MOLECULAR AND CELLULAR BIOLOGY, July 1984, p. 1420–1424
0270-7306/84/071420-05$02.00/0

Vol. 4, No. 7

Expression of the Rous Sarcoma Virus *src* Gene in Avian Macrophages Fails to Elicit Transformed Cell Phenotype

LEAH LIPSICH,[1] JOAN S. BRUGGE,[1]* AND DAVID BOETTIGER[2]

Department of Microbiology, School of Medicine, State University of New York at Stony Brook, Stony Brook, New York 11794,[1] and Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104[2]

Received 21 December 1983/Accepted 20 April 1984

Infection of avian macrophages with Rous sarcoma virus does not induce any changes in the morphology, growth behavior, or expression of macrophage-specific proteins. The absence of cellular transformation does not result from a block in the synthesis of viral proteins, since infectious viruses are released from a majority of cells in the culture. In this report, we examine the synthesis, processing, and functional activity of pp60^{src} in Rous sarcoma virus-infected macrophages to determine whether the absence of transformation is due to an alteration in the functional expression of pp60^{src}. Although the absolute level of pp60^{src} was reduced compared with fibroblasts, the protein exhibited the same phosphorylation pattern and subcellular distribution and was able to phosphorylate immunoglobulin in the immune complex-protein kinase assay. These results imply that the failure of Rous sarcoma virus to transform macrophage may be due to a restriction in the cellular response to a functional *src* protein, perhaps due to the absence of cellular products which are essential for mediating pp60^{src}-induced transformation.

In general, inoculation of Rous sarcoma virus (RSV) into avian species results in the production of tumors with a narrow pathogenic spectrum. Regardless of the site of injection, RSV usually induces sarcomas. The histology of these sarcomas is dependent on the organization of mesodermal cells in the tumor (2). Attempts to induce other types of tumors by intravenous or intracerebral injection of chickens produced only tumors of connective tissue near the inoculation site. In contrast to this rather restricted host-cell range observed in vivo, infection of cells in culture has revealed a wider range of cell types which are susceptible to transformation, including neural retinal cells (32), retinal melanoblasts (8), iris epithelial cells (21), and skin epidermal cells (39). Given this wide range of cell types capable of responding to the transforming function of RSV in vitro, it is not clear whether the tumor specificity of RSV is due to a restricted distribution of the virus in vivo or other factors which might restrict the expression of RSV in specific cells in the chicken.

Recently, it was demonstrated that macrophages could be infected by RSV, and the majority of the infected macrophages produced infectious RSV; however, no alterations in growth capacity, morphology, or expression of differentiated cell parameters were detected (20). This suggested that either the macrophages failed to express the transforming function of RSV properly or these cells failed to respond to this RSV function.

Transformation by RSV is mediated by the protein product of a single RSV-encoded gene product denoted pp60^{src} (9, 12, 33; reviewed in reference 4). This protein functions as a tyrosine-specific protein phosphotransferase, and it is believed that RSV-induced transformation involves the phosphorylation of cellular proteins on tyrosine (15, 26, 28, 35). The precise cellular substrates of pp60^{src}-mediated phosphorylation which are responsible for eliciting cellular transformation are not known; however, a large number of candidate substrates have been identified (reviewed in M. Weber, Adv. Viral Oncol., in press).

The identification of the transforming protein of RSV and the assignment of a functional activity associated with this protein has made it possible to assay several aspects of the functional expression of pp60^{src} in RSV-infected macrophages. These studies could provide insight into the basis for the restricted expression of pp60^{src} in these cells and provide information concerning the requirements for the functional expression of pp60^{src} or the cellular substrates which mediate the transformed phenotype. In this report, we have examined the synthesis and phosphorylation of pp60^{src}, the binding of pp60^{src} to two cellular proteins believed to be involved in the processing of the *src* protein, and the phosphotransferase activity of this protein extracted from RSV-infected macrophages.

