JOURNAL OF VIROLOGY, Nov. 1982, p. 467–474
0022-538X/82/110467-08$02.00/0

Vol. 44, No. 2

Antiserum Specific for the Carboxy Terminus of the Transforming Protein of Rous Sarcoma Virus

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Received 28 April 1982/Accepted 16 July 1982

An antiserum specific for the carboxy terminus of $p60^{src}$, the transforming protein of Rous sarcoma virus, was produced by immunization of rabbits with a conjugate of bovine serum albumin and the synthetic peptide NH_2-Tyr-Val-Leu-Glu-Val-Ala-Glu-COOH. The carboxy-terminal six amino acids of this peptide correspond in sequence to that deduced for the carboxy terminus of the $p60^{src}$ of the Schmidt-Ruppin strain of Rous sarcoma virus of subgroup A. The $p60^{src}$ proteins of the several strains of Rous sarcoma virus and the cellular homolog of the viral transforming protein, $p60^{c\text{-}src}$, comprise a polymorphic family of polypeptides. The anticarboxy-terminal serum reacted readily with the $p60^{src}$ proteins of three different strains of Rous sarcoma virus. In contrast, no precipitation of cellular $p60^{c\text{-}src}$ could be detected with this serum. This suggests that the viral $p60^{src}$ proteins have identical carboxy termini and that the carboxy terminus of cellular $p60^{c\text{-}src}$ may be different from that of viral $p60^{src}$. The anticarboxy-terminal serum reacted poorly with the subpopulation of viral $p60^{src}$ which is present in a complex with two cellular phosphoproteins. Apparently, the presence of the two cellular proteins interferes with the recognition of $p60^{src}$ by the anticarboxy-terminal serum. It seems likely, therefore, that these two cellular proteins bind to the carboxy-terminal domain of $p60^{src}$.

Cellular transformation by Rous sarcoma virus (RSV) results from the activity of a single viral protein, $p60^{src}$ (21). It is now clear that this phosphoprotein is a protein kinase which phosphorylates its protein substrates at tyrosine residues (10, 11, 15–17). The modification of cellular proteins by $p60^{src}$ almost certainly plays a fundamental role in the malignant transformation of cells infected by this virus (26).

The study of $p60^{src}$ in vivo was impossible until the discovery by Brugge and Erikson (5) of a protocol for the production of anti-RSV tumor sera in newborn rabbits. Such sera have proved to be powerful and invaluable reagents. In several respects, however, these antitumor sera are not ideal. First, they are in no sense monospecific, containing in almost all cases antibodies directed against structural proteins of RSV (5). Furthermore, evaluation of whether irrelevant reactivities are present in the sera is made difficult by the absence of preimmune sera.

In many cases, antisera produced by using as an immunogen a peptide which corresponds in sequence to a defined portion of a polypeptide have been shown to be reactive with the native protein (13, 18, 28, 30). Sera produced in this manner are particularly useful in that they are potentially monospecific and recognize a predetermined portion of the protein of interest. Also, they can be purified by affinity chromatography, using an adsorbent to which the peptide used for immunization has been bound. Finally, since they are produced in adult rabbits, preimmune sera can be obtained readily.

We describe here results obtained with sera produced by immunization of rabbits with a synthetic heptapeptide which includes the sequence of six amino acids at the carboxy terminus of $p60^{src}$, which has been deduced from the nucleotide sequence of the *src* gene of the Schmidt-Ruppin strain of RSV, subgroup A (SR-RSV-A) (12).

MATERIALS AND METHODS

Preparation of anticarboxy-terminal sera. The heptapeptide NH_2-Tyr-Val-Leu-Glu-Val-Ala-Glu-COOH was purchased from Bachem (Bubendorf, Switzerland). It was at least 95% pure as determined by high-performance liquid chromatography analysis. Only the six carboxy-terminal amino acids of this peptide correspond in sequence to the deduced carboxy terminus of $p60^{src}$. The amino-terminal tyrosine was added to facilitate coupling of the peptide via its amino terminus, using the procedure of Bassiri et al. (1). To prepare the peptide-bovine serum albumin (BSA) conjugate, 50 mg of BSA was dissolved in 10 ml of 0.16 M borate–0.13 M NaCl, pH 9, at 0°C; 20 mg of peptide

was then added. The molar ratio of peptide to BSA was 32. To this solution was then added, dropwise, 2 ml of bis-diazotized benzidine in 0.2 M HCl with stirring at 0°C. The pH was adjusted by the addition of 0.5 M NaOH, and stirring was continued in the cold for 2 h. The conjugate was dialyzed against water for 1 day and against 0.15 M NaCl for 1 day. To measure the efficiency of coupling, a small amount of ^{125}I-labeled peptide was included in the coupling reaction. The extent of coupling was approximately 43%. This corresponds to 14 molecules of peptide per molecule of BSA. Antisera were obtained by immunizing rabbits with the peptide-BSA conjugate as described previously (30).

