Journal of Cell Science, Supplement 18, 121-126 (1994)
Printed in Great Britain © The Company of Biologists Limited 1994

Specificity in recognition of phosphopeptides by src-homology 2 domains

Lewis C. Cantley[1,2] and Zhou Songyang[1,3]

[1]Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA
[2]Division of Signal Transduction, Beth Israel Hospital, Boston, MA 02115, USA
[3]Dept of Physiology, Tufts University School of Medicine, Boston, MA 02111, USA

SUMMARY

SH2 domains and SH3 domains, found in a number of protein-tyrosine kinases and substrates of protein-tyrosine kinases, provide specificity in downstream signaling. Both of these domains bind to relatively short linear sequences of peptides to provide specific interactions between proteins. The SH2 domains directly bind to phosphotyrosine residues of proteins in a specific sequence context. We have devised a phosphopeptide library technique that allows us to rapidly determine the sequence specificity of individual SH2 domains on the basis of amino acids selected at position +1, +2 and +3 C-terminal of the phosphotyrosine. The optimal motif for 22 distinct SH2 domains has been determined and used to predict likely sites of in vivo interaction. A second phosphopeptide library was devised in which the amino acids N-terminal of the phosphotyrosine were also varied. The residues N-terminal of phosphotyrosine had little influence on binding to the N-SH2 domain of the 85 kDa subunit of phosphoinositide 3-kinase. These results indicate that for this SH2 domain, specificity is determined by sequences carboxy-terminal of the phosphotyrosine moiety. Knowledge of the specificity of SH2 domains allows predictions about likely downstream targets on the basis of primary sequence of proteins. Some of these predictions will be discussed.

Key words: SH2 domain, SH3 domain, tyrosine kinase, phosphopeptide

INTRODUCTION

A breakthrough in understanding how protein-tyrosine kinases are regulated and specifically associate with downstream targets was provided by the discovery that SH2 and SH3 domains recognize specific short peptide sequences. These domains were originally pointed out by Tony Pawson as regions of homology conserved among cytosolic protein-tyrosine kinases that are not part of the kinase domain. As additional signaling proteins were found to contain SH2 and/or SH3 domains without protein kinase domains, it became clear that these domains have functions separate from the kinase domain. Later work from Pawson's laboratory and from Hanafusa's laboratory (Matsuda, 1990; Anderson, 1990; reviewed by Cantley et al., 1991) showed that SH2 domains mediate binding to heterologous proteins in a manner that depends on tyrosine phosphorylation of the target proteins. In fact, the binding could be blocked with phosphotyrosine indicating that the SH2 domain directly binds to the phosphotyrosine moiety of the proteins. In addition we noticed a short region of similarity between the sequence around a phosphotyrosine residue in polyoma middle t implicated in binding to phosphatidylinositol (PtdIns) 3-kinase (Whitman et al., 1985) and two tyrosine phosphorylation sites in the PDGF receptor implicated in binding this same enzyme (Kazlauskus and Cooper, 1989; Escobedo et al., 1991) that suggested a consensus site for binding of the PtdIns 3-kinase SH2 domains (phosphoTyr-Met/Val-Xxx-Met; Cantley et al., 1991). On the basis of these and other observations we suggested that SH2 domains from different signaling proteins bind phosphotyrosine in distinct sequence contexts such that the sequence immediately surrounding a site of tyrosine-phosphorylation dictates which SH2-containing protein will bind in vivo. This hypothesis has been supported by additional studies discussed below.

Like SH2 domains, SH3 domains recognize relatively short (10-12 amino acid) sequences of proteins or peptides. The abl SH3 domain was used to screen an expression library and cDNA clones of two proteins with regions rich in proline were isolated (Cicchetti et al., 1992). The proline-rich domains of the two proteins were shown to mediate the binding to the abl SH3 domain and a consensus sequence for binding to the abl SH3 domain was determined from studies with synthetic peptides (Ren et al., 1993). A number of other proteins with proline-rich regions have now been shown to bind to various SH3 domains. We discovered that in stimulated lymphocytes, the src-family members, $p60^{fyn}$ and $p56^{lck}$ utilize their SH3 domains to associate with PtdIns 3-kinase (Prasad et al., 1993; Kapeller et al., 1994). This association involves two proline-rich regions of the p85 regulatory subunit of PtdIns 3-kinase (Kapeller et al., 1994).

In this manuscript the results obtained using the phosphopeptide library to explore SH2 domains are discussed.

MATERIALS AND METHODS

Construction of peptide libraries

The phosphopeptide library with degeneracy carboxy-terminal of the phosphotyrosine was constructed as described by Songyang et al.

