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TRANSMEMBRANE SIGNALING PROTOCOLS

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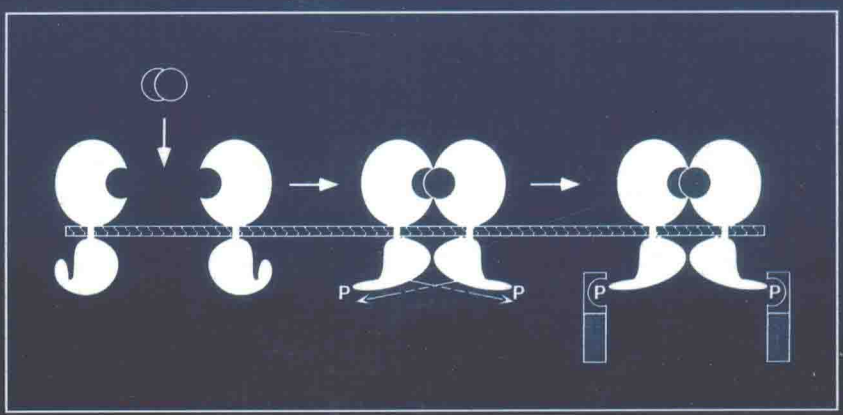

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I

OVERVIEWS

1

Peptide Recognition Mechanisms of Eukaryotic Signaling Modules

Chi-Hon Lee, David Cowburn, and John Kuriyan

1. Introduction

The formation of specific protein–protein interactions is one of the key mechanisms for signal transduction mediated by tyrosine phosphorylation. These intermolecular interactions target signaling proteins to particular cellular locations and modulate the enzymatic activities that further propagate the signal. A distinctive characteristic of the pathways that are initiated by tyrosine phosphorylation is that target recognition and catalytic activity are usually functions of separate domains within the signaling molecules that participate in these pathways. Each of the signaling molecules contains one or more of a set of modular peptide-binding domains that are responsible for generating protein–protein interactions. Such peptide-recognition domains are modular in both structural and functional respects: They are capable of folding correctly when removed from the parent protein, and they can usually recognize their targets even when isolated.

The first peptide-recognition modules to be identified were the Src homology 2 and 3 domains (SH2 and SH3 domains), so named because they share sequence similarity with two separate noncatalytic regions of the Src family tyrosine kinases *(1,2)*. SH2 and SH3 domains are now well-known for their critical roles in eukaryotic signal transduction, and they function by recognizing sites that contain phosphotyrosyl residues (for SH2) and proline-rich sequences (for SH3) (reviewed in **refs. *3–5***).

Several other peptide-binding domains have been discovered recently, and the determination of their three-dimensional structures have provided some surprises. The phosphotyrosine binding/phosphotyrosine interaction (PTB/PI)

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domain binds to phosphopeptides containing NPXY* motifs (Y*, phosphotyrosine) *(6,7)*. The architecture and mode of peptide recognition of the PTB domains is unrelated to that of the SH2 domains, although both recognize phosphotyrosine. Most strikingly, the architecture and topology of the PTB domains resemble closely that of another signaling module, the plekstrin homology (PH) domain, although there is no sequence similarity between these domains *(8–10)*. Furthermore, the newly discovered PDZ domains, which recognize non-phosphorylated peptide sequences at the carboxyl-terminus of ion-channel proteins, have a core topology and peptide-binding mechanism with elements in common with the PTB domains *(11)*. The WW domains, whose structure has been determined recently, represent an alternative mode of recognizing proline-containing motifs when compared to the well-known SH3 domains *(12)*. Again, the SH3 and WW domains are unrelated in sequence or structure.

In this chapter, we focus on the structural aspects of these peptide-binding domains, with emphasis on the sequence-specific recognition of targets. Much of the discussion is focused on the SH2 and SH3 domains, because more is known about them. The PTB and PDZ domains are discussed briefly in the context of their structural resemblance to PH domains. Newly characterized domains, such as the WW domain and the 14-3-3 protein, are not discussed.

