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Structure and function of SH2 domains

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SUMMARY

In order for cells to respond to their environment, a series of regulated molecular events has to take place. External signalling molecules bind to cellular receptors and thereby trigger the activation of multiple intracellular pathways, which modify cellular phenotypes. The cell-surface receptors for a wide range of polypeptide hormones possess protein tyrosine kinase activity, which is induced by binding of the appropriate extracellular ligand. Tyrosine phosphorylation can act as a molecular switch, by initiating the recruitment of cytoplasmic effector molecules containing Src homology (SH) 2 domains, to activated receptors. These SH2-containing proteins, in turn, regulate intracellular signalling pathways. Here, we discuss the role of tyrosine phosphorylation in triggering signalling pathways, as well as the functions of SH2 domains, which mediate these events through phosphotyrosine-dependent protein-protein interactions.

Key words: protein tyrosine kinase, signal transduction

RECEPTOR PROTEIN TYROSINE KINASES

Receptor protein tyrosine kinases (RPTKs) are membrane-spanning molecules, which function as regulators of cell growth and differentiation. RPTKs contain an extracellular ligand-binding domain, a transmembrane element, and an intracellular catalytic region. The extracellular portion is characterized by specific motifs such as cysteine-rich sequences, immunoglobulin-like loops, fibronectin repeats, and others, which are apparently involved in growth factor binding. The transmembrane domain is hydrophobic and plays a crucial role in receptor dimerization, while the intracellular region contains the tyrosine kinase domain and non-catalytic sequences that, following RPTK activation, serve as transphosphorylation substrates (Yarden and Ullrich, 1988; Ullrich and Schlessinger, 1990; van der Geer and Hunter, 1994). These characteristics are common to all RPTKs, but specific features can vary, such as the type of repeats in the extracellular domain, or the structure of the kinase domain, and these differences have been used to define subfamilies of RPTKs (Fig. 1) (van der Geer and Hunter, 1994). For example, receptors such as the epidermal growth factor receptor (EGFR), the platelet-derived growth factor receptor (PDGFR), the insulin receptor (IR), the nerve growth factor receptor (NGFR), and the fibroblast growth factor receptor (FGFR) constitute five subfamilies of RPTK. The EGFR, PDGFR, NGFR, and FGFR vary mostly in their extracellular ligand-binding domains, although the PDGFR has an additional kinase insert within its catalytic domain, while the IR has a different receptor architecture altogether (Fig. 1).

RPTK activation is achieved in the following fashion: binding of the growth factor to the extracellular portion of a RPTK induces receptor dimerization, and stimulates kinase activity, thereby permitting intermolecular autophosphorylation, which largely occurs within non-catalytic intracellular sequences. Mitogenic responses mediated by activated RPTKs are dependent upon receptor tyrosine kinase activity. Receptors in which the kinase domain is mutated and rendered inactive can no longer induce a mitogenic signal in response to growth factor stimulation. The importance of tyrosine kinase activity has also been shown in vivo. Loss-of-function (LOF) mutations in genes encoding RPTKs, such as *c-kit, torso, der, sevenless*, and *let23* drastically affect development of distinct species, such as the mouse, *Drosophila*, and *Caenorhabditis elegans* (Pawson and Bernstein, 1990). LOF mutations in the mouse *kit* gene affect hair pigmentation, hematopoiesis, and fertility depending on the severity of the mutated allele (Russel, 1979; Reith et al., 1990). The most severe *kit* allele, known as W^{42}, induces a substitution of an aspartic acid within the kinase domain, thought to be the catalytic base, leading to a complete loss of tyrosine kinase activity. In *Drosophila*, mutations in the *torso* tyrosine kinase gene affect terminal embryonic structure development (Nusslein-Volhard et al., 1987); while LOF mutations in the *Drosophila* gene *der*, affect head and central nervous system development (Schejter and Shilo, 1989; Price et al., 1989). The *sevenless* LOF mutation specifically affects the development of photoreceptor cell R7, which normally differentiates into a neuronal retinal cell (Tomlinson et al., 1987). In *C. elegans*, *let23* mutations affect the development of the vulval precursor cells, which contribute to the formation of the hermaphrodite vulva (Ferguson et al., 1987; Aroian et al., 1991). Consistent with this view, gain-of-function (GOF) mutations in *let23*, which positively affect its tyrosine kinase activity, contribute to an increase in differentiated vulval precursor cells, and lead to the formation of multiple vulvae.

