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Biochemistry

Cellular information in the genome of recovered avian sarcoma virus directs the synthesis of transforming protein

(Rous sarcoma virus/*src* gene product/cell transformation)

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ABSTRACT **Recovered avian sarcoma viruses, whose sarcomagenic information is largely derived from cellular sequences [Wang, L.-H., Halpern, C. C., Nadel, M. & Hanafusa, H. (1978) *Proc. Natl. Acad. Sci. USA* 75, 5812–5816], produce the transforming protein p60^{src} in infected cells, in amounts comparable to the amount found in cells transformed by standard strains of avian sarcoma virus. Though displaying some virus-specific differences in electrophoretic mobility, p60^{src}s from these viruses are similar to those of other avian sarcoma virus strains by the criteria of (*i*) antigenicity, (*ii*) partial proteolysis mapping, and (*iii*) association with protein kinase activity. We also find that p60^{sarc}, a protein present in normal cells at a low level, is associated with a protein kinase activity, and thus it too is similar by the above criteria to p60^{src} of avian sarcoma virus. Possible causes for the pathogenicity of p60^{src} are discussed in light of these similarities.**

The *src* gene of avian sarcoma viruses (ASVs) is responsible for the ability of these agents to induce tumors in chickens and to transform fibroblasts in tissue culture (1, 2). This gene is not required for virus replication, because transformation-defective (td) mutants of ASV, lacking all or a part of *src*, are still capable of normal growth (1–3). The identification of *src*-related sequences in the genome and cytoplasmic RNA of normal uninfected chicken cells has led to the definition of a presumptive genetic element in normal cells denoted *sarc* (4–6).

Erikson and coworkers have identified a protein (7), called p60^{src}, that is encoded by the *src* region of the ASV genome (8). It is a phosphoprotein (9) of 60 kilodaltons (kDal) that is found closely associated with, and may be identical to, a protein kinase activity (10–12). An antigenically and structurally related protein has been found in small amounts in immunoprecipitates of normal chicken cells (13). Because this protein is presumed to be coded by the endogenous *sarc* gene, it has been denoted p60^{sarc}. However, no associated protein kinase activity was detected (13).

We have reported the isolation of a number of ASVs from chicken tumors produced after long latent periods following injection of certain td mutants of the Schmidt–Ruppin strain of Rous sarcoma virus (SR) of subgroup A (SR-A) (14). Virus recovered from these tumors had regained the capacity to transform cells in culture and to rapidly induce tumors in chickens at the site of injection. The genomes of these recovered avian sarcoma viruses (rASVs) had acquired *src*-specific genetic information (15, 16). It is likely that the recovered sarcomagenic information was obtained through a recombination event between the parental td virus and endogenous *sarc* sequences of normal cells (16).

We report here that rASVs possess information directing the production of p60^{src}, and show it to be enzymatically and structurally similar to the p60^{src} of the SR-A strain of ASV. In addition we show that p60^{sarc} of normal cells shares both structural and enzymatic properties with the transforming proteins of ASV.

MATERIALS AND METHODS

Cell Culture and Viruses. Chicken embryo fibroblasts were cultured as described (17), except that Ham's F-10 medium with 5% calf serum was used for all secondary cultures. The isolation and characterization of td mutant 108 (td 108) of SR-A was reported previously (3). Preliminary description of rASVs is the subject of earlier reports (14–16). The recovered virus rASV 1441, used in this study as the prototype of the group, was derived from td 108 (14).

Isotopic Labeling of Cells and Preparation of Cell Extracts. Transformed cells grown in 100-mm tissue culture plates were incubated for 4 hr in 2 ml of medium containing either 50 μCi of L-[^{35}S]methionine (900–1200 Ci/mmol; Amersham) per ml in methionine-free minimal essential medium (GIBCO) with 2% calf serum, or 1 mCi of carrier-free [^{32}P]phosphate (Amersham) in phosphate-free medium 199 containing 2% dialyzed calf serum (1 Ci = 3.7×10^{10} becquerels). Nontransformed cells were labeled with 150 μCi of [^{35}S]methionine per ml, or 4 mCi of [^{32}P]phosphate. The cultures were washed twice and lysed in slightly modified RIPA buffer (0.05 M Tris·HCl, pH 7.4/0.15 M NaCl/1% Triton X-100/1% sodium deoxycholate/0.1% sodium dodecyl sulfate/1 mM EDTA) containing 1% Trasylol (FBA Pharmaceutical, New York) as described (7). Transformed cultures received 1 ml of RIPA buffer per 100-mm plate; nontransformed cells were lysed in 0.5 ml, to make the final protein concentrations more comparable. Lysed cells were scraped from the plate with a rubber policeman and transferred to a 1.5-ml Eppendorf micro test tube (Brinkmann). After Vortex mixing vigorously for 30 sec, the sample was placed on ice for 5 min. The sample was mixed again and centrifuged in a precooled Beckman Microfuge B for 4 min. The resulting clarified supernatant was used in all subsequent manipulations.