Rabbit anti-RSV tumor sera. Rabbit anti-RSV tumor sera were produced in newborn rabbits by the protocol of Brugge and Erikson as described before (23, 25).

Cells and viruses. The preparation and growth of cultures of chick cells transformed by RSV have been described previously (23). The Schmidt-Ruppin strain of RSV of subgroup D (SR-RSV-D), the Prague strain of RSV of subgroup B (PR-RSV-B), and the NB77 line of normal rat kidney (NRK) cells (7) all originated in the laboratory of P. K. Vogt, The University of Southern California. The NB77 cells were grown in Dulbecco-Vogt modified Eagle medium (DMEM) supplemented with 10% fetal calf serum at 37°C.

Radioactive labeling and immunoprecipitation. RSV-transformed chick cells were labeled with $^{32}P_i$ by incubation in phosphate-free DMEM containing 4% calf serum, dialyzed against phosphate-free saline, and $^{32}P_i$ (1 mCi/ml, carrier-free; ICN, Irvine, Calif.) or with [^{35}S]methionine in DMEM containing 5% the normal concentration of methionine, [^{35}S]methionine (>500 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) at a concentration of 100 μCi/ml, and 4% complete calf serum. In both cases, labeling was for 16 h at 41°C. Uninfected chick cells were labeled with $^{32}P_i$ in phosphate-free DMEM containing 4% calf serum, dialyzed against phosphate-free saline, and $^{32}P_i$, at a concentration of 1 mCi/ml, for 2 h at 41°C. The NB77 cells were labeled with [^{35}S]methionine by incubation in methionine-free DMEM containing 10% complete fetal calf serum and [^{35}S]methionine at a concentration of 100 μCi/ml for 16 h at 37°C. All of the ^{32}P-labeled cells and the NB77 cells labeled with [^{35}S]methionine were prepared for immunoprecipitation by lysis in phosphate-buffered RIPA buffer (23) containing 2 mM EDTA. Chick cells labeled with [^{35}S]methionine were dissolved in the same buffer lacking EDTA. All other details of the immunoprecipitation procedures have been described previously (23).

Protein kinase assay. To test for protein kinase activity in immune complexes, immunoprecipitates were prepared from unlabeled cells in phosphate-buffered RIPA buffer containing 2 mM EDTA essentially as described before (24). The resulting immune complexes were incubated with 2 μCi of [γ-^{32}P]ATP (3,000 Ci/mmol; Amersham) in 20 μl of 0.01 M sodium phosphate (pH 6.8)–0.005 M $MgCl_2$ for 10 min at 30°C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. All immunoprecipitates were dissolved by boiling in 2% sodium dodecyl sulfate–0.1 M dithiothreitol–10% 2-mercaptoethanol–5 mM sodium phosphate (pH 7.0)–10% glycerol. Analysis was by electrophoresis in gels containing 15% acrylamide and 0.09% bisacrylamide. Detection of the [^{35}S]methionine-labeled samples was accelerated by impregnating the gels with diphenyloxazole. The ^{32}P-labeled proteins were detected with the aid of an intensifying screen.

Analysis of partial proteolysis. Gel slices containing p60^{src} were isolated from a preparative gel which had not been prepared for fluorography and placed in the wells of a 15% polyacrylamide gel. Digestion was with 25 ng of *Staphylococcus aureus* V8 protease as de-

