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Cell Biology

Identification of a Rous sarcoma virus transformation-related protein in normal avian and mammalian cells

(anti-tumor serum/peptide maps/protein kinase/endogenous tumor virus protein)

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ABSTRACT **Avian sarcoma viruses (ASV) contain a gene (*src*) whose protein product mediates sarcomagenic transformation. This product is a 60,000-M_r phosphoprotein designated pp60^{src}. We have found that normal uninfected frog, chicken, rat, and human cells contain a 60,000-M_r phosphoprotein related to the product of the ASV *src* gene and have designated that protein pp60. A phosphoprotein of similar size was not detectable in *Drosophila* cells. The pp60 proteins were detected by immunoprecipitation with rabbit antitumor serum containing broad spectrum antibodies to pp60^{src}. Peptide maps of [^{35}S]-methionine-labeled pp60 and pp60^{src} indicated major similarities as well as some differences in amino acid composition. Peptide maps of the ^{32}P-labeled proteins demonstrated that the phosphopeptides of all endogenous pp60 molecules tested were identical. However, some differences were noted between the phosphopeptide patterns of pp60 and viral pp60^{src}. The kinase activity associated with pp60^{src} was measured in the immunocomplex and resulted in the transfer of radioactive phosphorus from [γ-^{32}P]ATP to the immunoglobulin heavy chain as well as to an 80,000-M_r phosphoprotein. The pp60 of chicken, rat, and human origin also contained an associated kinase activity. These results are consistent with the notion that the pp60 molecules are the protein products of endogenous *sarc* sequences found in vertebrate cells.**

The single-stranded RNA genome of nondefective avian sarcoma viruses (ASV) contains independent coding regions involved with virus multiplication and with sarcomagenic transformation (see refs. 1 and 2 for recent reviews). The viral *gag*, *pol*, and *env* genes specify proteins that constitute the internal structural proteins, reverse transcriptase, and the envelope glycoproteins, respectively (1–3), whereas the genetic element known as *src*, located near the 3′ end of the genome, encodes a 60,000-M_r phosphoprotein (pp60^{src}) that possesses kinase activity and probably mediates cellular transformation (4–7).

Genes analogous to viral sequences specifying the replicative functions (*gag*, *pol*, *env*) are present in normal uninfected chicken cells (see ref. 8 for review). The existence of these endogenous genes was first recognized by the cellular expression of two virus-related protein products termed the chicken helper factor and the group-specific antigen. The endogenous chicken helper factor complements *env*-defective ASV but can be antigenically distinguished from exogenous viral *env* gene products (9, 10). The group-specific antigen has been characterized as a 120,000-M_r protein derived in part from the endogenous *gag* gene and at least half the endogenous *pol* gene (11). The missing peptide segments are thought to preclude the posttranslational processing required to generate mature virus particles. Therefore, chicken helper factor is functionally similar to but structurally different from the exogenous viral envelope antigens, whereas the group-specific antigen demonstrates both structural and functional differences with the cognate viral protein products.

Endogenous genes encoding replication-related functions are found only in chicken cells. In contrast, DNA sequences related to the *src* gene encoding the transformation function have been detected in a wide variety of avian and mammalian cells and are designated *sarc* (12–14). In avian cells the endogenous *sarc* sequences are present in a few copies per cell, reside on chromosomes that are different from those the endogenous viral replication genes reside on (15), and are transcribed independently (13). Poly(A)-containing *sarc* RNA has been found on polyribosomes (16) and presumably directs the synthesis of an endogenous *sarc* protein. Hybridization data suggest that the viral *src* gene product must be related to the putative endogenous *sarc* gene product with less than about 10% divergence (13, 14). The widespread distribution of the *sarc* sequences within vertebrate cells and their highly conserved nature might suggest a fundamental intracellular function for the information encoded in this genetic segment.

In this paper we report the identification and partial characterization of an endogenous *src*-related protein having the same electrophoretic mobility as pp60^{src}. These experiments were stimulated by the initial finding of a *src*-related protein in normal rat kidney (NRK) cells (17). While this research was in progress, Collett *et al.* (18) reported on an endogenous *sarc* polypeptide lacking an associated kinase activity and detectable only in avian cells. In contrast, we demonstrate here that an endogenous *sarc* protein is present in frog, chicken, rat, and human cells; possesses kinase activity; and is highly related, but not identical, to ASV pp60^{src}.

