Proc. Natl. Acad. Sci. USA
Vol. 78, No. 3, pp. 1906–1910, March 1981
Medical Sciences

Evidence for three classes of avian sarcoma viruses: Comparison of the transformation-specific proteins of PRCII, Y73, and Fujinami viruses

(retrovirus/tryptic peptide maps/protein kinase/phosphopeptides)

JAMES C. NEIL, JOHN F. DELAMARTER, AND PETER K. VOGT

Department of Microbiology, University of Southern California, School of Medicine, 2011 Zonal Avenue, Los Angeles, California 90033

Contributed by Peter K. Vogt, November 17, 1980

ABSTRACT **The *gag*-linked transformation-specific proteins (polyproteins) of PRCII, Fujinami, and Y73 avian sarcoma viruses have been compared by tryptic peptide mapping. In addition to shared *gag* peptides, PRCII polyprotein p105 and FSV polyprotein p140 were found to have seven methionine-containing and five cysteine-containing tryptic peptides in common. These represent the majority of the non-*gag* peptides for each virus. In contrast, no overlap was detected with the non-*gag* peptides of the Y73 polyprotein p90. Examination of the tryptic phosphopeptides of p105, p140, and p90 labeled by their associated protein kinases gave similar results. Although the major phosphopeptides of p105 and p140 comigrated, they were distinct from the phosphopeptide of p90. Three classes of transformation-specific proteins can now be identified among known avian sarcoma viruses. After the pp60^{src} of Rous sarcoma virus and B77 virus, the proteins of PRCII and Fujinami virus form a second class and Y73—currently the only representative—characterizes the third. Despite their structural differences, these viruses may share a common mechanism of transformation, effected by their associated protein kinases.**

Three recently characterized avian sarcoma viruses, Y73, PRCII, and Fujinami sarcoma virus (FSV), share fundamental features (1–5). All three isolates were found to be mixtures of replication-competent helper viruses and replication-defective transforming viruses. Nonproducer transformed cells generated by infection with the transforming virus alone synthesize none of the characteristic helper virus gene products: Pr76^{gag}, gPr95^{env}, and Pr180$^{gag-pol}$. Instead, these cells were shown to contain polyproteins composed of helper virus *gag* protein sequences fused to nonstructural, transformation-specific sequences: in FSV-transformed cells a M_r 140,000 transformation-specific protein, p140 is synthesized; in PRCII cells it is p105; and in Y73 it is p90 (1–4). These nonproducer cells do not contain the *src* gene product pp60^{src} of Rous sarcoma virus (RSV).

From these results and from studies on the virion RNA of the defective sarcoma viruses (1–3) emerges a model for genome structure similar to that of the avian acute leukemia viruses MC29 and AEV. The acute leukemia viruses have extensive deletions and cell-derived, transformation-specific inserted sequences leaving residual *gag* and *env* gene sequences flanking the cellular inserts (6–10). In contrast to the acute leukemia viruses, and like RSV, however, the new sarcoma viruses have tyrosine-specific protein kinases associated with their transformation-specific proteins (3, 11–16). This enzymatic activity is labile in RSV mutants that are temperature sensitive for the maintenance of transformation, suggesting that it is essential for oncogenesis (13, 14, 17).

We have previously analyzed the PRCII transformation-specific polyprotein p105 and compared it to the RSV *src* gene product pp60^{src} (4). No overlap in methionine-containing tryptic peptides was detected. We now report on a comparison of p105 with the polyproteins of the recently characterized avian sarcoma viruses FSV and Y73. Our results show extensive overlap between the non-*gag* portions of the PRCII and FSV polyproteins but no relatedness with the Y73 polyprotein.