For the experiments described in this report, primary yolk sac cells from 12-day-old chicken embryos were infected with RSV 2 to 4 h after preparation. In a previous report on infection of macrophage cells with RSV, primary yolk sac cells were allowed to differentiate in vitro to produce a culture of mature macrophages (20). These cultures were first examined for the expression of macrophage markers and then infected with RSV. Since this procedure was quite tedious and since all of the cells, except infrequent fibroblast-like contaminating cells, expressed macrophage markers, we infected the primary yolk sac cells shortly after removal from the embryo. The adherent macrophage population which was produced was passaged twice to ensure complete infection by the virus. Before use, the cultures were examined, and those containing fibroblast contaminants were discarded. As previously observed (20), infection with either the Schmidt-Ruppin A (SRA) or Schmidt-Ruppin D strains of RSV did not produce any alteration in the morphology or growth behavior of the cells.

As a further test of the ability of RSV to transform yolk sac cells, the freshly prepared yolk sac cells were infected with RSV or avian myeloblastosis virus (AMV) and assayed for transformation in agar suspension. Under these conditions, the normal macrophages produce colonies of dispersed cells, whereas the transformed macrophages produce compact colonies (19, 20). The RSV-infected yolk sac cells

* Corresponding author.

TABLE 1. Colony Assay for RSV-infected yolk sac cells[a]

Treatment	No. of colonies per 10^6 cells	
	Normal	Transformed
Control YSC	838	0
RSV-infected YSC	738	0
AMV-infected YSC	NT[b]	97

[a] Yolk sac cells (YSC) were infected with 0.1 focus-forming unit per cell of RSV LA24A, 0.1 CFU per cell of AMV, or mock infected; 24 h later, cells were assayed for agar colony formation in the presence of chicken serum as a colony-stimulating factor (CSF) source as described previously (7). No colonies were detected in the absence of a CSF. Transformed colonies were recognized by compact morphology.

[b] NT, Not tested.

produced only normal macrophage-like colonies which retained their dependence on the colony-stimulating activity present in chicken serum, whereas cells infected with AMV produced compact colonies with a transformed morphology (Table 1).

To determine whether the RSV-infected macrophages synthesized pp60^{src}, cell lysates were prepared from infected macrophages and infected fibroblasts labeled with ^{32}P. Figure 1 shows the immunoprecipitation of these cell lysates with normal and tumor-bearing rabbit (TBR) serum. The TBR serum precipitated pp60^{src} from both the RSV-infected fibroblasts and the RSV-infected macrophages (lanes 2 and 4). The other prominent phosphate-labeled protein precipitated by the TBR serum is Pr76, the initial translation product of the *gag* structural protein gene. In addition, minor proteins of 90,000 and 50,000 daltons (pp90 and pp50) were precipitated by the TBR serum from both cell types. These proteins have been previously shown to be cellular proteins which associate with newly synthesized pp60^{src} in a short-lived protein complex (10, 11, 30). These results indicate that pp60^{src} was synthesized in RSV-infected macrophages and was phosphorylated and associated with pp50 and pp90.

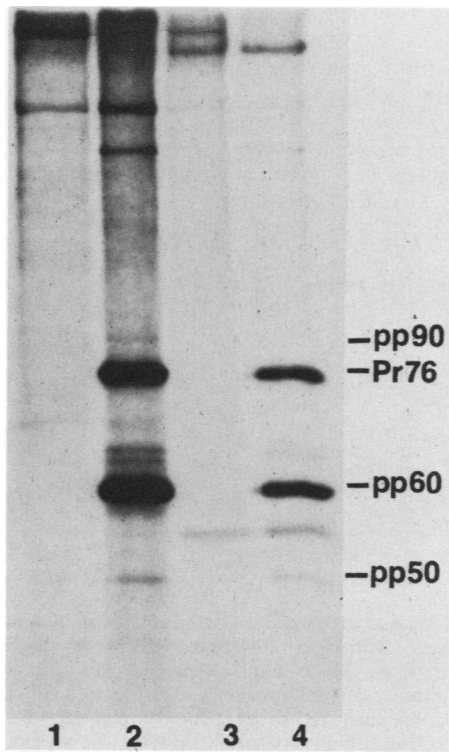

FIG. 1. Proteins immunoprecipitated from $^{32}P_i$-labeled RSV-infected fibroblasts and macrophages. Cells from a 60-mm petri dish were labeled for 4 h with $^{32}P_i$ (1 mCi/ml; ICN Pharmaceutical, Irvine, Calif.), and the lysates were prepared and immunoprecipitated as described previously (12). The types of cells and sera used are as follows: SRA-RSV-infected chicken embryo fibroblast cells (lanes 1 and 2), SRA-RSV-infected macrophages (lanes 3 and 4), normal rabbit serum (lanes 1 and 3), and TBR serum (lanes 2 and 4) (exposure, 2 days). Electrophoresis was performed on a 7.5% sodium dodecyl sulfate-polyacrylamide gel.

