Proc. Natl. Acad. Sci. USA
Vol. 76, No. 4, pp. 1804–1808, April 1979
Cell Biology

Uninfected vertebrate cells contain a protein that is closely related to the product of the avian sarcoma virus transforming gene (*src*)

(protein kinase/phosphoprotein/immunoprecipitation/peptide maps/phylogenetic conservation)

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Communicated by David Baltimore, December 26, 1978

ABSTRACT **Neoplastic transformation of cells by avian sarcoma virus is mediated by a single viral gene (*src*), which encodes a phosphoprotein (pp60^{src}) with the enzymatic activity of a protein kinase. The DNAs of vertebrate species contain a highly conserved homologue of *src* that is also represented in the polysomal RNA of uninfected cells and, hence, may specify a normal cellular protein. We have used antisera directed against pp60^{src} to isolate a closely related phosphoprotein (denoted vertebrate pp60) from uninfected chicken, quail, rat, and human cells. Our data indicate that vertebrate pp60 is a homologue of pp60^{src}, highly conserved both antigenically and chemically. Moreover, the cellular protein may possess protein kinase activity similar to that associated with pp60^{src}. We conclude that the product of *src* is a slightly modified analogue of a normal cellular protein.**

A single viral gene (*src*) mediates neoplastic transformation of cells infected with avian sarcoma virus (ASV) (1). Nucleotide sequences related to *src* (denoted "endogenous sarc") are present in the DNA of chickens (2), where they may have served as progenitor of the viral gene (2, 3). Several findings indicate that endogenous sarc may encode an essential cellular protein: (*i*) nucleotide sequences homologous to endogenous sarc are highly conserved throughout the vertebrate kingdom (4); (*ii*) these sequences belong to the family of low-frequency DNA that characteristically encodes functional proteins (2, 4); and (*iii*) polyribosomes of avian cells contain transcripts of endogenous sarc (5). In view of these findings, we sought to identify a protein encoded by endogenous sarc with the expectation that comparison of this protein to the product of ASV *src* would provide insight into the mechanism of viral oncogenesis. Our strategy derived from the discovery by Brugge, Erikson, and coworkers (6, 7) that the sera of rabbits bearing ASV-induced tumors could be used to isolate a protein encoded in *src*; this protein (denoted pp60^{src}) has a molecular weight of 60,000 (6), is phosphorylated (8, 9), and displays the enzymatic properties of a cyclic nucleotide-independent protein kinase (9, 10).

On the assumption that pp60^{src} and the presumed product of endogenous sarc would share at least some of their antigenic determinants, we examined extracts of uninfected vertebrate cells for a protein that could react with rabbit antisera directed against pp60^{src}. Our results indicate that uninfected cells from a variety of vertebrate species contain a highly conserved phosphoprotein that possesses antigenic, chemical, and functional homology with pp60^{src}.

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MATERIALS AND METHODS

Cells and Virus. The source of virus strains, the propagation of cells and virus, and the isotopic labeling of macromolecules have all been described (9). Cells were labeled with [^{35}S]methionine (1000 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) at 2 mCi/ml for 5 hr, or with [^{32}P]orthophosphate at 1–10 mCi/ml for 5 hr. The subgroup D Schmidt–Ruppin strain of ASV was used throughout. Uninfected NRK cells (rat kidney fibroblasts) and NRK cells transformed by ASV (ASV-NRK) were provided by P. Vogt. Suspension cultures of Kc *Drosophila* cells were obtained from B. McCarthy. Human foreskin diploid fibroblasts in their 14th passage were obtained from the Cell Center at this university.

Preparation of Antisera. Antisera were raised in newborn rabbits by inducing sarcomas with the Schmidt–Ruppin strain of ASV as described (6, 9). Additional antisera were prepared by injecting newborn rabbits with embryonic chicken fibroblasts transformed by and producing ASV. These antisera had exceptionally high titers against pp60^{src} but were otherwise similar to antisera raised by induction of tumors with ASV alone.