(1993). The sequence of the library mixture is GlyAspGly(phospho)TyrXxxXxxXxxSerProLeuLeuLeu where Xxx indicates all amino acids but Cys or Trp.

A second library with degeneracy amino-terminal of the phospho-Try was also made. This library has the sequence GlyAlaXxxXxxXxx(phospho)TyrXxxXxxXxxAlaLysLysLys where Xxx indicates all amino acids but Cys or Trp. The degeneracy of this library is 34,012,224.

GST-SH2 domains and affinity purification of peptides

cDNA clones of the various SH2 domains were expressed as glutathione S-transferase fusion proteins by expression in pGEX as described by Songyang et al. (1993). The GST-SH2 domain was harvested on glutathione beads and packed in a 1 ml syringe. The beads were washed with 1 ml PBS (150 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.2). The peptide mixture (~0.3 mg) was added to the column and allowed to stand for 10 minutes. The column was then quickly washed twice with 1 ml ice-cold PBS (containing 10 mg/ml blue dextran and 0.5% NP-40) and once with 1 ml ice-cold PBS (without blue dextran or detergent) using a plunger to force the solution through. Elution of the specifically associated peptides was accomplished with 200 μ l of 20 mM sodium phenylphosphate solution (pH 7.8). The solution was collected, concentrated by evaporation, and sequenced on the Applied Biosystems 477A protein sequencer.

RESULTS

We wished to test the hypothesis that SH2 domains provide specificity for downstream signaling by protein-tyrosine kinases because of their ability to distinguish between tyrosine-phosphorylated sites on the basis of the sequence immediately surrounding the phosphotyrosine. We had shown that relatively short phosphopeptides (as short as 6 amino acids) containing the motif phosphoTyr-Met-Pro-Met (based on Y315 of polyoma middle t) can bind to the N- or C-terminal SH2 domains of the p85 subunit of PtdIns 3-kinase in competition assays with K_i values of 5-20 nM (Auger et al., 1992; Carpenter et al., 1993). Scrambled phosphotyrosine-containing peptides bound orders of magnitude weaker. These results were consistent with studies done by Lewis Williams' laboratory using peptides based on sequences in the PDGF receptor (Fantl et al., 1992; Escobedo et al., 1991).

Despite these findings it was still not clear that phosphoTyr-Met/Val-Xxx-Met was the optimal sequence for binding to these SH2 domains since only a few amino acid replacements at the +1 and +3 positions were tested. In addition, no consensus motifs for the other ~30 SH2 domains known to exist had been determined.

In order to determine whether phosphoTyr-Met/Val-Xxx-Met is truly the optimal sequence for binding to the SH2 domains of p85, we constructed a partially degenerate phosphopeptide library. Every peptide in this library began with the sequence Gly-Asp-Gly-phosphoTyr-. The next three residues following the phosphotyrosine were degenerate such that all amino acids except Cys or Trp (left out because of problems in sequencing/disulfide bond formation) were present. In all peptides the C-terminal sequence was Ser-Pro-Leu-Leu-Leu. The Ser-Pro motif was added because it was detected downstream of many potential tyrosine-phosphorylation sites in proteins. The poly-Leu tail prevented wash-out during sequencing. The procedure for synthesis of this library and the rational are discussed in detail in the Experimental Procedures of Songyang et al. (1993). The degeneracy of the library was $18^3 = 5832$ distinct peptides.

The N-terminal SH2 domain of p85 was expressed as a glutathione S-transferase fusion protein in bacteria, purified on glutathione beads and used to construct an affinity column. Washing and elution conditions were optimized using a radiolabeled phosphopeptide (^{125}I-labeled Arg-Glu-Asn-Glu-phosphoTyr-Met-Pro-Met-Ala-Pro-Gln-Ile-His) known to bind with high affinity to this SH2 domain. The column was then used to specifically retain peptides from the degenerate mixture that bound with highest affinity to the SH2 domain. (Phenylphosphate was used to elute the specifically-bound peptides.) Rather than attempt to separate distinct peptides in the mixture of affinity-purified peptides, we sequenced the complete mixture. Since in every case the phosphotyrosine is the 4th residue in the peptide, the relative abundance of the various amino acids detected at cycle 5 is an indication of the amino acid preference of this SH2 domain at the +1 site C-terminal of the phosphoTyr moiety. (The slight variation in relative abundance of the 18 amino acids at this cycle in the starting mixture was divided out). In this way we were able to show that the N-SH2 domain of p85 prefers Met, Val, Ile or Glu (in that order) at the +1 site and strongly prefers Met at the +3 site (Songyang et al., 1993). Little specificity was detected at the +2 site. These results were reassuring that the technique works since they agreed with the consensus sequence arrived at by comparing in vivo sites for binding of PtdIns 3-kinase (Cantley et al., 1991) and competition experiments done using synthetic phosphopeptides (Fantl et al., 1992; Carpenter et al., 1993).

