[CANCER RESEARCH 47, 6586–6589, December 15, 1987]

Apparent DNA-binding Protein Specific for Cells Transformed by Avian Acute Leukemia Viruses[1]

Stephen H. Benedict[2]

Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206

ABSTRACT

An apparent DNA-binding protein is described that is specific for cells transformed by avian acute leukemia viruses. This protein cannot be demonstrated in cells transformed by any of the avian sarcoma viruses or in cells infected with nontransforming avian retroviruses. The protein also is not detectable in noninfected quail or chicken embryo primary cultures or in noninfected chicken hematopoietic cells. The apparent molecular weight of this protein is 105,000 and it is referred to as DBP105.

INTRODUCTION

Avian retroviruses can be subdivided into three groups (reviewed in Ref. 1). Two of these groups, avian sarcoma viruses and avian acute leukemia viruses, carry oncogenic information in their genomes and transform cells in tissue culture. Both of these groups of viruses are rapidly carcinogenic, causing tumors within 2 weeks of injection. The acute leukemia viruses all cause tumors of hematopoietic origin and some also induce nonhematopoietic tumors. The avian sarcoma viruses cause only sarcoma. Lymphoid leukosis viruses represent the third group of avian retroviruses. These viruses carry no oncogenic information and infect but do not transform cells in culture. Lymphoid leukosis viruses cause tumors *in vivo* only after a long latent period.

Each member of both groups of rapidly carcinogenic retroviruses carries in its genome at least one oncogene, a piece of cell-derived genetic information that plays an important role in the process of oncogenic transformation (for reviews, see Refs. 1 and 2). There exist more than 25 different oncogenes; each codes for a transformation-specific protein that appears to interact with cellular target(s) to effect transformation. Some oncogenes such as the ones carried by avian sarcoma viruses are protein kinases. Other oncogenes such as *myc* or *myb*, carried by certain avian acute leukemia viruses, are localized in the nucleus and may bind to chromatin (3, 4). Elucidation of the events which lead to cell transformation require study of the cellular proteins which directly interact with oncogene products as well as study of those cellular proteins induced by oncogene expression. This report describes a cellular protein with a molecular weight of 105,000, DBP105, which apparently binds DNA and which is found in cells transformed by avian acute leukemia viruses but not in cells transformed by avian sarcoma viruses and not in normal cells or in cells infected with nontransforming avian leukosis viruses.

MATERIALS AND METHODS

Cell Culture and Viruses. Avian acute leukemia viruses used in this study were avian myelocytomatosis viruses MH2, MC29, OK10, and CMII; avian erythroblastosis viruses AEV and S13; AMV[3]; and REV-T. Avian sarcoma viruses used were Schmidt-Ruppin strain Rous sarcoma virus (SR-RSV), Prague strain Rous sarcoma virus, Fujinami sarcoma virus, Yamaguchi sarcoma virus (Y73), Esh sarcoma virus, and PRCII and PRCIV sarcoma viruses. Avian leukosis viruses tested were RAV-1 and S13-associated virus S13AV. With the exception of S13 and S13AV (5, 6) all avian retroviruses used in this study are described by Weiss *et al.* (1). Preparation of chick embryo and quail embryo cultures, viral transformation and maintenance of transformed cultures, metabolic labeling of cultures with [^{35}S]methionine (7), preparation of lysates, and immunoprecipitation (6) have been described. MH2-transformed quail nonproducer cells have been described previously (8). MH2-12 cells are a subclone of such cells. Other cell lines used were QT6, a methylcholanthrene-transformed quail tumor cell line (9), the transplantable lymphoid tumor of Olsen (10, 11) which originated from a natural case of lymphoid leukosis in a chicken, and the 1104X-5 cell line (12) which originated from a lymphoid leukosis induced by ALV. Finally, MSB-1 is a cell line (13) derived from an ALV-negative lymphoma induced by Marek's disease herpesvirus.

Labeling and Preparation of DNA. Cells were incubated overnight in phosphate-free medium and then placed in the same medium containing 10 mCi/ml H_3 $^{32}PO_4$ (ICN, Irvine, CA) for 12 h. DNA was extracted by standard methods using two overnight digestions with Pronase and one with RNase A.

