Cell, Vol. 80, 237–248, January 27, 1995, Copyright © 1995 by Cell Press

# Modular Binding Domains in Signal Transduction Proteins

Review

**George B. Cohen, Ruibao Ren,* and David Baltimore**
Department of Biology
Massachusetts Institute of Technology
77 Massachusetts Avenue
Cambridge, Massachusetts 02139

The transduction of a signal is a change in form of the signal as it is passed from one carrier to another. The root "duce" means "to lead" in Latin; thus, a signal is led through a cell by steps of transduction (the same root is in the words seduce and duct as well as Il Duce). The earliest transduction steps that were elucidated involved massive release of small molecule "second messengers", originally cAMP, that flooded a cell with information. With the understanding that such proteins as tyrosine kinases and Ras relatives are signal transducers, came the realization that many signaling pathways are more precise, sending controlled and probably weakly amplified signals to specific targets. These intracellular signals are often maintained in macromolecular form rather than being passed to small molecules.

The proteins that carry these signals are not acting in the classic fashion of enzymes that are designed to modify large numbers of substrate molecules. These signal transducers, even if they catalyze an event such as phosphorylation, generally affect small numbers of target molecules and have often separated their catalytic function from their binding regions, which can bring substrates to the catalytic centers, link the signal transducers to upstream proteins, and localize protein complexes to particular cellular subregions. The binding domains are often modular ones constructed with a common core recognition ability coupled to a fine specificity control. They modulate the interaction of proteins with other proteins and therefore determine the paths of signal transduction systems. They are generally controlled, also, so that the aggregates they form are transient, pathways forming only when the signal is being transmitted and then disaggregating when the signal has passed. The signal transduction protein must be highly integrated, with all of the elements working together to send just the appropriate quanta of signal for the specific need.

The preceding paragraphs are generalizations based on scanty and fragmentary evidence. With many transduction pathways known only in outline and more sure to be found, there is a deeply exciting richness yet to be elucidated. But many investigators are now working within the framework presented above, trying to find the relevant units and understand their integration.

Protein–protein interaction has long been studied, but the realization of the importance of defined binding modules came with the recognition that the Rous sarcoma virus oncogene, *src*, has not only a tyrosine kinase catalytic domain but also two other domains that are held in common with proteins that either do or do not have kinase domains. First came the SH2 and then the SH3 domains (for Src homology modules) (Koch et al., 1991), which were later shown to be specific binding domains. Later still, the Pleckstrin homology (PH) domains were recognized (Mayer et al., 1993; Haslam et al., 1993). Many investigators have their computers trained on recognizing other such domains, but no other large, modular families have been reported, suggesting that there may be few left to find.

SH2, SH3, and PH domains have a few common properties. First, they are true protein domains: they form compact units that maintain their structures in isolation, and each has its N- and C-termini in close apposition so that they can be plugged into the surface of proteins. They are not restricted to particular types of signal transduction proteins; they occur in protein kinases, lipid kinases, protein phosphatases, phospholipases, Ras-controlling proteins, and even transcription factors, although they are rarely found in receptors. They are also found in adapter proteins such as Crk, which have no enzymatic function and appear to act solely to aggregate other proteins. SH3 and PH domains are also seen in cytoskeleton proteins, where they may mediate the action of signal transduction pathways on cellular architecture and cell movement. There is no pattern to the number of these domains that occur in proteins, nor in their location within proteins (Figure 1). PH and SH3 domains are found in all eukaryotic organisms, including yeast, while SH2 domains are not found in yeast. None of these domains are seen in prokaryotes (Koch et al., 1991; Mayer and Baltimore, 1993; Pawson and Schlessinger, 1993; Musacchio et al., 1994b).

This review will present our current understanding of how SH2, SH3, and PH domains are constructed, how they function, and how their activities are integrated into the general scheme of signal transduction.

## SH2 Domains

In cells that respond to growth and differentiation factors through receptor tyrosine kinases (RTKs), among the most heavily phosphorylated proteins are the kinases them-

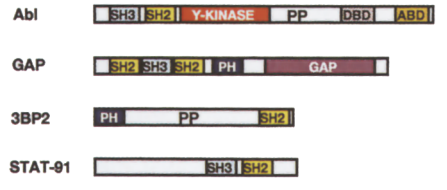


Figure 1. Examples of Proteins with SH2, SH3, and PH Domains

Y-kinase, tyrosine kinase domain; PP, Pro-rich region that binds to SH3 domains; DBD, DNA-binding domain; ABD, actin-binding domain; GAP, GTPase-activating domain.

*Present address: Department of Biology, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02254.

selves. Autophosphorylation of the kinase is critical for transmitting signals to downstream molecules. In the early 1990s, it became clear that most of the molecules that stably bind to phosphorylated RTKs contain SH2 domains. Subsequent work showed that SH2 domains by themselves stably associate with Tyr-phosphorylated, but not unphosphorylated, RTKs (Mayer and Baltimore, 1993). The question then became, how specific are SH2–phosphoprotein interactions? In other words, can an isolated SH2 domain, immersed in a sea of phosphoproteins, choose a specific binding partner? And if specific, how is the specificity encoded within the phosphoprotein and SH2 domain structures? The answers soon followed.

Different SH2s bind to distinct phosphotyrosine (pTyr)-containing regions of the RTK. Mapping of the binding sites revealed that SH2 specificity is largely determined by the three residues immediately carboxyl to the pTyr, pTyr-X-X-X, where X-X-X varies for different SH2s. For example, a consensus binding site for the two SH2 domains of phosphatidylinositol 3′-kinase (PI3K) is p-Tyr-(Val/Met)-X-Met. Site-directed mutations in the platelet-derived growth factor receptor (PDGFR) at sites containing the consensus sequence—$Y^{740}$MDM and $Y^{751}$VPM—severely disrupt binding of PI3K to PDGFR. These mutations, however, do not affect the affinity of other SH2-containing molecules for the receptor, such as RasGAP (Cantley et al., 1991; Kazlauskas et al., 1992; Pawson and Schlessinger, 1993).

Songyang et al. devised a systematic approach for determining the optimal peptide ligand for specific SH2 domains (Songyang et al., 1993, 1994). They synthesized an 8 amino acid phosphotyrosyl peptide library, randomized at positions Y+1 to Y+3 (the nomenclature refers to the residues carboxyl to the pTyr as +1, +2, etc.). Of the 22 SH2 domains tested, each selected a unique peptide sequence, except for those from Src family proteins, all of which selected the sequence pYEEI (which might suggest that the Src family has redundant SH2 functions). Most SH2 domains fall into one of two broad categories: group I SH2s prefer pTyr-hydrophilic-hydrophilic-hydrophobic; group II SH2s select pTyr-hydrophobic-X-hydrophobic. In proteins that contain two SH2 domains, the SH2s may have either similar or different specificities (Songyang et al., 1993; Panayotou et al., 1993). On the basis of the unique motif recognized by an individual SH2 domain, protein sequence data bases were searched for potential ligands. In some cases, the proteins predicted to interact were found later to do so.

Shoelson and colleagues have quantified the relative binding affinity of various ~12-mer phosphotyrosyl peptides for the N-terminal SH2 domains of the Syp phosphatase and PI3K (Piccione et al., 1993; Case et al., 1994). Using a competition binding assay they demonstrated that the peptides display a continuum of affinities for SH2 domains ranging over three orders of magnitude (pTyr alone binds ~$10^4$-fold less tightly than the highest affinity peptides). When the lengths of the peptides were shortened from 12 amino acids to 6, there was little loss in affinity for the PI3K SH2 domain, as long as the N- and C-termini were blocked (Ac-X-pY-X-X-X-X-$CONH_2$). However, the Syp SH2 domain behaved differently. Similar truncations in Syp-specific peptides severely diminished binding to the SH2 domain. We explore the physical basis for these different behaviors later. Measurements of the dissociation constant ($K_d$) for SH2 domains and high affinity peptide ligands have ranged from <nM to the μM range (Mayer and Baltimore, 1993); it appears that values of 20–100 nM are probably correct.

What is the in vivo significance of different SH2 specificities? Work by Pawson and coworkers begins to answer this question (Marengere et al., 1994). By changing a single residue in the Src SH2 domain, they switched its selectivity toward that of a SEM-5 SH2 domain. When the altered SH2 domain was spliced into the *sem-5* gene, it provided SEM-5-like activity in Caenorhabditis elegans better than did a construct containing the Src SH2, indicating a correlation of binding specificity with biological activity.

Therefore, although all SH2 domains bind to phosphotyrosyl proteins, they are not promiscuous in choosing partners. Ligand specificity is largely determined by the linear sequence surrounding the pTyr. However, these specificities are not absolute, and there may be more than one SH2 within a cell with high affinity for a particular ligand. Therefore, in vivo, the ability of an SH2 domain to engage a particular phosphoprotein may depend critically on the local concentration of proteins as well as the modulating effect of other domains found on interacting proteins. We take up these complications in later sections. In the section that follows, we discuss the structural basis for SH2 specificity.

