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Structure and specific sequences of avian erythroblastosis virus RNA: Evidence for multiple classes of transforming genes among avian tumor viruses

(cloning of defective virus/RNA electrophoresis/RNA·DNA hybridization/oligonucleotide fingerprinting/*onc* genes)

KLAUS BISTER AND PETER H. DUESBERG

Department of Molecular Biology and Virus Laboratory, University of California, Berkeley, California 94720

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ABSTRACT **Two major RNA species were found in several clonal isolates of avian erythroblastosis virus (AEV) and avian erythroblastosis-associated helper virus (AEAV) complexes: one of 8.7 kilobases (kb), the other of 5.5 kb. The 5.5-kb species was identified as AEV RNA because (*i*) it was absent from nontransforming AEAV isolated from the same virus complex, (*ii*) it was present in complexes of AEV and different helper viruses, and (*iii*) its structure is similar to that of avian acute leukemia viruses of the MC29 group. Molecular hybridization indicated that 54% of AEV RNA is specific and 46% is related to other viruses of the avian tumor virus group, particularly to AEAV, therefore termed group-specific. The genetic structure of AEV RNA was deduced by mapping oligonucleotides representing specific and group-specific sequences and by comparing the resulting map to maps of AEAV and of other avian tumor viruses derived previously. AEV RNA contains a *gag* gene-related, 5′ group-specific section of 1 kb, an internal AEV-specific section of 3 kb unrelated to any other viral RNA tested, and a 3′ group-specific section of 1.5 kb. The 5′ section of AEV RNA is closely related to analogous 5′ sections of the MC29 group viruses and is homologous with a 5′ RNA section that is part of the *gag* gene of AEAV. The 3′ section is also shared with AEAV RNA and includes a variant C-oligonucleotide near the 3′ end that is different from the highly conserved counterparts of all other exogenous avian tumor viruses. By analogy with Rous sarcoma virus and the acute leukemia viruses of the MC29 group, the internal specific section of AEV RNA is thought to signal a third class of *onc* genes in avian tumor viruses. Comparisons with AEAV and the MC29 group viruses suggest that both the 5′ *gag*-related and the internal specific RNA sections of AEV are necessary for *onc* gene function.**

Avian erythroblastosis virus (AEV) has a broad oncogenic spectrum including erythroblastosis, carcinoma, and sarcoma formation in the animal and morphological transformation of chicken fibroblasts in cell culture (1–6, 9). Because AEV is defective in replicative genes of avian tumor viruses, it requires a nondefective helper virus for replication (4–6). On the basis of these properties, AEV has been classified as a defective avian acute leukemia virus (5). The oncogenic spectrum of AEV overlaps with the spectra of a group of three other avian acute leukemia viruses, named after the group's prototype MC29 (7), and also with the spectrum of avian sarcoma viruses (3, 5, 6, 8). Each of these viruses may cause sarcomas in the animal and transform fibroblasts in culture. In addition, both AEV and MC29 may cause erythroblastosis and carcinomas in the animal (3, 9). Thus, different viruses cause similar transformation, albeit with specific parameters (3). Therefore, the question as to whether the transforming *onc* gene of AEV is related to the *onc* genes of the above viruses or is AEV specific remained open.

The *onc* genes of several strains of avian sarcoma viruses have been identified by deletion and recombination analyses involving nondefective viruses and have been shown to belong to a related class of ribonucleotide sequences termed *src* genes (10–12). By contrast, the *onc* genes of acute leukemia viruses have not as yet been genetically defined because deletion and recombination analyses involving secondary biological markers could not be carried out due to the defectiveness of these viruses in all three replicative genes (5, 13). However, the genetic structure of the MC29 group viruses has been determined by identifying viral RNA and comparing it to RNAs of nondefective helper and sarcoma viruses (7, 14–16). A typical RNA measures about 6 kilobases (kb) and consists of three RNA sections: a 5′ and a 3′ group-specific section of 1.2 and 2.5 kb, respectively, which are defined as being sequence-related to nondefective viruses of the avian tumor virus group (15) and an internal specific section of 2 kb, which is unrelated to genetic elements of nondefective helper viruses and to the *src* genes of sarcoma viruses. Their internal specific sections are closely related structural trademarks of the acute leukemia viruses of the MC29 group (7, 16) and likely to encode specific oncogenic properties together with the 5′ helper virus-related RNA sections (7, 15, 24).

