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# Src homology region 2 domains direct protein–protein interactions in signal transduction

(Src protein homology/p21$^{ras}$ GTPase-activating protein/v-*crk*/epidermal growth factor receptor/tyrosine phosphorylation)

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**ABSTRACT** **Cytoplasmic proteins that regulate signal transduction or induce cellular transformation, including cytoplasmic protein-tyrosine kinases, p21$^{ras}$ GTPase-activating protein (GAP), phospholipase C$\gamma$, and the v-*crk* oncoprotein, possess one or two copies of a conserved noncatalytic domain, Src homology region 2 (SH2). Here we provide direct evidence that SH2 domains can mediate the interactions of these diverse signaling proteins with a related set of phosphotyrosine ligands, including the epidermal growth factor (EGF) receptor. In *src*-transformed cells GAP forms heteromeric complexes, notably with a highly tyrosine phosphorylated 62-kDa protein (p62). The stable association between GAP and p62 can be specifically reconstituted *in vitro* by using a bacterial polypeptide containing only the N-terminal GAP SH2 domain. The efficient phosphorylation of p62 by the v-Src or v-Fps tyrosine kinases depends, in turn, on their SH2 domains and correlates with their transforming activity. In lysates of EGF-stimulated cells, the N-terminal GAP SH2 domain binds to both the EGF receptor and p62. Fusion proteins containing GAP or v-Crk SH2 domains complex with similar phosphotyrosine proteins from *src*-transformed or EGF-stimulated cells but with different efficiencies. SH2 sequences, therefore, form autonomous domains that direct signaling proteins, such as GAP, to bind specific phosphotyrosine-containing polypeptides. By promoting the formation of these complexes, SH2 domains are ideally suited to regulate the activation of intracellular signaling pathways by growth factors.**

The Src homology region 2 (SH2) domain was initially identified as a noncatalytic region of ≈100 amino acids that is conserved among cytoplasmic (nonreceptor) tyrosine kinases (1, 2). Genetic and biochemical analysis of the Fps and Src tyrosine kinases suggests that their SH2 domains interact with and regulate the adjacent kinase domain and may also form binding sites for proteins phosphorylated by the kinase domain (3–10). Consistent with this view, the v-Crk retroviral oncoprotein, which consists primarily of an SH2 domain (11, 12), activates an endogenous tyrosine kinase and forms complexes with several phosphotyrosine proteins in v-*crk*-transformed cells (11, 13, 14). The v-*crk* gene product also possesses a distinct motif, Src homology region 3 (SH3), found in a number of cytoskeletal and SH2-containing proteins (11, 12, 15, 16) (Fig. 1).

Phospholipase C$\gamma$ (PLC$\gamma$) and p21$^{ras}$ GTPase-activating protein (GAP) each contain two adjacent SH2 domains related in structure to one another and to the SH2 domains of tyrosine kinases (9, 17–19). These enzymes occupy primary regulatory positions in signaling pathways. PLC$\gamma$ catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol triphosphate and, therefore, plays a central role in the activation of protein kinase C and the mobilization of intracellular calcium (20). GAP stimulates the intrinsic GTPase activity of p21$^{ras}$ and, hence, returns p21$^{ras}$ from the active GTP-bound state to the inactive GDP-bound form (21). GAP interacts with the presumed effector region of p21$^{ras}$ (22, 23), suggesting that it might also be the Ras target or might modify the association of p21$^{ras}$ with its target.

PLC$\gamma$ forms a physical complex with activated epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors (EGFRs and PDGFRs), is a substrate for receptor-catalyzed tyrosine phosphorylation, and apparently couples the PDGFR to phosphatidylinositol turnover (24–27). GAP is phosphorylated by a variety of oncogenic tyrosine kinases, as well as by the EGFR and PDGFR (28–31). GAP forms a complex with the PDGFR, which is dependent upon hormone-binding and receptor kinase activity and is potentiated by receptor autophosphorylation (30, 31). These results indicate that GAP may link tyrosine kinases to the Ras