In fibroblasts, pp60^{src} is phosphorylated on both serine and tyrosine; it can be cleaved with V8 protease to produce a 34,000-dalton fragment containing the phosphoserine site and a 26,000-dalton fragment containing the phosphotyrosine site (14, 31, 37). To determine whether the pp60^{src} produced in macrophages and fibroblasts had similar phosphorylation patterns, ^{32}P-labeled pp60^{src} derived from each cell type was subjected to partial proteolytic cleavage with V8 protease by the method of Cleveland et al. (13). The ratio of phosphate incorporation into the two protease-generated fragments was similar for pp60^{src} derived from each cell type (Fig. 2). This suggests that the phosphorylation pattern of pp60^{src} is similar in both cell types; however, phospho amino acid analysis is essential to demonstrate the exact amino acid linkage.

It has been previously demonstrated that pp60^{src} is capable of transferring the γ-phosphate of ATP to the heavy chain of the immunoglobulin present in the immune complex formed with TBR (15, 28). Hunter and Sefton (26)

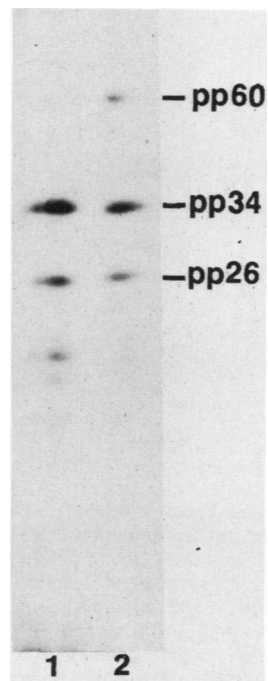

FIG. 2. Partial proteolytic digestion of pp60^{src} immunoprecipitated from RSV-infected fibroblasts and macrophages. $^{32}P_i$-labeled pp60^{src} which had been excised from the gel shown in Fig. 1 was reelectrophoresed in the presence of 20 ng of staphylococcus V8 protease (Miles Laboratories, Inc., Elkhart, Ind.) as described by Cleveland and co-workers (13). Lane 1, pp60^{src} from SRA-RSV-infected chicken cells; lane 2, pp60^{src} from SRA-RSV-infected macrophages (exposure, 14 days).

further demonstrated that this phosphate was specifically transferred to a tyrosine residue. This immune complex-kinase assay was utilized to determine whether pp60^{src} present in macrophages was active as a protein kinase. Proteins in both uninfected and RSV-infected fibroblasts and macrophages were labeled with [^{35}S]methionine, and lysates were prepared. Equal trichloroacetic acid-precipitable [^{35}S]methionine counts from each cell type were immunoprecipitated with either normal rabbit serum or TBR serum. After immunoprecipitation, the samples were divided in half (except the uninfected macrophages in which there were insufficient counts); one-half was electrophoresed in a sodium dodecyl sulfate-polyacrylamide gel (Fig. 3A), and the other half was assayed in the immune complex-kinase assay (Fig. 3B). Densitometric tracing of the bands in Fig. 3A showed that the levels of Pr76 in the infected fibroblasts (lane 4) and infected macrophages (lane 8) were similar, whereas the levels of Pr180 and pp60^{src} were reduced ca. 7- to 10-fold in macrophages in comparison with fibroblasts. The radiolabeled immunoglobulin heavy-chain bands were excised from the gel (Fig. 3B), and the amount of phosphorylation was determined by scintillation spectroscopy. There was ca. nine-fold greater ^{32}P incorporation in the immunoglobulin molecules phosphorylated by pp60^{src} from infected fibroblasts (lane 6) than infected macrophages (lane 3). No kinase activity was detected in the uninfected cells since this TBR serum does not recognize the normal cell pp60$^{c\text{-}src}$ protein and, therefore, does not allow phosphorylation of immunoglobulin G by pp60$^{c\text{-}src}$. Since there was a similar decrease in the level of [^{35}S]methionine-labeled pp60^{src} and in kinase activity in the macrophages relative to fibroblasts, it appears that the difference was due to reduced pp60^{src} synthesis in the infected macrophages rather than to a change in the activity of the kinase. The decrease is unlikely to be caused by incomplete infection of the macrophage population since the levels of Pr76 remained similar and does not appear to be caused by an increase in the production of nontransforming virus, since Pr180 would be produced equally by both transforming and nontransforming viruses.