This investigation is to identify and isolate AEV RNA and to determine its genetic structure and sequences necessary for oncogenic function by comparing it to the RNAs of its nontransforming helper AEAV and of the MC29 group viruses.

MATERIALS AND METHODS

Virus. Stocks of AEV(AEAV) strain ES4 of subgroup B (AEAV is avian erythroblastosis-associated helper virus) were obtained from H. Hanafusa (New York) and J. H. Chen and C. Moscovici (St. Petersburg and Gainesville, FL); a stock of AEV(RAV-1) (RAV is Rous-associated helper virus) was obtained from G. S. Martin (Berkeley, CA). A focus assay of AEV(AEAV)-transformed cells under an agar overlay was as described for Rous sarcoma virus (17). After 5 days at 38°C, a second agar overlay (5 ml) was applied and foci were counted about 10 days after infection. The focus titer of our AEV(AEAV) stock was about 5×10^5. Foci were picked from under the agar overlay and seeded on chicken fibroblasts to prepare clonal colonies of AEV-infected cultures. All cultures so obtained produced virus, indicating an initial excess of helper virus in our stocks of AEV.

RNA. Preparation and analysis of radioactive viral RNA followed published procedures (7, 14–16).

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Abbreviations: AEV, avian erythroblastosis virus; AEAV, avian erythroblastosis-associated helper virus; PR-B, Prague strain of Rous sarcoma virus B; RAV, Rous-associated helper virus; MCAV, MC29-associated helper virus; RPV, ring-necked pheasant virus; td, transformation-defective; kb, kilobase.

RESULTS

RNA of AEV. To identify AEV and helper virus RNA, the monomer RNA species of different pseudotypes of AEV, propagated in chicken embryo fibroblasts, were electrophoretically analyzed. Initial analysis (not shown) indicated the presence of a helper virus-like 8.7-kb RNA species and of an avian acute leukemia virus-like (14, 15) 5.5-kb RNA species present at a molar ratio between 4 and 6 in AEV(AEAV) stocks and at molar ratios of over 10 in AEV(RAV-1) stocks. To obtain an AEV pseudotype in which the 5.5-kb RNA species was present at near equimolar ratio, as is necessary for biochemical analysis (14), clonal colonies of transformed cells were prepared by infecting chicken embryo fibroblast cultures with foci of AEV-transformed cells, picked from cultures infected at low multiplicity of infection. Electrophoretic analysis of monomer RNA species of AEV(AEAV) clone 78-3 is shown in Fig. 1*A*. It can be seen that the large RNA species migrated slightly slower than the 8.5-kb RNA of MC29-associated helper virus (MCAV) and that the smaller RNA species migrated slightly ahead of the 5.7-kb MC29 RNA standard (14, 15). On the basis of their electrophoretic mobilities relative to those of the MC29 standards, the large RNA species of AEV(AEAV) was estimated to measure 8.7 kb and the smaller one to measure 5.5 kb (18). The molar ratio of the 8.7-kb to 5.5-kb species was about 0.5 in this stock and varied between 0.5 and 2 in five other clonal stocks of AEV(AEAV) analyzed under the same conditions (not shown). The molar ratios of 8.7-kb to 5.5-kb RNA were much higher in six clonal isolates of AEV(RAV-1), ranging between 3 (Fig. 1*B*) and 5 (others not shown). It would appear that the ratio of the two RNA species in AEV pseudotypes varies with different infections of the same AEV pseudotype, but is also influenced by specific combinations of AEV and helper virus. AEAV, obtained by serial dilution of AEV(AEAV) beyond the end-point of fibroblast-transforming activity (19), contained only 8.7-kb RNA (Fig. 1*C*). On this basis, and by analogy with the RNAs of MC29 group viruses (7, 14–16), it is concluded that the 8.7-kb RNA species is the RNA of AEAV and that the 5.5-kb species is the RNA of AEV.

Specific and Group-Specific Sequences of AEV RNA. The 5.5-kb AEV RNA and 8.7-kb AEAV RNA were prepared electrophoretically (Fig. 1*A*) (14, 15) and digested with RNase T1. The T1 oligonucleotides were analyzed by two-dimensional fingerprinting. It can be seen in Fig. 2 that AEV RNA contained about 24 and AEAV RNA about 35 large T1 oligonucleotides.