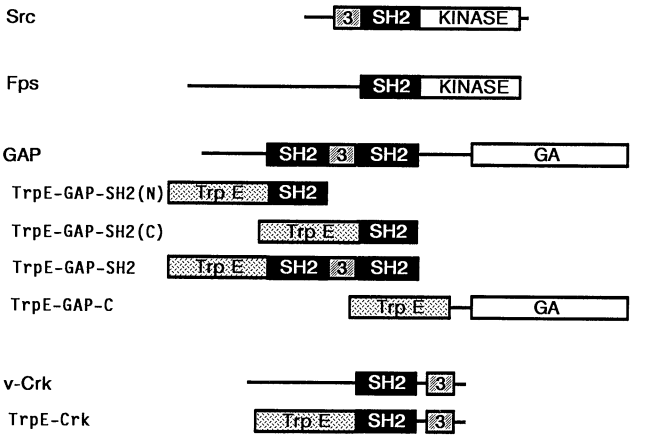


FIG. 1. Locations of SH2 and SH3 domains in signaling and transforming proteins. The catalytic domains of tyrosine kinases and GAP are indicated by open boxes. The bacterial TrpE fusion proteins employed in binding experiments are also illustrated. These contained the following residues: TrpE–GAP-SH2, human GAP 171–448; TrpE–GAP-SH2(N), GAP 178–278; TrpE–GAP-SH2(C), GAP 348–445; TrpE–GAP-C, GAP 670–1047; TrpE–v-Crk, p47$^{gag\text{-}crk}$ 206–327; TrpE–PLC$\gamma$, bovine PLC$\gamma$1 956–1291. 3, SH3 domain; GA, GTPase activating region of GAP.

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Abbreviations: GAP, GTPase-activating protein; PLC, phospholipase C; EGF, epidermal growth factor; EGFR, EGF receptor; PDGFR, platelet-derived growth factor receptor; SH2 and SH3, Src homology regions 2 and 3, respectively.

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signaling pathway. Activated tyrosine kinases that phosphorylate GAP also phosphorylate two proteins of 62 and 190 kDa (p62 and p190), which coimmunoprecipitate in a complex with GAP (28). Here we provide direct evidence that SH2 domains endow proteins such as GAP, v-Crk and $p60^{v\text{-}src}$ with the potential to form complexes with specific tyrosine phosphorylated ligands. We suggest that SH2 domains are critical in regulating the intracellular response to growth factors.

## MATERIALS AND METHODS

**Cell Culture and Antibodies.** Growth conditions, $^{32}P_i$ labeling, EGF treatment, and immunoprecipitation of R1hER (obtained from M. Weber, University of Virginia, Charlottesville), Rat-2, and Rat-2 cells expressing v-*src* or v-*fps* genes were as described (7, 9, 28). Polyclonal rabbit antibodies against human GAP residues 171–448 or phosphotyrosine have been described (28, 32). Anti-TrpE rabbit antiserum was raised against the N-terminal 323 residues of *Escherichia coli* TrpE protein. Rabbit antibodies directed against residues 1176–1186 of human EGFR (33) were a gift of B. Margolis and J. Schlessinger (New York University).

**Complex Formation with TrpE Fusion Proteins.** Restriction fragments from human GAP, bovine PLCγ, or v-*crk* cDNAs were subcloned into pATH bacterial TrpE expression vectors, using both natural and engineered restriction sites (ref. 28; D.A., unpublished results). Fifty-milliliter cultures of *E. coli* strain RR1 with the parental pATH expression plasmid or a derivative encoding one of the various TrpE fusion proteins were grown and induced with indole acrylic acid as described (34). Cells were washed with 1 ml of 50 mM Tris·HCl, pH 7.5/10% (wt/vol) sucrose. Collected cells were resuspended in 1 ml of ice-cold PLCLB (50 mM Hepes, pH 7.0/150 mM NaCl/10% glycerol/1% Triton X-100/1.5 mM $MgCl_2$/1 mM EGTA/100 mM NaF/10 mM $NaPP_i$/1 mM $Na_3VO_4$/1 mM phenylmethylsulfonyl fluoride/aprotinin and leupeptin each at 10 μg/ml), sonicated 6 times for 10 sec each, and clarified by centrifugation at 15,000 × *g* for 15 min. Sonication and all subsequent steps were done at 4°C. Supernatants were incubated with 40 μl of anti-trpE serum and 30 μl of protein A–Sepharose beads. After being gently mixed for 90 min, the immune complexes were washed 3 times with HNTG (20 mM Hepes, pH 7.0/150 mM NaCl/0.1% Triton X-100/10% glycerol/1 mM $Na_3VO_4$) and divided into four equal aliquots. Similar amounts of the different TrpE fusion proteins were detected in these immune complexes by immunoblotting with anti-TrpE antiserum.

