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Avian Retroviruses That Cause Carcinoma and Leukemia: Identification of Nucleotide Sequences Associated with Pathogenicity

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Avian myelocytomatosis virus (MC29V) is a retrovirus that transforms both fibroblasts and macrophages in culture and induces myelocytomatosis, carcinomas, and sarcomas in birds. Previous work identified a sequence of about 1,500 nucleotides (here denoted onc_{MCV}) that apparently derived from a normal cellular sequence and that may encode the oncogenic capacity of MC29V. In an effort to further implicate onc_{MCV} in tumorigenesis, we used molecular hybridization to examine the distribution of nucleotide sequences related to onc_{MCV} among the genomes of various avian retroviruses. In addition, we characterized further the genetic composition of the remainder of the MC29V genome. Our work exploited the availability of radioactive DNAs (cDNA's) complementary to onc_{MCV} ($cDNA_{MCV}$) or to specific portions of the genome of avian sarcoma virus (ASV). We showed that genomic RNAs of avian erythroblastosis virus (AEV) and avian myeloblastosis virus (AMV) could not hybridize appreciably with $cDNA_{MCV}$. By contrast, $cDNA_{MCV}$ hybridized extensively (about 75%) and with essentially complete fidelity to the genome of Mill Hill 2 virus (MH2V), whose pathogenicity is very similar to that of MC29V, but different from that of AEV or AMV. Hybridization with the ASV cDNA's demonstrated that the MC29V genome includes about half of the ASV envelope protein gene and that the remainder of the MC29V genome is closely related to nucleotide sequences that are shared among the genomes of many avian leukosis and sarcoma viruses. We conclude that onc_{MCV} probably specifies the unique set of pathogenicities displayed by MC29V and MH2V, whereas the oncogenic potentials of AEV and AMV are presumably encoded by a distinct nucleotide sequence unrelated to onc_{MCV}. The genomes of ASV, MC29V, and other avian oncoviruses thus share a set of common sequences, but apparently owe their various oncogenic potentials to unrelated transforming genes.

Chickens are subject to a variety of malignant diseases induced by oncogenic retroviruses whose targets for transformation include fibroblastic, hematopoietic, and epithelial cells. Prototypic avian retroviruses presently under study include: avian sarcoma virus (ASV), which transforms fibroblasts; avian myelocytomatosis virus (MC29V), which induces primarily myelocytomatosis carcinomas of liver and kidney; Mill Hill 2 virus (MH2V), which induces carcinomas similar to those caused by MC29V; avian myeloblastosis virus (AMV), which induces acute myeloblastic leukemia; and avian erythroblastosis virus (AEV), which induces erythroblastic leukemia. With the exception of ASV, these viruses are defective and can replicate only in the presence of helper viruses.

Current evidence suggests that the diverse oncogenic capacities of avian retroviruses may result from the distribution of dissimilar transforming genes among a group of otherwise closely related viruses (8, 11, 26, 27; D. Stehelin, personal communication). In support of this hypothesis, it has been recently shown that the gene *src*, which is responsible for the sarcomagenic capacity of ASV, is absent from the genomes of several retroviruses that cause occasional sarcomas, but generally induce malignancies other than sarcomas (8, 27, 29). Moreover, a candidate for a transforming gene unrelated to *src* has recently been identified in the genome of MC29V (11, 27). This putative MC29V-transforming gene comprises about 1,500 nucleotides of the MC29V genome and has been tentatively designated "onc_{MCV}." However, there is currently no direct genetic evidence that demon-

strates the participation of onc_{MCV} in tumorigenesis.

In an effort to further implicate onc_{MCV} in tumorigenesis, we have examined the genomes of several avian oncogenic retroviruses for the presence of onc_{MCV} and have compared the nucleotide sequences of the MC29V and ASV genomes. These experiments were performed by hybridization of viral RNAs to radioactive complementary DNAs (cDNA's) corresponding to onc_{MCV} or to specific regions of the ASV genomes. Our hybridization experiments have detected a homolog of onc_{MCV} in the genome of MH2V, a virus whose pathogenicity resembles that of MC29V. By contrast, nucleotide sequences homologous to onc_{MCV} were not detectable in the genomes of either AEV or AMV, viruses whose pathogenicities are distinct from those of MC29V and MH2V. These findings confirm and extend the previous proposal that onc_{MCV} is a specific transforming gene whose presence in a retroviral genome determines the targets for transformation by that virus (10). Hybridization of RNA from MC29V-infected cells to ASV cDNA's demonstrated that, except for onc_{MCV}, essentially all remaining nucleotide sequences in the MC29V genome correspond to nucleotide sequences in the genome of ASV. It therefore seems possible that ASV and MC29V share a common phylogenetic origin, but have developed their dissimilar oncogenic capacities by the acquisition of different transforming genes (26, 31).

