Biological and Biochemical Activity of v-Crk Chimeras Containing the SH2/SH3 Regions of Phosphatidylinositol-Specific Phospholipase C- γ and Src

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The chicken CT10 virus oncogene product, P47^{gag-crk}, contains SH2/SH3 domains that have been identified as conserved domains among proteins involved in signal transduction. We studied the functional similarity of the SH2/SH3 domains by replacing those of v-Crk with those of phosphatidylinositol-specific phospholipase C- γ , v-Src, or c-Src. The transforming activity of v-Crk was partially retained in a mutant with a v-Src SH3 domain but not in the other mutants with heterologous SH2/SH3 domains. Mutant viruses with Crk-SH2/SH2' domains induced tyrosine phosphorylation of cellular proteins, but mutants with phosphatidylinositol-specific phospholipase C- γ or Src SH2/SH2' domains did not. However, the mutant proteins with heterologous SH2/SH2' regions were able to weakly associate with some phosphotyrosine-containing proteins *in vitro*. These results indicate that in the context of the P47^{gag-crk} structure, the requirement of Crk-SH2/SH3 is more stringent for its activity to induce cell transformation than to cause phosphorylation of cellular proteins. The substitution with heterologous sequences least perturbs the capacity to bind phosphotyrosine-containing proteins. In each case, the SH3 domain is more flexible to substitution than is the SH2 domain.

The *src* homology domains SH2, SH2', and SH3 have been identified in many proteins involved in signal transduction (28), including nonreceptor-type tyrosine kinases (35), the *crk* oncogene product (21), phosphatidylinositol-specific phospholipase C- γ (PLC- γ) (5, 38, 39), *ras* GTPase-activating protein (GAP) (42, 44), and the Nck protein, the function of which is unknown (16). Although most of the proteins containing SH2 also possess SH3, there are many with SH3 alone, some of which are associated with the membrane cytoskeleton (4, 16, 17, 33, 40, 43, 45, 46). We and other groups recently demonstrated that a function of the SH2/SH2' domains is to bind to phosphotyrosine (ptyr)-containing proteins in a ptyr-dependent manner (1, 19, 20, 25, 26). In this paper, the term SH2/SH3 indicates the entire region containing SH2, SH2', and SH3, which correspond to the B, C, and A boxes, respectively, as proposed by Stahl et al. (38). The term SH2/SH2', denoting the SH2 and SH2' domains, will be used when these domains are dealt with together.

The oncogene product of the CT10 virus, P47^{gag-crk}, consists of a retroviral Gag domain fused to a v-Crk domain, the latter consisting of SH2/SH3 and intervening regions (31). Cells transformed by CT10 have an elevated level of ptyr, and immunoprecipitates of P47^{gag-crk} protein have both tyrosine kinase activity and ptyr-containing proteins (22, 24). P47^{gag-crk} may activate tyrosine kinases or inhibit tyrosine phosphatases in transformed cells; however, the mechanism responsible for the increase in tyrosine phosphorylation is not clear.

Previous analysis of P47^{gag-crk} has shown that deletions or point mutations in the SH2/SH3 domains impair both binding to ptyr-containing proteins and the ability to induce cell transformation. This implies that these regions play a crucial role in Crk function (20, 23). In this paper, we describe the

biological and biochemical properties of mutant v-Crk proteins in which the SH2/SH3 regions have been deleted or substituted by the equivalent regions of PLC- γ or Src. This sheds light on the functional equivalence of these regions from different proteins.

MATERIALS AND METHODS

Cells and viruses. Chicken embryo fibroblasts (CEF) were prepared, maintained, and infected as described previously (9). Preparation of avian sarcoma virus CT10, which encodes wild-type (wt) v-Crk, and helper virus UR2AV (27) from molecularly cloned DNA has been described elsewhere (23). Transfected cells were maintained in medium containing 0.375% agar at 40°C. Crk-3Y1 (18) and SR-3Y1 (13) are rat 3Y1 cells (14) expressing P47^{gag-crk} and p60^{v-src}, respectively. To assay anchorage independence, CEF were trypsinized 5 days posttransfection, and 10⁶ cells per 10-cm bacterial plastic dish were plated in 10 ml of medium containing 11% calf serum, 1% chicken serum, 1% dimethyl sulfoxide, and 0.4% agar over another layer of 15 ml of the same medium containing 0.7% agar. The plates were incubated at either 37 or 40°C.

