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Biochemistry

Association of the v-*crk* oncogene product with phosphotyrosine-containing proteins and protein kinase activity

(transformation/tyrosine kinases/SH2, SH3 homology domains)

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ABSTRACT **An antiserum specific for P47$^{gag-crk}$, the oncogene product of avian sarcoma virus CT10, was used to investigate possible crk-binding proteins. In *in vitro* kinase assays, four proteins were phosphorylated in anti-crk immunoprecipitates. Tyrosine, serine, and threonine residues were phosphorylated. A number of tyrosine-phosphorylated proteins were identified in anti-crk immunoprecipitates of ^{32}P-labeled cells, including the three major phosphotyrosine-containing proteins of CT10-infected cells. These three proteins also bound to bacterially synthesized crk protein. These results suggest that the crk transforming protein can stably associate with both endogenous kinases and cellular kinase substrates.**

Avian sarcoma virus CT10 (CT10) encodes a 47-kDa *gag*-fusion oncogene product, P47$^{gag-crk}$ (1, 2). The *crk* oncogene contains two blocks of significant sequence similarity to putative regulatory domains found in the nonreceptor class of protein-tyrosine kinases, a phosphatidylinositol-specific phospholipase C (PLC-γ), the ras GTPase activator protein (GAP), and several submembranous matrix proteins such as α-spectrin (3). Since the *crk* gene has no homology to any known catalytic domain, it is likely that P47$^{gag-crk}$ transforms cells in which it is expressed by modulating the activity of endogenous cellular enzymes. Previous work demonstrated that CT10-infected cells had greatly increased phosphotyrosine on at least three proteins when compared with control cells. In addition, a tyrosine kinase activity could be detected in immunoprecipitates of CT10-infected cells by using antisera that recognize the gag portion of P47$^{gag-crk}$ (1, 2). These results suggested that a tyrosine kinase activity is modulated by the *crk* gene product and that this kinase may actually be bound to P47$^{gag-crk}$.

We have raised a crk-specific antiserum to a bacterially expressed crk protein, and using this serum we have confirmed that a tyrosine kinase is apparently associated with P47$^{gag-crk}$. We also found that all of the major phosphotyrosine-containing proteins of CT10-infected cells could be coimmunoprecipitated in apparent association with the crk protein. These three phosphotyrosine-containing proteins were also bound to bacterially synthesized crk protein that was covalently coupled to beads. Our results are consistent with a model in which P47$^{gag-crk}$ exerts its biological activity by mediating the association of an endogenous protein-tyrosine kinase with critical cellular substrates.

MATERIALS AND METHODS

Cells and Viruses. Chicken embryo fibroblasts (CEFs) were cultured and maintained essentially as described (4). CEFs were transfected with molecularly cloned CT10 and UR2AV helper virus DNA as described (1). Cells were routinely used ≈14 days posttransfection or 7 days postinfection.

Generation of crk-Specific Antiserum. The crk peptide was expressed in *Escherichia coli* BL21 (DE3) pLysS using the T7 RNA polymerase expression system (5, 6). An 0.8-kilobase *Sty* I/*Hae* II fragment containing the entire c-crk-derived portion of v-crk in the absence of viral gag sequences was cloned into pET-3a using *Bam*HI linkers. Plasmid-bearing bacteria were induced with isopropyl β-D-thiogalactopyranoside for 3 hr, spun out, and lysed by boiling in SDS sample buffer; proteins were separated by SDS/PAGE. The crk peptide band was excised from the Coomassie blue-stained gel, washed with 10% methanol, dried, and homogenized in phosphate-buffered saline (PBS). Approximately 50 μg of antigen was used to immunize rabbits and 1/4th this amount was used for booster injections.

Protein Analysis. Isotopic labeling, cell lysis, immunoprecipitation, *in vitro* kinase assay, and immunoblotting have been described (1, 7). CEFs were labeled for 4–6 hr with 1–5 mCi of carrier-free [^{32}P]orthophosphate or 0.5 mCi of [^{35}S]-methionine (800 Ci/mmol; 1 Ci = 37 GBq) in 1.0 ml of medium per 6-cm plate. Kinase assays contained 20 μCi of [γ-^{32}P]ATP in 30 μl of Mn^{2+} kinase buffer. Generally, anti-crk antiserum was used at 1:100 dilution for immunoprecipitation and 1:400 dilution for immunoblotting. All immunoprecipitates were washed three times in RIPA buffer containing 300 mM NaCl and twice with RIPA containing 10 mM NaCl before boiling in sample buffer. For immunoprecipitation of ^{32}P-labeled lysates, 1 mM Na_3VO_4 and 0.1 mM Na_2MoO_4 were included in lysis and immunoprecipitation buffers to inhibit phosphatases.