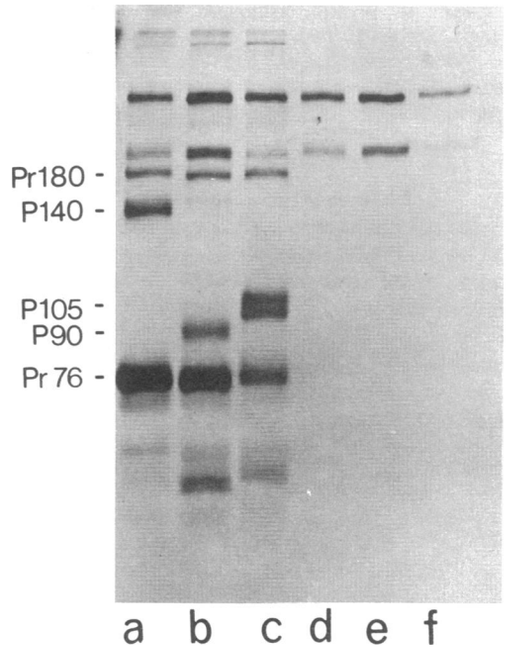

FIG. 1. Comparison of sarcoma virus *gag*-related polyproteins by $NaDodSO_4$/polyacrylamide gel electrophoresis. [^{35}S]Cysteine-labeled proteins were separated on a 10% acrylamide gel with an acrylamide/bisacrylamide ratio of 30:0.39. The proteins were precipitated from detergent extracts of cells transformed by PRCII (lanes a and d), Y73 (lanes b and e), and FSV (lanes c and f) with antiserum to *gag* protein p19 (lanes a, b, and c) (the gift of D. P. Bolognesi, Duke University, NC) or normal rabbit serum (lanes d, e, and f).

MATERIALS AND METHODS

Cells and Viruses. The propagation of PRCII in chicken embryo fibroblasts has been described (5). FSV was kindly provided by H. Hanafusa (Rockefeller University, New York); Y73

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: RSV, Rous sarcoma virus; FSV, Fujinami sarcoma virus; PRCII, PRCII avian sarcoma virus; Y73, Y73 avian sarcoma virus; *gag*, *pol*, *env*, and *src*, retrovirus genes coding for internal virion proteins, reverse transcriptase, virion surface glycoproteins, and transforming protein pp60^{src}, respectively.

