Isolation and Partial Characterization of a Monoclonal Antibody to the Rous Sarcoma Virus Transforming Protein pp60^{src}

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Transformation of cells by Rous sarcoma virus is mediated by the product of the viral *src* gene, pp60^{src}. A hybridoma cell line producing an immunoglobulin G₃ antibody to pp60^{src} was isolated after lymph node cells from immune mice were fused with mouse myeloma cells (P3-NS1-1). Mice were immunized with pp60^{src} purified from *Escherichia coli* cells expressing the *src* gene product. The monoclonal antibody immunoprecipitated pp60^{src} from Rous sarcoma virus-transformed cells and recognized an antigenic determinant located in the amino-terminal third of the pp60^{src} protein.

Cellular transformation by Rous sarcoma virus (RSV) requires the functional expression of the RSV *src* gene (19, 40). Brugge and co-workers first identified the RSV *src* gene product, showing that sera from rabbits bearing RSV-induced tumors (TBR sera) immunoprecipitated a 60,000-molecular-weight phosphoprotein (pp60^{src}) from RSV-transformed cells but not from normal cells or from cells infected with transformation-defective RSV (1, 2, 31). The RSV *src* gene product is a protein kinase (7, 24). pp60^{src} isolated as an immune complex with TBR sera exhibits a unique phosphotransferase activity, catalyzing the phosphorylation of tyrosine in the immunoglobulin heavy chain (7-9, 11, 21, 25, 37, 38). This activity appears to be an intrinsic property of pp60^{src}, since purified preparations of it readily phosphorylate tyrosine in a variety of substrates (11, 25, 30). In RSV-transformed cells, the cellular expression of pp60^{src} results in the phosphorylation of tyrosine in several specific cellular proteins (10, 12, 32, 36). These unique phosphorylation events, coupled with the intrinsic kinase activity of pp60^{src}, suggest that phosphorylation of defined cellular target proteins plays a critical role in cellular transformation.

The biochemical characterization of pp60^{src} has thus far been carried out with TBR sera (1, 2, 8, 21, 24, 34, 35) or sera from mice bearing avian sarcoma virus (ASV)-induced tumors (29); both are heterogeneous, polyclonal antisera with specificities for several antigenic determinants of *src*. However, a detailed analysis of the structure and function of pp60^{src} would be greatly facilitated by the availability of monoclonal anti-

bodies specific for defined antigenic determinants of the *src* protein. Unfortunately, the isolation of such monoclonal antibodies has been hindered by the low levels of pp60^{src} present in RSV-transformed cells and the difficulty of obtaining adequate amounts of purified *src* protein for immunization. Recently, recombinant DNA techniques have been used to construct bacterial plasmid vectors that direct the synthesis of high levels of p60^{src} in *Escherichia coli* (16, 26). The p60^{src} partially purified from bacterial extracts exhibits protein kinase activity (14, 26) and is immunogenic in rabbits, yielding antisera (rabbit anti-p60^{src}) that react with pp60^{src} from RSV-transformed cells (15).

In this paper we describe the isolation and initial characterization of a monoclonal antibody to pp60^{src}. We used Prague A strain (PrA) RSV p60^{src} purified from *E. coli* (14, 15) as an antigen for both the immunization of BALB/c mice and the screening of hybridoma culture fluids. The monoclonal antibody that was isolated immunoprecipitated pp60^{src} from RSV-transformed cells and recognized an antigenic determinant located in the amino-terminal third of the pp60^{src} protein.