Protein Blotting and DNA Binding. SDS-polyacrylamide gel electrophoresis was by the method of Laemmli (14) and has been described (6). Lysates run without immunoprecipitation were heated at 60°C in sample buffer for 20 min just prior to electrophoresis. Proteins were blotted from gels to nitrocellulose sheets essentially as described by Bowen *et al.* (15). Briefly, the gel was treated in 4 M urea, 50 mM NaCl, 10 mM Tris (pH 7), 2 mM EDTA, and 0.1 mM dithiothreitol (pretreatment buffer) for 3 h at room temperature and then sandwiched between two sheets of nitrocellulose (GSWP; Millipore) held in place by thin sheets of foam rubber and clamped between two stainless steel screens. The assembly was submerged in 4 liters of transfer buffer (10 mM Tris (pH 7), 50 mM NaCl, 2 mM EDTA, and 0.1 mM dithiothreitol). Transfer of proteins to nitrocellulose was allowed to take place by diffusion in both directions for 3 days, after which nitrocellulose sheets were air dried and stored at ambient temperature.

Binding of DNA to proteins on nitrocellulose was essentially as described by Bowen *et al.* (15). Blots were preincubated for 15 min at room temperature with gentle mixing in binding buffer consisting of 10 mM Tris (pH 7), 50 mM NaCl, 1 mM EDTA, and 0.02% each of Ficoll, bovine serum albumin, and polyvinylpyrrolidone. Binding of DNA was effected by a 60-min incubation with mixing in binding buffer containing 10^5 cpm/ml [^{32}P]DNA. After binding, blots were rinsed three times for 20 min each in binding buffer, air dried, and exposed to Kodak XAR-5 film in the presence or absence of intensifying screens (Dupont Conex; Lightning Plus).

Quantitation of Protein. Total protein in cell lysates was measured by the procedure of Bradford (16). Proteins were visualized on nitrocellulose blots as described by Bowen *et al.* (15). Blots were prewet with binding buffer and stained for 15 min with 0.1% aniline blue black in 43% methanol, 10% glacial acetic acid, and 45% H_2O). Blots were destained with 90% methanol, 2% acetic acid, and 8% H_2O, three times for 30 min each.

Received 3/24/87; revised 9/10/87; accepted 9/21/87.

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

[1] This work was supported by USPHS Research Grants CA 29777 and CA 13213 to Peter Vogt.

[2] Supported by a postdoctoral fellowship from the American Cancer Society and by a new Projects Research Grant from the University of Southern California School of Medicine.

[3] The abbreviations used are: AMV, avian myeloblastosis virus; RAV, Rous-associated virus; ALV, avian leukosis virus; SDS, sodium dodecyl sulfate; p100, M_r 100,000 protein; REV-T, transforming reticuloendotheliosis virus.

RESULTS

MH2-transformed quail nonproducer cells contain a DNA-binding protein that is absent from normal controls. Fig. 1 shows a representative experiment with proteins of MH2-transformed quail nonproducer cells clone number 12 (MH2-12) and a normal control. Lysates of nonlabeled MH2-12 (Fig. 1, *lane 2*) and normal quail (Fig. 1, *lane 3*) were separated by SDS-polyacrylamide gel electrophoresis and blotted to nitrocellulose. The resulting filter was incubated with [^{32}P]DNA (Fig. 1*A*) and exposed for autoradiography. Replicate experiments demonstrated that the strong band visible in *Lane 2* at an apparent molecular weight of 105,000 was consistently demonstrable in MH2-12 lysates but never in normal cell lysates. Other bands which seem to differ in this representative experiment were not consistently different in other gels (see Figs. 2 and 3). Also consistently demonstrable by this technique in both normal and transformed cells is a strong band at M_r 43,000 and a series of bands between M_r 25,000 and M_r 35,000 which probably include the cellular H1 or H5 histones. As indicated, the presence of certain proteins other than DBP105 seems to vary with lysates of the same cell type. This transient appearance was not significantly improved by varying NaCl concentration. However, increased ionic strength did affect the binding interactions. Compared with the standard binding buffer (0.05 M NaCl), rinsing of filters in 0.075 M NaCl removed most bands other than DBP105 (50% diminished), the band at 43,000 (undiminished), and the histone-like bands at 25,000–35,000 (50% diminished). Rinsing with 0.1 M NaCl reduced the binding by the M_r 43,000 band to 50% and binding by DBP105 and the M_r 25,000–35,000 bands to ~10%.