For *in vitro* binding experiments, ≈5 × $10^6$ nonradioactive or $^{32}P$-labeled cells were lysed in 1 or 2 ml of PLCLB and clarified as described below. One milliliter of clarified lysate was incubated with one aliquot of an anti-trpE immune complex. After being mixed by gentle inversion for 90 min at 4°C, the immune complexes were recovered by centrifugation, washed three times with HNTG, resuspended in 40 μl of SDS sample buffer, and heated at 100°C for 3 min.

**Immunoblotting.** Cell lysates (prepared as in ref. 9; 25 μg of protein per lane), immunoprecipitates, and bacterial complexes were resolved by SDS/PAGE and transferred to nitrocellulose in a semi-dry blotting apparatus at 0.8 $mA \cdot cm^{-2}$ for 60 min. Blots were analyzed by autoradiography ($^{32}P$-labeled samples) or were blocked and then probed with anti-EGFR antiserum (1:200 dilution) or antiphosphotyrosine antibodies as described (9). Antiphosphotyrosine blots of whole-cell lysates were probed with 10 μCi of $^{125}I$-labeled protein A (2–10 μCi/μg; 1 Ci = 37 GBq; New England Nuclear), whereas all other blots were probed with 5 μCi of high-specific-activity $^{125}I$-labeled protein A (35 μCi/μg, Amersham). Blots were exposed to Kodak XAR film at −75°C with an intensifying screen.

## RESULTS

**GAP and Crk SH2 Domains Bind a Related Set of Phosphotyrosine-Containing Proteins.** The disposition of SH2 and SH3 domains within several signaling and transforming proteins is shown in Fig. 1. We initially used GAP to test whether these regions might be involved in protein–protein interactions. Different regions of GAP were expressed in bacteria as fusion proteins joined to a 37-kDa TrpE protein (Fig. 1). TrpE–GAP-SH2 contains almost precisely the two GAP SH2 domains and the intervening SH3 sequence. In contrast, TrpE–GAP-C contains the C-terminal half of GAP, including all residues required to stimulate $p21^{ras}$ GTPase activity (35). As controls we used the TrpE protein by itself and a TrpE–PLCγ fusion protein containing C-terminal PLCγ catalytic sequences. These TrpE fusion proteins were immunoprecipitated with anti-TrpE antiserum. To investigate whether these polypeptides could form specific complexes with proteins from *src*-transformed cells, the immunoprecipitates were incubated with a lysate of Rat-2 v-*src* cells and analyzed for associated proteins by immunoblotting with antiphosphotyrosine antibodies. TrpE, TrpE–PLCγ, and TrpE–GAP-C, which lack SH2 sequences, did not retain any phosphotyrosine-containing proteins from the Rat-2 v-*src* lysate. However, TrpE–GAP-SH2 bound a 62-kDa phosphotyrosine protein, as well as variable amounts of a 130-kDa protein (Fig. 2*A*). The 62-kDa protein comigrated with p62 immunoprecipitated with anti-GAP antibodies from Rat-2 v-*src* cells. As a more direct test of their binding activities, the TrpE fusion proteins were incubated with a lysate of Rat-2 v-*src* cells that had been metabolically labeled with $^{32}P_i$. Again, TrpE–GAP-SH2 specifically bound a 62-kDa phosphoprotein that comigrated with GAP-associated p62 (Fig. 2*A*). The same result was obtained using $^{32}P$-labeled v-*fps*-transformed cells (data not shown). Tryptic phosphopeptide analysis confirmed the identity of the 62-kDa SH2-binding protein as p62 (data not shown). p62 is not obviously related to $p60^{src}$ and lacks detectable protein kinase activity *in vitro* (C.A.K. and M.M., unpublished results). The 130-kDa protein that bound to TrpE–GAP-SH2 may correspond to a protein (p130) whose phosphorylation by activated $p60^{src}$ requires the Src SH2 domain, with which it complexes *in vivo* (refs. 10 and 36; C.A.K., unpublished results).