To isolate hybridoma cell lines producing antibody specific for pp60^{src}, we first immunized BALB/c mice by injection into the hind footpads with p60^{src} emulsified in complete Freund adjuvant. Each mouse received approximately 20 µg of p60^{src} purified from extracts of *E. coli* (bearing the plasmid pLac-*src*) by the method of Gilmer and Erikson (15). Two weeks after this immunization, popliteal lymph nodes were removed, and the lymphocytes were fused with

P3-NS1-1 mouse myeloma cells (27). Culture fluids from the hybrid cells were assayed for the presence of antibody to *src* protein by a solid-phase radioimmunoassay with bacterial pp60^{src} as antigen and ^{125}I -rabbit antimouse Fab (39). Forty-one hybrid cultures (of a total of 182) were judged positive for the production of cross-reactive antibody. These hybrids were subcloned by limiting dilution, and individual culture supernatants were tested for the ability to immunoprecipitate proteins from extracts of [^{35}S]methionine- or ^{32}P -labeled PrA RSV-infected chicken embryo (CE) cells. Three clones produced antibodies directed against proteins from RSV-infected CE cells; clone R2D2 immunoprecipitated a 60,000- M_r phosphoprotein, identified as pp60^{src} , clone G2K9 immunopreci-

pitated a 120,000- M_r phosphoprotein, and clone C3P0 immunoprecipitated a 56,000- M_r phosphoprotein. R2D2 antibody was of the immunoglobulin G_3 isotype, with kappa light chains. G2K9 and C3P0 antibodies were of the immunoglobulin M isotype with kappa light chains. Preliminary data indicate that both G2K9 and C3P0 react with normal cell phosphoproteins that may be antigenically related to pp60^{src} .

Three criteria established R2D2 as a monoclonal antibody to pp60^{src} . First, R2D2 culture fluid, ascites fluid, or culture fluid from a representative subclone, R2D2-E4, immunoprecipitated a 60,000- M_r phosphoprotein from extracts of either [^{35}S]methionine- or ^{32}P -labeled PrA RSV-infected CE cells (Fig. 1A and B). The 60,000- M_r protein comigrated with pp60^{src} im-

FIG. 1. Immunoprecipitation of RSV *src* protein with R2D2 monoclonal antibody. (A) Immunoprecipitation of [^{35}S]methionine-labeled extracts of PrA RSV-infected CE cells. Cells were labeled for 4 to 5 h in Dulbecco modified Eagle medium lacking methionine but supplemented with 5% dialyzed fetal calf serum and 200 μCi of [^{35}S]methionine (Amersham Corp.) per ml. Cell extracts were prepared and incubated with antisera as previously described (3, 29). Immune complexes formed with mouse monoclonal antibodies were incubated with rabbit anti-mouse Fab to ensure complete adsorption of immunoglobulin classes that do not bind protein A. Immune complexes were boiled in sample buffer and subjected to electrophoresis on 10.5% polyacrylamide gels (23). Stained gels were equilibrated in En^3Hance (New England Nuclear Corp.) to provide fluorographic enhancement. Immunoprecipitation with (lane 1) culture fluid from clone R2D2, (lane 2) culture fluid from subclone R2D2-E4, (lane 3) culture fluid from mouse myeloma cells (P3-NS1-1), (lane 4) rabbit anti- pp60^{src} , (lane 5) normal rabbit serum. Molecular weights were determined from the relative migration rates of a standard mix of peptides containing myosin (200,000), β -galactosidase (116,000), phosphorylase A (92,500), bovine serum albumin (66,000), human gamma globulin heavy chain (55,000), ovalbumin (42,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and cytochrome *c* (12,500). (B) Immunoprecipitation of ^{32}P -labeled extracts of PrA RSV-infected CE cells. Cells were incubated for 1.5 h with 500 μCi of carrier-free $^{32}\text{P}_i$ (ICN) per ml in phosphate-free medium 199 (Gibco Diagnostics) supplemented with 5% dialyzed fetal calf serum. Cell extracts were immunoprecipitated with antibody preparations as described for panel A. Immunoprecipitations were carried out with the following culture fluids or antisera: lane 1, R2D2; lane 2, subclone R2D2-E4; lane 3, P3-NS1-1; lane 4, rabbit anti- pp60^{src} ; lane 5, normal rabbit serum. (C) Immunoprecipitation of [^{35}S]methionine-labeled extracts of *tsCH119* RSV-infected CE cells. Cells were labeled and extracts were prepared and incubated with antibody as described for panel A. Lanes: 1, R2D2; 2, P3-NS1-1; 3, rabbit anti- pp60^{src} ; 4, normal rabbit serum.

