

Genetic structure of avian myeloblastosis virus, released from transformed myeloblasts as a defective virus particle

(RNA and protein electrophoresis/oligonucleotide mapping/RNA-cDNA hybridization/unique c-region/specific internal RNA sequence)

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ABSTRACT Chicken myeloblasts transformed by avian myeloblastosis virus (AMV) in the absence of nondefective helper virus (termed nonproducer cells) were found to release a defective virus particle (DVP) that contains avian tumor viral *gag* proteins but lacks envelope glycoprotein and a DNA polymerase. Nonproducer cells contain a Pr76 *gag* precursor protein and also a protein that is indistinguishable from the Pr180 *gag-pol* protein of nondefective viruses. The RNA of the DVP is 7.5 kilobases (kb) long and is 0.7 kb shorter than the 8.2-kb RNAs of the helper viruses of AMV, MAV-1 and MAV-2. Comparisons based on RNA-cDNA hybridization and mapping of RNase T1-resistant oligonucleotides indicated that DVP RNA shares with MAV RNAs nearly isogenic 5'-terminal *gag* and *pol*-related sequences of 5.3 kb and a 3'-terminal c-region of 0.7 kb that is different from that found in other avian tumor viruses. Adjacent to the c-region, DVP RNA contains a contiguous specific sequence of 1.5 kb defined by 14 specific oligonucleotides. Except for two of these oligonucleotides that map at its 5' end, this sequence is unrelated to any sequences of nondefective avian tumor viruses of four different envelope subgroups as well as to the specific sequences of fibroblast-transforming avian acute leukemia and sarcoma viruses of four different RNA subgroups. The specific sequence of the DVP RNA is present in infectious stocks of AMV from this and other laboratories in an AMV-transformed myeloblast line from another laboratory, and it is about 70% related to nucleotide sequences of E26 virus, an independent isolate of an AMV-like virus. Preliminary experiments show DVP to be leukemogenic if fused into susceptible cells in the presence of helper virus. We conclude that DVP RNA is the leukemogenic component of infectious AMV and that its specific sequence, termed AMV, may carry genetic information for oncogenicity. Thus we have found here a transformation-specific RNA sequence, unrelated to helper virus, in a highly oncogenic virus that does not transform fibroblasts.

RNA of sarcoma and acute leukemia viruses, which transform fibroblasts in cell culture, contains an internal specific sequence in addition to terminal sequences that are allelic with the essential virion genes of a given group of retroviruses and therefore termed group-specific (1). The internal specific RNA sequences are the structural hallmark of the fibroblast-transforming retroviruses and code alone or together with various amounts of group-specific sequences for nonstructural proteins thought to have transforming function (1, 2).

To date, contiguous specific nucleotide sequences that are not allelic with sequences of helper viruses have not been identified in the RNAs of acute leukemia viruses that fail to transform fibroblasts, such as the avian myeloblastosis virus (AMV) and the murine Friend virus (3). The replication-defective leukemogenic component of AMV has not yet been identified or isolated free of helper virus (4, 5). Major obstacles are that in typical infectious AMV stocks helper viruses are present at a large excess over the defective AMV (5, 6), which

complicates biochemical and biological analysis of AMV. Nevertheless specific sequences that were not found in isolated helper viruses were detected in several stocks of infectious AMV by subtractive hybridization of AMV cDNA with helper viral RNA (6, 7) or by restriction enzyme analyses of proviral DNA from virus-infected cells (8). These presumably AMV-specific nucleotide sequences have been associated with a 7.8-kilobase (kb) RNA (6) or proviral DNA (8) component that was only 5-10% smaller than the 8- to 8.5-kb helper viral RNA or DNA components (1). However, because (i) a contiguous nucleotide sequence that is not allelic with helper viral RNA was not identified in AMV, (ii) the complexity of the AMV-specific sequences was not determined, and (iii) obviously each distinct viral strain, defective or nondefective, must have specific sequence elements detectable by the above techniques (1), these results are not definitive proof for the presence in AMV of a specific sequence analogous to the sequences found in fibroblast-transforming viruses. Moreover, because the RNAs of known defective, avian acute leukemia viruses are over 30% smaller (5-6 kb) than those of their helper viruses (1, 2), the small size difference reported between the specific RNA (6) or DNA (8) component from AMV stocks and those of respective helper viral RNAs or DNAs is not sufficient to identify the specific component as the genome of a defective virus.