2. SH2 Domains

The SH2 domain was first recognized as a phosphotyrosine-binding module during studies of the mechanisms of viral oncogenes that interfere with cellular signaling *(1,13,14)*. Subsequent experiments demonstrated that an individual SH2 domain binds to specific regions of tyrosine-phosphorylated proteins, such as particular sequences in the cytoplasmic regions of activated receptor tyrosine kinases (reviewed in **ref. *15***). The first three-dimensional structures of SH2 domains confirmed that the module corresponds to a well-folded domain with a defined peptide-binding surface *(16–18)*. In addition, the crystal structure of the Src tyrosine kinase SH2 domain complexed with low-affinity phosphotyrosyl peptides revealed the mechanism of phosphotyrosine recognition that has subsequently been found to be conserved in general terms among all SH2 domains of known structure *(18)*.

Comparisons of SH2-target sequences in tyrosine-phosphorylated proteins such as platelet-derived growth-factor (PDGF) receptor and the polyoma-virus middle-T antigen indicated that residues immediately surrounding the phosphotyrosine determine the binding specificity of SH2 domains *(19–22)*. However, a general picture of SH2-target specificity did not emerge until an exhaustive investigation was carried out using a peptide library approach

(23,24). This established that the three residues immediately C-terminal to the phosphotyrosine are the key determinants of specificity. The determination of the structures of high-affinity peptide complexes of Src and the closely related Lck-SH2 domains provided the first view of sequence-specific peptide recognition *(25,26)*. By combining the structural information with selectivity data from the peptide-library study, the sequence preference can be correlated with particular residues in the SH2 domain *(23,27)*. Subsequently, the structures of peptide complexes of the SH2 domains of the tyrosine phosphatase SH-PTP2 *(28)*, phospholipase C-γ *(29)* and the adapter proteins GRB2 *(30)* and Shc *(31)* have further clarified the mechanism of peptide recognition and have extended our understanding of SH2 specificity.

An additional level of complexity was added when the biochemical and structural analysis extended toward larger components of signaling molecules, containing more than one domain. Structures of the adapter-protein GRB2 *(32)* and the regulatory unit of Abl tyrosine kinase *(33)* have provided insights into spatial arrangements of multiple domains. Furthermore, structural analysis of multi-domain constructs of ZAP-70 *(34)*, Lck tyrosine kinase *(35)*, and the tyrosine phosphatase SH-PTP2 *(36)* revealed the cooperative recognition of peptides by larger-signaling molecules of which these domains are component parts.

2.1. General Architecture

The SH2 domain is a compact α-/β-structure comprised of around 100 residues (*see* **Fig. 1** for a sequence alignment). The central scaffold is an antiparallel β-sheet formed by strands A, B, C, D, and G. Two α-helices, αA and αB, flank the central β-sheet (*see* **Fig. 2** for a schematic diagram and the notation used). This β-sheet runs perpendicular to the peptide-binding surface, and divides the domain into two functionally distinct regions. One region, comprising helix αA, loop BC (the phosphate-binding loop), and the adjacent face of the central β-sheet, provides residues that interact with the phosphotyrosine. The other region includes helix αB, loops EF, BG, and the other face of the central β-sheet, and interacts with peptide residues immediately following the phosphotyrosine; this region accounts for the sequence-specific recognition.