Tumorigenicity was assayed by injection of 0.1 ml of virus into each wing web of newborn (10 days posthatching) White Leghorn chickens (SPAFAS, Inc.).

Construction of v-*crk* mutants. The structures of v-*crk* mutants are diagrammed in Fig. 1. The first three mutants were deletions of either SH2 (pOCC), SH2' (pCOC), or SH3 (pCCO) from pCT10, which encodes the entire CT10 genome (23). p10-SMH was derived from pCT10 and had a unique *Hind*III site at the *Sma*I site of pCT10 shown in Fig. 1 (23). pOCC was constructed by ligating a *Cla*I octamer linker (New England BioLabs, Inc.) to the 6.0-kb *Sfi*I-*Hind*III fragment of p10-SMH that was blunt-ended by T4 DNA polymerase. pCOC was constructed by blunt-end self-ligation of the end-filled, 6.1-kb *Hind*III-*Mst*II fragment of p10-SMH. pCCO was constructed by ligating a *Hind*III

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FIG. 1. Structures of mutant v-Crk proteins. Black boxes indicate the Gag region; oval, round, and rectangular boxes indicate the SH2, SH2', and SH3 regions, respectively; open boxes indicate those of v-Crk. The amino acid numbers shown here are from references 21, 38, and 3 for v-Crk, PLC- γ , and v-Src and c-Src, respectively. Restriction enzyme sites shown were used to construct the following mutants: SAB, M-1 to D-52; CT10, wt P47^{gag-crk}; CH, a three-amino-acid substitution (L-235-M-236-A-237 to PSI) and a four-amino-acid insertion (P-316 to PPSLG) compared with CT10, which changes the restriction sites from *SfiI* to *ClaI* and *SmaI* to *HindIII*, respectively; OCC, a deletion from L-235 to P-316; COC, a deletion from G-317 to L-365; and CCO, a deletion from R-366 to C-426. Hatched and stippled regions indicate the amino- and carboxyl-terminal PLC SH2/SH2' regions, respectively. PLC- γ -derived sequences are as follows: P4C, G-540 to G-765; P3C, G-540 to V-717; nPPC, G-540 to H-663; cPPC, P-658 to G-765; and CCP, R-790 to E-848. Vertically striped regions indicate SH2/SH3 regions of either v-Src or c-Src. Note that the amino acid sequences of the SH2/SH2' domains are identical, but those of SH3 are different between v-Src and c-Src (indicated by the letters v and c). The Src protein-derived sequences are as follows: SSC, A-138 to G-254; CSC, I-204 to G-254; and CCSv and CCSc, A-80 to P-139.

octamer linker (New England BioLabs) to the end-filled 6.0-kb *MstII*-*AccI* fragment of pCT10.

v-Crk chimeras were constructed by first amplifying the DNA sequences encoding the SH2/SH2' or SH3 region of PLC- γ or Src by using the polymerase chain reaction (PCR) (37) and then inserting these into pCT10-derived DNAs lacking either SH2/SH2' or SH3.

The template used for amplification of pCT10-derived sequences was p10-SMH (23); for PLC- γ , it was pPLC148 (38); for v-src, it was pSR-XD2 (3); and for c-src, it was pSR-5H (10). Oligonucleotides for DNA amplification (Fig. 2) were designed to include restriction sites and to preserve in the chimeras the same spacing between SH regions as that found in unmutated v-Crk protein.

SH2/SH2'-substituted chimeras and the primers used for amplification were as follows: pCH, primers 1 and 2; pP4C, primers 3 and 8; pP3C, primers 3 and 7; pNPPC, primers 3

FIG. 2. Binding sites and sequences of the oligonucleotides used as primers for the PCR reaction. The symbols used here are the same as for Fig. 1. Restriction sites for the sequence encoding P47^{gag-crk} (Δ) were those of pCH and were used to exchange the domains. Arrows show binding sites of the primers. Sequences marked by double underlines are the recognition sites of restriction enzymes listed on the right.

and 6; pcPPC, primers 4 and 8; and pSSC, primers 11 and 13. Amplified DNAs were digested with *ClaI* and *MstII* and ligated to the 5.9-kb *ClaI*-*MstII* fragment of pOCC, which lacks SH2/SH2'. pP3C was incidentally obtained, despite the lack of an *MstII* recognition site in primer 7. In this plasmid, nucleotides 1472 to 1478 of pCT10 were missing and the 3' end of the PLC- γ DNA fragment was ligated to nucleotide 1479 of pOCC (ahead of SH3).