RESULTS

DVP Released by AMV-Transformed Nonproducer Cells. Three lines of AMV-transformed nonproducer chicken myeloblasts, which do not release infectious virus or virus particles with functional DNA polymerase (5, 9), were chosen to study AMV free of associated helper viruses. Transformation of these lines is confirmed by the criteria that they are capable of growing but are unable to undergo terminal differentiation in cell culture and that they induce myeloblastosis when inoculated into chicken together with (but not without) helper viruses (unpublished data). Initial analyses of these cells showed that the *gag*-gene precursor protein Pr76 and a protein electrophoretically and serologically indistinguishable from the polymerase precursor Pr180 were present (Fig. 1 A and B). (Small amounts of a *gag* and *pol* gene-related protein of 150,000 daltons were occasionally observed in producer and nonproducer myeloblasts infected by AMV or E26.) To verify that these cultures were indeed nonproducers, we have examined whether virus particles are released. It was found that [³H]-uridine-, ³²P₀₄-, or [³⁵S]methionine-labeled ribonucleoprotein particles banding in sucrose density gradients between 1.15 and 1.19 g/ml with noncoinciding peaks between 1.160 and 1.175

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Abbreviations: AMV, avian myeloblastosis virus; kb, kilobase(s); DVP, defective virus particle; MAV, myeloblastosis-associated virus; RSV, Rous sarcoma virus; PR RSV-C, Prague C strain of RSV; SR RSV, Schmidt-Ruppin strain of RSV; RPV, ring-necked pheasant virus.