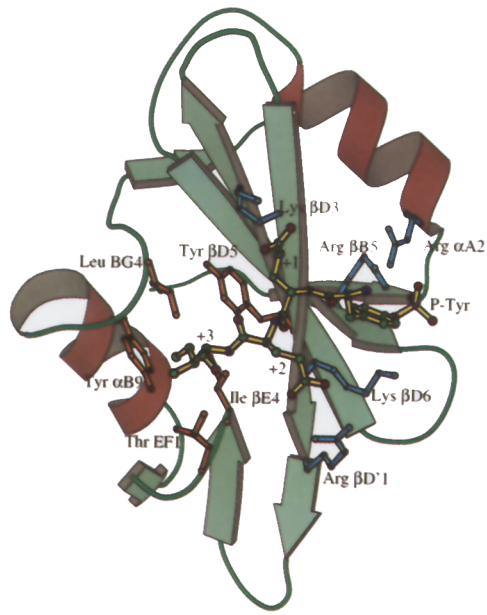


Figure 2. Schematic Diagram of the Src SH2 Domain, Illustrating the Orientation of the Bound Phosphopeptide

The phosphopeptide backbone has been highlighted in yellow, while some SH2 residues in the ligand-binding site have been highlighted in orange and blue (see text for details). This figure is based on a figure that appeared in Waksman et al. (1993) and was redrawn by Chi-Hon Lee and John Kuriyan.

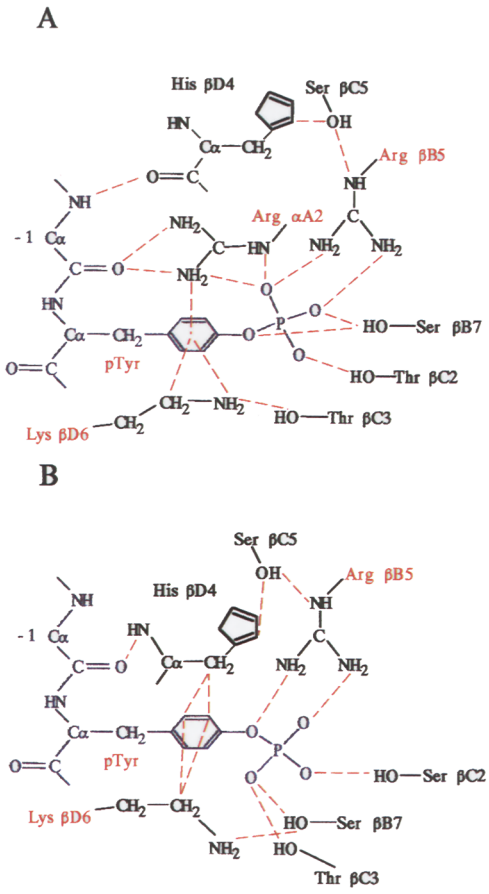


Figure 3. pTyr-Binding Sites

The Src pTyr-binding site (A) and the Syp pTyr-binding site (B). SH2 domain and phosphopeptide residues have been drawn in black and blue, respectively. Residues that are mentioned in the text have their names rendered in red, while noncovalent interactions are indicated by broken red lines (see text for details). For the sake of clarity, not all contacts between the pTyr and SH2 domain have been included. These figures are based on figures that appeared in Waksman et al. (1993) and Lee et al. (1994) and are reproduced by permission of the authors and publishers.

## SH2 Structure

All SH2 domains solved to date contain a large central antiparallel β sheet, two flanking α helices, and follow a general pattern of β-α-β-β-β-β-α-β. Given the similarities in SH2 secondary structure, a nomenclature was adopted to facilitate comparisons (Eck et al., 1993): the β sheets are lettered βA, βB, et cetera; the α helices are αA and αB; and loops are labeled by the 2° structures they connect. Residues are numbered consecutively within the 2° structures. Figure 2 shows a schematic diagram of the Src SH2 domain bound to a high affinity phosphopeptide and illustrates the nomenclature.

### *The pTyr-Binding Site*

SH2 domains bind specifically to pTyr-containing proteins and show little or no affinity for phosphoserine/threonine sequences. Structures of SH2 domains bound to pTyr peptides give a structural basis for this preference (Waksman et al., 1992). The pTyr side chain projects into a deep pocket. At the bottom of the crevice, the negatively charged phosphate is coordinated by a bidentate interaction with the positively charged side chain of Arg βB5 (Figure 3). Since Arg βB5 is 7.5 Å removed from the α-carbon atom of the pTyr (in the Syp structure) (Lee et al., 1994), it is inaccessible to the shorter side chains of phosphoserine or Thr. This Arg is invariant, and mutation of the Arg, even to Lys, abolishes the ability of the Abl SH2 domain to recognize phosphoproteins (Mayer and Baltimore, 1993).

The pTyr recognition site also contains an extensive network of hydrogen bonds to the phosphate and a number of hydrophobic contacts to the phenol ring. While one might think the architecture of the pTyr-binding site is strictly conserved, there is in fact some flexibility in its design. For Src and Lck, there are two amino-aromatic contacts to the phenol ring (one is supplied by Lys βD6 and the other by Arg αA2; Figure 3A) (Eck et al., 1993; Waksman et al., 1993). Bonds between positively charged atoms and benzene rings have recently been described in a number of protein structures (Waksman et al., 1993; Miller, 1994); therefore, it was natural to think that these two amino-aromatic interactions would be critical for pTyr recognition. Apparently, at least for some SH2 domains, this is not the case. Syp is rare among SH2 domains in lacking Arg αA2. Instead, it has a Gly. Furthermore, in the Syp structure, Lys βD6, which supplied the other amino-aromatic contact in Src, contacts the phenol ring solely with its hydrocarbon chain and not with the amine (Figure 3B). Despite the loss of the amino-aromatic interactions there are no major structural alterations in Syp's pTyr pocket (although Arg βB5, which binds to the two terminal oxygens in Src, binds to the phenolic oxygen and a terminal phosphate oxygen in Syp; Figure 3) (Lee et al., 1994). A further demonstration of the plasticity of the pTyr-binding site is seen in the nuclear magnetic resonance (NMR) structure of the SH2 domain of PLC-γ1, where the BC loop, which makes important hydrogen-bond contacts in the Src, Lck, and Syp structures, no longer directly contacts the phosphate (PLC-γ may replace these contacts with stronger associations between the phosphate and positively charged residues found in the PLC-γ pocket) (Pascal et al., 1994). Thus, although certain residues may contribute greatly to pTyr-binding interactions in a given SH2 domain, they are replaceable in others. Within the SH2 domain kingdom, only Arg βB5 is invariant.

### *The Peptide-Binding Site: Act I (Enter Src and Lck)*

The first structure of a ligated SH2 domain was solved using a low affinity peptide (Waksman et al., 1992). In this structure, there were few contacts to the peptide chain. Now that there are structures of both low and high affinity ligands complexed to SH2 domains, it is clear that what differs between these ligands is not how they bind to pTyr, which is relatively unaltered, but rather the extent of interactions to the peptide chain. High affinity ligated structures were determined for Src and Lck bound to an 11-mer peptide, derived from hamster middle T antigen, that contained the Src family–binding motif pYEEI (Eck et al., 1993; Waksman et al., 1993). Four residues in the peptide, pYEEI, make much more extensive contact with the protein than the other seven. Of these four residues, the most

elaborate pockets cradle the pTyr and Ile +3. Binding of this peptide to the Src SH2 domain has therefore been described as "a two-pronged plug engaging a two-holed socket" (Waksman et al., 1993). The Ile +3–binding pocket is lined, as expected, primarily with hydrophobic residues (see Figure 2). The EF and BG loops form part of the Ile +3–binding pocket and have been described as a "set of jaws" that clamp down on the Ile +3 residue (Eck et al., 1993).

The glutamate residues (Y+1 and Y+2) are both solvent-exposed in the Src and Lck structures, and there is no obvious glutamate-binding pocket. There are positively charged residues in the vicinity of the glutamates that may contribute to selectivity (e.g., Lys βD3, Lys βD6, and Arg βD'1; see Figure 2). However, some of these residues are also found on SH2 domains that select for hydrophobic amino acids at the +1 and +2 sites. Moreover, the distance from these residues to the glutamates is too large for there to be a strong interaction. This is somewhat disturbing, since for Lck, the selectivity for a Glu at the +1 site is as high as it is for Ile +3 (Songyang et al., 1993). Furthermore, in the PDGF receptor, a likely binding site for Src is $pY^{579}I$-$pY^{581}V$ (where both Tyrs are phosphorylated), while for the CSF-1 receptor, the site may be $Y^{559}TFI$ (Mori et al., 1993). Both of these sites significantly deviate from the putative consensus, pYEEI. Careful biochemical analysis and systematic mutations of the consensus ligand motif and SH2 domain are needed to assess better the contribution of individual residues toward binding specificity.

### *The Peptide-Binding Site: Act II (Enter Syp and PLC-γ1)*

Recently, the Syp and PLC-γ1 SH2 structures were determined (Lee et al., 1994; Pascal et al., 1994). In contrast with Src and Lck, which are classified as group I ligand binders (pTyr-hydrophilic-hydrophilic-hydrophobic), Syp and PLC-γ1 are group II binders (pTyr-hydrophobic-X-hydrophobic). What is most strikingly similar about these four structures, besides the obvious pTyr-binding site, is the manner in which the peptide is held by the protein. A direct comparison of the Syp and Src ligated peptides reveals that when the peptides' α-carbon atoms are compared, the backbone conformation from the pTyr to the +3 position are virtually identical (Lee et al., 1994). This conservation had been predicted by Harrison and coworkers, who had noted in the Lck structure that the extended peptide–protein interface contained extensive contacts to the peptide's backbone that "suggest tight binding will generally require the peptide conformation seen in this structure" (Eck et al., 1993).