The peptide ligand lies across the surface of the domain approximately orthogonal to the central β-sheet (**Fig. 2**). The peptide ligands are usually in an extended conformation and do not participate in secondary-structure formation with the domain. The phosphotyrosine residue appears to be the main anchor point of the SH2-peptide complex, allowing the domain to read out the three to six residues immediately following the phosphotyrosine. The peptide residues N-terminal to the phosphotyrosine make limited and nonspecific inter-

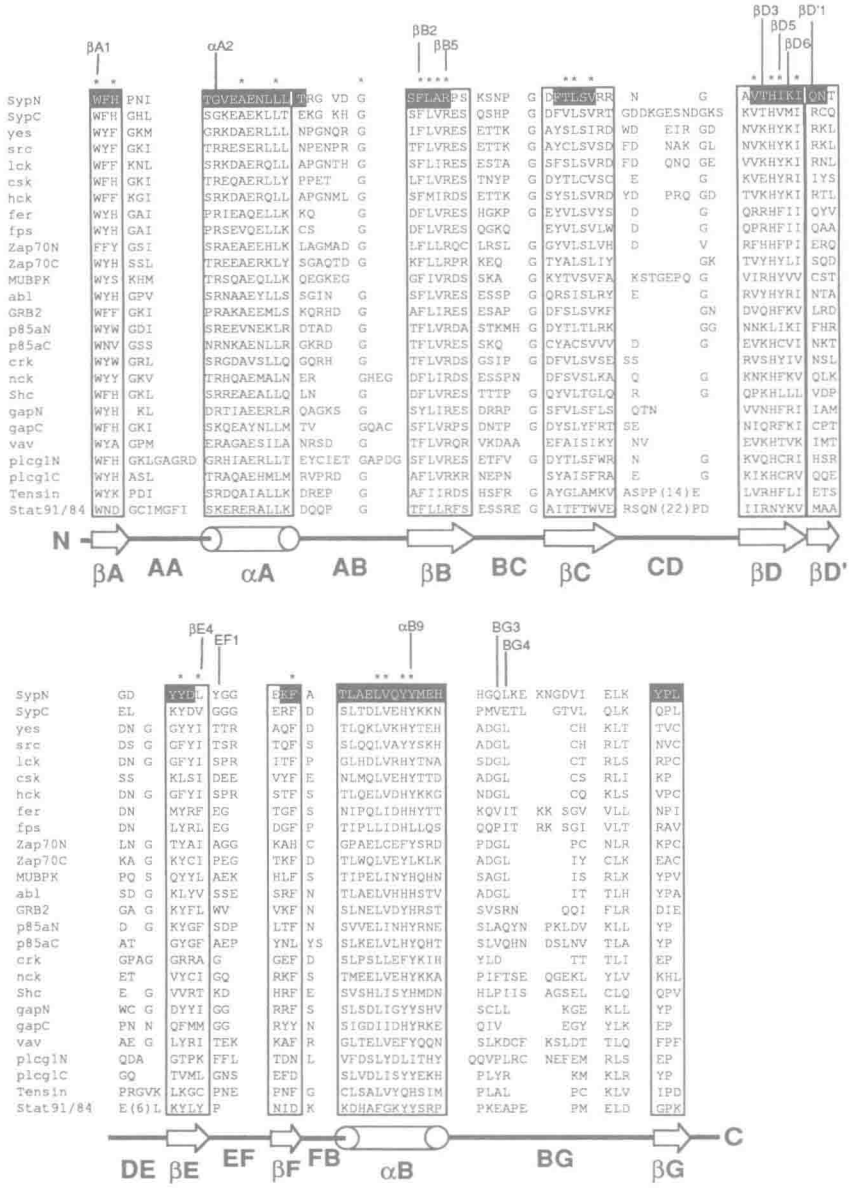

Fig. 1. Alignment of SH2 sequences and definition of the residue notation. The sequences of different SH2 domains are aligned, based on the secondary-structure definitions of Src and Lck *(26)*. The boundaries of the secondary structural elements of Src are shown by solid boxes, and the notation for these elements is shown schematically at the bottom. The important residues are indicated by vertical lines at the top. (Adapted with permission from **ref. *28***.)

actions with the domain, and therefore most likely contribute little to the binding specificity. The N- and C-termini of the SH2 domain are located on the side of the domain opposite to the peptide-binding surface. For this reason, the domain can be readily inserted into different molecular contexts without affecting the peptide-binding ability.