We went on to use this library to determine the optimal motif for 21 additional SH2 domains (Songyang et al., 1993, 1994). We found that SH2 domains from src-family members all recognized a common motif (phosphoTyr-Glu-Glu-Ile) while each SH2 domain outside the src-family had a unique optimal motif. We synthesized a phosphopeptide based on this motif and showed that it has a very high affinity for the src SH2 domain. High affinity (10-50 nM K_d) was also demonstrated for the lck SH2 domain (Payne et al., 1993). Stephen Harrison's laboratory and John Kuriyan's laboratory used phosphopeptides based on this motif to form co-crystals with the lck (Eck et al., 1993) and src (Waksman et al., 1993) SH2 domains with the phosphoTyr-Glu-Glu-Ile peptides bound. The crystal structures revealed the regions of the SH2 domains that made contact with the side chains of the +1, +2 and +3 residues. These residues are summarized in Table 1. The amino acids selected at these positions could be rationalized based on the structure of the complex. Consistent with our finding that all src-family members bind the same optimal motif, the residues that made contact with the side chains were conserved in this family. In contrast, all other SH2 domains having one or more of the residues predicted to make contact with the side chains varied from the src structure. Thus, these results provide a clear structural explanation for the sequence specificity of SH2 domains. We also noted that the SH2 domains thus far known fall into 4 distinct groups on the basis of the residues at the peptide-binding sites (Songyang et al., 1993, 1994). General predictions about the likely motifs of SH2 domains can be made on the basis of which group they fall into.

This phosphopeptide library technique is extremely rapid.