munoprecipitated by rabbit antiserum to bacterial p60^{src} (rabbit anti-p60^{src}) (Fig. 1A and B) or TBR sera (data not shown). To confirm that the 60,000-*M_r* protein recognized by R2D2 was structurally identical to pp60^{src}, we subjected the ³²P-labeled 60,000-*M_r* proteins immunoprecipitated by TBR sera and R2D2 to partial *Staphylococcus aureus* V-8 protease digestion (4), and the products were resolved by polyacrylamide gel electrophoresis. In each case, identical ³²P-labeled polypeptide products with molecular weights of 34,000 (p34) and 26,000 (p26) were observed (Fig. 2).

Second, clone R2D2 immunoprecipitated a structurally altered form of the *src* protein present in CE cells infected with RSV tsCH119, an RSV mutant containing a deletion within the *src* gene (3). CE cells infected with tsCH119 synthesize a 53,000-molecular-weight *src* protein (p53^{src}) containing a deletion of amino acid residues 202 through 255 (3). Both R2D2 and rabbit anti-p60^{src} immunoprecipitated a p53^{src} from extracts of [³⁵S]methionine-labeled, tsCH119-infected cells (Fig. 1C).

Third, clone R2D2 was used to immunoprecipitate a hybrid protein of p60^{src} and β -galacto-

FIG. 2. Partial V-8 protease digestion patterns of pp60^{src} immunoprecipitated by monoclonal antibody R2D2 and TBR sera. Extracts of ³²P-labeled PrA RSV-infected CE cells were immunoprecipitated with either R2D2 monoclonal antibody or TBR sera, and the immune complexes were subjected to electrophoresis on a 10.5% polyacrylamide gel. Radioactive proteins migrating with an apparent molecular weight of 60,000 were excised from the gel, digested with *S. aureus* V-8 protease, and subjected to polyacrylamide gel electrophoresis by the method of Cleveland et al. (4). (A) Samples immunoprecipitated with R2D2. Lanes: 1, undigested; 2 and 3, incubated with 10 μ g of V-8 protease. (B) Samples immunoprecipitated with TBR sera. Lanes: 1, undigested; 2 and 3, incubated with 10 μ g of V-8 protease. Molecular weights of the labeled peptides were determined as described in the legend to Fig. 1.

FIG. 3. Immunoprecipitation of *src*- β -gal hybrid protein (p130) by monoclonal antibody R2D2. Cultures of *E. coli* carrying plasmid pLSZ and expressing an *src*- β -gal hybrid protein of 130,000 molecular weight were labeled for 2 to 3 h in sulfate-depleted growth medium containing 100 μ Ci of [³⁵S]sulfate per ml (16). The cells were washed two times in 1 mM EDTA-10 mM Tris-hydrochloride (pH 8.0), and the extracts were prepared by the method of Gilmer and Erikson (15). As a negative control, cultures of *E. coli* containing a plasmid with the *src*- β -gal coding sequence but lacking the lactose operator-promoter (pSZ) were grown and labeled in the same manner. Odd-numbered lanes, immunoprecipitations of extracts of *E. coli* (pLSZ) cells; even-numbered lanes, immunoprecipitations of the pSZ cells. Immunoprecipitations were carried out with (lanes 1 and 2) monoclonal antibody R2D2, (lanes 3 and 4) monoclonal antibody G2K9, (lanes 5 and 6) P3-NS1-1, (lanes 7 and 8) rabbit anti-p60^{src}, (lanes 9 and 10) tumor-bearing mouse sera, (lanes 11 and 12) normal rabbit serum, and (lanes 13 and 14) normal mouse serum. The molecular weight of p130 was determined as described in the legend to Fig. 1.

