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The Same Normal Cell Protein Is Phosphorylated After Transformation by Avian Sarcoma Viruses with Unrelated Transforming Genes

ELEANOR ERIKSON,* ROBERT COOK, GARY J. MILLER, AND R. L. ERIKSON

Department of Pathology, University of Colorado Health Sciences Center, School of Medicine, Denver, Colorado 80262

The phosphorylation of a normal cellular protein of molecular weight 34,000 (34K) is enhanced in Rous sarcoma virus-transformed chicken embryo fibroblasts apparently as a direct consequence of the phosphotransferase activity of the Rous sarcoma virus-transforming protein pp60^{src}. We have prepared anti-34K serum by using 34K purified from normal fibroblasts to confirm that the transformation-specific phosphorylation described previously occurs on a normal cellular protein and to further characterize the nature of the protein. In this communication, we also show that the phosphorylation of 34K is also increased in cells transformed by either Fujinami or PRCII sarcoma virus, two recently characterized avian sarcoma viruses whose transforming proteins, although distinct from pp60^{src}, are also associated with phosphotransferase activity. Moreover, comparative fingerprinting of tryptic phosphopeptides shows that the major site of phosphorylation of 34K is the same in all three cases.

The process of fibroblast transformation in culture by Rous sarcoma virus (RSV) has been extensively studied and has resulted in the characterization of the viral transforming gene *src* and a phosphoprotein product of *src*, pp60^{src} (1, 2, 8, 13, 24, 27). Two other avian sarcoma viruses, Fujinami (FSV) and PRCII, have been recently characterized and shown to be defective in their replication (15, 21; M. L. Breitman, J. C. Neil, C. Moscovici, and P. K. Vogt, Virology, in press; J. C. Neil, M. L. Breitman, and P. K. Vogt, Virology, in press). Unlike most strains of RSV, which have no helper requirement, FSV and PRCII need an associated helper virus for the synthesis of the structural proteins necessary to produce infectious progeny virus. The middle portion of the transforming ribonucleic acid genome of each of these viruses carries unique sequences unrelated to RSV *src* sequences, with helper viral ribonucleic acid sequences at both the 5′ and 3′ ends. The transforming gene was apparently generated by fusion of the helper virus *gag* gene and sequences derived from the host cell. Molecular hybridization shows that the genes of these two transforming viruses are related but apparently not identical (15). The only known protein product of these transformation-specific sequences is apparently a product of this fused gene, since it can be immunoprecipitated with antibody against viral *gag* gene-encoded polypeptides. The molecular weights of the transformation-specific protein are 140,000 for FSV and 105,000 for PRCII (15; Neil et al., in press).

The RSV *src* gene product, pp60^{src}, has been shown to be closely associated with a protein kinase activity (6, 7, 11, 12). Although they are antigenically different from pp60*src*, p140 and p105 in specific immunoprecipitates also have an associated protein kinase activity which results in phosphorylation of p140 or p105 as well as of rabbit immunoglobulin G of the immune complex (J. C. Neil, personal communication; R. A. Feldman, T. Hanafusa, and H. Hanafusa, Cell, in press).

Recently we identified a normal cellular protein of 34,000 daltons (34K) that appears to be a substrate of the phosphotransferase activity of pp60^{src} in RSV-transformed avian and mammalian cells (10). Antibody against 34K has been prepared to aid in completion of its characterization and to gain insight about the general significance of this protein and its phosphorylation. Furthermore, in view of the potentially similar function of the RSV- and FSV/PRCII-transforming gene products, we have examined cells transformed by the latter two viruses for the phosphorylated form of 34K. The results of these studies show that 34K is a relatively abundant protein in normal uninfected cells and that it is phosphorylated at a similar site in cells transformed by each of the three viruses.

MATERIALS AND METHODS

Cells and viruses. Chicken embryo fibroblasts were prepared from 11-day-old embryos (Spafas, Inc., Roanoke, Ill.). The Schmidt-Ruppin strain of RSV,

subgroup D (SRD), was originally obtained from J. Wyke. FSV was obtained from H. Hanafusa. PRCII sarcoma virus was obtained from P. K. Vogt. Transformed chicken cells were used for experiments several passages after they had been infected.

