Figure 1.1) and bind preferentially to doubly phosphorylated sites in which the two pTyr residues are closely spaced. Tandem SH2 domains appear to bind such bisphosphorylated motifs with significantly increased affinity and specificity, as compared to the interaction of a single SH2 domain with a monophosphorylated peptide [152]. This principle is demonstrated by the Syk and ZAP-70 tyrosine kinases, which mediate signaling by immunoreceptors, notably the B-cell and T-cell antigen receptors (BCR/TCR), and Fc receptors [153]. Upon activation of antigen receptors, Src family tyrosine kinases phosphorylate BCR or TCR signaling subunits on ITAM sequences (for immunoreceptor tyrosine-based activation motif). ITAMs have the consensus YxxI/L-$x_{6–8}$-YxxI/L, and their efficient binding to the Syk or ZAP-70 tandem SH2 domains requires that both tyrosines be phosphorylated [154]. Each of the two pTyr motifs within an ITAM binds in a conventional orientation to a single SH2 domain, such that the N-terminal pTyr associates with the C-terminal SH2 domain, and the C-terminal pTyr with the N-terminal SH2 domain. Strikingly, the pTyr binding pocket of the N-terminal SH2 domain of ZAP-70 is formed in part by residues from the C-terminal SH2 domain [155]. As a consequence, binding of the tandem ZAP–70 SH2 domains to doubly phosphorylated ITAM motifs in the TCR complex is strongly dependent on the spacing of the two phosphorylated tyrosines. The Syk SH2 domains are autonomous and more flexible and can bind a wider range of ITAM motifs with variable spacing between the pTyr residues [156, 157]. These data make the point that multiple interaction domains can be joined to create more-specific protein–protein interactions than those displayed by any one domain alone.

Indeed, many signaling proteins contain several distinct interaction domains, often with quite different binding properties, which can potentially bind cooperatively to their targets.

1.9 Composite and Complex Interaction Domains

The c-Cbl protein is an E3 protein–ubiquitin ligase with an N-terminal SH2 domain, which binds specific pTyr motifs on autophosphorylated RTKs, and a central ring domain that recruits an E2 ubiquitin ligase and thus induces the monoubiquitination of receptors associated with the SH2 domain. The c-Cbl SH2 domain is unusual, in the sense that it is embedded in a larger folded structure that also contains a four-helix bundle and an EF hand [158]. Although phosphopeptides bind the SH2 domain in a conventional fashion, the associated subdomains may participate in target recognition.

The SH2 domain of STAT transcription factors is also part of a larger structural unit, since, unlike most SH2 domains, those from STAT proteins are not functional when expressed in isolation from their surrounding sequences. The core region of STATs contains a helical coiled-coil sequence, a DNA binding region, and a linker region followed by the SH2 domain; C-terminal to this core lies a transactivation sequence that regulates transcription. Activated cytokine receptors at the plasma membrane induce phosphorylation of a STAT tyrosine residue located immediately C-terminal to the SH2 domain, and this results in STAT dimerization through reciprocal SH2–pTyr interactions. STAT dimers are recruited to the nucleus, where they bind specific promoters to regulate gene expression. Structural analysis of the dimeric core region shows that the four domains (coiled-coil, DNA binding, linker, SH2) are interlocked through a common hydrophobic core and extensive interfaces between the domains [159, 160]. Of interest, the phosphate binding loop of the SH2 domain interacts directly with the linker domain, which in turn associates with the DNA binding domain; thus, there is potential for direct coupling between pTyr binding to the SH2 domain and DNA binding. The sequence following the SH2 domain of one monomer is organized so that, upon phosphorylation, it can engage only the SH2 domain of a partner STAT, rather than undergoing intramolecular interaction. STAT proteins therefore provide an elegant example of a functionally and structurally complex polypeptide built from smaller protein–protein and protein–nucleic acid interaction domains.