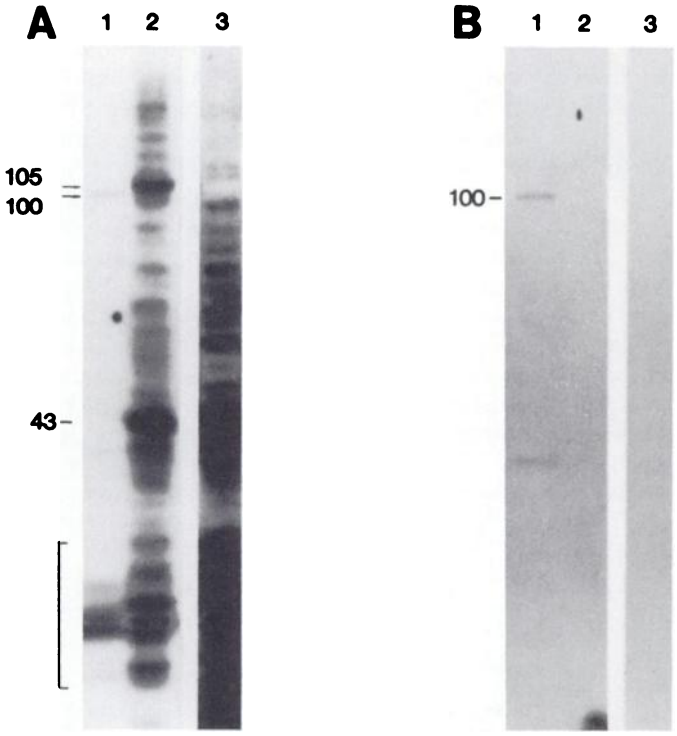

Fig. 1. DNA binding protein has apparent molecular weight of 105,000 and is not the same as the MH2 transformation-specific protein p100. SDS-polyacrylamide gel electrophoresis was run on the indicated samples, and the gel was blotted to nitrocellulose as described in "Materials and Methods." *A* and *B* represent the same nitrocellulose filter before (*B*) and after (*A*) binding to [^{32}P]-DNA. Autoradiographic exposure was identical for each panel, for 24 h at −80°C with an intensifying screen. Only [^{35}S]methionine contributes to the autoradiographic image seen in *B*, whereas both ^{35}S and ^{32}P contribute to *A*. *Lanes A1* and *B1* show MH2-transformed quail nonproducer cells (MH2-12) labeled with [^{35}S]methionine and immunoprecipitated with antiserum to Rous sarcoma virus. *Lanes 2* and *3* contain whole cell lysates of nonradioactively labeled MH2-12 cells and normal quail primary cells, respectively, run without immunoprecipitation.