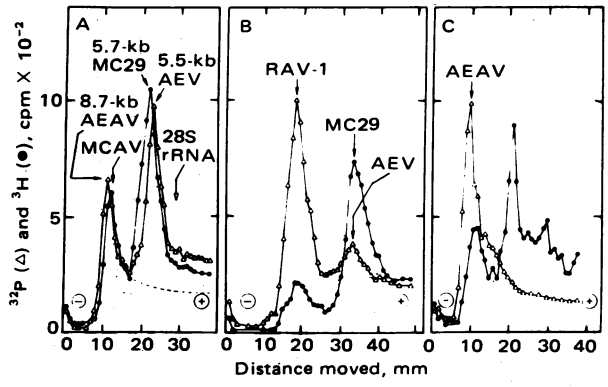

FIG. 1. RNA monomers of AEV(AEAV) (Δ, *A*), AEV(RAV-1) (Δ, *B*), and AEAV (Δ, *C*) after electrophoresis in 2% polyacrylamide gels, in the presence of MC29(MCAV-A) (●) RNA standards. Preparation of 50–70S RNA complexes, heat dissociation into RNA monomers, and electrophoretic conditions are described in the text and in previous publications (7, 14–16). Sizes of viral RNAs in kb were estimated (18) from electrophoretic mobilities relative to the known standards (14, 15). The broken line in *A* is a hypothetical base line of degraded AEAV RNA under the peak of 5.5-kb AEV RNA.

Their compositions in terms of RNase A-resistant sequences are reported in Table 1. The fingerprint of AEAV obtained by endpoint dilution was as in Fig. 2*A*. AEV contained 14 specific oligonucleotides and shared 10 oligonucleotides with AEAV RNA which by definition (15) represent group-specific sequence elements of AEV RNA. None of the AEV-specific oligonucleotides (Fig. 2*B*) had homologous counterparts in the RNAs of viruses from the MC29 group or in the RNAs of avian sarcoma viruses, indicating that the specific sequences of AEV are unrelated to those of these viruses (Table 1). Shared oligonucleotides of AEV and AEAV RNAs were given identical numbers (Fig. 2, Table 1). Some, but not all, AEAV-specific oligonucleotides were also present in the fingerprint of AEV RNA at low molar ratios compared to the major AEV oligonucleotides (Fig. 2*B*). They were parenthesized and numbered as in Fig. 2*A*. These probably reflect degraded AEAV RNA coelectrophoresing with AEV RNA. This contamination of AEV RNA was estimated as 25% from the hypothetical distribution of degraded AEAV RNA in Fig. 1*A* (broken line) and from quantitation (not shown) of AEAV-specific oligonucleotides in AEV RNA. Fingerprint analyses of the RNAs of AEV-(RAV-1) indicated that the 5.5-kb component was closely related to its counterpart in Fig. 2*B*, whereas the 8.5-kb RAV-1 RNA component was different from 8.7-kb AEAV RNA (not shown). This confirmed that the 5.5-kb species is the RNA of AEV.

To determine whether AEV contains RNA sequences that are more distantly related to the specific sequences of the MC29 group or to *src* than would be detected by the criterion of shared T1 oligonucleotides (a criterion that is sensitive to single base changes), AEV RNA was hybridized to cDNAs of these viruses. The cDNAs used here are the same as those described previously and hybridized about 90% of their original RNA templates under our conditions—i.e., at 20- to 80-fold excess over RNA (14–16). About 55% of AEV RNA was hybridized by cDNA of Prague Rous sarcoma virus B (PR-B) or by transformation-defective (td) PR-B, which lacks the *src* gene of avian sarcoma viruses (10), indicating that AEV lacks *src*-related sequences. In addition, no more AEV RNA was hybridized by a combination of PR-B and MC29 [ring-necked pheasant virus (RPV)] cDNAs than was hybridized by each cDNA alone (Table 2), which indicates that no sequences related to the specific sequences of the MC29 group are present in AEV RNA. If present in 5.5-kb AEV RNA, the *src* sequences (1.5 kb; ref. 10) or the specific sequences of the MC29 group (2 kb; refs. 7 and 14–16) would have represented at least 25% of AEV RNA and would have been detected by our assay. The T1 oligonucleotides of AEV RNA that were hybridized by PR-B cDNA (nos. 102, 108, 113, 114, and 117) (Fig. 2*C*) and those that were hybridized by td PR-B or PR-B and MC29(RPV) cDNAs were identical (not shown) and were a subset of those that AEV shares with AEAV RNA (Fig. 3). As expected, the hybrid analyzed in Fig. 2*C* also included AEAV-specific oligonucleotides from AEAV RNA contaminating our AEV RNA. These experiments confirm that AEV and the above viruses share group-specific sequences and that AEV is more closely related to AEAV than it is to PR-B, td PR-B, and MC29(RPV), because fewer oligonucleotides were recovered than are shared with AEAV.

