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The RNA of avian acute leukemia virus MC29

(genetic defectiveness/oncogenic genes/avian sarcoma virus/nucleotide fingerprinting/nucleic acid hybridization)

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ABSTRACT **The RNA of myelocytoma virus MC29, a replication-defective avian acute leukemia virus, was investigated. Sedimentation and electrophoretic analyses indicated that the virus contains a distinct 28S RNA with about 5700 nucleotides. It is the smallest avian tumor virus RNA detected to date. The small size of the RNA suggests that the defectiveness of the virus is due to deletions in replicative genes. The RNA shared 3 to 5 of 30 large RNase T_1-resistant oligonucleotides with the RNA of other avian leukosis and sarcoma viruses. Hybridization indicated that 61% of the viral RNA contains sequences in common with other avian sarcoma and leukosis viruses. At least 32% of the RNA (about 1800 nucleotides) appear to be MC29-specific and may represent the transforming information of the virus. Sequences of the conserved transforming gene of avian sarcoma viruses were not detected in MC29 RNA. It was concluded that the transforming sequences of MC29 RNA define a new class of avian tumor viral transforming genes.**

Avian oncoviruses fall into several groups on the basis of their pathological properties: sarcoma viruses, lymphatic leukemia viruses, and acute leukemia viruses (1–4). Sarcoma viruses produce solid mesodermal tumors in animals and induce neoplastic transformation of fibroblasts in tissue culture. Lymphatic leukemia viruses cause, after prolonged latent periods, lymphoid leukemia and leukosis in animals and usually fail to induce an overt transformation in either hematopoietic or fibroblastic tissue cultures (4). Acute leukemia viruses cause myeloid and erythroid leukemias and carcinomas, and some of these viruses also induce fibrosarcomas (1–4). The corresponding effects in tissue culture are neoplastic transformation of hematopoietic cells and sometimes of fibroblasts (4–13). Transformation in culture by acute leukemia viruses suggests the presence of a distinct transforming gene, perhaps analogous to that found in sarcoma viruses (4, 14–18).

Avian oncoviruses have also been divided on the basis of their genetic complexity. All acute leukemia viruses studied to date, as well as some sarcoma viruses, are defective and can produce infectious progeny only with the aid of a lymphatic leukemia helper virus (4, 7, 19–21). By contrast, lymphatic leukemia viruses and certain sarcoma viruses are nondefective and replicate independently (4, 22).

We have investigated the RNA of MC29, an avian acute leukemia virus that causes leukemias and carcinomas and transforms hematopoietic cells as well as fibroblasts in culture (8–12). Nonproducer cells transformed in single infection by MC29 lack the expression of functional envelope glycoprotein (23, 24), viral DNA polymerase, and certain internal virion proteins (24). Instead, a large polyprotein (110,000 daltons) is seen in infected cells that carries some but not all serological determinants of the viral *gag* gene and that has no counterpart in cells infected by helper-independent viruses (24). MC29 may therefore have defects covering all three genes necessary for replication of RNA tumor viruses—namely, *env*, *pol*, and *gag* (22).

We have investigated the RNA of MC29 to determine whether it has unique transforming sequences responsible for acute leukemia and transformation of tissue culture cells or shares transforming sequences with avian sarcoma viruses (14–18). In addition we have analyzed the size and complexity of the viral RNA in an attempt to define the defectiveness of MC29.

MATERIALS AND METHODS

All cells and viruses used in this study have been described in detail (24, 25). Procedures for the preparation and analyses of viral RNAs are referenced and all modifications are described in the *text*.

RESULTS

Identification and Physical Properties of MC29 RNA. MC29 was prepared from the growth medium of Japanese quail fibroblast cell lines that had been transformed in single infection by MC29. Two nonproducer sublines (Q5 and Q8) and one producer subline (Q10) were used. For production of infectious MC29, Q8 and Q5 were superinfected with ring-necked pheasant virus (RPV) of subgroup F (24). Q10 was a producing Japanese quail cell line that originated from double infection with MC29 and its associated helper virus (MCAV). The resulting virus preparations, MC29(RPV) and MC29(MCAV), contained free helper virus as well as the corresponding MC29 pseudotype.