The binding sites for p62 and p130 were more precisely ascribed to the N-terminal SH2 domain of GAP (GAP-SH2(N), Fig. 1), which efficiently bound p62 and p130 from Rat-2 v-*src* cells (Fig. 2*B*). To investigate whether these phosphotyrosine-containing proteins might be more general ligands for SH2-containing proteins, similar experiments were done with a TrpE–v-Crk fusion protein (Fig. 1). TrpE–v-Crk also bound two phosphotyrosine-containing proteins when incubated with a Rat-2 v-*src* lysate, which likely correspond to p62 and p130 (Fig. 2*B*). TrpE–v-Crk bound p130 more efficiently than did TrpE–GAP-SH2 and also associated with a distinct 70-kDa tyrosine phosphorylated protein (p70). In lysates of normal Rat-2 cells TrpE–GAP-SH2 bound a small amount of p62, whereas TrpE–v-Crk formed more readily detectable complexes with p130 and p70 (Fig. 2*B*). It is of interest that phosphotyrosine-containing proteins of this size are associated with $p47^{gag\text{-}crk}$ in v-*crk*-transformed chicken embryo fibroblasts and bind bacterial v-Crk in lysates of v-*crk*-transformed cells (11, 13, 14). These results indicate that the GAP and Crk SH2 domains have distinct but overlapping binding specificities. They bind common phosphotyrosine-containing ligands but apparently with different efficiencies.

**The N-Terminal GAP SH2 Domain Binds Activated EGF Receptor *in Vitro*.** We investigated the binding activity of TrpE–GAP bacterial proteins in lysates of Rat-1 cells expressing the human EGFR (≈2.5 × $10^5$ per cell). No phos-