Immunoprecipitation and Analysis of Virus-Specific Proteins. We modified our previously described procedure (11) as follows in order to augment the efficiency of the formation of specific immune complex and to reduce the nonspecific background of proteins in the immunoprecipitates. The bacteria used as immunoadsorbents were prepared and stored according to the revised protocol of Kessler (12). Extracts of isotopically labeled cells were prepared as described (9, 11), using as lysis buffer 0.1 M NaCl/0.001 M EDTA/0.01 M Tris·HCl (pH 7.2)/1% (vol/vol) Nonidet-P40 (Shell Chemical Corp.)/bovine serum albumin (Sigma), 1 mg/ml. The clarified lysates were passed through a 200-μl bed volume of DE52 DEAE-cellulose (Whatman) previously equilibrated against lysis buffer; under these conditions, neither pp60^{src} nor its vertebrate homologue binds to the column (unpublished observations). The eluate was preadsorbed with a standard aliquot of bacteria, then supplemented with ATP (2.0 mM) and NaCl (0.5 M); ATP was used to dilute the pool of radioactive ATP in ^{32}P-labeled samples, thereby reducing the phosphorylation of proteins by intrinsic kinases during the manipulations (unpublished observation).

Abbreviations: ASV, avian sarcoma virus; *src*, the gene responsible for neoplastic transformation induced by ASV; pp60^{src}, a phosphoprotein encoded in *src*; pp60, a phosphoprotein found in normal cells; Pr76^{gag}, a polyprotein precursor of structural proteins of ASV; NRK cells, normal rat kidney fibroblasts; ASV-NRK, NRK cells transformed by ASV.

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A suitable portion of antiserum was added (the final volume after addition never exceeded 200 μl), followed by a second aliquot of bacterial immunoadsorbent, previously suspended in a lysate (0.5 ml) of *ca.* 10^7 uninfected chicken fibroblasts prepared as above in buffer supplemented to 0.5 M NaCl. The bacteria with adsorbed immune complexes were collected by centrifugation and washed sequentially with: (*i*) 1.0 M NaCl/0.01 M Tris·HCl (pH 7.2)/0.1% (vol/vol) Nonidet-P40; (*ii*) 0.1 M NaCl/0.001 M EDTA/0.01 M Tris·HCl (pH 7.2)/1% (vol/vol) Nonidet-P40/0.3% (wt/vol) sodium dodecyl sulfate; and (*iii*) 0.01 M Tris·HCl (pH 7.2)/0.1% (vol/vol) Nonidet-P40. After washing, absorbed proteins were released from the bacteria by suspension in sample buffer for subsequent electrophoresis in polyacrylamide gels as described (9, 11). After electrophoresis, radioactive proteins were located in the gels by autoradiography; when necessary, portions of the gels containing desired polypeptides were excised and used for partial hydrolysis with proteases as described by Cleveland *et al.* (13). We have described our protocols for these procedures (9). As a convenience, we frequently used pp60^{src} isolated from rat cells transformed by ASV; we have shown that this protein is identical to pp60^{src} found in permissive cells infected with the same strain of ASV (ref. 9; unpublished data).

Assay for Protein Kinase. Immunoprecipitates were used in phosphotransfer reactions as described (9), then processed as follows in order to reduce the background of nonspecifically bound radioactivity. Immune complexes were eluted from the immunoadsorbent in 4 M $MgCl_2$/0.1 M NaCl/0.001 M EDTA/0.01 M Tris·HCl (pH 7.2)/1% (vol/vol) Nonidet-P40. After centrifugation to remove the bacteria, the supernatant was adjusted to 0.25 M $MgCl_2$ by dilution; the immune complexes were collected by adsorption to a fresh sample of bacteria, washed twice with 0.1 M NaCl/0.001 M EDTA/0.01 M Tris·HCl (pH 7.2), and then eluted into sample buffer for electrophoresis in polyacrylamide gels.