METHODS AND MATERIALS

Cells and Viruses. Chicken embryo fibroblasts (CEF) of phenotype C/E and negative for the expression of chicken helper factor and group-specific antigen were obtained from H and N Laboratories (Redmond, WA). The Schmidt–Ruppin strain of Rous sarcoma virus subgroup D (SR-RSV-D) used to transform CEF was from a recently cloned laboratory stock. The NRK cells were from a described epithelioid clone (17). A line of SR-RSV-D-transformed NRK cells (SR-NRK clone A4B5) was isolated by growth in methylcellulose. Early passage human lung fibroblasts (HL) were obtained from C. Berglund, *Drosophila* cells were from C. Laird, and *Xenopus laevis* kidney cells were from M. L. Pardue. Chicken cells were cultured in Ham's F10 medium as described (17) and mammalian cells were grown in Eagle's minimal essential medium plus 10% calf serum. The insect cells were grown in Schneider's *Drosophila* medium plus 15% fetal calf serum. Cells were labeled for 4–6 hr with [^{35}S]methionine (50 μCi/ml; 1 Ci = 3.7 × 10^{10} becquerels) or [^{3}H]methionine (300 μCi/ml) in methionine-free

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Abbreviations: ASV, avian sarcoma virus; SR-RSV-D, Schmidt–Ruppin strain of Rous sarcoma virus subgroup D; CEF, chicken embryo fibroblasts; NRK, normal rat kidney; HL, human lung fibroblasts; pp60^{src}, the 60,000-M_r phosphoprotein product of the ASV transforming gene; pp60, a 60,000-M_r phosphoprotein.

Eagle's medium plus 5% calf serum, and for 2–3 hr with ^{32}P (750 μCi/ml) in phosphate-free Eagle's medium plus 5% dialized fetal calf serum.

Immunoprecipitation and Gel Electrophoresis. Antisera to the ASV transforming protein was prepared as described (17). Marmoset antitumor serum was obtained from an animal immunized with autochthonous SR-RSV-D-transformed cells and kindly supplied by R. Massey.

Transforming and antigenically related proteins were detected by immunoprecipitation and polyacrylamide gel electrophoresis as described (17). All cell extracts were precleared by incubation with 15 μl of normal rabbit serum and 30 mg of formalin-fixed *Staphylococcus aureus* (19) for 30 min at 4°C and subsequent centrifugation (10,000 rpm for 10 min). Aliquots of the cleared extract were then mixed with normal rabbit serum or antitumor serum and immunocomplexes were formed with *S. aureus* as detailed (17). We deviated from the described immunoprecipitation method by employing more extensive washing procedures to reduce nonspecific binding to the immunocomplex. Each immunocomplex was washed five times: twice with RIPA buffer (150 mM NaCl/10 mM Tris·HCl, pH 7.2/1% sodium deoxycholate/1% Triton X-100/0.1% sodium dodecyl sulfate/300 Kallikrein inhibitor units of Trasylol per ml); once with high-salt buffer (2 M NaCl/10 mM Tris·HCl, pH 7.2/0.5% sodium deoxycholate/1% Nonidet P-40); once with phosphate-buffered saline/Nonidet P-40; and once with lysis buffer (10 mM Tris·HCl, pH 7.2/50 mM NaCl/0.5% Nonidet P-40). Antigens in the immunocomplex were electrophoresed on 10–20% linear gradient polyacrylamide gels.

Proteolytic Peptide Analyses. Two methods were utilized to compare proteolytic peptides of antigenically related proteins excised from dried polyacrylamide gels. One method used exhaustive digestion with trypsin and analysis by cation exchange chromatography (11). A second method employed partial proteolysis with *S. aureus* V8 protease (Miles) during reelectrophoresis to generate comparative peptide patterns (7, 20).