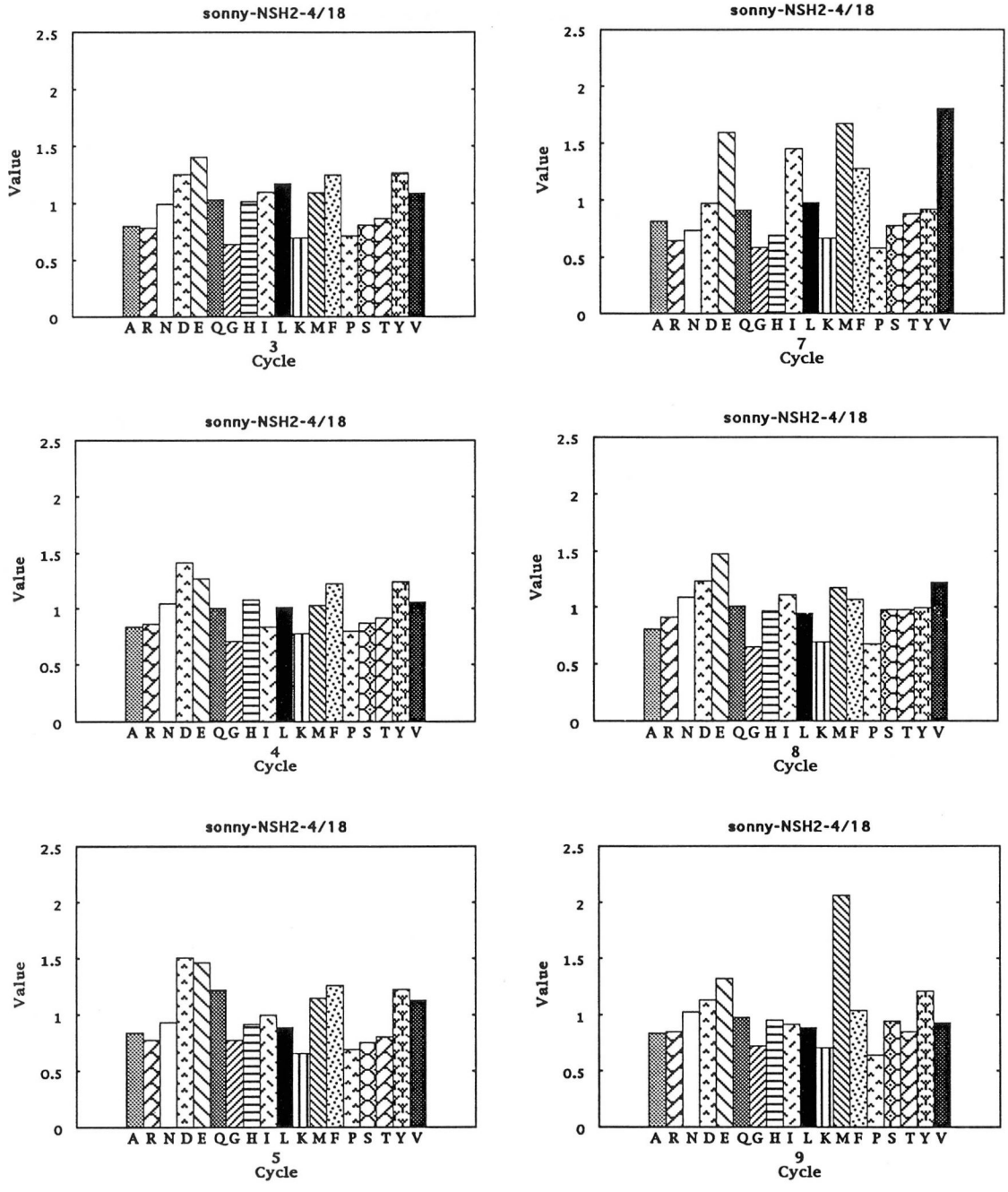

Fig. 1. The selectivity of the N-SH2 domain of p85 for amino acids at position, N-terminal and C-terminal of the phosphoTyr moiety of associated peptides. The GST-N-SH2 domain of p85 was used to affinity-purify peptides from a library with the sequence GAXXXpYXXXAKKK. The affinity-purified peptide mixture was sequenced and the data plotted as described by Songyang et al. (1993). The histogram in the top left corner indicates the relative amino acid abundance in the 3rd cycle of the sequence (the first degenerate position of the library) and the histogram under it represents the 4th cycle and etc. Cycle 6 (not shown) is phosphoTyr. Notice that significant selectivity is only observed at cycle 7 (the +1 position) and cycle 9 (the +3 position). Amino acids are indicated by single letter codes.

preference for Asp residues at the −1 and −2 positions but the major selection was still for Met/Val/Ile/Glu at +1 and for Met at +3 (Fig. 1). In this library, the sequences N-terminal of the phosphotyrosine and C-terminal from the region of degeneracy are different from those in the original library yet the same motif was selected, indicating that for the p85 N-SH2 domain the motif selected is not significantly affected by the residues outside the phosphoTyr-Xxx-Xxx-Xxx region.

DISCUSSION

Using a peptide library technique we have investigated the specificity of SH2 domains for linear sequences of phospho-Tyr-containing peptides. Twenty two distinct SH2 domains have been investigated using a library that varies the residues at positions +1, +2 and +3 amino acids carboxy-terminal of the phosphoTyr (Table 1). These SH2 domains can be distributed

Table 1. Phosphopeptide motifs for SH2 domains: residues predicted to interact with the side chains of the associated phosphopeptides

