Molecular Events in Cells Transformed by Rous Sarcoma Virus

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ABSTRACT The Rous sarcoma virus (RSV) transforming gene product has been identified and characterized as a phosphoprotein with a molecular weight of 60,000, denoted pp60^{src}. Partially purified pp60^{src} displays a closely associated phosphotransferase activity with the unusual specificity of phosphorylating tyrosine residues in a variety of proteins. That the enzymatic activity observed is actually encoded by the RSV-transforming gene is indicated by the comparison of the pp60^{src}-protein kinase isolated from cells transformed by a wild-type RSV or by a RSV temperature-sensitive transformation mutant; these experiments revealed that the latter enzyme had a half-life of 3 min at 41°C, whereas that of the wild-type enzyme was 20 min. Evidence is now beginning to accumulate showing that viral pp60^{src}expresses its protein kinase activity in transformed cells as well as in vitro because at least one cellular protein has been identified as a substrate for this activity of pp60^{src}.

Although the protein kinase activity associated with pp60^{src} is itself cyclic AMP (cAMP) independent, the molecule contains at least one serine residue that is directly phosphorylated by the cellular cAMP-dependent protein kinase, thus suggesting that the viral transforming gene product may be regulated indirectly by the level of cAMP. The significance of this latter observation must be regarded from the point of view that the RSV *src* gene is apparently derived from a normal cellular gene that seemingly expresses in normal uninfected cells a phosphoprotein structurally and functionally closely related to pp60^{src}. This cellular protein, found in all vertebrate species tested, also is a substrate for a cAMP-dependent protein kinase of normal cells, and, therefore, may have evolved to function in a regulatory circuit involving cAMP.

Rous sarcoma virus (RSV) causes rapid and efficient transformation of cells in culture and tumor formation in several species. The malignant changes are the consequence of the expression of a single viral gene termed *src* for sarcoma (1, 2). The *src* gene is not required for virus replication; spontaneous deletions of the *src* gene can occur in the viral genome resulting in the generation of nonconditional transformation-defective viruses that replicate normally (3). Furthermore, a number of RSV temperature-sensitive (ts) mutants that have an altered capacity to transform cells have also been isolated (4–6). Avian or mammalian cells infected with a ts mutant can be reversibly switched from the normal to the transformed phenotype by the appropriate shift in the temperature at which the cells are maintained. These and other features of this tumor virus-host system make it useful for the study of the biochemistry of one form of malignant transformation.

Many of the phenotypic changes characteristic of cell transformation have been reviewed by Hanafusa (1). They include altered cellular morphology and the ability of transformed cells, but not normal cells, to grow in soft agar. At the morphological level, scanning electron microscopy reveals increased cell surface activity (7) and modification of the cytoskeletal components in transformed cells (8). Various other biochemical events also occur, such as increased hexose transport, increased secretion of plasminogen activator, and loss from transformed cells of the surface glycoprotein fibronectin (1, 9, 10).

To follow the sequence of events in RSV-infected cells that lead to cell transformation, identification of the product of the *src* gene was required. Hopefully, extensive characterization of this product and of its functions will lead to a detailed description of the molecular changes in RSV-transformed cells.

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Identification of the src Gene Product

The genetic evidence cited above had indicated by 1972 that the *src* gene product was likely to be a protein, however, it has only recently been identified. We used two approaches in our search for the *src* gene product. One utilized serum from mammals bearing virus-induced tumors for immunoprecipitation of radiolabeled proteins from transformed cells; a technique that had proved successful in the identification of nonstructural virus-encoded polypeptides of DNA tumor viruses (11, 12). The other was cell-free translation of the subgenomic 3′-third of the RSV genome, the region of the genome that had previously been shown to contain the *src* gene (13). These techniques led to the identification of a transformation-specific antigen in all RSV-transformed cells examined (14, 15) and to the identification of a polypeptide among the products of cell-free translation that was synthesized only when *src*-containing RNA was translated (16). The transformation-specific antigen found by immunoprecipitation of extracts of transformed cells and the *src*-specific cell-free translation product both had the same molecular weight, 60,000, and similar methionine-containing tryptic peptides (17). Similar results have been reported from other laboratories as well (18, 19). The transformation-specific polypeptide immunoprecipitated from transformed cells proved to be a phosphoprotein (15, 20) and was, thus, designated pp60^{src} to denote its size, secondary modification, and genetic origin. These results are summarized, in part, by the data shown in Fig. 1.