Kinase Assay. The ability of pp60 proteins to transfer ^{32}P from [γ-^{32}P]ATP to the immunoglobulin heavy chain was measured in the immunocomplex (6, 7). Extracts of unlabeled cells were mixed with antitumor serum or normal serum and immunocomplexes were prepared and extensively washed as described above. Each immunoprecipitate was suspended in 30 μl of kinase buffer (0.15 M NaCl/0.01 M Tris·HCl, pH 7.2/5 mM $MgCl_2$/0.5% Nonidet P-40) and made 0.5 μM in [γ-^{32}P]ATP. After 10 min at room temperature, the reaction vials were centrifuged and the immunoprecipitates were washed once with RIPA buffer containing 1 mM EDTA, once with high-salt buffer, and once with lysis buffer. Samples were electrophoresed on 10–20% gradient gels, dried, and exposed to film with or without an intensifying screen.

RESULTS

As previously shown (17), rabbit antitumor serum precipitated the pp60^{src} from NRK cells and CEF transformed with SR-RSV-D (Fig. 1). Precipitation of pp60^{src} was not blocked by preabsorbing the antitumor serum with unlabeled SR-RSV-D virions, whereas all viral structural proteins (such as the *gag* protein precursor Pr76^{gag}) could be eliminated from the immunocomplex by this preabsorption step (Fig. 1, lane B, SR-CEF). Consistent with our recent results, NRK cells transformed with SR-RSV-D did not appear to express significant levels of the viral structural proteins, as judged by immunoprecipitation.

The results in Fig. 1 also demonstrate that rabbit antitumor serum precipitated an $\approx$60,000-M_r protein from uninfected CEF, NRK cells, and HL. This 60,000-M_r protein was not

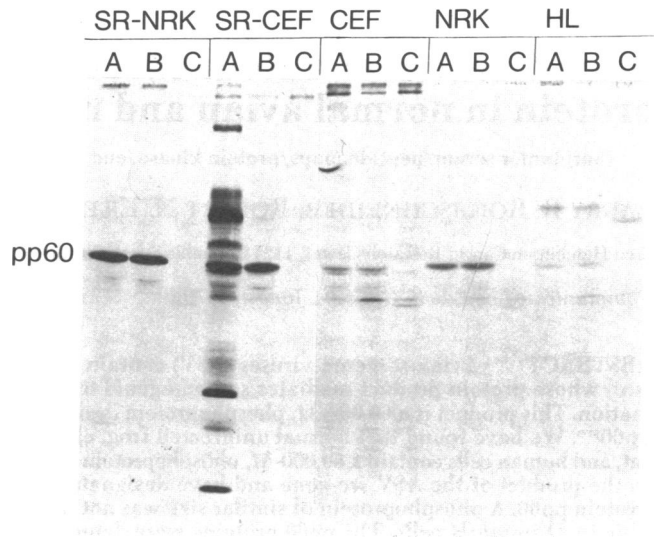

FIG. 1. Detection of [^{35}S]methionine-labeled pp60 in various uninfected and SR-RSV-D-transformed cells. Cells were labeled with [^{35}S]methionine for 4–6 hr and detergent extracts were prepared. Immunoprecipitates from each extract were electrophoresed on 10–20% polyacrylamide gels. The immunoprecipitates were made with 5 μl of rabbit antitumor serum B20II (lane A), 5 μl of rabbit antitumor serum B20II preadsorbed with 200 μg of unlabeled SR-RSV-D virus (lane B), and 5 μl of normal rabbit serum (lane C). SR-, SR-RSV-D-transformed cells.

eliminated by preabsorbing the antitumor serum with unlabeled virus (lane B) and was not precipitated by normal rabbit serum (lane C). Quantitative analyses indicated that pp60^{src} in SR-NRK cells was present in a 22-fold excess over the 60,000-M_r protein in uninfected NRK cells. Our results confirm those of Collett *et al.* (18) in avian cells and indicate that antigenically similar proteins exist in rat and human cells.

Possible similarities in amino acid sequence among the 60,000-M_r proteins of uninfected and SR-RSV-D-transformed cells were examined by the method of Cleveland *et al.* (20). The proteolytic polypeptide patterns of pp60^{src} are shown in Fig. 2 and compared with those of the antigenically related 60,000-M_r protein of uninfected CEF, NRK cells, and HL. The majority of polypeptide fragments generated from each of these 60,000-M_r proteins formed an identical pattern in the polyacrylamide gel. Therefore, these results suggest that the primary structure of the viral pp60^{src} is closely related to the primary structure of the endogenous 60,000-M_r protein of CEF, NRK cells, and HL.