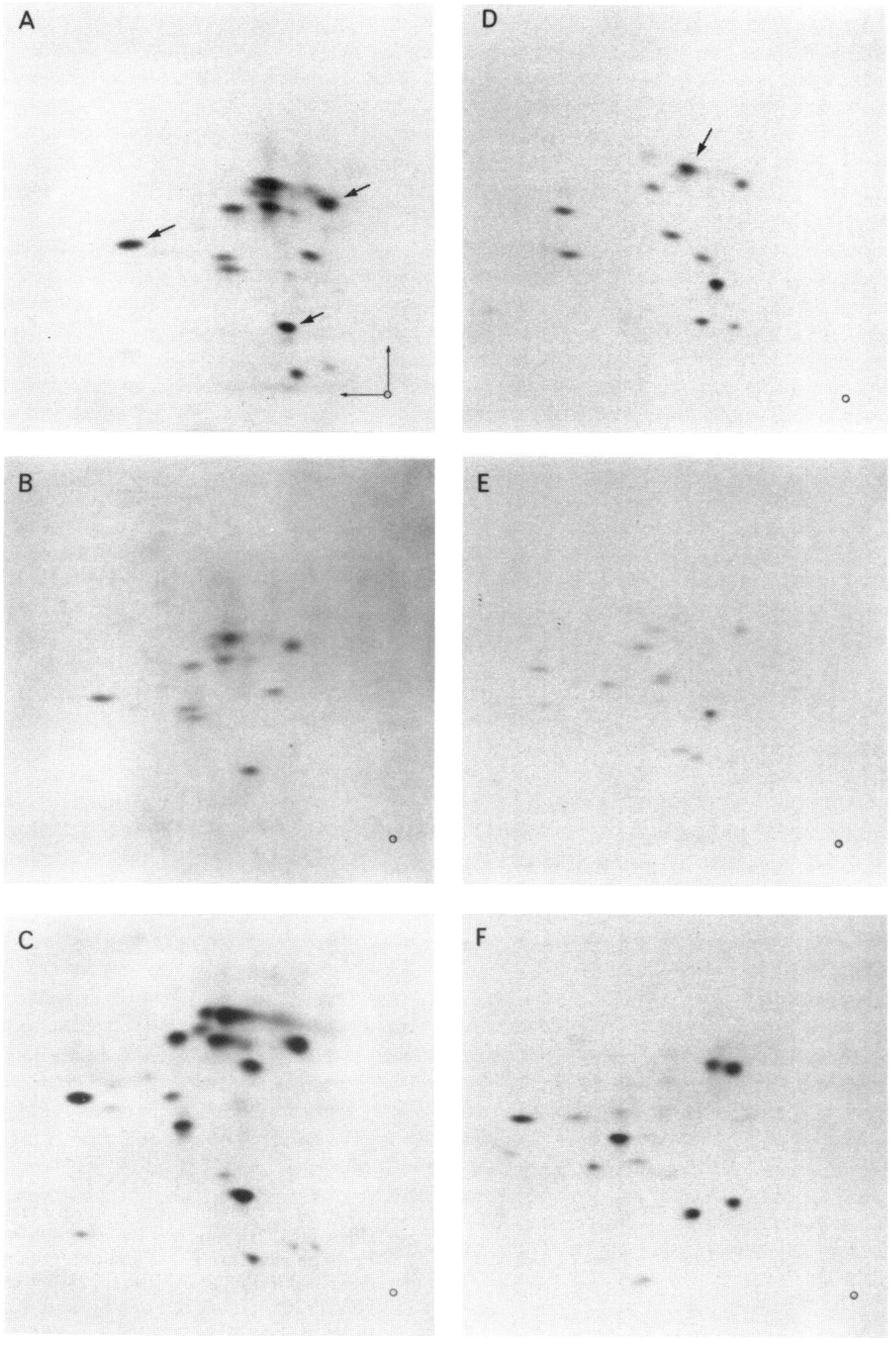

FIG. 2. Two-dimensional tryptic peptide maps of [^{35}S]methionine-labeled proteins. Proteins were separated on Na-DodSO$_4$/polyacrylamide gels and the bands were located by autoradiography and excised. The proteins were eluted, precipitated with carrier protein, oxidized with performic acid, and digested with TPCK-trypsin. The resultant peptides were separated by thin-layer electrophoresis at pH 4.5 (600 V, 100 min) in the first dimension and chromatography in *n*-butanol/pyridine/acetic acid/water, 75:60:15:60 (vol/vol) in the second dimension. The peptides were visualized by fluorography (20) on Kodak XR film. (*A*) PRCII Pr76^{gag}; (*B*) FSV Pr76^{gag}; (*C*) Y73 Pr76^{gag}; (*D*) PRCII p105; (*E*) FSV p140; and (*F*) Y73 p90. Peptides indicated by arrows in *A* are contained in the helper *gag* protein p19 and the peptide in *D* is in p27.

virus was the gift of K. Toyoshima (University of Tokyo, Japan). For all three viruses, stocks with high titers of transforming virus were generated by several rounds of picking transformed cell foci and seeding them onto uninfected cells.

Labeling and Immunoprecipitation. Labeling of cells with [^{35}S]methionine, extraction with buffers containing detergents (Triton X-100, sodium deoxycholate, and NaDodSO$_4$), and immunoprecipitation with fixed *Staphylococcus aureus* cells (18) were performed as described (4). [^{35}S]Cysteine labeling was performed similarly but in Ham's F-10 medium deficient in cysteine. All radioisotopes were purchased from Amersham. Labeling of proteins in immune complexes with [γ-^{32}P]ATP was essentially as described by Collett and Erikson (13) but with the cell lysis conditions described by Sefton and coworkers (17). We have recently described this procedure for PRCII (12). Labeled proteins were separated on NaDodSO$_4$/polyacrylamide gels with the buffer system described by Laemmli (19).

Tryptic Peptide Mapping. We have described our procedure for peptide mapping in detail (4). For phosphopeptide analysis, however, the first-dimension separation was at pH 8.9 in 1% ammonium carbonate as described by Hunter and Sefton (15). Chromatography was in *n*-butanol/pyridine/water/acetic acid, 75:60:60:15 (vol/vol).

RESULTS

For the peptide mapping studies described here, the viral polyproteins were first purified by immunoprecipitation and NaDodSO$_4$/polyacrylamide gel electrophoresis. The relative mobilities of the proteins on such gels is illustrated in Fig. 1, where [^{35}S]cysteine-labeled immunoprecipitates of cells infected with PRCII, FSV, and Y73 are compared on the same gel.