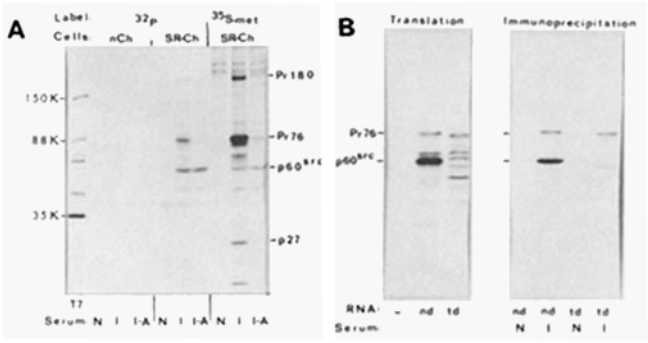

FIGURE 1 Identification of the RSV *src* gene product. (*A*) Normal chicken embryo fibroblasts (*nCh*) and chicken embryo fibroblasts infected and transformed by the Schmidt-Ruppin strain of RSV (*SR-Ch*) were labeled in culture with either [^{32}P]orthophosphate (*^{32}P*) or [^{35}S]methionine (*^{35}S-met*). Cell extracts were then prepared and aliquots were immunoprecipitated with normal rabbit serum (*N*), immune RSV tumor-bearing rabbit serum (*I*), and immune serum that had been preadsorbed with RSV structural proteins (*I-A*). The immunoprecipitated materials were then subjected to electrophoresis in SDS-polyacrylamide gels followed by fluorography. Pr180, Pr76, and p27 represent products of RSV structural genes. Phage T7 virion proteins are included as molecular weight references. p60^{src} represents the product of the RSV *src* gene and appears as a ^{32}P-labeled and [^{35}S]methionine-labeled nonstructural protein specifically precipitated from RSV-transformed cells by immune, but not normal, rabbit serum. (*B*) Polyacrylamide gel electrophoretic analysis of [^{35}S]methionine-labeled polypeptides synthesized in cell-free extracts programmed by virion RNA. Subgenomic (21S in size) poly(A)-containing virion RNA generated from nondefective (*nd*) or transformation-defective (*td*) RSV was translated in a messenger-dependent reticulocyte lysate system. Immunoprecipitation of the translation products was carried out with normal (*N*) or immune (*I*) rabbit serum. -, no RNA added to the system.

Characterization of the Phosphorylation Sites in pp60^{src}

When pp60^{src} was purified by immunoprecipitation from transformed cells that had been radiolabeled in culture with [^{32}P]orthophosphate and then partially digested with the proteolytic enzyme V8 from *Staphylococcus aureus*, two large fragments were generated, both of which contained phosphorous radiolabel. Experiments showed that these two fragments corresponded to the amino and carboxy terminal portions of the molecule, and, thus, we reported that pp60^{src} had two major sites of phosphorylation (21). Phosphoamino acid analysis of the amino-terminal fragment revealed that it contained a phosphoserine residue, as judged by electrophoresis at pH 1.9, whereas the same analysis applied to the carboxy-terminal fragment showed that the phosphorylated residue comigrated with phosphothreonine. More recently, it was demonstrated that the nonserine phosphoamino acid is tyrosine not threonine (22), and we have subsequently confirmed these results. Two-dimensional fractionation is required to separate phosphotyrosine from phosphothreonine because they comigrate at pH 1.9.

In our original studies (21), we demonstrated that the serine residue is phosphorylated by a cyclic AMP (cAMP)-stimulated protein kinase activity in cell-free extracts. This is of considerable interest because precedent established with other substrates of the cAMP-dependent protein kinase indicates that their function is influenced by their phosphorylation state (23), and the same may prove true for pp60^{src}. Furthermore, our recent unpublished results show that partially purified pp60^{src} is a direct substrate of the catalytic subunit of the cAMP-dependent protein kinase in vitro. Thus, an enzyme potentially able to regulate the activity of the transforming gene product of RSV is present in both normal and transformed cells.

In contrast to the serine residue, our cell-free phosphorylation studies of the tyrosine residue showed that it is phosphorylated by a cAMP-independent protein kinase activity (21). Furthermore, this same series of experiments revealed that the phosphotyrosine residue on the carboxy-terminal V8 fragment is not phosphorylated in pp60^{src} encoded by ts transformation mutants of RSV when ts mutant-transformed cells are grown at the nonpermissive temperature (normal morphology), whereas phosphorylation is similar to that of pp60^{src} encoded by wild-type virus when ts mutant-transformed cells are grown and radiolabeled at the permissive temperature (transformed morphology). These data are summarized in Fig. 2.

