SH2 and SH3 Domains: Elements That Control Interactions of Cytoplasmic Signaling Proteins

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Src homology (SH) regions 2 and 3 are noncatalytic domains that are conserved among a series of cytoplasmic signaling proteins regulated by receptor protein–tyrosine kinases, including phospholipase C–γ, Ras GTPase (guanosine triphosphatase)–activating protein, and Src-like tyrosine kinases. The SH2 domains of these signaling proteins bind tyrosine phosphorylated polypeptides, implicated in normal signaling and cellular transformation. Tyrosine phosphorylation acts as a switch to induce the binding of SH2 domains, thereby mediating the formation of heteromeric protein complexes at or near the plasma membrane. The formation of these complexes is likely to control the activation of signal transduction pathways by tyrosine kinases. The SH3 domain is a distinct motif that, together with SH2, may modulate interactions with the cytoskeleton and membrane. Some signaling and transforming proteins contain SH2 and SH3 domains unattached to any known catalytic element. These noncatalytic proteins may serve as adaptors to link tyrosine kinases to specific target proteins. These observations suggest that SH2 and SH3 domains participate in the control of intracellular responses to growth factor stimulation.

A COMMON MECHANISM BY WHICH GROWTH FACTORS REGulate cellular proliferation and differentiation is through transmembrane receptors with inducible protein–tyrosine kinase activity (*1*). Indeed the mitogenic effects of growth factors such as epidermal growth factor (EGF) or platelet–derived growth factor (PDGF) absolutely require the tyrosine kinase activity of their receptors (*2*). Growth factors induce receptors to cluster, which is followed by intermolecular tyrosine phosphorylation of the oligomerized receptors (*3*). Autophosphorylation of the PDGF receptor (PDGFR) is important both for its subsequent interactions with substrates and for the induction of DNA synthesis (*4, 5*).

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A second group of tyrosine kinases, for which Src, Fps, and Abl are the prototypes, are entirely intracellular (*6*). In the case of the Src-like tyrosine kinase Lck, which is specifically expressed in T cells, the NH_2-terminal region of the kinase associates with the short cytoplasmic tails of the cell adhesion molecules CD4 and CD8 (*7*). In addition, Src and the related kinases Fyn and Yes physically associate with, and are phosphorylated by, the β-PDGFR (*8*). PDGF stimulation is associated with a three- to five-fold increase in Src kinase activity, which may serve to amplify the tyrosine kinase signal (*8, 9*). Hence the Src-like kinases also appear to participate in signal transduction.

Many structural alterations have been documented for both receptor-like and cytoplasmic tyrosine kinases, which induce constitutive tyrosine kinase activity and simultaneously activate oncogenic potential (*1, 10*). The biological activities of transforming tyrosine kinases, like their normal counterparts, are generally dependent on their kinase activity. A logical conclusion of these observations is that activated tyrosine kinases phosphorylate intracellular proteins, which in turn regulate signaling pathways that control gene expression, cell division, cytoskeletal architecture, and cell metabolism. Identifying the immediate targets of tyrosine kinases and defining the mechanisms by which they interact with tyrosine kinases and with one another are therefore critical to understanding the regulation of signal transduction by growth factors.

Association of Activated Receptor Tyrosine Kinases with Intracellular Signaling Proteins

After stimulation with PDGF or EGF several proteins become physically associated with, and phosphorylated by, the activated PDGFR or EGF receptor (EGFR). A number of these receptor-binding proteins have been identified, including phospholipase C(PLC)–γ1 (*11*), $p21^{ras}$ GTPase-activating protein (GAP) (*5, 12*), phosphatidylinositol (PI) 3′-kinase (PI3K) (*4*), Src and Src-like tyrosine kinases (*8*), and Raf (*13*). These associated proteins are likely targets of receptor activity.

PLC-γ1 is one of several PLC isoforms that cleaves the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) to the second messengers diacyglycerol and inositol triphosphate, which in turn stimulate protein kinase C and raise intracellular calcium (*14*). PDGF stimulates PI turnover in cells where PLC-γ1 is the principal PLC isoform (*11*), and overexpression of PLC-γ1 enhances the

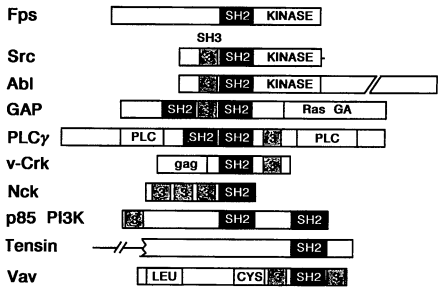

Fig. 1. The SH2 family of signaling proteins. 3, SH3 domain; Ras GA, Ras GTPase activating region of GAP; PLC, catalytic sequences of PLC-γ1; gag, retroviral coat protein sequence; CYS, cysteine-rich domain of Vav; LEU, leucine-rich region of Vav.

accumulation of inositol phosphates in response to PDGF (*15*). Thus, PLC-γ may couple PDGF stimulation to the breakdown of PIP_2.