Immunoprecipitation. Serum from tumor-bearing rabbits (TBR serum) was prepared as described (7), by injecting newborn New Zealand White rabbits with 0.2 ml of 12-hr culture medium from a confluent transformed culture of SR-D-infected cells. Two sera were used in these experiments. Both displayed broad crossreactivity for p60^{src} from different sources. For quantitation studies (Fig. 4, Table 1), a single serum was used

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Abbreviations: ASV, avian sarcoma virus; td, transformation-defective; kDal, kilodaltons; SR-A, Schmidt–Ruppin strain ASV, subgroup A; SR-D, Schmidt-Ruppin strain ASV, subgroup D; TBR, tumor-bearing rabbit; rASV, recovered ASV.

throughout. Preabsorbed TBR serum was prepared by adding 50 μg of protein from disrupted Rous-associated virus-2 in modified RIPA buffer to 5 μl of TBR serum, incubating at room temperature 60 min, and spinning out the precipitate in a Beckman Microfuge. Aliquots of cell extracts were incubated with 5 μl of TBR serum for qualitative studies or with predetermined saturating amounts of TBR serum for quantitative studies. Extracts of labeled or unlabeled cells were incubated with serum for 60 min on ice. Four serum volumes of staphylococcal protein A-Sepharose CL 4B [a 50% (wt/vol) slurry in modified RIPA buffer; Sigma] was added and mixed for 30 min at 4°C to adsorb immune complexes by the method of Kessler (18). The Sepharose pellet was washed five times in modified RIPA buffer and twice in 0.05 M Tris·HCl (pH 7.4). The pellet was then suspended in 40 μl of gel sample buffer (7) or assayed for protein kinase activity.

Protein Kinase Assay. To the packed pellet of immune complex–protein A-Sepharose was added 25 μl of reaction mixture: 50 mM Hepes (pH 7.0)/0.15 M KCl/5 mM dithiothreitol/10 mM magnesium acetate/0.1 μM [γ-^{32}P]ATP (3000 Ci/mmol, Amersham). The tube was Vortex mixed briefly and incubated for 10 min at 30°C. The reaction was stopped by the addition of 40 μl of gel sample buffer.

Polyacrylamide Gel Electrophoresis. Polyacrylamide slab gels (30:0.8, acrylamide/bisacrylamide) containing 0.1% sodium dodecyl sulfate were prepared according to the procedure of Laemmli (19). Immunoprecipitates or kinase assay samples in sample buffer were heated in boiling water for 3 min. The Sepharose beads were spun out, and the supernatant was loaded into the gel slots. Gels were run at 15 or 20 mA constant current, until bromophenol blue tracking dye reached the bottom of the gel. Gels were fixed and stained, dried down onto filter paper, and exposed for autoradiography as described (20). Gels containing samples to be used in peptide analysis were not fixed, but were dried down directly and exposed.

Partial Hydrolysis of Proteins with Protease. Peptide maps were prepared from protein samples in gel slices by partial digestion with *Staphylococcus aureus* V8 protease (Miles) by using the procedure described by Levinson and Levine (21). Aliquots of sample were incubated with increasing amounts of protease for 15 min at 30°C, and the products of digestion were displayed on a 12% polyacrylamide gel.

Quantitation of p60^{src} and p60^{sarc} in Cell Extracts. Determinations were essentially as described by Collett *et al.* (13) by comparing ^{35}S radioactivity per p60 band precipitated by excess antibody with the specific activity of the [^{35}S]methionine-labeled protein from the cell extracts, except that (*i*) the cells were labeled as described above, and (*ii*) the gel slices containing p60 were solubilized in 1 ml of NCS tissue solubilizer (Amersham) for 2 hr at 55°C prior to measurement of radioactivity. Protein content was assayed by the method of Lowry *et al.* (22).