To compare more precisely the endogenous 60,000-M_r protein with pp60^{src}, we analyzed their methionine-containing tryptic peptides by double-label cation-exchange chromatography. [^{3}H]Methionine-labeled pp60^{src} from SR-RSV-D-transformed NRK cells and [^{35}S]methionine-labeled pp60 from uninfected NRK cells were digested with *N*-tosylphenylalanine chloromethyl ketone-treated trypsin, oxidized, and cochromatographed on a Chromobeads P column (Technicon). The results in Fig. 3 show resolution of three major tryptic peptides from both 60,000-M_r proteins within the pH range of the column. Two of the three peptides (60a and 60b) coeluted from the column and the more basic 60d peptide of pp60^{src} did not appear to be present in the NRK 60,000-M_r protein. Instead, a third peptide of pp60 (60c) eluted at a lower pH. A fourth [^{35}S]methionine-labeled peptide (fraction 65) also appeared in this chromatograph, but because it was not routinely present it may be a contaminant. These data clearly indicate that, although these two proteins share polypeptide regions, they are not identical. However, the extent of difference cannot be estimated because a single amino acid change could be expected to alter the elution of peptides from the column.

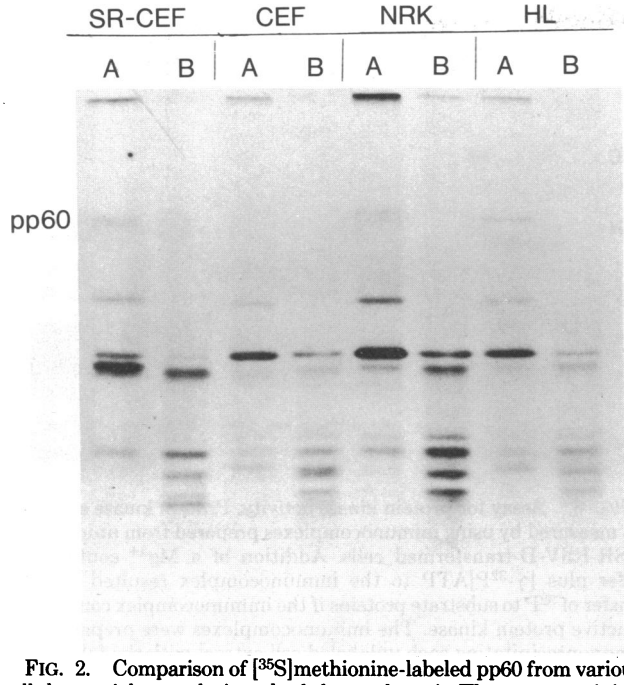

FIG. 2. Comparison of [^{35}S]methionine-labeled pp60 from various cells by partial proteolysis and gel electrophoresis. The area containing the [^{35}S]methionine-labeled pp60 was cut from dried gels, rehydrated, homogenized, and reelectrophoresed on a 13–20% polyacrylamide gel in the presence of *S. aureus* V8 protease. The pp60 from each cell type was digested with either 50 μg (lane A) or 1000 μg (lane B) of protease.

Further similarities among the viral pp60^{src} and the endogenous 60,000-M_r proteins of CEF, NRK cells, and HL were sought by comparing the incorporation of ^{32}P into these proteins. The viral pp60^{src} has been shown to be a phosphoprotein (7, 21). The results in Fig. 4 show the pp60^{src} immunoprecipitated from SR-RSV-D-transformed NRK cells and CEF. A phosphoprotein of approximately 60,000-M_r (pp60) also was immunoprecipitated from uninfected CEF, NRK cells, and HL (Fig. 4, lane A). A similar phosphoprotein was not detectable in extracts of *Drosophila* cells but was found in chicken embryo macrophages and in extracts of ^{32}P-labeled *Xenopus laevis* kidney cells (data not shown). The pp60s were not precipitated by normal rabbit serum (Fig. 4, lane C) and preabsorbing the antitumor serum with unlabeled virus neither eliminated nor reduced precipitation of pp60 molecules (Fig. 4, lane B). Unlabeled virus did block precipitation of viral phosphoproteins Pr180, Pr76, and p19 and of various intermediates in the *gag*-