Analysis of phosphorylated amino acids was performed as described (7), except rehydrated gel slices were digested in 1 ml of proteinase K (50 μg/ml) prior to acid hydrolysis. Partial proteolytic mapping was essentially as described (8); each gel slice was digested with 100 ng of V8 protease.

Bacterial crk Coupled to Beads. crk peptide-expressing bacteria and bacteria containing only the pET-3a vector were spun out 3 hr after induction and lysed by freeze–thawing in ME buffer (100 mM Mops, pH 7.5/1 mM EDTA) containing 1 mM phenylmethylsulfonyl fluoride. $MgCl_2$ was added to 10 mM, DNase I to 50 μg/ml, and RNase A to 20 μg/ml, and lysates were incubated on ice for 30 min to digest nucleic acids. EDTA was added to 15 mM, and lysates were cleared by spinning twice at 10,000 rpm for 30 min in a Sorvall SS34 rotor. Supernatant was dialyzed extensively against 100 mM Mops (pH 7.5). By Coomassie blue staining, we estimate that crk protein comprised 5–10% of total soluble protein prepared from crk-expressing bacteria.

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Abbreviations: CT10, avian sarcoma virus CT10; PLC, phospholipase C; GAP, ras GTPase activator protein; CEF, chicken embryo fibroblast; anti-ptyr, phosphotyrosine-specific antiserum; PDGF, platelet-derived growth factor.

Bacterial protein (30 mg per ml of Affigel-10) (Bio-Rad) was coupled following the manufacturer's instructions. Beads were washed extensively after coupling with ME buffer, then with RIPA containing 150 mM NaCl, then with PBS. Beads (packed volume, 25 μl) were mixed with ^{32}P-labeled lysate (1/5th 6-cm plate) in RIPA containing 150 mM NaCl including vanadate and molybdate, and rotated at 4°C for 1 hr. Beads were washed as for immunoprecipitation prior to boiling in SDS sample buffer and SDS/PAGE. Gel was washed with 1 M KOH for 2 hr at 55°C to enhance detection of phosphotyrosine-containing proteins (9).

RESULTS

crk-Specific Antiserum. We have shown previously that P47$^{gag\text{-}crk}$ is bound by antisera, such as tumor-bearing rabbit serum, that recognize the retroviral gag protein (1, 2). However, these sera give high backgrounds due to the large amount of retroviral structural proteins in infected cells. We raised a crk-specific antiserum against the c-crk-derived portion of P47$^{gag\text{-}crk}$ expressed in bacteria. This antiserum specifically immunoprecipitates the native v-crk protein from infected cell lysates and recognizes the denatured form in immunoblots (Fig. 1).

crk-Associated Kinase Activities. When unlabeled CT10-infected CEFs were lysed in RIPA buffer, immunoprecipitated with anti-crk antiserum, and incubated with [γ-^{32}P]ATP and manganese, four proteins were specifically phosphorylated in the *in vitro* reaction (Fig. 2*A*). The major band of 135–155 kDa was the same apparent size as the protein observed previously in *in vitro* kinase assays of tumor-bearing rabbit serum immunoprecipitates (refs. 1 and 2; Fig. 2*A*). The other three proteins had apparent masses of 47, 52, and 62 kDa. Analysis of phosphorylated amino acids showed that the proteins in the lower (135 kDa) and upper (140–155 kDa) parts of the 135- to 155-kDa band were phosphorylated predominantly on tyrosine, while the 47- and 52-kDa proteins were more highly phosphorylated on serine and threonine. The 62-kDa protein was phosphorylated almost exclusively on threonine.