The maximal percentage of group-specific sequences in AEV RNA was estimated at 46% on the following basis: Our preparation of AEV RNA was hybridized 55% by PR-B and td PR-B cDNAs (Table 2). Because this RNA was about 25% contaminated by AEAV RNA, which hybridized 82% with the same cDNAs (Table 2), we calculate that pure AEV RNA was 46% hybridized. Again, this result is in agreement with the finding that 10 out of 24 AEV oligonucleotides were shared with AEAV and some of these also with PR-B and MC29(RPV) and thus represent group-specific sequences of AEV RNA.

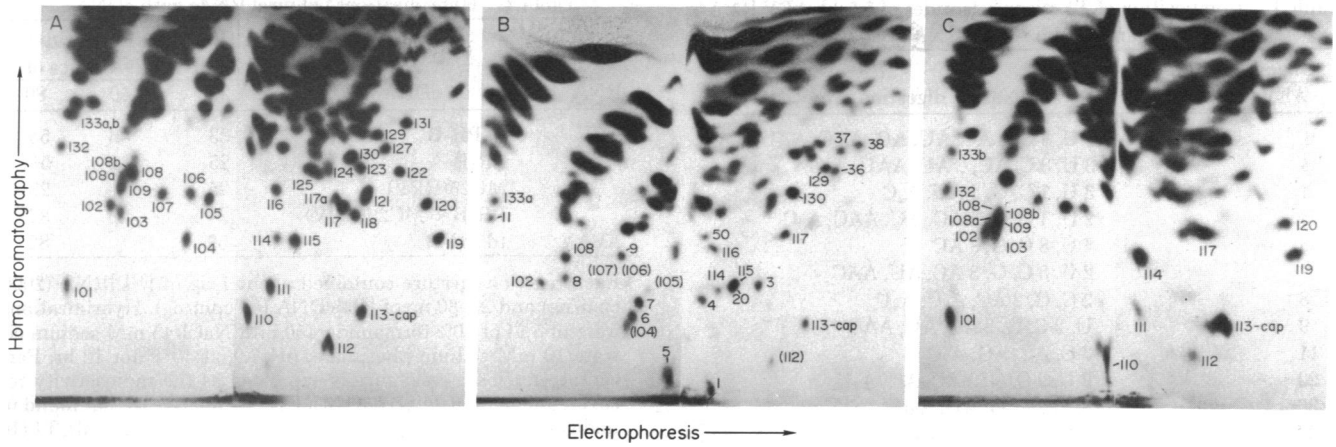

FIG. 2. Electrophoretic–chromatographic fingerprint analyses of RNase T1-digested 8.7-kb (*A*) and 5.5-kb (*B*) [^{32}P]RNA components isolated from AEV(AEAV), and of RNA isolated from a hybrid formed between 5.5-kb AEV RNA and cDNA from the Prague strain of Rous sarcoma virus B (PR-B) (*C*). (*A, B*) The 8.7- and 5.5-kb RNAs of AEV(AEAV) were prepared electrophoretically as described for Fig. 1 and fingerprinted by using a pH 2.5 formic acid buffer in the electrophoretic dimension (20) and otherwise as described previously (7, 14–16). Numbers refer to T1 oligonucleotides whose RNase A-resistant fragments were determined (Table 1). AEAV oligonucleotides thought to derive from degraded AEAV RNA contaminating our preparation of 5.5-kb AEV RNA are in parentheses in *B*. (*C*) Hybridization of 0.1 μg of 5.5-kb AEV [^{32}P]RNA (0.5×10^6 cpm) with 1 μg of PR-B cDNA was in 50% formamide/0.45 M NaCl/0.045 M sodium citrate/0.01 M sodium phosphate, pH 7.0, at 40°C for 8 hr and otherwise as described (14–16). Unhybridized RNA was digested for 30 min in 200 μl of 0.15 M NaCl/0.015 M sodium citrate containing 5 units of RNase T1 and was then separated from hybrid by chromatography on a Bio-Gel P-150 (Bio-Rad) column (15 × 1 cm) in 0.1 M NaCl/0.01 M Tris, pH 7.4/1 mM EDTA/0.1% sodium dodecyl sulfate/0.1% β-mercaptoethanol. The hybrid eluted in the void volume was extracted with phenol, precipitated, and, after heat dissociation, digested with RNase T1 and fingerprinted as for *A* and *B*.

Oligonucleotide Maps of AEV and AEAV RNA Compared. To determine the distribution in AEV RNA of AEV-specific oligonucleotides and oligonucleotides that are shared with AEAV RNA and with RNAs of other avian tumor viruses mapped previously (7, 11, 12, 15, 21, 22), oligonucleotide maps of AEV and AEAV RNAs were prepared. The order of the oligonucleotides relative to the 3′-poly(A) coordinate of viral RNA was deduced from the size of the smallest poly(A)-tagged RNA fragment in which a given oligonucleotide could be detected by fingerprinting (21). The resulting AEV map showed an internal cluster of 14 AEV-specific oligonucleotides (hatched in Fig. 3) between 1.5 and 4.5 kb. The external 1-kb 5′ and 1.5-kb 3′ map segments of AEV RNA had homologous counterparts in analogous map locations in AEAV RNA (Fig. 3, Table 1). All oligonucleotides of the 5′ map segment of AEV also had counterparts in the 5′ map segments of the MC29 group (Table 1, refs. 7, 