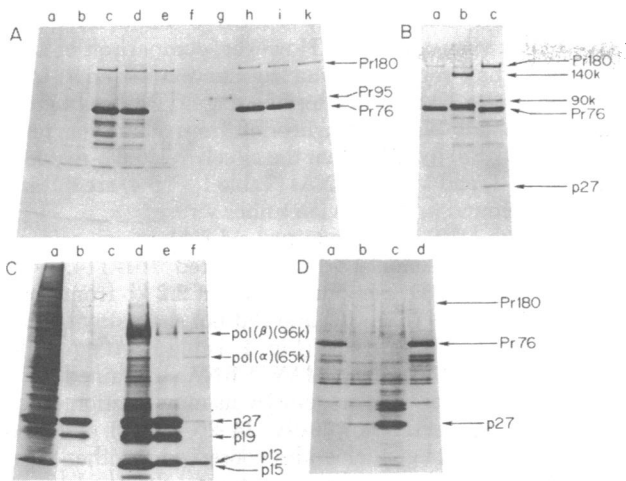


FIG. 1. Avian tumor viral proteins associated with the DVP and with AMV(MAV-2) and with myeloblasts releasing DVP or AMV(MAV-2). Labeling of cells and virus with [³⁵S]methionine, preparation of cellular lysates, immunoprecipitation, gel electrophoresis in sodium dodecyl sulfate, 6–18% polyacrylamide gradient gels, and fluorography were carried out as described (2, 10, 11). (A) Intracellular viral proteins in myeloblasts producing DVP (a–e) or AMV(MAV-2) (f–k). Labeling with [³⁵S]methionine was for 20 min, and immunoprecipitation from cellular lysates was with the following rabbit sera: normal serum (a, f), serum against envelope glycoproteins of the Prague C strain of Rous sarcoma virus (PR RSV-C) (b, g), serum against Nonidet P40-disrupted PR RSV-C (c, h), serum against p27 and p19 gag proteins (d, i), and serum against viral DNA polymerase (e, k). (B) Size comparison (molecular weights in thousands) of gag and pol gene-related proteins in DVP-releasing myeloblasts (a) to those in fibroblasts infected with sarcoma virus FSV(FAV) (12) (b) or leukemia virus CMII(CMIIAV) (11) (c). Immunoprecipitation was with serum against disrupted PR RSV-C. (C) Proteins associated with the DVP (a–c) or AMV(MAV-2) virions (d–f), purified from the culture fluid of the chase experiments described below, were analyzed in total (a, d), and after immunoprecipitation with antiserum against Nonidet P40-disrupted PR RSV-C (b, e) or against viral DNA polymerase (c, f). Positions and molecular weights (in thousands) of polymerase α and β chains are indicated. (D) Pulse-chase analysis of viral protein synthesis in myeloblasts releasing DVP (a, d) or AMV(MAV-2) (b, c). Proteins were immunoprecipitated from lysates of cells labeled for 2 hr with [³⁵S]methionine (c, d) or labeled for 2 hr and chased with complete medium for 16 hr (a, b). The relative amount of cellular lysate used was increased 2-fold in a and b.

g/ml could be isolated from the culture media of each of these cell lines by the same procedures that are used for purification of infectious virus (13) (Fig. 2A). The particles contained among other nonviral proteins the gag proteins p27, p19, p15, and p12 (Fig. 1C) of infectious avian tumor viruses (14). However, the particles lacked detectable polymerase protein (Fig. 1C), even after prolonged autoradiography, and activity as assayed by using either the endogenous reaction or poly(A)-oligo(dT) as template. The particles also failed to incorporate detectable [³⁵S]methionine (Fig. 1C) or [³H]glucosamine or [³H]fucose (Fig. 2A) into viral glycoprotein under conditions that showed positive results with virus released by myeloblasts producing AMV(MAV-2) (MAV, myeloblastosis-associated virus). The particles were not found to be infectious when plated on chicken embryo cell cultures. It is concluded that the ribonucleoprotein is a defective avian tumor virus particle (DVP). Judged on the basis of the amounts of the three isotopes incorporated into DVP (Fig. 2A) per isotope added to a culture, DVP production was 5–10% of AMV(MAV-2) production by myeloblasts (not shown). The noncoincidence of the ³⁵S and ³²P peaks with the [³H]uridine peak (Fig. 2A) reflects contamination of DVP with cellular protein (Fig. 1C, lane a) and probably cellular phospholipid material, which would scarcely be detected under the higher virus peaks from producer cells.