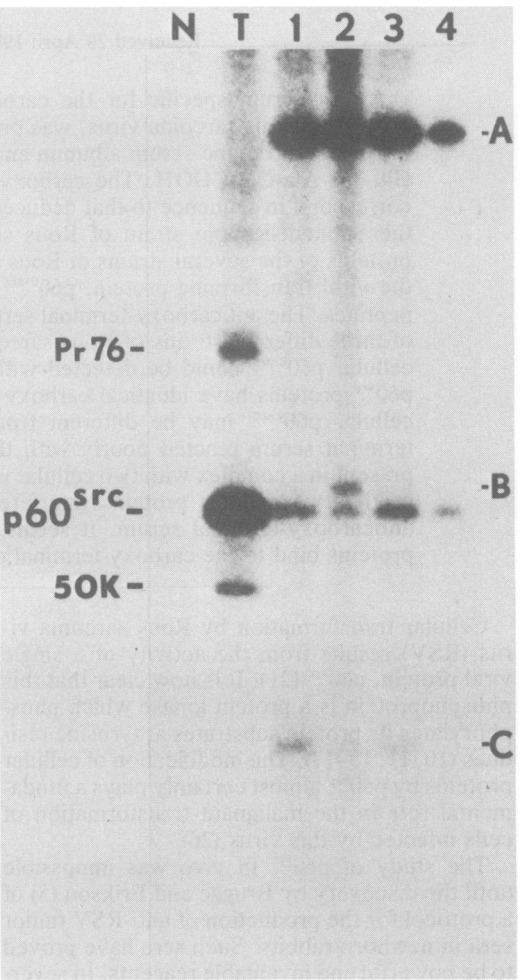
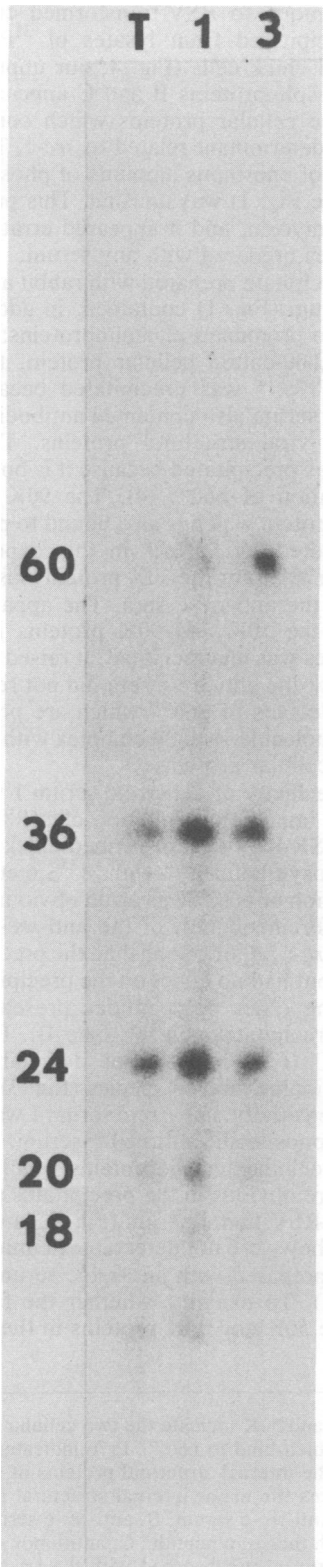


FIG. 1. Characterization of anti-*src*-c sera by immunoprecipitation from a lysate of SR-RSV-D-transformed cells. Chick cells transformed by SR-RSV-D were labeled with $^{32}P_i$ for 16 h at 41°C and dissolved in RIPA buffer containing 2 mM EDTA. Immunoprecipitates were then prepared with preimmune serum, rabbit anti-RSV tumor serum, and four anti-*src*-c sera. The immunoprecipitates were analyzed by electrophoresis on a 15% polyacrylamide gel. All other experimental details are presented in the text. Pr76 indicates the position of the precursor to the internal structural proteins of RSV. 50K denotes the position of the 50,000-dalton cellular protein bound to p60^{src}. N, Preimmune serum; T, antitumor serum; 1, anti-*src*-c serum 1; 2, anti-*src*-c serum 2; 3, anti-*src*-c serum 3; 4, anti-*src*-c serum 4.



scribed elsewhere (8; B. M. Sefton, J. A. Cooper, I. S. Trowbridge, and E. M. Scolnick, Cell, in press).

Affinity purification. Antibodies specific for the carboxy terminus of $p60^{src}$ were isolated by binding immunoglobulin G from the anticarboxy-terminal sera to a column to which the synthetic peptide had been linked covalently. The specific antibodies were then eluted with glycine buffer, pH 2.5. The details of this preparation are described elsewhere (18a).

RESULTS

The nucleotide sequence of the *src* gene of SR-RSV-A (12) predicts that the carboxy terminus of the $p60^{src}$ of this isolate of RSV has the sequence -Val-Leu-Glu-Val-Ala-Glu-COOH. To prepare an anti-$p60^{src}$ serum, a synthetic peptide with the sequence NH_2-Tyr-Val-Leu-Glu-Val-Ala-Glu-COOH was coupled to BSA via its amino-terminal tyrosine, using bis-diazotized benzidine as described by Bassiri et al. (1). This synthetic peptide will be referred to hereafter as *src*-c. Four rabbits were injected with the *src*-c–BSA conjugate, and the resulting sera were tested for reactivity with $p60^{src}$ by immunoprecipitation. A lysate of chick cells transformed by SR-RSV-D and labeled biosynthetically with $^{32}P_i$ was used as a source of $p60^{src}$. Precipitation of a 60,000-dalton phosphoprotein which comigrated precisely with bona fide $p60^{src}$ was apparent in each case (Fig. 1). Comparison of the protein precipitated by the anti-*src*-c sera with $p60^{src}$ by partial proteolytic mapping revealed that it was indeed $p60^{src}$ (Fig. 2).