The buoyant density of MC29(RPV) was 1.2 g/ml in sucrose gradients, which is higher than that of avian oncoviruses propagated in chicken cells (26) and confirms an observation by Bister and coworkers (24). Glycerol gradient sedimentation resolved the RNA of MC29(RPV) produced by Q8 into a 50–70S and a 4–12S component, which is the characteristic pattern obtained with oncoviruses (26). The RNA monomers of the 50–70S complex were obtained by heat dissociation and were resolved by sedimentation into a 34S species with an estimated molecular weight (M_r) of 2.55×10^6 and a 28S species with an estimated M_r of 1.7×10^6 (27) (Fig. 1). The sedimentation coefficients were determined by the method of Martin and Ames (28) with 28S and 18S ribosomal RNAs as standards.

Complete separation of the 28S and 34S MC29(RPV) RNA species was accomplished by electrophoresis in polyacrylamide gels. The viral 28S RNA was clearly separated from the 28S

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Abbreviations: MC29, avian myelocytoma virus, strain 29; RPV, ring-necked pheasant virus; MCAV, MC29 associated helper virus; $NaDodSO_4$, sodium dodecyl sulfate; M_r, molecular weight; PR-A, Prague strain Rous sarcoma virus of subgroup A; *td*, transformation defective; PR-B, Prague strain Rous sarcoma virus of subgroup B.

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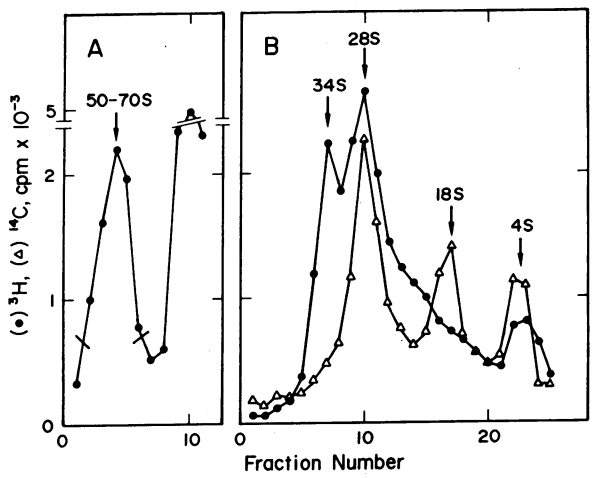

FIG. 1. Sedimentation analyses of MC29(RPV) RNA. (*A*) A culture of quail cells Q8 (24) infected and transformed by MC29(RPV) was propagated in the presence of 50 μ Ci of [^{3}H]uridine (50 Ci/mmol) per 10-cm petri dish containing 6 ml of Eagle's medium supplemented with 10% tryptose phosphate broth, 4% fetal calf serum, 2% chicken serum, and 1% dimethyl sulfoxide. Medium was changed at 6–8-hr intervals for a total of 2 days. Labeling was repeated once or twice during this period. Virus was purified as described (26) with the modification that initial virus concentration was by pelleting in a Beckman no. 19 rotor (2 hr at 19,000 rpm and 5°) instead of ammonium sulfate precipitation. RNA was prepared by the phenol method (26) with the modification that about 25% $CHCl_3$ (vol/vol) was included in the phenol phase and that only two extractions were performed. After ethanol precipitation, the [^{3}H]RNA was dissolved in 0.3 ml of 0.1 M NaCl/0.01 M Tris, pH 7.4/1 mM EDTA/0.2% sodium dodecyl sulfate ($NaDodSO_4$) and incubated for 3 min at 40°. It was then centrifuged in a glycerol gradient (15–30%) in the same buffer but with 0.1% $NaDodSO_4$ for 60 min at 50,000 rpm in a Spinco SW 50.1 rotor at 20°. Radioactivity was assayed on an aliquot of each fraction in a Tri-Carb scintillation counter (25, 26). (*B*) Sedimentation of the RNA monomer species of MC29(RPV). The 50–70S RNA obtained in *A* was pooled (fractions marked in *A*), dissolved as in *A*, and heated at 100° for 45 sec in the presence of rat cell [^{14}C]RNA to provide 28S and 18S ribosomal RNA markers. Sedimentation of the RNAs was for 105 min at 60,000 rpm in a Spinco SW 60 rotor at 20° and otherwise as for *A*. MC29(RPV) RNA was resolved into a 34S and a 28S monomer species.