GAP stimulates the ability of $p21^{ras}$ (Ras) to hydrolyze GTP to GDP (guanosine disphosphate) (*16*) and thereby acts as a negative regulator by returning Ras from the active GTP-bound state to the inactive GDP-bound conformation. Because GAP interacts with the effector region of Ras, which is thought on genetic grounds to contact the downstream Ras target, a GAP-associated activity or protein may be the Ras target (*17*). The notion that Ras might lie downstream of tyrosine kinases in a mitogenic signaling pathway was first suggested by the effects of microinjecting antibodies to Ras into cells. Antibodies that neutralize the biological activity of Ras proteins also block the mitogenic effects of PDGF and serum and transiently revert the phenotype of *src*-transformed cells (*18*). PDGF stimulation or v-*src* transformation increase the amount of cellular Ras in the GTP-bound form by two- to threefold (*19*). GAP is a candidate for the molecule that couples tyrosine kinases to Ras activation, because it is a substrate for a number of activated tyrosine kinases (*20, 21*). Tyrosine kinases also induce GAP to complex with two other tyrosine phosphorylated proteins (p62 and p190), which might be regulators or targets of Ras (*21–23*). Indeed GAP associated with p190 in v-*src*–transformed cells is impaired in its ability to promote Ras GTPase activity (*23*). Inhibition of GAP activity should increase the fraction of Ras in the GTP-bound state.

PI3K phosphorylates the inositol ring of PI in the D-3 position (*24*). PI3K activity is associated with a variety of activated tyrosine kinases and correlates with the presence of a tyrosine phosphorylated 85-kilodalton (kD) protein (p85) (*25*). Purified PI3K is a heterodimeric complex that contains p85 and a 110-kD protein (p110) (*26*). The purified p85 subunit has no detectable PI3K activity, but binds tightly to activated PDGFR or EGFR in vitro. This suggests that p85 may be a regulatory subunit responsible for receptor binding, whereas p110 is probably the catalytic element (*27*). PDGF stimulation induces accumulation of PI-3,4-P_2 and PI-3,4,5-P_3, confirming that PI3K is regulated by tyrosine kinases in vivo (*28*).

Raf is a protein-serine/threonine kinase that complexes with the PDGFR after PDGF stimulation, although it is unclear whether this is a direct interaction (*13*). In addition to these proteins, several unidentified polypeptides bind to activated PDGFR (*4, 29*).

Signaling and Transforming Proteins That Share Noncatalytic SH2 and SH3 Domains

The proteins that associate with activated PDGFR have quite different enzymatic properties and are structurally distinct within their catalytic regions. However, with the exception of Raf, they share conserved noncatalytic domains termed Src homology (SH) regions 2 and 3 (Fig. 1). The SH2 domain is a sequence of ~100 amino acids, originally identified in the v-Fps and v-Src cytoplasmic tyrosine kinases, by virtue of its effects on both catalytic activity and substrate phosphorylation (*6, 30*). Several v-Fps and v-Src mutants with alterations in the SH2 domain are temperature-sensitive, defective, or host-dependent in their transforming activities (*30–38*). These genetic and biochemical data have implicated the SH2 domain in regulating protein-protein interactions. Additional clues to the functions of SH2 domains came from the sequences of GAP (*39*) and PLC-γ1 (*40*), each of which contains two closely spaced SH2 domains (Fig. 1). This suggested that SH2 domains might be a common feature of proteins that bind activated receptor tyrosine kinases. Consistent with this prediction, the p85 subunit of PI3K also possesses two SH2 domains (*27, 41, 42*). More direct evidence that SH2 domains might mediate protein-protein interactions was provided by the v-*crk* retroviral oncogene. The $P47^{gag\text{-}crk}$ oncoprotein contains an SH2 domain, but lacks any identifiable catalytic region (*43*). $P47^{gag\text{-}crk}$ has the remarkable property of forming stable complexes with several tyrosine phosphorylated proteins in v-*crk*–transformed fibroblasts (*43–47*). As discussed below, SH2 domains apparently regulate protein-protein interactions by recognizing peptide sequences that encompass tyrosine phosphorylation sites. Tyrosine phosphorylation of the relevant ligand acts as a switch to induce high-affinity SH2 binding.