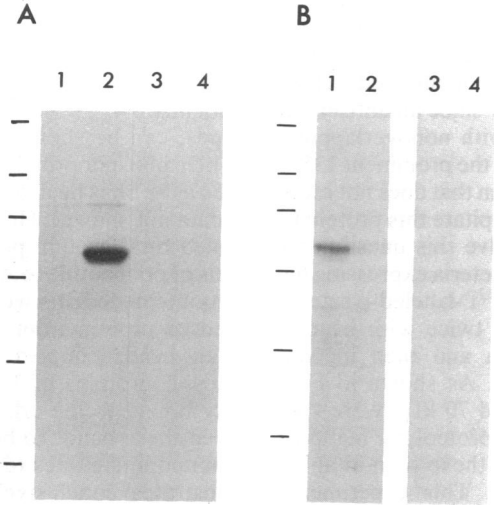

FIG. 1. Specificity of anti-crk antiserum. (*A*) [^{35}S]Methionine-labeled lysates from CEFs infected with CT10 (lanes 1 and 2) or UR2AV (lanes 3 and 4), immunoprecipitated with anti-crk antiserum (lanes 2 and 4) or preimmune serum (lanes 1 and 3). (*B*) Immunoblots of CT10-infected (lanes 1 and 3) or UR2AV-infected (lanes 2 and 4) cell lysates probed with anti-crk antiserum (lanes 1 and 2) or preimmune serum (lanes 3 and 4). Gels were 10% acrylamide; markers were 220, 100, 68, 43, 27, and 18 kDa.

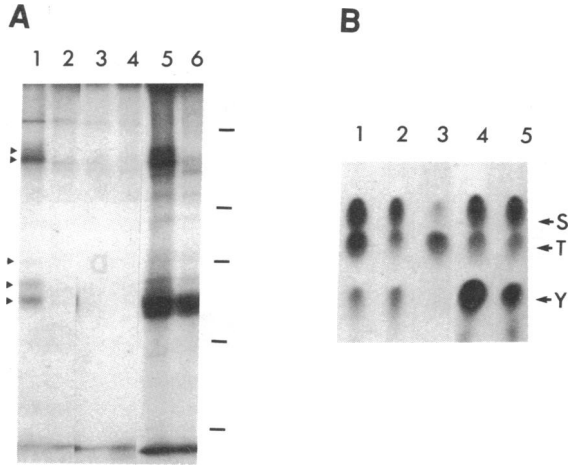

FIG. 2. *In vitro* kinase activity of crk immunoprecipitates and analysis of proteins phosphorylated *in vitro*. (*A*) *In vitro* kinase assays performed on CEFs infected with CT10 (lanes 1, 3, and 5) or UR2AV (lanes 2, 4, and 6) and immunoprecipitated with anti-crk antiserum (lanes 1 and 2), preimmune rabbit serum (lanes 3 and 4), or tumor-bearing rabbit serum (lanes 5 and 6). Locations of proteins analyzed for content of phosphorylated amino acids are indicated by arrowheads. Gel was 8.5% acrylamide; markers are the same as in Fig. 1. (*B*) Phosphorylated amino acid content of proteins indicated in *A*. Lanes: 1, 47 kDa; 2, 52 kDa; 3, 62 kDa; 4, 135 kDa; 5, 140–155 kDa. Positions of phosphorylated amino acid standards are indicated on the right: S, phosphoserine; T, phosphothreonine; Y, phosphotyrosine.

These results demonstrate that both tyrosine and serine-threonine kinase activities were present in anti-crk immunoprecipitates. The most likely explanation for these activities is that the kinases were bound to P47$^{gag\text{-}crk}$ directly or to other proteins bound to P47$^{gag\text{-}crk}$. It is unlikely that the kinase activity observed is nonspecific, since only antisera that recognize P47$^{gag\text{-}crk}$ (including tumor-bearing rabbit and anti-gag monoclonal antibodies) give *in vitro* kinase activity. However, it is possible that kinases nonspecifically bound to immune complexes were phosphorylating proteins specifically immunoprecipitated by the anti-crk antiserum. This is unlikely in the case of the tyrosine kinase, since increased tyrosine phosphorylation of exogenous substrates has also been observed in crk immunoprecipitates (M. Matsuda and H.H., unpublished observation).