ribosomal marker (Fig. 2*A*), presumably reflecting differential responses of the viral and ribosomal RNAs to the different ionic conditions used in the gradient and in the polyacrylamide gel (26). Judging from its electrophoretic mobility relative to that of 28S ribosomal RNA, the 28S MC29(RPV) RNA appears to be larger than estimated from its sedimentation coefficient. Therefore, the above M_r of 28S MC29(RPV) RNA is considered a minimal estimate. Class *a* RNA of the Prague strain of Rous sarcoma virus subgroup A (PR-A) propagated in secondary cultures of Japanese quail fibroblast migrated more slowly (i.e., was larger) than the 34S RNA of MC29(RPV) (Fig. 2*B*) (25). The 34S RNA of MC29(RPV) migrated with the class *b* RNA of transformation-defective (*td*) deletion mutants of PR-A present in our stock (25). Simultaneous electrophoresis of the monomers of 50–70S MC29(RPV) RNA and of RPV RNA showed that RPV contained only a single RNA species that coincided exactly with the 34S RNA of MC29(RPV) (Fig. 2*C*). This result suggested that the 34S RNA component of MC29(RPV) was the RNA of the helper RPV and that the 28S RNA was MC29-specific. The RNA of MC29 had been previously resolved into two electrophoretic peaks, but at that time the possible significance of this observation was not recognized (29).

The ratio of 34S to 28S viral RNA components varied in virus

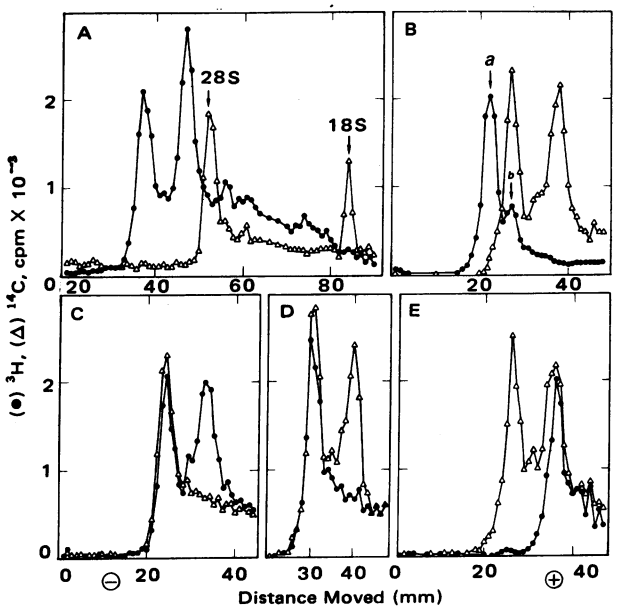

FIG. 2. Simultaneous electrophoresis of MC29 RNAs and other viral and cellular RNAs. (*A*) 50–70S [^{3}H]RNA of MC29(RPV) propagated in Q8 was prepared as in Fig. 1 and was mixed with rat cell [^{14}C]RNA in electrophoresis sample buffer. The mixture was heated to 100° for 45 sec and subjected to electrophoresis in a 2% polyacrylamide gel for 6 hr at 8 V/cm as described (25). (*B*) Electrophoresis of MC29(RPV) [^{14}C]RNA as in *A* together with the [^{3}H]RNA of subgroup A Prague strain Rous carcinoma virus (PR-A), also propagated in quail cells. The class *a* RNA is the genome of PR-A and the class *b* species is the RNA of transformation-defective PR-A (*td*PR-A) (25). (*C*) Electrophoresis of MC29(RPV) [^{3}H]RNA as in *A* together with the [^{14}C]RNA of RPV also propagated in quail cells. (*D*) Electrophoresis of the [^{3}H]RNA of MC29(RPV) propagated in Q5 (24) together with the [^{14}C]RNA of MC29(RPV) from Q8. (*E*) Electrophoresis of the [^{3}H]RNA of MC29(MCAV) propagated in Q10 (24) together with the [^{14}C]RNA of MC29(RPV) from Q8.