The identification of cell-surface receptors for growth

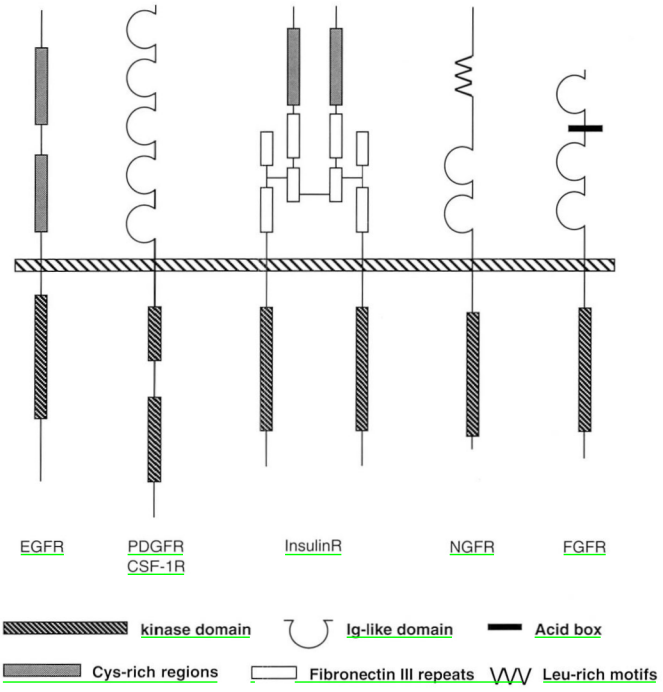

Fig. 1. Schematic representation of five subfamilies of receptor protein tyrosine kinases.

factors with intrinsic tyrosine kinase activity, and the discovery of the role of these receptors in cell growth, differentiation, and development have triggered great interest in determining their mechanism of action.

SIGNALLING MOLECULES

The initial molecular event mediated by RPTKs after binding their ligand is autophosphorylation and stimulation of tyrosine phosphorylation of cellular proteins. Stimulation of quiescent fibroblasts by PDGF is accompanied by autophosphorylation of the PDGFR and increased tyrosine-phosphorylation of cellular proteins (Kazlauskas and Cooper, 1989). The PDGFR kinase domain contains an insertion relative to the other tyrosine kinases, termed the kinase insert (Fig. 1), which together with other sites on the PDGFR intracellular domain, become tyrosine-phosphorylated. Autophosphorylation sites on the human βPDGFR also serve as docking sites for signalling molecules. Phosphatidylinositol (PI) 3′-kinase activity associates specifically with tyrosine-phosphorylated sites within the kinase insert of the activated βPDGFR. This association is dependent on tyrosine phosphorylation (Kazlauskas and Cooper, 1989; Coughlin et al., 1989). The binding of PI3′-kinase to the βPDGFR was mapped to tyrosine residues 740 and 751 (Y^{740} and Y^{751}) within the kinase insert, and substituting these residues for phenylalanine was shown to abolish the ability of the PDGFR to bind PI3′-kinase (Kazlauskas and Cooper, 1990; Escobedo et al., 1991). The PDGFR binds other signalling molecules, including p21ras GTPase-activating protein (GAP), phospholipase C-γ (PLCγ1) and the Syp phosphotyrosine phosphatase. These interactions involve the SH2 domain(s) of the signalling molecules and specific receptor phosphotyrosine sites. The SH2-containing proteins become tyrosine-phosphorylated as a consequence of binding to the activated PDGFR (Molloy et al., 1989; Meisenhelder et al., 1989; Kazlauskas et al., 1990; Kaplan and Cooper, 1990; Morrison et al., 1990; Kazlauskas et al., 1993). In addition, members of the Src family of cytoplasmic tyrosine kinases, as well as Shc and Nck, all of which contain SH2 domains, can also bind the activated PDGFR (Kypta et al., 1990; Mori et al., 1993; Nishimura et al., 1993; Yokote et al., 1994). The colony-stimulating factor 1 receptor (CSF-1R) (Fig. 1) can induce proliferation of mouse fibroblasts engineered to express the receptor, in response to CSF-1. Consistent with the view that activated RPTKs, which have undergone tyrosine-autophosphorylation, can bind signalling molecules, the activated CSF-1R associates with PI3′-kinase, and Grb2 in a phosphotyrosine-dependent fashion (Downing et al., 1989; Reedijk et al., 1990, 1992; van der Geer and Hunter, 1993).