		SRC-SH2			SRC-SH2		SRC-SH2					
SH2 domain	+1	200 βD3	202 βD5	+2	205 βD'1	+3	202 βD5	214 βE4	215 EF1	230 αB9	237 BG4	
SRC	E	K	Y	E	R	**I**	Y	I	T	Y	L	
FYN	**E**	K	Y	**E**	R	**I**	Y	I	T	Y	L	
LCK	**E**	K	Y	E	R	**I**	Y	I	S	Y	L	GROUP 1A
FGR	**E**	K	Y	**E**	R	IV	Y	I	T	Y	L	
LYN		K	Y		R		Y	I	S	Y	L	
YES		K	Y		R		Y	I	T	Y	L	
HCK		K	Y		R		Y	I	S	Y	L	
Dsrc		K	Y		K		Y	L	S	Y	L	
SYK N		H	Y		E		Y	I	S	H	L	GROUP 1B
SYK C	QTE	L	Y	eqt	D	**L**	Y	I	P	Y	L	
ZAP70 C		Y	Y		S		Y	I	P	L	L	
TEC		R	Y		K		Y	L	A	H	L	
ATK		R	Y		C		Y	L	A	H	L	
ITK		K	Y		K		Y	V	A	H	L	
ABL	E	Y	Y	**N**	N	P	Y	V	S	H	L	
ARG		Y	Y		N		Y	V	T	H	L	
CSK	**T**	E	Y	n	M	mr	Y	I	D	Y	?	
CRK	D	S	Y	H	N	**P**	Y	A	G	Y	T?	
NCK	**D**	K	F	**E**	Q	**P**	F	I	G	Y	T?	
fes/fps	**E**	R	F	-	Q	vi	F	R	L	L	G	
ZAP70 N		H	F		E		F	I	A	Y	L	
SEM5	LV	Q	F	**N**	L	vp	F	L	W	H	R?	
DGBR2	y	Q	F	**N**	L	-	F	L	W	H	R?	
GRB2	qy	Q	F	**N**	L	y	F	L	W	H	R?	
GAP C		Q	F		C		F	M	G	Y	I?	
GAP N		N	F		I		F	I	G	Y	L?	
Tensin		R	F		T		F	?	?	H	?	
3BP2	**E**	R	Y	**N**	F	-	Y	E	G	Y	P?	
VAV	**M**	K	T	E	I	P	T	I	T	Y	?	GROUP 2
p85aN	MIVE	K	I	-	F	**M**	I	F	S	Y	A?	GROUP 3
p85bN		K	I		F		I	F	S	Y	A?	
p85aC	mli	K	C	-	N	**M**	C	F	A	Y	V?	
p85bC		K	C		Y		C	F	A	Y	V?	
PLC g1C	VI	K	C	IL	N	**PIV**	C	L	G	Y	Y?	
PLC g2C		K	C		Q		C	L	G	Y	Y?	
PLC g1N	**LIV**	Q	C	Ed	H	LIV	C	K	F	Y	L?	
PLC g2N		Q	C		R		C	K	Y	Y	L?	
SHPTP1 N	F	T	I	-	Q	**F**	I	D	L	Y	V	
SHPTP2 N	IV	T	I	-	Q	VI	I	D	L	Y	L	
CSW N		T	I		Q		I	D	L	Y	L	
SHPTP1 C		T	I		M		I	T	V	F	E	
SHC	EI	K	L	-	V	**ILM**	L	T	K	H	P?	
ShB		M	M		A		M	L	G	Y	?	GROUP 4
SHPTP2 C		T	V		R		V	D	V	Y	E	
CSW C		T	V		R		V	D	V	Y	E	
113 TF		F	A		P		A	L?	S?	Y	?	
91 TF		I	S		P		S	L?	Q?	Y	?	

Columns +1, +2 and +3 comprise the 1st, 2nd and 3rd residues C-terminal to P-tyrosine of the optimal phosphopeptide selected by each SH2 domain (e.g. P-YEEI for src SH2). SRC-SH2 200 and 202 indicate the residues of src (and residues at analogous positions of other SH2 domains) predicted to contact the +1 residue side chain of the associate peptide. SRC-SH2 205 is predicted to be near the +2 side chain and SRC 202, 214, 215 and 237 are predicted to form a hydrophobic pocket to bind the +3 residue side chain. The alignments were made on the basis of work by Waksman et al. (1992).

Bold letters indicate strong selection. Upper case without bold indicates medium selection. Lower case indicates weak selection. A hyphen indicates no selection. Motifs not yet determined or not submitted for publication are left blank.

Once the SH2 domain is expressed in bacteria as a GST-fusion protein it only requires 6 hours from the time of bacterial lysis until the affinity-selected peptide mixture is added to the micro sequencer. Each SH2 domain is evaluated at least twice from two different bacterial lysates to ensure reproducibility.

We also tested whether some SH2 domains may also be sensitive to the amino acids N-terminal of the phosphoTyr. This does not appear to be the case for the SH2 domains of the p85 subunit of PtdIns 3-kinase. We constructed a library with the following sequence: Gly-Ala-Xxx-Xxx-Xxx-phosphoTyr-Xxx-Xxx-Xxx-Ala-Lys-Lys-Lys in which Xxx indicates all possible amino acids but Trp or Cys. The SH2 domains were used to affinity-purify optimal phosphopeptides from this library as before. For the p85 N-SH2 domain there was a slight

into 4 distinct groups on the basis of the amino acid at the βD5 position (Songyang et al., 1993). From the crystal structures of src and lck SH2 domains it is clear that this residue contacts the side chains of the amino acids +1 and +3 residues carboxy-terminal of the phosphoTyr (Eck et al., 1993; Waksman et al., 1993). In about half the known SH2 domains, this residue is either Tyr or Phe (Group I). Vav is the single known member of a group (Group II) in which βD5 is a Thr residue. Group III SH2 domains have a Cys or Ile at βD5 and the remaining SH2 domains have other residues at βD5.