In transformed fibroblasts, pp60^{src} is located primarily in the particulate fraction isolated by homogenization in low-ionic-strength buffers (18, 27). A portion of pp60^{src} in the soluble fraction is bound to the cellular pp50 and pp90 proteins (11, 17). To determine whether the localization of pp60^{src} in infected macrophages differed from infected fibroblasts, $^{32}P_i$-labeled macrophages were separated into particulate and soluble fractions by a modification of the method of Krueger and co-workers (27) as described previously (11). Figure 4 represents the immunoprecipitation of the total cell lysate, the soluble fraction, and the particulate fraction with normal rabbit serum (lanes 1, 4, 7), TBR serum (lanes 2, 5, 8), and a monoclonal antibody to pp90 (lanes 3, 6, 9). The majority of the pp60^{src} was found in the particulate fraction (lane 8). The cellular proteins pp90 and pp50 were coprecipi-

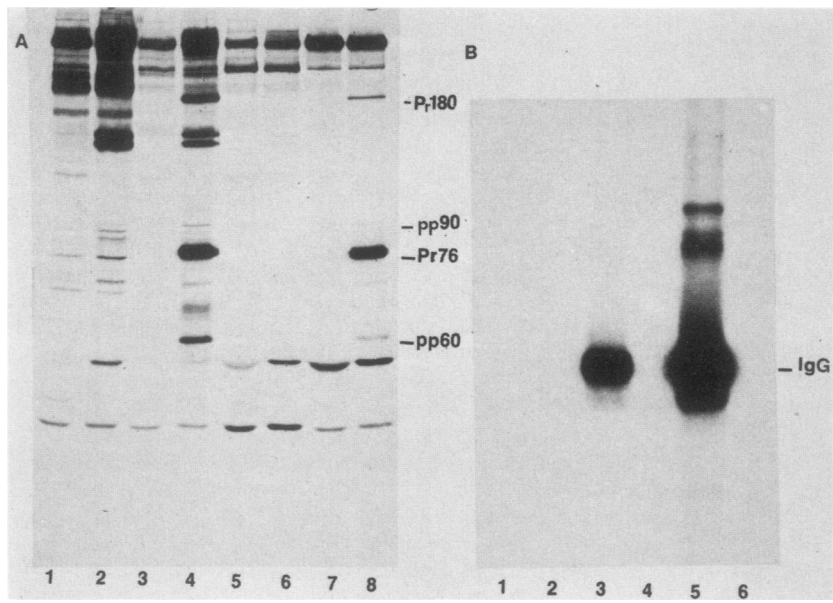

FIG. 3. Synthesis and protein kinase activity of pp60^{src} in RSV-infected fibroblasts and macrophages. (A) Proteins immunoprecipitated from [^{35}S]methionine-labeled normal or RSV-infected fibroblasts and macrophages. Normal or RSV-infected chicken fibroblasts and macrophages were labeled for 4 h with [^{35}S]methionine (50 μCi/ml, 100 Ci/mmol, Amersham Corp., Arlington Heights, Ill.) in methionine-free medium. Lysates were prepared as described previously (6), and an equivalent number of trichloroacetic acid-precipitable counts were incubated with antibody; the immunoprecipitated proteins from each cell lysate were analyzed as described previously (6). The figure represents an autoradiogram of fluorographed 7.5% sodium dodecyl sulfate-polyacrylamide gel (exposure, 21 days). The types of cells and sera used are as follows: normal chicken cells (lanes 1 and 2); SRA-RSV-infected chicken cells (lanes 3 and 4); normal macrophages (lanes 5 and 6); SRA-RSV-infected macrophages (lanes 7 and 8); normal serum (lanes 