The IR has a similar mechanism for activating effector molecules upon insulin stimulation. Although the IR has tyrosine kinase activity, SH2-containing signalling molecules do not associate directly with the activated receptor. Activation of the IR leads to autophosphorylation and to tyrosine-phosphorylation of the insulin receptor substrate (IRS) 1, which in turn binds SH2-containing signalling molecules such as PI3′-kinase, Grb2, Syp and Nck (Lavan et al., 1992; Myers et al., 1992; Yamamoto et al., 1992; Backer et al., 1992; Kuhne et al., 1993; Lee et al., 1993; Tobe et al., 1993; Pronk et al., 1994). The association of effector molecules with specific tyrosine-phosphorylated sites on activated RPTKs suggests a general mechanism by which RPTKs couple to intracellular signalling molecules.

SH2 BINDING

Receptor autophosphorylation acts as a switch to induce physical association between activated receptor and signalling molecules. Although these signalling proteins vary in their catalytic activities, structures, and cellular functions, they all share a common region termed the SH2 domain. The SH2 domain was initially identified as a common 100 amino acid sequence in the Src and Fps oncoproteins (Sadowski et al., 1986; Pawson, 1988). SH2 domains are highly conserved (approximately 35% identical amongst all SH2 domains), associate specifically with phosphotyrosine in a sequence-dependent manner, and are found in one or two copies in many cytoplasmic signalling molecules (Pawson and Gish, 1992). These SH2-containing proteins can be classified into two groups; the first group includes signalling proteins that contain intrinsic catalytic activity, and includes the Src, Fps and Abl families of intracellular tyrosine kinases, PLCγ1 and 2, GAP and tyrosine-specific phosphatases such as the SH2-containing tyrosine phosphatase Syp, amongst others. The second group includes molecules such as Grb2, SHC, Nck, Crk and the p85 subunit of PI3′-kinase, which do not have detectable intrinsic catalytic activity but apparently function as molecular adaptors to couple RPTKs to signalling proteins that themselves may lack SH2 domains (Fig. 2).

The SH2 domains of proteins such as GAP, PLCγ1, PI3′-kinase, and Src were shown to be directly involved in protein-protein interactions with activated receptors. The binding sites of these and other SH2-containing molecules have been

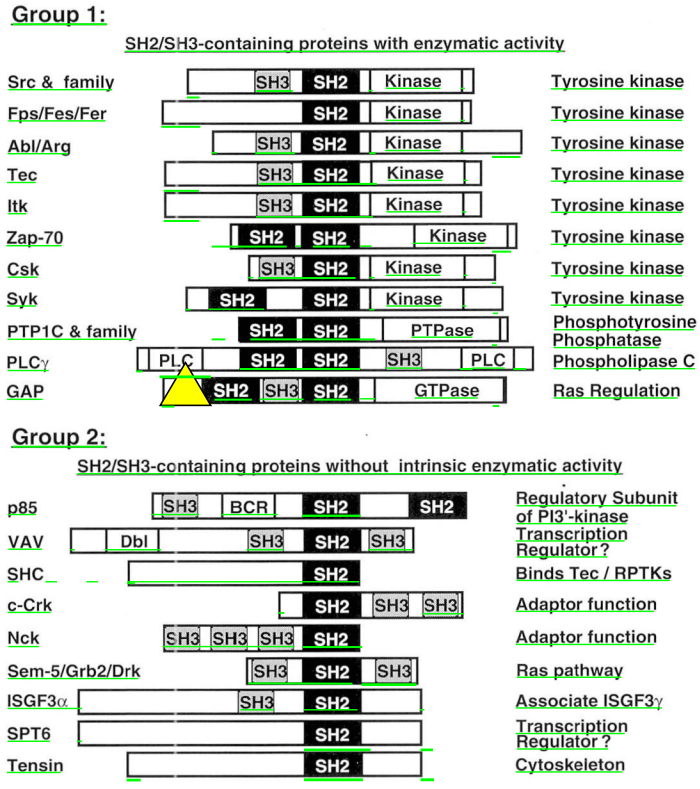

Fig. 2. Structures of SH2-containing proteins. These molecules are divided into two groups: Group 1 includes proteins with intrinsic catalytic activity, while Group 2 includes proteins without intrinsic catalytic activity, serving an adaptor function, coupling RPTKs to downstream effector molecules. The catalytic activities, and adaptor functions are listed on the right. Kinase, the tyrosine kinase domain; PTPase, the phosphotyrosine phosphatase domain; PLC, the phospholipase domain; GTPase, Ras GTPase-activating domain; BCR, the G-binding protein Rac/Rho GTPase-activating domain; Dbl, a guanine-nucleotide exchange domain.