2.2. Peptide-Binding Specificity and Affinity

Several lines of evidence indicate that different SH2 domains bind to distinct phosphotyrosine containing sites of their target proteins in vivo, and that the linear sequence surrounding the specific phosphotyrosine determines the binding specificity *(19–22)*. To illustrate, a point mutation (Tyr 739 to Phe) in the PDGF receptor selectively eliminates the binding of the Ras GTPase activating protein (GAP) to the activated receptors, but the binding of other SH2-containing proteins (such as PLC-γ and PI-3 kinase) remains intact *(37)*. It appears that the local sequence, rather than the tertiary structure, of the SH2-targets dominates the binding specificity. Tyrosine-phosphorylated peptides that contain sequences resembling the local sequence of the target protein (the Tyr 739 of PDGF receptor in this case) compete efficiently for the binding of the target protein (PDGFR) to a particular SH2 domain (GAP) *(37)*. In addition, the observation that a mutant PDGF receptor containing a deletion near the GAP-SH2 binding site binds to the GAP-SH2 domain with nearly the same affinity as the wild-type PDGF receptor suggested that the tertiary structure is not a primary factor in determining binding affinity *(38)*. These observations establish the relevance of studies using isolated peptides.

A systematic search for optimal peptide sequences for SH2 domains had been carried out by screening a random phosphopeptide library *(23,24)*. Of over 20 different SH2 domains tested, each showed distinct selectivity in the three residues immediately C-terminal to phosphotyrosine in the peptide ligand. Such sequence preference could be correlated with the side-chains of residues at several critical positions of the SH2 domain *(24)*. The clearest example of this correlation is provided for the residue at the βD5 position of the SH2 domain, which contacts the peptide side chains at position +1 and +3. Certain SH2 domains, including Src-family tyrosine kinases as well as GAP and the adapter proteins GRB2 and Nck, have aromatic residues at βD5, and preferentially bind to peptides containing polar side chains at +1. In contrast, other SH2 domains (p85, phospholipase C-γ, the tyrosine phosphatases) contain hydrophobic side chains at βD5, and select for hydrophobic residues at +1.

Quantitative analysis using isothermal-titration calorimetry and surface-plasma resonance *(39)* indicated that the SH2-peptide interaction is of only moderate strength (K_d ~0.1–3.0 μ*M*) compared with strong interactions