Nucleic Acid Hybridization. The number of copies of *src*-specific DNA per cell was determined by comparing the $C_0t_{1/2}$ for the annealing of cDNA$_{src}$ to cell DNA with the $C_0t_{1/2}$ for reassociation of unique sequence cellular DNA (23) ($C_0t_{1/2}$ is the product of DNA concentration and the incubation time necessary for 50% hybridization). DNAs were fragmented to an average length of 150 nucleotides by depurination at pH 4.2 (23). Concentrations of *src*-specific RNA were determined by hybridization kinetics, as described (24). Values shown in Table 2 for SR-A- or rASV-infected cells reflect only the putative *src* mRNA species (21S), which represents 15–20% of the total *src*-specific viral RNA in the infected cell (24). The preparation and characterization of cDNA$_{src}$ have been described (24).

RESULTS

Identification of p60 in rASV-Infected and Nontransformed Cells. Chicken embryo fibroblasts infected with different rASVs were labeled with [^{35}S]methionine or [^{32}P]phosphate and immunoprecipitated with serum raised in rabbits bearing regressing tumors induced by the SR-D strain of ASV (TBR serum). A protein migrating closely to the p60^{src} of SR-A was found in each case, as well as some viral structural proteins and their precursors (Fig. 1 *left*). The latter proteins, however, were not precipitated when TBR serum was preabsorbed with unlabeled disrupted virus. The 60-kDal proteins will be collectively called p60^{src}, although they displayed some virus-specific variation in their apparent molecular weights. None of these antigens was detected when nonimmune serum was employed. Similar results were obtained with [^{32}P]phosphate labeled cells, indicating that p60^{src} from rASV was a phosphoprotein, as has been found for p60^{src} from other viruses (9, 11, 12).

Immunoprecipitates of nontransformed chicken cells, either uninfected or infected with the transformation-defective virus td 108, contained small amounts of a protein of 60 kDal, specifically precipitated by TBR serum (Fig. 1 *right*). This protein displayed strong structural homology with authentic p60^{src} from SR-A-infected cells (see below). We therefore believe it to be the p60^{sarc} of normal cells recently described by Collett *et al.* (13).

Partial proteolysis mapping of the [^{32}P]phosphate-labeled p60^{src}s of rASV 1441- and SR-A-infected cells, and p60^{sarc} from normal cells, by the V8 protease of *S. aureus* is shown in Fig. 2. The p60s from these three sources were nearly identical in their digestion patterns, although the smaller primary cleavage fragment of normal cell p60^{sarc} (labeled b in Fig. 2) displayed a slightly slower mobility than the corresponding peptides of p60^{src} from SR-A and rASV 1441. Essentially identical patterns were found for several other rASV p60^{src}s (data not shown).

Protein Kinase Activity Is Associated with p60^{src} of rASV and p60^{sarc} of Nontransformed Cells. A protein kinase activity associated with p60^{src} of ASV-infected cells has been described (10–12). To investigate whether immunoprecipitates of p60^{src} from rASV-infected cells possessed protein kinase activity, they were incubated with [γ-^{32}P]ATP, and the reaction products