1.10 Allosteric Regulation

In enzymes such as the Src and Abl cytoplasmic tyrosine kinases or the Shp-2 protein-tyrosine phosphatase (PTP), reversible intramolecular interactions between the SH2 and catalytic domains provide the basis for autoinhibition of enzymatic

activity and for switch-like activation after these repressive contacts are broken. The Shp-2 tyrosine phosphatase has two tandem SH2 domains, followed by a phosphatase domain and a C-terminal tail. In the autoinhibited state, the N-terminal (N-) SH2 domain undergoes an extensive intramolecular interaction with the active site of the phosphatase domain [161]. The D′E loop of the SH2 domain buries into the PTP catalytic cleft, directly engages the catalytic Cys nucleophile and the phosphate binding cradle of the active site, and locks the PTP domain in an open, inactive conformation. This interaction also distorts the conformation of the N-SH2 domain by imposing movements of the αB helix and the EF loop that occlude the phosphopeptide binding cleft; as a consequence, although the phosphopeptide binding site of the N-SH2 domain is exposed to solvent in the autoinhibited state, it is not properly configured for ligand recognition. The C-SH2 domain, in contrast, makes minimal contact with the N-SH2 or PTP domains, but could promote Shp-2 activation by binding one pTyr site on a bisphosphorylated peptide. This would increase the local concentration of the doubly phosphorylated motif and promote binding of the second pTyr site to the N-SH2 domain. The resulting reorganization of the N-SH2 domain would then break its interaction with the PTP domain, resulting in activation of the phosphatase. In this scheme, the N-SH2 domain has two distinct ligand binding sites (for a phosphopeptide or the PTP domain), but, in contrast to the examples discussed above, these interactions display negative cooperativity and are mutually incompatible. In Shp-2, the specific recognition of a bisphosphorylated motif by the tandem SH2 domains is thus precisely coupled to activation of the phosphatase. This device ensures that Shp-2 phosphatase activity is turned on only as the SH2 domains bind an appropriate target.

A related mechanism is exploited by the Src and Abl tyrosine kinases, although the details are very different. In both Src and Abl, an SH3 domain is followed by an SH2 domain, which precedes the kinase domain. In Src family kinases, autoinhibition involves phosphorylation of a Tyr (Tyr527) in the extreme C-terminal tail by another tyrosine kinase, Csk [124]. This induces an intramolecular interaction with the SH2 domain and positions the SH3 domain to bind the linker region between the SH2 and kinase domains (Figure 1.9) [162, 163]. In this autoinhibited conformation, the conventional binding clefts of both the SH2 and SH3 domains engage internal ligands and are therefore protected from adventitious interactions with other polypeptides. At the same time, these interactions inactivate the kinase domain, although in contrast to Shp-2, this is achieved indirectly. Dynamic simulations and mutagenesis experiments have suggested that the linker between the SH2 and SH3 domains forms a rigid clamp in the autoinhibited state [164]. Since the SH2 domain interacts with the large lobe of the kinase and the SH3 domain with the small lobe, this clamp prevents flexibility at the intervening active site required for the exchange of ATP and ADP. Also, in this autoinhibited conformation, the regulatory tyrosine in the activation loop is sequestered and therefore less prone to autophosphorylation and resulting kinase activation, and the C helix in the small lobe undergoes a rotation that removes a key glutamate from the active site.

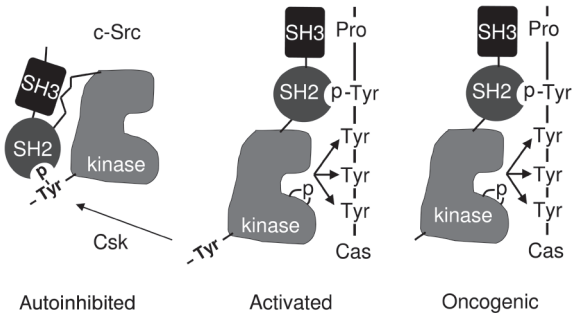


Figure 1.9 The SH2 and SH3 domains of the c-Src tyrosine kinase regulate its activity and substrate specificity. Activated c-Src can be targeted to its substrates through SH2/SH3-mediated interaction, which promotes the progressive phosphorylation of proteins such as Cas. Loss of the regulatory C terminus converts Src into a constitutively active tyrosine kinase.