1, 3, 5, and 7); and TBR serum (lanes 2, 4, 6, and 8). The TBR serum used in this analysis does not recognize pp60$^{c\text{-}src}$. The cells used in this experiment were assayed for the production of virus by an infectious center assay; 90 to 95% of the cells were found to produce focus-inducing virus. (B) Phosphorylation of TBR immunoglobulin G (IgG) by pp60^{src} bound in an immune complex. Immune complexes derived from panel A were suspended in 20 mM Tris-hydrochloride (pH 7.2)–5 mM $MgCl_2$–10 μCi of [γ-^{32}P]ATP and subjected to the immune complex-protein kinase assay as described previously (13). The phosphorylated proteins were analyzed on a 7.5% sodium dodecyl sulfate-polyacrylamide gel. A single piece of X-ray film was used to block exposure of this autoradiogram to the radioactivity from [^{35}S]methionine (exposure, 7 h). The types of cells and sera used are as follows: normal chicken cells (lanes 1 and 2); SRA-RSV-infected macrophages (lanes 3 and 4); SRA-RSV-infected chicken cells (lanes 5 and 6); normal serum (lanes 1, 3, and 5); and TBR serum (lanes 2, 4, and 6).

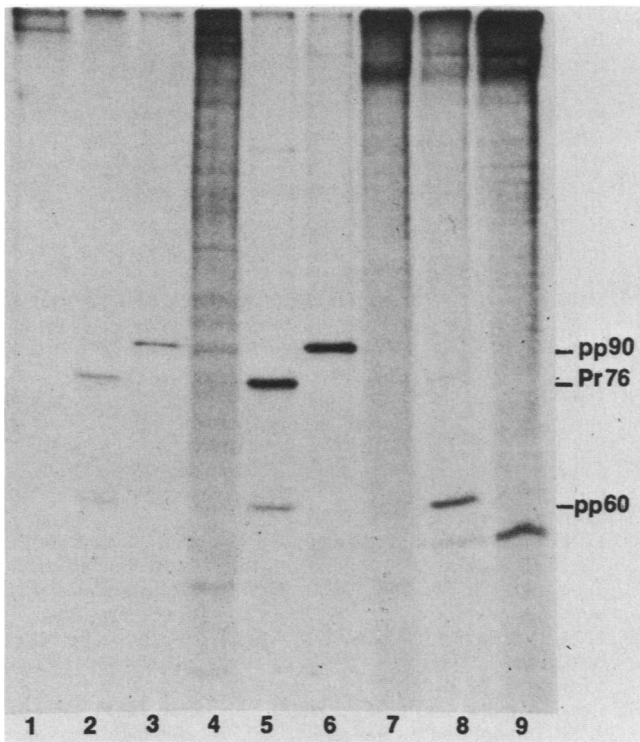

FIG. 4. Fractionation of SRA-RSV-infected macrophages into soluble and particulate fractions. SRA-RSV-infected macrophages were fractionated as described previously (27). One-tenth of the lysate was removed before homogenization and diluted with an equal volume of RIPA buffer (12). This represented the "total" fraction. Each fraction was divided into three parts and immunoprecipitated with normal serum (lanes 1, 4, and 7), TBR serum (lanes 2, 5, and 8), or anti-pp90 antibody (lanes 3, 6, and 9). Lanes 1, 2, and 3, Total cell lysate; lanes 4, 5, and 6, soluble fraction; lanes 7, 8, and 9, particulate fraction.

tated with $pp60^{src}$ in the soluble fraction. This pattern is very similar to that obtained from the fractionation of fibroblasts. In this autoradiogram, $pp60^{src}$ was not detectable in the anti-pp90 immunoprecipitates.