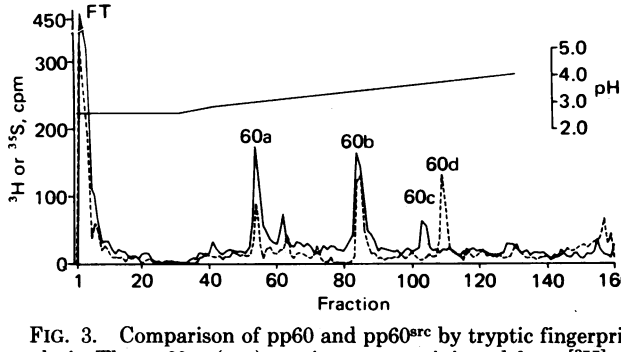

FIG. 3. Comparison of pp60 and pp60^{src} by tryptic fingerprint analysis. The pp60^{src} (---) was immunoprecipitated from [^{3}H]methionine-labeled SR-NRK cells and pp60 (—) was obtained from [^{35}S]methionine-labeled NRK cells by the same method. The appropriate pp60 bands were cut from dried polyacrylamide gels, digested with trypsin, oxidized with performic acid, combined, and chromatographed on a chromobeads P column.

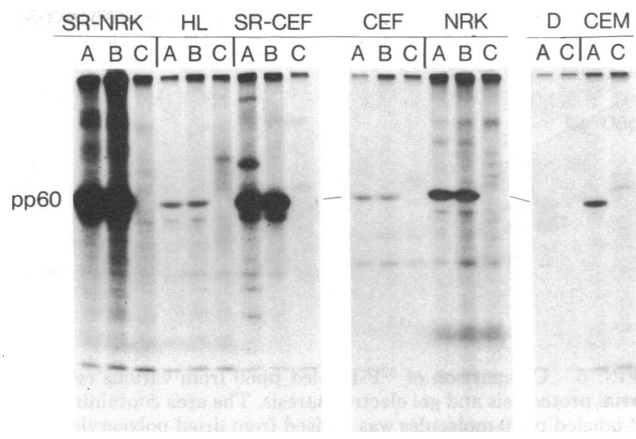

FIG. 4. Detection of ^{32}P-labeled pp60 in various uninfected and SR-RSV-D-transformed cells. Cells were labeled with ^{32}P for 2–3 hr and detergent extracts were prepared. Immunoprecipitates were then made from each extract and electrophoresed on a 10–20% polyacrylamide gel. The immunoprecipitates were prepared with 4 μl of rabbit antitumor serum B20II (lane A); 4 μl of rabbit antitumor serum B20II preabsorbed with 250 μg of unlabeled SR-RSV-D virus (lane B); 4 μl of normal rabbit serum (lane C). D, *Drosophila* cells; CEM, chicken embryo macrophages.

protein cleavage pathway leading to viral structural proteins (Fig. 4, SR-CEF, lane B). These results indicate that pp60 molecules are phosphorylated in uninfected *Xenopus*, CEF, NRK cells, and HL but cannot be detected in *Drosophila* cells.

In addition to pp60, a phosphoprotein of about 50,000-M_r was detectable upon electrophoresis of immunoprecipitates from most cell extracts (Fig. 4). Fingerprint analysis by partial proteolysis indicated that pp50 was identical to its corresponding pp60 (data not shown) and, moreover, comigrated with pp60 when eluted and reelectrophoresed. We believe, therefore, that pp50 may result from an artifact related to the high concentration of immunoglobulin heavy chain in that region of the gel.

Fingerprint analysis of ^{32}P-labeled pp60^{src} from SR-RSV-D-transformed CEF is shown in Fig. 5. This analysis reveals phosphorylated polypeptide fragments and these fragments are compared with those generated under identical conditions from the ^{32}P-labeled endogenous pp60 of CEF, NRK cells, and HL. Several interesting features of these polypeptides are evident from the results in Fig. 5. First, by comparing the ^{32}P-labeled polypeptide fragments from SR-CEF pp60^{src} with the [^{35}S]methionine-labeled fragments of the same molecule (Fig. 2), it appears that some phosphorylated fragments lack methionine (indicated by asterisks in Fig. 5). Second, at least three of the phosphopeptide fragments from SR-CEF pp60^{src} appear identical to phosphopolypeptides from uninfected CEF pp60, and an equal number of phosphopolypeptide fragments from SR-CEF pp60^{src} are clearly not found in the corresponding fragment pattern generated from CEF pp60 (indicated by arrow and asterisks in Fig. 5). Third, the phosphopolypeptide fragments derived from CEF, NRK, and HL (Fig. 5) pp60s are virtually identical. Therefore, whereas differences exist between the phosphorylated peptides of viral pp60^{src} and the endogenous CEF pp60, no differences were found in the phosphorylated peptides from the endogenous pp60s of chicken, rat, and human cells.