MATERIALS AND METHODS

Cells and viruses. Our stock of MC29V-associated virus subgroup A (MCAV-A) and our mixed stock of MC29V/MCAV-A were obtained as previously described (27). We used a stock of AEV/MCAV-A obtained by co-infection of C/O chick fibroblasts with the ES4 strain of AEV (obtained from Thomas Graf and containing subgroup B helper virus) and with MCAV-A; the progeny of this co-infection were grown in C/BE chick cells. Titers of transforming virus in stocks of AEV/MCAV were determined by end-point dilution and by direct focus assay on avian fibroblasts. Both procedures gave titers of approximately 10^6 infectious or focus-forming units per ml. Hence, the stocks contained a minimum of 10^6 particles of AEV per ml, although this is probably a conservative estimate because of the inefficiency of bioassays. A mixed stock of MH2V and its associated helper virus, MHAV, was obtained from P. Vogt and grown in cultured quail macrophages; this mixed stock will be referred to as MH2V/MHAV. The BA1 strain of AMV was obtained originally from R. E. Smith. A clonal stock of subgroup C AMV was prepared by superinfecting a nonproducer clone of AMV-transformed myeloblasts with a transformation-defective variant of subgroup C B77 ASV. The infectious AMV recovered from this procedure was then propagated by infecting myeloblasts either in culture or in vivo. In work to be reported elsewhere, we used molecular hybridization with cDNA specific for AMV to demonstrate that our preparations of AMV RNA contained helper virus and AMV genomes in a ratio of about 8:1 (T. Gonda and J. M. Bishop, manuscript in preparation). The Schmidt-Ruppin (SR), Prague (Pr), and Bratislava 77 (B77) strains of ASV were obtained and grown as previously described (27). Fertile chicken eggs were supplied by H & N Farms, Redmond, Wash., and quail eggs were from Life Sciences, St. Petersburg, Fla. A clonal line of quail cells (Q8) transformed by MC29V but not producing virus particles was isolated as described previously (4).

Preparation of unlabeled RNA. Virus particles were concentrated by centrifugation and RNA was extracted from virus pellets as previously described (3). Total cellular RNA was prepared from cultures of chicken or quail fibroblasts according to the method of Weiss et al. (35). Polyadenylated RNA was prepared from total cellular RNA by binding to oligodeoxythymidine-cellulose, using previously described conditions (27).

Preparation of ^{3}H-labeled viral RNA. Infected cells were labeled with [^{3}H]uridine (26 Ci/mmol; New England Nuclear Corp., Boston, Mass.) at 250 μCi/ml, and labeled viral RNA was purified from the tissue culture medium as previously described (27).

Electrophoresis and fluorography in agarose gels of viral RNA. ^{3}H-labeled viral RNA was fractionated by electrophoresis in gels of 1.5% agarose containing methylmercuric hydroxide as described by Bailey and Davidson (2). The gels were processed for fluorography according to a modification of the method of Bonner and Laskey (6). The molecular weights of viral RNAs were computed by reference to the electrophoretic mobility of MCAV genomic RNA (3.1×10^6; 27) and ribosomal RNAs used as internal standards in the analyses.

Preparation of virus-specific cDNA's. [^{32}P]-$cDNA_{B77}$ (10^5 cpm/ng) was synthesized by the endogenous reverse transcriptase of detergent-activated B77 ASV and purified as described previously (30). The majority of cDNA made under these reaction conditions consists of a 101-nucleotide sequence complementary to the 5′ terminus of the B77 genome; a small proportion of $cDNA_{B77}$ represents the entire ASV genome (12).

[^{32}P]$cDNA_{td}$ (10^5 cpm/ng) was transcribed from RNA of Pr-C ASV which contained a deletion for the *src* gene. The transcription was carried out with purified reverse transcriptase of AMV in a reaction in which oligomers of calf thymus DNA were used as primers; cDNA made by this method approximates a uniform representation of the template RNA (25). Reverse transcriptase was obtained from Joseph Beard, Life Sciences Inc. (through the auspices of The Office of Program Resources and Logistics, National Cancer Institute). Reaction conditions were as described previously (27, 34).

[^{3}H]$cDNA_{gp}$ (2×10^4 cpm/ng) was prepared as described by Tal et al. (32) and was complementary to most or all of the *env* deletion in the replication-defective Bryan high-titer strain of ASV. Since this

deletion includes most or all of the ASV *env* gene (9), $cDNA_{gp}$ is complementary to at least the majority of *env*.