What was unexpected in the new structures is the length of the peptide that contacts the protein. For Syp, there are contacts out to the +5 position, and for PLC-γ1 they extend out to +6. Furthermore, the binding site for the peptide in both of these structures is a shallow groove—in contrast with the "two-holed socket" of Src and Lck. A number of features account for the different binding pockets. All group I SH2s (Src and Lck) contain bulky side chains at position βD5 (Tyr or Phe) (Songyang et al., 1994). In contrast, group II SH2s (Syp and PLC-γ1) contain smaller, nonaromatic side chains at βD5. Tyr βD5 acts as the divider between the pTyr and Ile +3–binding pockets (see Figure 2). In its absence, a slender channel is created between the two pockets. This groove is hydrophobic and provides to hydrophobic residues at the +1 position a place to bury their side chains partially.

Further selectivity for hydrophobic residues by the Syp and PLC-γ1 SH2 domains is imparted by the BG loop. The conformation and length of this loop varies among the SH2 domains, as does the extent to which it contacts the ligated peptides. Therefore, this loop may play an important role in determining peptide selectivity. In the Src and Lck structures, the BG and EF loops seal off the Ile +3-binding pocket. This action forces the peptide chain away from the protein and precludes significant interactions beyond Ile +3 (see Figure 2). In the Syp and PLC-γ1 structures, these loops are less constraining, and a groove opens up beyond the +3 site, which allows for the more extended contacts to the peptide chain.

Consistent with the structural studies, mutation of the +5 residue in the Syp peptide results in a >100-fold decrease in the peptide's affinity. This result explains why truncation of Syp-specific peptides to 6-mers (mentioned above) severely diminishes binding to the protein. Similar biochemical studies for PLC-γ1 suggest that the extended contacts (+5 and +6) are not critical for high affinity interactions (Case et al., 1994). It is unclear why these results and the NMR structure are at odds. Others have reported that residues on the N-terminus of the pTyr alter the binding affinity of peptides to some SH2 domains (Bibbins et al., 1993; Nishimura et al., 1993). These results suggest that although much has been learnt about SH2 domain specificity, there is still more to go. The future will no doubt see more structures, more bound ligands, and, hopefully, quantitative analysis of the binding interactions between whole phosphoproteins and SH2 domains.

### *Kinetics of Ligand Binding*

High affinity phosphopeptides bind to SH2 domains rapidly: $k_{on}$, $\sim 1 \times 10^5\ M^{-1}s^{-1}$ to $2 \times 10^6\ M^{-1}s^{-1}$, but dissociation is also moderately fast, even for high affinity ligands: $k_{off}$, $\sim 0.1\ s^{-1}$ ($t_{½\ dissociation}$ = 5–10 s) (Panayotou et al., 1993). Since the response time of cells to growth factors is minutes to hours, even after SH2-containing proteins have engaged an RTK, the signal emanating from the receptor may remain open to the modulatory effect of phosphatases as well as competing SH2-containing proteins. Interestingly, the difference between high and low affinity ligands appears to be due to differences in $k_{on}$, with little variation in $k_{off}$ (Felder et al., 1993; Panayotou et al., 1993; Marengere et al., 1994). This seems surprising in light of the crystal structures, which show that high affinity ligands make more extensive contacts to the protein than do low affinity ligands. However, binding to the SH2 domain by a phosphopeptide may be a two-step process. In the first step, the phosphopeptide may become weakly associated with the protein to form an unstable intermediate complex. The rate-limiting transition state may occur subsequently, as the pTyr becomes tightly locked into its binding pocket. Therefore, during dissociation of the peptide, the rate-limiting step could be disengagement of the pTyr. Since the pTyr is held in the binding pocket in a similar manner

for both high and low affinity ligands, $k_{off}$ would be similar for both ligands.

### *Do SH2 Domains Bind in a Non-pTyr-Dependent Manner?*

A number of reports claim that SH2 domains interact with proteins in a non-pTyr-dependent manner (Muller et al., 1992; Bibbins et al., 1993; Cooper and Kashishian, 1993). In at least one case, the interaction described (binding of the SH2 domains from the noncatalytic p85α subunit of PI3K to the catalytic p110 subunit) could not be reproduced by another lab and subsequently has been ascribed to a different region of p85α (Hu and Schlessinger, 1994; Klippel et al., 1994). Furthermore, the pTyr-independent interactions described so far are weaker than SH2–pTyr-dependent interactions (Muller et al., 1992; Bibbins et al., 1993). However, if the pTyr-independent interactions occur intramolecularly, then even relatively weak interactions could be used by a protein as a means of allosteric regulation. Therefore, the possibility that some SH2 domains have an effector region outside the pTyr-binding site is an intriguing but at this time speculative idea.

## SH2 Domain Function

How does the binding of an SH2-containing protein to a Tyr-phosphorylated protein help transmit a signal? First, binding may alter the subcellular localization of a protein, bringing it closer to its substrate, or closer to a protein that modifies it. Second, binding may induce conformational changes that alter the catalytic activity of the interacting proteins. SH2-containing proteins use both of these methods.

### *Propagation of Signals by a Change in Subcellular Localization*

The RTK-mediated Ras activation pathway has been the paradigm for activation by localization (Pawson and Schlessinger, 1993). Ras, a GTPase associated with the plasma membrane, is activated by the guanine nucleotide exchange factor SOS. Even in quiescent cells, SOS is found in the cytoplasm engaged with the adaptor molecule Grb2 via the latter molecule's two SH3 domains. Activation and autophosphorylation of the epidermal growth factor receptor (EGFR) creates a binding site for the SH2 domain of Grb2 and recruits the Grb2–SOS complex to the membrane, bringing SOS into proximity with its Ras substrate. At the membrane, SOS may be Thr- or Ser-phosphorylated. However, the intrinsic catalytic activity of SOS in activated or quiescent cells is indistinguishable, leaving the biological relevance of SOS phosphorylation unknown (in yeast, phosphorylation of a Ras exchanger, Cdc25, by a cAMP-dependent kinase releases Cdc25 from the membrane; Rozakis-Adcock et al., 1993). Therefore, in EGF-treated cells, Ras activation is due to the increased local concentration of SOS. Localization is also important for the pathways that activate PLC-γ1, PI3K, and RasGAP, because like SOS, these molecules are cytosolic, and their substrates, phospholipids and Ras, are membrane bound.

A variation on the EGFR/Grb2 pathway is used by PDGFR. PDGFR does not bind directly to Grb2 but instead contains a binding site, $pY^{1009}$, for the tyrosine phosphatase Syp (Lechleider et al., 1993; Li et al., 1994). Syp, upon binding to the PDGF receptor, is Tyr-phosphorylated, and it is thought that phosphorylation of Syp provides a docking site for the Grb2–SOS complex. In PDGFR mutants that carry multiple Tyr to Phe mutations, the presence of $Y^{1009}$ is sufficient but not necessary for Ras activation by PDGF (Valius and Kazlauskas, 1993). Therefore, Syp-independent pathways for Ras activation must also exist. There is also genetic evidence that the PDGFR/Syp/Grb2/SOS pathway is physiologically relevant. In Drosophila melanogaster, the Syp homolog, corkscrew, positively transmits signals from the putative RTK, torso, on a pathway that mediates cell fate determination for the head and tail regions of Drosophila embryos. SOS and Ras are also essential for this pathway. The ability of Syp to act as an adaptor molecule and bring Grb2 to the kinase may explain the apparent paradox of a phosphatase aiding the signaling pathway of a kinase (Li et al., 1994). The function of Syp's phosphatase activity on this pathway, however, remains a mystery.

Grb2 appears to be (almost) a universal adaptor protein in tyrosine kinase signaling pathways. Besides Syp and EGFR, it also binds to Tyr-phosphorylated forms of Shc and insulin receptor substrate 1 (IRS-1) (Pawson and Schlessinger, 1993). However, phosphorylated Shc and IRS-1 do not stably associate with the membrane, which suggests that they may use a presently unknown mechanism to bring about Ras activation.

### *Phosphorylation of SH2-Containing Proteins*

The binding of an SH2-containing protein to a tyrosine kinase increases the likelihood that the SH2-containing protein is going to get phosphorylated (kinases that modify proteins frequently stably associate with their substrates, e.g., rhodopsin kinase and the stress-activated protein kinase family; Lefkowitz, 1993; Dérijard et al., 1994; Kyriakis et al., 1994). Phosphorylation of SH2-containing molecules may recruit other molecules to the activated complex; e.g., phosphorylation of Syp by PDGFR created a new binding site for Grb2. Alternatively, phosphorylation may alter the intrinsic catalytic activity of the SH2-containing molecule; e.g., EGFR phosphorylation of PLC-γ1 increases the catalytic activity of PLC-γ1 toward profilin-bound phosphatidylinositol (4,5)-bisphosphate ($PtdInsP_2$; hydrolysis of $PtdInsP_2$ frees profilin, which then regulates actin polymerization) (Aderem, 1992).

The JAK/STAT pathway provides another rich example of how phosphorylation of an SH2-containing molecule is used in signal transduction pathways (Darnell et al., 1994). JAKs are members of a family of nonreceptor tyrosine kinases, the Janus kinases, that contain two putative tyrosine kinase domains. Their name is derived from that of the Roman god, Janus, god of gates and doorways and the porter of heaven; he is often portrayed with two faces looking in opposite directions (thus, the first month of the year is January). JAKs associate with membrane-bound receptors, e.g., PDGFR and the interferon α receptor. When a cell is treated with growth factors or cytokines, JAK family members may be activated and then phosphorylate members of the STAT protein family. Phosphorylated STATs form homo- and probably heterodimers, held to-

gether by a reciprocal interaction of the SH2 domain on one STAT with a pTyr on the opposing STAT. Unphosphorylated STATs are cytoplasmic, but upon phosphorylation and dimerization they, along with other molecules that bind to the dimer, translocate to the nucleus. In the nucleus, the complex binds to specific DNA sites and activates transcription. STATs are an example of transcription factors regulated by Tyr phosphorylation.