Viral pp60^{src} appears to be, or have associated with it, an enzyme capable of transferring radioactive phosphorus from [γ-^{32}P]ATP to a protein substrate. This protein kinase activity has been measured in the immunocomplex that contains *S. aureus* cells, antibody, and pp60^{src}. We have measured the

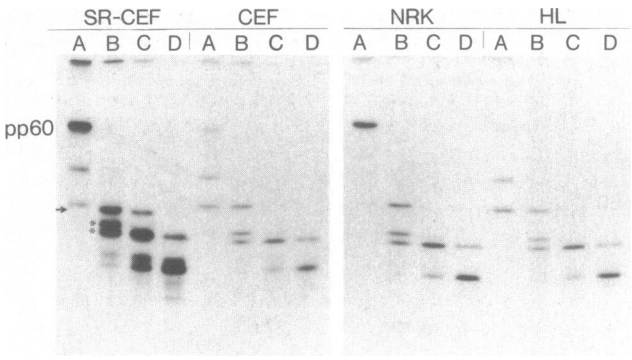

FIG. 5. Comparison of ^{32}P-labeled pp60 from various cells by partial proteolysis and gel electrophoresis. The area containing the ^{32}P-labeled pp60 molecules was excised from dried polyacrylamide gels, rehydrated, homogenized, and reelectrophoresed on 13–20% gels in the absence or presence of *S. aureus* V8 protease. Lanes: A, no protease; B, 0.2 μg of protease; C, 1 μg of protease; D, 5 μg of protease. The partial proteolytic fragments in lane A probably result from cross-contamination with *S. aureus* V8 protease while loading samples on the gel. The asterisks mark peptide fragments that lack methionine (compared with the results in Fig. 2) and the arrow and asterisks mark fragments of pp60^{src} not found in endogenous pp60 molecules.

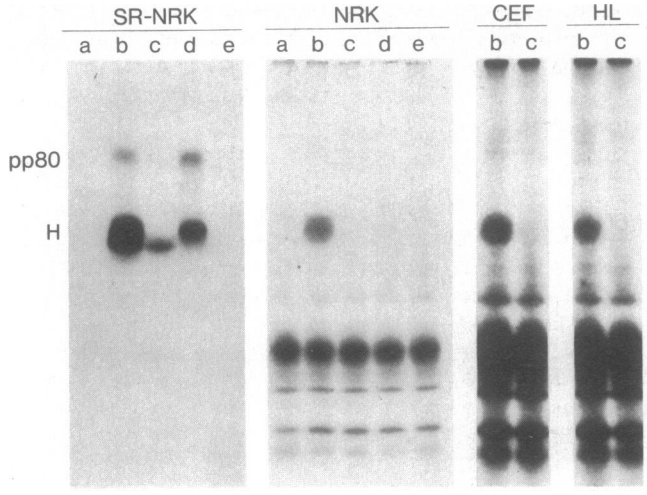

FIG. 6. Assay for protein kinase activity. Protein kinase activity was measured by using immunocomplexes prepared from uninfected or SR-RSV-D-transformed cells. Addition of a Mg^{2+}-containing buffer plus [γ-^{32}P]ATP to the immunocomplex resulted in the transfer of ^{32}P to substrate proteins if the immunocomplex contained an active protein kinase. The immunocomplexes were prepared by immunoprecipitating each unlabeled cell extract with the following sera. Lanes: a, serum BY-1 from a marmoset bearing an SR-RSV-D-induced tumor; b, rabbit antitumor serum B20II; c, rabbit antitumor serum RD2 progressor; d, rabbit antitumor serum B37I; e, normal rabbit serum. The cells are described in the legend to Fig. 1. The letter H marks the position of immunoglobulin heavy chains detected by Coomassie blue staining. The background radioactivity was identical for each gel but more pronounced in some due to the long exposure required to reveal weak kinase activity.

ability of various antitumor sera to immunoprecipitate such a protein kinase from SR-RSV-D-transformed NRK cells. These sera also were tested for their ability to immunoprecipitate a similar kinase activity from uninfected NRK cells, CEF, and HL.