Preparation of antisera. Serum from rabbits bearing tumors induced by SRD (TBR serum) was prepared as originally described by Brugge and Erikson (1). This serum has antibodies against the avian sarcoma virus structural proteins (*gag*), as well as against the RSV-transforming protein, $pp60^{src}$. The TBR serum used in these studies was chosen for its high titer of anti-*gag* activity. Serum against the 34K protein was prepared in rabbits by injection of approximately 100 μg of 34K, purified from normal chicken embryo fibroblasts by ion-exchange chromatography (10), in complete Freund adjuvant into the popliteal lymph nodes. Subsequent injections of the same amount of protein in incomplete Freund adjuvant were administered intramuscularly and subcutaneously at 3-week intervals. The serum used in these studies was obtained 12 days after the first boost.

Immunoprecipitation of cell lysates. Cultures were radiolabeled with 50 to 100 μCi of [^{35}S]methionine per ml (700 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) for 2 to 4 h in methionine-free medium or with 0.5 to 1 mCi of [^{32}P]orthophosphate per ml (carrier-free; ICN, Irvine, Calif.) for 2 to 3 h in phosphate-free medium. Cells were washed, scraped from the dishes, and lysed in 100 mM NaCl–10 mM tris(hydroxymethyl)amino-methane (Tris)-hydrochloride (pH 6.5)–1 mM ethylenediamine-tetraacetate–1% Nonidet P-40–0.5% sodium deoxycholate. After clarification at 100,000 × *g* for 30 min, samples of the supernatants were immunoprecipitated with preimmune or anti-34K serum with the use of protein A-containing *Staphylococcus aureus* strain Cowan I (19) as detailed previously (1). The bacteria-immune complexes were washed once with 1 M NaCl-10 mM Tris-hydrochloride (pH 7.2)–0.1% Nonidet P-40 and three times with 150 mM NaCl–10 mM Tris-hydrochloride (pH 7.2)–1% sodium deoxycholate–1% Triton X-100–1 M urea. Immunoprecipitated proteins were released from the bacteria by treatment at 95°C for 1 min in electrophoresis sample buffer (70 mM Tris-hydrochloride [pH 6.8], 11% glycerol, 3% sodium dodecyl sulfate [SDS], 0.01% bromophenol blue, 5% 2-mercaptoethanol) and resolved on SDS-polyacrylamide gels.

To assay for immune-complex kinase activity, unlabeled cells were lysed in RIPA buffer (14) (150 mM NaCl, 10 mM Tris-hydrochloride [pH 7.2], 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS) and clarified, and the supernatants were immunoprecipitated as described above with TBR serum. The immune complexes were washed several times, suspended in buffer containing adenosine [γ-^{32}P]triphosphate (5,000 Ci/mmol; prepared by the method of Johnson and Walseth [18]), and incubated at 22°C for 15 min, as originally described (6) except that 10 mM $MnCl_2$ was used instead of 5 mM $MgCl_2$. The products of the reaction were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography.

Polyacrylamide gel analyses and peptide mapping. Proteins were resolved by electrophoresis through a discontinuous slab gel system (10% acrylamide, 0.26% bisacrylamide) with the buffer systems described by Laemmli (20). Before electrophoresis, samples were heated at 95°C for 1 min in electrophoresis sample buffer. Two-dimensional fractionation of proteins was performed by non-equilibrium pH gradient gel electrophoresis, using pH 3.5 to 10 ampholines in the first dimension (23). Polyacrylamide gels, 10%, were used for the second dimension. Gels were stained, destained, and dried as described previously (9). ^{32}P-labeled proteins were located by autoradiography with the aid of DuPont Lightning-Plus intensifying screens. Fluorography with sodium salicylate (3) was used to enhance detection of ^{35}S-labeled proteins.

For two-dimensional peptide fingerprinting, polypeptides were eluted from preparative gels, precipitated, and digested with tolylsulfonyl phenylalanyl chloromethyl ketone-treated trypsin (4). The peptides were resolved by ascending chromatography in *n*-propanol–*s*-butanol–isoamyl alcohol–pyridine–water (1:1:1:3:3, by volume) followed by electrophoresis in the second dimension at pH 3.5 (pyridine-acetic acid-water [1:10:189, by volume]).