produced by the different transformed sublines of Japanese quail fibroblasts: MC29(RPV) from Q8 contained more 28S than 34S RNA (Fig. 1*A*). Virus produced by Q5 superinfected with RPV showed predominantly 34S RNA and no electrophoretically detectable 28S species (Fig. 2*D*). MC29(MCAV) obtained from Q10 contained mostly 28S and very little 34S RNA (Fig. 2*E*) but the yield of Q10 was at least 50-fold lower than that of the other quail lines, prohibiting further analysis of the viral RNA. There appeared to be a correlation between parameters of transformation in MC29-infected quail fibroblast lines and the ratio of 28S MC29 RNA to 34S helper virus RNA in the virus yield. Q10 cells grew faster and to a higher density and were more round and refractile than cells of Q8 which, in turn, appeared more transformed than those of Q5 (24).

Comparing RNase T_1-Resistant Oligonucleotides of MC29(RPV) RNA, RPV RNA, and Subgroup B Prague Strain (PR-B)RNA. The RNase T_1-resistant ^{32}P-labeled oligonucleotides of the 28S and 34S RNA species of MC29(RPV) and of the 50–70S RNA of RPV, resolved by fingerprinting, are shown in Fig. 3. Fingerprinting was by two-dimensional electrophoretic-chromatographic analyses of RNase T_1-digested RNA followed by autoradiography (15, 16). These fingerprints show that the 28S (Fig. 3*A*) and the 34S RNA (Fig. 3*B*) of MC29(RPV) have distinct sequences and that the 34S RNA of MC29(RPV) and authentic RPV RNA (Fig. 3*C*) have identical oligonucleotide patterns. These assessments were confirmed by analyses of the RNase A-resistant fragments of the large oligonucleotides (numbered in Fig. 3) of 28S and 34S MC29(RPV) RNA and of RPV RNA (Tables 1 and 2). The