The residue at βD5 is predictive of the type of phospho-peptide that will associate with the SH2 domain. For example, most Group I SH2 domains select peptides with the general motif phosphoTyr-Hydrophilic-Hydrophilic-Hydrophobic. In contrast most Group III SH2 domains select peptides with the sequence phosphoTyr-Hydrophobic-Xxx-Hydrophobic. Within these groups, the individual SH2 domains recognize specific sequences on the basis of diversity in the other residues that make up the +1, +2 and +3 binding sites. The possibility that additional contact is made between the SH2 domain and residues amino-terminal of the phosphoTyr-binding site was explored using the new library first described in this paper. The results with the N-SH2 domain of p85 indicate that this SH2 domain does not significantly interact with the side chains of residues amino-terminal of the phosphoTyr moiety. The fact that, using this library, a similar motif was observed to that observed with the original library in which only residues carboxy-terminal of phosphoTyr were varied (phosphoTyr-Val/Met/Ile/Glu-Xxx-Met; Fig 1 and Table 1) indicates that the procedure works. The results are in agreement with the crystal structures of Eck et al. (1993) and Waksman et al. (1993) in which the src and lck-SH2 domains had little contact with side chains of residues amino-terminal of the phosphoTyr moiety of the associated peptide.

Perhaps the most exciting outcome from determination of these motifs is the ability to predict likely sites in proteins for assembly of specific SH2 domain-containing proteins in vivo. For example, we correctly predicted the sites on the c-fms and c-kit receptor tyrosine kinases where PtdIns 3-kinase binds in vivo on the basis of the phosphoTyr-Met/Val-Xxx-Met motif (Cantley et al., 1991; Songyang et al., 1993). We also correctly predicted PtdIns 3-kinase-binding sites on proteins involved in B-cell and T-cell activation: CD19 (Tuveson et al., 1993) and CD 28 (Prasad et al., unpublished data) and on ERB B3 (Soltoff et al., 1994). Our peptide library results predicted that T-cell receptor Zeta, would be a good binding site for SHC and this was shown to occur in vivo (Ravichandran, 1993). Several other sites predicted by Songyang et al. (1993) have also been confirmed by us or other laboratories, including binding sites for sem5/grb-2 on the EGF receptor, SHPTP2 and on SHC (Pawson and Schlessinger, 1993).

It is now clear from these studies that specificity in signaling downstream of protein-tyrosine kinases is at least partially determined by the ability of SH2 domains to specifically interact with unique sites of tyrosine phosphorylation. In this way, by autophosphorylating on tyrosine residues that are within motifs optimal for the SH2 domains of PtdIns 3-kinase, PtdIns-PLC-g, ras-GAP, SHPTP2, and pp60$^{c\text{-}src}$, the PDGF receptor can specifically associate with these downstream targets (Fig. 2). The association with the receptor alone may activate some of these enzymes, as we have shown for PtdIns

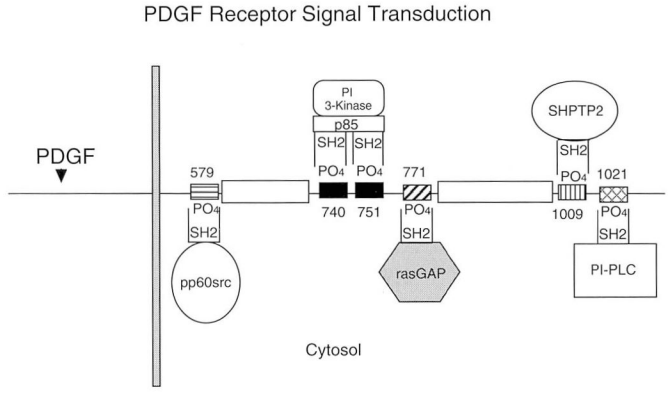

Fig. 2. A model for PDGF receptor interaction with cytosolic proteins bearing SH2 domains.

3-kinase (Carpenter et al., 1993). In most cases the associated proteins become phosphorylated on tyrosine. This tyrosine phosphorylation may regulate the activities of these proteins or provide binding sites for SH2 domains of yet additional signaling molecules.

This model raises the question of how much specificity in downstream signaling is mediated by the substrate specificity of the kinase domain of the protein-tyrosine kinase. It seems unlikely that the kinase domain will randomly phosphorylate any protein-tyrosine residue that is locally available and that specificity will be determined entirely by SH2 domain selection. In fact, the model that has arisen from studies of the PDGF receptor appears not to apply to many other receptors. For example, truncation or Tyr-Phe mutants of the EGF receptor that eliminate all the known autophosphorylation sites, including the binding sites for Grb-2, SHC, PLC-gamma and ras-GAP are not compromised in their ability to mediate EGF-dependent mitogenesis (Wells et al., 1990; Decker, 1993). These results indicate that some aspects of EGF receptor signaling are mediated directly by the kinase domain. We are currently using a peptide library technique to determine the specificity of kinase domains for linear peptide sequences. These results should provide further information about specificity in downstream signaling.