RESULTS

Immunoprecipitation of 34K. After injection of the 34K protein into rabbits as described in Materials and Methods, the serum obtained was tested for anti-34K activity by conventional immunoprecipitation of biosynthetically [^{35}S]methionine-labeled total cellular proteins. One prominent protein was immunoprecipitated from both normal and transformed cells (Fig. 1, tracks 1 to 4). This protein comigrated with radiolabeled 34K prepared according to procedures detailed earlier (10) (data not shown). The results depicted here were obtained with a limiting amount of antibody. Immunoprecipitation experiments with a constant amount of antiserum versus various amounts of cell lysate and quantification of the radioactivity recovered in the 34K region of the gel revealed that 34K constituted approximately 0.9% of the protein in the clarified lysate for both normal and transformed cells. For this determination cells were radiolabeled for either 7 h or 18 h. In the latter case, unlabeled methionine was added to the medium at 1/10 the normal concentration of methionine. The value for the percentage of 34K is based on the assumption that the specific activity of 34K was the same as that of the total cellular proteins.

When extracts of cells which had been radiolabeled with [^{32}P]orthophosphate were immunoprecipitated and similarly analyzed, the amount of radiolabel in the 34K region of the gel was greatly increased (about fivefold) in 34K immunoprecipitated from transformed cells (Fig. 1, tracks 5 to 8). These results were also obtained with a limiting amount of antiserum; therefore, the ^{32}P radiolabel in this region of the gel is an indication of the specific activity of the

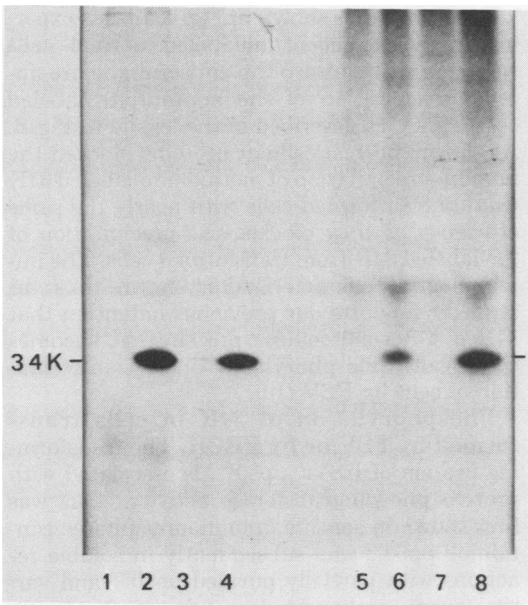

FIG. 1. *Immunoprecipitation of 34K from normal and transformed chicken embryo fibroblasts. Normal and SRD-transformed chicken embryo fibroblasts were labeled with either [^{35}S]methionine or [^{32}P]orthophosphate, lysed, clarified, and immunoprecipitated with either preimmune (tracks 1, 3, 5, and 7) or anti-34K (tracks 2, 4, 6, and 8) serum as described in the text. A 2.5-μl portion of serum was used to immunoprecipitate approximately 70 μg of protein in the clarified lysate, a ratio determined to result in antigen excess. The immunoprecipitated proteins were resolved by electrophoresis through an SDS-polyacrylamide gel and visualized by fluorography or autoradiography. Tracks: 1 and 2, [^{35}S]methionine-labeled normal cells; 3 and 4, [^{35}S]methionine-labeled SRD-transformed cells; 5 and 6, ^{32}P-labeled normal cells; 7 and 8, ^{32}P-labeled SRD-transformed cells.*

protein with respect to ^{32}P. The amount of ^{32}P radiolabel in 34K from normal cells varied, with some embryos showing higher levels than others. The results depicted here were obtained from normal and transformed cells of the same embryo.

To confirm that the protein obtained by immunoprecipitation was indeed the 34K protein described previously, we carried out two further methods of characterization, non-equilibrium pH gradient gel electrophoresis and tryptic phosphopeptide analysis. The transformation-specific phosphorylation of 34K was first detected (10, 12, 25) by the fractionation of total cellular proteins from ^{32}P-labeled cells by a two-dimensional procedure consisting of non-equilibrium pH gradient electrophoresis in the first dimension followed by SDS-polyacrylamide gel electrophoresis in the second dimension to resolve proteins on the basis of both charge and molecular weight (23). Our subsequent experiments permitted the localization of unphosphorylated 34K in this two-dimensional gel system by either [^{35}S]methionine labeling or Coomassie blue staining (10).