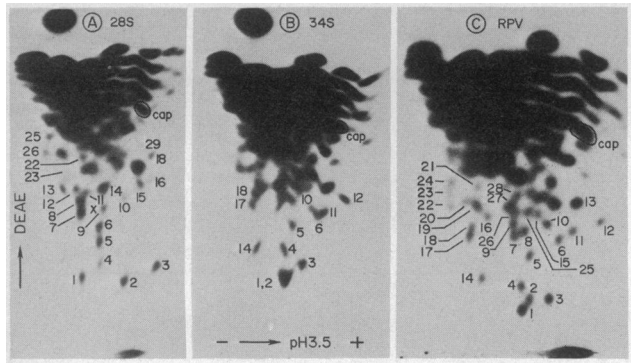

FIG. 3. Autoradiographs of RNase T_1-digested 28S (*A*) and 34S (*B*) MC29(RPV) [^{32}P]RNAs and 50–70S RPV [^{32}P]RNA (*C*) after two-dimensional electrophoresis/chromatography (fingerprint analysis). (*A* and *B*) Heat-dissociated monomers of about 30 × 10^6 cpm of 50–70S MC29(RPV) [^{32}P]RNA were resolved electrophoretically as described for Fig. 2. The radioactivity of 1-mm gel slices was determined by Cerenkov radiation with an efficiency of about 40% in plastic scintillation vials using settings optimal for ^{3}H in a Tri-Carb scintillation counter. About seven gel slices containing the 28S and 34S RNA peaks were pooled. The RNA was eluted by magnetic stirring for 12 hr at room temperature in 7 ml of 0.4 M LiCl/0.01 M Tris, pH 7.4/2 mM EDTA/0.15% $NaDodSO_4$ containing 100 μg of yeast RNA. After pelleting of the polyacrylamide at 15,000 × *g*, the supernatant contained about 80% of the radioactivity. The RNA was ethanol-precipitated after the addition of NaCl to 0.25 M and freed of soluble polyacrylamide by glycerol gradient sedimentation as in Fig. 1. Each RNA, recovered as a symmetrical 28S or 34S peak (not shown), was ethanol-precipitated with 50 μg of yeast RNA. The pellets were washed once with 75% ethanol and digested for 45 min at 40° with 5 units of RNase T_1. The digests were resolved by electrophoresis at pH 3.5 and then homochromatography on DEAE-cellulose as described (15, 16). The large oligonucleotides are numbered and their RNase-resistant fragments are reported for 28S RNA in Table 1 and for 34S RNA in Table 2. The large spot in the lower right corner is the viral poly(A). × in *A* denotes the location expected for the *src*-specific oligonucleotide 9 of PR-B (15, 16). The oligonucleotide spot marked "cap" contained the 5′-terminal 7mGpppGmC structure of the viral RNA. (*C*) The 50–70S [^{32}P]RNA of RPV, prepared as described for Fig. 1, was fingerprinted as for *A*. The RNase A-resistant fragments of the large oligonucleotides are listed in Table 2.

compositions of most RPV oligonucleotides (Table 2) were obtained from 50 to 70S RPV RNA rather than from electrophoretically prepared 34S MC29(RPV) RNA, but independent analysis of all those oligonucleotides of 34S RNA that are numbered in Fig. 3*B* showed them to have homologous counterparts in RPV RNA. Homologous oligonucleotides of 34S and RPV RNA were given the same numbers.

In order to detect common oligonucleotides and to determine whether MC29 contains *src* gene-specific oligonucleotides, the oligonucleotides of each RNA were compared to those of the other and to those of PR-B (15, 16). Several common oligonucleotides were identified and are listed by their respective numbers in Table 1. In cases in which identification was tentative, the numbers are in parentheses. MC29 RNA shared the 5′-terminal 7mGpppGmC-capped oligonucleotide with RPV RNA (marked "cap" in Fig. 3) which is different from that of PR-B RNA (30). The observation that 28S MC29 RNA shared only certain oligonucleotides with RPV RNA (e.g., no. 4 of MC29 and no. 1 of RPV) suggests that these were genuine MC29 oligonucleotides and not contaminants derived from RPV RNA. However, the 28S RNA prepared from MC29 rescued by other helper viruses needs to be examined to confirm this conclusion. The fingerprints showed that MC29 lacked the *src*-specific oligonucleotide no. 9 of PR-B (15, 16). Its expected location, estimated by triangulation with common oligonucleotides of MC29 and PR-B, is indicated by × in Fig. 3. Whether MC29 RNA contained the other *src*-specific double-oligonucleotide of PR-B (no. 12 ab) could not be determined because MC29 RNA contained, in the appropriate map position, a triple oligonucleotide, no. 14. Although all RNase A-resistant fragments of the *src*-oligonucleotides no. 12ab were found in MC29 spot no. 14, individual oligonucleotides need to be analyzed to establish difference or identity between spots.

Table 1. Composition of RNase T_1-resistant oligonucleotides[a] of 28S MC29 RNA

Spot[b]	RNase A digestion products	PR-B[c] spot	RPV[d] spot
1	2U,3C,G,2(AC),(AU),2(AAC)		
2	7U,7C,G,(AC),3(AU),(AAC),(AAU)		
3	8U,2C,G,(AAC),<1(AAAN)		
4	5U,8C,2(AC),(AU),(AAC),(AAG),(AAAN)		1
5	3U,5C,1.5(AC),(AU),(AAU),(AAAC),(A_4G)		
6	2U,8C,3(AU),(AG),(AAAU)		
7	5U,14C,G,3(AC),(AU),(AAC),(AAG)		
8	3U,6C,G,3(AC),(AG),(AAC),2(AAU),(A_5N)		
9	5U,6C,(AAG),(A_5C)	8	5
10	3U,2C,(AC),2(AU),(AG),(AAU)		
11	2U,4C,G,2(AC),(AU),(AAC)	14	
12	U,4C,2(AC),(AU),(AG),(AAC)	(15)	
13	U,3C,G,2(AC),(A_4N)		
14	12U,11C,2G,5(AC),4(AU),(AG),2(AAC)	(12ab)	(8)
15	4U,4C,G,1.5(AC),2(AU),(AAU)		
16	8U,6C,G,(AU)	11	12
18	5U,3C,G,(AU),(AAU)	(18)	
22	3U,6C,G,(AAC)		
23	U,6C,2(AC),(AU),(AG),(A_6N)		
25	8C,4(AC),(AG)		
26	5C,2(AC),(AG),(AAC)		
29	2U,C,G,3(AU),(AAU)		