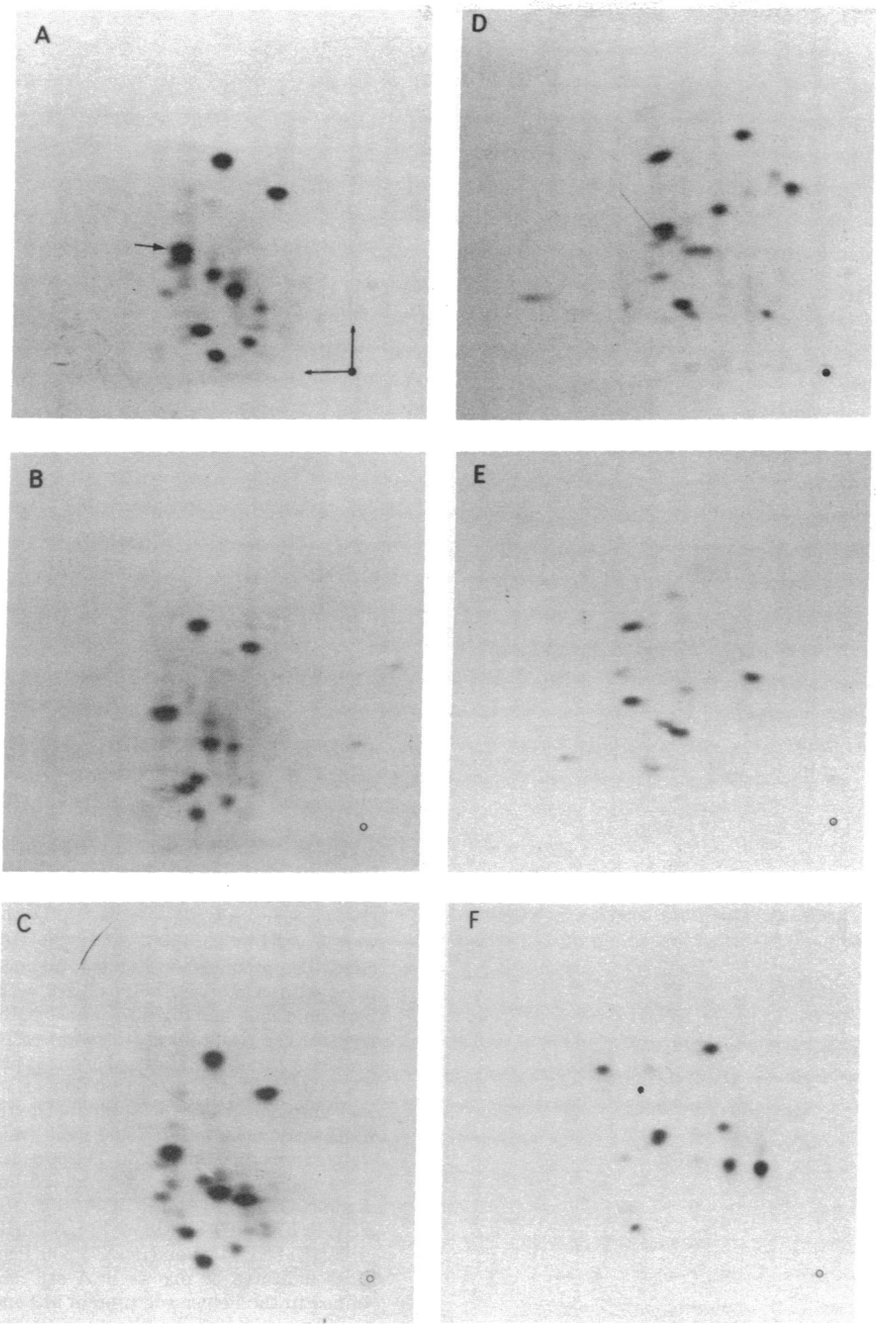

FIG. 3. Two-dimensional tryptic peptides of [^{35}S]cysteine-labeled proteins. Proteins were processed and analyzed as described in Fig. 2. (*A*) PRCII Pr76^{gag}; (*B*) FSV Pr76^{gag}; (*C*) Y73 Pr76^{gag}; (*D*) PRCII p105; (*E*) FSV p140; and (*F*) Y73 p90.