Our unpublished results indicate that the turnover of the phosphate on each residue is very rapid. In the case of the tyrosine site, the half-life of the phosphate is <1 h. Therefore, pp60^{src} may exist in several forms with regard to its state of phosphorylation. Thus far, we have not measured the quantity of each form because we have been unable to resolve discrete species of the molecule based on phosphate content.

A Function Associated with pp60^{src}

Early results from our laboratory indicated that most pp60^{src} is located in the cytoplasm of transformed cells (24) and is present at relatively low levels. These observations, together with the variety of alterations produced by the expression of pp60^{src}, suggested that it may have an enzymatic function resulting in the modification of several substrates. Because

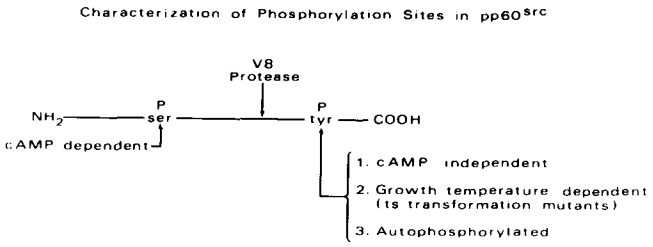

FIGURE 2 Diagrammatic representation of the phosphorylation sites in pp60^{src}.

phosphorylation-dephosphorylation reactions are apparently involved in the regulation of many cellular processes (25–27), we searched for a protein kinase activity associated with pp60^{src}. In our initial experiments (28), when we tested specific immunoprecipitates that contained pp60^{src} for protein kinase activity we found that no substrates added exogenously, such as histone or casein, were phosphorylated, whether or not pp60^{src} was present. However, immunoprecipitates that contained pp60^{src} did catalyze the transfer of radiolabel from [γ-^{32}P]ATP to the heavy chain of IgG. Immunoprecipitates that lacked pp60^{src} showed no activity in this assay. In addition, we found a similar activity associated with pp60^{src} generated by cell-free translation of subgenomic viral RNA that contained the *src* gene (29). Others have also reported similar results from their studies on pp60^{src} (20, 30). Thus, it appeared that the presence of pp60^{src} was an obligatory requirement for the IgG kinase activity. This unexpected observation has proven to be a useful assay for additional studies on pp60^{src}. This assay is diagrammed in Fig. 3. These data indicated that phosphotransferase activity was not inhibited in the immune complex and, in this regard, it is interesting that other results from this laboratory have shown that some antisera from RSV tumor-bearing mammals could immunoprecipitate viral DNA polymerase without inhibiting its enzymatic activity.

Fractionation by conventional biochemical procedures using ion-exchange chromatography together with the assay of fractions for both biosynthetically radiolabeled pp60^{src} and IgG kinase activity revealed that pp60^{src} and IgG kinase activity coeluted under all conditions examined to date (31, 32). Ion-exchange and immunoaffinity chromatography yielded preparations of pp60^{src} sufficiently pure to permit the characterization of the protein kinase activity during conventional soluble reactions. When substrates routinely used in such assays were surveyed, only casein showed detectable phosphate-acceptor activity. Moreover, as shown in Fig. 4, this casein kinase activity was inhibited by anti-pp60^{src} IgG, but not normal IgG, added before the reaction. Our interpretation of this result is that pp60^{src}, when sequested by antibody, is unable to interact with the casein substrate properly and no phosphorylation occurs. Although no information was presented regarding the quantity or presence of pp60^{src}, others using the criterion of IgG kinase activity arrived at a similar conclusion (33). It is also evident in Fig. 4 that the phosphotransferase reaction carried out on pp60^{src} with no additions resulted in an apparent "autophosphorylation." In this case, radiolabel is incorporated into the same tyrosine-containing phosphopeptide as is found after biosynthetic labeling (31). This result has been noted in Fig. 2.