### *IRS-1: Phosphorylation of a Signaling Molecule That Does Not Contain an SH2 Domain*

In insulin-stimulated cells, a major substrate for the insulin receptor is the cytosolic protein IRS-1. It is not entirely clear how IRS-1, which lacks an SH2 domain to direct it to the membrane, gets phosphorylated. The insulin receptor kinase is specific for certain IRS-1 Tyr-containing motifs (Shoelson et al., 1992). However, in general, tyrosine kinases are not as specific as serine and threonine kinases. Moreover, IRS-1 has 21 potential Tyr phosphorylation sites, at least eight of which can be phosphorylated (Myers et al., 1994). Whether the insulin receptor is specific for all of these phosphorylation sites is not known. In any event, once IRS-1 is phosphorylated it associates with a number of proteins, including PI3K, Grb2, Syp, and Nck. Because IRS-1 interacts with so many proteins, it has been dubbed a "docking protein". The docking protein links the insulin receptor to many SH2-containing signaling pathways, and in this respect it is analogous to the cytoplasmic tail of the PDGFR. In these signaling complexes, many opportunities might exist for interactions between neighboring molecules. However, to date, there is little evidence for such interactions, and it is not even known whether signaling complexes containing proteins from multiple pathways generally form under physiological conditions.

### *Allosteric Activation of SH2-Containing Proteins*

Both the Syp phosphatase and PI3K show enhanced catalytic activity upon binding to phosphopeptides (Carpenter et al., 1993; Shoelson et al., 1993; Sugimoto et al., 1994). For Syp, the increase in activity can be more than 50-fold; for PI3K it is about 5-fold. While it is conceivable that phosphoprotein binding induces a conformational change in the SH2 domain that is then transmitted to the catalytic domain, the crystal structures suggest that this is unlikely. Very little structural alteration is seen within SH2 domains upon ligand binding (Lee et al., 1994). However, although SH2s may not be materially altered by binding, their relation to the rest of the protein could be changed. Thus, an internal interaction of the SH2 with the protein could be modified when the SH2 binds to an exogenous ligand. The best example of this comes from the Src family, where a pTyr residue in the carboxyl tail, $pY^{527}$, interacts with the Src SH2 domain to regulate the kinase negatively (Superti-Furga et al., 1993). A somewhat similar SH2/carboxyl tail repression mechanism might be used by the Syp phosphatase as limited protease digestion of Syp's carboxyl tail activates the phosphatase and mitigates the increase in activity seen upon addition of phosphopeptides (Sugimoto et al., 1994). Both Syp and PI3K contain two SH2 domains, and there is evidence that engagement of both domains is required for maximal stimulation of the catalytic domain.

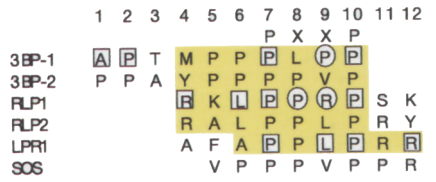


Figure 4. SH3 Peptide Ligands

3BP-1, 3BP-2 , RLP1, and RLP2 are class I ligands, while LPR1 and the SOS-derived peptide are class II ligands (see text for details). Regions of these ligands that are contained in the PPII helix in ligated structures have been highlighted in yellow (a structure for the ligated form of the SOS-derived peptide has not been determined, so this peptide is not shaded). Residues that have been identified as critical for high affinity interactions with SH3 domains have been boxed, while residues that contribute less to binding have been circled (thorough mutagenic analysis has not yet been done for 3BP-2, RLP2, and the SOS-derived peptide, so these peptides have not been marked).

### SH3 Domains

While the biological functions of SH2 domains are fairly well understood, understanding the biology of SH3 domains has lagged behind. A key for deciphering the function of SH3 domains came when two SH3-binding proteins, 3BP1 and 3BP2, were identified by screening a cDNA expression library with the Abl SH3 domain (Cicchetti et al., 1992). The location of the possible binding regions on 3BP1 and 3BP2 was narrowed to ~10 amino acid Pro-rich sequences (Figure 4) (Ren et al., 1993). Since then, a number of in vivo SH3 ligands have been identified, e.g., SOS, PI3K, and $p47^{phox}$, and the binding sites have similarly mapped to Pro-rich sequences (Finan et al., 1994; Musacchio et al., 1994b). Other high affinity SH3 peptide ligands were identified by screening with a chemically synthesized peptide library (Chen et al., 1993; Yu et al., 1994). Schreiber and coworkers started with a biased random peptide library, XXXPPXPXX, where X is any amino acid other than Cys, and found that both the Src and PI3K SH3s selected for two classes of ligands (the bias was based on residues found in 3BP1 and 3BP2; without the bias, no high affinity ligands were found). Class I ligands are RXLPPδPXX, where δ is L for Src and R for PI3K. Class II are XXXPPLPXR for both Src and PI3K. In class I ligands for Src and PI3K, an Arg is N-terminal to the Pro-rich sequence, while for class II ligands it is C-terminal. Examples of class I and II ligands used in structural studies are listed in Figure 4. Mutational analysis has identified residues that are particularly important for binding, and these residues are boxed in Figure 4; less important residues have been circled (Ren et al., 1993; Yu et al., 1994; Feng et al., 1994). All high affinity SH3 ligands identified so far contain a PXXP motif, and the sequences in Figure 4 have been aligned by this motif. In the following section, we consider the structural basis for SH3 selectivity.

### SH3 Structure

Structures for eight SH3 domains have been reported, four of these with ligands (PI3K, Abl, Fyn, Src) (Feng et al., 1994; Musacchio et al., 1994a; Yu et al., 1994). Although there are variations among these structures, the overall topology is well conserved. The basic fold consists

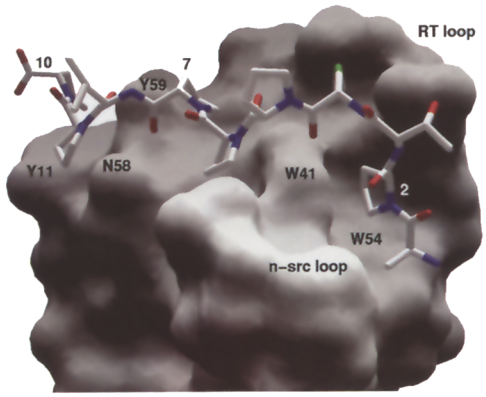


Figure 5. The 3BP-1 Peptide Bound to the Abl SH3 Domain

The numbering on the residues of the peptide corresponds to the numbering for the 3BP-1 peptide in Figure 4. Residues 4–10 of the peptide adopt a PPII-helical conformation (see text for details). Pepide color coding: nitrogen, blue; oxygen, red; and carbon, white. Some residues and regions of the Abl SH3 domain that define the ligand-binding surface are also highlighted (see Musacchio et al., 1994a, for a more complete description of the interactions). This figure previously appeared in the paper by Musacchio et al. (1994a) and is reproduced by permission of the authors and publisher.

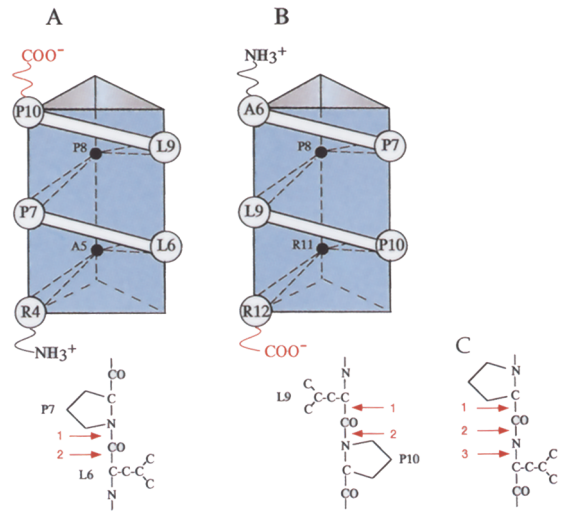


Figure 6. Schematic Diagram Illustrating PPII Helices for Src-Binding Peptides

RLP2 (A) and LPR1 (B). Below each illustration is a stick figure for two residues that are found in each helix. Notice that two backbone bonds (numbered in red) separate the Pro and non-Pro side chains, while in (C), three backbone bonds separate the side chains (see text for details).

of five antiparallel β strands (named "A" to "E") that pack to form two perpendicular β sheets. A hydrophobic patch that contains a cluster of conserved aromatic residues and is surrounded by two charged and variable loops (the AB and BC loops) forms the ligand-binding pocket.

The first ligated structure was of the PI3K SH3 bound to the peptide RLP1 (see Figure 4; Yu et al., 1994). As with the SH2 domain, very little structural alteration in the SH3 is detected upon ligand binding. The central portion of this peptide, residues Arg4 to Pro10, adopts a polyproline type II (PPII) helix (the numbering of these residues refers to the numbers shown above the peptide sequences in Figure 4). PPII helices appear in all bound SH3 ligands examined so far. Figure 5 illustrates the PPII helical conformation of the 3BP-1 peptide bound to the Abl SH3 domain (the PPII helical regions of other SH3 ligands have been highlighted in yellow in Figure 4). Though the PPII motif was first identified in 1955 in crystals of L-polyproline, their ubiquity in globular proteins has only recently been recognized (Adzhubei and Sternberg, 1993). In contrast with their more renowned counterparts, the $3_{10}$ and α helices, which are predominantly right handed, PPII helices are left handed. The number of residues per turn in a perfect PPII helix is ~3.0; therefore, we can imagine the PPII portion of the bound peptide as a trigonal prism (Figure 6A is drawn for the Src-binding peptide RLP2). Two edges of the prism contact the protein (the SH3 domain would be where your face is) (Yu et al., 1994). This agrees well with the critical residues identified on RLP1 by mutational analysis. In globular proteins, most of the amide nitrogens and oxygens in the PPII helix form hydrogen bonds to water. PPII helices therefore tend to be solvent-exposed and mobile, ideal sites for interactions with other proteins. No hydrogen bonds between the peptide backbone and SH3 domain were seen in the PI3K structure, and only two hydrogen bonds were seen in the Abl and Fyn structures (Musacchio et al., 1994a). Therefore, the driving force for bonding comes primarily from interactions between the ligand side chains and the protein.