[^{3}H]$cDNA_{MCV}$ (2×10^4 cpm/ng), formerly called $cDNA_{MC29}$, was prepared as described previously and was complementary to a sequence of 1,500 nucleotides present in the genome of MC29V, but not MCAV (27).

Molecular hybridization of nucleic acids in solution. Hybridization reactions were performed in solutions containing 0.6 M NaCl, 2 mM EDTA, 40 mM Tris (pH 7.0), 5 μg or more of calf thymus DNA as carrier, about 1,000 cpm (0.01 to 0.5 ng) of cDNA, and various amounts of RNA. Samples were incubated at 68°C to the desired value of C_rt (molar concentration of RNA × time in seconds, corrected to standard conditions [7]) or V_0t (see below). One half of each sample was digested with S1 single-strand nuclease, and hybridization was measured as percentage of resistance to S1 nuclease (20). V_0t is defined as original volume of tissue culture medium/reaction volume × time in hours (24, 27). With this definition of V_0t, the value for $V_0t_{1/2}$ is a constant for a virus stock with a given titer; the calculations for correlating focus titer and V_0t are described in detail elsewhere (27). Kinetics of hybridization were measured by varying the duration of incubation, reaction volume, or amount of RNA to achieve the desired values of C_rt or V_0t.

Thermal denaturation of RNA-DNA hybrids. Viral RNA was incubated with [^{3}H]$cDNA_{MCV}$ under conditions that ensured maximal hybridization. Samples were removed for measurement of initial S1 nuclease resistance; then reaction mixtures were diluted to final concentrations of 0.1 M NaCl and 0.005 M Tris (pH 7.4) and to final volumes of 2.0 ml. As an internal standard, portions of a mixture containing SR-D ASV RNA hybridized to [^{32}P]$cDNA_{B77}$ were added to each reaction tube. The mixtures were covered with a layer of mineral oil and placed at 50°C, and then the temperature raised in 4 to 5°C increments; portions for S1 assay were removed after 8 min at each temperature. The percentage of cDNA remaining in hybrid at each temperature was determined by hydrolysis with S1 nuclease. T_m was defined as the temperature at which 50% of the radioactivity originally in hybrid became susceptible to S1 nuclease.

RESULTS

Examination of several avian oncoviruses for the presence of nucleotide sequences homologous to onc_{MCV}. RNAs from several avian oncoviruses that induce different spectra of tumors were tested for their ability to hybridize with cDNA corresponding to the putative MC29V transforming gene ($cDNA_{MCV}$). Less than 10% of $cDNA_{MCV}$ reacted with RNA of AEV and its associated helper virus, even at values for V_0t well past the point where the control reaction with $cDNA_{B77}$ had reached completion (Fig. 1A). According to focus assay, the stock of AEV used for this analysis contained at least 10^6 particles of transforming virus per ml (see Materials and Methods); consequently, cDNA reactions with the genome of AEV prepared from this stock would have a maximum $V_0t_{1/2}$ of 4×10^4 (27; see Materials and Methods). Since we carried the reactions with $cDNA_{MCV}$ to a value of V_0t in excess of 5×10^5 without detecting significant hybridization, we conclude that the genome of AEV does not contain appreciable homology with onc_{MCV}.

The slight amount of hybridization we observed between $cDNA_{MCV}$ and the mixture of RNA from AEV and its helper virus is of unknown significance, although RNA purified from the AEV helper virus reacts to a similar extent with $cDNA_{MCV}$ (data not shown). The reaction with $cDNA_{MCV}$ seen in Fig. 1A therefore may have resulted from hybridization of AEV helper virus RNA with a minor contaminant present in $cDNA_{MCV}$ (27).

In similar experiments, we failed to detect any significant reaction between $cDNA_{MCV}$ and RNA from AMV (Fig. 1D). The preparation of RNA used in these analyses contained the genomes of helper virus and AMV in ratios of about 8:1 (see Materials and Methods). The $V_0t_{1/2}$ for the reaction of this RNA with $cDNA_{B77}$ (Fig. 1D) reflects the overall concentration of viral RNA present during these reactions (24, 27). If AMV RNA had any homology with $cDNA_{MCV}$, it should have reacted with a value for $V_0t_{1/2}$ 10-fold higher than that for the reaction with $cDNA_{B77}$. Yet this RNA failed to react significantly with $cDNA_{MCV}$ even at values for V_0t 10^5-fold greater than the $V_0t_{1/2}$ for the reaction with $cDNA_{B77}$. We therefore conclude that the genome of AMV does not contain nucleotide sequences similar to those for onc_{MCV}.