To identify the non-*gag* peptides of the transformation-specific proteins, it was necessary to compare the Pr76^{gag} proteins of the respective helper viruses first, in case the *gag*-derived peptides of the polyproteins differed significantly. The Pr76^{gag} maps were similar (Fig. 2 *A*, *B*, and *C*). Comparison of the Pr76^{gag} maps of the *gag*-linked polyproteins allowed identification of peptides contributed by the residual *gag* protein sequences of the defective virus proteins. In each viral polyprotein the p19 *gag* peptides were present, as reported (4) for PRCII (Fig. 2*A*, arrows). Similarly, the cysteine-containing *gag* peptides of the polyproteins were identified. The major p19 cysteine peptide is indicated by an arrow in Fig. 3*A*. Unlike Y73 p90 and FSV p140, p105 of PRCII contained the strongly labeled p27 peptide indicated by an arrow in Fig. 2*D*. However, p27 is relatively deficient in methionine and cysteine, and it may be premature to conclude that PRCII p105 contains more residual *gag* sequences than does p90 or p140.

The data in Figs. 2 and 3 and of a number of additional mixing experiments are summarized in Fig. 4. Significant overlap in the non-*gag* peptides of PRCII p105 and FSV p140 is illustrated by the shared methionine and cysteine peptides. Three heavily labeled and four relatively weakly labeled non-*gag* methionine-containing peptides and two heavily labeled and three weakly labeled cysteine-containing peptides comigrated. However, FSV p140 and PRCII p105 are not identical, and the peptide pattern of p105 is not a subset of that of p140. Several peptides unique to each protein were detected. In contrast to the overlap of PRCII and FSV polyproteins, Y73 p90 showed no relatedness to either protein, as demonstrated by both methionine- and cysteine-containing tryptic peptides. We also compared the methionine maps of pp60^{src} with those of p90, p105, and p140 and found no significant overlap between the polyproteins and

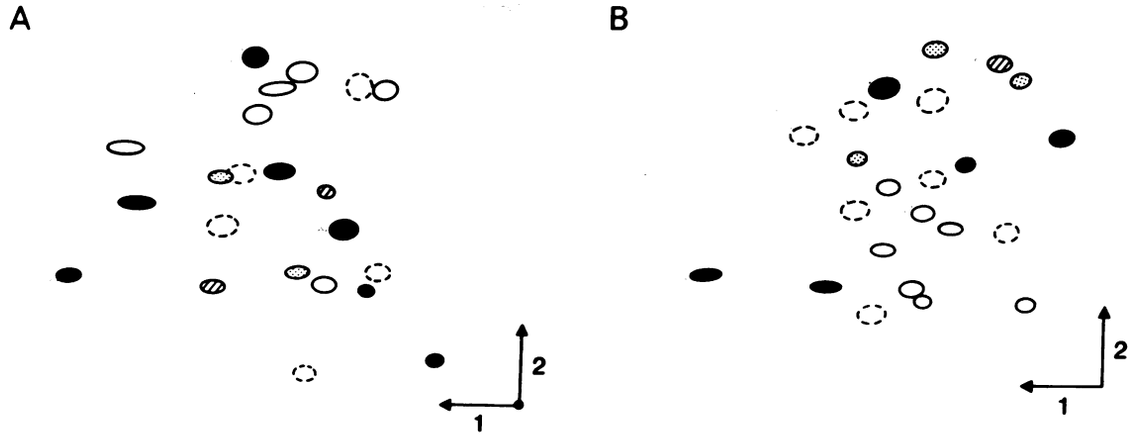

FIG. 4. Diagrams showing *gag* and non-*gag* peptides of PRCII, FSV, and Y73 polyproteins based on mixing experiments with the proteins analyzed in Figs. 2 and 3. (*A*) [^{35}S]Methionine-labeled tryptic peptides. (*B*) [^{35}S]Cysteine-labeled tryptic peptides. ○, Of Pr76^{gag}; ●, shared between FSV p140 and PRCII p105; ○, contained only in PRCII p105; ○, contained only in FSV p140; ○, specific for Y73 p90. Non-*gag* peptides that could not be clearly assigned have been omitted. Only major *gag* peptides have been shown, to simplify the diagrams.

pp60^{src} (refs. 4 and 21; data not shown).

Y73, FSV, and PRCII all have associated protein kinases that phosphorylate tyrosine residues on acceptor proteins, including the polyproteins themselves (3, 11, 12). To compare the kinase target sites on the three polyproteins, we immunoprecipitated the proteins and added [γ-^{32}P]ATP to the immune complexes (13). P140, p105, and p90 labeled in this manner and analyzed by two-dimensional tryptic peptide mapping are shown in Fig. 5. PRCII and FSV polyproteins showed the same two peptides, one heavily labeled and one weakly labeled. These two phosphopeptides migrated identically on electrophoresis but were separated by chromatography. Y73 p90 showed a single strongly labeled tryptic peptide that migrated differently from peptides of PRCII and FSV. Thus, the relatedness in the methionine-labeled and cysteine-labeled peptides of p140 and p105 was further reflected in the relatedness of the target sites of their respective protein kinases. The lack of similarity of the Y73 polyprotein to either p105 or p140 was extended to include its p90 kinase target site.