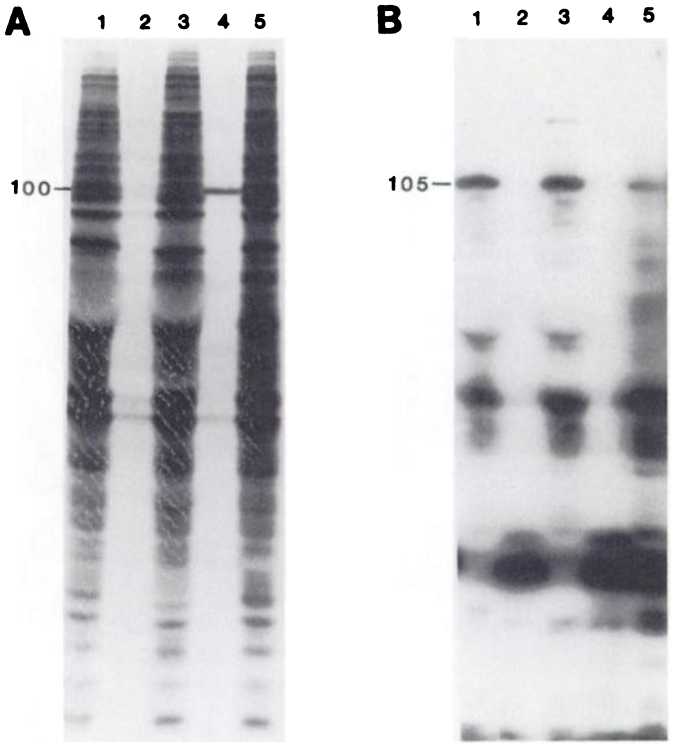

Fig. 2. DBP105 does not immunoprecipitate with antiviral sera. MH2-12 cells were labeled with [^{35}S]methionine lysed, and immunoprecipitated. Both the immunoreactive proteins and those remaining in the supernatant were run in adjacent lanes of an 8% SDS-polyacrylamide gel and blotted, and the blots were exposed directly for 10 days at room temperature (*A*) or incubated with [^{32}P]DNA and exposed for 24 h at −80°C with an intensifying screen (*B*). *Lanes 1* and *2* show the supernatant and pellet, respectively, of normal rabbit serum. *Lanes 3* and *4* show the supernatant and pellet of anti-whole virus serum. *Lanes A5* and *B5* show the MH2-12 lysate run directly without immunoprecipitation.

DBP105 was not identical with the transformation-specific protein p100 encoded by the avian acute leukemia virus MH2. Fig. 1, *Lane A1*, shows this protein labeled with [^{35}S]methionine, precipitated with antivirion antibody, electrophoresed, blotted, and treated with [^{32}P]DNA. In replicate gels, the apparent molecular weight of p100 was always smaller than that of DBP105. In addition, under these conditions, MH2 p100 did not bind detectable amounts of DNA; the intensity of the p100 band was the same after exposure to [^{32}P]DNA (Fig. 1, *Lane A1*) as it was before (Fig. 1, *Lane B1*). Under the conditions used in these experiments, DBP105 bound double stranded DNA three times better than single stranded DNA, bound slightly to RNA, and did not bind to [^{32}P]dATP, or to $^{32}P_i$ (data not shown).

No Reaction of DBP105 with Antiviral Sera. Despite the difference in apparent molecular weight between MH2 p100 and DBP105, the possibility existed that, like p100, DBP105 might consist of some viral and some nonviral information. Fig. 2 shows that this is not the case. MH2-transformed quail nonproducer cells (MH2-12) were labeled with [^{35}S]methionine, allowed to react with antiserum against Rous sarcoma virus virions, and electrophoresed. Supernatants of the immunoprecipitation were also processed in parallel. The gels were blotted, and autoradiograms were immediately obtained (Fig. 2*A*). The same nitrocellulose blots were then incubated with [^{32}P]DNA and autoradiographed a second time with much shorter exposures to show only the ^{32}P label (Fig. 2*B*). Fig. 2*A*, *Lane 4*,