In contrast to our findings for AEV and AMV, RNA from stocks containing MH2V and its helper virus hybridized to an average value of 75% with $cDNA_{MCV}$ (Fig. 1B). $cDNA_{MCV}$ did not hybridize to RNA of MHAV (Fig. 1C); therefore the reaction shown in Fig. 1B must represent the hybridization of $cDNA_{MCV}$ with the MH2V genome. Examination of ^{3}H-labeled MH2V/MHAV RNA (Fig. 2) confirmed that the MH2V component, identified according to its previously measured molecular weight (1.6×10^6; 11), was present in our MH2V/MHAV stock in sufficient amounts relative to MHAV RNA to account for the reaction with $cDNA_{MCV}$ illustrated in Fig. 1B. We conclude that the genome of MH2V, unlike the genomes of AEV and AMV, contains a major fraction of the nucleotide sequences comprising the putative transforming gene of MC29V.

Thermal denaturation of hybrids formed between $cDNA_{MCV}$ and MH2V RNA. The fidelity of base pairing in hybrids formed between $cDNA_{MCV}$ and MH2V RNA was evaluated by thermal denaturation. [^{3}H]$cDNA_{MCV}$ was hy-

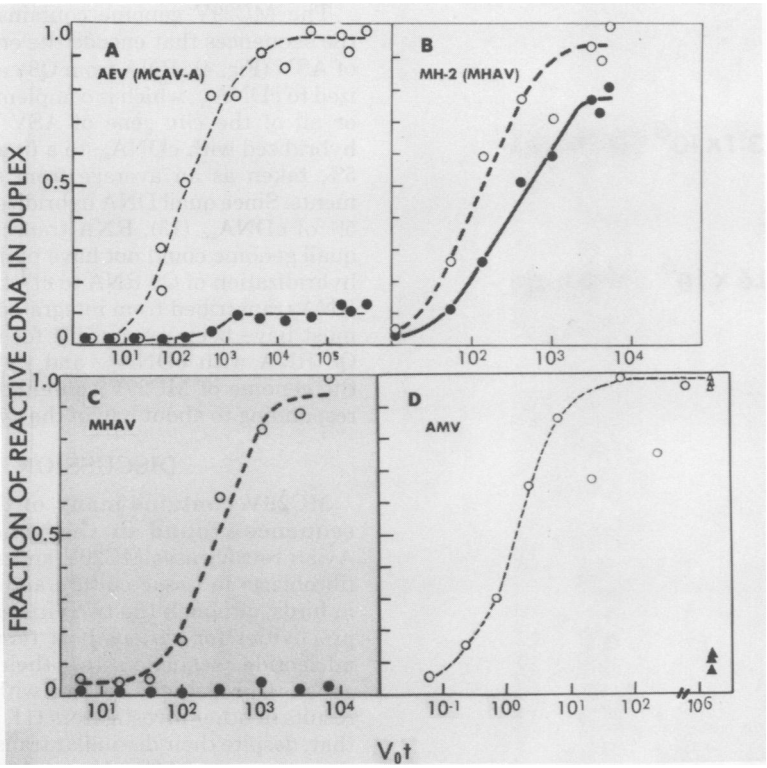

FIG. 1. *Hybridization analysis of RNAs from several avian oncoviruses for homology with* onc_{MCV}. *RNAs of AEV/MCAV, AMV/MAV, MH2V/MHAV, and MHAV were extracted from virus that was pelleted by high-speed ultracentrifugation (3). Hybridization reaction mixtures contained at least 5 ng of viral RNA and* 10^3 *cpm each of* $[^{32}P]cDNA_{B77}$ *and* $[^3H]cDNA_{MCV}$ *in volumes ranging from 10 to 200 μl. Incubations at 68°C varied from 5 min to 24 h. The extent of hybridization was measured by hydrolysis with S1 nuclease. The percentage of hybridization with* $[^{32}P]cDNA_{B77}$ *of each RNA was normalized to the final extent of reaction of* $[^{32}P]cDNA_{B77}$ *with that RNA; percentage of hybridization with* $[^3H]cDNA_{MCV}$ *was normalized in all cases to the final extent of hybridization to MC29V RNA. Symbols: (○, △)* $[^{32}P]cDNA_{B77}$; *(●, ▲)* $[^3H]cDNA_{MCV}$. *The two types of symbols indicate data taken from experiments done at different times. (A) AEV/MCAV RNA; (B) MH2V/MHAV RNA; (C) MHAV RNA; (D) AMV/tdB77 RNA.*

bridized with MH2V or MC29V RNA, and an internal standard consisting of duplex formed between $[^{32}P]cDNA_{B77}$ and SR-A ASV RNA was added to each reaction mixture. Samples were placed initially at 50°C and then subjected to a gradual increase in temperature as described in Materials and Methods. There were no differences in the thermal stabilities of hybrids formed between $cDNA_{MCV}$ and either MH2V or MC29V RNA (Fig. 3). It is apparent from these data that a high degree of similarity must exist between the sequences corresponding to onc_{MCV} in the MH2V and the MC29V genomes.