sidase (*src*- β -gal) from extracts of *E. coli*. The *src*- β -gal hybrid protein contains the amino-terminal 204 amino acids of p60^{src} fused with β -galactosidase and is encoded by a plasmid (pLSZ) in which the first 612 base pairs of the PrA RSV *src* gene (20) are joined to the gene for β -galactosidase (17). The fused coding sequence is under the control of the lactose operator-promoter (17, 33). The details of this construction will be presented elsewhere (J. T. Parsons, A. Fenton, and V. Wilkerson, manuscript in preparation). A protein of 130,000 molecular weight (p130) was immunoprecipitated from extracts of [³⁵S]sulfate-labeled *E. coli* containing pLSZ by tumor-bearing mouse sera, rabbit anti-p60^{src}, and R2D2 monoclonal antibody (Fig. 3). Incubation of the same extracts with normal rabbit serum, culture fluid from mouse myeloma cells, or G2K9 cells (producing an antibody directed against a normal cellular protein) did not immunoprecipitate p130. Similarly, incubation of the antibodies

with [35 S]sulfate-labeled extracts from *E. coli* containing a fused *src*- β gal coding sequence but lacking the lactose operator-promoter (*E. coli* [pSZ]) did not result in the immunoprecipitation of p130 (Fig. 3). These data not only confirm that R2D2 recognizes a pp60^{src} determinant, but localize that determinant within the amino-terminal third of the pp60^{src} sequence.

To investigate the reactivity of R2D2 monoclonal antibody with *src* proteins encoded by other ASVs, we used R2D2 to immunoprecipitate pp60^{src} from extracts of [35 S]methionine-labeled PrA RSV-, Schmidt-Ruppin (SR) strain RSV-, or B₇₇ ASV-infected CE cells. R2D2 antibody immunoprecipitated B₇₇ ASV pp60^{src} (Fig. 4A) and to a lesser extent SR RSV pp60^{src} (Fig. 4B). In each case, rabbit anti-p60^{src} immunoprecipitated a comparable amount of pp60^{src}. We observed no immunoprecipitation of normal cell pp60^{src} (5, 28) or of the transforming proteins of Fujinami ASV (p140) (13) or Y-73 ASV (p90) (18, 22) by R2D2 (data not shown).

Here we have reported the isolation and partial characterization of a monoclonal antibody to the *src* gene product, pp60^{src}, of PrA RSV. Several facets of the isolation and characterization of this monoclonal antibody rely on bacterial plasmids that express all or part of the p60^{src} protein. First, the plasmid pLac-*src* expresses high levels of the *src* protein (16), thereby facilitating the isolation of purified p60^{src}. Using the bacterial *src* protein, we readily hyperimmunized mice, whereas repeated attempts to hyperimmunize mice with pp60^{src} prepared from CE or vole cells (6) have been unsuccessful. The isolation of the monoclonal antibody R2D2 demonstrates the utility of this approach. Secondly, bacterial plasmids containing a part of the *src* coding sequence fused, in frame, to *E. coli* β -galactosidase provide useful reagents for charac-

terizing the determinants recognized by individual monoclonal antibodies. Construction of similar plasmids bearing discrete regions of the *src* gene sequence should provide a bank of plasmids that specify hybrid proteins useful for the identification and mapping of unique determinants.

The isolation of the clone R2D2 represents an initial step in the establishment of a panel of monoclonal antibodies with multiple specificities for pp60^{src}. Such antibodies will facilitate analysis of the structure and function of pp60^{src} as well as other transforming proteins encoded by related sarcoma viruses.

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ADDENDUM IN PROOF

Using methods similar to those described above, we have isolated an additional 15 stable hybridoma cell lines producing monoclonal antibodies specific for PrA RSV pp60^{src}. The characterization of these monoclonal antibodies is in progress.