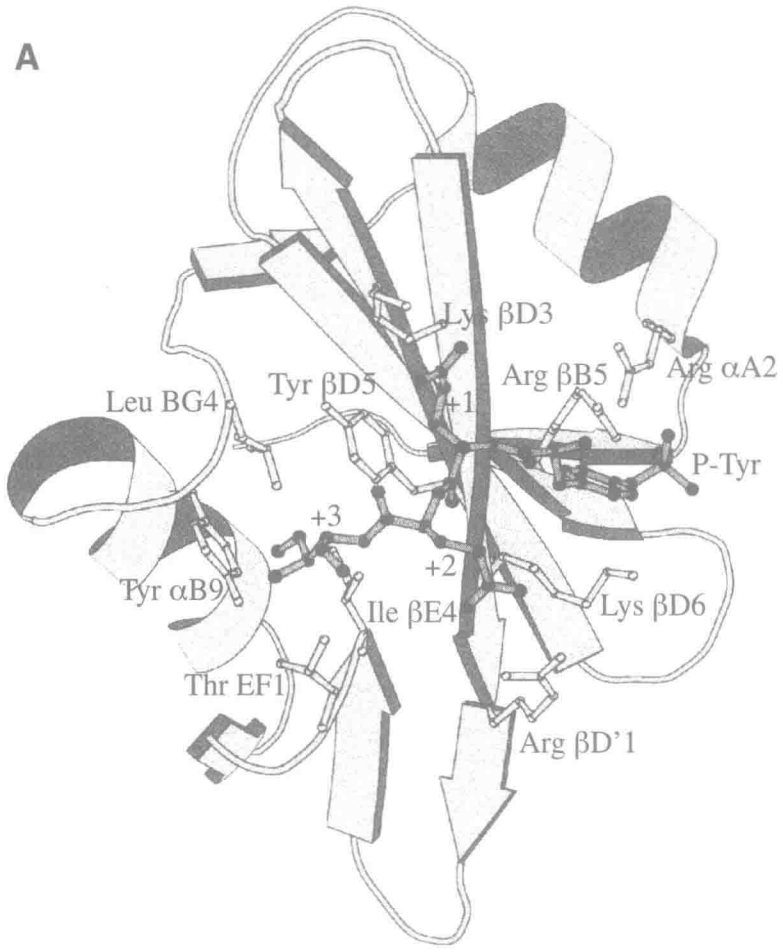

Fig. 2. (*see also facing page*) Schematic diagram of two SH2-peptide complexes (**A**) The Src-YEEI complex and (**B**) the N-terminal SH2 domain of SH-PTP2 complexed with a peptide derived from Tyr 895 of IRS-1. The view is from the peptide-binding surface and illustrates the secondary-structure elements and the notation used. The peptide is shown in a ball-and-stick representation and comprises phosphotyrosine (p-Tyr), residue +1, residue +2, and so on. α-helices and β-strands are shown as ribbons and arrows, respectively.

such as those between transcription factors and their specific DNA targets (K_d <1 n*M*). The phosphotyrosine is absolutely required for binding to SH2 domains ***(40)***.

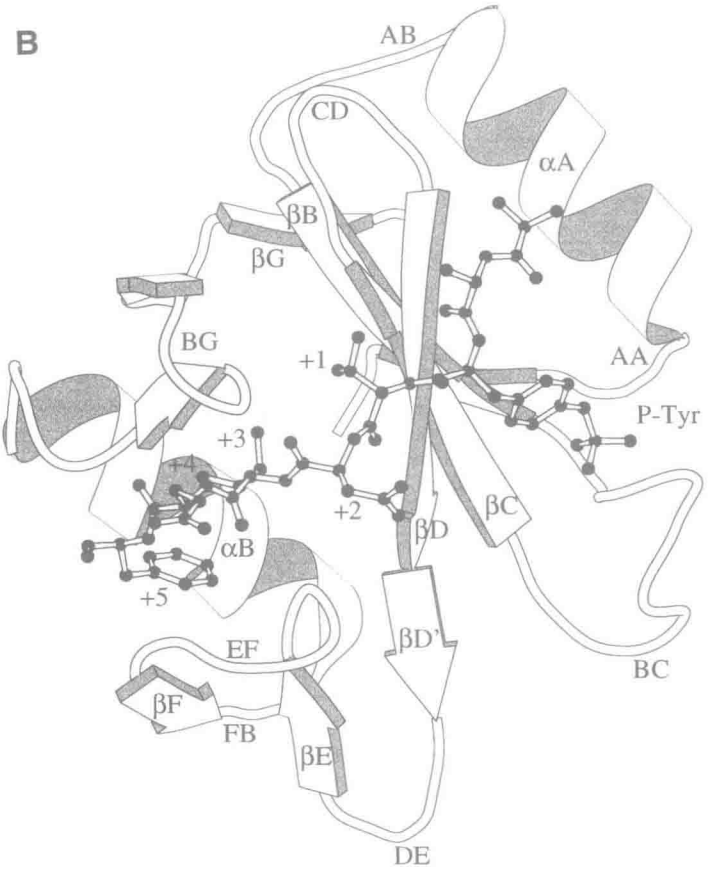

Fig. 2.