15, and 16). This segment also included oligonucleotide no. 114, which is associated with the *gag* genes of PR-B and of other nondefective avian tumor viruses (22, 23) (Table 1). The 3′ group-specific map segment of AEV RNA did not share a large T1 oligonucleotide with the 3′ segments of other acute leukemia viruses of the MC29 group or with known nondefective viruses other than AEAV. However, the map location and composition of no. 108, the 3′-most oligonucleotide of AEV RNA, suggests that it is a variant of the C-oligonucleotide (21), which is highly conserved among all exogenous avian tumor viruses (7, 15, 21, 22).

It is shown in Fig. 3 that AEAV RNA contained a large internal map segment that is not shared with AEV RNA. This segment of AEAV RNA shared with nondefective avian tumor viruses mapped previously (7, 11, 12, 21, 22) highly conserved oligonucleotides of *pol* and *env* genes and probably also conserved *gag*-oligonucleotides not shared with AEV. This result explains the defectiveness of AEV in these genes.

DISCUSSION

Genetic Structures of AEV and of MC29 Group Viruses Are Related. Our results indicate that the RNA of AEV is a 5.5-kb polynucleotide that consists of an internal specific section flanked by two external group-specific sections. The 5′ group-specific section of AEV RNA is *gag* gene related and also closely related to the 5′ sections of the viruses of the MC29 group. The 3′ group-specific section of AEV is identified as group-specific by its close relationship to AEAV, with which it shares all T1 oligonucleotides, and by a variant C-oligonucleotide (no. 108), which has a closely related counterpart, C, in all exogenous avian tumor viruses studied (7, 15, 21–23). In addition, the 3′ section is indirectly identified as group-specific by hybridization with cDNAs of other viruses, which hybridize more of AEV RNA (45%) than would be expected from a shared 5′ section of 1 kb (about 20%). However, the hybrids formed between the 3′ section of AEV RNA and cDNAs of PR-B and MC29(RPV) were apparently not matched well enough to allow large T1 oligonucleotides other than the C-oligonucleotide, no. 108, to survive the RNase T1 treatment used here to remove unhybridized RNA (Fig. 2*C*). Thus, the 3′ section of AEV RNA is not closely related to the avian tumor viruses tested, except AEAV. This appears typical also of the acute leukemia viruses of the MC29 group, which have 3′ sections that are not closely related to each other but are closely related to RNAs of their helper viruses (7, 24). Hence, the basic genetic structure of AEV RNA is very similar to that of the acute leukemia viruses of the MC29 group. However, the internal RNA sequence of AEV is unrelated to, and about 1 kb larger than, the conserved, internal 2-kb sequences of the MC29 group viruses. It is not yet clear whether AEV RNA contains *env*-related sequences as the MC29 group viruses do. Conceivably, the specific sequence of AEV is larger than that of MC29 because it includes *env*-sequences shared with presently unknown viruses.