The only clone not constructed by using the PCR was pCSC, in which the SH2' of Src replaces that of Crk. It was made by cleaving pSSC with *XhoII*, blunting with the Klenow fragment of DNA polymerase, and ligating to a 12-mer *HindIII* linker (New England BioLabs). This DNA was then cleaved with *HindIII* and *MstII*. A 150-bp fragment containing the *src*-SH2' region was purified and ligated to the 6.1-kb *HindIII*-*MstII* fragment of p10-SMH, which lacks SH2'.

SH3-substituted chimeras were constructed by amplification, digestion with *MstII* and *AccI*, and ligation to the 6.0-kb *MstII*-*AccI* fragment of pCT10, which lacks SH3. Primers used were 5 and 9 for pCCP and 10 and 12 for pCCSv (v-Src SH3) and pCCSc (c-Src SH3). Although v-Src and c-Src have identical SH2/SH2' regions, they differ by four amino acids in their SH3 regions (41).

After construction, all DNAs amplified by the PCR were sequenced by the dideoxy-chain termination method (36).

Expression of v-crk mutants in *Escherichia coli*. A T7 RNA polymerase expression system was used to produce v-Crk proteins (34). The construction of the expression vectors encoding v-crk mutants, expression of the peptides, and their coupling to protein A-Sepharose by means of an anti-gag monoclonal antibody (MAb 3C2) have been de-

scribed previously (20). The resulting complex is called Crk-beads.

Protein analysis of *v-crk* mutants. Immunoblotting was performed as described previously (19), by using anti-tyr antibody (8) or anti-p19^{gag} MAb 3C2 (29). In some experiments, we used an anti-mouse immunoglobulin G antibody conjugated with alkaline phosphatase (TAGO, Inc.) instead of ¹²⁵I-protein A. The antibodies bound to the membrane were visualized with the chromogenic substrate bromochloroindolyl phosphate-nitroblue tetrazolium (BCIP/NBT).

The association of tyr-containing proteins with Crk-beads was examined as described elsewhere (19, 20). Crk-3Y1 cells were lysed with RIPA buffer (10 mM Tris-hydrochloride [pH 7.5], 5 mM EDTA, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 10% glycerol, 50 μ M Na₃VO₄, 5 μ M Na₂MoO₄, 10 mM NaF), and SR-3Y1 cells were lysed with Triton X-100 lysis buffer (RIPA without SDS and sodium deoxycholate). Cell lysates containing approximately 100 μ g of protein were incubated for 2 h with 5 μ l of Crk-beads, which contained 0.5 μ g of wt or mutant v-Crk proteins. Beads were washed sequentially with RIPA or Triton X-100 lysis buffers containing 300 mM and 10 mM NaCl. Proteins associated with Crk-beads were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and then immunoblotting using anti-tyr antibody.

Pulse-chase experiment. Fourteen days after transfection, CEF were labeled in methionine-free medium for 1 h with 100 μ Ci of [³⁵S]methionine (Amersham Corp.). Cells were fed with complete medium and lysed with RIPA buffer at 0, 5, 10, and 20 h. The wt and mutant v-Crk proteins were immunoprecipitated with MAb 3C2, separated by 10% SDS-PAGE, and detected by fluorography. Protein half-lives were calculated from densitometry measurements. Because of different levels of protein expression, exposure times were adjusted for each protein to maintain densitometry readings in the linear range.

RESULTS

Construction of *v-crk* mutants. The mutants, constructed as mentioned above, are diagrammed in Fig. 1. The amino-terminal region of p19^{gag} was expressed in *E. coli* from the pET-*gag* construct. As a positive control for the domain exchange experiment, we made CH by using the amplified DNA of SH2/SH2' regions of p10-SMH, which is derived from pCT10 and encodes a mutant protein as transforming as the wt. pOCC, pCOC, and pCCO have a deleted SH2, SH2', or SH3 domain of pCT10. By using DNAs amplified by the PCR, the SH2/SH3 regions of *v-crk* were substituted with those of PLC- γ , *v-src*, and *c-src* without changing the distance between each domain (P4C, P3C, nPPC, cPPC, CCP, SSC, CCSv, and CCSs). The sequence in the SH2/SH2' domain from Gly-238 to Ile-364 of v-Crk, and that in the SH3 domain from Gln-367 to Lys-425 of v-Crk, were exchanged with equivalent regions of other proteins. One mutant, CSC, was constructed with a linker ligation method.