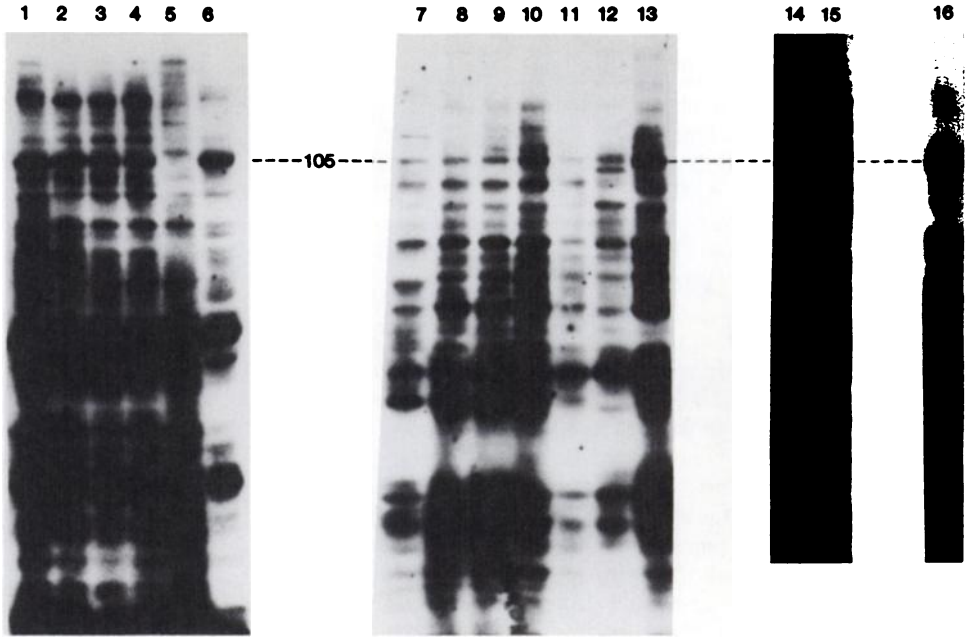

Fig. 3. DBP105 is demonstrable in cells transformed by avian leukemia viruses. Nonlabeled cells were lysed, total protein in each lysate was quantitated, and an equal amount of protein was run in each well of the 8% SDS-polyacrylamide gels. Gels were blotted and nitrocellulose was incubated with [^{32}P]DNA. *Lanes 1, 6, 7,* and *14* contain lysates of MH2-12 cells. The following lanes show secondary quail embryo cultures transformed with the indicated viruses: *Lanes 2* and *9*, MC29; *Lanes 3* and *10*, OK10; *Lanes 4* and *8*, MH2; *Lane 11*, CMII; and *Lane 13*, avian erythroblastosis virus. *Lane 12*, transforming reticuloendotheliosis virus-transformed bone marrow; *Lane 15*, MSB-1 cells; *Lane 16*, transplantable lymphoid tumor of Olsen; *Lane 5*, chemically transformed QT6 cells used as negative control.

shows that antivirion serum precipitated p100 of MH2-12 but not DBP105. Under conditions used here, the antivirion serum removed all p100 from the cell lysate (Fig. 2*A*, *Lanes 3* and *4*) whereas normal rabbit serum left p100 in the supernatant (Fig. 2, *Lanes A1* and *A2*). When the same filter was incubated with [^{32}P]DNA (Fig. 2*B*), DBP105 was always found undiminished in the supernatants (Fig. 2, *Lanes B1* and *B3*) after removal of the immunoprecipitates (Fig. 2, *Lanes B2* and *4*). DBP105 also was not precipitated by sera against RAV-O, AMV reverse transcriptase, or virion M_r 85,000 glycoprotein (not shown). These results show that DBP105 is immunologically unrelated to the virion proteins of avian retroviruses. Three proteins appear to bind DNA in immunoprecipitates of MH2-12; however, the same proteins are present in both normal serum and antiviral serum precipitates and are therefore nonspecific.

DBP105 is demonstrable in cells transformed by several avian acute leukemia viruses. In Fig. 3, unlabeled extracts from various cells were electrophoresed, blotted, and treated with [^{32}P]-DNA. Lysates positive for DBP105 (Fig. 3) were from cells transformed by avian acute leukemia viruses MH2, MC29, OK10, CMII, avian erythroblastosis virus, REV-T, S13, and AMV. Data for the last two viruses are not shown. Positive lysates were obtained with transformed cells of both chicken and quail origin. DBP105 was present in both fibroblasts and macrophages transformed by MH2 and MC29 and in various hematopoietic cell types transformed by AMV, S13, or REV-T. In addition to DBP105, a smaller protein was consistently observed only in lysates of REV-T-transformed cells (Fig. 3, *Lane 12*). Also positive for DBP105 were two lymphoid tumor cell lines, the transplantable lymphoid tumor of Olsen (Fig. 3, *Lane 16*) and 1104 X-5 (not shown). Finally, the MSB1 cell line from a tumor induced by Marek's disease herpesvirus was also positive for DBP105.