The finding of isogenic 5′ and 3′ RNA sections in AEV and AEAV (Fig. 3) suggests that each of these viruses may have been generated from the other by an internal substitution. The internal section of AEV could have been picked up by a hypothetical AEAV parent from an unknown viral or cellular source. The internal section of AEAV could have been picked up by a hypothetical AEV parent from any nondefective avian tumor virus.

The Transforming Gene of AEV. A definition of the *onc* gene of AEV can be derived by identifying nucleotide sequence elements that correlate with oncogenic properties. In the ab-

Table 1. Composition of T1 oligonucleotides of 5.5-kb AEV RNA and 8.7-kb AEAV RNA

Oligonucleotide no. AEV	Oligonucleotide no. AEAV	RNase A digestion products
1		3 U, 5 C, 2 AC, AU, AG, AAC, AAU, A_3U
3		3 U, 3 C, AC, 2 AU, AAU, A_3G
4		3 U, 7 C, G, 2 AU, A_4C
5		2 U, 4 C, G, 2 AC, AU, AAC, A_3C
6		4 U, 8 C, G, 3 AC
7		2 U, 5 C, G, 3 AC, AU, AAC
8		2 C, G, 2 AC, A_3C, A_4U
9		U, 2 C, G, 2 AC, AU, AAC
11		3 C, AC, AG, A_3C
20		2 U, 2 C, G, 2 AC, AU, A_4U
36		3 U, 2 C, G, AU, A_3U
37		4 U, 2 C, 2 AU, AG
38		5 U, C, AG, AAC
50		3 U, 6 C, G, AC, AU
	101†	C, G, AC, A_4C, A_5C
102*†	102*†	U, 4 C, G, 2 AC, A_4C
	103	4 C, 2 AC, AU, A_4G
	104*†	2 U, 8 C, G, 3 AC, AU
	105‡	U, 4 C, G, 2 AC, AU, AAC
	106	U, C, G, 2 AC, AU, A_5C
	107*	U, 3 C, 2 AC, AU, AG, AAC
108§	108§	G, 2 AC, AAU, A_3C
	108a	U, 4 C, AC, AG, AAC
	108b†‡	3 C, AU, AG, A_5C
	109*†	U, 6 C, 3 AC, AAG
	110‡	4 U, 8 C, 1.5 AC, AU, AAC, AAG
	111†‡	4 U, 7 C, G, AC, AU, A_3C
	112	7 U, 5 C, G, AC, 2 AU, 2 AAC, AAU
113-cap*†‡	113-cap*†‡	6 U, 6 C, G, 3 AC, 2-3 AU, $m^7GpppGmpC$
114*†‡	114*†‡	4 U, 4 C, AAG, A_4C
115	115	U, 3 C, 2 AU, AG, AAU, A_3U
116	116	2 U, 2 C, G, 2 AC, 2 AAU
117*†‡	117*†‡	5 U, 8 C, G, AU
	117a	4 U, 3 C, G, 2 AC, AU
	118	4 U, 2 C, G, 2 AC, 3 AU
	119†‡	6 U, 2 C, G, 3-4 AU, AAU
	120†‡	8 U, 6 C, G, AU
	121¶	9 U, 4 C, 2 G, 2 AC, AU, AAC, 2 AAU
	122*†‡	5 U, 4 C, G, AU, AAU
	123	6 U, 6 C, G, AC, AU
	124	3 U, 3 C, G, AC, 2 AU
	125¶	5 U, 8 C, 2 G, AC, 1.5 AU, AAU
	127†	2 U, C, G, 2 AU, AAU
129	129	4 U, 2 C, G, AU, AAC
130¶	130¶	4 U, 4 C, AC, 3 AU, AG, AAU, AAG
	131	4 U, 2 C, G, 2 AU
	132	4 C, G, 3 AC
133a	133a	2 C, G, 3 AC
	133b	2 C, AC, AG, AAC