Although Pro residues are not strictly required for PPII helices (~25% of PPII helices in globular proteins do not contain Pro [Adzhubei and Sternberg, 1993]), the propensity of Pro to adopt this conformation readily explains their presence in SH3 ligands (SH3-binding, Pro-rich peptide ligands may exist as PPII helices free in solution; Musacchio et al., 1994b). The two critical Pros in the PXXP motif directly pack against the SH3-binding protein, lodging themselves into a host of conserved, mostly aromatic, residues. The N-terminal Arg found on the class I ligands RLP1 and RLP2 (Arg4; see Figure 4) engages in a critical electrostatic interaction with Asp-99 (numbering is for Src); mutation of either Arg4 or Asp-99 severely disrupts ligand binding (Yu et al., 1994). However, Asp-99 also binds the C-terminal Arg (Arg-12) in class II ligands LPR1 and SOS. Therefore, the orientations of class I and II ligands on Src are opposite! Compare Figures 6A and 6B (Feng et al., 1994). Furthermore, the positions of the critical Pros on the face of the trigonal prism are exchanged with the non-Pro residues. This exchange keeps the distance between the prolyl ring and the non-Pro side chain constant (notice that in the stick figures in Figures 6A and 6B the two side chains are separated by two backbone bonds; if you change the position of the Pro and non-Pro within a stick figure, the side chains become separated by three backbone bonds; e.g., compare Figures 6B and 6C). Apparently, the SH3-binding pocket is flexible enough to accommodate both ligand orientations, and the tightest-binding class I and class II ligands have similar affinities for the SH3 of PI3K (8 μM and 13 μM, respectively; Chen et al., 1993). The

lack of substantial hydrogen bonding to the peptide backbone may further contribute to the orientation androgyny. Whether either orientation predominates in nature remains to be determined; the SOS peptide (see Figure 4) is likely to interact with Grb2 in a class II interaction (Feng et al., 1994), while the 3BP1–Abl interaction is class I oriented (Musacchio et al., 1994a).

Like that of SH2, the specificity of SH3 is thought to be determined by the variable amino acids that surround invariant residues. This specificity is evident in that Abl SH3 does not bind well to Src-specific ligands, while Src, in turn, binds poorly to Abl-specific peptides (Rickles et al., 1994). However, Asp-99, the residue that may dictate Src's specificity for Arg4, is found in virtually every SH3 domain (except Abl, which has a Thr). Moreover, the Fyn SH3 domain, which selected for a Src-like peptide (RXLPXXP) in a phage display library (Rickles et al., 1994), has a residue homologous to Asp-99, yet binds to the Abl-specific 3BP-2 peptide with fairly high affinity, ~35 mM (Musacchio et al., 1994a). Therefore, specificity could be influenced by residues lying outside the PPII helix; for instance, in the Abl–3BP1 and Fyn–3BP2 structures, Pro2 (which lies outside the PPII helix; see Figure 4) makes significant contact with the SH3 domain (Musacchio et al., 1994a). However, if longer peptides are needed, then perhaps in vivo specificity will depend upon nonlinear sequences, i.e., tertiary structure. There is evidence that disparate regions of the human inmmunodeficiency virus (HIV) protein Nef mediate interactions with the SH3 domain of Hck (Saksela et al., 1995). Nonlinear sequences might mimic the PPII helix and also provide additional regions that interact with the SH3 domain. Alternatively, SH3 domains may carry only crude specificity constraints, while the fine tuning of protein–protein interactions could be determined by other modular domains on the interacting proteins.

### Interactions between SH2 and SH3 Domains

SH2 and SH3 domains are found together on many proteins; might their activities be coordinated? There is evidence to suggest so. For instance, the c-Src kinase is negatively regulated by interactions between its SH2 domain and its Tyr-phosphorylated C-terminal tail. However, this inhibition also requires a functional SH3 domain. Mutation of the SH3 activates the kinase and increases the accessibility of the SH2 domain to exogenous substrates (Superti-Furga et al., 1993). In addition, a phosphoprotein that associates with Src during mitosis, p68, has been isolated. p68 interacts with both the isolated Src SH2 and the SH3 domains, but neither domain competes for p68 as well as the tandem SH2–SH3 module (Fumagalli et al., 1994; Taylor and Shalloway, 1994). A similar synergism is observed in the binding of the 110 kDa actin filament–associated protein to Src's SH2 and SH3 domains (Flynn et al., 1993).

Structural evidence in support of SH2–SH3 cooperativity comes from Eck et al. (1994), who crystallized a fragment containing the Lck SH2–SH3 domains (Lck is a Src family tyrosine kinase member). In the Lck structure there are few intramolecular contacts between the SH2 and SH3 domains; however, the fragment crystallized as a dimer and there are extensive intermolecular SH2–SH3 contacts. Interestingly, an SH2 Pro lies in the binding pocket of the opposing SH3 domain and is bound in an orientation similar to that of a PXXP Pro. The Lck SH2–SH3 fragment was also crystallized in the presence of a phosphorylated peptide that corresponds to Lck's carboxyl tail. While the peptide's pTyr binds in an orientation similar to that observed in SH2–peptide complexes, the peptide chain does not; it lies in the crease of the intermolecular SH2–SH3 interface. This result is intriguing, and if c-Src exists as a dimer in vivo, it may explain why both a functional SH2 and an SH3 are required for the negative regulatory effect of Src's phosphocarboxyl tail. To date, there is no evidence that c-Src does exist as a dimer in solution but, as Eck et al. point out, in vivo, Src is a membrane-bound protein, and membrane localization may favor dimer formation. Moreover, membrane-bound Src may further be concentrated within the membrane at signaling center complexes (Lisanti et al., 1994). The consequences of cellular organization on signal transduction pathways has been a largely unexplored area of cell biology.

### SH3 Function

Mutational analysis first demonstrated the biological importance of the SH3 domain: mutation of the SH3 domain of the adaptor molecule v-Crk mitigates this protein's transforming potential. For the nonreceptor tyrosine kinases Abl and Src, deletion of the SH3 domain activates the transforming potential of the proto-oncogene products. The importance of SH3 domains in signal transduction pathways was highlighted in C. elegans, where mutation of either of SEM-5's two SH3 domains blocked vulval development (Mayer and Baltimore, 1993). Although our understanding of the physiological role of SH3-mediated interactions is still at an early stage, recent studies suggest various places where SH3 interactions mediate critical protein–protein interactions. These interactions are used to organize protein complexes within the cell, bring substrates to enzymes, and regulate enzymatic activities, as discussed in the following sections.

#### *Role of SH3 in Cellular Localization*

Compartmentalization of proteins plays an important role in the regulation of signal transduction processes. Many of the proteins that localize to the plasma membrane or the cytoskeleton contain SH3 domains (e.g., the actin-binding protein α-spectrin, nonmuscle myosin Ib, and the S. cerevisiae protein ABP-1), suggesting that SH3 domains mediate localization to these regions (Mayer and Baltimore, 1993). Using a microinjection approach, Bar-Sagi et al. demonstrated that the SH3 domain of PLC-γ is responsible for the targeting of that protein to cytoskeletal microfilaments, while both SH3 domains of Grb2, but not its SH2 domain, are required for Grb2 localization to membrane ruffles (Bar-Sagi et al., 1993). Although many SH3-containing molecules associate with actin filaments, SH3 domains probably do not directly bind to actin. It is also likely that SH3-mediated interactions between α-spectrin and the Pro-rich C-terminal tail of the amiloride-sensitive $Na^+$ channel dictates that channel's localization to the apical

membrane of polarized epithelial cells. Since many ion channels contain Pro-rich sequences, this mechanism of localization may be generally applicable to ion channels (Rotin et al., 1994).

There are also many examples of SH3-mediated interactions influencing signaling pathways containing G proteins of 21 kDa. For example, the Grb2–SOS interaction modulates Ras activity (see above), and the C-terminal SH3 domain of Grb2 binds the N-terminal SH3 domain of Vav, a hematopoietic-specific guanine nucleotide exchange factor (Ye and Baltimore, 1995). In S. cerevisiae, genetic analysis suggests that the SH3-containing protein Bem1p, which regulates cell polarity, interacts with the guanine nucleotide exchange factor BUD5 and a GTPase-activating protein, Bem2p. In addition, some guanine nucleotide exchange factors (e.g., Vav and CDC25) and guanosine triphosphatase–activating proteins (e.g., RasGAP) contain their own SH3 domains (Mayer and Baltimore, 1993). While the frequency of SH3 domains on small GTP-binding protein pathways suggests a common mechanism of regulation, what that mechanism might be is not known.