[a] Elution of RNase T_1-resistant oligonucleotides from DEAE-cellulose, RNase A digestion, and electrophoretic analysis of the digests have been described (15).
[b] See Fig. 3.
[c] PR-B oligonucleotides have been described (15). Numbers in parentheses are tentative identifications.
[d] See Table 2.

Preliminary quantification of the large MC29 oligonucleotides after elution and RNase A digestion (Table 1) as well as by comparison of their autoradiographic intensities (Fig. 3) with their M_r (Table 1) suggests that most MC29-specific oligonucleotides including no. 4 were present at approximately equimolar ratios. It would follow that the complexity of the viral RNA equals its physical size (31).

Hybridization of 28S MC29 RNA with cDNA of Avian Sarcoma and Leukemia Viruses. To determine whether 28S MC29 RNA contains the *src*-specific sequences of avian sarcoma viruses and to investigate its relationship to the RNAs of helper-independent avian oncoviruses, 28S MC29 RNA was hybridized with cDNA prepared from several related viruses. All hybridizations were carried out with an excess of cDNA and at different cDNA-to-RNA ratios to reach plateau values of maximal hybridization (32). Under the conditions used, 28S MC29 RNA was 93% hybridized by homologous MC29(RPV) cDNA at an approximately 100-fold excess of cDNA to RNA (Table 3). About 61% of the RNA was hybridized at saturating concentration of PR-B cDNA. The same percentage of 28S MC29 RNA was hybridized by cDNA of *td*PR-B and a slightly higher percentage by RPV cDNA. Both *td*PR-B and RPV are known to lack *src*-specific sequences (4, 14–18, 22, 33). The

Table 2. Composition of RNase T_1-resistant oligonucleotides[a] of RPV RNA

Spot	RNase A digestion products
1	5U,8C,2(AC),(AU),(AAC),(AAG),(AAAN)
2	3U,3C,(AU),(A_8NG)
3	8U,8C,G,2(AC),2(AU),(AAC)
4	3U,6C,G,2(AC),(AU),(AAC),(AAU)
5	5U,6C,(AAG),(A_5C)
6	5U,3C,2(AC),3(AU),(AG)
7	6U,6C,G,5(AC),(AU),(AAC)
8	3U,4C,G,3(AC),2(AU)
9	4U,4C,G,(AC),(AAC),(AAAC)
10	6U,8C,G,(AU)
11	7U,4C,G,(AAC),(AAU)
12	8U,6C,G,(AU)
13	11U,4C,2G,2(AC),(AU),2(AAU),(AAAC)
14	U,6C,(AC),(AU),(AG),(A_5N)
15	4U,6C,G,(AU)
16	2U,4C,G,3(AC),(AAU)
17	2U,6C,G,3(AC),(A_5N)
18	U,5C,3(AC),(AAG)
19	5C,(AU),(AG),.5(AAAC),(A_5G)
20	2U,10C,G,3(AC),(AAG),(AAAC)
21	U,4C,(AC),(AAAG)
22	5C,G,6(AC)
23	5C,2(AC),(AG)
24	5C,2(AC),(AG),(AAC),(AAAN)
25	6U,6C,G,2(AC),(AU),(AAC),(AAG)
26	4U,2C,4(AC),(AAAG)
27	2U,C,G,4(AC),(AU),(AAU),(AAAC),(AAAN),(AAAG)
28	2U,4C,G,1.5(AC),1.5(AU),(AAC)

[a] Same as Table 1.