* Shared with defective acute leukemia viruses of the MC29 group (7, 14–16, 24).
† Shared with nondefective helper viruses of the MC29 group (7, 14–16, 24).
‡ Shared with PR-B (21–23).
§ Related to the C-oligonucleotide of exogenous avian tumor viruses (23).
¶ Combined composition of unresolved double oligonucleotides.

sence of *onc* deletion mutants, comparison of AEV with AEAV, which lacks the oncogenic properties of AEV, identifies the internal specific sequence of AEV RNA as a specific determinant of *onc* gene function. Further comparisons among AEV and the structurally and functionally related viruses of the MC29 group can be used in place of as-yet-unavailable AEV

Table 2. Hybridizations* of viral RNAs with cDNAs

RNA†	cDNA‡	% of RNA in hybrid§ at cDNA/RNA ratios of: 20	40	80
AEV	td PR-B	33	56	55
	PR-B	25	41	56
	MC29(RPV)	25		28
	PR-B + MC29(RPV)	24	40	37¶
AEAV	td PR-B	59		82

* Each reaction mixture contained about 1 ng of [^{32}P]RNA (2000 cpm/ng) and 20–80 ng of [^{3}H]cDNA (50 cpm/ng). Hybridizations were in 5 μl of 50% formamide/450 mM NaCl/45 mM sodium citrate/10 mM sodium phosphate, pH 7.0, at 40°C for 12 hr. Percentage nuclease resistance is expressed as the radioactivity recovered in aliquots digested with RNases relative to that found in undigested aliquots. Digestion was with RNases A (5 μg/ml), T1 (10 units/ml), and T2 (10 units/ml) for 30 min at 40°C in 0.15 ml of 300 mM NaCl/30 mM sodium citrate, pH 7.0, and otherwise as described (14).
† RNAs were prepared as described for Fig. 1 and in refs. 14–16.
‡ cDNAs were the same as those described previously (14) or were prepared in the same way.
§ Numbers are normalized based on the 90% efficiency of our cDNAs in hybridizing to their original RNA templates (14).
¶ At very high cDNA concentrations, hybridization of RNA decreased in this and other experiments.

recombinants to define sequences correlating with *onc* function. From such comparisons, we deduce that the 5′ *gag*-related RNA section shared among these viruses corresponds to essential nonspecific elements of *onc*. The internal, MC29 group-specific and AEV-specific RNA sections would correspond to essential specific determinants of *onc* and would explain differences between the oncogenic spectra of AEV and the viruses of the MC29 group. Although the 3′ group-specific RNA section of AEV is distinct from related counterparts in the MC29 group viruses, it is assumed to be analogous to them and therefore not likely to be a specific element of *onc*. The 3′ RNA sections of MC29 group viruses are thought to play indirect or no roles in oncogenicity, because they are variable and altered readily by recombination with helper viruses (7, 24). Genetic variants of AEV need to be studied to confirm this for AEV. However, the above comparison with AEAV supports this view and has already shown that the 3′ section of AEV RNA is not a specific determinant of *onc*.

We conclude that the *onc* gene of AEV includes sequence elements of the 5′ *gag*-related and the internal specific sections of AEV RNA. The notion that both the 5′ *gag*-related and the internal specific RNA sections of AEV and of the MC29 group viruses are necessary for oncogenicity is consistent with the findings that these RNA sections together code for presumably transforming, nonvirion, *gag*-related proteins (7, 15, 25, 26).

Multiple Transforming Genes in Avian Tumor Viruses. Two classes of transforming *onc* genes have been distinguished in avian tumor viruses: the *src* genes of sarcoma viruses, and the *onc* genes of the MC29 group diagnosed by their related specific sequences (7, 14–16, 27). Because the specific sequence of AEV is unrelated to the sequences of the other two groups of *onc* genes, it represents a third class of transforming gene. An additional class of specific sequences may be expected in avian myeloblastosis virus, which differs from the viruses compared here in its inability to transform fibroblasts in cell culture (3, 8). Hence, we conclude that multiple transforming genes exist in the avian tumor virus family.