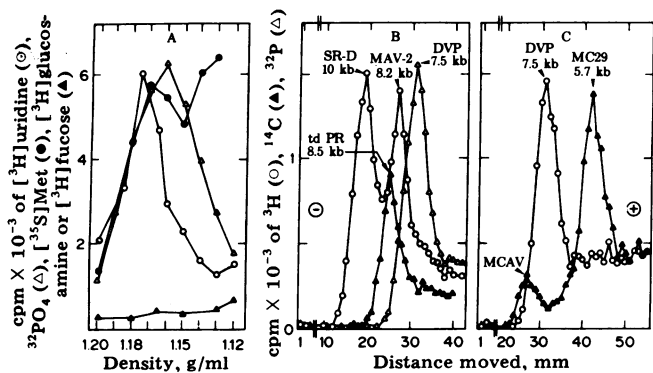


FIG. 2. Density and RNA and protein components of the DVP (A) and electrophoretic size analysis of DVP RNA (B and C). (A) Distribution of [³H]uridine (○), ³²PO₄ (△), [³⁵S]methionine (●), and [³H]glucosamine or [³H]fucose (▲) associated with the DVP in a sucrose density gradient (20–60% wt/vol) prepared as described (13). AMV-transformed nonproducer myeloblast lines releasing DVP were propagated in F10 medium supplemented with 10% tryptose phosphate broth, 5% chicken serum, 5% calf serum, and 1.6 μ g of folate per ml in 75-cm² plastic flasks containing 36 ml of medium. Media were changed at 3-day intervals. Line 727 grew in suspension to a density of about 5×10^6 cells per ml, line 5YS (used in most experiments) and line BM-2 grew as near-confluent monolayers of adherent cells and cell clumps in addition to cells suspended in medium. Labeling with [³H]uridine, [³H]glucosamine or [³H]fucose, and [³⁵S]methionine was as described (10, 11, 13–15). Propagation of ³²P-labeled DVP was in six to eight plastic flasks each containing about 150×10^6 cells in 15 ml of PO₄-free medium supplemented with 6–8 mCi (1 Ci = 3.7×10^{10} becquerels) of H₃³²PO₄ and 10% PO₄-free chicken serum and folate. After 12 hr at 40°C, cells were pelleted and relabeled for two additional 12-hr intervals in the same media. H₃³²PO₄ uptake ranged from 30% to 50% during the first interval and declined to 20–30% during the last interval. Virus was purified by centrifuging media for 2 hr at $36,000 \times g$ at 20°C and banding the resulting pellets in 20–60% sucrose density gradients (13–15). (B and C) Electrophoresis of the heat-dissociated RNA of the DVP and of MAV-2 in 2% polyacrylamide gels in the presence of 10-kb RNA from Schmidt-Ruppin strain D of Rous sarcoma virus (SR-D), 8.5-kb RNA of transformation-defective (td) PR RSV, and 5.7-kb MC29 and 8.5-kb MC29-associated virus (MCAV) RNA standards. Electrophoresis and sizing of the DVP RNA at 7.5 kb and of MAV-2 RNA at 8.2 kb followed published procedures (15–18).

Further analysis of intracellular virus-specific proteins in nonproducer myeloblasts failed to show mature gag proteins and indicated that Pr76 was not effectively processed into such proteins in 2-hr [³⁵S]methionine pulse, 16-hr chase experiments (Fig. 1D). By contrast, processing of Pr76 was readily observed in AMV(MAV-2)-producing myeloblasts, in which Pr76 was detected only with short (20-min) pulses of [³⁵S]methionine (Fig. 1A and D). This implies that in nonproducer myeloblasts processing of Pr76 must occur during or after release of DVPs from the cell and that, once assembled, DVPs do not remain associated with cells, possibly due to the absence of envelope glycoprotein. The presence of the polymerase precursor Pr180 in nonproducer cells (Fig. 1A and B) and the apparent absence of polymerase from DVP must reflect either a defective pol gene (for which we have no evidence) or a failure of DVP to package polymerase. Antisera against group-specific determinants of viral envelope glycoprotein (19) that detected viral glycoprotein precursor Pr95 in AMV(MAV-2)-producing cells failed to detect viral env proteins in nonproducer cells (Fig. 1A).

RNA of DVP: Size and Relationship to Other Avian Tumor Viruses. The RNA of [³H]uridine- or ³²PO₄-labeled DVP was resolved by sedimentation into a 60S RNA complex (15) and a 4–12S component. Electrophoretic analysis of the heat-dissociated 60S DVP RNA complex showed a major single RNA

subunit with an estimated (18) size of 7.5 kb based on its electrophoretic mobility relative to mobilities of 10-kb Schmidt-Ruppin (SR) RSV RNA, 8.5-kb transformation-defective Prague (td PR) RSV RNA (20–22), and 5.7-kb MC29 avian acute leukemia virus RNA (17) (Fig. 2). The RNA of MAV-2 (Fig. 2) and MAV-1 was about 8.2 kb, indicating that DVP RNA is about 0.7 kb shorter than that of MAV-1 and MAV-2.