Most SH2-containing proteins also possess a distinct motif of ~45 amino acids termed the SH3 domain (*43*). SH3-like sequences have been identified in a variety of proteins that comprise or associate with the cytoskeleton and membrane (*48*). These include non-erythroid α-spectrin (*49*), several isoforms of myosin 1b, and a yeast actin binding protein (ABP1p) implicated in the regulation of cytoskeletal assembly (*50*). These cytoskeletal proteins lack SH2 domains, indicating that SH2 and SH3 are not obligate partners. SH3 domains have also been identified in two neutrophil NADPH oxidase–associated proteins p47 and p67 (*48, 51*), which are each defective in distinct forms of chronic granulomatous disease, in the *Saccharomyces cerevisiae* proteins CDC25, IRA1, and FUS1, which control cell growth and nuclear fusion (*48*), and in a presumptive human transcription factor HS1 (*51*). Several proteins, such as p47, p67, and the normal c-*crk* gene product (*52*) contain multiple SH3 domains. The function of the SH3 domain is not clear; however, its identification in an eclectic group of proteins that share the common property of associating with membranes implies a role in subcellular localization. If so, it is intriguing to note that SH2 domains may be important in regulating the cytoskeleton. The SH2 domain of v-Src is required for its association with the detergent insoluble cellular matrix (*53*). Furthermore, tensin, a cytoskeletal protein that links actin filaments to the membrane in adhesion plaques, contains an SH2 domain (*54*). This finding suggests that tensin may mediate some of the effects of tyrosine kinases on cytoskeletal architecture and cell-substrate interaction. It seems likely that SH2 and SH3 domains, acting in combination, allow multimeric protein complexes to form at a subcellular location appropriate to the activation of specific signal transduction pathways.

Subfamilies of SH2 Domains

The sequences of several known SH2 domains are aligned in Fig. 2. A number of residues are invariant (*) or highly conserved (shaded). An inspection of the aligned SH2 sequences reveals the presence of five well-conserved sequence motifs (designated I to V in Fig. 2), which are separated by more variable sequence elements (i to iv). The variable regions generally contain one or more glycine or proline residues, suggesting that they form turns or hinges that connect the conserved subdomains. Because it is useful to divide the

SH2 domain into subregions, a system based on conserved motifs appears to be most logical. The majority of reported mutations that affect SH2 biological activity, usually measured in the context of the transforming activities of cytoplasmic tyrosine kinases or v-Crk, alter conserved residues in motifs I to IV.

Construction of a phylogenetic tree indicates that SH2 domains fall into subfamilies whose members are particularly closely related. These relations are consistent with the idea that an ancestral SH2 domain was duplicated and acquired by different genes during evolution, with the most similar SH2 domains having diverged most recently. The similarities of sequence and function displayed by the different SH2 domains suggests that they are indeed homologous, rather than analogous, structures. Clearly the sequence differences between distinct SH2 domains could affect the affinity with which they bind different tyrosine phosphorylated ligands. As discussed below, it is apparent that different SH2 domains do have distinct binding specificities.

Binding of SH2 Domains to Autophosphorylated Growth Factor Receptors

The formation of stable complexes between growth factor receptors and SH2-containing proteins such as PLC-γ1, GAP, or Src is dependent on prior growth factor binding and is generally potentiated by autophosphorylation (*4, 5, 8, 11, 12*). SH2 and SH3 domains are the only common features of a variety of polypeptides that bind activated receptors and therefore are candidates for mediating these associations. In addition, PLC-γ and GAP belong to larger gene families, whose other members lack SH2 and SH3 domains and apparently do not interact with tyrosine kinases (*11, 14, 55*). Direct evidence that the SH2 domains of cytoplasmic signaling proteins such as PLC-γ1, GAP, Src, and Crk are sufficient for in vitro binding to activated growth factor receptors has been obtained (*56, 57*). In the case of both GAP and PLC-γ1, the NH_2-terminal SH2 domain binds more strongly to activated receptors than the COOH-terminal domain, and the two SH2 domains together bind synergistically to the PDGFR (*56*). This may represent cooperative binding to multiple sites within the activated receptors. Similar properties are likely to hold for the two SH2 domains of the p85 subunit of PI3K, although this has not been directly tested. The PLC-γ1 SH3 domain, in contrast, does not have independent receptor-binding activity.

Where are the SH2 binding sites on activated receptors located? The β-PDGFR, α-PDGFR, the macrophage colony-stimulating factor (CSF-1) receptor, c-Kit, and a number of other receptor-like tyrosine kinases, contain an "insert" within the kinase domain that is dispensable for kinase activity and likely forms a loop on the surface of the protein (*1, 4, 5, 58, 59*). The human β-PDGFR contains at least two sites of autophosphorylation, one at Tyr^{751} within the kinase insert and a second at Tyr^{857} in the kinase domain (*4*). An additional autophosphorylation site in the kinase insert at Tyr^{740} has been predicted, although not formally demonstrated (*60, 61*). When the autophosphorylation site at Tyr^{751} within the β-PDGFR kinase insert is replaced by phenylalanine or the insert is deleted, the activated receptor loses its ability to bind PI3K in vivo and in vitro (*4, 12*). Conversely, a short peptide that corresponds to a sequence in the mouse β-PDGFR kinase insert that contains the mouse equivalents of Tyr^{740} and Tyr^{751} can block PI3K binding to autophosphorylated β-PDGFR, provided that the peptide contains phosphotyrosine at either position (*60*). These results imply that the SH2 domains of the p85 PI3K subunit recognize a short peptide sequence within the PDGFR kinase insert. The affinity of PI3K for this site is low when it is unphosphorylated, but phosphorylation of the relevant tyrosine induces high-affinity binding of the p85 SH2 domains. A possible consensus sequence for PI3K binding has been proposed (*61*).