Whereas all four rabbits produced antibodies reactive with $p60^{src}$, three other phosphoproteins, designated A, B, and C, were also precipitated by some of these sera (Fig. 1). Like the precipitation of $p60^{src}$ (Fig. 3), the precipitation of phosphoproteins B and C was prevented by preincubation of the serum with the *src*-c peptide (data not shown). These phosphoproteins

FIG. 2. Partial proteolytic mapping of the 60,000-dalton polypeptide precipitated with anti-*src*-c sera. Immunoprecipitates were prepared with either antitumor serum or anti-*src*-c sera from ^{32}P-labeled chick cells transformed with SR-RSV-D and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Gel slices containing the precipitated 60,000-dalton polypeptides or $p60^{src}$ were isolated from the preparative gel, placed in the wells of a second gel, and subjected to digestion with 25 ng of *S. aureus* V8 protease. 60 indicates the position of undigested $p60^{src}$. 36, 24, 20, and 18 indicate the positions of the principal products of digestion of $p60^{src}$ with *S. aureus* protease. T, $p60^{src}$ precipitated with antitumor serum; 1, 60,000-dalton polypeptide precipitated by anti-*src*-c serum 1; 3, 60,000-dalton polypeptide precipitated by anti-*src*-c serum 3.

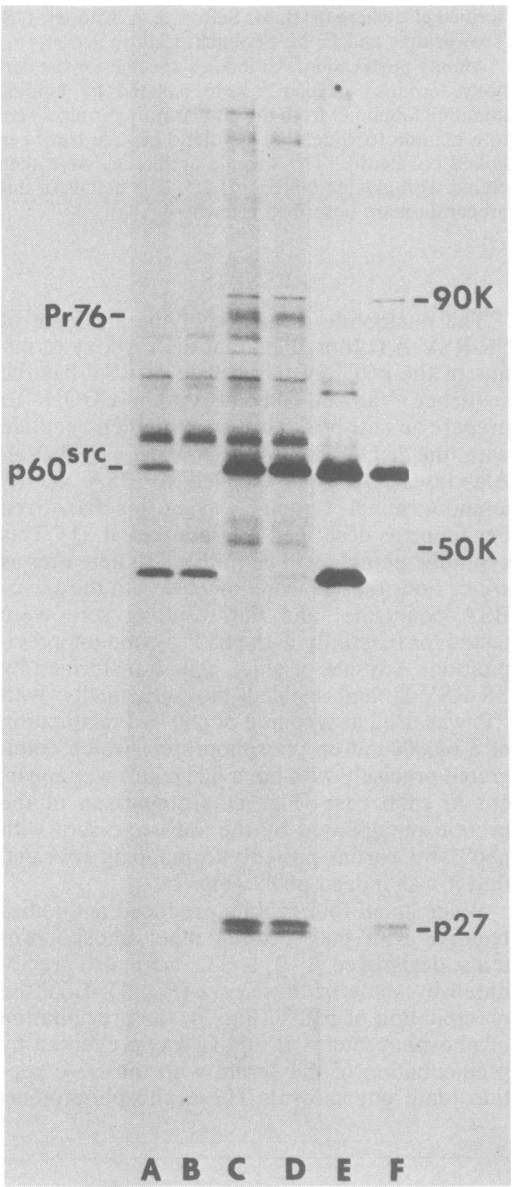


FIG. 3. Characterization of the anti-*src*-c serum by immunoprecipitation from a lysate of RSV-transformed chick cells labeled with [^{35}S]methionine. Chick cells transformed by SR-RSV-D were labeled with [^{35}S]methionine for 16 h at 41°C and then dissolved in RIPA buffer. Immunoprecipitates were then prepared with either anti-*src*-c serum 1 or rabbit antitumor serum. In addition, precipitates were prepared with these sera (2 μl) which had been preabsorbed with 10 μg (1 μl) of the *src*-c peptide, dissolved in 0.1 M NH_4HCO_3, pH 8.3. The resulting precipitates were then analyzed by electrophoresis on a 15% polyacrylamide gel. The figure is a composite of samples from a single gel. Tracks A, B, C, and D were exposed to prefogged film for 7 days. Tracks E and F contain samples from a separate experiment. They were exposed to film which had not been prefogged for 30 days. 90K and 50K indicate the two cellular phosphoproteins which bind to p60^{src}. Pr76 indicates the precursor to the internal structural proteins of the virus. p27 indicates the major internal structural protein of RSV. A, Anti-*src*-c serum; B, anti-*src*-c serum preabsorbed with the *src*-c peptide; C, antitumor serum; D, antitumor serum preabsorbed with the *src*-c peptide; E, anti-*src*-c serum; F, antitumor serum.

are not unique to RSV-transformed cells. All were precipitated from lysates of ^{32}P-labeled uninfected chick cells (Fig. 4; our unpublished data). Phosphoproteins B and C appear, therefore, to be cellular proteins which contain an antigenic determinant related to *src*-c. The precipitation of enormous amounts of phosphoprotein A (see Fig. 1) was unusual. This protein is probably myosin, and it appeared erratically in precipitates prepared with any serum.