RESULTS

Immunoprecipitation of a Phosphoprotein Related to pp60^{src} from Extracts of Uninfected Vertebrate Cells. In previous experiments (9), we failed to detect any specific reaction between the proteins of uninfected cells and antisera from rabbits bearing sarcomas induced by ASV. However, using improved procedures for immunoprecipitation, we have been able to identify a protein in extracts of uninfected chicken embryo fibroblasts that was precipitated specifically by many of our rabbit tumor antisera and had an electrophoretic mobility indistinguishable from that of pp60^{src} (Fig. 1*A*, lanes b and d). As demonstrated previously (8, 9), pp60^{src} is a phosphoprotein and can consequently be labeled with ^{32}P (Figs. 1*B*, lane d); we found the same to be true of the analogous protein precipitated from uninfected chicken cells (Fig. 1*B*, lane b) and therefore denoted it chicken pp60. Sera from eight different normal rabbits failed to precipitate pp60^{src} and chicken pp60 from extracts of cells labeled with either [^{35}S]methionine or ^{32}P (Fig. 1, lanes a and c of *A* and *B*, and unpublished data). On the basis of the exposure times required to achieve equal intensities for the autoradiographic bands representing pp60^{src} and chicken pp60, we estimate that the amount of the viral gene product in infected cells may be 100-fold greater than the amount of serologically related phosphoprotein in uninfected chicken cells.

On occasion, the immunoprecipitates prepared with immune sera contained proteins other than pp60^{src} and chicken pp60; some of these were previously identified virus-specific proteins (such as the polyprotein Pr76^{gag}); others have yet to be identified and may not be viral in origin. In the present study, we restricted our analyses to pp60 because of its potential homology with pp60^{src}.

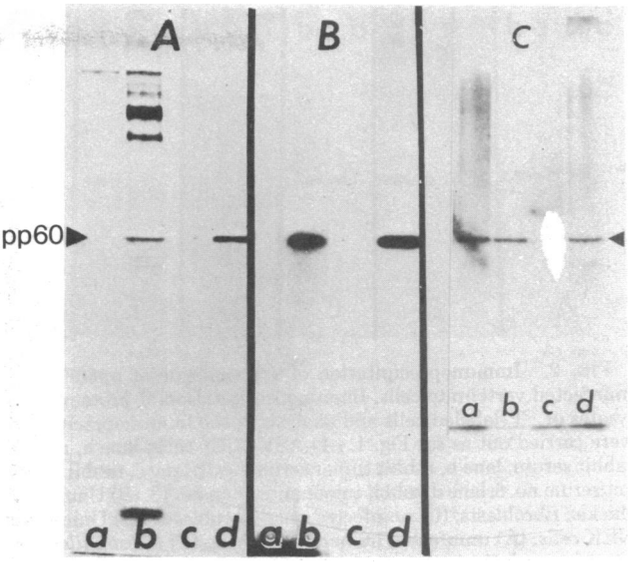

FIG. 1. Immunoprecipitation of pp60^{src} from infected cells and a homologous protein from uninfected chicken cells. Sera from normal or tumor-bearing rabbits were used to precipitate proteins from cells labeled with either [^{35}S]methionine or [^{32}P]orthophosphate. The immunoprecipitates were analyzed by electrophoresis in gels of 9% polyacrylamide. Fractionated proteins were visualized by autoradiography; exposure times were adjusted to obtain equivalent intensities. (*A*) ^{35}S-Labeled proteins: lane a, normal serum and uninfected chicken cells; lane b, tumor antiserum and uninfected chicken cells; lane c, normal serum and ASV-NRK cells; lane d, tumor antiserum and ASV-NRK cells. (*B*) ^{32}P-Labeled proteins: lane a, normal serum and uninfected chicken cells; lane b, tumor serum and uninfected chicken cells; lane c, normal serum and ASV-NRK cells; lane d, tumor antiserum and ASV-NRK cells. (*C*) Results of immunological competitions, performed as described in the text. ^{32}P-Labeled proteins were precipitated from 4×10^6 uninfected chicken cells with rabbit tumor antiserum in the absence of competitor (lane a), in the presence of a lysate of 2×10^7 uninfected NRK cells (lane b), in the presence of a lysate of 2×10^7 ASV-NRK cells (lane c), or in the presence of 100 μg (protein) of purified ASV (lane d).