It is unclear which, if any, of the proteins phosphorylated *in vitro* are kinases. Most tyrosine kinases autophosphorylate in *in vitro* reactions, but this might not necessarily be the case in v-crk immunoprecipitates, where it is likely that natural kinase substrates are present (see below). Partial proteolytic mapping demonstrated that the 47-kDa phosphorylated protein was P47$^{gag\text{-}crk}$ (see below and Fig. 4*B*); the 52-kDa band may be a modified form of P47$^{gag\text{-}crk}$, since a band of this size could often be seen in long exposures of anti-crk immunoblots (data not shown). A 135- to 155-kDa protein can be labeled *in vivo* with [^{32}P]orthophosphate and is associated with P47$^{gag\text{-}crk}$ (ref. 2; see below). Partial proteolytic mapping demonstrated that this protein is related to the 135- to 155-kDa protein phosphorylated *in vitro* (2). However, glycerol gradient sedimentation analysis showed that *in vitro* kinase activity (assayed by 135- to 155-kDa phosphorylation) did not correlate precisely with the presence of *in vivo*-labeled 135- to 155-kDa protein (2), suggesting that this protein is not itself a kinase.

crk-Associated Phosphoproteins. To determine what phosphoproteins might be associated with P47$^{gag\text{-}crk}$, CT10-infected CEFs were labeled with [^{32}P]orthophosphate and immunoprecipitated with anti-crk antiserum (Fig. 3*A*). P47$^{gag\text{-}crk}$ itself was highly phosphorylated; analysis of phos-

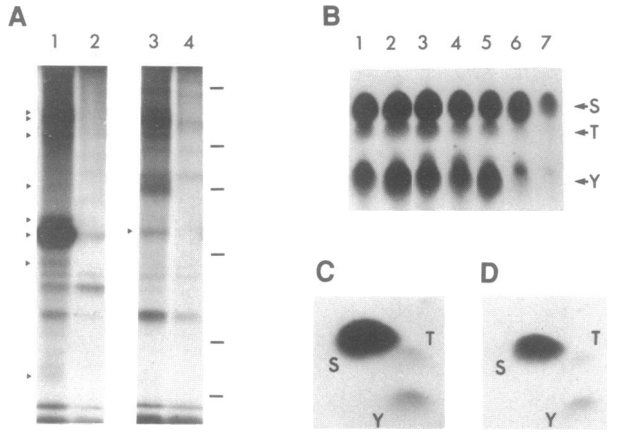

FIG. 3. Phosphoproteins coimmunoprecipitating with P47$^{gag-crk}$. (*A*) RIPA lysates of $^{32}P_i$-labeled CEFs infected with CT10 (lanes 1 and 3) or UR2AV (lanes 2 and 4) immunoprecipitated with anti-crk (lanes 1 and 2) or anti-ptyr (lanes 3 and 4) antisera. Bands analyzed for content of phosphorylated amino acids are indicated by arrowheads. Gel was 9% acrylamide; markers are the same as in Fig. 1. (*B*) One-dimensional analysis of phosphoproteins immunoprecipitated by anti-crk antiserum. Lanes: 1, 140–155 kDa; 2, 135 kDa; 3, 120 kDa; 4, 70 kDa; 5, 52 kDa; 6, 40 kDa; 7, 21 kDa. (*C* and *D*) Two-dimensional analysis of phosphorylated amino acids of P47$^{gag-crk}$ immunoprecipitated by anti-crk (*C*) or anti-ptyr (*D*) antisera. Positions of phosphorylated amino acid standards are indicated: S, phosphoserine; T, phosphothreonine; Y, phosphotyrosine.