precisely mapped on several receptors. For example, Src, PI3′-kinase, GAP, Syp, and PLCγ1 bind tyrosine-phosphorylated sites Y^{579}/Y^{581}, Y^{740}/Y^{751}, Y^{771}, Y^{1009}, and Y^{1021}, respectively, on the PDGFR (Fig. 3). These SH2-binding sites were mapped using two main approaches. The first approach involves in vivo expression of the wild-type (wt) receptor, or variant forms of the receptor in which specific tyrosine phosphorylation sites are substituted with phenylalanine. These receptor-expressing cells are then stimulated with the appropriate ligand necessary for receptor activation. The wt or mutant receptors are immunoprecipitated and assayed for the presence of specific co-immunoprecipitated SH2-containing proteins. In the case of the PDGFR, specific receptor autophosphorylation sites are required for binding of defined SH2-containing proteins. In vitro, the autophosphorylated receptor can bind SH2 signalling proteins. These interactions can be efficiently competed by short tyrosine-phosphorylated peptides corresponding to specific receptor autophosphorylation sites. Together these approaches have identified specific receptor-binding sites for SH2-containing molecules (Kazlauskas and Cooper, 1989, 1990; Molloy et al., 1989; Downing et al., 1989; Kaplan et al., 1990; Morrison et al., 1990; Anderson et al., 1990; Escobedo et al., 1991; Fantl et al., 1992; Kashishian et al., 1992; Kazlauskas et al., 1992, 1993; van der Geer et al., 1993).

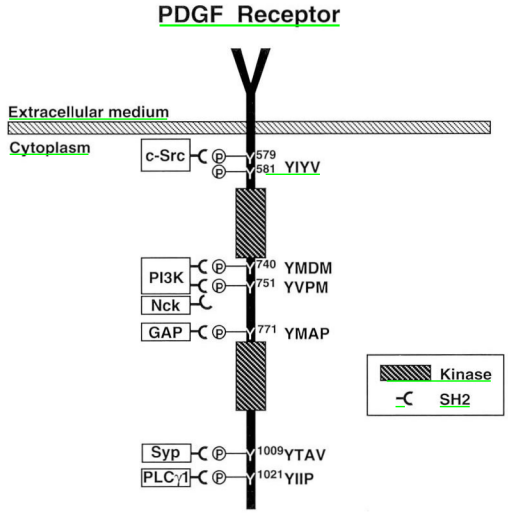

Fig. 3. SH2 domain binding sites on the PDGFR. The sequences C-terminal to the autophosphorylated tyrosine binding sites are indicated on the left of the receptor, in single letter amino acid code.

The ability of SH2 domains to mediate phosphotyrosine-dependent interactions is not limited to receptors. For example, GAP, Grb2, Src, and other signalling proteins can also associate, via their SH2 domain(s) with tyrosine-phosphorylated cytoplasmic molecules (Moran et al., 1990; Koch et al., 1991; Lowenstein et al., 1992; Schaller et al., 1992; Cobb et al., 1994). This was demonstrated by the ability of v-Crk and v-Abl SH2 domains to bind a spectrum of tyrosine-phosphorylated proteins in solution, and in filter-binding assays (Matsuda et al., 1990; Mayer and Hanafusa, 1990; Mayer et al., 1991, 1992). The N-terminal SH2 domain of GAP was also shown to bind predominantly tyrosine-phosphorylated proteins p62 and p190 in vivo and in vitro (Moran et al., 1990; Marengere and Pawson, 1992). These proteins have been suggested to have RNA-binding ability, and a GTPase activity towards the small GTP-binding protein Rho, respectively (Wong et al., 1992; Settleman et al., 1992a,b). These experiments revealed that binding to phosphotyrosine-containing sites is a fundamental property of all SH2 domains.