The SH2 domains of PI3K, GAP, and PLC-γ do not recognize identical sites on the β-PDGFR. For example, binding of GAP is reduced by alteration of Tyr^{751} in the insert, but is also impaired by substitution of Tyr^{857} with phenylalanine, which has no obvious effect on PI3K binding (*5*). Binding of PLC-γ1 to the PDGFR is not affected by partial deletion of the kinase insert, which entirely abrogates PI3K association (*62*). Further, the tyrosine phosphoryl-

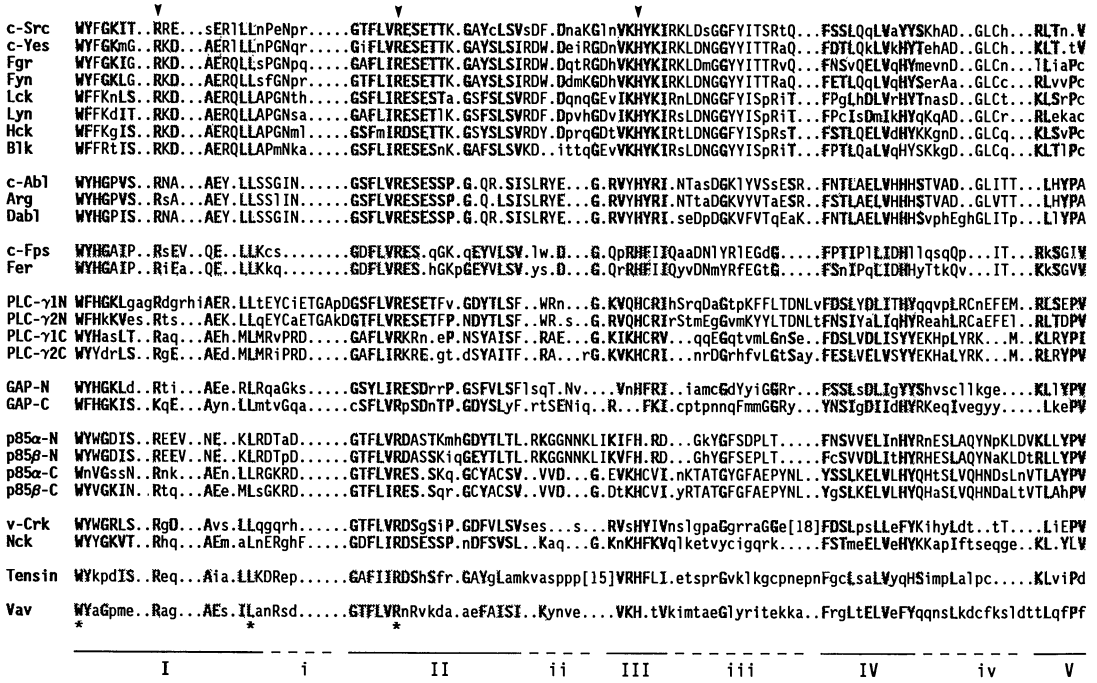

Fig. 2. Amino acid sequences of SH2 domains (*87*). SH2 domains are grouped into subfamilies of closely related sequences. Residues that are conserved within at least three subfamilies of SH2 domains are capitalized and shaded. Residues that are conserved within one or two groups are capitalized. Residues that are poorly or not at all conserved are in lowercase. Invariant residues are indicated by asterisks. Conserved basic amino acids that might participate in interactions with phosphotyrosine are arrowed. Conserved motifs I to V are indicated by solid lines, whereas the connecting variable regions i to iv are indicated by broken lines. The suffix N indicates the more NH_2-terminal SH2 domain of PLC-γ, GAP or p85 whereas C indicates the more COOH-terminal domain. The SH2 domain of two isoforms of PLC-γ (γ1 and γ2) and p85 (α and β) are shown (*27*). Sequences were aligned by eye. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

ated peptide that blocks PI3K binding to the β-PDGFR has no effect on GAP and PLC-γ association with the receptor (*60*). Although the SH2 domains of PI3K, GAP, and PLC-γ1 all probably recognize tyrosine phosphorylated sites, their different binding properties suggest that the amino acids that surround the phosphorylated tyrosine confer specificity for distinct SH2 domains. The sequence alignment of SH2 domains (Fig. 2) reveals highly conserved residues that are likely to participate in interactions with phosphotyrosine and in protein folding. The variable residues might interact with amino acids adjacent to the phosphotyrosine and hence be responsible for specificity.