Peptide residues immediately following the phosphotyrosine (+1 to +6) are the critical determinants for binding to individual SH2 domains; however, a varying range of amino acids are tolerated at each site. Although the selectivity of individual SH2 domains is not sharply defined, the specificity and affinity can increase dramatically when cooperative binding interactions occur (*see* discussion **Subheading 5.1.** for tandem SH2 domains of ZAP-70). Kinetic analysis of SH2-peptide interaction has shown that the association and dissociation rates (k_{on} and k_{off}) are both very rapid even for high-affinity peptide ligands ***(41)***. Fast turnover rates could allow the rapid sampling of different binding sites and are observed for many protein–protein interactions involved in signal transduction.

2.3. Recognition of Phosphotyrosine

The recognition of phosphotyrosine is the defining feature of the SH2-peptide interface. Although the details vary slightly from one SH2 complex to another, the overall features of the interaction are strikingly conserved. Residues from αA, βB, βD, and the BC loop form the phosphotyrosine-binding pocket and provide hydrophobic interactions with the phenolic ring of phosphotyrosine and hydrogen-bonding interactions with the phosphate group (**Figs. 3A,B**).

The most critical interaction with the phosphotyrosine is provided by Arg βB5, which forms a bidentate-ionic interaction with the phosphate group. This arginine is located at the bottom of the binding pocket and becomes completely inaccessible to solvent upon binding. Arg βB5 is strictly conserved in all SH2 domains, and even the conservative mutation of this residue to lysine abolishes binding *(42)*. With the backbone of the phosphotyrosine residue held in position by the outer strand of the central β-sheet (βD), the ionic interaction between the phosphate group and Arg βB5 provides a stereochemical "ruler" that appears to be the key for discriminating between phosphotyrosine and other residues. The location of Arg βB5 is such that, in a fully extended conformation, this side chain is just long enough to interact with the phosphate group of a fully extended phosphotyrosine side chain, thus excluding phosphoserine or phosphothreonine.

An interesting feature often observed in the SH2-peptide complexes is the presence of an amino-aromatic interaction between an amino nitrogen of Arg αA2 and the phosphotyrosine ring *(18)*. Amino-aromatic interactions have been observed in a number of protein structures as well as in some small molecules structures *(43)*. The imino nitrogen of this arginine hydrogen bonds with the phosphate group and the backbone-carbonyl group of the peptide. These interactions mediated by Arg αA2 appears to be optimal for phosphotyrosine and were first identified in the Src *(18)* and Lck structures *(26)* and later in other SH2-peptide structures, including ZAP-70 *(34)*. However, the SH2 domains of the tyrosine phosphatases do not have Arg αA2 (it is replaced by glycine). In the SH2 structure of the phosphatase SH-PTP2 *(28)*, the phosphate group rotates by ~180°, facing toward the BC loop (**Fig. 3B**), and the number of hydrogen bond with the phosphate group is almost the same as in Src or Lck. The amino-aromatic interaction is also not seen in other SH2 structures (such as p85 **ref.** *44*) even when Arg αA2 is present.

2.4. Peptide Recognition

The structures of the closely-related Src and Lck SH2 domains *(25,26)* in complex with a high-affinity peptide containing the Tyr-Glu-Glu-Ile (YEEI) motif provided the first piece of structural information on sequence-specific