SH2 SPECIFICITY

As noted above, autophosphorylated growth factor receptors possess multiple phosphotyrosine sites that bind to distinct SH2 domains (Fig. 3) (Fantl et al., 1992; Rotin et al., 1993; Mohammadi et al., 1991; Reedijk et al., 1990, 1992). The systematic mapping of PI3-5α, GAP, and PLCγ1 binding site(s) on the PDGFR and CSF-1R has suggested that the sequence C-terminal to the phosphotyrosine regulates SH2-binding specificity (van der Geer and Hunter, 1993; Mohammadi et al., 1991; Cantley et al., 1991; Panayotou et al., 1992; Kashishian et al., 1992; Kazlauskas et al., 1992). For example, binding sites for the SH2-containing p85 protein on the polyoma virus middle T-antigen, PDGFR, CSF-1R, c-Kit, and IRS-1 have the consensus sequence pTyr-(Met/Val)-(Asp/Glu)-(Pro)-(Met)

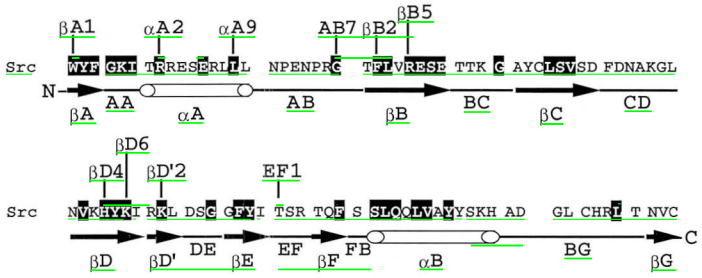

Fig. 4. Sequence of the Src SH2 domain with conserved residues highlighted, and respective positions indicated above. The locations of the α-helices, β-sheets and loops are indicated below according to the nomenclature developed by Eck et al. (1993).

depicted in single letter code as [pY(M/V)-(D/E/P)-(M)] (Escobedo et al., 1991; Auger et al., 1992; McGlade et al., 1992; Backer et al., 1992). Furthermore, the p85 SH2 domains can bind to phosphopeptides containing the consensus sequence pYM/V-X-M with high affinity (Felder et al., 1993; Panayotou et al., 1993). Based on the data for the p85 SH2 domain-selectivity, a degenerate phosphopeptide library screen was developed in order to determine the specificity of individual SH2 domains (Songyang et al., 1993, 1994). Ideally, the p85 SH2 domains expressed as fusion proteins were incubated with phosphopeptides, containing the sequence GDGpTyrX^{+1}X^{+2}X^{+3}SPLLL (single letter amino acid code), where X represents a degenerate position at the +1, +2, and +3 residues. The p85 N- and C-terminal SH2 domains selected amino acid motifs very similar to the consensus binding sites in the physiological targets mentioned above. Consequently, the assay was expanded to other SH2 domains, in order to investigate their respective potential specificity (Songyang et al., 1993, 1994).

Based on their binding specificity, SH2 domains can be classified into two groups (Songyang et al., 1994). The first group selects mostly hydrophilic residues at the two residues C-terminal to the phosphotyrosine (the +1 and +2 positions), and a hydrophobic residue at +3. The second group preferentially selects hydrophobic residues. (Songyang et al., 1993, 1994).

The ability of this assay to predict SH2-binding sequences implies that SH2 domain can independently select for phosphotyrosine and residues at the +1, +2, and +3 positions. This selection must therefore be performed by residues strategically located within the SH2 domain, which specifically interact with these positions. Evidence of SH2 domain binding-specificity was first provided by structural analysis of the v-Src SH2 domain complexed to the pYEEI peptide, and will be discussed in the next section.

SH2 STRUCTURE

NMR solutions of the uncomplexed Abl SH2, and p85α N-SH2 domains provided information about the overall topology of these modular domains, which comprise a central β-sheet flanked by two α-helices (Overduin et al., 1992; Booker et al., 1992).