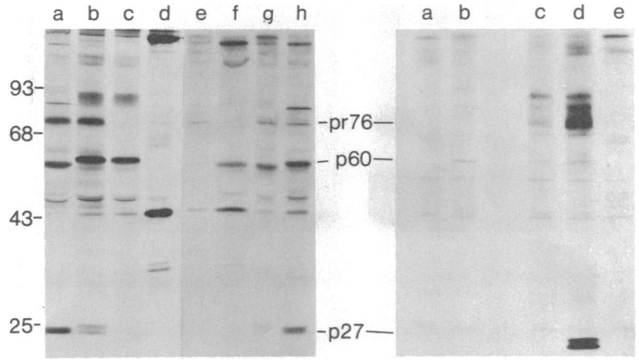

FIG. 1. Autoradiogram of immunoprecipitates of [^{35}S]methionine-labeled transformed (*Left*) and nontransformed (*Right*) cells after electrophoresis in a 10% polyacrylamide gel. (*Left*) SR-A-infected cells precipitated with TBR serum (a, h). rASV 157-infected cells precipitated with TBR (b), preabsorbed TBR (c), and nonimmune (d) serum. rASV 1441 infected cells precipitated with nonimmune (e), preabsorbed TBR (f), and TBR (g) serum. (*Right*) Uninfected cells precipitated with nonimmune (a), and TBR (b) serum. td 108-infected cells, precipitated with preabsorbed TBR (c), TBR (d), and nonimmune (e) serum. Some virus-specific proteins are noted in the center. Marker proteins are indicated in kDal in the left margin: phosphorylase a, 93 kDal; bovine serum albumin, 68 kDal; ovalbumin, 43 kDal; chymotrypsinogen A, 25 kDal.

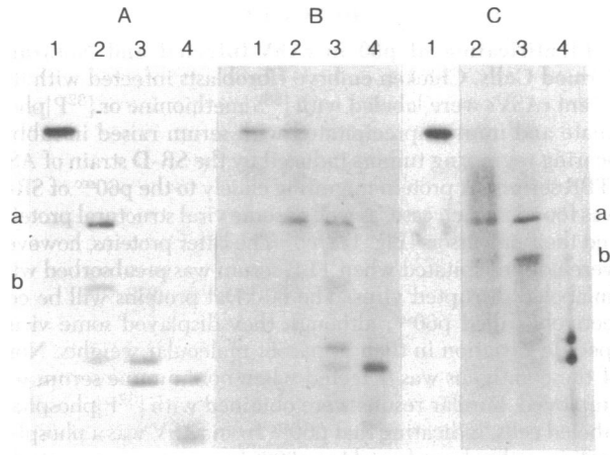

FIG. 2. *S. aureus* V8 protease partial digest patterns of [^{32}P]-phosphate-labeled p60^{src} of rASV 1441 (*A*), SR-A (*B*), and p60^{sarc} from normal cells (*C*). Lanes 1–4 in each case represent increasing concentrations of protease, incubated for 15 min at 30°C. Lane 1, no protease; 2, 0.2 μg/ml; 3, 4 μg/ml; 4, 80 μg/ml. The bands marked a and b in the margins indicate the probable primary cleavage products of p60 (13).

were analyzed by polyacrylamide gel electrophoresis (Fig. 3). An activity phosphorylating the 52-kDal heavy chain of IgG was easily detected in immunoprecipitates of SR-A, rASV 1441, and rASV 157 (Fig. 3*A*, lanes b, c, d), while nonimmune serum precipitated no such activity (lane a). The prominent minor band at approximately 60 kDal we believe to be p60^{src} phosphorylated by the same kinase activity responsible for the IgG heavy chain phosphorylation (unpublished data).

Immunoprecipitates of nontransformed cells were subjected to the protein kinase assay. A weak activity phosphorylating IgG heavy chain in TBR serum precipitates was detected (Fig. 3*B*). This activity was not found in immunoprecipitates with non-

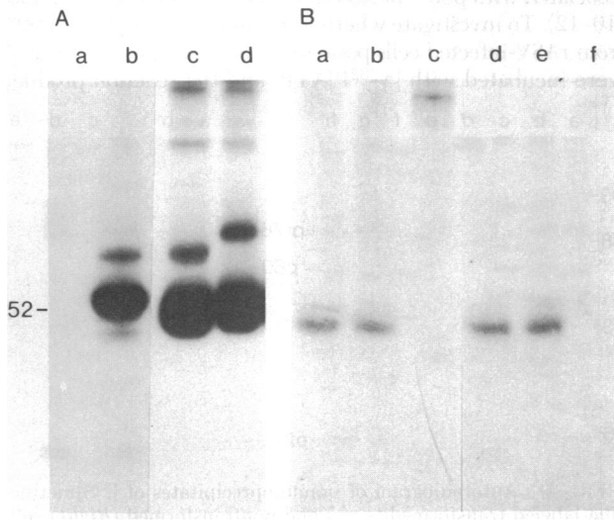

FIG. 3. Reaction products of protein kinase assay performed on immunoprecipitates of transformed (*A*) and nontransformed (*B*) cells, displayed on an 8.5% polyacrylamide gel. (*A*) Kinase activity immunoprecipitated from: lane a, SR-A-infected cells with nonimmune serum; lane b, SR-A-infected cells with TBR serum; lane c, rASV 1441-infected cells with TBR serum; lane d, rASV 157-infected cells with TBR serum. (*B*) Kinase activity immunoprecipitated from uninfected cells with: lane a, TBR serum; lane b, preabsorbed TBR serum; lane c, nonimmune serum. Kinase activity immunoprecipitated from td 108-infected cells with: lane d, TBR serum; lane e, preabsorbed TBR serum; lane f, nonimmune serum.