This autoinhibited state can be broken in several ways. Most simply, dephosphorylation of Tyr527 leads both to dissociation of the SH2 and SH3 domains, which can then bind exogenous proteins with appropriate motifs, and to activation of the kinase domain, which can phosphorylate targets engaged by the adjacent interaction domains. Alternatively, an exogenous protein with high-affinity binding motifs for the Src SH3 or SH2 domains can compete for the inhibitory intramolecular interactions and thereby activate and localize the kinase domain. Therefore, as with Shp-2, the autoinhibited state both sequesters the interaction domains and inactivates the catalytic domain, and activation involves coordinate stimulation of the kinase domain and tethering of the SH2/SH3 domains to potential substrates. In the v-Src oncoprotein, deletion of the C-terminal tail removes the autoinhibitory Tyr527 site, and the tyrosine kinase becomes constitutively active (Figure 1.9).

The Abl kinase lacks an inhibitory phosphorylation site C-terminal to the kinase domain, but undergoes a very similar mode of autoinhibition as Src. In the Abl1b isoform, an N-terminal myristate group is inserted into the large helical lobe of the kinase, inducing a conformational distortion that creates a direct interface between the large lobe of the kinase domain and the SH2 domain [165]. This interaction, which involves the αA helix of the SH2 domain, is incompatible with phosphopeptide binding and positions the SH3 domain for interaction with the SH2-kinase linker, in a similar fashion to Src. The precise mechanism through which this inactivates the kinase domain is somewhat different for Abl than for Src, but the principles are the same. Interestingly, the specificity of the kinase inhibitor Gleevec (STI-571/Imanitib) for Abl as compared with Src is due to the distinct autoinhibited conformation imposed on the Abl kinase by its associated SH2 domain [165]. Gleevec binds exclusively to Abl in the autoinhibited conformation and therefore disturbs the equilibrium between inactive and active kinases, with beneficial effects in the treatment of chronic myelogenous leukemia. Under physiological conditions, Abl could be activated through loss of the N-terminal myristate group, which would

abolish the conformation of the large lobe required for SH2 binding, or through competition by exogenous proteins for interactions with the SH2 and SH3 domains [166].

The regulatory device in which interaction domains undergo an intramolecular interaction with a catalytic domain or another interaction surface, resulting in reversible autoinhibition, is emerging as a common theme in intracellular regulatory proteins. Similarly, signaling enzymes are typically localized within the cell and docked onto their substrates through noncatalytic protein–protein and protein–phospholipid interactions.

1.11 SH2 Domains and Disease

Mutations in either SH2 domains or their binding motifs are associated with a number of human disorders. Missense mutations in the gene for human Shp-2 (*PTPN11*) cause Noonan's syndrome (NS), a developmental disorder associated with cardiac defects, facial dysmorphism, and skeletal abnormalities [167]. The substitutions that cause NS map to the autoinhibitory interface between the N-SH2 and PTP domains. As a consequence, these mutations abrogate the inhibitory interaction between the SH2 and phosphatase domains without disrupting pTyr binding or catalytic activity. NS therefore results from activating mutations that selectively block the ability of the N-SH2 domain to inhibit the PTP domain. Somatic mutations that activate Shp-2 are also common in juvenile myelomonocytic leukemia (JMML) and are present in other myeloid leukemias [168, 169]. The Shp-2 mutations in JMML are almost all in the N-SH2 domain and block autoinhibition, resulting in aberrant phosphatase activation. Although it is counterintuitive that a tyrosine phosphatase would promote malignant cell proliferation, Shp-2 activates signaling through growth stimulatory pathways such as Erk MAP kinase by dephosphorylating inhibitory pTyr sites.