Transforming activity of *v-crk* mutants. To examine the transforming activity of *v-crk* mutants, CEF were transfected with pCT10-derived DNA and UR2AV helper virus DNA and were assayed by three criteria: morphological alteration, anchorage-independent growth of transfected CEF, and tumorigenicity in newborn chickens (Table 1). Only two viruses, wt CT10 and CH, fulfilled all three criteria for transformation (Fig. 3 and 4; Table 1). Another mutant that retained some transforming activity was CCSv, which

TABLE 1. Tumorigenicity of *v-crk* mutants^a

Virus	No. of sites (tumor/total)
UR2AV.....	0/4
CCO + UR2AV	0/4
CCP + UR2AV	0/4
CCSv + UR2AV	0/8
CCSc + UR2AV	0/8
CH + UR2AV	8/8
CT10 + UR2AV	6/8

^a Assayed by inspection of each wing web of newborn chickens at 14 days postinjection. Values shown are the number of sites with palpable tumors of the total number of sites injected.

had the v-Src SH3 region. Although CEF infected with CCSv did not show morphological alteration, they grew into colonies in soft agar. The number of colonies induced by CCSv was almost comparable to those induced by CT10 and CH; however, the colonies were larger and less compact than those of the fully transforming viruses (Fig. 4). All other mutants were negative for transformation by the criteria of morphological alteration and colony formation. Because SH2-substituted chimeras were negative for transformation and had low binding activity for tyr-containing proteins (see below), we did not test them for tumorigenicity in chickens.

Analysis of mutant v-Crk proteins. The expression of mutant proteins was examined by immunoblotting with anti-Gag MAb 3C2. Mutant proteins of the expected molecular weights were observed in all cases (Fig. 5A). The level of expression of wt and mutant proteins was highest in CEF transfected with the two fully transforming viruses (CT10 and CH), followed by CCSv, a partially transforming virus, and was lowest with the mutants with the substituted SH3 regions (CCO, CCP, and CCSc). The pr76^{gag} protein was expressed at comparable levels in all mutants, suggesting that cells were fully infected with UR2AV helper virus. Two

FIG. 3. Morphological alteration of CEF transfected with wt and mutant CT10 viruses. CEF were transfected with calf thymus DNA only (1), UR2AV (2), UR2AV plus CH (3), or UR2AV plus CT10 (4) and maintained in monolayer culture under soft agar at 40°C. Photomicrographs were taken after the second passage. Magnification, $\times 200$.

FIG. 4. Induction of anchorage-independent growth in CEF transfected with *v-crkr* mutants. CEF were transfected with UR2AV (1), CCSv plus UR2AV (2), CH (3), or CT10 (4). Transfected cells were plated in suspension 5 days posttransfection and incubated at 40°C. Photomicrographs were taken 21 days after plating. Magnification, $\times 200$.

days after transfection (Fig. 5B), the level of expression of mutant proteins encoded by the transforming viruses (CT10, CH, and CCSv) was not higher than that encoded by nontransforming viruses.

Because the expression of mutant proteins varied with the different SH3 regions rather than with the SH2/SH2' regions (Fig. 5A), we examined the stability of mutant *v-crkr* proteins of various SH3 regions; proteins labeled in the pulse-chase

FIG. 6. Pulse-chase labeling of *v-Crk* mutant proteins. Transfected CEF were pulse-labeled with [35 S]methionine; chased for 5, 10, and 20 h; lysed; and immunoprecipitated with MAb 3C2 (anti-Gag). Proteins were separated on 10% SDS-PAGE and detected by fluorography. The relative amounts of proteins were quantitated by densitometry to calculate their half-lives. Exposure times are shown at the bottom. *pr76gag* and *P47gag-crkr* are indicated by open and closed arrowheads, respectively.

experiment are shown in Fig. 6. Some mutant *v-Crk* proteins had somewhat shorter half-lives than *P47gag-crkr* (4.3, 3.8, 7.4, and 7.0 h for CCP, CCSv, CCSs, and CT10, respectively), but these differences do not appear to explain the differences in protein levels. Since the nucleotide sequences of these mutants are the same except for the SH3-coding region, large differences in the viral transcriptional rate or mRNA stability are also unlikely. Moreover, the plasmids encoding transforming viruses did not induce higher levels of protein expression than those encoding nontransforming viruses on the second day after transfection (Fig. 5B). These results suggest that the low level of expression of nontransforming mutants may be due to selection against nontransforming mutant viruses. This selection is often seen in CEF transfected with a replication-defective retrovirus carrying an oncogene together with helper virus when the oncogene is incapable of transforming cells (23).