Marmoset serum similar to that described by Collett *et al.* (18) did precipitate [^{35}S]methionine-labeled pp60^{src} (data not shown), but this product did not contain an active kinase activity (Fig. 6, SR-NRK lane a). On the other hand, three different rabbit antitumor sera precipitated active kinase as judged by the transfer of radioactive phosphorus from [γ-^{32}P]ATP to the heavy chain of IgG in the immunocomplex (Fig. 6, SR-NRK lanes b, c, and d). The variation in size of the heavy chains in each serum probably stems from different classes of IgG specific for pp60^{src}. The immunocomplex prepared from SR-NRK cells with normal rabbit serum was devoid of kinase activity.

In addition to the heavy chain of the immunoglobulin molecules, the pp60^{src} immunoprecipitated by all three rabbit sera transferred ^{32}P to an 80,000-M_r protein (pp80). Other phosphoproteins of M_r about 40,000 and 17,000 were seen when the gel of the kinase reaction was exposed to the film for longer periods. These smaller phosphoproteins may result either from heavy chain degredation or phosphorylation of other proteins in the immunocomplex. Their exact nature has not yet been established. These results on the viral pp60^{src}-associated kinase are similar to those reported by others (6, 7) with the exception that a pp80 also was phosphorylated in our assay.

The same battery of sera were next used to prepare immunocomplexes from detergent lysates of unlabeled NRK cells, CEF, and HL. The results in Fig. 6 (NRK, CEF, and HL, lane b) indicate that a functional kinase similar to that found in SR-RSV-D-transformed cells was detectable in the uninfected cells of each species tested. Only one antitumor serum precipitated an active kinase enzyme from the uninfected cells and the activity was much weaker than that found in SR-RSV-D-transformed cells. Similar assays have failed to detect kinase activity in immunocomplexes prepared from *Drosophila* cell extracts. Therefore, these results suggest that the endogenous pp60 molecules of avian and mammalian cells contain a kinase activity similar to that associated with viral pp60^{src}. Because the detectable activity of the endogenous pp60 is relatively low, it has not yet been possible to determine definitively whether the endogenous proteins also phosphorylate an 80,000-M_r phosphoprotein.

DISCUSSION

We have presented several lines of evidence demonstrating that a protein highly related to the transforming protein of ASV (pp60^{src}) is synthesized in normal uninfected cells from a wide variety of vertebrate species. By using an antiserum from a rabbit bearing SR-RSV-D-induced tumors, we have been able to precipitate from uninfected CEF, chicken macrophages, NRK cells, *Xenopus laevis* kidney cells, and HL a 60,000-M_r phosphoprotein (Figs. 1 and 4). Electrophoretic analysis of the [^{35}S]methionine-containing fragments generated by partial proteolysis of ASV pp60^{src} and the endogenous protein from CEF, NRK, and HL cells indicated that these proteins were highly related (Fig. 2). Similar analysis of the phosphopeptides showed again a high degree of relatedness with some differences apparent between the endogenous pp60 and the ASV pp60^{src}. No differences were apparent among the various endogenous pp60 proteins tested. Cation-exchange chromatography of the [^{35}S]methionine tryptic peptides of pp60^{src} synthesized in SR-RSV-D-transformed NRK cells and the pp60 synthesized in uninfected NRK cells indicated similarities as well as some differences in amino acid sequence. Therefore, an endogenous pp60 of several vertebrate species was antigenically and structurally related to the ASV pp60^{src}.