The precipitate prepared with rabbit anti-RSV tumor serum (Fig. 1) contained, in addition to p60^{src}, two prominant phosphoproteins: Pr76^{gag} and a 50,000-dalton cellular protein, the 50K protein. Pr76^{gag} was precipitated because the antitumor serum also contained antibodies reactive with viral structural proteins. The 50K protein was precipitated because it is bound to a subpopulation of p60^{src} (4). The 90K cellular phosphoprotein which is also bound to p60^{src} (4) was obscured by Pr76^{gag} in this experiment. Neither Pr76^{gag} nor the 50K protein was precipitated by the anti-*src*-c sera. The apparent absence of the 50K and 90K proteins in these precipitates was unanticipated. It raised the possibility that the anti-*src*-c sera did not recognize those molecules of p60^{src} which are present in the high-molecular-weight complex with the 50K and 90K cellular proteins.

The specificity of anti-*src*-c serum 1 was also tested by immunoprecipitation of p60^{src} from a lysate of SR-RSV-D-transformed chick cells labeled biosynthetically with [^{35}S]methionine. Precipitation of p60^{src} was again obvious (Fig. 3, lane A). Preincubation of the anti-*src*-c serum with the *src*-c peptide abolished the precipitation of p60^{src} but had no effect on the precipitation of any of the other polypeptides present in the immunoprecipitates (Fig. 3, lane B). Phosphoprotein C (Fig. 1) was not detectable as a [^{35}S]methionine-labeled species (Fig. 3). At this level of sensitivity, anti-*src*-c serum 1 was essentially a monospecific anti-p60^{src} serum. The 50K and 90K cellular phoshoproteins which bind to p60^{src} were obvious in the precipitates prepared with anti-RSV tumor serum (Fig. 3, lanes C, D, and F). They were not detectable in immunoprecipitates prepared with anti-*src*-c serum 1 (Fig. 3, lane A). To examine whether the failure to detect the 50K and 90K proteins in the precipi-

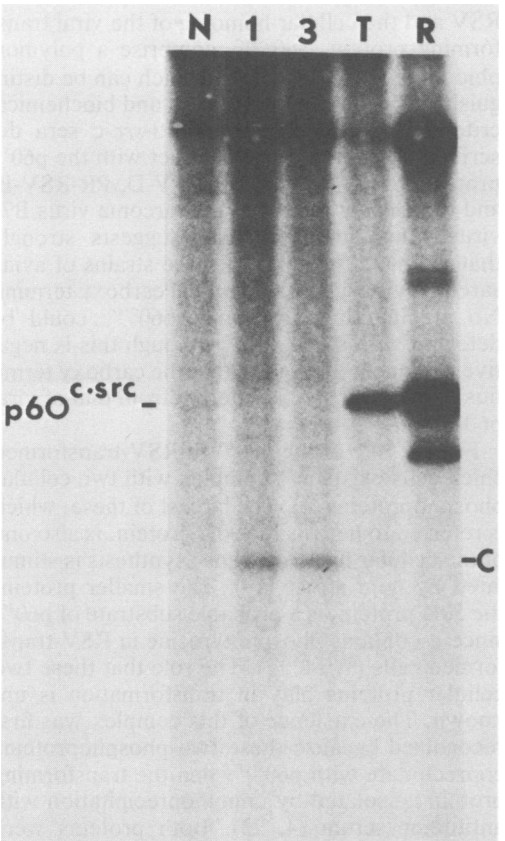


FIG. 4. Anti-*src*-c sera do not precipitate detectable amounts of $p60^{c\text{-}src}$. Uninfected chick cells were labeled with $^{32}P_i$ for 2 h, and then immunoprecipitates were prepared with preimmune serum, two anti-*src*-c sera, and an antitumor serum which reacts with $p60^{c\text{-}src}$. The precipitates were analyzed by electrophoresis on a 15% polyacrylamide gel. C indicates cellular phosphoprotein C which is recognized by some of the anti-*src*-c sera. N, Uninfected cells, preimmune serum; 1, uninfected cells, anti-*src*-c serum 1; 3, uninfected cells, anti-*src*-c serum 3; T, uninfected cells, antitumor serum; R, ^{32}P-labeled RSV-transformed cells, antitumor serum.

tate prepared with the anti-*src*-c serum was due simply to the fact that the antitumor serum precipitated $p60^{src}$ more efficiently, precipitates prepared with the two sera, in a separate experiment, were compared under conditions in which more $p60^{src}$ was present in the anti-*src*-c precipitate. Here, too, neither the 50K nor the 90K protein was detectable in the anti-*src*-c precipitate (Fig. 3, lane E).