Homology of MC29V RNA with specific regions of the ASV genome. Previous studies have indicated that onc_{MCV} may be the only unique portion of the MC29V genome; the remainder of the genome is closely related to nucleotide sequences found in all avian leukosis and sarcoma viruses (4, 10, 18, 22). We have substantiated this conclusion by the use of molecular hybridization. In particular, we confirmed the previous conclusion that the MC29V genome contains a large fraction of the gene *env* (18), despite the failure of infection with MC29V to generate any proteins known to be encoded by this gene (4). As a convenience, in these studies we used total cellular RNA from the Q8 line as a source of MC29V genomic RNA that does not contain genomic RNA of MCAV (4). Q8 cells contain a species of RNA similar in size to the 5,300-nucleotide genome of MC29V (D. Sheiness and J. M. Bishop, unpublished data), and infectious MC29V can be rescued from Q8 by superinfection with nondefective avian retroviruses (4). Thus, RNA from Q8 cells probably contains all of the nucleotide sequences present in the MC29V genome itself.

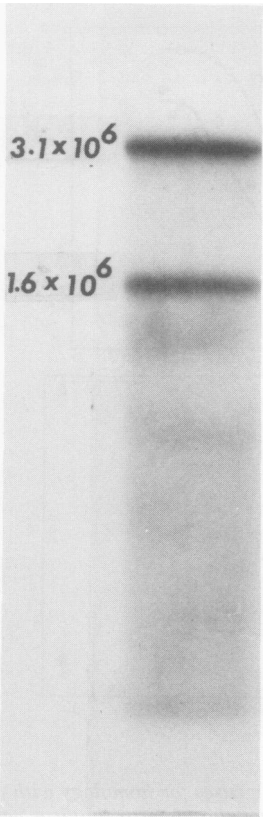

FIG. 2. *Electrophoresis of ^{3}H-labeled MH2/MHAV RNA in agarose gels. Infected cells were labeled with [^{3}H]uridine, and then viral RNA was purified from the tissue culture medium and its size was measured by gel electrophoresis as described in Materials and Methods. Shown here is an autoradiogram of a dried gel. Molecular weights of viral RNA are indicated in the figure and were determined by comparison with rRNA markers included in the gel.*

RNA was extracted from Q8 cells and found to hybridize to about 40% of $cDNA_{td}$, which uniformly represents the genome of transformation-defective Pr-C ASV (25). In three separate experiments, hybridization reactions were carried to plateaus in which the values for C_rt were $\geq 3 \times 10^4$ for Q8 and ≥ 2 for Pr-C RNA. The averaged value for hybridization with duplicate samples of Q8 RNA was normalized to the percentage of hybridization of $cDNA_{td}$ with Pr-C RNA (45 to 55%). Since $cDNA_{td}$ corresponds to about 8,500 nucleotides, hybridization of Q8 RNA to 40% of $cDNA_{td}$ indicates that roughly 3,500 nucleotides of the 5,300-nucleotide MC29V genome are homologous in sequence to the genome of ASV. Nucleotide sequences homologous to the ASV genome therefore account for about 65% of the genome of MC29V.

The MC29V genome contains about half of the sequences that encode the envelope protein of ASV (Fig. 4). RNA from Q8 cells was hybridized to $cDNA_{gp}$, which is complementary to most or all of the *env* gene of ASV (32). Q8 RNA hybridized with $cDNA_{gp}$ to a final value of 45 ± 5%, taken as an average from several experiments. Since quail DNA hybridizes to only about 5% of $cDNA_{gp}$ (13), RNA transcribed from the quail genome could not have participated in the hybridization of Q8 RNA to $cDNA_{gp}$. Therefore, RNA transcribed from integrated MC29V DNA must have been responsible for the reaction of Q8 RNA with $cDNA_{gp}$, and we conclude that the genome of MC29V contains sequences corresponding to about half of the *env* gene of ASV.