Our previous analyses of cellular DNA indicated that all vertebrates may contain a homologue of ASV *src* (4). Consequently, we analyzed quail, rat, and human fibroblasts for a phosphoprotein analogous to pp60^{src} (Fig. 2 *C*, *D*, and *E*). In each instance, certain rabbit tumor antisera precipitated a phosphoprotein with an electrophoretic mobility identical to that of both pp60^{src} (Fig. 2*A*) and the pp60 from chicken cells (Fig. 2*B*). By contrast, an analogous protein could not be identified in insect cells (Fig. 2*F*); this finding mirrors our previous failure to find nucleotide sequences homologous to *src* in insects, echinoderms, and prokaryotes (4). As before, neither pp60^{src} nor cellular pp60 was precipitated by normal rabbit sera (Fig. 2, lanes a of *A*–*E*).

Not all of our rabbit tumor antisera displayed complete crossreactivity with pp60 from uninfected vertebrate cells. Some antisera precipitated only the viral protein pp60^{src} (Fig. 2, lanes b of *A* and *B*); a second set of antisera precipitated both pp60^{src} and avian pp60, but not the pp60 of mammalian cells (Fig. 2, lanes c of *A*–*E*); a third set of antisera precipitated pp60^{src} and the pp60 of all the vertebrates examined (Fig. 2, lanes d of *A*–*E*). These findings indicate that the antigenic homologies among pp60^{src} and the various forms of pp60 from uninfected cells are not perfect and provide a useful test of serological specificity to be applied below.

In order to document further the antigenic relationship between pp60^{src} and chicken pp60, we exploited the phenomenon

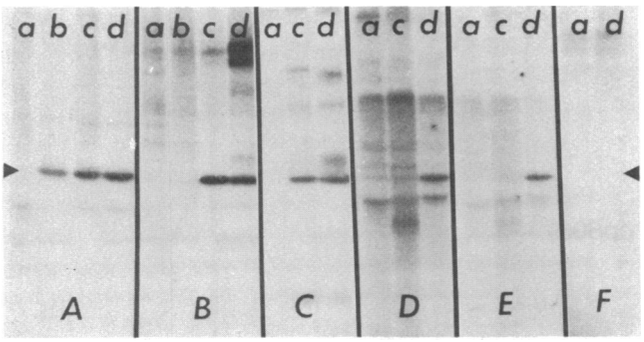

FIG. 2. Immunoprecipitation of a homologue of pp60^{src} from uninfected vertebrate cells. Immunoprecipitation of proteins from lysates of ^{32}P-labeled cells and analysis of the immunoprecipitates were carried out as for Fig. 1. (*A*) ASV-NRK cells; lane a, normal rabbit serum; lane b, rabbit tumor serum no. 3; lane c, rabbit tumor antiserum no. 6; lane d, rabbit tumor antiserum no. 13. (*B*) Uninfected chicken fibroblasts; (*C*) uninfected quail fibroblasts; (*D*) Uninfected NRK cells; (*E*) uninfected human fibroblasts; (*F*) *Drosophila* cells. In each panel, the lanes were as described for *A*.