Induction of ptyr-containing proteins. Ptyr levels in mutant-transfected CEF were assayed by immunoblotting with anti-ptyr antibody (Fig. 7). In addition to CT10 virus, CH virus induced three major ptyr-containing proteins (130 to 150, 120, and 70 kDa) as described previously (21). Three viruses with *v-crkr* SH2/SH2' regions (CCO, CCP, and CCSv) also induced two proteins (130 and 120 kDa), al-

FIG. 5. Expression of mutant *v-Crk* proteins. (A) CEF transfected with calf thymus DNA only (mock), UR2AV, and UR2AV plus pCT10-derived plasmids (listed above each lane) were maintained in monolayer culture under soft agar at 40°C. After the second passage (10 days after transfection), cells were lysed in sample buffer, separated on 10% SDS-PAGE, and then analyzed by immunoblotting with anti-Gag MAb 3C2 and 125 I-protein A. Filters were exposed for 16 h. (B) Two days after the transfection of pCT10-derived plasmids listed above each lane, CEF were lysed and analyzed as in panel A. The bound MAb 3C2 was detected with an anti-mouse immunoglobulin G antibody conjugated with alkaline phosphatase and then visualized with BCIP/NBT. Bars are prestained molecular size markers: 110, 70, and 46 kDa (from top to bottom). Open and closed arrows indicate *pr76gag* and *P47gag-crkr*, respectively.

FIG. 7. Ptyr-containing proteins in CEF infected with mutant CT10 viruses. CEF were transfected with calf thymus DNA only (mock), UR2AV, and UR2AV plus pCT10-derived plasmids as shown above each lane. Lysates were prepared, and proteins were separated on 10% SDS-PAGE and analyzed by immunoblotting with anti-ptyr antibody and 125 I-protein A. Filters were exposed for 48 h. Bars are prestained molecular mass size markers: 205, 116, and 77 kDa (from top to bottom). The 130- to 150- and the 120-kDa ptyr-containing proteins are indicated by arrowheads on the right.

though the level of induction was less than with the wt virus. Phosphorylation of the 70-kDa protein was less obvious in these cultures, but this is not conclusive because of the low level of protein expression, particularly in CCO- and CCP-transfected cultures. The other mutants did not induce significant levels of tyrosine phosphorylation.

Association of mutant proteins with ptyr-containing proteins. Bacterially expressed v-Crk mutant proteins were used to examine association with ptyr-containing proteins. Cell lysates from Crk-3Y1 and SR-3Y1 were incubated with mutant proteins in the form of Crk-beads (mutant proteins-MAb 3C2-protein A-Sepharose). The ptyr-containing proteins bound to Crk-beads were analyzed by SDS-PAGE and immunoblotting using anti-ptyr antibody (Fig. 8). The mutants with v-crk SH2/SH2' regions (CCO, CCP, CCSv, and CCSs) associated with ptyr-containing proteins to an extent similar to the parental (CT10 and CH) proteins. The mutant proteins without SH2 (SAB and OCC) did not bind any ptyr-containing proteins. The other mutant proteins with various SH2/SH2' domains (COC, P4C, P3C, nPPC, cPPC, and SSC) associated weakly with the 135-kDa protein from Crk-3Y1. Among them, OCC, COC, P4C, cPPC, and SSC weakly associated also with the 65-kDa protein from SR-3Y1. Among the four PLC-derived SH2/SH2' mutants, P4C had the highest affinity for ptyr-containing proteins.

DISCUSSION

We have compared the biological and biochemical activities of various SH2/SH3 domains by replacing the v-crk domains of CT10 virus with those of PLC- γ , v-Src, or c-Src. The use of the PCR enabled us to construct v-crk chimeras without changing the distance between the domains, thus minimizing the possible changes in protein conformation. The results shown here demonstrate that v-Crk SH2/SH2' cannot be substituted by those of PLC- γ and Src, despite their common property of binding to ptyr-containing proteins.