Our data also demonstrate that immunoprecipitates of endogenous pp60 possess a kinase activity similar to that previously observed for ASV pp60^{src} (Fig. 6; ref. 6). In contrast, Collett *et al.*, although able to precipitate endogenous pp60, were unable to detect an accompanying kinase activity in uninfected cells (18). These results may suggest that some antitumor sera contain neutralizing antibodies that block the activity of the kinase enzyme. In addition, we found several sera that precipitated ASV pp60^{src} kinase activity but failed to precipitate similar activity from uninfected cells (Fig. 6). This would suggest either real differences between the endogenous

pp60 and ASV pp60^{src} or may simply be due to the inability to detect low levels of pp60 kinase in the normal cells. In any case, the similarity between endogenous pp60s and ASV pp60^{src} included an enzymatic kinase activity immunoprecipitable by at least one antitumor serum.

Our kinase assay with viral pp60^{src} transferred ^{32}P not only to the immunoglobulin heavy chain, but also to a phosphoprotein of M_r 80,000 (pp80). Previous reports on pp60^{src} kinase activity have demonstrated phosphorylation of the immunoglobulin heavy chain only, presumably because of its close association with pp60^{src} in the immunocomplex. Because exogenously added phosphoprotein acceptors do not serve as substrates (6), pp80 also must be closely associated with pp60^{src} in the immunocomplex. Phosphorylation of pp80 was dependent upon pp60^{src} kinase activity because immunocomplexes containing marmoset antiserum or normal rabbit serum but no active pp60^{src} failed to phosphorylate either immunoglobulin or pp80. This pp80 may be the same host protein that Sefton *et al.* (22) found coprecipitating with pp60^{src}. Therefore, if pp60^{src} does mediate transformation by phosphorylating host protein(s), then pp80 is a likely candidate for the host protein target. On the other hand, it is not known whether pp60^{src} elicits transformation via host protein phosphorylation or whether autophosphorylation activates an as yet unknown activity of pp60^{src}.

Overall, the results on *src*-related proteins in normal cells are compatible with the notion that the pp60 of each species represents the protein product synthesized from an endogenous *sarc* gene. DNA sequences closely related to the viral *src* gene are present in uninfected vertebrate cells and are absent from insect cells (14). Hybridization experiments first indicated that DNA of all uninfected avian cells tested contained sequences related to the viral *src* gene (12), and these endogenous sequences have been designated "*sarc*." More recent hybridization studies have now shown that probably all vertebrate cells, including human, contain related *sarc* DNA sequences (14). The antigenic, structural, and enzymatic relationship of the endogenous rat, chicken, and human pp60 to the viral pp60^{src} as well as the presence of *src* related sequences in uninfected cells of these species suggest that pp60 is the endogenous analogue of the pp60^{src}. We therefore designate these endogenous phosphoproteins pp60^{sarc}.

Recent studies by Hanafusa and colleagues (23, 24, 25) suggest that endogenous *sarc* may be important in the generation of nondefective ASV from at least certain types of transformation-defective viruses lacking part of the *src* region. A critical question then is whether the viral pp60^{src} is functionally identical to the endogenous pp60^{sarc}. If these two proteins are identical, then viral transformation may simply be envisioned as an overdose or inappropriate expression of a normal cellular protein now under viral control. Indeed, the large amount of pp60^{src} protein in ASV-infected cells, compared to endogenous pp60 in uninfected cells, could be considered consistent with such a dose "threshold" model. However, the detection of equivalent amounts of polyribosome-associated 21S *src* mRNA in ASV-transformed mammalian cells and their revertant subclones would suggest that the absolute level of pp60 proteins may not be sufficient to maintain the transformed state, and that posttranslational modification may also play a role (25–27). Alternatively, if ASV-pp60^{src} and endogenous pp60^{sarc} are not identical, then viral transformation may be envisioned as the introduction of a modified cellular protein having an altered function. The viral pp60^{src} could differ from pp60^{sarc} in either the active site, affecting substrate specificity, or a regulatory site mediating host cell control.

The data presented in this paper, although demonstrating both antigenic and structural differences between pp60^{src} and pp60^{sarc}, do not indicate significant functional differences. Indeed, the differences we have found between these proteins may not be greater than the variation observed between the pp60^{src} proteins synthesized by different strains of RSV (22). Thus, it remains to be ascertained what role, if any, these differences might have in determining the function of pp60^{src} and the mechanism of transformation.

Note Added In Proof. Recently we detected a polypeptide with properties of pp60^{sarc} in human melanoma cells and in a human B cell line (unpublished results). Oppermann *et al.* (28) have also reported a pp60^{sarc}-associated kinase activity in various uninfected cells.

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