The EGFR has no kinase insert, but has a long COOH-terminal tail that contains several sites of tyrosine autophosphorylation. Full-length PLC-γ1 or a polypeptide that contains only the GAP SH2 domains binds in vitro to a COOH-terminal EGFR phosphopeptide that contains four of these tyrosine phosphorylated residues but lacks the catalytic domain (*63*). Hence, the EGFR also has a noncatalytic tyrosine phosphorylated domain that binds cytoplasmic SH2-containing signaling proteins (Fig. 3). The finding that autophosphorylation sites on activated growth factor receptors serve as magnets to attract signaling proteins explains a long-standing puzzle. That is, in cells stimulated with growth factors or transformed with oncogenic tyrosine kinases, one of the most highly tyrosine phosphorylated proteins is usually the activated tyrosine kinase itself.

These results suggest the following scheme for the interactions of PLC-γ1 with the EGFR (Fig. 3), which can probably be generalized for the binding of other SH2 proteins to receptors. Addition of EGF induces receptor autophosphorylation, which in turn creates high-affinity binding sites for the PLC-γ1 SH2 domains. Autophosphorylated tyrosines may interact directly with conserved positively charged residues within the PLC-γ1 SH2 domain. Several basic amino acids are highly conserved within SH2 domains (Fig. 2). The SH2 domain probably also contacts residues adjacent to phosphotyrosine. Association between the receptor and PLC-γ1 may account for the redistribution of PLC-γ1 from the cytosol to the membrane (*64*), a phenomenon that has also been observed for GAP in PDGF-stimulated or v-*src*–transformed cells (*20, 23*). In vivo, it is likely that recruitment of PLC-γ1 to the membrane, where its phospholipid substrate is located, is important for activation of PI turnover. Similar considerations apply to P13K (*65*). The association of PLC-γ1 with the EGFR precedes PLC-γ1 tyrosine phosphorylation (*12, 28, 56, 66*). Bound PLC-γ1 is then phosphorylated by the receptor, leading to stimulation of PLC activity and PIP_2 hydrolysis. In vitro phosphorylation of immunoprecipitated PLC-γ1 by the EGFR induces PLC activity (*67*). The EGFR phosphorylates PLC-γ1 on tyrosine residues 472, 771, 783, and 1254 in vitro, of which Tyr^{771} and Tyr^{783} are phosphorylated most rapidly (*68*). Tyr^{771} and Tyr^{783} are located immediately COOH-terminal to the second PLC-γ1 SH2 domain. Phosphorylation of these residues might directly affect catalytic activity; alternatively, phosphorylation might modulate the interactions of PLC-γ1 with regulatory proteins that bind to the SH2 or SH3 domains.

Binding of SH2 Domains to Cytoplasmic Tyrosine Phosphorylated Proteins

The binding of SH2 domains to activated growth factor receptors is a specific example of a more general ability to interact with tryosine phosphorylated proteins, as exemplified by the $P47^{gag\text{-}crk}$ oncoprotein. The *crk*-derived sequences of $P47^{gag\text{-}crk}$ are restricted to an SH2 and an SH3 domain (Fig. 1). Despite the absence of a tyrosine kinase domain in Crk, the tyrosine phosphorylation of

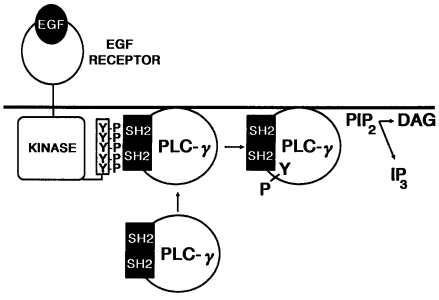

Fig. 3. Model for the interactions of PLC-γ1 with the activated EGF receptor.

cellular polypeptides of 60, 70, 120, and 135 to 155 kD is elevated in v-*crk*–transformed cells. In addition, these proteins are complexed with $P47^{gag\text{-}crk}$ in lysates of transformed cells (*43–47*). A group of similarly sized tyrosine phosphorylated proteins associated with $P47^{gag\text{-}crk}$ in v-*crk*–transformed rat cells (*47*). The Crk SH2 domain is both necessary and sufficient for binding. Mutant v-Crk proteins with alterations in SH2 are defective in both transforming activity and complex formation (*46*). Furthermore, proteins that contain the Crk SH2 domain complex in vitro with several tyrosine phosphorylated proteins from normal fibroblasts or v-*crk*– or v-*src*–transformed cells. These proteins appear similar or identical to those associated with $P47^{gag\text{-}crk}$ in vivo (*45, 47, 57*). Although the v-Crk SH2 domain can associate with multiple tyrosine phosphorylated proteins, each individual SH2 domain probably engages a single ligand.