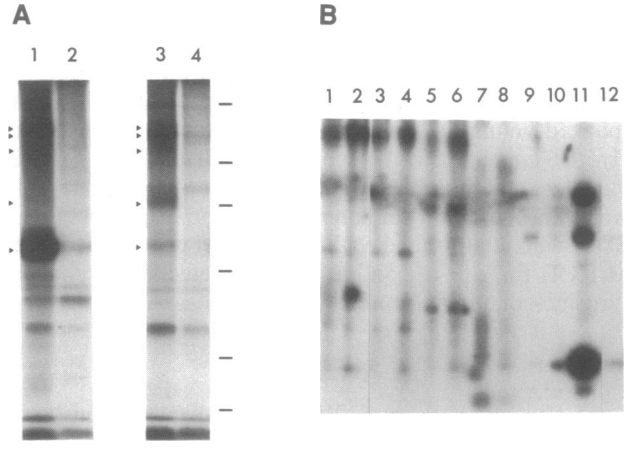

FIG. 4. Partial proteolytic mapping of phosphoproteins immunoprecipitated by anti-crk and anti-ptyr antisera. (*A*) Same gel as in Fig. 3*A*. $^{32}P_i$-labeled proteins from CEFs infected with CT10 (lanes 1 and 3) or UR2AV (lanes 2 and 4) immunoprecipitated with anti-crk (lanes 1 and 2) or anti-ptyr (lanes 3 and 4) antisera. Positions of bands mapped in *B* are indicated by arrowheads. (*B*) Partial proteolytic mapping of proteins indicated in *A* and P47$^{gag-crk}$. Lanes 1, 3, 5, 7, and 10 are from anti-ptyr immunoprecipitates (*A*, lane 3); lanes 2, 4, 6, 8, and 11 are from anti-crk immunoprecipitates (*A*, lane 1). Lanes: 1 and 2, 140–155 kDa; 3 and 4, 135 kDa; 5 and 6, 120 kDa; 7 and 8, 70 kDa; 10 and 11, 47 kDa. Lane 9, 47-kDa protein phosphorylated in *in vitro* kinase reactions of anti-crk immunoprecipitates. Lane 12, [^{35}S]-methionine-labeled P47$^{gag-crk}$ immunoprecipitated by anti-crk antiserum. Gel was 12.5% acrylamide.

phorylated amino acids demonstrated that the majority of phosphate was on serine, although ≈5% was on tyrosine (Fig. 3*C*). A number of other ^{32}P-labeled proteins that were not visible in helper virus-infected controls were also observed. Each of these coprecipitating proteins was found to contain detectable amounts of phosphotyrosine, in most cases approaching 50% of total phosphorylated amino acids (Fig. 3*B*). Since phosphotyrosine is such a rare modification [<1% of total phosphorylated amino acids, even in CEFs transformed by Rous sarcoma virus (10)], the high levels of phosphotyrosine on each of these coprecipitating proteins is significant.

When ^{32}P-labeled CT10-infected cell lysates were immunoprecipitated with an antiserum specific for phosphotyrosine (anti-ptyr) (7, 11), a faint 47-kDa band was observed (Fig. 3*A*). This protein was P47$^{gag-crk}$ (see below), and analysis of phosphorylated amino acids demonstrated that this fraction of P47$^{gag-crk}$ had similar relative levels of phosphorylated amino acids compared to the whole population immunoprecipitated by anti-crk (compare Fig. 3 *C* and *D*). It is likely that at least some of the P47$^{gag-crk}$ immunoprecipitated by anti-ptyr is precipitated by virtue of its association with other phosphotyrosine-containing proteins. This is supported by the observation that a 47-kDa band is usually not seen in anti-ptyr immunoblots (ref. 1; data not shown).

When the pattern of phosphoproteins immunoprecipitated by anti-crk antiserum and anti-ptyr are compared, several similarities are apparent (Fig. 3*A*). In each case, ^{32}P-labeled bands are seen at 135–155, 120, and 70 kDa. These molecular masses correspond to the three major bands seen in anti-ptyr immunoblots of CT10-infected cell lysates (1, 2). Immunoprecipitation with anti-ptyr, while less sensitive than immunoblotting, has the advantage that phosphotyrosine-containing proteins can be recovered from the gel and proteolytically mapped. The profiles generated by partial digestion with V8 protease (8) of the major bands immunoprecipitated by anti-ptyr and anti-crk were compared (Fig. 4). In each case, the proteolytic maps were essentially identical, demonstrating that the three major phosphotyrosine-containing proteins of CT10-infected cells could be immunoprecipitated in apparent association with P47$^{gag-crk}$. The data in Fig. 4 also demonstrate that the 135-kDa protein and the 140- to 155-kDa material that migrated above it are closely related or identical.

The ^{32}P-labeled 47-kDa bands phosphorylated in *in vitro* kinase assays, immunoprecipitated by anti-crk or by anti-ptyr antisera, were found by partial proteolytic mapping to be identical to authentic [^{35}S]methionine-labeled P47$^{gag-crk}$ (Fig. 4*B*). It is interesting that P47$^{gag-crk}$ can be phosphorylated *in vitro* on tyrosine, albeit at low levels, suggesting that it might also be a substrate for the crk-associated tyrosine kinase *in vivo*.