DISCUSSION

MC29V contains many of the nucleotide sequences found in the genome of ASV. Avian retroviruses MC29V and ASV transform fibroblasts in tissue culture and induce tumors in birds, although the two viruses differ in their proclivities for various host tissues and in the nucleotide sequences of their transforming genes. Our present results with $cDNA_{td}$ and results of other investigators (11, 18, 22) indicate that, despite their dissimilar transforming genes, the genomes of MC29V and ASV contain con-

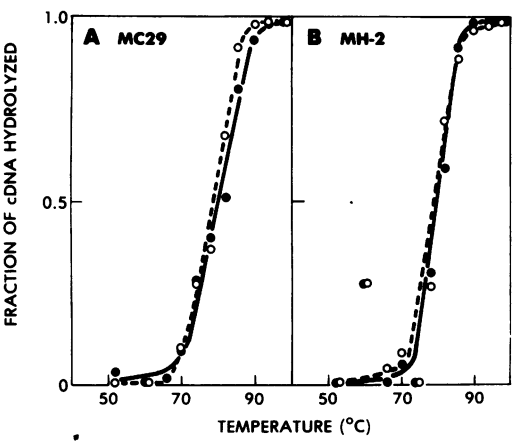

FIG. 3. *Thermal denaturation of hybrids formed between $cDNA_{MCV}$ and MH2V and MC29V genomic RNAs. RNAs were extracted from virus and hybridized with [^{3}H]$cDNA_{MCV}$ (5,000 cpm, 0.25 ng; ●) as described in Materials and Methods. Hybridized samples were placed at 50°C; the temperature was raised in 4 to 5°C increments, and denaturation was assayed with S1 nuclease as described in Materials and Methods. As an internal standard, duplexes between SR-D RNA and [^{32}P]$cDNA_{B77}$ were included in each denaturation (○). (A) MC29V/MCAV RNA; (B) MH2V/MHAV RNA.*

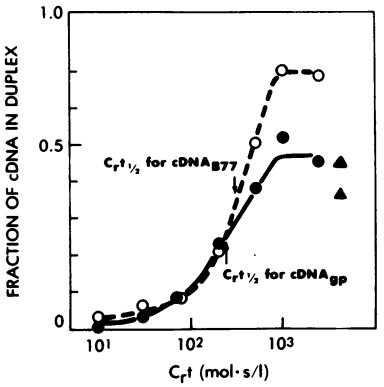

FIG. 4. *Hybridization of [^{3}H]cDNA$_{gp}$ and [^{32}P]-cDNA$_{B77}$ to MC29V RNA from Q8 cells. Hybridizations were performed as described in Materials and Methods, using 125 μg of unfractionated cellular RNA from quail cells nonproductively infected with MC29V and 10^3 cpm each of [^{32}P]cDNA$_{B77}$ and [^{3}H]cDNA$_{gp}$ per sample. Reactions were carried out in volumes of 25 μg, and periods of incubation at 68°C varied from 2 min to 32 h. The extent of hybridization was determined by hydrolysis with S1 nuclease. Since the cDNAs were transcribed from ASV RNA, the values for percentage of cDNA in hybrid with Q8 RNA were normalized to the final extent of control hybridizations of each cDNA with RNA of Pr-C ASV; the values for normalization were 73% for cDNA$_{gp}$ and 66% for cDNA$_{B77}$. Symbols:* (○) *[^{32}P]cDNA$_{B77}$;* (●) *[^{3}H]cDNA$_{gp}$.*

siderable nucleotide sequence homology and may have evolved from a common viral ancestor. In preliminary experiments, we also demonstrated that nucleotide sequences found at the 3′ and 5′ ends of ASV are present in MC29V (data not shown). Part of the homology between the MC29V and ASV genomes can be accounted for by the fact that MC29V encodes a protein with a molecular weight of 110,000 (P110) that shares antigenic determinants with some of the *gag* proteins of ASV; therefore, the MC29V genome must contain part of the *gag* gene of ASV (4). P110, however, does not correspond in molecular weight or in function to any ASV-encoded protein (4). Recent experiments have demonstrated that sequences corresponding to onc$_{MCV}$ are adjacent to the *gag*-related sequences in the MC29V genome (18, 22); it follows that the non-*gag* portion of P110 must be encoded by the putative MC29V-transforming gene. P110 is thus a possible candidate for the MC29V-transforming protein.

Our experiments indicate that about half the sequences in the *env* gene of ASV are present in the MC29V genome (Fig. 4), although cells infected with MC29V in the absence of helper virus do not contain any envelope proteins detectable by either biological or biochemical assays (4, 5). Previous experiments of Hu et al. (18) are in accord with our finding that MC29V contains a portion of the ASV *env* gene. Either the *env*-related sequences found in MC29V are unexpressed, or they encode a protein of unknown function that does not share antigenic determinants with the ASV envelope proteins.