Hanafusa and colleagues have shown that $P47^{gag\text{-}crk}$ will complex with $p60^{v\text{-}src}$ in vitro and that this association is dependent on $p60^{v\text{-}src}$ tyrosine phosphorylation (*47*). Hence, interaction of $P47^{gag\text{-}crk}$ with specific cellular proteins apparently requires the Crk SH2 domain and is likely dependent on the phosphorylation of Crk ligands. The mechanism by which v-Crk enhances the tyrosine phosphorylation of cellular proteins is not known. $P47^{gag\text{-}crk}$ apparently disturbs an equilibrium of phosphorylation and dephosphorylation, either by activating an endogenous tyrosine kinase or blocking the action of a tyrosine phosphatase. The former possibility is consistent with the observation that tyrosine kinase activity is associated with $P47^{gag\text{-}crk}$ in v-*crk*–transformed cells (*44*). The v-Crk SH2 domain may therefore transactivate the catalytic domain of a cellular tyrosine kinase and enhance its ability to phosphorylate SH2-binding substrates. Alternatively, by complexing with specific tyrosine phosphorylated ligands, v-Crk may protect them from dephosphorylation or proteolysis, and hence increase their intracellular concentration. These two models are not mutually exclusive. Several of the v-*crk*–associated proteins also become tyrosine phosphorylated in v-*src*–transformed cells (*69*), suggesting that they are common targets of the v-Crk and v-Src.

$P47^{gag\text{-}crk}$ is not the only SH2-containing protein with the potential to stably associate with a spectrum of tyrosine phosphorylated polypeptides. GAP, Src, and Crk SH2 domains bind with different efficiencies to a similar set of phosphotyrosine-containing proteins in lysates of v-*src*–transformed cells (*57, 70*). The GAP SH2 domain interacts efficiently with p62 in vitro, and intact GAP is associated with p62 in vivo. The Src and Crk SH2 domains complex more tightly with a 130-kD protein (*57, 70*) that likely corresponds to a tyrosine phosphorylated protein with which $p60^{v\text{-}src}$ is stably associated in *src*-transformed cells (*71*). These results underline the concept that the sequence differences between SH2 domains may result in distinct, albeit overlapping, binding specificities.

Recombinant Abl SH2 domain binds a wide range of tyrosine phosphorylated proteins from lysates of *abl*-transformed mouse fibroblasts (*72*). This binding can be detected even after the phosphoproteins have been separated by electrophoresis on a denaturing

SDS-polyacrylamide gel and transferred to a filter. The ability of the Abl SH2 domain to bind denatured proteins is consistent with the idea that SH2-binding sites are formed by short linear peptide sequences that contain tyrosine phosphorylation sites, rather than by conformational determinants. Similar results have been obtained for the GAP, Crk, and Src SH2 domains.

SH2 and SH3 Adaptor Proteins

The p85 subunit of PI3K is a prototype for a class of SH2- and SH3-containing proteins that lack obvious catalytic domains. p85 associates with activated receptors (probably through its SH2 domains) and with p110, the presumptive PI3K catalytic subunit (*26*). Hence p85 links tyrosine kinases to the PI3K catalytic domain. In this arrangement, a single SH2 adaptor might potentially interact with multiple targets, and it is therefore more flexible than one in which the catalytic domain is covalently attached to SH2 and SH3 domains, as in GAP and PLC-γ1. The v-Crk oncoprotein acts in a similar fashion to p85 in the sense that it functions as an adaptor to juxtapose an activated tyrosine kinase with target proteins. In addition to p85, a number of small proteins that contain SH2 and SH3 domains unattached to any known catalytic domain have been described. These include the c-*crk* gene product, which is very similar in structure to v-Crk, with the exception that it contains an additional COOH-terminal SH3 domain (*52*). The *nck* human complementary DNA (cDNA) encodes a small protein that like c-Crk contains SH2 and SH3 domains but has no evident catalytic sequences (*73*). The *SHC* cDNA, which was cloned from a leukemic cell line by virtue of its sequence similarity to the *fps* SH2 coding region, encodes a small protein with a COOH-terminal SH2 domain and an NH_2-terminal region related in sequence to collagen (*74*). The functions of these proteins are unknown. However, the precedents set by p85 and v-Crk suggest that they control the association of tyrosine kinases and presently unidentified targets during signal transduction. These proteins could therefore act as a kind of molecular glue to promote the formation of signaling complexes.