The apparent inability of the anticarboxy-terminal serum to precipitate $p60^{src}$ which is in a complex with the 50K and 90K cellular proteins was not absolute. The $p60^{src}$ protein of SR-RSV-A forms a complex with these two cellular proteins somewhat more efficiently than does that of SR-RSV-D (4). We have, on occasion, observed both the 50K and 90K cellular proteins in immunoprecipitates containing $p60^{src}$ of SR-RSV-A prepared with anti-*src*-c serum (data not shown). When measurable, the precipitation of the population of $p60^{src}$ which is present in the high-molecular-weight complex with the anticarboxy-terminal serum was found to be, at most, one-third as efficient as with antitumor serum.

The *src*-c peptide corresponds in sequence to that predicted for the carboxy terminus of the $p60^{src}$ of SR-RSV-A. $p60^{src}$ is, however, a polymorphic protein (2, 6). The $p60^{src}$ proteins of different strains of RSV and the cellular homolog of viral $p60^{src}$, $p60^{c\text{-}src}$, are readily distinguishable both immunologically and biochemically (2, 6, 9, 20). It was of interest, therefore, to determine whether the anti-*src*-c sera would react with the $p60^{src}$ proteins of other strains of RSV and with its cellular homolog $p60^{c\text{-}src}$. Anti-*src*-c serum was able to precipitate the $p60^{src}$ of PR-RSV-B from a lysate of transformed chick cells (Fig. 5, lane B). Also, anti-*src*-c antibody, purified by adsorption and elution from a column containing the *src*-c peptide, precipitated the $p60^{src}$ of B77 virus from a lysate of the NB77 line of normal rat kidney cells transformed by B77 virus (Fig. 5, lane G).

To examine whether the anti-*src*-c sera would recognize cellular $p60^{c\text{-}src}$, uninfected chick cells were labeled with $^{32}P_i$ for 2 h, and then immunoprecipitates were prepared with two anti-*src*-c sera and a rabbit antitumor serum which recognizes $p60^{c\text{-}src}$ efficiently (Fig. 4). Even after deliberate overexposure of the fluorogram, no precipitation of $p60^{c\text{-}src}$ was detectable with either of the anti-*src*-c sera. The inability of the anti-*src*-c sera to precipitate $p60^{c\text{-}src}$ is also apparent in Fig. 5. The $p60^{src}$ of PR-RSV-B is sufficiently different in size from $p60^{c\text{-}src}$ (25) that the two proteins can be resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The band just below viral $p60^{src}$ in lane C of Fig. 5 is $p60^{c\text{-}src}$. It was not detectable in the sample prepared with the anti-*src*-c serum (Fig. 5, lane B).

$p60^{src}$ is a protein kinase and is able to phosphorylate some precipitating antibodies when immune complexes are incubated with [γ-^{32}P]ATP (10). The anti-*src*-c serum also served as a substrate for $p60^{src}$ (Fig. 6). The extent of phosphorylation of the anti-*src*-c serum was, however, extremely variable and never as pronounced as that obtained with rabbit anti-RSV tumor serum. The protein kinase assay in the immune complex is a very sensitive means by which to detect $p60^{c\text{-}src}$. No precipitation of a protein kinase capable of phosphorylating the heavy chain of the anti-*src*-c antibodies was