Immunoprecipitates were prepared with anti-34K serum from [^{35}S]methionine-labeled normal cells and from ^{32}P-labeled transformed cells. Proteins were released from the immunoadsorbent by treatment with urea and mercaptoethanol, as described in the legend to Fig. 2, and fractionated by the two-dimensional gel procedure described above. First, proteins immunoprecipitated from [^{35}S]methionine-labeled normal cells were mixed with a lysate of unlabeled normal cells and subjected to two-dimensional analysis. The gel was stained, destained, dried, and exposed to film without the use of fluorography. The [^{35}S]methionine-labeled, immunoprecipitated 34K protein comigrated with the stainable spot previously identified as unphosphorylated 34K (data not shown). Furthermore, in this gel system ^{32}P-labeled 34K immunoprecipitated from transformed cells migrated relative to [^{35}S]methionine-labeled 34K immunoprecipitated from normal cells in a manner similar to that of the corresponding 34K proteins prepared from cells by ion-exchange chromatography (Fig. 2). To determine the percentage of 34K in the phosphorylated form, [^{35}S]methionine-labeled transformed cells were lysed in 2% SDS and heated at 95°C for 2 min in an attempt to inactivate phosphatases or kinases. The lysates were then diluted with 20 volumes of RIPA buffer lacking SDS, clarified, and immunoprecipitated as described above. The immunoprecipitated proteins were analyzed by two-dimensional gel electrophoresis. Under these conditions 5 to 10% of the radiolabel migrated at the position corresponding to the phosphorylated form of 34K.

The two-dimensional trypic phosphopeptide map of 34K immunoprecipitated from extracts of ^{32}P-labeled transformed cells is shown in Fig. 3B. This map is very similar to that of ^{32}P-labeled 34K prepared as described previously (10; Fig. 3A). Longer exposure reveals that immunoprecipitated 34K also contains the minor phosphotyrosine-containing peptide evident in Fig. 3A. Analysis of a mixture of the two preparations confirmed that the major spots comigrated (Fig. 3C). Direct analysis has previously determined that the spots in Fig. 3A marked TYR are phosphotyrosine-containing peptides, whereas that marked SER is a phosphoserine-containing peptide. 34K immunoprecipitated from ^{32}P-labeled normal cells (as shown in Fig. 1, track 6) and analyzed by this procedure

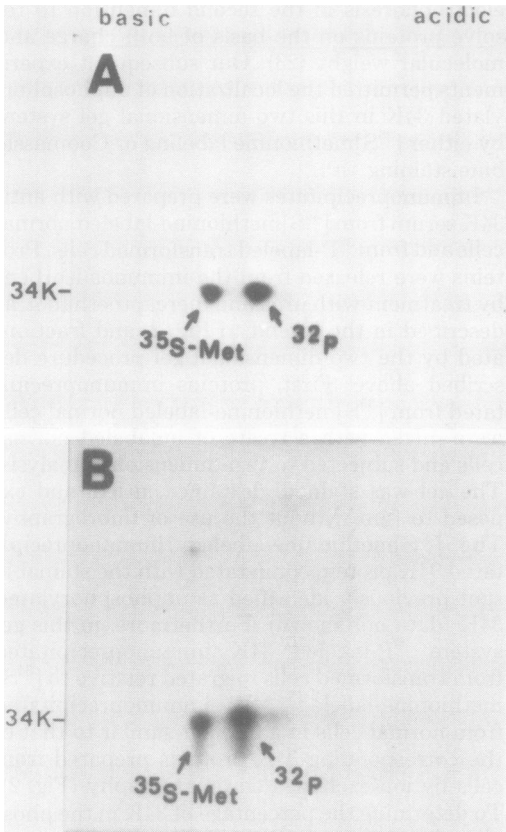

FIG. 2. *Non-equilibrium pH gradient gel electrophoresis of immunoprecipitated 34K proteins. Extracts of [^{35}S]methionine (^{35}S-Met)-labeled normal cells or ^{32}P-labeled SRD-transformed cells were immunoprecipitated with anti-34K serum as described in the text and the legend to Fig. 1. The immunoprecipitated proteins were released from the bacterial adsorbent by treatment with urea-containing lysis buffer (9.5 M urea, 5% 2-mercaptoethanol, 2% Nonidet P-40, 2% ampholines [22]), and samples were fractionated by non-equilibrium pH gradient electrophoresis in the first dimension (right to left) and SDS-polyacrylamide gel electrophoresis in the second dimension (top to bottom). In addition, [^{35}S]methionine- and ^{32}P-labeled 34K proteins were purified from normal and transformed cells, respectively, by ion-exchange chromatography (10) and fractionated as described above. (A) [^{35}S]methionine- and ^{32}P-labeled 34K prepared by ion-exchange chromatography; (B) [^{35}S]methionine- and ^{32}P-labeled 34K prepared by immunoprecipitation.*

yielded radiolabel that comigrated with the phosphoserine peptide and as a streak along the origin of the second dimension (data not shown).