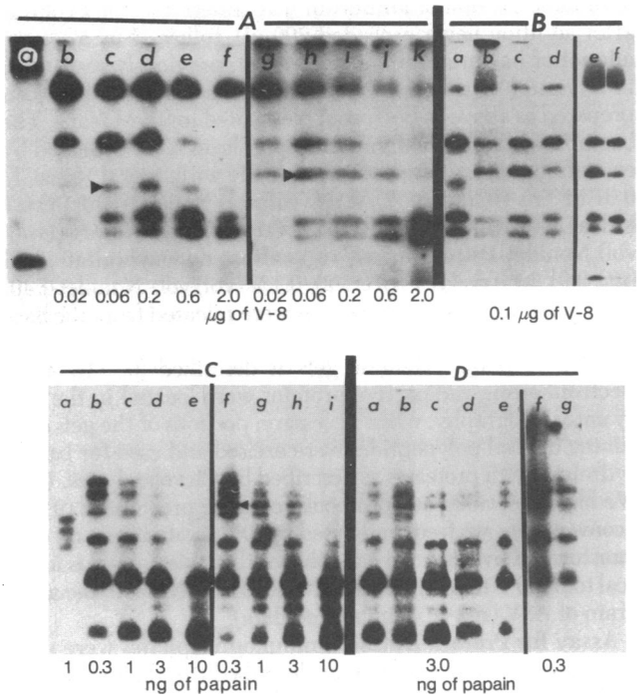

FIG. 3. Partial hydrolysis of immunoprecipitated proteins with proteases. Immunoprecipitated ^{32}P-labeled proteins were isolated from polyacrylamide gels and analyzed by partial hydrolysis with either staphylococcal protease V-8 (*A* and *B*) or papain (*C* and *D*). (*A*) Lane a, Pr76^{gag}; lanes b–f, pp60^{src}; lanes g–k, pp60 from uninfected chicken fibroblasts. (*B*) Lane a, pp60^{src}; lane b, pp60 from chicken fibroblasts; lane c, pp60 from quail fibroblasts; lane d, pp60 from human fibroblasts; lane e, pp60 from chicken fibroblasts, analyzed in a separate experiment with lane f, pp60 from NRK cells. (*C*) Lane a, Pr76^{gag}; lanes b–e, pp60^{src}; lanes f–i, pp60 from chicken fibroblasts. (*D*) Lane a, pp60^{src}; lane b, pp60 from chicken fibroblasts; lane c, pp60 from quail fibroblasts; lane d, pp60 from NRK cells; lane e, pp60 from human fibroblasts; lane f, pp60 from chicken fibroblasts, analyzed in a separate experiment with lane g, pp60 from NRK cells.

of immunological competition. Samples of a tumor antiserum that reacted with avian pp60 but not with pp60 from uninfected rat cells (as in Fig. 2, lanes c) were first exposed to extracts of either uninfected rat cells or rat cells transformed by ASV (and therefore containing pp60^{src}), then used for immunoprecipitation with an extract of uninfected chicken cells. Pretreatment of the antiserum with the extract of uninfected rat cells had no effect on the precipitation of pp60 (Fig. 1*C*; compare lanes a and b), whereas the extract of ASV-infected rat cells blocked most of the antiserum's reactivity with pp60 (Fig. 1*C*, lane c). Exposure of the antiserum to disrupted virions of ASV also failed to block reactivity with chicken pp60 (Fig. 1*C*, lane d). We conclude that the pp60 identified in uninfected chicken cells shares at least some of the antigenic determinants of the viral gene product pp60^{src}. Similar results were obtained with pp60 isolated from NRK cells (data not shown).

Chemical Similarity between pp60^{src} and pp60 Isolated from Vertebrate Cells. Partial hydrolysis with proteases can be used to demonstrate identity or similarity among related proteins (13). We employed this approach to explore further the relationship between pp60^{src} and pp60 isolated from vertebrate cells. Two considerations caused us to perform these studies principally with ^{32}P-labeled proteins, although similar results were obtained in limited analyses of ^{35}S-labeled proteins. First, labeling with ^{32}P proved to be more efficient and economical. Second, we had found previously that hydrolysis of ^{32}P-labeled pp60^{src} produces more numerous detectable peptides than does hydrolysis of the ^{35}S-labeled protein and, hence, provides a more persuasive test of similarity (9).

We first compared pp60^{src} to chicken pp60, using hydrolysis with low, intermediate, and high concentrations of staphylococcal protease V-8 and papain; the products of hydrolysis were analyzed by electrophoresis in polyacrylamide gels (Fig. 3 *A* and *C*). As expected, the extent of hydrolysis varied at the different concentrations of proteases, but the particular pattern of products obtained at each concentration of either protease was virtually identical for pp60^{src} and chicken pp60. Only single differences (marked with arrowheads in Fig. 3) reproducibly distinguished the patterns of peptides obtained from the two proteins by hydrolysis with either enzyme. By contrast, hydrolysis of a viral protein (Pr76^{gag}) unrelated to pp60^{src} and vertebrate pp60 produced entirely different products (Fig. 3, lanes a of *A* and *C*).