MC29 RNA sequences hybridized by PR-B and *td*PR-B were not additive, because a mixture of these two cDNAs did not hybridize more than each by itself (Table 3). It follows that MC29 RNA is about 61% related to the RNAs of PR-B and *td*PR-B and lacks the *src*-specific sequences of PR-B. At least 32% (i.e., 93 minus 61) of the RNA would appear to be MC29-specific. This is considered a minimal estimate because the electrophoretically or hydrodynamically purified 28S MC29 RNA includes about 20–30% of degraded 34S RPV RNA (see Figs. 1 and 2). This may also explain why the RNA hybridized about 8% better with RPV cDNA than with *td*PR-B or PR-B cDNAs and why different preparations of 28S MC29 RNA showed slightly different degrees of homologies to cDNAs as reflected by the deviations in Table 3. The failure to detect *src*-specific sequences in MC29 RNA was not due to the absence of *src*-specific cDNA in our cDNA probe. It is shown in Table 3 that our PR-B cDNA specifically hybridized 11–13% of PR-B RNA that could not be hybridized by *td*PR-B cDNA—i.e., the same sequences that were originally defined as *src*-specific (14, 18).

DISCUSSION

Defectiveness of MC29. The 28S MC29 RNA is the smallest avian tumor virus RNA observed to date. Its estimated size, 1.7 $\times 10^6$ daltons, corresponds to 5700 nucleotides of which 61% are closely related to the sequences of helper-independent avian oncoviruses, and at least 32% (1800 nucleotides) appear to be MC29-specific. Because the RNA of lymphatic leukemia viruses or *td* viruses is thought to have the minimal complexity of 8500 nucleotides needed to code for infectious progeny virus in single infection, it would follow that MC29 lacks about half of that genetic complement essential for virus reproduction. Hence,

Table 3. Hybridizations of viral RNAs with viral cDNAs

RNA	cDNA[a]	% hybridization at various cDNA/RNA ratios[b], mean ± SD 5:1	20:1	50:1	100:1
28S MC29[c]	MC29(RPV)	65 ± 6	84 ± 4	88 ± 1	93 ± 7
	RPV	46 ± 3	61 ± 0	66 ± 3	69 ± 4
	PR-B[d]	40 ± 2	54 ± 7	61 ± 8	61 ± 8
	*td*PR-B[d]	50 ± 2	55 ± 6	61 ± 6	62 ± 7
	PR-B + *td*PR-B[d,e]	50	62 ± 6	60	61
PR-B[f]	PR-B[d]			93 ± 0	93 ± 1
	*td*PR-B			81	81
	PR-B[d]			94 ± 0	92 ± 1
	*td*PR-B			79	81

[a] Viral cDNA was synthesized by the following modification of a procedure described previously (32). Purified virus (2–5 A_{260} units, measured in 0.2% sodium dodecyl sulfate at pH 7.0) was incubated at 40° for 12 hr in 1.5 ml of 3 mM Tris, pH 7.4/0.3 mM EDTA/15 mM $MgCl_2$/7 mM dithiothreitol/15–20% glycerol/actinomycin D at 30 μg/ml/0.022% Triton X-100 containing dATP, dGTP, dTTP, and dCTP at 3 mM each and supplemented with 100 μCi of [^{3}H]-dCTP to produce [^{3}H]cDNA with 50 cpm/ng. After incubation the solution was made 0.1 M in EDTA, diluted with buffered saline to 6 ml, and extracted twice with phenol. The cDNA was precipitated with two volumes of ethanol and incubated for 4 hr at 40° in 0.3 M NaOH to degrade viral RNA. It was neutralized with acetic acid, ethanol-precipitated, and redissolved in 300 μl of 0.01 M Tris, pH 7.4/1 mM EDTA/0.05% sodium dodecyl sulfate, and chromatographed on a Bio-Gel P-100 column (12 × 0.6 cm; Bio-Rad, Richmond, CA). The [^{3}H]cDNA eluted in the void volume was precipitated with carrier yeast RNA and used for hybridization. The yields of cDNA per A_{260} unit of purified virus were 0.8 μg for MC29(RPV) and 0.1–0.6 μg for RPV, PR-B, and *td*PR-B.