Additional support for the immunological relationship of the ^{32}P-labeled protein in the immunoprecipitates to the normal-cell methionine-labeled protein is shown in Fig. 4. In this experiment an extract of unlabeled normal cells was used to preadsorb the antiserum before immunoprecipitation of the appropriate labeled cell extract, as described in the legend to Fig. 4. As shown, normal cellular proteins blocked the immunoprecipitation of methionine-labeled 34K from untransformed cells with nearly the same efficiency as they blocked the precipitation of ^{32}P-labeled 34K from transformed cells. The immunological characterization shown thus far strongly supports our previous contention that 34K is a normal cellular protein that becomes specifically phosphorylated upon transformation of cells by RSV (10).

Phosphorylation of 34K in cells transformed by FSV or by PRCII. The transforming protein of RSVs, pp60^{src}, is associated with protein phosphotransferase activity. This was first shown in specific immunoprecipitates containing pp60^{src} and subsequently in soluble reactions with partially purified pp60^{src} and various exogenously added proteins (6, 7, 11, 12). This observation gave impetus to the studies which led to the detection of the transformation-specific phosphorylation of 34K.

Two recently characterized avian sarcoma viruses, FSV and PRCII, encode the transformation-specific proteins p140 and p105, respectively, as mentioned above. Because specific immunoprecipitates of these putative transforming proteins also reveal protein phosphotransferase activity (Neil, personal communication; Feldman et al., in press), we wished to determine whether the phosphorylation of 34K was increased in cells transformed by these viruses. We repeated the immune-complex phosphotransferase assay, using the anti-*gag* activity of TBR serum to immunoprecipitate p140 and p105. The results shown in Fig. 5 reveal that when specific immunoprecipitates containing either p140 (FSV) or p105 (PRCII) are incubated with adenosine [γ-^{32}P]triphosphate, protein kinase activities result in the phosphorylation of p140 and p105 as well as the phosphorylation of immunoglobulin G from rabbits bearing RSV-induced fibrosarcomas, thus confirming the results obtained in the laboratories of H. Hanafusa (Feldman et al., in press) and P. K. Vogt and coworkers (Neil, personal communication). The low level of phosphorylation of immunoglobulin G in the case of the normal cell lysate and TBR serum (track 2) results from a small degree of immune cross-reactivity of this particular TBR serum with normal cell pp60^{src} (5).

Immunoprecipitation of lysates of ^{32}P-radiolabeled FSV- or PRCII-transformed cells with anti-34K serum indicated that the phosphorylation of 34K was increased in those cells (Fig.

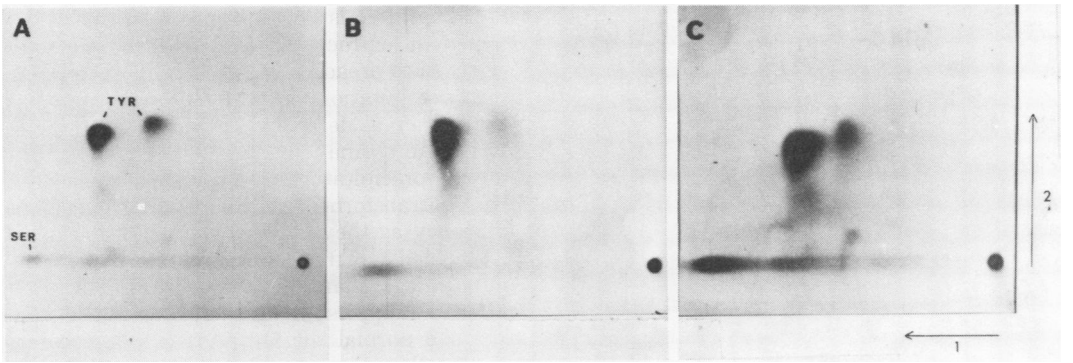

FIG. 3. *Tryptic phosphopeptide analysis of immunoprecipitated 34K. ^{32}P-labeled 34K was isolated from an extract of SRD-transformed cells by passage through diethylaminoethyl Sephacel and polyacrylamide gel electrophoresis as described previously (10). In addition, ^{32}P-labeled 34K was purified from SRD-transformed cells by immunoprecipitation with anti-34K serum and polyacrylamide gel electrophoresis, as shown in Fig. 1, track 8. The two radiolabeled 34K proteins were localized in preparative gels by autoradiography, excised, eluted from the gels, precipitated, digested with trypsin, and fractionated by chromatography in the first dimension and by electrophoresis at pH 3.5 in the second dimension. (A) ^{32}P-labeled 34K prepared by biochemical techniques; (B) ^{32}P-labeled 34K prepared by immunoprecipitation; (C) mix of the two preparations shown in (A) and (B).*