Loss-of-function mutations in the Btk cytoplasmic tyrosine kinase cause a B-cell immunodeficiency termed X-linked agammaglobulinemia (XLA), which is associated with a deficiency in mature B cells and susceptibility to bacterial infection. Btk has an N-terminal PH domain, followed by SH3, SH2, and kinase domains, which adopt an extended conformation with little interaction between the domains [170]. Btk is activated by the binding of its PH domain to PI-3,4,5-P_3 at the membrane [171] and by tyrosine phosphorylation, and associates with the phosphorylated Blnk/SLP-65 scaffold through its SH2 domain [172]. Btk in turn stimulates a number of downstream pathways involved in B cell activation, for example, through the phosphorylation of PLC-γ2 and consequent calcium release [173]. XLA missense mutations can affect either the PH, SH3, SH2, or kinase domains and result in a loss of ligand binding, kinase activity, or protein stability [174, 175]. A distinct inherited immune defect, X-linked lymphoproliferative syndrome (XLP; also called Purtilo's syndrome or Duncan's disease), results from mutations in the *SAP/SH2D1A* gene [135, 176, 177]. Young boys with XLP are unable to control infections

of Epstein–Barr virus (EBV) and typically develop a fulminant infectious mononucleosis, which is usually fatal as a result of severe tissue necrosis and organ failure. Surviving patients commonly develop malignant lymphomas. Many SAP mutations cause substitutions in the SH2 domain, resulting in either a loss of binding to SLAM or protein destabilization (or both) (Figures 1.6 and 1.7) [178, 179]. In the absence of SAP, the Fyn tyrosine kinase cannot be recruited to SLAM or related receptors, and this aspect of lymphoid regulation is lost.

SH2 domains are also indirectly influenced by a wide range of disease-causing mutations. Notably, oncogenic mutations in RTKs or cytoplasmic tyrosine kinases activate ectopic pTyr–SH2 domain interactions that promote intracellular signaling and lead to a malignant state. An example is the Tyr177 site located in the BCR region of the chimeric Bcr–Abl oncoprotein that causes chronic myelogenous leukemia. In the context of Bcr–Abl, Tyr177 is phosphorylated, and because it lies in a Tyr-x-Asn motif, recruits the SH2 domain of the Grb2 adaptor [180, 181], which in turn interacts through its SH3 domains with Sos and Gab-2 (Figure 1.10). These latter proteins stimulate the Ras-MAP kinase and PI 3′ kinase pathways, in a fashion that correlates with malignancy [182].

Similarly, pathogenic proteins encoded by human viruses or bacteria can form aberrant interactions with the SH2 domains of host cell proteins and thus rewire cellular functions, to the advantage of the pathogen. Examples include the latent membrane protein (LMP) 2A of EBV, which spans the membrane of infected B cells 12 times and has an N-terminal cytoplasmic region with three sites of tyrosine phosphorylation; these pTyr motifs bind to Src family tyrosine kinases and to the tandem SH2 domains of Syk. By recruiting these B-cell tyrosine kinases, LMP2A appears to interfere with normal signaling through the BCR and at the same time provide a survival signal [183, 184]. This helps to maintain viral latency by simultaneously blocking activation of the infected B cell, which would induce a lytic state, and keeping the cell alive [185]. In a related vein, the CagA protein of *Helicobacter pylori* is phosphorylated by Src family kinases at multiple tyrosine motifs