SH3-mediated interactions also play a role in the assembly of the phagocyte NADPH oxidase system. Phagocyte NADPH oxidase catalyzes reduction of molecular oxygen to superoxide. The NADPH oxidase consists of a membrane-bound flavocytochrome b, composed of two subunits, gp91$^{phox}$ and p22$^{phox}$. Activation of the oxidase occurs when a small G protein, p21$^{rac}$, and three cytosolic SH3-containing proteins, p47$^{phox}$, p67$^{phox}$, and p40$^{phox}$, translocate to the membrane. The two SH3 domains of p47$^{phox}$, both of which are required for the assembly of the oxidase complex, bind to p22$^{phox}$ and p67$^{phox}$ (Sumimoto et al., 1994). In quiescent cells, the p47$^{phox}$ SH3 domains may be blocked by an intramolecular interaction. They are unmasked in vitro by activators of the oxidase, such as arachidonic acid and sodium dodecyl sulfate. Interestingly, the point mutation P156Q, found in a patient with chronic granulomatous disease, occurs in a Pro-rich region of p22$^{phox}$ and abolishes interaction with the p47$^{phox}$ SH3 domain. The C-terminal SH3 domain of p67$^{phox}$ also binds to p47$^{phox}$ (Finan et al., 1994). These results reveal that a complex SH3 network directs the assembly of the phagocyte NADPH oxidase system. Apparently, in this system, SH3-mediated interactions are regulated in contrast with the Grb2/SOS SH3–mediated contacts, which are constitutive.

***Role of SH3 in Regulating Enzymatic Activities***

As was previously mentioned for c-Src, SH3-mediated interactions negatively regulate the kinase activity of nonreceptor tyrosine kinases. The Abl tyrosine kinase is also negatively regulated by its SH3 domain (Mayer and Baltimore, 1993). Although c-Src is activated by mutations in the SH3 domain, in the context of a constitutively active variant of c-Src, Y527F, mutations in the SH3 domain *decrease* transforming activity. However, in another activated variant of c-Src, E378G (a mutation within the kinase domain), the SH3 domain is not required for the transforming activity (Seidel-Dugan et al., 1992). It is not clear why there are different requirements for the SH3 domains in different contexts.

An SH3-mediated interaction is also implicated in the activation of dynamin (Gout et al., 1993). Dynamin is a 100 kDa microtubule-activated GTPase that plays a critical role in the initial stages of endocytosis. Dynamin's GTPase activity is activated by the binding of SH3 domains from Grb2, Src, Fgr, and Fyn (although this activation is weaker than that stimulated by microtubules). This suggests that the association of SH3-containing signaling proteins with dynamin may regulate endocytosis.

***Role of SH3 in Recruiting Specific Substrates to the Enzyme***

Besides SH2-mediated interactions, nonreceptor tyrosine kinases may also use SH3-mediated interactions to target themselves to some of their substrates. For instance, the Abl tyrosine kinase has binding sites selective for the SH3 domains of the Crk, Grb2, and Nck adaptor molecules. It is only upon binding to Abl that Crk becomes phosphorylated (Ren et al., 1994; Feller et al., 1994). The SH3 domain of Src family tyrosine kinases may target Src to its substrates: AFAP-110 and p68 (Flynn et al., 1993; Fumagalli et al., 1994; Taylor and Shalloway, 1994). Stable binding of these substrates to Src requires both the Src SH3 and the SH2 domains. This has led to the suggestion that binding of Src to these substrates is initiated by SH3-mediated interactions, followed by Tyr-phosphorylation, and ends with stable complex formation that is mediated by both SH3 and SH2 interactions. The stable association of tyrosine kinases with their substrates through noncatalytic domains may block the access of other substrates to the kinase. Signaling by tyrosine kinases may therefore involve pathways that require phosphorylation of only stoichiometric amounts of substrates.

***Role of SH3 in Interacting with Viral Proteins***

Because SH3-mediated interactions are critical for many cellular processes, it is no wonder that many viruses have pirated modified forms of SH3-containing proteins for their own devices (e.g., the Abelson murine leukemia virus). However, some viruses have evolved novel proteins to tap into tyrosine kinase signaling pathways. Recently, the HIV Nef protein was found to have a conserved PXXP motif that mediates Nef interaction with the SH3 domains of Hck and Lyn (Saksela et al., 1995). The PXXP motif is required for the higher replicative potential of Nef-bearing viruses, suggesting that the virus uses the SH3-mediated interaction to regulate a signaling pathway that facilitates viral growth.

**PH Domains**

The PH domain is a region of approximately 100 amino acids found in a wide variety of signaling and cytoskeletal proteins. The pleckstrin protein, the major protein kinase C substrate in platelets, has two such domains and was the first protein in which the domain was recognized (Haslam et al., 1993; Mayer et al., 1993). The sequence homology of the PH domain among proteins is generally low (Musacchio et al., 1993). The structure of three PH domains—the N-terminal PH domain and those from spectrin and dynamin—have been solved by NMR analysis (Downing et al., 1994; Macias et al., 1994; Yoon et al., 1994; Ferguson et al., 1994). Despite the low sequence homol-

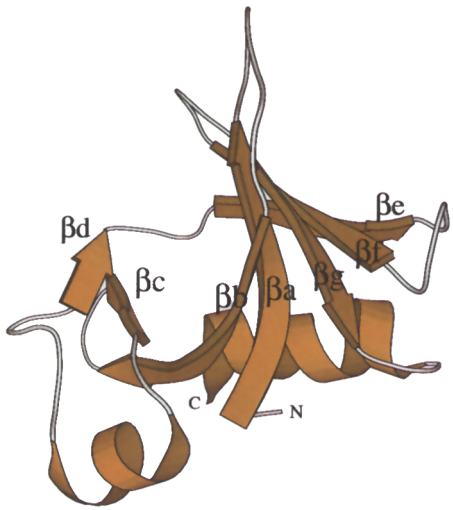


Figure 7. Schematic Diagram of the Spectrin PH Domain

This figure previously appeared in the paper by Gibson et al. (1994) and is reproduced by permission of the authors and publisher.

ogy among these domains, the peptide fold seen in these structures is virtually the same and provides structural evidence that the PH domain is a real functional unit. The basic PH fold contains two antiparallel β sheets and a long C-terminal α helix (Figure 7). The PH structures differ most in the loop regions between β strands. In particular, the CD loop tolerates large insertions (Gibson et al., 1994). Interestingly, for the PH domain of PLC-γ, the protein's two SH2 domains and one SH3 domain are predicted to insert into this loop. Many of the conserved hydrophobic residues found in PH domains are located in the interior of the protein (in contrast with the SH3 domain, where they form the aromatic-rich PPII-binding pocket), while the highly conserved charged residues are solvent-exposed. On one side of the PH domain there are a narrow cleft, three variable loops, and clusters of positive charges. This side of the molecule has been hypothesized to be the site of ligand binding (Gibson et al., 1994).

The function of PH domains is not known, although the presence of PH domains in a wide variety of proteins suggests that, like SH2 and SH3 domains, they mediate protein–protein interactions. Several PH domains bind to the βγ subunits of heterotrimeric G proteins. However, these interactions only require the C-terminal portion of the PH domain and also require residues that lie outside the PH domain, so it is not clear whether these interactions can be generalized for other PH domains. Mutations in the N-terminal portion of the PH domain found in the nonreceptor tyrosine kinase Btk result in the immune deficiency disorder agammaglobulinemia and illustrate the importance of this region of the PH domain (Gibson et al., 1994). Recently, it was suggested that PH domains might not serve as mediators of protein–protein interactions, but rather as mediators of protein–lipid interactions. Using a centrifugation assay, Harlan et al. (1994) reported that a number of PH domains associate specifically with lipid vesicles doped with $PtdInsP_2$. This interaction involves the previously mentioned positively charged area on the PH domain. The generality and biological relevance of PH–$PtdInsP_2$ interactions—and whether PH domains display other binding functions—remains to be determined.

## Perspectives

We have now reached the point in our understanding of these three modular domains where their occurrence and their structures are well characterized. For SH2 and SH3, but not PH, binding sites have been well delineated, and the structural basis of recognition and specificity is moderately clear. However, many questions remain open. These have been highlighted above and will not be reiterated. The issues for PH are much more open than for the others, because its binding specificity is not yet apparent. It is the most variable of the three in sequence; it may not have a stereotyped core-binding activity and could depend more on three-dimensional protein structure for its binding specificity. It should be remembered that the prototype binding protein, the immunoglobulin, binds to either linear epitopes or ones composed of segments from various parts of the linear structure, and there is nothing in common among the sites of binding.

There is sure to be much excitement in the future about the roles that these domains play in particular biological processes. Having already seen that they can mediate dimerization, bring proteins to their substrates at membranes, bring substrates to enzymes, modify the activities of enzymes, and aggregate subunits of multiprotein complexes, we can expect new classes of functions to emerge. Ultimately, the recognition of modular binding domains will take its historic place as one of the key events that will have allowed biologists to comprehend the full variety of mechanisms inherent in the signal transduction pathways of cells.

After submission of this review, there were three other reports of high-resolution structure determinations of SH3 domains bound to class II ligands: Lim et al. (1994), Terasawa et al. (1994), and Goudreau et al. (1994). These reports also demonstrate that class I and II ligands bind to SH3 domains in opposite orientations. In addition, a solution structure for the PH domain of dynamin has been reported: Fushman et al. (1995).

### Acknowledgments

Correspondence should be addressed to D. B. We would like to thank Martyn Botfield, John Kuriyan, Daniel Oprian, Matti Saraste, Joseph Schlessinger, Stuart Schreiber, Steven Shoelson, and Michael Waterfield for helpful discussions on this manuscript and for communicating results that are in press. We also thank Chi-Hon Lee, John Kuriyan, Andrea Musacchio, and Matti Saraste for redrawing figures for this manuscript, and *Structure*, *Trends in Biochemical Sciences*, and *Nature Structural Biology*, for permission to use previously published figures. We are also grateful to members of the Baltimore laboratory, especially Liz Alcamo and Marty Scott for comments on this manuscript. This work was supported by postdoctoral fellowships from the Helen Hay Whitney Foundation (G. B. C.) and the Cancer Research Institute (R. R.), and National Institutes of Health grant 5RO1 CA51462-06 (D. B.). Space limitation has precluded citation of all primary references, which are included in reviews cited.