The intermediate concentrations of the proteases produced the most complex and, hence, most informative set of products (Fig. 3 *A* and *C*); for convenience and economy of materials, we focused on these concentrations to extend the comparisons to other samples. Using intermediate concentrations of staphylococcal protease and papain, we found that preparations of pp60 isolated from chicken, quail, rat, and human cells were indistinguishable (Fig. 3 *B* and *D*). We conclude that the pp60 identified in vertebrate cells by reaction with rabbit tumor antisera is a highly conserved protein that is closely related, albeit not identical, to pp60^{src}.

Protein Kinase Activity Affiliated with pp60 Isolated from Vertebrate Cells. pp60^{src} isolated from infected cells (9, 10) or synthesized *in vitro* (14) displays the enzymatic properties of a protein kinase. The enzymatic activity had been identified by the phosphorylation of immunoglobulin heavy chains in immunoprecipitates containing pp60^{src} (9, 10). We employed the same approach to demonstrate that protein kinase activity is also affiliated with pp60 precipitated from uninfected vertebrate cells (Fig. 4). Immunoglobulin molecules could be phosphorylated in immunoprecipitates prepared by reacting rabbit tumor antisera with extracts of uninfected chicken, rat, and human cells under conditions previously demonstrated to precipitate pp60 (Fig. 4 *B–E*). We obtained the following evidence that the phosphotransferase activity is affiliated with pp60. (*i*) No phosphorylation was observed in precipitates prepared with sera from ten different normal rabbits (Fig. 4*A* and unpublished data). (*ii*) The immunoprecipitation of protein kinase activity from extracts of vertebrate cells faithfully re-

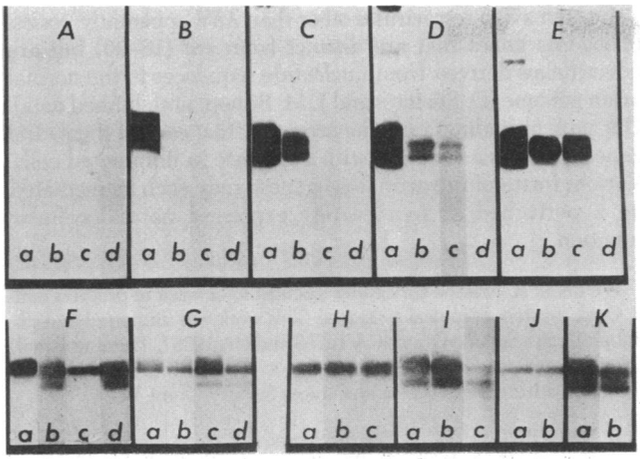

FIG. 4. Protein kinase activity in immunoprecipitates. Immunoprecipitates were prepared with cellular lysates and then assayed for protein kinase activity. The sizes of samples used for immunoprecipitation and the phosphate transfer reactions were identical in each instance. In *A–E*, the autoradiograms were exposed for a standard period of time in order to provide a rough assessment of the relative amounts of phosphate transfer; in *F* and *H–K*, exposures were varied in order to obtain autoradiograms of nearly equal intensity. (*A*) Normal serum: lane a, ASV-infected chicken fibroblasts; lane b, uninfected chicken fibroblasts; lane c, human fibroblasts; and lane d, *Drosophila* cells. (*B*) Tumor serum no. 3, lanes as in *A*. (*C*) Tumor serum no. 6, lanes as in *A*. (*D*) Tumor serum no. 1, lanes as in *A*. (*E*) Tumor serum no. 13, lanes as in *A*. (*F*) ASV-infected chicken fibroblasts: lane a, tumor serum no. 13; lane b, tumor serum no. 1; lane c, tumor serum no. 8; lane d, tumor serum no. 6. (*G*) Protein-stained gel: lanes as in *F*. (*H*) Tumor serum no. 13: lane a, ASV-infected chicken fibroblasts; lane b, uninfected chicken cells; lane c, human fibroblasts. (*I*) Tumor serum no. 1, lanes as in *H*; (*J*) tumor serum no. 8, lanes as in *H*; (*K*) tumor serum no. 6, lanes as in *H*.