DISCUSSION

Three avian sarcoma viruses have recently been shown to lack the *src* gene of RSV and to carry, instead, transformation-specific genetic information that is expressed in the form of *gag*-linked polyproteins (1–5). We have demonstrated structural relatedness in the transformation-specific regions of polyproteins of two of these viruses, PRCII and FSV. Functional relatedness is also suggested by our demonstration that the protein kinases associated with FSV p140 and PRCII p105 catalyze phosphorylation of indistinguishable tyrosine-containing tryptic peptides on these two polyproteins. In contrast, Y73 p90 showed no relatedness to FSV p140 or PRCII p105 by either criterion. Although some small region of homology between Y73 and PRCII/FSV might have been missed, our observations are supported by parallel DNA hybridization studies on transformation-specific sequences of these viruses. No relationship was detected among Y73, RSV, and FSV, but strong homology was shown between FSV and PRCII (22, 23). Furthermore, FSV proved to be related in its transformation-specific sequences to two isolates of feline sarcoma virus (refs. 22 and 24; K. Beemon, personal communication). Also, the feline viral polyproteins show relatedness to their counterparts in PRCII and FSV by tryptic peptide mapping (K. Beemon, personal communication). These results suggest that independently isolated avian and feline sarcoma viruses may have acquired related cellular genes.

Three distinct classes of transformation-specific genetic in-

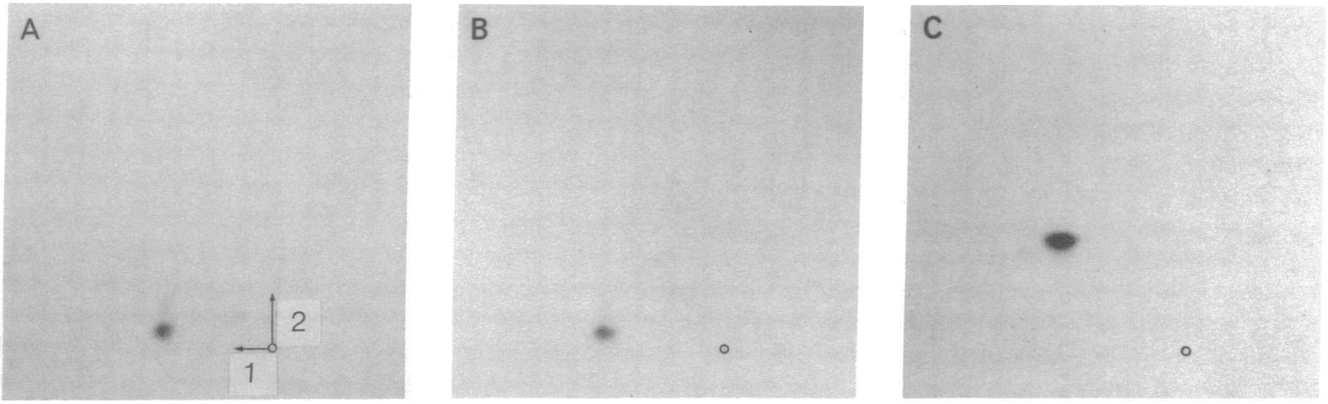

FIG. 5. Tryptic phosphopeptide maps of sarcoma virus polyproteins. Proteins were labeled with [γ-^{32}P]ATP in immune complexes (12) and processed as described in Fig. 2. The peptides were separated by electrophoresis in 1% ammonium carbonate (pH 8.9) for 27 min at 1000 V in the first dimension (15) and by chromatography as described in Fig. 2 in the second dimension. (*A*) PRCII p105; (*B*) FSV p140; (*C*) Y73 p90.

serts have now been identified in avian sarcoma: (*i*) the *src* gene of RSV which is cell-derived (25, 26); (*ii*) the transformation-specific sequences of FSV and PRCII, probably of cellular origin (22); and (*iii*) those of Y73, also derived from normal cells (23). Despite their structural diversity, all three virus groups may encode tyrosine-specific protein kinases. So far, only RSV *src* has been shown to possess a cellular homologue with a functionally similar product (27, 28). Similar cellular homologues of PRCII/FSV and Y73 transformation-specific proteins may be identified when suitable antisera become available. Conceivably, these may be kinases associated with distinct steps in a phosphorylation pathway responsible for regulation of fibroblast growth. Alternatively, functionally similar enzymes of the three virus classes may be directed at an identical target critical for cell growth.