The SH2 and SH3 Domains of Cytoplasmic Tyrosine Kinases

The SH2 domains of the Src, Abl, and Fps cytoplasmic tyrosine kinases have multiple functions. They interact with their own kinase domains to modulate catalytic activity and are involved in substrate recognition. The function of the Src SH2 domain may be similar to that proposed above for Crk, with the exception that in Src the SH2 domain is already tethered to a kinase domain.

Evidence exists that suggests the SH2 domain of Src-like kinases contributes to the repression of kinase activity. c-Src enzymatic and transforming capacity can be modestly increased by the substitution of conserved residues in regions I, II, or IV (Fig. 2) (*37, 75*), although an extensive deletion within the c-Src SH2 and SH3 domains is not activating (*76*). Similarly, human Fyn is weakly activated by a deletion that encompasses regions III to V (*77*). Conversely, in v-Fps or v-Src the SH2 domain generally has a positive effect on kinase activity, is required for the phosphorylation and binding of specific substrates, and stimulates transforming activity (*30, 32, 33, 36*). The effects of SH2 on kinase activity can be rationalized if the SH2 domain can recognize tyrosine phosphorylated sites within or adjacent to the kinase domain (*34, 47*). c-Src kinase activity could then be repressed if SH2 folds over the kinase domain and binds a site that contains phosphorylated Tyr^{527} (Fig. 4A). Dephosphorylation of Tyr^{527}, which activates c-Src, would then release the SH2 domain to interact with substrates or autophosphorylated Tyr^{416}, and would also derepress the kinase domain (Fig. 4, B and C). Consistent with this model, the NH_2-terminal region of Src is required for the phosphorylated COOH-terminal tail to exert its regulatory effects (*78*).

The SH2 and SH3 domains of activated Src are also required for the recognition of specific substrates. Mutant v-Src or v-Fps proteins with deletions in SH2 regions I or II do not induce tyrosine phosphorylation of p62 or GAP, although they retain fairly high kinase activity (*22, 34, 57*). One explanation for these observations is that the Src SH2 domain recognizes unphosphorylated p62 and thereby acts as a substrate binding site (Fig. 4C). Alternatively, the Src SH2 domain might stabilize phosphorylated p62, perhaps by preventing its rapid dephosphorylation. Once it is tyrosine phosphorylated, p62 binds stably to the NH_2-terminal GAP SH2 domain (*22, 57*), suggesting a network of interactions in which SH2 ligands can interact with multiple SH2-containing proteins.

Activated Src forms stable complexes with at least two phosphoproteins, of 130 (p130) and 110 kD (p110), whose association with Src is dependent on their tyrosine phosphorylation (*71*) (Fig. 4B). (There is no evidence that this p110 corresponds to the P13K catalytic subunit.) p130 binds to the SH2 domain, while p110 apparently associates with SH3, although it also requires SH2 for stable binding. One function of these complexes might be to retain phosphorylated p110 and p130 at the plasma membrane; formation of a ternary Src-p110-p130 complex might allow p130 and p110 to interact with one another. Mutations in v-Src SH2 that inhibit the phosphorylation or binding of p62, p130, p110, and GAP also impair transforming activity, suggesting that the phosphorylation and interactions of these proteins contributes to the transformed phenotype (*32, 34, 57, 71*). Unlike wild-type v-Src, which induces a collapse of the cytoskeletal architecture, mutant v-Src proteins with alterations in SH3 fail to completely depolymerize the actin filaments (*79, 80*). The tyrosine phosphorylation of p110 and its association with v-Src SH3 may contribute to the destabilization of the cytoskeleton. p110 in normal cells appears to be weakly associated with actin filaments, whereas v-*src* transformation induces a substantial fraction of tyrosine phosphorylated p110 to relocate to the plasma membrane, probably in a complex with v-Src (*71, 81*).

The Src SH2 and SH3 domains apparently stimulate the phosphorylation of specific proteins, which subsequently bind with high affinity either to Src itself or to other SH2- and SH3-containing

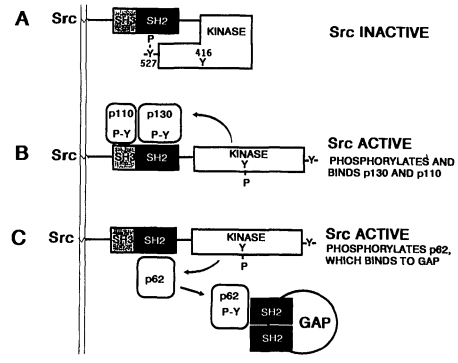

Fig. 4. Possible protein-protein interactions of Src SH2 and SH3 domains. These are models designed to rationalize a large body of data and should be taken as speculative. (**A**) The Src SH2 domain is suggested to interact in cis with phosphorylated Tyr^{527}, a site of negative regulation, thereby repressing kinase activity. (**B**) Dephosphorylation of Tyr^{527} leads to a conformational change in the interactions of the Src SH2 and kinase domains with a consequent stimulation of kinase activity. The activated kinase domain phosphorylates proteins such as p130 and p110, which subsequently bind with high affinity to the SH2 and SH3 domains, respectively. (**C**) The Src SH2 domain is also required for the tyrosine phosphorylation of p62, possibly acting as a substrate binding site. Tyrosine phosphorylated p62 subsequently binds with high affinity to the NH_2-terminal GAP SH2 domain.