Despite the accumulation of experimental data, biochemical evidence alone remained insufficient to establish the origin of

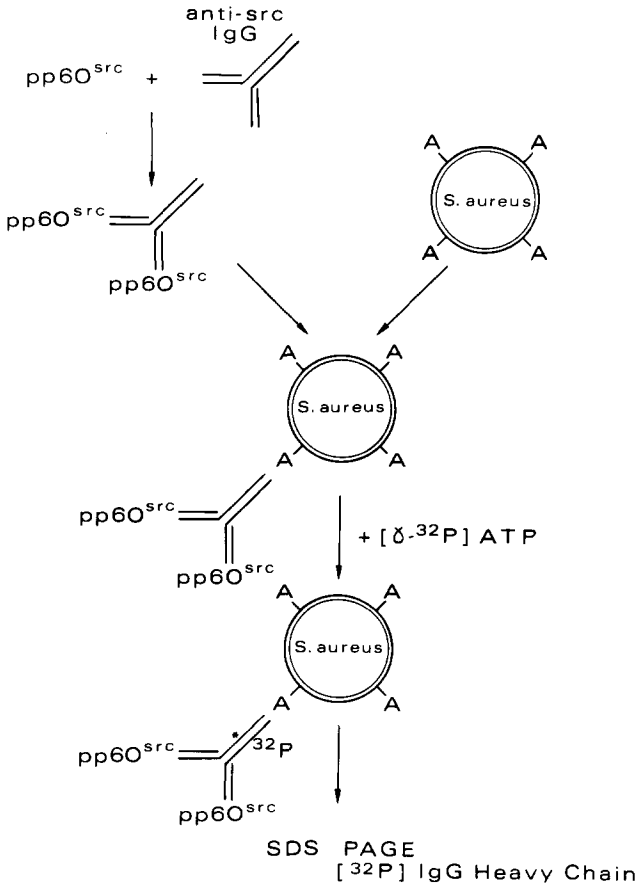

FIGURE 3 Illustration of the immune complex assay for protein kinase activity associated with pp60^{src}. Extracts of cells containing pp60^{src} are prepared and mixed with antiserum from tumor-bearing rabbits and the immune complexes are collected with protein A-bearing *S. aureus*. After extensive washing, the *S. aureus*-immune complex is suspended in 5 mM $MgCl_2$, 10 mM Tris pH 7.2, and 1 μM [γ-P^{32}]ATP. After incubation to allow phosphorylation to proceed, the IgG and all adsorbed proteins are eluted with SDS-containing buffer and analyzed by polyacrylamide gel electrophoresis (PAGE). (See reference 28 for details.)

the protein kinase because the possibility remained that another polypeptide undetectable by our techniques was the actual kinase acting in conjunction with pp60^{src}. Therefore, we also used mutants of RSV that were ts in their transforming function on the assumption that the function of the transforming protein encoded by these mutants should be more thermolabile than that of the wild-type parental virus. Protein kinase activity associated with pp60^{src} was partially purified from chicken cells transformed by NY68, a ts transformation mutant (5), and compared with that isolated from cells transformed by its wild-type parent. These assays showed that the protein kinase activity of the mutant in both the soluble reaction and in the immune complex IgG phosphorylation was at least sevenfold more thermolabile than that of wild-type virus, demonstrating more directly that the RSV *src* gene product is likely to have a protein kinase function (32). Using ts mutants but different experimental approaches, others have reached similar conclusions (20, 34). It must be emphasized, however, that this result does not eliminate the possibility that pp60^{src} may have other functions important for its role in transformation.

During their studies which led to the identification of phos-

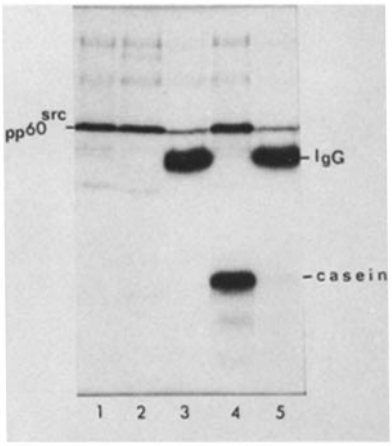

FIGURE 4 Phosphorylation of casein by partially purified pp60^{src}-kinase. Immunoaffinity column-purified pp60^{src} was incubated in standard protein kinase reaction mixtures with the indicated additions. After termination of the reaction, samples were subjected to SDS-polyacrylamide gel electrophoresis followed by autoradiography. Track *1*, partially purified pp60^{src}-kinase alone, *2* partially purified pp60^{src}-kinase with normal rabbit serum IgG, *3* with Immune IgG from tumor-bearing rabbits (TBR IgG), *4* with casein, and *5* with TBR IgG and casein.