Onc$_{MCV}$ may encode the oncogenic capacity and target cell specificity of MC29V. The genomes of avian retroviruses with pathogenicities similar to those of MC29V might be expected to contain sequences related to onc$_{MCV}$, the putative MC29V-transforming gene. Accordingly, we detected a sequence homologous to onc$_{MCV}$ in the genome of MH2V, a virus whose spectrum of target cells for malignant transformation in vivo and in vitro resembles that of MC29V (1). Recent results from Duesberg and Vogt agree with our conclusion that MH2V contains nucleotide sequences closely related to onc$_{MCV}$ (10). The presence in MH2V of sequences related to onc$_{MCV}$ supports the designation of onc$_{MCV}$ as the MC29V-transforming gene; further support for this identification derives from our failure to find homology with onc$_{MCV}$ in the genomes of AEV and AMV, whose pathogenicities differ from those of MC29V and MH2V. At least three other avian oncoviruses have been isolated (CMII, OK10, and Furth's strain) whose target cells for transformation in vivo are similar to those of MC29V and MH2V (14, 21, 23). On the basis of our present results, we would expect that each of these viruses contains nucleotide sequences homologous to part or all of onc$_{MCV}$; unpublished findings of M. J. Hayman, D. Stehelin, and co-workers conform to this expectation (personal communication). We conclude that the onc$_{MCV}$ probably encodes the oncogenic capacity of MC29V and related viruses and therefore represents a newly identified type of retrovirus-transforming gene.

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LITERATURE CITED

1. **Alexander, R. W., C. Moscovici, and P. K. Vogt.** 1979. Avian oncornavirus MH2: pathogenicity in chickens. J. Natl. Cancer Inst. **62**:359–366.
2. **Bailey, J. M., and N. Davidson.** 1976. Methylmercury as a reversible denaturing agent for agarose gel electrophoresis. Anal. Biochem. **70**:75–85.
3. **Bishop, J. M., W. E. Levinson, N. Quintrell, D. Sulli-**