proteins. The ability of a noncatalytic domain within a protein kinase to recognize a substrate and bind the modified substrate in a stable complex is unusual. These findings suggest that Src has at least two important functions. One is to phosphorylate proteins that regulate cell phenotype; the second is to promote the formation of membrane-associated complexes that contain these signaling proteins.

The Future: Protein-Protein Interactions in Signal Transduction

The binding of a growth factor such as PDGF to its receptor induces a ferment of intracellular activity at the plasma membrane and in the cytoplasm, resulting in the stimulation of multiple signaling pathways. A principal mechanism by which these events are controlled appears to be the relocalization of cytoplasmic signaling proteins into heteromeric membrane-associated complexes, whose formation is directly mediated by SH2 and possibly SH3 domains. We suggest that a primary function of tyrosine phosphorylation is to regulate the affinity with which these signaling proteins interact. Tyrosine phosphorylation thereby acts as a switch to induce the assembly of signaling complexes.

While this is an attractive hypothesis, there are several problems that must be resolved before it can be substantiated. Foremost among these involves the precise mechanisms by which signaling pathways are activated. For PLC-γ1, tyrosine phosphorylation is important for stimulation of PI turnover, but the details of this activation are obscure. Even less is known about the GAP-Ras and PI3K pathways; the means by which they are activated by tyrosine kinases are unclear, as are the downstream targets of Ras-GTP or PI-3-P. In addition, we have no information as to the functions of proteins such as Crk, Nck, and SHC in normal cells. As is often the case, the identification of SH2 and SH3 family members may proceed more rapidly than their biological characterization. The cDNA cloning of SH2 proteins has been facilitated by a technique devised by Schlessinger and colleagues (*42*). In this approach, the autophosphorylated COOH-terminal tail of the EGFR, which binds with high affinity to SH2 domains (see above), has been used to screen a cDNA expression library, resulting in the isolation of cDNAs for both known and novel SH2 proteins. It is possible that this procedure could be applied to other receptor tyrosine kinases as a means of identifying their targets.

There is little direct evidence that the protein complexes observed after cell lysis or formed in vitro with bacterially expressed SH2 and SH3 polypeptides actually exist in the intact cell, nor is it certain that these complexes are critical to signal transduction in the living cell. In some cases, however, receptor mutations that impair SH2 binding also block signal transduction; the most striking example is the substitution of Tyr^{751} in the β-PDGFR kinase insert, which coordinately reduces PI3K and GAP binding and impairs mitogenesis in response to PDGF. These issues can also potentially be addressed by the introduction of SH2 and SH3 antibodies or peptides into cells, which might inhibit the formation of endogenous complexes and disturb intracellular signaling. The interactions of SH2 domains with receptors and other tyrosine phosphorylated ligands might be an effective point at which to block signal transduction. To address this possibility, it is important to know the structures of SH2 and SH3 elements. The SH2 and SH3 domains are sufficiently small that their three-dimensional structures, with or without ligands, should be readily amenable to either x-ray crystallography or nuclear magnetic resonance. The sequences of SH2 domains, with conserved motifs interspersed with glycine and proline-rich elements, suggest that SH2 domains may have a mobile, floppy structure which may become fixed upon ligand binding.

There appears to be considerable specificity to the signal transduction pathways activated by different growth factor receptors. For example, the activated CSF-1R binds PI3K and induces the production of PI-3-P, but fails to bind PLC-γ1 or GAP, and apparently does not stimulate hydrolysis of PI-4,5-P_2 (*82, 83*). In contrast, activated c-Kit binds PI3K and PLC-γ1, but not GAP (*84*), whereas the β-PDGFR binds all three of these SH2 proteins. If distinct SH2 domains recognize phosphotyrosine in the context of different surrounding amino acids, then the potential of a receptor to bind signaling proteins will be dictated by the sequences around its autophosphorylation sites. Hence, the diversity in the cellular responses to growth factors is likely to be regulated by the affinities with which different SH2 domains and receptors interact. The results discussed in this review provide a preliminary conceptual framework for considering the mechanisms by which protein-protein interactions are regulated in the cytoplasm during signal transduction. In addition, SH2 and SH3 domains have been identified in the *vav* proto-oncogene product (Figs. 1 and 2) (*85*), a protein that has several motifs found in transcription factors (*86*). There is clearly much to discover about the functions of SH2 and SH3 domains.

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