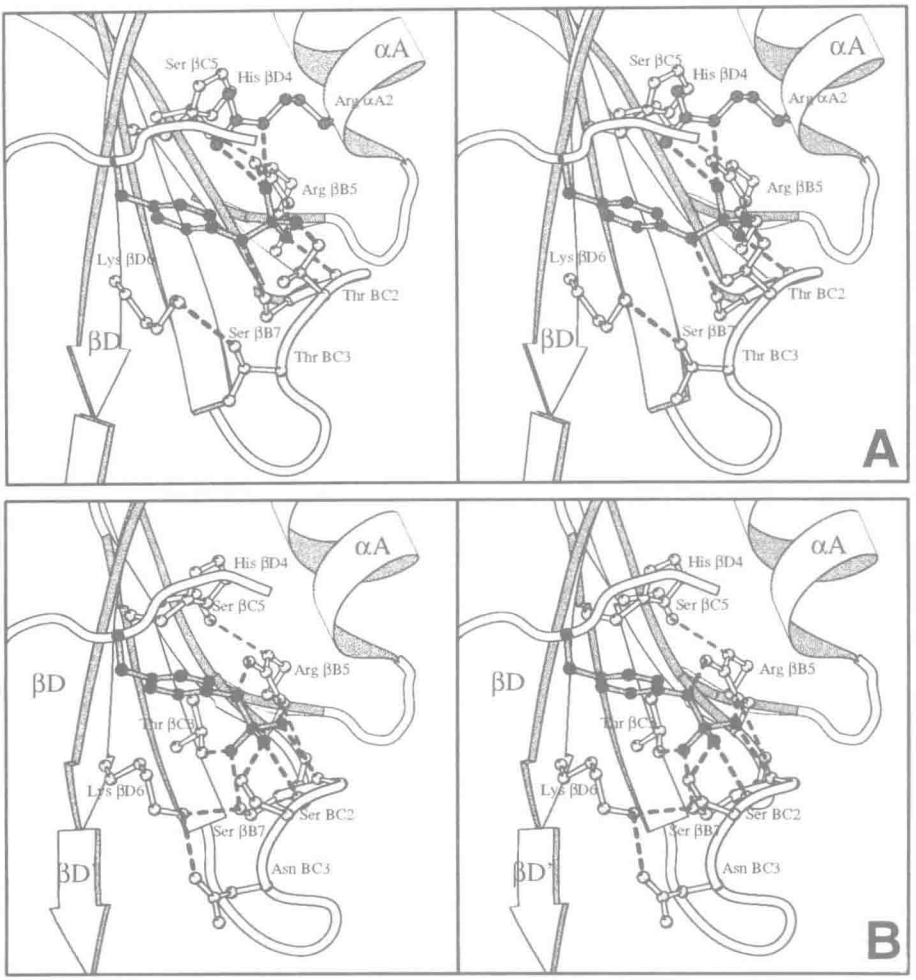

Fig. 3. Stereoviews of the phosphotyrosine binding sites of (**A**) Src and (**B**) N-terminal SH2 domains of SH-PTP2. The polypeptide backbone of the peptide is shown as a tube and the phosphotyrosine side chain is shown in black. Hydrogen bonds are indicated by dashed lines. (Adapted with permission from **ref. *28***.)

recognition by the SH2 domain (**Fig. 3A**). In these structures, the peptide binds to the SH2 domain in an extended conformation and the interaction resembles a two-pronged plug (the peptide) engaging a two-holed socket (the SH2 domain). The two prongs refer to the phosphotyrosine and the Ile +3 residue of the peptide, which fit into the corresponding pockets on the SH2 surface. This type of interaction is also observed in several other SH2-peptide complexes.

2.4.1. Type 1: Src and Lck

Previous studies using random-peptide libraries indicated that the Src-family SH2 domains strongly select large hydrophobic residues at +3 and, to a lesser extent, prefer nonbasic polar residues at +1 and +2, with the optimal motif being YEEI (two glutamates and one isoleucine following the phosphotyrosine) *(24)*. A peptide containing this optimal motif, derived from the hamster polyomavirus middle-T antigen, binds to Src-family SH2 domains with high affinity and has been used for the structural studies.

The most important feature in the Src and Lck structures is that the Ile +3 residue of the YEEI peptide engages a well-defined hydrophobic pocket of the SH2 domain. This interaction is responsible for the selection of large hydrophobic residues at +3 position. The residues lining this pocket (which arise from βD, βE, loops EF and BG) are rather divergent. In particular, the two variable loops, BG and EF, shape the surface topography of this pocket. Mutations as well as large insertions/deletions are often found in this region, and these have been shown to be important for binding specificity. The glutamate residues at +1 and +2 do not form extensive interactions with the SH2 domain, but are in the vicinity of basic residues that may account for the moderate selectivity against basic residues at these positions in the peptides (**Fig. 2A**).