**References**

Aderem, A. (1992). Signal transduction and the actin cytoskeleton: the roles of MARCKS and profilin. Trends Biochem. Sci. *17*, 438–443.

Adzhubei, A. A., and Sternberg, M. J. E. (1993). Left-handed polyproline II helices commonly occur in globular proteins. J. Mol. Biol. *229*, 472–493.

Bar-Sagi, D., Rotin, D., Batzer, A., Mandiyan, V., and Schlessinger, J. (1993). SH3 domains direct cellular localization of signaling molecules. Cell *74*, 83–91.

Bibbins, K. B., Boeuf, H., and Varmus, H. E. (1993). Binding of the Src SH2 domain to phosphopeptides is determined by residues in both the SH2 domain and the phosphopeptides. Mol. Cell. Biol. *13*, 7278–7287.

Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R., and Soltoff, S. (1991). Oncogenes and signal transduction. Cell *64*, 281–302.

Carpenter, C. L., Auger, K. R., Chanudhuri, M., Yoakim, M., Schaffhausen, B., Shoelson, S., and Cantley, L. C. (1993). Phosphoinositide 3-kinase is activated by phosphopeptides that bind to the SH2 domains of the 85-kDa subunit. J. Biol. Chem. *268*, 9478–9483.

Case, R. D., Piccione, E., Wolf, G., Benett, A. M., Lechleider, R. J., Neel, B. G., and Shoelson, S. E. (1994). SH-PTP2/Syp SH2 domain binding specificity is defined by direct interactions with platelet-derived growth factor β–receptor, epidermal growth factor receptor, and insulin receptor substrate-1-derived phosphopeptides. J. Biol. Chem. *269*, 10467–10474.

Chen, J. K., Lane, W. S., Brauer, A. W., Tanaka, A., and Schreiber, S. L. (1993). Biased combinatorial libraries: novel ligands for the SH3 domain of phosphatidylinositol 3-kinase. J. Am. Chem. Soc. *115*, 12591–12592.

Cicchetti, P., Mayer, B. J., Thiel, G., and Baltimore, D. (1992). Identification of a protein that binds to the SH3 region of Abl and is similar to Bcr and GAP-rho. Science *257*, 803–806.

Cooper, J. A., and Kashishian, A. (1993). In vivo binding properties of SH2 domains from GTPase-activating protein and phosphatidylinositol 3-kinase. Mol. Cell. Biol. *13*, 1737–1745.

Darnell, J. E., Jr., Kerr, I. M., and Stark, G. R. (1994). Jak–STAT pathways and transcriptional activation in response to INFs and other extracellular signaling proteins. Science *264*, 1415–1421.

Dérijard, B., Hibi, M., Wu, I-H., Barrett, T., Su, B., Deng, T., Karin, M., and Davis, R. J. (1994). JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. Cell *76*, 1025–1037.

Downing, A. K., Driscoll, P. C., Gout, I., Salim, K., Zvelebil, M. J., and Waterfield, M. D. (1994). Three-dimentional solution structure of the pleckstrin homology domain from dynamin. Curr. Biol. *4*, 884–891.

Eck, M. J., Shoelson, S. E., and Harrison, S. C. (1993). Recognition of a high affinity phosphotyrosyl peptide by the Src homology 2 domain of p56lck. Nature *362*, 87–91.

Eck, M. J., Atwell, S. K., Shoelson, S. E., and Harrison, S. C. (1994). Structure of the regulatory domains of the Src-family tyrosine kinase Lck. Nature *368*, 764–769.

Felder, S., Zhou, M., Hu, P., Urena, J., Ullrich, A., Chaudhuri, M., White, M., Shoelson, S. E., and Schlessinger, J. (1993). SH2 domains exhibit high-affinity binding to tyrosine-phosphorylated peptides yet also exhibit rapid dissociation and exchange. Mol. Cell. Biol. *13*, 1449–1455.

Feller, S. M., Knudsen, B., and Hanafusa, H. (1994). c-Abl kinase regulates the protein binding activity of c-Crk. EMBO J. *13*, 2341–2351.

Feng, S., Chen, J. K., Yu, H., Simon, J. A., and Schreiber, S. L. (1994). A general model for SH3–ligand interactions involving dual binding modes. Science *266*, 1241–1247.

Ferguson, K. M., Lemmon, M. A., Schlessinger, J., and Sigler, P. B. (1994). Crystal structure at 2.2 Å resolution of the pleckstrin homology domain from human dynamin. Cell *79*, 199–209.

Finan, P., Shimizu, Y., Gout, I., Hsuan, J., Truong, O., Butcher, P., Bennett, P., Waterfield, M. D., and Kellie, S. (1994). An SH3 domain and proline-rich sequence mediate an interaction between two components of the phagocyte NADPH oxidase complex. J. Biol. Chem. *269*, 13752–13755.

Flynn, D. C., Leu, T. H., Reynolds, A. B., and Parsons, J. T. (1993). Identification and sequence analysis of cDNAs encoding a 110-kilodalton actin filament–associated pp60src substrate. Mol. Cell. Biol. *13*, 7892–7900.

Fumagalli, S., Totty, N. F., Hsuan, J. J., and Courtneidge, S. A. (1994). A target for Src in mitosis. Nature *368*, 871–874.

Fushman, D., Cahill, S., Lemmon, M. A., Schlessinger, J., and Cowburn, D. (1995). Solution structure of Pleckstrin homology domain of dynamin by heteronuclear NMR stectroscopy. Proc. Natl. Acad. Sci. *92*, in press.

Gibson, T. J., Hyvonen, M., Birney, E., Musacchio, A., and Saraste, M. (1994). PH domain—the first anniversary. Trends Biochem. Sci. *19*, 349–353.

Goudreau N., Cornille, F., Duchesne, M., Parker, F., Tocqué, B., Garbay, C., and Roques, B. P. (1994). NMR structure of the N-terminal SH3 domain of GRB2 and its complex with a proline-rich peptide from Sos. Nature Struct. Biol. *1*, 898–907.

Gout, I., Dhand, R., Hiles, I. D., Fry, M. J., Panayotou, G., Das, P., Truong, O., Totty, N. F., Hsuan, J., Booker, G. W., Campbell, I. D., and Waterfield, M. D. (1993). The GTPase dynamin binds to and is activated by a subset of SH3 domains. Cell *75*, 25–36.

Harlan, J. E., Hajduk, P. J., Yoon, H. S., and Fesik, S. W. (1994). Pleckstrin homology domains bind to phosphatidylinositol-4,5-bisphosphate. Nature *371*, 168–170.

Haslam, R., Kolde, H. B., and Hemmings, B. A. (1993). Pleckstrin domain homology. Nature *363*, 309–310.

Hu, P., and Schlessinger, J. (1994). Direct association of p110b phosphatidylinositol 3-kinase with p85 is mediated by an N-terminal fragment of p110b. Mol. Cell. Biol. *14*, 2577–2583.

Kazlauskas, A., Kashishian, A., Cooper, J. A., and Valius, M. (1992). GTPase-activating protein and phosphatidylinositol 3-kinase bind to distinct regions of the platelet-derived growth factor receptor β subunit. Mol. Cell. Biol. *12*, 2534–2544.

Klippel, A., Escobedo, J. A., Hirano, M., and Williams, L. T. (1994). The interaction of small domains between the subunits of phosphatidylinositol 3-kinase determines enzyme activity. Mol. Cell. Biol. *14*, 2675–2685.

Koch, C. A., Anderson, D., Moran, M. F., Ellis, C., and Pawson, T. (1991). SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. Science *252*, 668–674.

Kyriakis, J. M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E. A., Ahmad, M. F., Avruch, J., and Woodgett, J. R. (1994). The stress-activated protein kinase subfamily of c-Jun kinases. Nature *369*, 156–160.

Lechleider, R. J., Sugimoto, S., Bennett, A. M., Kashishian, A. S., Cooper, J. A., Shoelson, S. E., Walsh, C. T., and Neel, B. G. (1993). Activation of the SH2-containing phosphotyrosine phosphatase SH-PTP2 by its binding site, phosphotyrosine 1009, on the human platelet-derived growth factor receptor β. J. Biol. Chem. *268*, 21478–21481.

Lee, C., Kominos, D., Jacques, S., Margolis, B., Schlessinger, J., Shoelson, S. E., and Kuriyan, J. (1994). Crystal structures of peptide complexes of the amino-terminal SH2 domain of the Syp tyrosine phosphatase. Structure *2*, 423–438.

Lefkowitz, R. J. (1993). G protein–coupled receptor kinases. Cell *74*, 409–412.

Li, W., Nishimura, R., Kashishian, A., Batzer, A. G., Kim, W. J. H., Cooper, J. A., and Schlessinger, J. (1994). A new function for a phosphotyrosine phosphatase: linking Grb2–SOS to a receptor tyrosine kinase. Mol. Cell. Biol. *14*, 509–517.

Lim, W. A., Richards, F. M., and Fox, R. O. (1994). Structural determinants of peptide-binding orientation and of sequence specificity in SH3 domains. Nature *372*, 375–379.