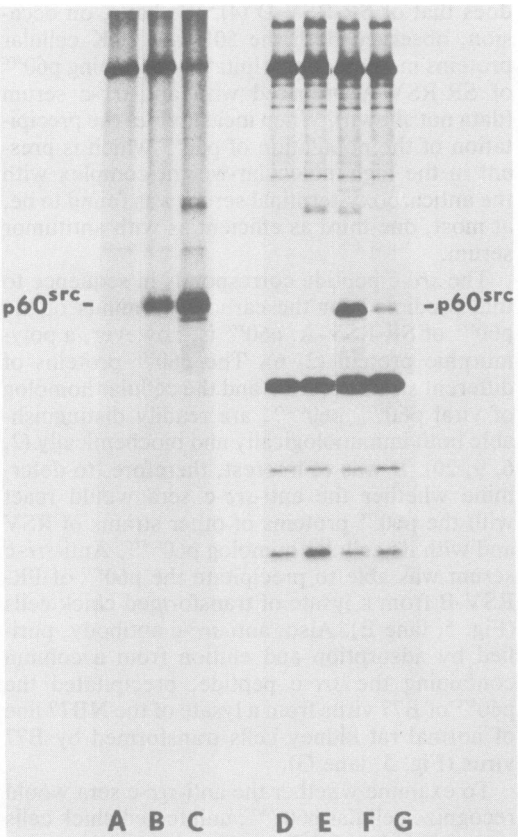


FIG. 5. Anti-*src*-c serum precipitates the $p60^{src}$ proteins of PR-RSV-B and B77 virus. Immunoprecipitates were prepared from lysates of PR-RSV-B-transformed chick cells labeled with $^{32}P_i$ for 16 h at 41°C and rat NB77 cells labeled with [^{35}S]methionine for 16 h at 37°C. The immunoprecipitates were analyzed on separate 15% polyacrylamide gels. The band just below $p60^{src}$ in track C is $p60^{c\text{-}src}$. A, PR-RSV-B-transformed cells, preimmune serum; B, PR-RSV-B-transformed cells, anti-*src*-c serum 3; C, PR-RSV-B-transformed cells, antitumor serum; D, NB77 cells, preimmune serum; E, NB77 cells, anti-RSV virion serum; F, NB77 cells, antitumor serum; G, NB77 cells, affinity-purified anti-*src*-c antibodies.

observed with uninfected chick cells. This lends further credence to the idea that these sera do not recognize $p60^{c\text{-}src}$.

DISCUSSION

We have shown here that immunization of rabbits with a conjugate of BSA and a synthetic peptide corresponding in sequence to that deduced for the carboxy terminus of $p60^{src}$ of SR-RSV-A produces antibodies reactive with native $p60^{src}$. This provides, therefore, indirect confirmation of the deduced amino acid sequence of this polypeptide and demonstrates that the carboxy terminus of native $p60^{src}$ is exposed.

The $p60^{src}$ proteins of the several strains of RSV and the cellular homolog of the viral transforming protein, $p60^{c\text{-}src}$, comprise a polymorphic family of polypeptides which can be distinguished by both immunological and biochemical criteria (2, 6, 9, 20). The anti-*src*-c sera described here were found to react with the $p60^{src}$ proteins of SR-RSV-A, SR-RSV-D, PR-RSV-B, and the closely related avian sarcoma virus B77 virus. This cross-reactivity suggests strongly that the $p60^{src}$ proteins of these strains of avian sarcoma virus possess identical carboxy termini. No precipitation of cellular $p60^{c\text{-}src}$ could be detected with these sera. Although this is negative evidence, it suggests that the carboxy terminus of $p60^{c\text{-}src}$ may be different from that of viral $p60^{src}$.

Five to 10% of the $p60^{src}$ in RSV-transformed chick cells exists in a complex with two cellular phosphoproteins (4). The largest of these, which is referred to here as the 90K protein, is also one of the cellular proteins whose synthesis is stimulated by mild stress (19). The smaller protein, the 50K protein, is a probable substrate of $p60^{src}$ since it contains phosphotyrosine in RSV-transformed cells (3, 14, 15). The role that these two cellular proteins play in transformation is unknown. The existence of this complex was first recognized because these two phosphoproteins coprecipitate with $p60^{src}$ when the transforming protein is isolated by immunoprecipitation with antitumor serum (4, 23). Both proteins were found to be noticeably less abundant in precipitates prepared with the anticarboxy-terminal serum than in precipitates prepared with antitumor serum. This suggests that the anti-*src*-c sera react inefficiently with those molecules of $p60^{src}$ to which the 90K and 50K proteins are bound. These two cellular phosphoproteins appear to either block anti-*src*-c binding directly or alter the conformation of $p60^{src}$ such that the carboxy terminus of the polypeptide is less exposed than it is in monomeric $p60^{src}$. It seems likely, therefore, that the 90K and 50K proteins interact with the carboxy-terminal domain of $p60^{src}$.

It is apparent in Fig. 1, 3, and 5 that the amount of $p60^{src}$ precipitated by the anti-*src*-c serum was less than that precipitated by antitumor serum. This is due in part to the fact that the anti-*src*-c serum poorly precipitates those molecules of $p60^{src}$ (5 to 10% of the total population of $p60^{src}$ [4]) to which the 50K and 90K cellular phosphoproteins are bound. Even under conditions of marked antibody excess, however, the difference in the efficiency of immunoprecipitation of $p60^{src}$ with these two sera was much greater than 10%. Perhaps the affinity of the anti-*src*-c serum for $p60^{src}$ is too low to allow quantitative immunoprecipitation of the polypeptide. Alternatively, another subclass of $p60^{src}$ molecules may exist in which the carboxy terminus, although not complexed with the 50K