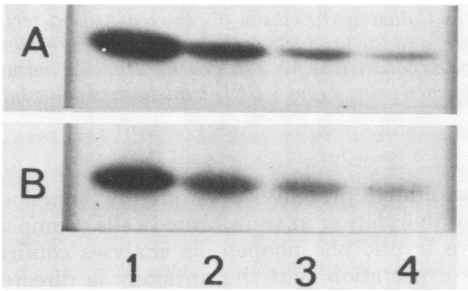

FIG. 4. *Effect of preadsorption of anti-34K serum with normal cellular proteins on the immunoprecipitation of [^{35}S]methionine- or ^{32}P-labeled 34K. Normal unlabeled chicken embryo fibroblasts were lysed and clarified as described in the text. One-microliter samples of anti-34K serum were incubated with various amounts of this lysate for 30 min at 4°C before the addition of the radiolabeled cell extracts. After further incubation for 30 min at 4°C, protein A-containing S. aureus was added, and the immunoprecipitated proteins were analyzed as described in the legend to Fig. 1. (A) [^{35}S]methionine-labeled normal cell extract; (B) ^{32}P-labeled transformed cell extract. Tracks: 1, no unlabeled lysate; 2, 20 μg of unlabeled lysate; 3, 100 μg of unlabeled lysate; 4, 500 μg of unlabeled lysate.*

6), as it was in RSV-transformed cells (Fig. 1, tracks 5 to 8). In addition, analysis by two-dimensional gel electrophoresis revealed that ^{32}P-radiolabeled 34K from these cells comigrated with that from SRD-transformed cells.

It has previously been reported that ^{32}P-labeled 34K from RSV-transformed cells contains radiolabel in both phosphoserine and phosphotyrosine (10). Phosphoamino acid analysis on ^{32}P-labeled 34K from cells transformed by either FSV or PRCII also revealed both phosphoserine and phosphotyrosine (data not shown).

To compare the specific sites phosphorylated in the 34K protein in cells transformed by either FSV or PRCII with those phosphorylated in cells transformed by SRD, these proteins were subjected to tryptic phosphopeptide analysis. Figure 7 demonstrates that ^{32}P-labeled 34K from these cells yielded tryptic phosphopeptide maps identical to that obtained from RSV-transformed cells (Fig. 3A and B). We have previously shown that partially purified pp60^{src} phosphorylates 34K in vitro at a site similar to the major phosphotyrosine-containing tryptic peptide shown here (10); thus, if the phosphorylation observed in FSV/PRCII-transformed cells is a direct consequence of p140 and p105 activities, the enzymes concerned have evolved to have the same substrate specificities.

DISCUSSION

We have previously argued, based on conventional biochemical results, that the RSV-transforming gene product pp60^{src} directly phosphorylates a normal cellular protein of 34,000 molecular weight. The new immunological data presented here provide additional strong support for this contention since we have now raised antibody against the protein purified from normal chicken embryo fibroblasts grown in culture. The antibody obtained produced specific immunoprecipitates of 34K from both normal and transformed cells, and moreover, the immuno-