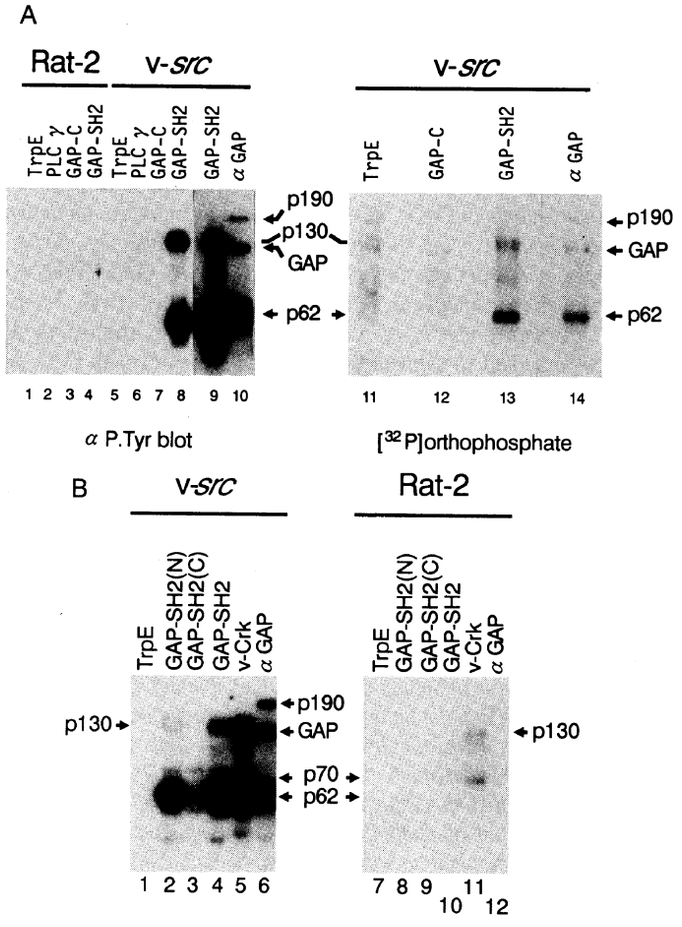


FIG. 2. GAP and v-Crk Src homology domains synthesized in bacteria complex *in vitro* with phosphotyrosine-containing proteins from v-*src*-transformed Rat-2 cells. (*A*) TrpE, TrpE–PLCγ, TrpE–GAP-C, or TrpE–GAP-SH2 fusion proteins immobilized with anti-TrpE antibodies were mixed with lysates of normal Rat-2 cells (lanes 1–4) or v-*src*-transformed Rat-2 cells (lanes 5–8) and analyzed for associated proteins by antiphosphotyrosine immunoblotting. Phosphoproteins bound to TrpE–GAP-SH2 from Rat-2 v-*src* cells (lane 9) are also compared directly with an anti-GAP immunoprecipitate from the same lysate (lane 10). Alternatively, lysates from $^{32}P_i$-labeled Rat-2 v-*src* cells were incubated with the indicated fusion proteins (lanes 11–13) or with anti-GAP antibodies (lane 14), and $^{32}P_i$-labeled proteins were visualized by autoradiography (*Right*). Exposure time was 3 hr, except for lane 14 (18 hr). P.Tyr, phosphotyrosine. (*B*) Immobilized TrpE (lanes 1 and 7), TrpE–GAP-SH2(N) (lanes 2 and 8), TrpE–GAP-SH2(C) (lanes 3 and 9), TrpE–GAP-SH2 (lanes 4 and 10), and TrpE–v-Crk (lanes 5 and 11) were incubated with lysates from Rat-2 v-*src* cells (lanes 1–6) or normal Rat-2 cells (lanes 7–12). For comparison, anti-GAP immunoprecipitations (lanes 6 and 12) were made from the same cell lysates. Samples were analyzed by immunoblotting with antiphosphotyrosine antibodies and $^{125}I$-protein A. Autoradiography was for 16 hr (lanes 1–6) or 72 hr (lanes 7–12).

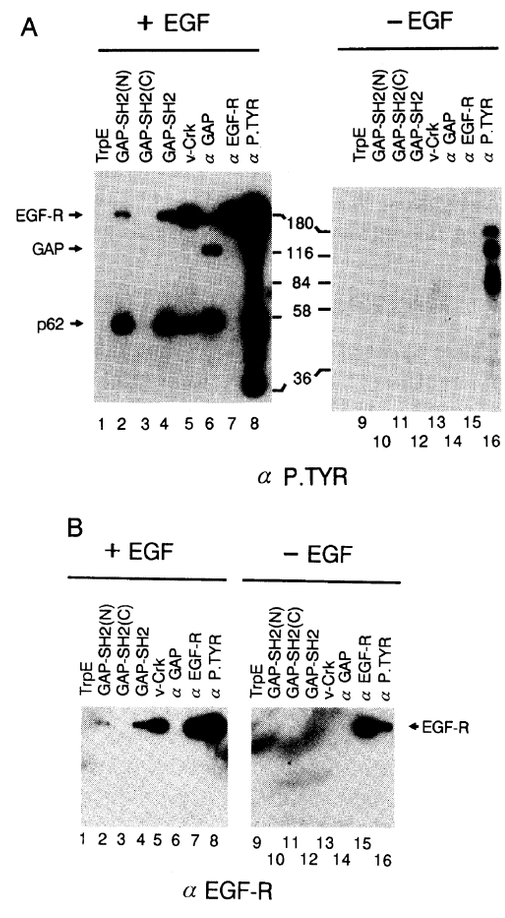


FIG. 3. EGF-dependent binding of GAP and Crk SH2 to the EGFR and p62. (*A*) Serum-starved (for 48 hr) Rat-1 cells overexpressing the human EGFR were stimulated with 0 (lanes 9–16) or 80 nM EGF (lanes 1–8) for 5 min at 37°C. Cell lysates were mixed with the indicated immobilized fusion proteins (lanes 1–5 and 9–13), or immunoprecipitated with anti-GAP (lanes 6 and 14), anti-EGFR (lanes 7 and 15), or antiphosphotyrosine (lanes 8 and 16) antibodies followed by antiphosphotyrosine immunoblotting. P.Tyr, phosphotyrosine. (*B*) Nitrocellulose filters containing duplicate samples of those in *A* were immunoblotted with anti-EGFR antibodies.