The results presented here have confirmed and extended previous studies on the inability of RSV to transform macrophages. Analysis of the RSV-infected, nontransformed macrophage population has revealed that *src* is expressed in these cells at appreciable levels and that it shows a similar subcellular distribution, phosphorylation pattern, and protein kinase activity as RSV-infected fibroblasts. Thus, by those criteria, we have been unable to detect any qualitative differences at the biochemical level in the action of $pp60^{src}$ in fibroblasts and macrophages. The only difference appears to be a reduction in the total level of $pp60^{src}$ produced. The concomitant reduction in Pr180 suggests that the mechanism of this reduction could lie in the splicing of the viral RNA in the macrophages, since these are both products of spliced mRNAs, whereas Pr76 is not (16, 38).

The failure of the macrophages to transform in response to the expression of the viral *src* gene may be explained either by the reduced level of $pp60^{src}$ in the macrophages as compared with transformed fibroblasts or in the inability of the macrophages to respond to the oncogenic activity of *src*. In either case, the implication of these results is that the failure of RSV to transform certain cell types cannot be explained on the basis of restrictions to virus entry and expression of viral products. It seems unlikely that the reduced synthesis of $pp60^{src}$ in macrophages is responsible for the absence of transformation, since the levels of $pp60^{v\text{-}src}$-specific kinase activity in infected macrophages were still high relative to the levels of normal cellular *src* kinase activity, and we have observed similar levels of $pp60^{src}$ in several lines of RSV-transformed mammalian cells (Brugge, unpublished data). However, it is possible that the relative amount of $pp60^{src}$ necessary to transform fibroblasts may be significantly lower than that necessary to transform macrophages. Thus, it appears more likely that macrophage cells are refractory to the transforming activity of $pp60^{src}$, possibly due to the absence of some developmentally regulated molecule which performs an essential function in the pathway leading to $pp60^{src}$-mediated cell transformation. The macrophage cell type could represent the equivalent of a mutant cell unable to respond to *src* activity. We have examined the phosphorylation of two proteins which are candidate targets of $pp60^{src}$-mediated phosphorylation. The pp50 protein found associated with newly synthesized $pp60^{src}$ is phosphorylated on tyrosine in RSV-infected macrophage, and the major 34- to 36-kilodalton phosphotyrosine-containing species found in RSV-infected fibroblasts was also detectable on two-dimensional gels of ^{32}P-labeled cell extracts from RSV-infected macrophage (data not shown). We are currently examining other phosphotyrosine-containing species in infected macrophage cells.

We have also infected murine bone marrow cells with a recombinant murine retrovirus which contains the *src* gene from RSV (1). In the 8 months during which these cells were cultured, there was no evidence of transformation of the murine macrophages by this virus (5). These results suggest that this restriction to transformation by the *src* gene is a common feature of macrophage cells.

There is precedent for the lineage-restricted action of oncogenes in the acute leukemia viruses, but this is the only described example for the sarcoma viruses. AMV is the most restricted, since it only transforms the morphologically identifiable members of the monocyte-macrophage lineage (19, 29). Avian erythroblastosis virus transforms specific stages of erythroid cell differentiation (22) but is also able to transform fibroblasts (3, 24, 34). It is of interest that Graf and co-workers (23) have previously shown that avian erythroblastosis virus, like RSV, is capable of replicating in macrophage cells in the absence of transformation. Both avian reticuloendotheliosis virus and murine Abelson virus transform primarily B cells (22, 36), although they are both also able to transform fibroblasts. Whether this lineage restriction will hold for all oncogenes awaits more extensive investigation.

This work was supported by grants CA30383 and CA2814604 from the National Cancer Institute. L.L. is supported by a postdoctoral fellowship from the Muscular Dystrophy Association.

We gratefully acknowledge the excellent secretarial assistance of Sandi Burns.

LITERATURE CITED

1. **Anderson, S. M., and E. M. Scolnick.** 1983. Construction and isolation of a transforming murine retrovirus containing the *src* gene of Rous sarcoma virus. J. Virol. **46**:594–605.
2. **Beard, J. W.** 1980. Biology of avian oncornaviruses, p. 55–87. *In* G. Klein (ed.), Viral oncology. Raven Press, New York.
3. **Beug, H., A. von Kirchbach, G. Doderlein, J.-F. Conscience, and T. Graf.** 1979. Chicken hematopoietic cells transformed by seven strains of defective avian leukemia viruses display three distinct phenotypes of differentiation. Cell **18**:375–390.