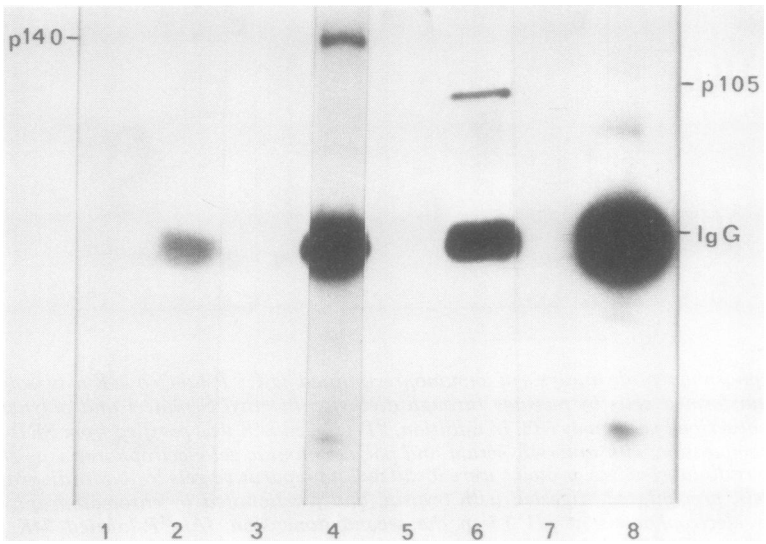

FIG. 5. *Protein kinase activity associated with immunoprecipitates containing pp60^{src}, p140, or p105. Extracts of normal chicken embryo fibroblasts or of chicken embryo fibroblasts transformed by FSV or PRCII or SRD avian sarcoma virus were immunoprecipitated with normal rabbit (tracks 1, 3, 5, and 7) or TBR (tracks 2, 4, 6, and 8) serum. As described in the text and by Collett and Erikson (6), the washed bacteria-immune complexes were suspended in kinase reaction mixture, and the products of the reaction were resolved by polyacrylamide gel electrophoresis and autoradiography. Exposure was for 3 h. Tracks: 1 and 2, normal cells; 3 and 4, FSV-transformed cells; 5 and 6, PRCII-transformed cells; 7 and 8, SRD-transformed cells. IgG, Immunoglobulin G.*

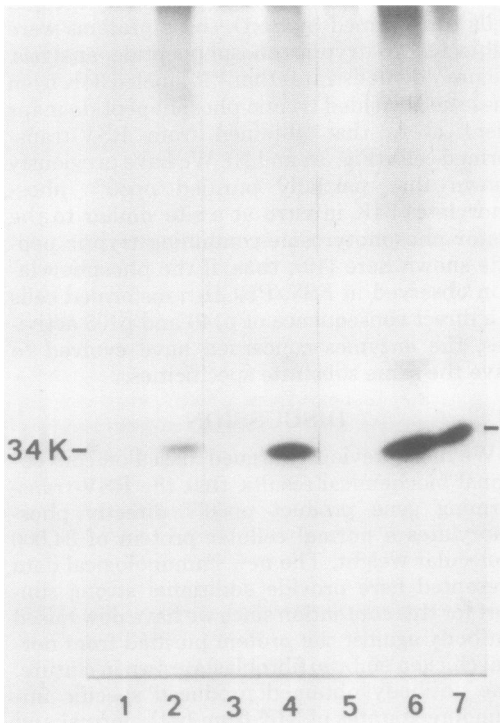

FIG. 6. *Transformation-specific phosphorylation of 34K in FSV- and PRCII-transformed chicken embryo fibroblasts. Normal or transformed cells were radiolabeled with [^{32}P]orthophosphate. Cell lysates were prepared and immunoprecipitated with preimmune (tracks 1, 3, and 5) or anti-34K (tracks 2, 4, 6, and 7) serum and analyzed by polyacrylamide gel electrophoresis as described in the legend to Fig. 1. All cells used for this experiment were from the same embryo. Tracks: 1 and 2, normal cells; 3 and 4, FSV-transformed cells; 5 and 6, PRCII-transformed cells; 7, SRD-transformed cells.*

precipitated protein shows greatly enhanced phosphorylation in transformed cells. Comparative tryptic phosphopeptide analyses confirm the expectation that the antibody is directed against the transformation-specific phosphoprotein characterized previously (10).

Additional characterization shows that 34K is relatively abundant: 0.9% of the total methionine content. Its specific location in fibroblasts and expression in other cell types are under investigation.