Note Added in Proof. We have recently found that another virus, Esh sarcoma virus (ESV), is closely related to Y73 (29).

We thank K. Beemon for discussion of unpublished work. This investigation was supported by U.S. Public Health Service Research Grants CA 13213 and CA 19725 awarded by the National Cancer Institute. J.C.N. is a fellow of the Leukemia Society of America.

1. Lee, W.-H., Bister, K., Pawson, A., Robins, T., Moscovici, C. & Duesberg, P. H. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2018–2022.
2. Hanafusa, T., Wang, L.-H., Anderson, S. M., Karess, R. E., Hayward, W. S. & Hanafusa, H. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3009–3013.
3. Kawai, S., Yoshida, M., Segawa, K., Sugiyama, H., Ishizaki, R. & Toyoshima, K. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6199–6203.
4. Neil, J. C., Breitman, M. L. & Vogt, P. K. (1981) *Virology* **108**, 98–110.
5. Breitman, M. L., Neil, J. C., Moscovici, C. & Vogt, P. K. (1981) *Virology*, in press.
6. Bister, K., Hayman, M. J. & Vogt, P. K. (1977) *Virology* **82**, 431–448.
7. Duesberg, P. H., Bister, K. & Vogt, P. K. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4320–4324.
8. Hu, S. S. F., Lai, M. M. C. & Vogt, P. K. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1265–1268.
9. Hayman, M. J., Royer-Pokora, B. & Graf, T. (1979) *Virology* **92**, 31–45.
10. Bister, K. & Duesberg, P. H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5023–5027.
11. Feldman, R., Hanafusa, T. & Hanafusa, H. (1981) *Cell*, in press.
12. Neil, J. C., Ghysdael, J. & Vogt, P. K. (1981) *Virology*, in press.
13. Collett, M. S. & Erikson, R. L. (1978) *Proc. Natl. Acad. Sci. USA* **76**, 2121–2024.
14. Levinson, A. D., Oppermann, D., Levintow, L., Varmus, H. E. & Bishop, J. M. (1978) *Cell* **15**, 561–572.
15. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1311–1315.
16. Collett, M. S., Purchio, A. F. & Erikson, R. L. (1980) *Nature (London)* **285**, 167–169.
17. Sefton, B. M., Hunter, T., Beemon, K. (1980) *J. Virol.* **33**, 220–229.
18. Kessler, W. (1975) *J. Immunol.* **115**, 1617–1627.
19. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
20. Bonner, W. M. & Stedman, J. D. (1978) *Anal. Biochem.* **87**, 247–256.
21. Vigne, R., Neil, J. C., Breitman, M. L. & Vogt, P. K. (1980) *Virology* **105**, 71–85.
22. Shibuya, M., Hanafusa, T., Hanafusa, H. & Stephenson, J. R. (1980) *Proc. Natl. Acad. Sci. USA*, in press.
23. Yoshida, M., Kawai, S. & Toyoshima, K. (1980) *Nature (London)* **287**, 653–654.
24. Barbacid, M., Breitman, M. L., Lauver, A. V., Long, L. K. & Vogt, P. K. (1981) *Virology*, in press.
25. Stehelin, D., Varmus, H. E., Bishop, J. M. & Vogt, P. K. (1976) *Nature (London)* **200**, 170–173.
26. Spector, D., Varmus, H. E. & Bishop, J. M. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4102–4106.
27. Collett, M. S., Brugge, J. S. & Erikson, R. L. (1978) *Cell* **15**, 1363–1369.
28. Oppermann, H., Levinson, A. D., Varmus, H. E., Levintow, L. & Bishop, J. M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1804–1808.
29. Ghysdael, J., Neil, J. C. & Vogt, P. K. (1981) *Proc. Natl. Acad. Sci. USA* **78**, in press.