The prototypical two-pronged interaction is also observed in two X-ray structures of the N-terminal SH2 domain of the p85 subunit of PI-3 kinase (p85N), complexed separately with two high-affinity peptides containing the optimal Y-M/V-X-M motif *(44)*. Although in p85N SH2 the position of Met +3 shifts slightly toward the central β-sheet, the interaction between this residue and the hydrophobic pocket is similar to that seen in Src and Lck. This resemblance is expected, because both SH2 domains favor large hydrophobic residues at this position (although p85N shows a higher preference for Met). A unique feature of the p85N SH2 domain is that this hydrophobic pocket is blocked by the side chain of Tyr BG5 in the absence of ligand, and this side chain has to move by 8 Å to open up the pocket for ligand binding. The large movement of the Tyr side chain might account for the changes in circular dichroism and fluorescence spectra that had been noted upon peptide binding, because no other large-conformational change induced by peptide binding was found.

A notable difference in the binding specificities of p85N and Src is that, at the +1 position, p85N SH2 prefers hydrophobic residues, whereas Src SH2 favors nonbasic polar residues. Ile βD5 (in p85N) appears to be the major determinant for this difference, since replacement of this residue by Tyr (found in Src) shifts the selectivity toward that of the Src-family SH2 domains *(27)*. In the p85N structure, the less bulky side chain of Ile βD5 opens up a shallow-

hydrophobic pocket for housing a hydrophobic residue such as Met or Val at the +1 position. Likewise, the SH-PTP2 and PLC-γ SH2 domains (which have Ile and Cys at βD5, respectively) also prefer a hydrophobic residue at the +1 position. In contrast, the bulky side chain of Tyr βD5 closes up this hydrophobic pocket in Src and Lck.

2.4.2. Type 2: SH-PTP2 and PLC-γ

The crystal structures of the N-terminal SH2 domains of the SH-PTP2 tyrosine phosphatase have been determined in separate complexes with two high-affinity peptides. A distinctive feature in these structures is that five residues following the phosphotyrosine of the peptide run through a hydrophobic groove on the SH2 domain *(28)*. Mutagenesis studies confirmed the strong selectivity for hydrophobic residues at the +5 position *(28)*; truncating the peptide or replacing the residue at +5 with a hydrophilic residue completely abolishes its interaction with the SH-PTP2 SH2 domain *(45)*. This selectivity was unexpected because this residue had not been randomized in the peptide-library study, and consequently this enhanced selectivity had not been predicted.

The differences in surface topography for the SH2 domains of Src and SH-PTP2 arise from the opening of pockets for housing peptide residues +1 and +5. The presence of a shallow-hydrophobic pocket for +1 in SH-PTP2 is primarily owing to the less bulky residue (Ile) at the βB5 position. The +5 binding site is flanked by the two variable loops, EF and BG, and these are opened up relative to their positions in Src and Lck. Such significant changes could perhaps have been anticipated by examining the primary sequences of the SH2 domains, but the precise structural details would be difficult to model without information from crystallography or nuclear magnetic resonance (NMR).

The binding of peptide to the C-terminal SH2 domain of phospholipase C-γ1 (PLC-γ) also involves extended interactions in a surface groove *(29)*. The solution structure of this SH2 domain has been determined, revealing an interaction involving six peptide residues following the phosphotyrosine. Surprisingly, a binding study indicated that residues at the +2 to +6 positions contribute little binding energy, although they make extensive contacts with the PLC-γ-SH2 domain; truncation of the peptide to just the three residue DYI resulted in only a 15-fold reduction in binding affinity *(46)*. The discrepancy between the structural results and the binding data has been further investigated by examining the changes in the dynamic properties of the SH2 domain upon peptide binding *(46)*. This analysis demonstrates that the residues contacting the phosphotyrosine (which contribute to binding energy) undergo a significant restriction in dynamic flexibility upon binding, whereas the residues interacting with C-terminal end of the peptide (which contribute little