Cell lysates which did not contain reactive DBP105 were from the chemically transformed quail tumor line QT6 (Fig. 3, *Lane 5*) and from cells transformed by two strains of Rous avian sarcoma virus, PRC IV virus, Y73 virus, and Esh sarcoma virus (data not shown). Also negative for DBP105 were normal chicken and quail fibroblasts, chicken bone marrow cells, and chicken macrophages. Infection of cells with lymphoid leukosis virus RAV-1 or S13AV, which do not carry oncogenes and do not transform fibroblasts in tissue culture, also did not lead to the appearance of DBP105, even after very prolonged autoradiographic exposures. Since it was possible that the proteins

Table 1 *Distribution of DBP105*

Virus group	Virus	Cell type	DBP105
Acute leukemia			
Myelocytomatosis	MH2, MC29, OK10, CMII	Fibroblast (T)[a]	+
	MH2, MC29	Macrophage (T)	+
Myeloblastosis	AMV	Myeloblast (T)	+
Erythroblastosis	AEV, S13	Fibroblast (T)	+
Reticuloendotheliosis	REV-T	Bone marrow (T)	+
Lymphoid leukosis	RAV-1, S13AV	Fibroblast (I)	−
	ALV Olsen, 1104X-5	Lymphoid leukosis (T)	+
Sarcoma	SR-RSV, Pr-RSV	Fibroblast (T)	−
	PRCII, PRCIV, FSV	Fibroblast (T)	−
	Y73, ESV	Fibroblast (T)	−
Herpesvirus	Marek's disease herpesvirus	T-cell (T)	+
None	None	Quail embryo (N)	−
		Chick embryo (N)	−
		Chick macrophage (N)	−
		Chick bone marrow (N)	−

[a] T, transformed; I, infected but not transformed; N, not infected; AEV, avian erythroblastosis virus; SR-RSV, Schmidt-Ruppin strain Rous Sarcoma virus; Pr-RSV, Prague strain Rous sarcoma virus; FSV, Fujinami sarcoma virus; ESV, Esh sarcoma virus.

from nontransformed cell lysates might undergo selective elution from the filter during processing, the filters were routinely stained with aniline blue black after DNA binding and washing to ensure that each lane retained the same amount of protein. No differences or changes were observed in any experiments. Other experiments (not shown) with increased amounts of protein did not show DBP105 in any nontransformed or ALV-infected cells.

Table 1 summarizes the results of studies on DBP105, which indicate that cells transformed by the avian acute leukemia viruses express DBP105 as do cells derived from three lymphoid tumors. Cells transformed by acutely transforming avian sarcoma viruses do not express DBP105 nor do normal cells or cell infected with nontransforming avian leukosis viruses.

DISCUSSION

The data presented in this communication suggest that DBP105 is a phenotypic marker for cells transformed by avian leukemia viruses. The oncogenes active in these viruses include v-*myc*, v-*myc*/*mil*, v-*erb* A and B, v-*rel*, v-*myb*, and v-*sea*. They also include the oncogenic information of Marek's disease herpesvirus. Normal cells, some chemically transformed cells, and cells transformed by avian sarcoma viruses containing the *src*, *fps*, or *yes* oncogenes lack functional DBP105. Further studies extending to additional viruses, cell types, and oncogenes will show whether this correlation has general validity. The avian leukosis viruses present an interesting case. When these nontransforming viruses are used to infect cells in culture, they do not induce DBP105. When incubated *in vivo* for extended periods, these viruses cause lymphoid tumors in the animals. Cells derived from such tumors express DBP105, and this suggests a correlation between DBP105 and tumorigenicity. Under current conditions of assay, DBP105 was the only protein which was both consistently demonstrable and specific for cells transformed by a particular group of viruses. Other DNA-binding proteins may exist but require different conditions of assay or may need to bind as a complex.