photyrosine in pp60^{src}, Hunter and Sefton (22) also found that the phosphorylation of IgG mediated by pp60^{src} in the immune complex assay occurred at tyrosine. Work in this laboratory with other potential substrates of pp60^{src} showed that all substrates examined were phosphorylated at a tyrosine residue during conventional soluble protein kinase reactions (35). It appears, therefore, that the protein kinase activity associated with pp60^{src} has an unusual specificity because many other protein kinases have been reported to phosphorylate serine or threonine. Moreover, a tyrosine-specific phosphotransferase activity present in immunoprecipitates results in phosphorylation of the apparent transforming gene product of Abelson murine leukemia virus (36), hence, kinase activity for tyrosine residues in proteins may have general significance in RNA tumor virus-induced oncogenesis. Furthermore, direct analyses indicate that phosphotyrosine is rare in normal cells (T. Hunter, personal communication), suggesting that few other tyrosine-specific kinases are present.

Identification of a Substrate for pp60^{src}

The demonstration of a protein kinase activity tightly associated with pp60^{src} had obvious implications with regard to cellular transformation because protein phosphorylation/dephosphorylation reactions are involved in various cellular regulation mechanisms (23, 25–27). For example, as was originally suggested (28), one might assume that pp60^{src} activity causes a quantitative or qualitative change in the phosphorylation patterns in the infected cell resulting in transformation that could be readily reversible by phosphatases. This could account for the observations made with ts transformation mutants (37), which show that at least normal morphology can be restored to the transformed cell in the absence of protein synthesis.

Demonstration of protein kinase activity associated with pp60^{src} in vitro does not establish that pp60^{src} acts in the infected cell as a protein kinase or that transformation is mediated via phosphorylation. To establish a role for phosphorylation-dephosphorylation in RSV-induced transformation, a cellular protein substrate(s) had to be identified that was phosphorylated directly by pp60^{src} activity in the cell. In addition, some functional modification associated with this phosphorylation must be characterized and shown to lead to at least one phenotypic change in the cell. Because there are so many changes in response to the *src* gene product, more than one substrate might be anticipated.

A number of experiments performed by several different groups (37, 38) have led to the frequent suggestion that the cellular cytoskeleton is likely to include one target of the *src* gene product essential for the transformation process. With the availability of anti-pp60^{src} serum, as stated above, it quickly became clear that pp60^{src} was located primarily in the cytoplasm of transformed cells; however, the issue of a subcytoplasmic localization appears open to debate. It has been reported that the location of pp60^{src} is primarily perinuclear (39), whereas others have shown it to be highly concentrated immediately under the plasma membrane (40). Some results suggest that it is rather tightly complexed to the cytoskeleton after detergent extraction of whole cells (41), whereas on the other hand, it is also found in cellular membrane preparations (42). More recently, Rohrschneider (43) has reported that pp60^{src} is located in adhesion plaques of transformed cells, certainly a pivotal location when considered with regard to the morphological changes induced by RSV. There may be no inconsistency in these results because the presence of pp60^{src} at several sites may be essential for the full spectrum of changes observed in transformed cells. Whether or not pp60^{src} is ultimately assigned to a precise and correct subcellular location(s), investigators are not, unfortunately, relieved of finding and suitably characterizing a substrate(s) among the large number of cellular polypeptides that remain with the subcellular fraction under study. Nor does localization at a particular site imply that pp60^{src} located elsewhere is silent in the transformation process.

Although attention is now directed at the cytoplasm and particularly at cytoskeletal or membrane components as primary targets for pp60^{src} activity, the results which reveal that changes in certain mRNA levels also occur in RSV-transformed cells should be considered. Transcriptional activation of cellular genes in RSV-transformed cells has been found, as judged by the presence of new mRNA transcripts and increased sensitivity of certain genes to deoxyribonuclease I, an assay for transcriptionally active chromatin (44, 45). Other studies, showing greatly decreased levels of collagen mRNAs, also imply alterations in transcriptional controls although other explanations are available (46). These changes may result from pp60^{src} action on primary targets in the cytoplasm that only indirectly alter transcription but, nevertheless, may have important consequences with regard to sarcoma formation.