flected the hierarchy of serological crossreactivities demonstrated above for pp60^{src} and vertebrate pp60: antisera that precipitated pp60 from both avian and mammalian cells also precipitated kinase activity from both sources (Fig. 4 *D* and *E*); antisera that failed to precipitate pp60 from mammalian cells did not precipitate kinase activity from these cells (Fig. 4*C*); and antisera that precipitated only pp60^{src} failed to precipitate kinase activity from any of the uninfected cells tested (Fig. 4*B*). (*iii*) The phylogenetic distribution of the immunoprecipitable kinase activity mirrored our previous findings with pp60 and with the DNA encoding endogenous sarc: none of our antisera precipitated kinase activity from extracts of *Drosophila* cells (Fig. 4 *B–E*, lanes d). (*iv*) Similar sets of antibody molecules react with the protein kinases affiliated with pp60^{src} and vertebrate pp60. This conclusion is based on the patterns of partially fractionated phosphorylated heavy chains illustrated in Fig. 4 *H–K*. Different sets of heavy chains were phosphorylated in immunoprecipitates prepared with different rabbit tumor antisera (Fig. 4*F*), yet the kinases affiliated with pp60^{src} and vertebrate pp60 phosphorylated similar sets of heavy chains in any single antiserum (Fig. 4 *H–K*). The patterns of total heavy chains (revealed by staining the gels) in the various sera were not notably different (Fig. 4*G*); distinctive patterns of fractionation were apparent only among the phosphorylated molecules. Because the principal targets for phosphorylation in the immunoprecipitates are apparently the heavy chains of the antibody molecules to which the protein kinase is bound in antigen–antibody complexes (9), phosphorylation serves to identify the particular antibodies directed against the enzyme. (We estimate that the amount of protein kinase activity attributable to pp60^{src} in infected cells is at least 100-fold greater than the amount of activity isolated from uninfected cells; consequently, we ascribe the vast majority of phosphate transfer in immune precipitates prepared with infected cells to the viral gene product rather than to the normal cellular kinase detected in the absence of pp60^{src}.) We conclude that the immunoprecipitable protein kinases in both ASV-infected and uninfected cells may form immune complexes with the same antibody molecules and may therefore be antigenically related proteins.

DISCUSSION

The Cellular Homologue of ASV *src* Encodes a Highly Conserved Phosphoprotein. Our previous analyses of cellular nucleic acids indicated that the genomes of all vertebrates harbor nucleotide sequences related to the transforming gene *src* of ASV (2, 4) and that this homologue of *src* may be expressed because it is represented in polysomal RNA of normal avian cells (5). We have now obtained evidence that corroborates the latter supposition by identifying a phosphoprotein in avian and mammalian cells that is immunologically and chemically related to the protein product of *src*. Moreover, this protein homologue of pp60^{src} was not detectable in insect cells, as expected from our previous failures to detect homology between *src* and the DNA of echinoderms, insects, and prokaryotes (4).

A puzzle emerges from our findings: *ca.* 2000 nucleotides are required to encode the vertebrate homologue of *src*, yet the polysomal RNA that carries this homologue contains at least 6500 nucleotides (5). We hope that direct analysis of the gene(s) and mRNA specifying endogenous sarc will resolve this conundrum.

The nucleotide sequences encoding the cellular homologue of *src* diverge appreciably over large phylogenetic distances (2, 4), whereas the presumed protein product of this homologue appears to be highly conserved (see Fig. 3). This apparent discrepancy could be due to a predominance of changes in the third nucleotides of codons; such changes can have large effects on measurements of divergence in nucleic acids without appreciably affecting amino acid sequences (15). In addition, the peptide mapping procedure used here can fail to detect at least minor divergence in amino acid sequence. For example, avian forms of tubulin and actin were virtually indistinguishable from their primate analogues when selectively cleaved with any of several proteases (unpublished data of H. Oppermann and D. Cleveland).

In previous studies (9) with rabbit tumor antisera, we failed to detect the protein homologue of pp60^{src} in uninfected cells. We attribute our present success to substantial reductions in the background noise due to nonspecific immunoprecipitation; these reductions were achieved by the protocol described under *Materials and Methods* and provided the level of sensitivity required to detect specific immunoprecipitation of vertebrate pp60. In addition, we now recognize that only some of our rabbit tumor antisera are crossreactive with pp60 from uninfected cells; many of our earlier studies were performed with antisera that are not crossreactive.