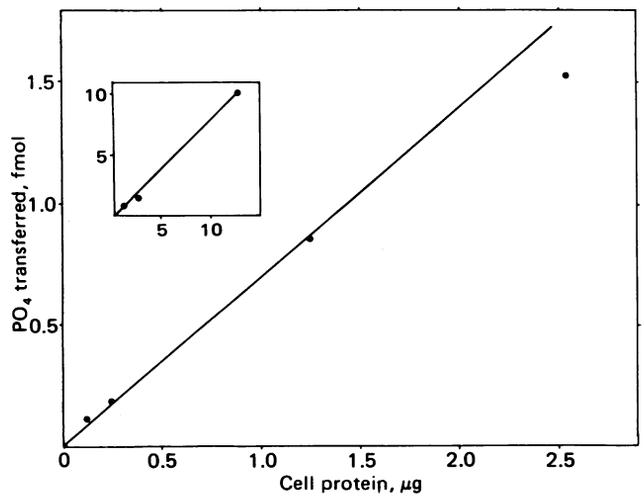

FIG. 4. Dependence of kinase activity on the amount of cell extract used in immunoprecipitation with TBR serum. Equal amounts of serum (2 μl) were added to dilutions of SR-A cell extract and brought up to 100 μl with buffer. Immunoprecipitation and kinase assay were otherwise as described in *Materials and Methods*. Reaction products were analyzed on a polyacrylamide gel. The heavy chain of Ig was cut out and its radioactivity was measured in Beckman NA scintillation cocktail. Background counts (110 cpm) from the Ig band of a nonimmune serum precipitate of 5 μg of extract protein were subtracted from each point. (*Inset*) Compressed scale plot of assay values for the three highest amounts of cell extract protein tested.

immune serum (lanes c, f), nor was it found when TBR serum alone was incubated with [γ-^{32}P]ATP. In addition, certain TBR sera were incapable of recognizing the p60^{sarc} in [^{35}S]methionine labeled normal cells, and these sera were unable to precipitate a detectable IgG phosphorylating activity from these cells (data not shown).

Amounts of p60^{src} in Normal and Transformed Cells. We wished to roughly quantitate the phosphotransferase activity associated with p60 from these different sources. Kinase activity was defined as the total fmol of phosphate transferred to the heavy chain of Ig, the major accepting species, per mg of cell extract protein, under conditions of saturating TBR antiserum and excess carrier-free [γ-^{32}P]ATP. Fig. 4 shows that kinase activity increased linearly with the amount of extract of SR-A-infected cells used in the immunoprecipitation, with an average value of 750 fmol phosphate transferred per mg of cell extract protein. The activity was detectable when as little as 100 ng of extract was employed. TBR-specific kinase activity in extracts of recovered ASV 1441-infected cells averaged 1300 fmol/mg. Extracts of nontransformed cells, however, either uninfected or infected with td 108, contained 1/10,000th as much precipitable kinase activity (Table 1).

The concentration of [^{35}S]methionine-labeled p60^{src} protein within the cell extracts was determined as described (ref. 13, and *Materials and Methods*). The values obtained for p60^{src} were comparable for rASV 1441- and SR-A-transformed cells, whereas the amount of p60^{sarc} in nontransformed cell extracts was 1/100th–1/200th of this level (Table 1).

Ratios of kinase activity to ng of p60 protein define a "specific activity" for the enzyme. The specific activity associated with the p60^{sarc} of normal cells appeared to be in a class distinct from that of cells transformed by either SR-A or rASV 1441, yielding a value approximately 1/100th that of their transformed cell counterparts.