Our present studies also extend the investigation of the phosphorylation of 34K in cells transformed by other types of avian sarcoma viruses. Recently, others have shown that FSV and PRCII sarcoma viruses represent new types of avian ribonucleic acid tumor virus that lack the RSV *src* gene but do contain unique ribonucleic acid sequences which are likely to encode

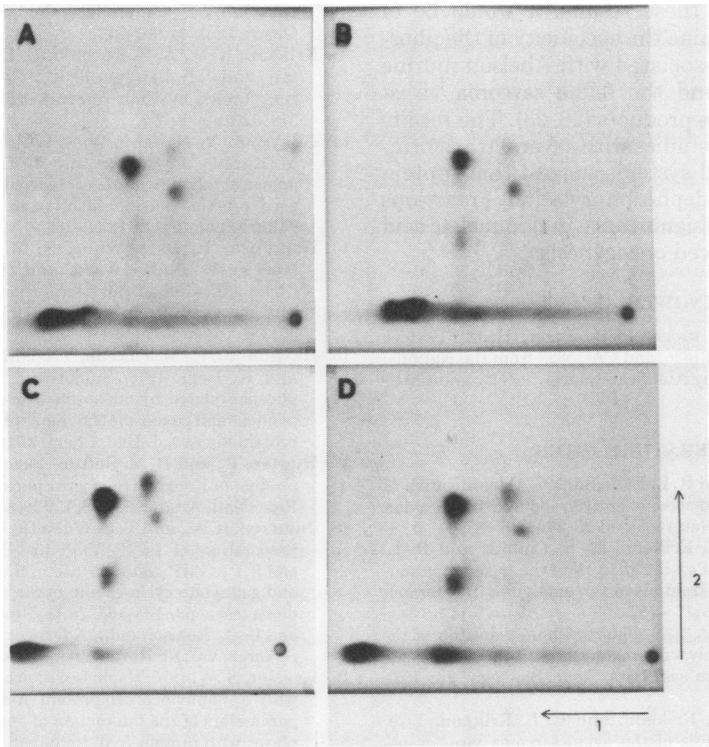

FIG. 7. *Tryptic phosphopeptide analysis of ^{32}P-labeled 34K from FSV- and PRCII-transformed chicken embryo fibroblasts. The 34K proteins were isolated from radiolabeled transformed cells by chromatography through diethylaminoethyl Sephacel and polyacrylamide gel electrophoresis. Tryptic digests were prepared and fractionated as described in the legend to Fig. 3. (A) ^{32}P-labeled 34K isolated from FSV-transformed cells; (B) ^{32}P-labeled 34K isolated from PRCII-transformed cells; (C) mix of the preparation shown in (A) with ^{32}P-labeled 34K isolated from SRD-transformed cells (see Fig. 3A); (D) mix of the preparation shown in (B) with ^{32}P-labeled 34K isolated from SRD-transformed cells.*

transforming proteins (15, 21; Breitman et al., in press; Neil et al., in press). Furthermore, these investigators have shown that the only transformation-specific protein so far identified appears to be associated with a protein kinase activity that results in phosphorylation of the respective transformation-specific proteins as well as immunoglobulin G in immunoprecipitates prepared with TBR antisera. We have confirmed these results as shown in Fig. 5 and with our other unpublished data.

The protein kinase activity associated with these putative transforming gene products takes on added significance with our current observation that 34K is phosphorylated in FSV- and PRCII-transformed cells in a manner similar to that found in RSV-transformed cells. As in the case of the RSV-encoded pp60^{src}, which mediates the phosphorylation of tyrosine in immunoglobulin G (17) and other substrates (7), FSV- and PRCII-mediated phosphorylation also occurs at tyrosine residues (Feldman et al., in press).

It is consistent with these observations that the major tryptic phosphopeptide in 34K taken from FSV- and PRCII-transformed cells is the same as that in 34K taken from RSV-transformed cells and the same as that phosphorylated by pp60^{src} in vitro. Furthermore, in each case this phosphopeptide contains phosphotyrosine. Although p140 and p105 are apparently antigenically distinct from pp60^{src}, they are associated with a protein kinase activity with a similar specificity. There is precedent for different protein kinases having similar specificities since the cyclic adenosine monophosphate- and cyclic guanosine monophosphate-dependent protein kinases may have similar substrate specificities, at least in vitro (16), while being immunologically distinct (J. A. Beavo and M. C. Mumby, in *Protein Phosphorylation, Cold Spring Harbor Conferences on Cell Proliferation,* vol. 8, in press). However, more complete characterization must await the availability of purified and soluble transformation-specific protein kinase from PRCII- and FSV-transformed

cells. In view of these results, it would be of interest to determine the specificity of the phosphotransferase associated with Abelson murine leukemia virus and the feline sarcoma virus-transforming gene products (26, 28). The results now available on studies with several transforming virus-host cell systems suggest that protein phosphorylation-dephosphorylation reactions may have general significance in ribonucleic acid tumor virus-induced oncogenesis.

ACKNOWLEDGMENTS

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