REFERENCES

Anderson, D., Koch, C. A., Grey, L., Ellis, C., Moran, M. F. and Pawson, T. (1990). Binding of SH2 domains of phospholipase C gamma 1, GAP, and Src to activated growth factor receptors. *Science* **250**, 979-82.

Auger, K. R., Carpenter, C. L. Shoelson, S. E., Piwnica-Worms, H. and Cantley, L. C. (1992). Polyoma virus middle T antigen/pp60$^{c\text{-}src}$ complex associates with purified phosphatidylinositol 3-kinase in vitro. *J. Biol. Chem.* **267**, 5408-5415.

Birge, R. B., Fajardo, J. E., Reichman, C., Shoelson, S. E., Songyang, Z., Cantley, L. C. and Hanafusa, H. (1993). Identification and characterization of a high-affinity interaction between v-Crk and tyrosine-phosphorylated paxillin in CT10-transformed fibroblasts. *Mol. Cell. Biol.* **13**, 4648-4656.

Cantley, L. C., Auger, K., 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., Chaudhuri, M., Schaffhausen B., Schoelson, S. and Cantley, L. C. (1993). Phosphoinositide 3-kinase is activated by phosphopeptides that bind to the SH2 domains of the 85 kD subunit. *J. Biol. Chem.* **268**, 9478-9483.

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**, 803-6.

Cobb, B. S., Schaller, M. D., Leu, T.-H. and Parsons, J. T. (1994). Stable association of $pp60^{src}$ and $pp59^{fyn}$ with the focal adhesion-associated protein tyrosine kinase, $pp125^{Fak}$. *Mol. Cell. Biol.* **14**, 147-155.

Courtneidge, S. A., Goutebroze, L., Cartwright, A., Heber, A., Scherneck, S. and Feunteun, J. (1991). Identification and characterization of the hamster polyomavirus middle T antigen. *J. Virol.* **65**, 3301-8.

Decker, S. J. (1993). *J. Biol. Chem.* **268**, 9176-9179.

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.

Escobedo, J. A., Kaplan, D. R., Kavanaugh, W. M., Turck, C. W. and Williams, L. T. (1991). A phosphatidylinositol-3 kinase binds to platelet-derived growth factor receptors through a specific receptor sequence containing phosphotyrosine. *Mol. Cell. Biol.* **11**, 1125-32.

Fantl, W. J., Escobedo, J. A., Martin, G. A., Turck, C. W., del, R. M., McCormick, F. and Williams, L. T. (1992). Distinct phosphotyrosines on a growth factor receptor bind to specific molecules that mediate different signaling pathways. *Cell* **69**, 413-23.

Fukui, Y. and Hanafusa, H. (1991). Requirement of phosphatidylinositol-3 kinase modification for its association with p60src. *Mol. Cell. Biol.* **11**, 1972-9.

Kapeller, R., Prasad, K. V. S., Janssen, O., Hou, W., Schaffhausen B. S., Rudd, C. E. and Cantley, L. C. (1994). Identification of two SH3-binding motifs in the regulatory subunit of phosphatidylinositol 3-kinase. *J. Biol. Chem.* **269**, 1927-1933.

Kazlauskas, A. and Cooper, J. A. (1989). Autophosphorylation of the PDGF receptor in the kinase insert region regulates interactions with cell proteins. *Cell* **58**, 1121-33.

Kim, J. W., Sim, S. S., Kim U.-H, Nishibe, S., Wahl, M. I., Carpenter, G. and Rhee, S. G. (1990). Tyrosine residues in bovine phospholipase C-g phosphorylated by the epidermal growth factor receptor in vitro. *J. Biol. Chem.* **265**, 3940-3943.

Liu, X., Brodeur, S. R., Gish, G., Songyang, Z., Cantley, L. C., Laudano, A. P. and Pawson, T. (1993). Regulation of c-Src tyrosine kinase activity by the Src SH2 domain. *Oncogene* **8**, 1119-26.

Marcote, M. J., Knighton, D. R., Basi, G., Sowadski, J. M., Brambilla, P., Draetta, G. and Taylor, S. S. (1993). A three-dimensional model of the Cdc2 protein kinase: localization of cyclin- and Suc1-binding regions and phosphorylation sites. *Mol. Cell. Biol.* **13**, 5122-31.

Matsuda, M., Mayer, B., Fukui, Y. and Hanafusa, H. (1990). Binding of transforming protein p47gag-crk to a broad range of phosphotyrosine-containing proteins. *Science* **248**, 1537-1539.

Mustelin, T. and Burn, P. (1993). Regulation of src family tyrosine kinases in lymphocytes. *Trends Biochem. Sci.* **18**, 215-220.