photyrosine-containing proteins associated with immobilized TrpE fusion proteins before EGF stimulation (Fig. 3*A*) or with TrpE–GAP-C after EGF addition (data not shown). However, TrpE–GAP-SH2, TrpE–GAP-SH2(N), and TrpE–v-Crk precipitated two tyrosine phosphorylated proteins from lysates of EGF-stimulated cells, with mobilities of 62 and 180 kDa (Fig. 3*A*). The 62-kDa protein comigrated with p62 precipitated from the EGF-stimulated lysate with anti-GAP antibodies. The 180-kDa band comigrated with EGFR immunoprecipitated from the same lysate and was recognized by anti-EGFR antibodies on an immunoblot (Fig. 3*B*) and was phosphorylated on tyrosine in an *in vitro* kinase reaction (data not shown). These data show that the 180-kDa protein is the EGFR and that its association with SH2 domains is clearly dependent on prior EGF stimulation (Fig. 3*B*). TrpE–v-Crk bound the EGFR more effectively than the GAP SH2 fusion proteins but was less efficient in p62-binding (Fig. 3 *A* and *B*, lanes 5).

**Fps and Src SH2 Domains Are Required for Tyrosine Phosphorylation of p62 and GAP.** p62 is rapidly and abundantly phosphorylated by activated v-Src and v-Fps tyrosine kinases (28). The v-Fps SH2 domain, and Glu-832 in particular, have been previously implicated in recognition of a 62-kDa protein whose phosphorylation correlates with transformation (9). We, therefore, tested whether this substrate corresponds to p62, which displays an affinity for SH2 domains *in vitro* (see above). Direct comparison revealed that GAP-associated p62, precipitated with anti-GAP antibodies from cells transformed by wild type v-*fps*, comigrated with the prominent SH2-dependent 62-kDa substrate identified in the whole-cell lysate. Furthermore, little phosphotyrosine-containing p62 could be detected in anti-GAP immunoprecipitates from cells expressing a v-*fps* mutant with a substitution of lysine for Glu-832 in the SH2 domain (Fig. 4*A*). GAP itself is a relatively poor substrate for $P130^{gag\text{-}fps}$ (28); prolonged exposure revealed that GAP tyrosine phosphorylation also depends on the v-Fps SH2 domain.

A series of in-phase linker-insertion and deletion mutations constructed in v-*src* has yielded several mutants that have relatively high levels of $p60^{v\text{-}src}$ kinase activity but are poorly transforming in Rat-2 cells (7). The XD6 and SHX13 mutants have alterations within highly conserved regions of the v-Src SH2 domain. XD6 has a deletion of residues 149–174, and the SHX13 mutation inserts Arg-Ala after residue 228. In con-