The X-ray structures of v-Src SH2 and Lck SH2 domains complexed to the high affinity peptide EPQpY^{0}E^{+1}E^{+2}I^{+3}PIYL (pYEEI) added information about SH2 interactions with phosphotyrosine and the +1Glu, +2Glu, and +3Ile residues within the phosphopeptide (Waksman et al., 1993; Eck et al., 1993). Following the structural analysis of these domains, a new nomenclature was adopted for SH2 residues based on secondary structures (see Fig. 4). X-ray crystallographic structures were also of higher resolution, showing two clefts, the first being the phosphotyrosine-binding site, and the second, a hydrophobic-binding pocket for the +3 residue. Both pockets are flexible; the phosphotyrosine-binding pocket closes upon association with phosphotyrosine, while the hydrophobic pocket opens after interaction with the +3 residue. As expected, well-conserved residues within the SH2 domains form the hydrophobic core and the phosphotyrosine-binding pocket, while the more variable residues are involved in interactions with the +1 to +3 residues, and therefore in conferring specificity. The phosphotyrosine moiety is stabilized mostly via interactions with ArgαA2, ArgβB5, and LysβD6, which contact the phenyl ring and the phosphate group. Residues within the BC loop also stabilize the phosphotyrosine structure through interactions with the terminal phosphate oxygens.

In contrast to the phosphotyrosine-binding site, the +1 and +2Glu residues lie on the surface of the SH2 domain. The +1Glu forms ionic interactions with TyrβD5 and LysβD3, while the +2Glu is stabilized by ionic interactions and water molecules with ArgβD′1, LysβD6 and the carbonyl oxygen of the +1Glu. The hydrophobic binding pocket specific for the +3Ile is formed by residues in the EF and BG loops, and engulfs the +3Ile. We have recently shown that changing the Thr at the EF1 position of the Src SH2 domain to Trp markedly alters its binding specificity and biological behaviour (Marengere et al., 1994).

The interior of the binding pocket is lined by helix αB, while the edges are formed by the EF and BG loops, and the βD strand. More specifically, IleβE4, TyrβD5, TyrαA9, LeuBG2, GlyBG3 and ThrEF1 are residues that directly interact with the +3Ile and may therefore be important in determining specificity at that position. These amino acids vary amongst SH2 domains, consistent with the possibility that they are major determinants in SH2 specificity, at least at the +3 position. The Src and Lck SH2 structures have identified one specific type of SH2/phosphopeptide interaction, in which the phosphopeptide can be represented as a two-pronged plug (the prongs being formed by phosphotyrosine and the +3Ile sidechain), while the SH2 domain is a socket with two accommodating holes.

The NMR structure of the PLCγ1 C-terminal SH2 (C-SH2) domain complexed to DNSpY^{0}I^{+1}I^{+2}P^{+3}LPDPK (termed pYIIP) phosphopeptide, has shown a second class of SH2 binding-specificity (Pascal et al., 1994). The PLCγ1 C-SH2 domain shares some topological features with the complexed Src/Lck SH2 domains, such as the hydrophobic core and the concentration of basic residues near the phosphotyrosine-binding pocket. Interestingly, the PLCγ1 C-SH2 domain differs with respect to its phosphotyrosine-stabilizing interactions, SH2-binding surface for positions C-terminal to the phosphotyrosine, and its additional ability to contact the phosphopeptide +4, +5, and +6 positions.

Also in contrast to the +3-binding pocket of the Src SH2 domain, the PLCγ1 C-SH2 domain binding surface, has an extended hydrophobic groove in which the +1, +3 and +5 residues are buried. One factor contributing to this difference

in binding surface is the SH2 position βD5, which is a Tyr in Src SH2 domain, but a Cys in the PLCγ1 C-SH2 domain. TyrβD5 in the Src SH2 domain interacts with the BG loop, and pinches that segment of the binding surface, closing the hydrophobic groove, and thereby forcing the +1 and +2 residues to bind at the surface of the SH2 domain. The other major difference between SH2 structures is the ability of the PLCγ1 C-SH2 domain to associate with the +4, +5, and +6 positions of the phosphopeptide. Although 85% of the NMR-detected interactions were between the SH2 domain and the phosphotyrosine +1, +2, and +3 positions, interactions between the +4Leu, +5Pro, and +6Asp of the phosphopeptide were also detected, mostly with SH2 residues within the EF and BG loops. SH2 binding to the +4, +5, and +6 residues may confer optimal binding affinity towards a physiological target.