4. **Bishop, J. M., and H. Varmus.** 1982. Functions and origins of retroviral transforming genes, p. 999–1108. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Boettiger, D., S. Anderson, and T. M. Dexter.** 1984. Effect of src infection on long-term marrow cultures: increased self-renewal of hemopoietic progenitor cells without leukemia. Cell **36**:763–773.
6. **Boettiger, D., and E. Durban.** 1984. Target cells for avian myeloblastosis virus in embryonic yolk sac and relationship of cell differentiation to cell transformation. J. Virol. **49**:841–847.
7. **Boettiger, D., and E. M. Durban.** 1980. Progenitor-cell populations can be infected by RNA tumor viruses, but transformation is dependent on the expression of specific differentiated functions. Cold Spring Harbor Symp. Quant. Biol. **44**:1249–1254.
8. **Boettiger, D., K. Roby, J. Brumbaugh, J. Biehl, and H. Holtzer.** 1977. Transformation of chicken embryo retinal melanoblasts by a temperature-sensitive mutant of Rous sarcoma virus. Cell **11**:881–890.
9. **Brugge, J., E. Erikson, M. S. Collett, and R. L. Erikson.** 1978. Peptide analysis of the transformation-specific antigen from avian sarcoma virus-transformed cells. J. Virol. **26**:773–782.
10. **Brugge, J., E. Erikson, and R. L. Erikson.** 1981. The specific interaction of the Rous sarcoma virus transforming protein, $pp60^{src}$, with two cellular proteins. Cell **25**:363–372.
11. **Brugge, J., W. Yonemoto, and D. Darrow.** 1983. Interaction between the Rous sarcoma virus transforming protein and two cellular phosphoproteins: analysis of the turnover and distribution of this complex. Mol. Cell. Biol. **3**:9–19.
12. **Brugge, J. S., and R. L. Erikson.** 1977. Identification of a transformation-specific antigen induced by an avian sarcoma virus. Nature (London) **269**:346–348.
13. **Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli.** 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252**:1102–1106.
14. **Collett, M. S., E. Erikson, and R. L. Erikson.** 1979. Structural analysis of the avian sarcoma virus transforming protein: sites of phosphorylation. J. Virol. **29**:770–781.
15. **Collett, M. S., and R. L. Erikson.** 1978. Protein kinase activity associated with the avian sarcoma virus *src* gene product. Proc. Natl. Acad. Sci. U.S.A. **75**:2021–2024.
16. **Cordell, B., S. R. Weiss, H. E. Varmus, and J. M. Bishop.** 1978. At least 104 nucleotides are transposed from the 5′ terminus of the avian sarcoma virus genome to the 5′ termini of smaller mRNAs cell. Cell **15**:79–91.
17. **Courtneidge, S. A., and J. M. Bishop.** 1982. Transit of $pp60^{v\text{-}src}$ to the plasma membrane. Proc. Natl. Acad. Sci. U.S.A. **79**: 7117–7121.
18. **Courtneidge, S. A., A. D. Levinson, and J. M. Bishop.** 1980. The protein encoded by the transforming gene of avian sarcoma virus ($pp60^{src}$) and a homologous protein in normal cells ($pp60^{proto\text{-}src}$) are associated with the plasma membrane. Proc. Natl. Acad. Sci. U.S.A. **77**:3783–3787.
19. **Durban, E. M., and D. Boettiger.** 1981. Replicating, differentiated macrophages can serve as in vitro targets for transformation by avian myeloblastosis virus. J. Virol. **37**:488–492.
20. **Durban, E. M., and D. Boettiger.** 1981. Differential effects of transforming avian RNA tumor viruses on avian macrophages. Proc. Natl. Acad. Sci. U.S.A. **78**:3600–3604.
21. **Ephrussi, B., and H. M. Temin.** 1960. Infection of chick iris epithelium with Rous sarcoma virus *in vitro*. Virology **11**:547–552.