Since mutations in the SH3 region activate the catalytic and transforming activity of c-Src and c-Abl (6, 7, 11, 12, 30), this region is regarded as a negative regulatory element. However, the removal (CCO) or mutation (BSP [23]) of SH3 impairs the transforming activity of v-Crk. Compared with c-Crk, v-Crk has two point mutations in SH3, and a v-Crk mutant with c-Crk SH3 is less transforming than v-Crk (31). In addition, the SH3 region of v-Src (CCSv), but not of

FIG. 8. Association of mutant v-Crk proteins with ptyr-containing proteins in vitro. The mutant v-Crk proteins expressed from pET3-derived vectors were covalently bound to protein A-Sepharose via MAb 3C2 (anti-Gag). These Crk-beads were preincubated with peptide expressed from pET-SAB and then incubated with Crk-3Y1 and SR-3Y1 cell lysates as described previously (20). Proteins associated with the Crk-beads were analyzed by 7.5% SDS-PAGE and immunoblotting with anti-ptyr antibody and 125 I-protein A. Bars are prestained molecular mass size markers: 200, 97, 69, and 46 kDa (from top to bottom).

c-Src, partially restored the anchorage-independent growth of CT10. These results suggest that SH3 can regulate the transforming activity in both positive and negative ways and that mutations in the SH3 regions of v-Src and v-Crk enhance the positive effect on transformation.

Expression of v-Crk SH2/SH2' regions was found to be sufficient to elevate phosphorylation of two major proteins (130 to 150 and 120 kDa) but not to achieve transformation (CCO, CCP, and CCSs) (Fig. 7). In previous studies (23), we have shown that v-Crk mutants without Gag can also induce phosphorylation of these proteins, indicating that the SH2/SH2' regions alone are responsible for the induction of ptyr-containing proteins. As mentioned previously (23) and in this paper, the levels of mutant proteins reflect primarily the number of infected cells rather than the expression in each infected cell; therefore, we could not assess the efficiency of induction of ptyr-containing proteins by each mutant. Although we have found that a function of the Crk SH2/SH2' domains is to bind ptyr-containing proteins (19, 20), the mechanism of induction of tyrosine phosphorylation by CT10 virus is as yet unclear.

In vitro binding experiments revealed that different SH2/SH2' domains have different affinities for each ptyr-containing protein. As expected from the previous observations that SH2/SH2' of v-Crk is necessary and sufficient for association with ptyr-containing proteins (19, 20), all the mutant proteins with v-Crk SH2/SH2' domains (CH, CCO, CCP, CCSv, and CCSs) bound to the ptyr-containing proteins from crk- and src-transformed cells. The other mutant proteins with heterologous SH2/SH2' domains (P4C, P3C, nPPC, cPPC, and SSC) weakly associated with the ptyr-containing proteins in a manner similar to the product of COC, a SH2' deletion mutant of v-Crk. It is possible that other undefined domains

of PLC- γ and Src are necessary to fully bind ptyr-containing proteins. Although a 120- to 130-kDa, ptyr-containing protein of SR-3Y1 cells coimmunoprecipitates with the v-Src protein (15, 32), we could not detect the in vitro association of this protein with the v-Crk chimera containing Src SH2/SH2' domains. Another possibility is that the SH2/SH2' domains of v-Crk have a higher affinity for ptyr-containing proteins induced in *src*- and *crk*-transformed cells than do those of PLC- γ and Src. The mutant proteins with v-Crk SH2/SH2' domains (CCO, CCP, CCSv, and CCSs) bound well to ptyr-containing proteins but failed to strongly stimulate tyrosine phosphorylation of cellular proteins. The relatively poor expression of these mutant proteins in transfected cells may explain these differences, and their inability to induce cell transformation may need further study.

The SH2/SH2' domains of several proteins, including Crk, PLC- γ , and Src, have been found to bind to activated epidermal growth factor and platelet-derived growth factor receptors (1), but their affinities for other ptyr-containing proteins present in *src*- or *crk*-transformed cells appear to vary (20, 26). It should be pointed out that the affinity can be influenced by the structural context in which the SH2/SH2' domains reside. In these studies, Gag-Crk fusion proteins (20; this study) and bacterial TrpE fusion proteins (1, 26) were used to express the SH2/SH2' domains, and thus these results cannot be directly compared. In addition, regions intervening cloning sites and SH2/SH2' regions were substituted along with the SH2/SH2' regions; these could affect chimera structure. Nevertheless, the observed divergent affinities of each of the SH2/SH2' domains for ptyr-containing proteins are consistent with the idea that activated tyrosine kinases associate with, and probably transmit signals to, various combinations of SH2/SH2'-containing proteins (for a review, see reference 2).

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