Some progress has been made in the identification of substrates phosphorylated by pp60^{src} without regard to subcellular location. Total phosphoproteins from normal and transformed cells have been solubilized and analyzed by the techniques described by O'Farrell et al. (47). This procedure involves fractionation of proteins based on isoelectric point and molecular weight, permitting the identification of a newly phosphorylated protein by a shift in its isoelectric point. The transformation-specific phosphorylation of a protein has been detected by application of this procedure (31, 48). This protein has a molecular weight of ~34,000 and a pI of ~7.5. Radke and Martin (48) reported that this protein was phosphorylated

within 20 min after shifting ts transformation mutant-infected cells from the nonpermissive to permissive temperature. Thus, the phosphorylation of this protein is one of the earliest biochemical markers of transformation. We have recently purified the unphosphorylated form of this protein from normal cells and have shown that partially purified pp60^{src} will phosphorylate it in vitro (Fig. 5). Comparative phosphopeptide fingerprints reveal that the site phosphorylated in vitro occurs at a tyrosine residue in a tryptic peptide similar or identical to the major phosphopeptide found in the 34,000-dalton phosphoprotein isolated from transformed cells (49). Taken together, these results suggest that this normal cell protein is phosphorylated directly as a consequence of pp60^{src} activity. These data do not show, however, that this particular phosphorylation is crucial for transformation. Extension of these studies will require functional characterization of the phosphorylated and unphosphorylated forms of this protein. Nevertheless, these data do show more directly that the protein kinase activity associated with pp60^{src} in vitro is likely to be a biologically significant function of the molecule in the transformed cell.

Normal Cellular Homologue of the RSV src Protein

It is relevant to this discussion that both normal avian and mammalian cells contain a phosphoprotein antigenically, structurally, and perhaps functionally related to pp60^{src}. Molecular hybridization experiments with radioactive DNA specific for the RSV *src* gene have shown that normal uninfected vertebrate cells contain highly conserved nucleotide sequences, denoted *sarc*, that are related to the viral transforming gene and that are present in both DNA and RNA (50–53). Furthermore, *sarc*-containing RNA has been found to be associated with polyribosomes, suggesting that this RNA may be translated into a protein. The close relationship in nucleotide sequence between normal cell *sarc* and viral *src* implies that the putative *sarc* protein may be similar in structure and function to the viral pp60^{src}. We, therefore, attempted to identify a *src*-related protein in normal, uninfected cells. We (54, 55) and others (56–58) have found a 60,000-dalton phosphoprotein in normal avian and mammalian cells that is antigenically related to the viral *src* protein (Fig. 6). This protein was identified by immunoprecipitation of radiolabeled cell extracts with certain cross-reacting sera derived from RSV tumor-bearing mammals. In addition to being antigenically related, all three classes of proteins (viral, avian cell, and mammalian cell) appear to be structurally very similar but not identical.

Comparative studies of the sites of phosphorylation on these polypeptides have shown that the normal cell *src*-related protein also contains both phosphoserine and phosphotyrosine, with the former being located on the V8 amino-terminal peptide and the latter on the V8 carboxy-terminal fragment (59). Furthermore, the serine residue of the normal cell *src*-related protein is also a direct substrate of purified cAMP-dependent protein kinase and is located in a tryptic peptide similar, if not identical, to that from viral pp60^{src} (35, 59). Thus, the function of this protein, like that of viral pp60^{src}, is likely to be regulated indirectly by the level of cAMP in cells.

Because the normal cell 60,000-dalton protein is antigenically and structurally so similar to the viral *src* protein, it appears to fit the description expected of the protein product of the cellular *sarc* sequences, and we have, therefore, designated it pp60^{sarc}. The close relationship among the normal endogenous *sarc* polypeptides and the RSV *src* gene product suggests that these apparently highly conserved proteins may have a similar function. Along these lines, we (59) and others (56) have identified a protein kinase activity associated with pp60^{sarc} in a manner similar to that described for viral pp60^{src} (28). The similarity of the results obtained with both viral pp60^{src} and normal cell pp60^{sarc} in the immune complex protein kinase assay suggests that the two proteins may have similar functions resulting in the phosphorylation of tyrosine residues in polypeptides.