Pawson, T. and Gish, G. D. (1992). SH2 and SH3 domains: from structure to function. *Cell* **71**, 359-62.

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

Payne, G., Shoelson, S. E., Gish, G. D., Pawson, T. and Walsh, C. T. (1993). Kinetics of p56lck and p60src Src homology 2 domain binding to tyrosine-phosphorylated peptides determined by a competition assay or surface plasmon resonance. *Proc. Nat. Acad. Sci. USA* **90**, 4902-6.

Prasad, K. V. S., Janssen, O., Kapeller, R., Raab, M., Cantley, L. C. and Rudd, C. E. (1993a). SRC-homology 3 domain of protein kinase $p59^{fyn}$ mediates binding to phosphatidylinositol 3-kinase in T cells *Proc. Nat. Acad. Sci. USA* **90**, 7366-7370.

Prasad, K. V. S., Kapeller, R., Janssen, O., Repke, H., Duke-Cohan, J. S., Cantley, L. C. and Rudd, C. E. (1993b). Phosphatidylinositol 3-kinase and phosphatidylinositol 4-kinase binding to the CD4-p56lck complex: p56lck SH3 domain binding to PI 3-kinase but not PI 4-kinase. *Mol. Cell. Biol.* **13**, 7708-7717.

Pradsad, K. V., Cai, Y. C., Raab, M., Duckworth, B., Cantley, L., Shoelson, S. E., Rudd, C. E. (1994). T-cell antigen CD28 interact with the lipid kinase phosphatidylinositol 3-kinase by a cytoplasmic Tyr(p)-Met-Xaa-Met motif. *Proc. Nat. Acad. Sci. USA* **91**, 2834-2838.

Ravichandran, K. S., Lee, K. K., Songyang, Z., Cantley, L. C., Burn, P. and Burakoff, S. J. (1993). SHC interacts with the Zeta chain of the T cell receptor upon T cell activation. *Science* **262**, 902-905.

Ren, R., Mayer, B. J., Cicchetti, P. and Baltimore, D. (1993). Identification of a ten-amino acid proline-rich SH3 binding site. *Science* **259**, 1157-61.

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. Nat. Acad. Sci. USA* **89**, 2027-2031.

Soltoff, S. P., Carraway, K. L. III, Prigent, S. A., Gullick, W. G. and Cantley, L. C. (1994). ErbB3 is involved in the activation of phosphatidylinositol 3-kinase by epidermal growth factor. *Mol. Cell. Biol.* **14** (in press).

Songyang, Z., Shoelson, S. E., Chadhuri, M., Gish, G., Pawson, T., Haser, W. G., King, F., Roberts, T., Ratnofsky, S., Lechleider, R. J., Neel, B. G., Birge, R. B., Fajardom J. E., Chou, M. M., Hanafusa, H., Schaffhausen, B. and Cantley, L. C. (1993). SH2 domains recognize specific phosphopeptide sequences. *Cell* **72**, 767-778.

Songyang Z., Shoelson, S. E., McGlade, J., Olivier, P., Pawson, T., Bustelo, R. X., Barbacid, M., Sabe, H., Hanafusa, H., Yi, T., Ren, R., Baltimore, D., Ratnofsky, S., Feldman, R. A and Cantley, L. C. (1994). Specific motifs recognized by the SH2 domains of Csk, 3BP2, fps/fes, Grb-2, SHPTP1, SHC, Syk and Vav. *Mol. Cell. Biol.* **14** (in press).

Tuveson, D. A., Carter, R. H., Soltoff, S. P. and Fearon, D. T. (1993). CD19 of B Cells as a surrogate kinase insert region to bind Phosphatidylinositol 3-kinase. *Science* **260**, 986-989.

Wahl, M. I., Nishibe, S., Kim, J. W., Kim, H., Rhee, S. G. and Carpenter, G. (1990). Identification of two epidermal growth factor-sensitive tyrosine phosphorylation sites of phospholipase C-γ in intact HSC-1 cells. *J. Biol. Chem.* **265**, 3944-3948.

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. and Kuriyan, J. (1992). Crystal structure of the phosphotyrosine recognition domain SH2 of v-src complexed with tyrosine-phosphorylated peptides *Nature* **358**, 646-53.

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**, 779-90.

Wells, A., Welsh, J. B., Lazar, C. S., Wiley, H. S., Gill, G. N. and Rosenfeld, M. G. (1990). *Science* **247**, 962-964.

Whitman, M., Kaplan, D. R., Schaffhausen, B., Cantley, L. and Roberts, T. M. (1985). Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation. *Nature* **315**, 239-242.