Bacterially Synthesized crk Protein Binds to Cellular Phosphoproteins. We were concerned that the apparent association between P47$^{gag-crk}$ and the phosphotyrosine-containing proteins of infected cells might be nonspecific. This was unlikely since all antisera that recognize P47$^{gag-crk}$, including those with nonoverlapping epitopes, can be shown to precipitate the prominent 135- to 155-kDa phosphoprotein, while no serum that does not recognize P47$^{gag-crk}$ has been observed to precipitate this protein (ref. 2; data not shown). However, to resolve this question we coupled total protein prepared from bacteria expressing high levels of crk peptide to Affi-Gel beads. ^{32}P-labeled lysates of CT10-infected CEFs were first cleared twice with beads coupled to protein from normal bacteria and then incubated with beads coupled to crk protein. As shown in Fig. 5, phosphoproteins of 135–155, 120, and 70 kDa were specifically bound to the crk beads. Partial proteolytic mapping showed these bands to be identical to those seen in anti-crk immunoprecipitates (data not shown). This experiment demonstrates conclusively that P47$^{gag-crk}$ can associate *in vitro* with the major phosphotyrosine-containing proteins of CT10-infected cells. Since the bacterial peptide does not contain any gag sequences, this result also demonstrates that the association is mediated by the c-crk-derived portion of P47$^{gag-crk}$. The fact that association can be reconstituted *in vitro* with bacterially synthesized crk protein should simplify the purification of crk-associated phosphoproteins.

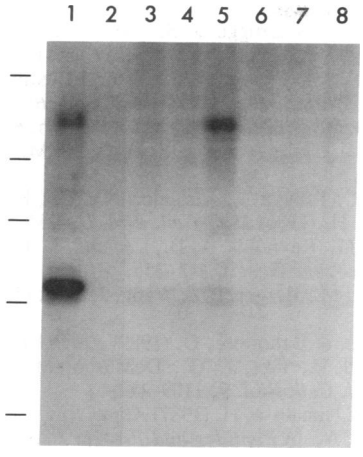

FIG. 5. Phosphotyrosine-containing proteins bound to bacterial crk protein. $^{32}P_i$-labeled lysates from CEFs infected with CT10 (lanes 1, 3, 4, and 5) or UR2AV (lanes 2, 6, 7, and 8) immunoprecipitated with anti-crk antiserum (lanes 1 and 2) or incubated with beads coupled to bacterial proteins (lanes 3–8). Lanes 3 and 6, proteins bound to nonspecific beads (first clear); lanes 4 and 7, proteins bound to a fresh aliquot of nonspecific beads added to the same lysate (second clear); lanes 5 and 8, proteins bound to crk-containing beads added to the cleared lysate. Gel was 8.5% acrylamide and was washed with 1 M KOH at 55°C (9).

DISCUSSION

In this study, we have used a crk-specific antiserum and bacterially produced crk protein to demonstrate that all of the major phosphotyrosine-containing proteins of CT10-infected cells can bind tightly to the crk protein *in vitro* (and presumably *in vivo*). We have also presented evidence that a tyrosine kinase and a serine-threonine kinase activity may be associated with $P47^{gag\text{-}crk}$. Since many growth factor receptors and oncogene products have intrinsic protein-tyrosine kinase activity (12), and since we have previously shown that increased phosphotyrosine is not an obligatory by-product of malignant transformation (2), we assume that the increased phosphotyrosine observed in CT10-infected cells is involved in the mechanism of transformation by the *crk* oncogene. This study addresses the question of how $P47^{gag\text{-}crk}$, which has no homology to any known catalytic domain, can increase cellular phosphotyrosine.

The v-*crk* gene has two regions of sequence homology, termed the SH2 and SH3 domains, found in the nonreceptor protein-tyrosine kinases, PLC-γ, GAP, and other proteins (1, 3, 13, 14). For the tyrosine kinases (15–17) and PLC (18, 19), it has been conclusively shown that the SH2 and SH3 domains are not required for catalytic activity. This suggests that these domains may function to regulate the activity of the catalytic domain. Transformation by v-crk, therefore, may reflect a perturbation of normal modulatory mechanisms involved in signal transduction pathways.