Src-Specific Messenger RNAs Correlate in Abundance with p60^{src} in Normal and Transformed Cells. ASV-transformed cells contain a 21S poly(A)-containing mRNA (24, 25)

Table 1. Quantitation of $p60^{src}$ protein and associated protein kinase activity in normal and transformed cells

Source	ng p60/ mg cell protein	fmol PO_4 transferred to Ig/mg cell protein	Specific activity, fmol PO_4/ ng p60
Uninfected cells	11	0.080	0.0072
Cells infected by			
td 108	12	0.085	0.0070
SR-A	1600	750	0.47
rASV 1441	2100	1300	0.62

believed to be the message for $p60^{src}$ (8, 26). A 26S species containing *sarc*-specific sequences, and presumed to be the messenger for $p60^{sarc}$, has been identified in the cytoplasm of normal cells (4, 5). A comparison of the *src*-specific nucleic acids in transformed and nontransformed cells is shown in Table 2. Two levels of *src*-specific RNA were found. The higher level was found in both SR-A and rASV. Normal and td-infected cells shared a considerably lower level of *src*-specific RNA. The differences in mRNA paralleled the concentration differences of $p60^{src}$ and $p60^{sarc}$; however, the magnitude of the difference was somewhat greater for the RNA. Genomic DNA copies of *src*-related sequences were only 2- to 3-fold higher in transformed cells, a level that did not approach the magnitude of the differences seen in their putative transcriptional and translational products.

DISCUSSION

We have previously shown that rASVs obtained most of their sarcomagenic information from *src*-related sequences in normal cells (16). We report here that rASVs can direct the synthesis of $p60^{src}$s that are antigenically, structurally, and enzymatically similar to the $p60^{src}$ of conventional ASV. In addition, we find that $p60^{sarc}$ of normal cells is specifically coprecipitated with an IgG-phosphorylating protein kinase activity. The demonstration of structural and enzymatic homology between $p60^{src}$ of rASV and $p60^{sarc}$ of normal cells raises the possibility that these proteins are functionally indistinguishable.

The RNA genomes of rASVs possess two or more *src*-specific RNase T1-resistant oligonucleotides not found in the SR-A genome (16). rASV 1441 has four unique oligonucleotides, yet comparision of V8 protease fragment patterns of $p60^{src}$ from rASV 1441 and from SR-A (Fig. 2) suggests retention of important features of the primary protein structure despite these differences in nucleotide sequence. Such homology is not surprising, however, because hybridization data obtained with *src*-specific cDNA probes indicate extensive homology of sequence between SR-A and rASV 1441 (15). We cannot say from the V8 protease maps whether recovered virus $p60^{src}$ is more closely related to the endogenous $p60^{sarc}$ than it is to $p60^{src}$ of SR-A. In preliminary studies of other rASV isolates we have recently identified $p60^{src}$ proteins that vary more dramatically in their apparent molecular weight.

Table 2. Comparison of transcriptional and translational products of *src*-specific genetic information in normal and transformed cells

Cell type	Copies per cell: DNA	Copies per cell: RNA	p60 molecules per cell*
Uninfected	2	5†	200
Infected by			
td 108	2	5†	200
SR-A	5	3000‡	40,000
rASV 165	ND	2000‡	ND
rASV 1441	4	ND	60,000

ND, not determined.

* Calculated by assuming M_r 60,000 and 10^7 cells per mg of cell protein.

† 26S *src*-specific RNA in gs^- chf^- cells (lacking viral group-specific antigens and chicken helper factor).

‡ 21S *src*-specific RNA.

Determinations of the absolute amounts of p60 in transformed and nontransformed cells are only approximate, for reasons mentioned elsewhere (13), and also because "fully transformed" cultures may have a substantial subpopulation of td-infected cells that are refractory to infection by transforming virus. Thus only order of magnitude differences should be considered meaningful. In this regard, we feel it is significant that we find that the concentration of $p60^{sarc}$ in normal cells is 1/100th that of $p60^{src}$ in transformed cells, in general agreement with the report of Collett *et al.* (13).