LITERATURE CITED

1. 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.
2. Brugge, J. S., P. J. Steinbaugh, and R. L. Erikson. 1978. Characterization of the avian sarcoma virus protein pp60^{src}. *Virology* **91**:131-140.
3. Bryant, D., and J. T. Parsons. 1982. Site-directed mutagenesis of the *src* gene of Rous sarcoma virus: construction and characterization of a deletion mutant temperature sensitive for transformation. *J. Virol.* **44**:683-691.
4. Cleveland, D., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli. 1978. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. *J. Biol. Chem.* **252**:1102-1106.
5. Collett, M., J. S. Brugge, and R. Erikson. 1978. Characterization of a normal cell protein related to the avian sarcoma virus transforming gene product. *Cell* **15**:1363-1370.
6. Collett, M. S., J. S. Brugge, R. L. Erikson, A. F. Lane, R. A. Krzyzek, and A. J. Faras. 1979. The *src* gene product of transformed and morphologically reverted ASV-infected mammalian cells. *Nature* (London) **281**:195-198.
7. 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.
8. 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.
9. Collett, M. S., A. F. Purchio, and P. L. Erikson. 1980. Avian sarcoma virus transforming protein, pp60^{src} shows protein kinase activity specific for tyrosine. *Nature* (London) **285**:167-169.
10. Cooper, J. A., and T. Hunter. 1981. Changes in protein phosphorylation in Rous sarcoma virus-transformed

FIG. 4. Reactivity of R2D2 monoclonal antibody with the *src* proteins of B₇₇ ASV, SR RSV, and PrA RSV. CE cells infected with B₇₇ ASV (A), SR RSV (B), or PrA RSV (C) were labeled with [35 S]methionine, and extracts were prepared and immunoprecipitated as described in the legend to Fig. 1. Lanes: 1, R2D2; 2, P3-NS1-1; 3, rabbit anti-p60^{src}; 4, normal rabbit antiserum.