Another example of an SH2 domain binding to a phosphotyrosine-containing sequence, is provided by the structural analysis of the Syp N-terminal SH2 domain (N-SH2) complexed to high affinity peptides (Lee et al., 1994). The Syp N-SH2 domain displays some unique features, while other facets resemble either the Src SH2 or the PLCγ1 C-SH2 domain structures. For example, the Gly at αA2, which replaces the Arg found at αA2 in the Src/Lck and PLCγ1 C-SH2 domains, does not contact the phosphotyrosine moiety. Instead, the invariant ArgβB5 of the Syp N-SH2 domain interacts with both the phenyl ring and the phosphate group terminal oxygens. In contrast to the PLCγ1 C-SH2 domain structure, no additional basic residues are found to bind the phosphotyrosine, which might compensate for the absence of the ArgαA2.

As found in the PLCγ1 C-SH2, IleβD5 of the Syp N-SH2 domain does not interact with the BG loop and is important for forming a hydrophobic binding channel in which the +1, +2, and +3 positions are deeply buried. Also consistent with this type of binding surface topology, Syp N-SH2 domain residues interact weakly with the +4 peptide residue, and tightly with the +5 residue. Although SH2 domains are well-conserved and display very similar backbone conformations, they vary in the details of their phosphotyrosine-binding pockets, and in their binding surfaces for the peptide residues C-terminal to the phosphotyrosine.

A growing body of evidence shows that residues both N-terminal of the phosphotyrosine, and C-terminal to +3 can affect SH2 binding-specificity. The ability of p85α N-SH2 to bind a phosphopeptide representing the IRS-1 Y^{628} binding site, was investigated by systematically substituting peptide positions −4 to +5, relative to the phosphotyrosine, with benzoylphenylalanine (Bpa) (Williams and Shoelson, 1993). Most changes had little effect on binding affinity but Bpa substitution for +1Met and +3Met greatly reduced the affinity of the p85α N-SH2 domain for these altered peptides. It was also shown that Bpa-substitution at positions −1 and +4 decreased the affinity of the p85α N-SH2 domain for these peptides. The −1 and +4 positions were cross-linked, upon photoactivation of the Bpa complex, to residues within the αA helix and BG loop, respectively, contributing to the overall affinity. These data are consistent with the NMR structure of PLCγ1 C-SH2 and the X-ray crystallographic structure of Syp N-SH2, which observed interactions between +4, +5, and +6 peptide positions, and residues within EF and BG loops (Pascal et al., 1994; Lee et al., 1994).

GENETIC EVIDENCE FOR THE ROLE OF SH2 DOMAINS IN SIGNAL TRANSDUCTION - SH2 DOMAINS REGULATE DEVELOPMENTAL PATHWAYS

The first genetic evidence describing a role for SH2 domains in development was provided by the *C. elegans* gene *sex myoblasts abnormal (sem)-5* (Clark et al., 1992). Disruptions within the *sem-5* gene affect hermaphrodite vulval development, and proper migration of sex myoblasts. *sem-5* mutations also affect the clear (clr) 1 phenotype, and larval viability (Horvitz and Sternberg, 1991).

The *sem-5* gene encodes a protein containing almost exclusively SH3 and SH2 domains (Fig. 2), and mutations affecting development map to these domains (Clark et al., 1992). A substitution at position BC1, within the BC loop of the SH2 domain, affects vulval development, sex myoblast migration, and the clr-1 phenotype, while a substitution at position BC2 affects the clr-1 phenotype and has a very minimal effect on vulval development. The BC1 mutation induces a substitution of the well-conserved Glu residue for Lys, while the BC2 mutation affects a more variable residue (Ser for Asn), possibly explaining the minimal effect on developmental processes compared to the BC1 mutation. A third mutation disrupts a splice acceptor site, and likely generates a null allele. This mutation results in a high level of larval lethality, and severe suppression of vulval development, sex myoblast migration and the clr-1 phenotype.

The *Drosophila downstream of receptor kinase* (*drk*) gene, which is homologous to the *C. elegans* gene *sem-5*, is required for proper differentiation of the R7 photoreceptor cell, leading to normal eye development. *drk* is also required for pupal viability, and is apparently involved in signalling pathways downstream of multiple receptor tyrosine kinases, including sevenless, the *Drosophila* EGFR homologue and torso (Olivier et al., 1993; Simon et al., 1993; Doyle and Bishop, 1993). Mutant alleles of *drk*, *E(sev)2B* and *Su(Sev^{s11})R1*, were initially identified by their effect on eye development (Simon et al., 1991), and later mapped as point mutations affecting well-conserved SH2 residues αA2 (substitution of Arg for His) and βD6 (substitution of His for Tyr) involved in phosphotyrosine binding (Olivier et al., 1993). Transheterozygous combinations of these mutant alleles result in pupal lethality (Olivier et al., 1993). Genetically, *drk* lies upstream of *son-of-sevenless (sos)*, which encodes a guanine nucleotide exchange factor for Ras (Simon et al., 1991). The SH3 domains of drk were shown to bind directly to the proline-rich tail of Sos (Olivier et al., 1993). drk therefore provides a direct link between activated receptors and Sos, which is able to directly convert Ras into the active GTP-bound state. These mutations affect the ability of the drk SH2 domain to bind activated receptor tyrosine kinases, thereby blocking signalling cascades and directly altering cellular responses (Olivier et al., 1993).