Possible mechanisms whereby the crk protein could elevate cellular phosphotyrosine can be evaluated in light of the available experimental evidence. The most straightforward possibilities are the activation of an endogenous protein-tyrosine kinase or the inactivation of a protein-tyrosine phosphatase. We consider the latter model unlikely given the presence of tyrosine kinase activity in anti-crk immunoprecipitates. If a tyrosine kinase were activated, this could be accomplished either directly by binding to $P47^{gag\text{-}crk}$ or indirectly by sequestering a putative tyrosine kinase inhibitor.

Direct activation by binding to src family tyrosine kinases has been proposed as the mechanism of transformation by polyoma virus middle-sized tumor antigen (mTAg) (20–22). Although the presence of tyrosine kinase activity in anti-crk immunoprecipitates is consistent with such a model, there are significant quantitative differences between transformation by the two oncogenes. First, the *in vitro* kinase activity of anti-crk immunoprecipitates is much weaker than that associated with mTAg (data not shown). In contrast, *in vivo* phosphotyrosine levels are much higher in crk-transformed cells (1) than in those transformed by mTAg, where the phosphatase inhibitor sodium orthovanadate must be added to the culture medium to observe increased phosphotyrosine (refs. 23 and 24; data not shown).

The possibility that $P47^{gag\text{-}crk}$ might transform by sequestering a kinase inhibitor is intriguing but is not yet supported by data. The observation that N-terminal mutations, especially within the SH3 domain, can activate the transforming activity of the normally nontransforming c-*src* and c-*abl* gene products (25–29) raised the possibility that SH3 might bind a kinase inhibitor. If this were the case, the overexpressed SH3 domain of $P47^{gag\text{-}crk}$ might be expected to compete away such an inhibitor, leading to kinase activation *in vivo*. The presence of kinase activity and the phosphotyrosine-containing substrates in anti-crk immunoprecipitates is not addressed by this model, however. Furthermore, we have expressed the SH2 + SH3 domains of $p60^{c\text{-}src}$ and PLC-γ in chicken cells and have not observed any increase in intracellular phosphotyrosine as would be expected if an inhibitor were sequestered (B.J.M., S. Kornbluth, and H.H., unpublished observations).

A model that incorporates all of the experimental data is one in which $P47^{gag\text{-}crk}$ increases phosphotyrosine by binding to both kinases and substrates in a ternary complex, leading to increased phosphorylation of the substrates due to increased local concentration and/or favorable conformation. In addition to the data presented here, there is other evidence that the SH2 and SH3 domains might be involved in binding to both kinase substrates and kinase catalytic domains. Mutant studies on the transforming nonreceptor tyrosine kinases suggest that the SH2 domain might bind cellular factors such as critical substrates (reviewed in refs. 3, 30, and 31). For example, mutation of the SH2 domain of c-src variants was shown to block binding to and tyrosine phosphorylation of two substrate proteins (32). Evidence that the N-terminal and catalytic domains of the nonreceptor tyrosine kinases are tightly associated includes the positive and negative effects of N-terminal mutants described above, protease-resistance data (33), and the isolation of a monoclonal antibody that recognizes an epitope assembled from N-terminal and catalytic domain determinants of src family kinases (34). It will be interesting to see whether either the SH2 or SH3 domain of v-crk is sufficient to bind phosphotyrosine-containing proteins or protein-tyrosine kinase activity.

This model is consistent with a more general picture that is beginning to emerge, in which many of the molecules implicated in signal transduction bind to and interact with each other. The platelet-derived growth factor (PDGF) and epidermal growth factor receptors, when stimulated by ligand, bind tightly to PLC-γ and phosphorylate it on tyrosine (35–39); GAP is also rapidly phosphorylated on tyrosine upon PDGF treatment (40). In addition to these SH2- and SH3-containing proteins, the raf-1 serine-threonine kinase and type I phosphatidylinositol kinase have been shown to bind to and/or be phosphorylated by the activated PDGF receptor (41, 42); a serine-threonine kinase activity and type I phosphatidylinositol kinase also apparently associate with $P47^{gag\text{-}crk}$ (Fig. 2; ref. 43). It may be that the SH2 and SH3 domains mediate the assembly of critical multiprotein complexes involved in the regulation of growth control.

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