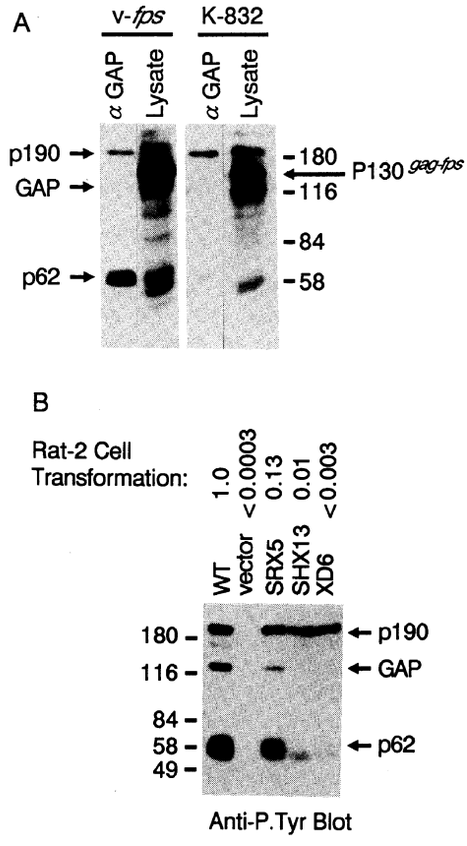


FIG. 4. Mutations in v-Src and v-Fps SH2 domains impair tyrosine phosphorylation of p62 and GAP. (*A*) Total cell lysates or anti-GAP immunoprecipitates from Rat-2 cells expressing either wild-type $P130^{gag\text{-}fps}$ (v-*fps*) or a Glu-832 → Lys SH2 mutant (K-832) were analyzed by immunoblotting with antiphosphotyrosine antibodies. (*B*) Anti-GAP immunoprecipitates from Rat-2 cells expressing wild-type v-*src* or the SRX5, SHX13, or XD6 mutants or containing empty vector were analyzed by immunoblotting with antiphosphotyrosine antibodies. The focus-forming activities in the v-*src* mutants on Rat-2 cells relative to wild type are indicated (7). P.Tyr, phosphotyrosine.

trast, the SRX5 mutation replaces the codon for the Tyr-416 autophosphorylation site in the catalytic domain with codons for Ser-Arg-Asp. Rat-2 cells expressing these v-*src* mutants contained similar levels of GAP and $p60^{v\text{-}src}$ compared with wild-type v-*src*-transformed cells (data not shown). However, anti-GAP immunoprecipitations showed that the tyrosine phosphorylation of GAP-associated p62, and of GAP itself, was greatly decreased in cells expressing the SHX13 and XD6 v-*src* SH2 mutants, correlating with their particularly low Rat-2 transforming activity (Fig. 4*B*). In contrast, the SRX5 autophosphorylation site mutant has an intact SH2 domain, retains 13% of wild-type transforming activity on Rat-2 cells, and still gives appreciable phosphorylation of p62 and GAP. Unlike p62, which is a minor but highly phosphorylated protein, p190 contains relatively little phosphotyrosine but is a major GAP-binding protein (28). p190 tyrosine phosphorylation was not affected by the v-*src* or v-*fps* SH2 mutations and, hence, does not require the tyrosine kinase SH2 domain and does not correlate with transformation. We did not observe binding of tyrosine phosphorylated p190 to GAP SH2 domains or C-terminal region *in vitro*, possibly because all the available p190 is already associated with GAP in cell lysates.

## DISCUSSION

**SH2 Domains of GAP and Crk Complex with Phosphotyrosine-Containing Proteins *in Vitro*.** SH2 sequences have been found within otherwise distinct proteins that have the common feature of regulating intracellular signaling pathways. Our results indicate that SH2 domains allow these proteins to interact with a related, but limited, set of tyrosine phosphorylated ligands. SH2-binding proteins include activated receptor tyrosine kinases, exemplified by the EGFR, and substrates such as p62, p70, and p130. The ability of bacterially derived GAP or v-Crk SH2 domains to form complexes *in vitro* suggests that their phosphorylation is not required for ligand binding. Rather, the tyrosine phosphorylation of SH2-binding proteins, such as growth factor receptors and p62, is likely to regulate complex formation by increasing their affinities for SH2-containing proteins. In support of this notion, substitution of the PDGFR autophosphorylation sites with phenylalanine inhibits the PDGF-dependent association of GAP with the receptor (30). We have subsequently observed that recombinant GAP and PLCγ SH2 domains associate with the wild-type PDGFR in lysates of PDGF-stimulated cells (37). The simplest explanation of these results is that SH2 domains directly contact phosphotyrosine-containing ligands. However, until these interactions have been reconstituted by using purified proteins, it remains possible that they involve additional unphosphorylated proteins.