Lisanti, M. P., Scherer, P. E., Tang, Z., and Sargiacomo, M. (1994). Caveolae, caveolin, and caveolin-rich membrane domains: a signalling hypothesis. Trends Cell Biol. *4*, 231–235.

Macias, M. J., Musacchio, A., Ponstingl, H., Nilges, M., Saraste, M., and Oschkinat, H. (1994). Structure of the pleckstrin homology domain from β-spectrin. Nature *368*, 675–677.

Marengere, L. E., Songyang, Z., Gish, G. D., Schaller, M. D., Parsons, J. T., Stern, M. J., Cantley, L. C., and Pawson, T. (1994). SH2 domain specificity and activity modified by a single residue. Nature *369*, 502–505.

Mayer, B., and Baltimore, D. (1993). Signalling through SH2 and SH3 domains. Trends Cell Biol. *3*, 8–13.

Mayer, B. J., Ren, R., Clark, K. L., and Baltimore, D. (1993). A putative modular domain present in diverse signaling proteins. Cell *73*, 629–630.

Miller, C. (1994). Potassium selectivity in proteins: oxygen cage or in the face? Science *261*, 1692–1693.

Mori, S., Rönnstrand, L., Yokote, K., Engström, A., Courtneidge, S. A., Claesson-Welsh, L., and Heldin, C. H. (1993). Identification of two juxtamembrane autophosphorylation sites in the PDGF β-receptor; involvement in the interaction with Src family tyrosine kinases. EMBO J. *12*, 2257–2264.

Muller, A. J., Pendergast, A.-M., Havlik, M. H., Puil, L., Pawson, T., and Witte, O. N. (1992). A limited set of SH2 domains binds BCR through a high-affinity phosphotyrosine-independent interaction. Mol. Cell. Biol. *12*, 5087–5093.

Musacchio, A., Gibson, T., Rice, P., Thompson, J., and Saraste, M. (1993). The PH domain: a common piece in the structural patchwork of signalling proteins. Trends Biochem. Sci. *18*, 343–348.

Musacchio, A., Saraste, M., and Wilmanns, M. (1994a). High-resolution crystal structures of tyrosine kinase SH3 domains complexed with proline-rich peptides. Nature Struct. Biol. *1*, 546–551.

Musacchio, A., Wilmanns, M., and Saraste, M. (1994b). Structure and function of the SH3 domain. Prog. Biophys. Mol. Biol. *61*, 283–297.

Myers, M. G. J., Sun, X. J., and White, M. F. (1994). The IRS-1 signaling system. Trends Biochem. Sci. *19*, 289–293.

Nishimura, R., Li, W., Kashishian, A., Mondino, A., Zhou, M., Cooper, J., and Schlessinger, J. (1993). Two signaling molecules share a phosphotyrosine-containing binding site in the platelet-derived growth factor receptor. Mol. Cell. Biol. *13*, 6889–6896.

Panayotou, G., Gish, G., End, P., Truong, O., Gout, I., Dhand, R., Fry, M. J., Hiles, I., Pawson, T., and Waterfield, M. D. (1993). Interactions between SH2 domains and tyrosine-phosphorylated platelet-derived growth factor beta-receptor sequences: analysis of kinetic parameters by a novel biosensor-based approach. Mol. Cell. Biol. *13*, 3567–3576.

Pascal, S. M., Singer, A. U., Gish, G., Yamazaki, T., Shoelson, S. E., Pawson, T., Kay, L. E., and Forman, K. J. (1994). Nuclear magnetic resonance structure of an SH2 domain of phospholipase C-γ1 complexed with a high affinity binding peptide. Cell *77*, 461–472.

Pawson, T., and Schlessinger, J. (1993). SH2 and SH3 domains. Curr. Biol. *3*, 434–442.

Piccione, E., Case, R. D., Domchek, S. M., Hu, P., Chaudhuri, M., Backer, J. M., Schlessinger, J., and Shoelson, S. E. (1993). Phosphatidylinositol 3-kinase p85 SH2 domain specificity defined by direct phosphopeptide/SH2 domain binding. Biochemistry *32*, 3197–3202.

Ren, R., Mayer, B. J., Cicchetti, P., and Baltimore, D. (1993). Identification of a 10-amino acid proline-rich SH3 binding site. Science *259*, 1157–1161.

Ren, R., Ye, Z., and Baltimore, D. (1994). Abl protein-tyrosine kinase selects the Crk adapter as a substrate using SH3-binding sites. Genes Dev. *8*, 783–795.

Rickles, R. J., Botfield, M. C., Weng, Z., Taylor, J. A., Green, O. M., Brugge, J. S., and Zoller, M. J. (1994). Identification of Src, Fyn, Lyn, PI3K, and Abl SH3 domain ligands using phage display libraries. EMBO J. *13*, 5598–5604.

Rotin, D., Bar-Sagi, D., O'Brodovich, H., Merilainen, J., Lehto, P. V., Canessa, C. M., Rossier, B. C., and Downey, G. P. (1994). A SH3 binding region in the epithelial $Na^+$ channel mediates its localization at the apical membrane. EMBO J. *13*, 4440–4450.

Rozakis-Adcock, M., Fernley, R., Wade, J., Pawson, T., and Bowtell, D. (1993). The SH2 and SH3 domains of mammalian Grb2 couple the EGF receptor to the Ras activator mSos1. Nature *363*, 83–85.

Saksela, K., Cheng, G., and Baltimore, D. (1995). Proline-rich (PxxP) motifs in HIV-1 Nef bind to SH3 domains of a subset of Src kinases and are required for the enhanced growth of $Nef^+$ viruses but not for downregulation of CD4. EMBO J. *14*, in press.

Seidel-Dugan, C., Meyer, B. E., Thomas, S. M., and Brugge, J. S. (1992). Effects of SH2 and SH3 deletions on the functional activities of wild-type and transforming variants of c-Src. Mol. Cell. Biol. *12*, 1835–1845.

Shoelson, S. E., Chatterjee, S., Chaudhuri, M., and White, M. F. (1992). YMXM motifs of IRS-1 define substrate specificity of the insulin receptor kinase. Proc. Natl. Acad. Sci. USA *89*, 2027–2031.

Shoelson, S. E., Sivaraja, M., Williams, K. P., Hu, P., Schlessinger, J., and Weiss, M. A. (1993). Specific phosphopeptide binding regulates a conformational change in the PI 3-kinase SH2 domain associated with enzyme activation. EMBO J. *12*, 795–802.

Songyang, Z., Shoelson, S. E., Chaudhuri, M., Gish, G., Pawson, T., Haser, W. G., King, F., Roberts, T., Ratnofsky, S., Lechleider, R. J., et al. (1993). SH2 domains recognize specific phosphopeptide sequences. Cell *72*, 767–778.

Songyang, Z., Shoelson, S. E., McGlade, J., Olivier, P., Pawson, T., Bustelo, X. R., Barbacid, M., Sabe, H., Hanafusa, H., Yi, T., et al. (1994). Specific motifs recognized by the SH2 domains of Csk, 3BP2, fps/fes, GRB-2, HCP, SHC, Syk, and Vav. Mol. Cell. Biol. *14*, 2777–2785.

Sugimoto, S., Wandless, T. J., Shoelson, S. E., Neel, B. G., and Walsh, C. T. (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.

Sumimoto, H., Kage, Y., Nunoi, H., Sasaki, H., Nose, T., Fukumaki, Y., Ohno, M., Minakami, S., and Takeshige, K. (1994). Role of Src homology 3 domains in assembly and activation of the phagocyte NADPH oxidase. Proc. Natl. Acad. Sci. USA *91*, 5345–5349.

Superti-Furga, G., Fumagalli, S., Koegl, M., Courtneidge, S. A., and Draetta, G. (1993). Csk inhibition of c-Src activity requires both the SH2 and SH3 domains of Src. EMBO J. *12*, 2625–2634.

Taylor, S. J., and Shalloway, D. (1994). An RNA-binding protein associated with Src through its SH2 and SH3 domains in mitosis. Nature *368*, 867–871.

Terasawa, H., Kohda, D., Hatanaka, H., Tsuchiya, S., Ogura, K., Nagata, K., Ishii, S., Mandiyan, V., Ullrich, A., Schlessinger, J., and Inagaki, F. (1994). Nature Struct. Biol. *1*, 891–897.

Valius, M., and Kazlauskas, A. (1993). Phospholipase C-γ1 and phosphatidylinositol 3 kinase are the downstream mediators of the PDGF receptor's mitogenic signal. Cell *73*, 321–334.

Waksman, G., Kominos, D., Robertson, S. C., Pant, N., Baltimore, D., Birge, R. B., Cowburn, D., Hanafusa, H., Mayer, B. J., Overduin, M., Resh, M. D., Rios, C. B., Silverman, L., and Kuriyan, J. (1992). Crystal structure of the phosphotyrosine recognition domain (SH2) of the v-*src* tyrosine kinase complexed with tyrosine phosphorylated peptides. Nature *358*, 646–653.

Waksman, G., Shoelson, S. E., Pant, N., Cowburn, D., and 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.

Ye, Z.-S., and Baltimore, D. (1995). Binding of Vav to Grb2 through dimerization of Src homology 3 domains. Proc. Natl. Acad. Sci. USA *92*, in press.

Yoon, H. S., Hajduk, P. J., Petros, A. M., Olejniczak, E. T., Meadows, R. P., and Fesik, S. W. (1994). Solution structure of a pleckstrin-homology domain. Nature *369*, 672–675.

Yu, H., Chen, J. K., Feng, S., Dalgarno, D. C., Brauer, A. W., and Schreiber, S. L. (1994). Structural basis for the binding of proline-rich peptides to SH3 domains. Cell *76*, 933–945.