- chicken embryo cells. *Mol. Cell. Biol.* 1:165-178.
11. Erikson, R. L. M. S. Collett, E. Erikson, and A. F. Purchio. 1979. Evidence that the avian sarcoma virus transforming gene product is a cyclic AMP-independent protein kinase. *Proc. Natl. Acad. Sci. U.S.A.* 76:6260-6264.
 12. Erikson, E., and R. L. Erikson. 1980. Identification of a cellular protein substrate phosphorylated by the avian sarcoma virus transforming gene product. *Cell* 21:829-836.
 13. Feldman, R. A., T. Hanafusa, and H. Hanafusa. 1980. Characterization of protein kinase activity associated with the transforming gene product of Fujinami sarcoma virus. *Cell* 22:757-765.
 14. Gilmer, T. M., and R. L. Erikson. 1981. Rous sarcoma virus transforming protein, $pp60^{src}$, expressed in *E. coli*, functions as a protein kinase. *Nature (London)* 294:771-773.
 15. Gilmer, T. M., and R. L. Erikson. 1982. Development of anti- $pp60^{src}$ serum with antigen produced in *Escherichia coli*. *J. Virol.* 45:462-465.
 16. Gilmer, T. M., J. T. Parsons, and R. L. Erikson. 1982. Construction of plasmids for expression of Rous sarcoma virus transforming protein, $pp60^{src}$, in *E. coli*. *Proc. Natl. Acad. Sci. U.S.A.* 79:2151-2156.
 17. Guarente, L., G. Lauer, T. M. Roberts, and M. Ptashne. 1980. Improved methods for maximizing expression of a cloned gene: a bacterium that synthesizes rabbit β -globin. *Cell* 20:543-553.
 18. Gysdael, J., J. C. Neil, and P. K. Vogt. 1981. A third class of avian sarcoma viruses defined by related transformation-specific proteins of Yamaguchi 73 and Esh sarcoma viruses. *Proc. Natl. Acad. Sci. U.S.A.* 78:2611-2615.
 19. Hanafusa, H. 1977. Cell transformation by RNA tumor viruses, p. 401-483. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), *Comprehensive virology*, vol. 10. Plenum Publishing Corp., New York.
 20. Highfield, P. E., L. F. Rafield, T. M. Gilmer, and J. T. Parsons. 1980. Molecular cloning of avian sarcoma virus closed circular DNA: structural and biological characterization of three recombinant clones. *J. Virol.* 36:271-279.
 21. 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.
 22. Kitamura, N., A. Kitamura, K. Toyoshima, Y. Hirayama, and M. Yoshida. 1982. Avian sarcoma virus Y73 genome sequence and structural similarity of its transforming gene product to that of Rous sarcoma virus. *Nature (London)* 297:205-208.
 23. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
 24. Levinson, A. D., H. Oppermann, L. Levintow, H. E. Varmus, and J. M. Bishop. 1978. Evidence that the transforming gene of avian sarcoma virus encodes a protein kinase associated with a phosphoprotein. *Cell* 15:561-572.
 25. Levinson, A. D., H. Oppermann, H. E. Varmus, and J. M. Bishop. 1980. The purified product of the transforming gene of avian sarcoma virus phosphorylates tyrosine. *J. Biol. Chem.* 255:11973-11980.
 26. McGrath, J. P., and A. D. Levinson. 1982. Bacterial expression of an enzymatically active protein encoded by RSV *src* gene. *Nature (London)* 295:423-425.
 27. Oi, V. T., and L. A. Herzenberg. 1980. Immunoglobulin-producing hybrid cell lines, p. 351-371. *In* B. Mishell and S. Shiigi (ed.), *Selected methods in cellular immunology—1980*. W. H. Freeman & Co., San Francisco, Calif.
 28. Oppermann, H., A. D. Levinson, H. E. Varmus, L. Levintow, and J. M. Bishop. 1979. Uninfected vertebrate cells contain a protein that is closely related to the product of the avian sarcoma virus transforming gene (*src*). *Proc. Natl. Acad. Sci. U.S.A.* 76:1804-1808.
 29. Parsons, S. J., S. C. Riley, E. E. Mullen, E. J. Brock, D. C. Benjamin, W. M. Kuehl, and J. T. Parsons. 1979. Immune response to the *src* gene product in mice bearing tumors induced by injection of avian sarcoma virus-transformed mouse cells. *J. Virol.* 32:40-46.
 30. Purchio, A. F. 1982. Evidence that $pp60^{src}$, the product of the Rous sarcoma virus *src* gene, undergoes autophosphorylation. *J. Virol.* 41:1-7.
 31. 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.
 32. Radke, K., T. Gilmore, and G. S. Martin. 1980. Transformation by Rous sarcoma virus: a cellular substrate for transformation-specific protein phosphorylation contains phosphotyrosine. *Cell* 21:821-828.
 33. Roberts, T. M., and G. D. Lauer. 1979. Maximizing gene expression on a plasmid using recombination *in vitro*. *Methods Enzymol.* 68:473-482.
 34. Rubsamen, H., R. R. Frilis, and H. Bauer. 1979. *Src* gene product from different strains of avian sarcoma virus: kinetics and possible mechanism of heat inactivation of protein kinase activity from cells infected by transformation-defective, temperature-sensitive mutant and wild-type virus. *Proc. Natl. Acad. Sci. U.S.A.* 76:967-971.
 35. Rubsamen, H., A. Ziemiecki, R. R. Frilis, and H. Bauer. 1980. The expression of $pp60^{src}$ and its associated protein kinase activity in cells infected with different transformation-defective temperature-sensitive mutants of Rous sarcoma virus. *Virology* 102:453-457.
 36. Sefton, B. M., T. Hunter, E. H. Ball, and S. J. Singer. 1981. Vinculin: a cytoskeletal target of the transforming protein of Rous sarcoma virus. *Cell* 24:165-174.
 37. Sefton, B. M., T. Hunter, and K. Beemon. 1980. Temperature-sensitive transformation by Rous sarcoma virus and temperature-sensitive protein kinase activity. *J. Virol.* 33:220-229.
 38. 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.
 39. Tsu, T. T., and L. A. Herzenberg. 1980. Solid phase radioimmune assays, p. 373-394. *In* B. Mishell and S. Shiigi (ed.), *Selected methods in cellular immunology—1980*. W. H. Freeman & Co., San Francisco, Calif.
 40. Vogt, P. K. 1977. The genetics of RNA tumor viruses, p. 341-455. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), *Comprehensive virology*, vol. 9. Plenum Publishing Corp. New York.