The genetic structures of defective murine sarcoma and acute leukemia viruses also suggest that multiple *onc* genes exist in the murine tumor virus family. Like the avian viruses studied here, the RNAs of the defective transforming murine viruses range between 5 and 7.5 kb in size and contain external helper

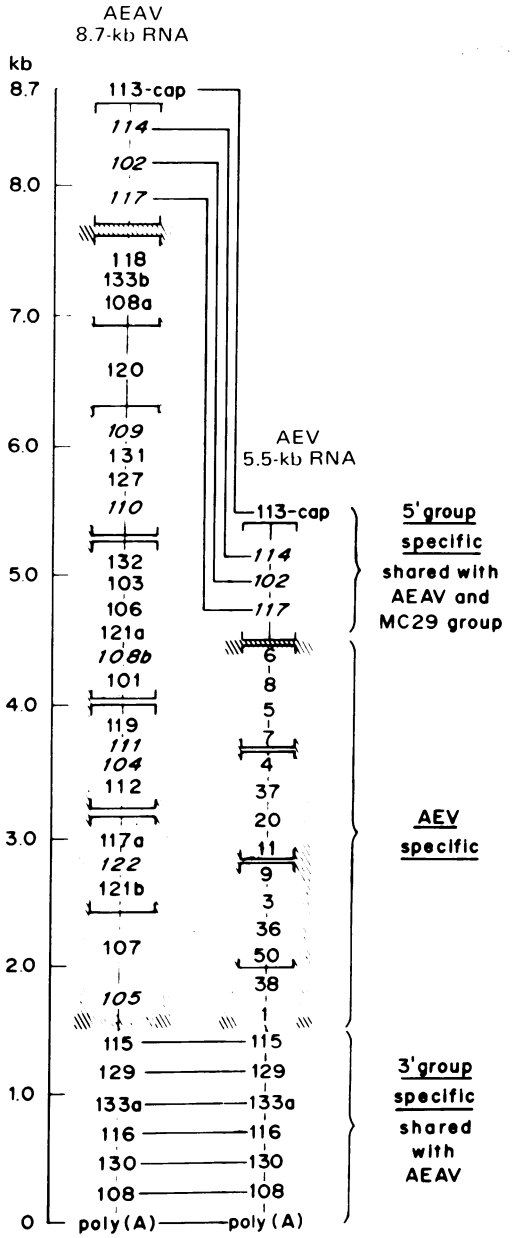

FIG. 3. Oligonucleotide maps of 8.7-kb AEAV and 5.5-kb AEV RNAs. AEV and AEAV RNAs were prepared electrophoretically from AEV(AEAV) as described for Fig. 1A. AEAV RNA was also obtained as 70S RNA complex from AEAV isolated from AEV(AEAV) by end-point dilution. The RNAs were degraded by incubating two equal aliquots for 1 and 4 min, in 0.05 M Na_2CO_3 at pH 11 and 50°C. Fragments were combined and neutralized with acetic acid, and poly(A)-tagged species were selected on oligo(dT)-cellulose, fractionated into discrete size classes, and fingerprinted as described (7, 15, 21). The resulting order of oligonucleotides, numbered as in Fig. 2 and Table 1, relative to the 3'-poly(A) coordinate of viral RNA, is plotted. AEV-specific oligonucleotides are on a hatched background and identified by one- and two-digit numbers. Group-specific AEV oligonucleotides that are shared with AEAV or PR-B and MC29(RPV) are identified by three-digit numbers and linked to their counterparts in AEAV RNA by solid lines. AEAV oligonucleotides not present in AEV RNA are on a hatched background. Oligonucleotides conserved for the *gag/pol* genes (nos. 114, 102, 117, 109, 110, 108b) and the *env* gene (nos. 111, 104, 122, 105) of avian tumor viruses (refs. 11, 12, and 23) are shown in italics.

virus-related sequences and at least four distinguishable classes of internal specific sequences (20,28–33).

Although the oncogenic spectra of AEV, MC29, and Rous sarcoma virus show considerable overlap (introduction), we find that each of these viral prototypes has a distinct *onc* gene. This implies that the functions of viral *onc* genes, their cellular targets, or both must not be very specific and may interact in cells of various differentiated states.

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