SH3 DOMAIN

Many signalling proteins that couple activated RPTKs to intracellular signalling events, contain single or multiple copies of SH3 domains, which can often be found in the same molecule as SH2 domains. SH3 domains are also well-conserved regions of approximately 50-75 residues, with no known catalytic

function, that are found both in signalling molecules with intrinsic catalytic activities and in adaptor proteins (Fig. 2) (Pawson, 1988; Gish and Pawson, 1992; Pawson and Gish, 1992). SH3 domains specifically recognize and bind with high affinity to proline-rich sequences. This was first demonstrated from the identification of the SH3-binding protein (3BP) 1, cloned from an expression library, using the SH3 domain of the tyrosine kinase c-Abl (Cicchetti et al., 1992). The c-Abl SH3 domain-binding site was later mapped to a proline-rich sequence within the C-terminal region of 3BP1. This SH3-binding site was further refined to a ten amino acid proline-rich motif with the sequence APTMPPPLPP (Ren et al., 1993). Furthermore, a second c-Abl SH3 domain binding protein was identified and termed 3BP2 (Ren et al., 1993). The binding site for c-Abl SH3 domain on 3BP2 was localized to the sequence PPAYPPPPVP (Ren et al., 1993). This suggested a binding specificity for SH3 domains and a role in signal transduction by mediating protein-protein interactions.

Genetic analyses of the mammalian Grb2 homologues *Drosophila* drk and *C. elegans* Sem-5 proteins, have revealed a role for SH3 domains in the conserved signalling pathway that couples activated receptor to Ras (Clark et al., 1992; Olivier et al., 1993). As discussed previously, mutations within the SH2 domains of drk and Sem-5, have defined their role in mediating signalling in both species. In *C. elegans*, Sem-5 SH3 mutations also disrupt normal signalling, and cause severe defects in vulval induction, sex myoblast migration, clr-1 suppression, and larval viability, showing a role for SH3 domains in cellular signalling (Clark et al., 1992). In contrast to mutations in the N-terminal SH3 domain of Sem-5, a substitution of Gly201 for Arg in the Sem-5 C-terminal SH3 domain, only results in a minor clr-1 suppression. This suggests that the N-terminal SH3 domain might play a more crucial role than the C-terminal SH3 domain in mediating proper signalling in this pathway. These genetically identified pathways, and the role played by drk and Sem-5 signalling molecules, were substantiated by biochemical studies in mammalian cells with their homologue Grb2 and mSos1/mSos2 (Lowenstein et al., 1992; Bowtell et al., 1992). As with drk, the SH3 domains of Grb2 form a stable cytoplasmic complex by binding the proline-rich sequences in the C terminus of the guanine nucleotide releasing protein mSos1 and mSos2 (the mouse homologues of *Drosophila* Son-of-sevenless). Upon activation and autophosphorylation of the EGFR, the Grb2-mSos1 complex binds directly to the receptor through recognition of binding site pYINQ (Tyr^{1068}) by the Grb2 SH2. As a consequence, it is hypothesized that mSos1 becomes co-localized with p21ras, and catalyses the exchange of GDP for GTP, activating p21ras and its signalling pathway (Pawson and Schlessinger, 1993; Gale et al., 1993; Rozakis-Adcock et al., 1993; Li et al., 1993; Buday and Downward, 1993; Egan et al., 1993). SH3 domains apparently have many other functions that are beyond the scope of this article. In particular, they are implicated in the subcellular localization of proline-rich proteins, and in the organization of signalling complexes.

In summary, SH2 and SH3 domains regulate a network of protein-protein interactions that are important for signalling downstream of receptors associated with tyrosine kinase activity.

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