Collett *et al.* (16) have also identified a protein homologue of pp60^{src} in uninfected avian cells, using antisera obtained from marmosets bearing tumors induced by ASV. However, these workers failed to detect an analogous protein in mammalian cells and found no protein kinase activity in immunoprecipitates containing the pp60 from avian cells. We explain the discrepancies between these findings and ours as follows. First, we have found that the serological crossreactivities of tumor antisera vary (Fig. 2). We suggest that the marmoset sera used by Collett *et al.* have serological reactivities similar to those of our rabbit tumor antisera that precipitate pp60 from avian cells but

not from mammalian cells. Second, the amounts of pp60 isolated from uninfected cells were extremely small, and reproducible detection of the accompanying kinase activity required modifications of the assay as described under *Materials and Methods*. Third, some antisera that react with the protein kinase affiliated with pp60 may inactivate the enzymatic activity of the protein. Although we cannot document this phenomenon, perhaps the marmoset sera used by Collett *et al.* are of this sort.

Functional Homology between pp60^{src} and pp60 from Vertebrate Cells: Implications for the Mechanism of Neoplastic Transformation. Our conclusion that the vertebrate pp60 may be a protein kinase is based on two points of evidence. First, enzymatic activity is observed only in immunoprecipitates known to contain pp60. Second, similar sets of immunoglobulin heavy chains are phosphorylated in immunoprecipitates containing either pp60^{src} or vertebrate pp60. This finding conforms to the previous conclusion that immunoprecipitated kinase phosphorylates the antibodies to which it is bound in antigen–antibody complexes (9) and provides circumstantial evidence that the same antibodies are immunologically reactive with both pp60^{src} and vertebrate pp60. As a further test of specificity, we attempted to phosphorylate our rabbit tumor antisera with two protein kinases purified from mammalian sources: the catalytic subunit of a cyclic nucleotide-dependent kinase of rabbit muscle, and a similar kinase from bovine heart (both provided by R. Steinberg). Neither of these enzymes phosphorylated rabbit immunoglobulins, either directly or after isolation of the immunoglobulins by our usual adsorption procedure (data not illustrated).

The chemical and possible functional similarities between pp60^{src} and vertebrate pp60 are not entirely unexpected: our previous evidence indicated that ASV *src* and its homologue in vertebrate genomes are closely related (2, 4). Nevertheless, the nucleotide sequence of *src* has diverged perceptibly from that of its cellular homologue (2, 4) and it is possible that the properties of pp60^{src} and the analogous vertebrate protein may not be identical. In particular, the protein kinase activity of pp60^{src} may have unique specificities for acceptor proteins that could account for the ability of this protein to alter the phenotype of ASV-infected cells. Alternatively, the enzymatic properties of pp60^{src} and vertebrate pp60 may be identical; if so, we would attribute neoplastic transformation by *src* to the mere overloading of the host cell with a normal cellular gene product. This is not an implausible mechanism: the amount of pp60^{src} exceeds that of its cellular homologue by a factor of at least 10 in every ASV-transformed cell we have examined, and our previous studies of phenotypically reverted ASV-infected cells have also signalled the possible importance of the dosage of *src* (17). We presume that these issues can be resolved by direct tests once purified preparations of both pp60^{src} and vertebrate pp60 are in hand.

Certain avian retroviruses other than ASV apparently possess oncogenic genes that are distinct from *src* (18–20) but are nevertheless derived from nucleotide sequences in the normal avian genome (D. Sheiness and J. M. Bishop, unpublished data). Our present findings raise the possibility that each of these viral genes specifies a function with a cognate in uninfected cells. Various forms of retroviral oncogenesis may each be mediated by a perturbed or inordinately expressed normal cellular function.

We thank R. Erikson for candid discussions of work in progress and B. Cook for stenographical assistance. This work was supported by U.S. Public Health Service Grants CA 12705 and CA 19287, Training Grant 1T32 CA 09043 within the Virus Cancer Program of the National Cancer Institute, and American Cancer Society Grant VC70.

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