[b] Each reaction mixture contained about 1 ng of [^{32}P]RNA (2000 cpm/ng) and 5–100 ng of [^{3}H]cDNA (50 cpm/ng). Hybridizations were in 4 μl of 70% deionized formamide/0.3 M NaCl/0.03 M Na citrate/1.5 mM Na phosphate, pH 7.0/0.05% $NaDodSO_4$ at 40° for 12 hr. Percentage nuclease-resistance is expressed as the radioactivity recovered in aliquots digested with nuclease relative to that found in undigested aliquots. Each value is the mean of two or three experiments using, in some cases, independent preparations of RNA and cDNA. Digestion was with RNases A (5 μg/ml), T_1 (10 units/ml), and T_2 (10 units/ml) for 30 min at 40° in 0.3 M NaCl/0.03 M Na_3 citrate, pH 7.0. The background of nuclease-resistance of an aliquot heated at 100° in 0.01 M Na^+ was <0.5%.

[c] Prepared by gel electrophoresis as described for Figs. 2 and 3 or by velocity sedimentation as in Fig. 1*B*.

[d] Several preparations of cDNAs were used.

[e] Each cDNA was present at the respective excess over RNA.

[f] Shown electrophoretically to be free of *td*PR-B RNA (25).

the defectiveness of the *gag*, *env*, and possibly *pol* genes of MC29 is probably due to a full or partial deletion of these genes. Further sequence and genetic analyses would be necessary to determine the extent of the deletions in these genes.

What is the Transforming Gene of MC29? Both hybridization and fingerprint analyses failed to detect *src*-specific sequences in MC29 RNA. However, the possibility that MC29 RNA may share one or two *src*-specific oligonucleotides with PR-B was not excluded. The presence of such oligonucleotides in MC29 RNA would not be incompatible with the failure to find *src*-sequences by hybridization, because hybridization is a much less sensitive measure of differences and of small similarities between nucleic sequences than is fingerprinting. Because *src*-specific sequences are highly conserved in avian sarcoma viruses (14–18), it would appear that our failure to detect such sequences in MC29 RNA by hybridization implies that this virus has unique transforming sequences.

Those sequences of 28S MC29 RNA that are not shared with

the other avian oncoviruses tested may represent the transforming sequences and perhaps a transforming gene of MC29, analogous to the *src* gene of avian sarcoma viruses. The complexity of 1800 nucleotides of these sequences is approximately the same as that of the *src*-specific sequences of avian sarcoma viruses (14, 15, 17). However, in order to understand their genetic function, it first will be necessary to determine whether the MC29-specific sequences belong to a continuous RNA segment, as is the case for *src*-specific sequences, or are scattered in small fragments over the RNA. Hence, the transforming gene of MC29 may consist only of MC29-specific sequences, or it may consist of a particular combination of MC29-specific sequences and sequences of MC29 RNA that are shared with other avian oncoviruses. Despite this uncertainty, it would appear that the transforming gene of MC29 is distinct from the *src* genes of avian sarcoma viruses and defines a new class of avian tumor virus transformation genes.

Preliminary evidence suggests that MH2, a defective avian carcinoma virus also containing a 28S RNA species, has specific sequences that are unrelated to those of MC29 and to the *src*-specific sequences of avian sarcoma viruses.

Analogies with Defective Murine Tumor Viruses. The structure of MC29 RNA appears to be analogous to that of the defective murine sarcoma and acute leukemia viruses. The RNA species of these viruses are also smaller than those of their lymphatic leukemia helper viruses and contain specific sequences not shared with helper virus RNA (34–37). As with MC29 RNA, it is not yet clear whether the transforming genes of the murine viruses are a specific and contiguous segment of their RNA or consist of specific as well as common elements shared with helper leukemia viruses. A further analogy between the two systems is that different clonal lines of murine cells produce defective sarcoma virus–helper virus complexes with different ratios of sarcoma to helper virus RNA (38), as was observed here with MC29 virus.

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