Our definition of kinase activity avoids the difficulties involved in performing meaningful kinetic studies with an enzyme bound to an immune complex. The levels of $p60^{src}$-associated protein kinase activity in transformed cells (Table 1) are somewhat higher than the level reported by Collett *et al.* (10). They found 20–40 fmol of phosphate transferred per mg of cell protein, whereas our value for SR-A-infected cells was 750 fmol per mg. Our reaction conditions differ slightly, and this might explain the greater activity. Alternatively, the difference might be ascribed to the antisera employed. We have found, in agreement with others (11, 12) that TBR sera vary widely in their ability to crossreact with $p60^{src}$ of different ASV strains and in their ability to detect *src*-associated kinase activity.

We estimate that the assay can detect as little as 0.02 fmol of phosphate transferred to IgG. For SR-A-infected cell extract, this corresponds to 40 pg of $p60^{src}$ protein. It is likely that the greater sensitivity of our kinase assay enabled us to detect the normal cell $p60^{sarc}$-associated kinase activity. [Recently R. Erikson (personal communication) has been able to detect $p60^{sarc}$-associated kinase activity in extracts of nontransformed cells.]

The specific activity (as defined in *Results*) of normal cell $p60^{sarc}$ kinase was considerably lower than that determined for $p60^{src}$ of SR-A and rASV 1441. Again, we do not consider our values more than order of magnitude estimates of the actual levels within these cells. Several considerations lessen the accuracy of these figures. (*i*) We do not know what factors regulate the *src*-specific kinase activity. Other protein kinases, for example, are highly regulated by degrees of phosphorylation (27). It is very possible that normal cell p60 kinase activity varies with the metabolic requirements of the cell, whereas the $p60^{src}$ of transforming virus has escaped this regulation, either by altered structure, or by its 100-fold overabundance. (*ii*) Although our assay for kinase is linear with the amount of $p60^{src}$-containing extract, it is by no means clear what factors are limiting the reaction. Because the extent of the reaction is highly dependent upon both the specific antibody and the antigen used, we suspect the reaction is limited by the number of suitable acceptor sites proximal to the active site of the enzyme, within the immune complex. Other substrates besides Ig heavy chain are evident in Fig. 3, including $p60^{src}$ itself. What effect the additional phosphates have on the activity of *src*-associated kinase is unknown.

Comparison of p60 in normal and transformed cells manifests two differences: the reduced ratio of kinase per unit $p60^{sarc}$ in normal cells and the 100-fold greater abundance of the $p60^{src}$ molecule in transformed cells. The maintenance of the trans-

formed state could be due to either of these two differences.

Recovered ASVs have obtained at least 75% of their sarcomagenic information from cellular sequences (15, 16). We have shown the basic structural similarity of p60 products from normal and rASV-infected cells. It is possible that the pathogenic nature of the rASV $p60^{src}$ resides in the 25% of the genetic information originally derived from the parental td virus, and which ultimately traces back to the SR-A from which the td ASV was isolated (3). However, we do not know how much of these sequences actually survived the recombination event that generated the rASVs.

Alternatively, the mere overproduction of $p60^{src}$ could be responsible for cellular transformation. There is a clear difference in the concentration of *src* sequence transcripts in ASV-infected and nontransformed cells (4, 5, 24, 25) and a roughly equivalent difference in the amounts of p60 protein produced (Table 2). Viral messages in infected cells represent about 2% of the total poly(A)-containing RNA (24). At 2000–3000 copies per cell, the *src* message belongs to the class of abundant mRNA (28). The increase in the number of *src* sequence DNA copies cannot alone account for the higher levels of *src* message in infected cells. It could be that the insertion of normal *sarc* sequences into the actively transcribed ASV genome is the source of the increased expression of *src* message and p60 protein levels in ASV infected cells.

It would appear then, that the td SR-A used in the generation of rASV is acting in a fashion similar to the specialized transducing phage of bacteria. It seems to be a vector for a normal host gene, carrying it from one cell to another, and inserting it in a way permitting its expression. If this is true, it might be possible to transform a cell by transfection with normal cell *sarc* DNA sequences, at a low frequency, corresponding to the chance of such a DNA fragment being inserted into the cistron of an active promoter. In this regard, a recent finding of Andersson *et al.* (29) is of interest. They reported the transformation of mammalian cells by transfection with subgenomic viral DNA from Moloney murine sarcoma virus, presumably without the assistance of viral transcriptional signals.

Note Added in Proof. Recently Oppermann *et al.* (30) have reported a kinase activity associated with $p60^{sarc}$ of uninfected avian cells.

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