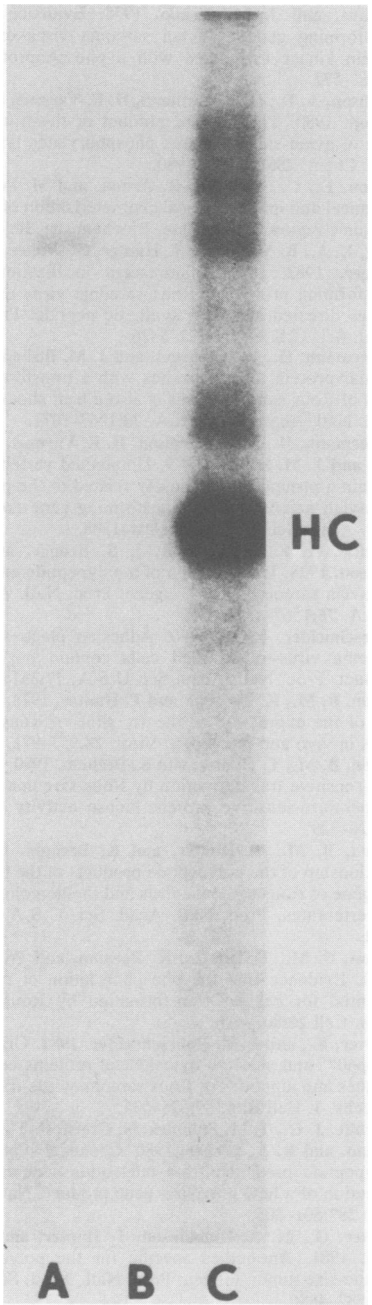


FIG. 6. p60^{src} phosphorylation of anti-*src*-c serum in the immune complex. Immunoprecipitates were prepared from unlabeled uninfected chick cells and chick cells transformed by SR-RSV-D. The precipitates were assayed for protein kinase activity by incubation with [γ-^{32}P]ATP as described in the text. HC indicates the position of the heavy chain of immunoglobulin. A, RSV-transformed cells, preimmune serum; B, uninfected cells, anti-*src*-c serum 1; C, RSV-transformed cells, anti-*src*-c serum 1.

and 90K proteins, is inaccessible to the anti-*src*-c serum.

The anti-*src*-c serum offers unique advantages in two lines of experimentation. First, it has already proved very useful in the localization of p60^{src} within RSV-transformed cells. Previous efforts to localize p60^{src} by immunofluorescence with antitumor serum were open to the criticism that the specificity of the sera was somewhat uncertain. We therefore reexamined the question of the intracellular location of p60^{src} by using the anti-*src*-c serum for immunofluorescent localization of the protein. Our results have corroborated fully earlier work with antitumor serum (6, 22, 27). We found p60^{src} to be located only in the cytoplasm of transformed cells, where some of it is concentrated in adhesion plaques, in rosette-like structures, and at cell-cell junctions in close association with the cytoskeletal proteins vinculin and α-actinin (18a).

The anti-*src*-c serum should also allow the rapid purification of p60^{src} in native form. In another study, we have shown that the medium T antigen of polyoma virus can be isolated with an antiserum against a synthetic peptide identical to the carboxy terminus of the protein and then eluted, in apparently native form, by competition with a great excess of the peptide (29a). This procedure allows a rapid and gentle 2,500-fold purification of the protein. A similar approach should be equally successful with p60^{src}.

In preparing these anti-p60^{src} sera, we chose a peptide identical to one of the termini of the protein because it increased the chance that the conformation of the peptide in the conjugate would resemble the conformation of the corresponding region of the native protein. Wong and Goldberg have shown recently (31) that it is possible to make an antiserum which recognizes p60^{src} by using a synthetic peptide corresponding to the internal region of p60^{src} which surrounds the single phosphorylated tyrosine in the polypeptide.

One of the attractions of antisera produced by immunization with a synthetic peptide is that they are potentially monospecific. The anti-*src*-c sera described here are preferable to antitumor sera in the sense that they clearly do not react with the polypeptide products of any other viral gene. To our surprise, however, three of the four sera reacted to various extents with two cellular phosphoproteins. As this interaction was inhibited by preabsorption of the antisera with the *src*-c peptide, the precipitation of these proteins must result from a genuine cross-reaction between a determinant on the cellular proteins and the carboxy terminus of p60^{src}. This cross-reactivity becomes much more apparent when affinity-purified antibodies are used; this is discussed in detail elsewhere (E. A. Nigg, G. Walter, and S. J. Singer, Proc. Natl. Acad. Sci. U.S.A., in

press). Similar unanticipated cross-reactivity has also been observed with sera designed to react specifically with the carboxy terminus of the middle T antigen of polyoma virus (29). The need for some caution in the use of sera such as these must therefore be kept in mind.

ACKNOWLEDGMENTS

We thank Peter Czernilovsky and Mike Bishop for providing us with their sequence of *src* prior to publication; Tony Hunter, Erich Nigg, and Tilo Patschinsky for many helpful discussions; and Claudie Berdot for help with the experiments.

This work was supported by Public Health Service grants CA 14195 and CA 17289 from the National Institutes of Health, a grant from the Deutsche Forschungsgemeinschaft, and a NATO Collaborative Research Grant.

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