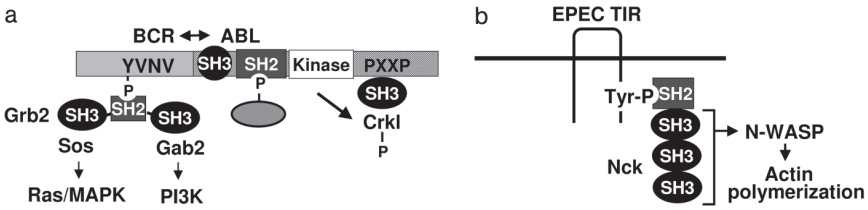


Figure 1.10 Aberrant SH2–pTyr interactions in cancer and bacterial infection. (a) Schematic of the Bcr–Abl tyrosine kinase. Tyr177 in the Bcr-encoded region is phosphorylated and binds Grb2. The Crkl adaptor binds a proline-rich motif in the C terminus and is itself a substrate for phosphorylation. (b) The TIR protein encoded by enteropathogenic *Escherichia coli* (EPEC) is phosphorylated at a tyrosine motif in its C-terminal cytoplasmic tail that binds the SH2 domain of the Nck adaptor, which recruits regulators of the actin cytoskeleton through its SH3 domains.

that bind the SH2 domains of Shp-2, which is important for morphological changes and increased motility of infected epithelial cells [186]. As a further example, the Tir protein of enteropathogenic *E. coli* is inserted into the host plasma membrane and is phosphorylated at a cytoplasmic Tyr site that recruits the Nck SH2/SH3 adaptor and its binding partner N-WASP to stimulate actin polymerization [187]. As a consequence, the bacterium induces abnormal actin polymerization (Figure 1.10), resulting in massive surface protrusions to which the bacterium adheres. In a striking example of convergent evolution, the A36R protein of vaccinia virus employs a similar motif to recruit Nck and induce actin polymerization, which drives the virus particle through the cell [188].

These observations regarding SH2 domains establish a precedent for the role of protein–protein interactions in disease states, and indeed there are now many examples of mutations that cause human disease by interfering with the functions of interaction domains. Another consideration is that naturally occurring non-synonomous polymorphisms might be a predisposing factor in disease by influencing protein–protein interactions. For example, the SKG strain of mice spontaneously develop chronic arthritis, caused by a polymorphism that results in a Trp-to-Cys substitution at the start of the C-SH2 domain of ZAP-70 [189]. This impairs the association of ZAP-70 with TCR and leads to attenuated T-cell signaling. This in turn favors the survival of autoreactive T cells that would otherwise be eliminated through negative selection and apoptosis.

Finally, it is likely that small molecules that modify protein–protein interactions will become more prominent in the treatment of disease. Such compounds could potentially block disease-associated protein interactions, stabilize existing complexes, or drive the interactions of proteins that are not normally associated. Indeed, immunosuppressant drugs such as cyclosporin and rapamycin function in the latter mode, by bridging an interaction between immunophilins and the protein phosphatase calcinuerin or the Tor protein kinase, respectively, which leads to inhibition of these enzymes and blockage of T-cell activation. Although protein–protein interactions are generally considered to be problematic drug targets, recent data have suggested the feasibility of inhibiting specific protein–protein interactions in therapeutically useful ways. For example, a small molecule that blocks the binding of p53 to Mdm2 stimulates the p53 pathway and has antitumor activity against cancer cells that retain wild-type p53 [190].

1.12 Summary

SH2 domains have a very simple function, namely, to recognize specific pTyr-containing sequences and thereby to couple tyrosine kinases to intracellular pathways that control cell growth, proliferation, metabolism, and differentiation. However, they also exhibit complex properties, through their ability to regulate both the substrate specificity and catalytic activity of enzymes in which they are embedded. In addition, by acting cooperatively with other interaction domains and

by providing the basis for networks of interacting proteins (as in activated lymphocytes), they can exert a broad effect on cellular behavior. Perhaps most importantly, these functional attributes of SH2 domains have yielded a framework for the discovery and analysis of a large family of interaction domains, which has crystallized a new way of thinking about the lexicon of cellular organization.

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