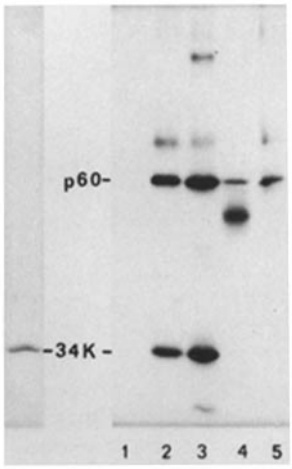

FIGURE 5 In vitro phosphorylation of the 34,000-dalton normal cell protein by pp60^{src}. A preparation of the 34,000-dalton protein purified from normal chicken embryo fibroblasts was incubated in the protein kinase reaction mixture and the products of the reaction were analyzed by polyacrylamide gel electrophoresis and autoradiography. Left panel, a Coomassie Blue-stained SDS-polyacrylamide gel after electrophoresis of the partially purified preparation. Autoradiogram of SDS-polyacrylamide gel analysis of protein kinase reaction products: track *1* 34,000-dalton protein preparation alone, *2* 34,000-dalton protein preparation with partially purified pp60^{src}, *3* with pp60^{src} and normal rabbit IgG, *4* with pp60^{src} and immune IgG from tumor-bearing rabbits, and *5* pp60^{src} preparation alone.

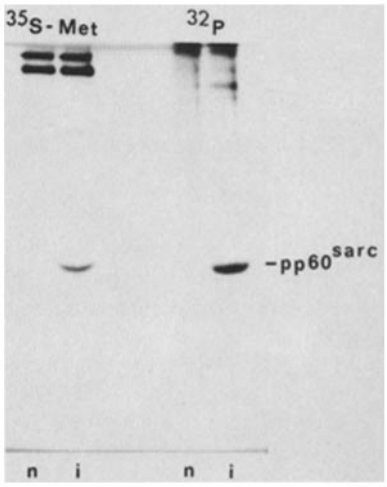

FIGURE 6 Identification of the normal cell homologue of pp60^{src}. Normal chicken embryo fibroblasts were labeled in culture with either [^{35}S]methionine (^{35}S-*met*) or [^{32}P]orthophosphate (^{32}*P*). Cell extracts were prepared and immunoprecipitated with normal rabbit serum (*n*) or cross-reacting tumor-bearing rabbit serum (*i*). The immunoprecipitated materials were analyzed by SDS-polyacrylamide gel electrophoresis and fluorography.

Supporting this possibility are the results obtained by Hanafusa and co-workers (60) involving the recovery of transforming viruses from tumors arising in chickens infected with transformation-defective *src*-gene deletion mutants of RSV. It appears that these recovered transforming viruses are generated by recombination between viral and cellular (presumably *sarc*) RNA or DNA sequences to create a functional *src* gene (61). These recovered viruses have obtained at least 75% of their transforming gene from cellular sequences and they produce a protein closely related to pp60^{src} during transformation of cells in culture. Moreover, the transforming gene product identified shows an association with a protein kinase activity, as judged by the immune complex assay (62).

Several explanations may be considered as to why pp60^{sarc} expression is compatible with normal cellular proliferation. First, as mentioned above, it is possible that the *src* and *src*-related polypeptides have functions other than protein phosphorylation, whereby the roles of viral and cellular pp60s differ. Alternatively, both the viral *src* protein and its normal cell homologue may function as protein kinases but they may have unique substrate specificities. Therefore, different cellular targets of protein phosphorylation may account for the lack of phenotypic transformation by pp60^{sarc}. It is relevant to note here that our unpublished experiments show that the level of cellular pp60^{sarc} is unchanged by RSV infection, although it has been shown that the viral gene product is present in substantially greater amounts (~50-fold) in transformed cells than is the normal cell protein in uninfected cells (54, 62). Thus, cellular transformation may merely be a consequence of a quantitative difference in expression of the two genes. In that case, biochemical events in RSV-induced oncogenesis may be qualitatively identical to those in normal cells, but perhaps occur to a greater degree, as the result of pp60^{src} expression, to produce the transformed phenotype. Finally, an intermediate possibility may exist such that the lower quantity of cellular pp60^{sarc} is present only at a precise subcellular location restricting its activity to a limited number of substrates, whereas the greater quantity of viral pp60^{src} with precisely the same specificity and function is distributed more widely throughout the cell, resulting in the phosphorylation of a wider variety of substrates. There is insufficient information at this time to argue strongly in support of any of the above possible explanations. Additional studies must be pursued to obtain further insights into the functional roles of pp60^{src} and pp60^{sarc} in neoplastic transformation and in normal cellular metabolism.

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