22. **Franklin, R. B., R. L. Maldonado, and H. R. Bose.** 1974. Isolation and characterization of reticuloendotheliosis virus transformed bone marrow cells. Intervirology **3**:342–352.
23. **Graf, T., H. Beug, and M. J. Hayman.** 1980. Target cell specificity of defective avian leukemia viruses: hematopoietic target cells for a given virus type can be infected but not transformed by strains of a different type. Proc. Natl. Acad. Sci. U.S.A. **77**:389–393.
24. **Graf, T., B. Royer-Pokora, G. E. Schubert, and H. Beug.** 1976. Evidence for the multiple oncogenic potential of cloned leukemia virus: *in vitro* and *in vivo* studies with avian erythroblastosis virus. Virology **71**:423–433.
25. **Hackett, P. B., R. Swanstrom, H. E. Varmus, and J. M. Bishop.** 1982. The leader sequence of the subgenomic mRNA's of Rous sarcoma virus is approximately 390 nucleotides. J. Virol. **41**:527–534.
26. **Hunter, T., and B. Sefton.** 1980. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. Proc. Natl. Acad. Sci. U.S.A. **77**:1311–1315.
27. **Krueger, J. G., E. Wang, and A. R. Goldberg.** 1980. Evidence that the *src* gene product of Rous sarcoma virus is membrane associated. Virology **101**:24–40.
28. **Levinson, A. D., H. Oppermann, L. Levintow, H. E. Varmus, and J. B. Bishop.** 1978. Evidence that the transforming gene of avian sarcoma virus encodes a protein kinase associated with phosphoprotein. Cell **15**:561–572.
29. **Moscovici, C., and M. Zanetti.** 1970. Studies on single foci of hematopoietic cells transformed by avian myeloblastosis virus. Virology **42**:61–67.
30. **Oppermann, H., A. D. Levinson, L. Levintow, H. E. Varmus, J. M. Bishop, and S. Kawai.** 1981. Two cellular proteins that immunoprecipitate with the transforming protein of Rous sarcoma virus. Virology **113**:736–751.
31. **Patschinsky, T., T. Hunter, F. S. Esch, J. A. Cooper, and B. M. Sefton.** 1982. Analysis of the sequence amino acids surrounding sites of tyrosine phosphorylation. Proc. Natl. Acad. Sci. U.S.A. **79**:973–977.
32. **Pessac, B., and G. Calothy.** 1974. Transformation of chick embyro neuroretinal cells by Rous sarcoma virus *in vitro*: induction of cell proliferation. Science **185**:709–710.
33. **Purchio, A. F., E. Erikson, J. S. Brugge, and R. L. Erikson.** 1978. Identification of a polypeptide encoded by the avian sarcoma virus *src* gene. Proc. Natl. Acad. Sci. U.S.A. **75**:1567–1571.
34. **Samarut, J., and L. Gazzolo.** 1982. Target cells infected by erythroblastosis virus differentiate and become transformed. Cell **28**:921–929.
35. **Sefton, B. M., T. Hunter, K. Beemon, and W. Eckhart.** 1980. Evidence that the phosphorylation of tyrosine is essential for cellular transformation by Rous sarcoma virus. Cell **20**:807–816.
36. **Siden, E. J., D. Baltimore, D. Clark, and N. Rosenberg.** 1979. Immunoglobulin synthesis by lymphoid cells transformed *in vitro* by Abelson murine leukemia virus. Cell **16**:389–396.
37. **Smart, J. E., H. Oppermann, A. P. Czernilofsky, A. F. Purchio, R. L. Erikson, and J. M. Bishop.** 1981. Characterization of sites for tyrosine phosphorylation in the transforming protein of Rous sarcoma virus ($pp60^{v\text{-}src}$) and its normal cellular homologue ($pp60^{c\text{-}src}$). Proc. Natl. Acad. Sci. U.S.A. **78**:6013–6017.
38. **Swartz, D., R. Tizard, and W. Gilbert.** 1983. Nucleotide sequence of Rous sarcoma virus. Cell **32**:853–869.
39. **Yoshimura, M., Y. Iwasaki, and A. Kaji.** 1981. *In vitro* differentiation of chicken embryo skin cells transformed by Rous sarcoma virus. J. Cell. Physiol. **109**:373–385.