**SH2 Domains May Regulate Protein–Protein Interactions *in Vivo*.** In the cell, the interactions of SH2 domains with phosphotyrosine-containing proteins may direct the formation of high-affinity complexes that facilitate signal transduction. Indeed, all the proteins identified as binding SH2 domains *in vitro* have been found in complexes with one or more SH2-containing proteins *in vivo*. For example, the ability of recombinant SH2 domains to associate with the activated EGFR suggests that they are responsible for the interactions of receptors with signaling proteins such as GAP and PLCγ (24, 25, 30, 31). In EGF-stimulated or *src*-transformed cells GAP forms a precipitable complex with a highly tyrosine phosphorylated protein, p62. p62 is an SH2-binding protein that associates efficiently with the N-terminal GAP SH2 domain *in vitro*. Tyrosine phosphorylation of p62 might increase its affinity for GAP-SH2 and allow it to displace other GAP-binding proteins, such as autophosphorylated receptors. The C-terminal region of GAP is known to associate with $p21^{ras}$; the data presented here suggest that the more N-terminal SH2 domain interacts in trans with phosphotyrosine proteins such as growth factor receptors and p62. One possibility is that these complexes affect $p21^{ras}$ activity, although this hypothesis remains to be established.

The v-Crk oncoprotein elevates cellular phosphotyrosine levels (11) and may transform cells by complexing with multiple phosphotyrosine-containing proteins (13, 14). Consistent with this suggestion, TrpE–v-Crk showed a remarkable ability to complex with the tyrosine phosphorylated proteins p70 and p130 in a lysate from normal Rat-2 cells (Fig. 2*B*).

Although SH2 domains from different proteins can interact with similar polypeptides *in vitro*, their binding specificities are not identical. The N-terminal GAP SH2 domain associated preferentially with p62, whereas v-Crk showed a higher affinity for p130 and p70. The functional complexes that form *in vivo* may reflect these different affinities. For example, GAP coimmunoprecipitates from *src*-transformed cells with p62 but not p130, which might be sequestered by other SH2-containing proteins. The various SH2 domains may also have distinct biological activities; thus, the high level expression of the GAP SH region does not transform fibroblasts, in contrast to v-*crk* (D.A. and G. Mbamalu, unpublished results). Protein–protein interactions induced by activated tyrosine kinases may, therefore, be regulated by two mechanisms. First, tyrosine phosphorylation may modulate the affinities of SH2-binding proteins for SH2 domains, thereby inducing the formation of complexes that initiate signal

transduction. In addition, specific SH2 domains bind some phosphotyrosine-containing ligands better than others. This apparent specificity in SH2–ligand interactions may determine which proteins associate within the cell.

**SH2 Domains of Cytoplasmic Tyrosine Kinases Interact in Cis with the Catalytic Domain and in Trans with Substrates.** The SH2 domain of $p60^{v\text{-}src}$ or $P130^{gag\text{-}fps}$ is important both for the regulation of kinase activity and the phosphorylation of specific substrates. The v-Fps SH2 domain apparently interacts in cis with the neighboring kinase domain to modulate its kinase activity (9). This association is augmented by autophosphorylation within the kinase domain (9), consistent with the notion that SH2 domains recognize phosphotyrosine sites. The Src and Fps SH2 domains are also required for phosphorylation of the p62 SH2-binding protein that complexes with GAP *in vivo*. Hence, the v-Src SH2 domain may recognize and bind unphosphorylated p62 to facilitate its phosphorylation by the kinase domain. Alternatively, Src SH2 might play no direct role in phosphorylation of p62 by the kinase domain but might bind and protect the tyrosine phosphorylated products. A subsequent intramolecular interaction between the Src SH2 and kinase domains might then destabilize p62–Src or GAP–Src complexes, and allow p62 to associate with the GAP SH2 domain. In this scheme, Src SH2 can complex with its own kinase domain or with substrates such as p62 and may thereby control kinase–substrate interactions. Regardless of the mechanism, the data indicate that p62 is recognized by v-Src *in vivo*. The ability of a noncatalytic protein kinase domain to recognize substrates of the enzymatic domain is unusual and sets cytoplasmic tyrosine kinases apart from their serine/threonine-specific counterparts. Furthermore, the v-*src* and v-*fps* mutants that fail to phosphorylate p62 and GAP are poorly transforming in Rat-2 cells, suggesting that one or both of these might be biologically important tyrosine kinase substrates.

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