van, L. Fanshier, and J. Jackson. 1970. The low molecular weight RNAs of Rous sarcoma virus. Virology 42:182-195.
4. Bister, K., M. J. Hayman, and P. K. Vogt. 1977. Defectiveness of avian myelocytomatosis virus MC29: isolation of long-term nonproducer cultures and analysis of virus-specific polypeptide synthesis. Virology 82:431-448.
5. Bister, K., and P. K. Vogt. 1978. Genetic analysis of the defectiveness in strain MC29 avian leukosis virus. Virology 88:213-221.
6. Bonner, W. M., and R. A. Laskey. 1974. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. 46:83-88.
7. Britten, R. J., and K. Smith. 1970. A bovine genome. Carnegie Inst. Washington Yearb. 68:378-386.
8. Duesberg, P. H., K. Bister, and P. K. Vogt. 1977. The RNA of avian acute leukemia virus MC29. Proc. Natl. Acad. Sci. U.S.A. 74:4320-4324.
9. Duesberg, P. H., S. Kawai, L.-H. Wang, P. K. Bogt, H. M. Murphy, and H. Hanafusa. 1975. RNA of replication-defective strains of Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. 72:1569-1573.
10. Duesberg, P. H., and P. K. Vogt. 1979. Avian acute leukemia viruses MC29 and MH2 share specific RNA sequences: evidence for a second class of transforming genes. Proc. Natl. Acad. Sci. U.S.A. 76:1633-1637.
11. Duesberg, P. H., P. K. Vogt, K. Bister, D. Troxler, and E. M. Scolnick. 1978. Oncogenic (onc) genes of sarcoma, leukemia, and carcinoma viruses, p. 95-111. *In* Avian RNA tumor viruses. Piccin Editore, Padva.
12. Friedrich, R., H. J. Kung, B. Baker, H. E. Varmus, H. M. Good, and J. M. Bishop. 1977. Characterization of DNA complementary to a nucleotide sequence at the 5' terminus of the avian sarcoma virus genome. Virology 78:198-215.
13. Fujita, D. J., J. Tal, H. E. Varmus, and J. M. Bishop. 1978. *Env* gene of chicken RNA tumor viruses: extent of conservation in cellular and viral genomes. J. Virol. 27:465-474.
14. Furth, J. 1934. Lymphomatosis, myelomatosis, and endothelcoma of chickens caused by a filterable agent. J. Exp. Med. 59:501-518.
15. Graf, T., and H. Beug. 1978. Avian leukemia viruses: interaction with their target cells *in vivo* and *in vitro*. Biochim. Biophys. Acta 516:269-299.
16. Haseltine, W. A., D. G. Kleid, A. Panet, E. Rothenberg, and D. Baltimore. 1976. Ordered transcription of RNA tumor virus genomes. J. Mol. Biol. 106:109-131.
17. Hayman, M. J., K. Bister, P. K. Vogt, B. Boyer-Pokova, and T. Graf. 1978. Viral polyprotein synthesis in cells infected with avian sarcoma-leukemia viruses, p. 214-226. *In* Avian RNA tumor viruses. Piccin Editore, Padua.
18. Hu, S. F., M. M. C. Lai, and P. K. Vogt. 1979. The genome of avian myelocytomatosis virus MC29 analyzed by heteroduplex mapping. Proc. Natl. Acad. Sci. U.S.A. 76:1265-1268.
19. Hu, S. S. F., C. Moscovici, and P. K. Vogt. 1978. The defectiveness of Mill Hill 2, a carcinoma-inducing avian oncovirus. Virology 89:162-178.
20. Leong, J. A., A. C. Garapin, N. Jackson, L. Fanshier, W. Levinson, and J. M. Bishop. 1972. Virus-specific ribonucleic acid in cells producing Rous sarcoma virus: detection and characterization. J. Virol. 9:891-902.
21. Loliger, H. C. 1964. Experimentelle untersuchungen zur Ubertranguang der Gefliigelleukose. II. Serienubertragungen mit einem Myelosestamm. III. Vergleichende untersuchungen Zwischen Zwei aus spontanfallen isolierten Myelosestammen und einem Lymphomatosestamm. Dtsch. Tieraerztl. Wochenschr. 71:207-212.
22. Mellon, P., A. Pawson, K. Bister, G. S. Martin, and P. H. Duesberg. 1968. Specific RNA sequences and gene products of MC29 avian acute leukemia virus. Proc. Natl. Acad. Sci. U.S.A. 75:5874-5878.
23. Oker-Blom, N., L. Hortling, A. Kallio, E. L. Numiaho, and H. Westermarck. 1978. OK 10 virus, an avian retrovirus resembling the acute leukemia viruses. J. Gen. Virol. 40:623-633.
24. Ringold, G., E. Y. Lasfargus, J. M. Bishop, and H. E. Varmus. 1975. Production of mouse mammary tumor virus by cultured cells in the absence and presence of hormones: assay by molecular hybridization. Virology 65:135-147.
25. Shank, P. R., J. C. Cohen, H. E. Varmus, K. R. Yamamoto, and G. M. Ringold. 1978. Mapping of linear and circular forms of mouse mammary tumor virus DNA with restriction endonucleases: evidence for a large specific deletion occurring at high frequency during circularization. Proc. Natl. Acad. Sci. U.S.A. 75: 2112-2116.
26. Sheiness, D., and J. M. Bishop. 1979. DNA and RNA from uninfected vertebrate cells contain nucleotide sequences related to the putative transforming gene of avian myelocytomatosis virus. J. Virol. 31:514-521.
27. Sheiness, D., L. Fanshier, and J. M. Bishop. 1978. Identification of nucleotide sequences which may encode the oncogenic capacity of avian retrovirus MC29. J. Virol. 28:600-610.
28. Shine, J., A. P. Czernilofsky, R. Friedrich, J. M. Bishop, and H. M. Goodman. 1977. Nucleotide sequence at the 5' terminus of the avian sarcoma virus genome. Proc. Natl. Acad. Sci. U.S.A. 74:1473-1477.
29. Stehelin, D., and T. Graf. 1978. Avian myelocytomatosis and erythroblastosis viruses lack the transforming gene *src* of avian sarcoma viruses. Cell 13:745-750.
30. Stehelin, D., R. V. Guntaka, H. E. Varmus, and J. M. Bishop. 1976. Purification of cDNA complementary to nucleotide sequences required for neoplastic transformation of fibroblasts by avian sarcoma viruses. J. Mol. Biol. 101:349-365.
31. Stehelin, D., H. E. Varmus, and J. M. Bishop. 1976. DNA related to the transforming gene(s) of avian sarcoma virus is present in normal avian DNA. Nature (London) 260:170-173.
32. Tal, J., D. J. Fujita, S. Kawai, H. E. Varmus, and J. M. Bishop. 1977. Purification of DNA complementary to the *env* gene of avian sarcoma virus and analysis of relationships among the *env* genes of avian leukosis-sarcoma viruses. J. Virol. 21:497-505.
33. Tal, J., H. J. Kung, H. E. Varmus, and J. M. Bishop. 1977. Characterization of DNA complementary to nucleotide sequences adjacent to poly(A) at the 3'-terminus of the avian sarcoma virus genome. Virology 79: 183-197.
34. Taylor, J. M., R. Illmensee, and I. Summers. 1976. Efficient transcription of RNA into DNA by avian sarcoma virus polymerase. Biochim. Biophys. Acta 442: 324-330.
35. Weiss, S. R., H. E. Varmus, and J. M. Bishop. 1977. The size and genetic composition of virus-specific RNA's in the cytoplasm of cells producing avian sarcoma-leukosis viruses. Cell 12:983-992.