Under the experimental conditions used in these studies, DBP105 is a DNA-binding protein. It could be argued that this property is the result of improper renaturation after denaturation induced by exposure to SDS and 4 M urea. Such a secondary acquisition of affinity to DNA cannot be ruled out on the basis of the available data. Although the DNA-binding properties of histones are not destroyed by the blotting procedures, those of p110 of MC29 apparently do not persist in the Western blotting. The question of whether the native DBP105 has DNA-binding properties must therefore await further studies.

DBP105 is not a virus-coded protein. There is no cross-reaction with the viral *gag* (internal proteins), *pol* (reverse transcriptase), or *env* (envelope glycoproteins) gene products. With minor variations the molecular weight of DBP105 is independent of the transforming virus. At least three explanations exist to account for the differential appearance of DBP105 in various cell types: (*a*) DBP105 may not be expressed in normal cells but may be induced as a result of acute leukemia virus transformation; (*b*) DBP105 may be expressed in a subpopulation of normal cells that contains the targets for transformation by avian acute leukemia viruses; (*c*) DBP105 may be present in all cell types but incapable of binding DNA in certain types of cells; only a transformation-dependent modification would induce the DNA-binding property.

REFERENCES

1. Weiss, R. A., Teich, N. M., Varmus, H., and Coffin, J. M. The Molecular Biology of Tumor Viruses. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982.
2. Bishop, J. M. Viral oncogenes. Cell, *42:* 23–28, 1985.
3. Donner, P., Greiser-Wilke, I., and Moelling, K. Nuclear localization and DNA binding of the transforming gene product of avian myelocytomatosis virus. Nature (Lond.), *296:* 262–265, 1982.
4. Klempnauer, K.-H., Symonds, G., Evan, G. I., and Bishop, J. M. Subcellular localization of proteins encoded by oncogenes of avian myeloblastosis virus and avian leukemia virus E26 and by the chicken c-*myb* gene. Cell, *37:* 537–547, 1984.
5. Beug, H., Hayman, M. J., Graf, T., Benedict, S. H., Wallbank, A. M., and Vogt, P. S13, a rapidly oncogenic replication-defective avian retrovirus. Virology, *145:* 141–153, 1985.
6. Benedict, S. H., Maki, Y., and Vogt, P. Avian retrovirus S13: properties of the genome and of the transformation-specific protein. Virology, *145:* 154–164, 1984.
7. Breitman, J. L., Neil, J. C., Moscovici, C., and Vogt, P. K. The pathogenicity and defectivenss of PRCII: a new type of avian sarcoma virus. Virology, *108:* 1–12, 1981.
8. Hu, S. S. F., Moscovici, C., and Vogt, P. K. The defectiveness of Mill Hill 2, a carcinoma-inducing avian oncovirus. Virology, *89:* 162–178, 1978.
9. Moscovici, D., Moscovici, M. G., Jiminez, J., Lai, M. M. C., Hayman, M. J., and Vogt, P. Continuous tissue culture cell lines derived from chemically induced tumors of Japanese quail. Cell, *11:* 195–203, 1977.
10. Olsen, C., Jr. A transmissible lymphoid tumor of the chicken. Cancer Res., *1:* 384–392, 1941.
11. Siegfried, L. M., and Olsen, C. Characteristics of avian transmissible lymphoid tumor cells maintained in culture. J. Natl. Cancer Inst., *48:* 791–795, 1972.
12. Hihara, H., Shimizu, T., and Yamamoto, H. Establishment of tumor cell lines culture from chickens with avian lymphoid leukosis. Natl. Inst. Anim. Health Q., *14:* 163–173, 1974.
13. Akiyama, Y., Kato, S., and Iwa, N. Continuous cell culture from lymphoma of Marek's disease. Bikens J., *16:* 177–179, 1973.
14. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.), *227:* 680–685, 1970.
15. Bowen, B., Steinberg, J., Laemmli, U. K., and Weintraub, H. The detection of DNA-binding proteins by protein blotting. Nucl. Acids Res., *8:* 1–20, 1980.
16. Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., *72:* 248–254, 1976.