Initial experiments measured sequence relationships between DVP RNA and the RNAs of other avian tumor viruses by quantitative hybridization (cf. Fig. 3), using DVP [³²P]RNA and saturating concentrations of nondefective SR RSV, MC29 (ring-necked pheasant virus [RPV]), MAV-2, or AMV (MAV-1 and MAV-2) cDNAs (at cDNA-to-RNA ratios of 20:1 to 50:1) followed by digestion with RNases A, T1, and T2 (17). Under these conditions $61 \pm 5\%$ (mean \pm SEM) of DVP RNA was hybridized by SR RSV or MC29 cDNAs, $81 \pm 5\%$ by MAV-2 cDNA, and $96 \pm 5\%$ by AMV cDNA. To locate on the DVP RNA shared and specific sequences identified by T1 oligonucleotides, an oligonucleotide map of DVP RNA was prepared and compared to the maps of MAV-1 and MAV-2 RNAs (see Figs. 3 and 4). The method of ordering oligonucleotides relative to the 3'-poly(A) coordinate of viral RNA by fingerprinting oligonucleotides of poly(A)-tagged RNA fragments of discrete sizes classes (see Fig. 3 for DVP RNA fragments) has been described (23). The numbering of oligonucleotides of certain contiguous RNA sequences (in Table 1 and Fig. 4) reflects the map order in the 5'-to-3' direction. The RNase A-resistant sequences of oligonucleotides are recorded in Table 1.

It can be seen in Fig. 4 that the oligonucleotide maps of 7.5-kb DVP RNA and the 8.2-kb MAV RNAs share virtually isogenic 5'-terminal sequences of 5.3 kb and 3'-terminal sequences of 0.7 kb. The shared 5'-terminal regions have the genetic complexities of the viral *gag* and *pol* genes and include known *gag* and *pol* oligonucleotides conserved in other avian tumor viruses (Table 1; refs. 24–26). The shared 3'-terminal

sequences have the complexity of the 3'-terminal c-region of avian tumor viruses (22–26). However, comparison of a fingerprint of the c-region oligonucleotides (numbered 51 to 57) prepared from a 1-kb poly(A) terminating DVP RNA fragment (Fig. 3C, Table 1) with the fingerprints from other avian tumor viruses (2, 23, 26) indicates that the closely related c-regions of DVP, MAV-1, and MAV-2 RNAs (Table 1, Fig. 4) are different from those found in other avian tumor viruses.

The 7.5-kb DVP RNA contained a 1.5-kb sequence represented by 14 oligonucleotides numbered 101–114, which mapped between the c-region and about 2.2 kb from the 3'-poly(A) coordinate (Fig. 3B) and did not share any oligonucleotide with the MAV RNAs (Fig. 4, Table 1). At equivalent map locations, MAV-1 and MAV-2 RNAs contained a 2-kb region identified as the *env* gene by its map location (Fig. 4) (24–26), by its genetic complexity (24–26, 29, 30), and by the presence of *env* oligonucleotides conserved in other viruses (24–26, 29). The following experiments were done to determine whether the 1.5-kb RNA sequence of DVP was related to the *env* genes or other genes of the MAVs and other avian tumor viruses. DVP [³²P]RNA was hybridized with cDNAs of MAV-1 of envelope subgroup A(5), MAV-2 of subgroup B(5), SR RSV of subgroup D and MC29(RPV) of subgroup F(10) and the RNA of the resulting RNase T1-resistant hybrids (28) was fingerprinted after dissociation of the hybrid and digestion with RNase T1. Only one (no. 102) of the 14 DVP-specific oligonucleotides was scarcely hybridized by three of these cDNAs—i.e., MC29(RPV), MAV-1 (Fig. 3 G and H), and MAV-2 (not shown)—which may signal a relationship between the *pol-env* border of these viruses and the 5' end of the DVP-specific sequence (Fig. 4). Hence the majority of the DVP-specific sequence is not related to genes of these viruses, which include the *src* gene of RSV and the *onc* gene of MC29 (1, 2). On the basis of comparisons of T1 oligonucleotides, the specific sequence of DVP is also unrelated to the RNA of Fujinami sarcoma virus (12) and the avian erythroblastosis virus (18).

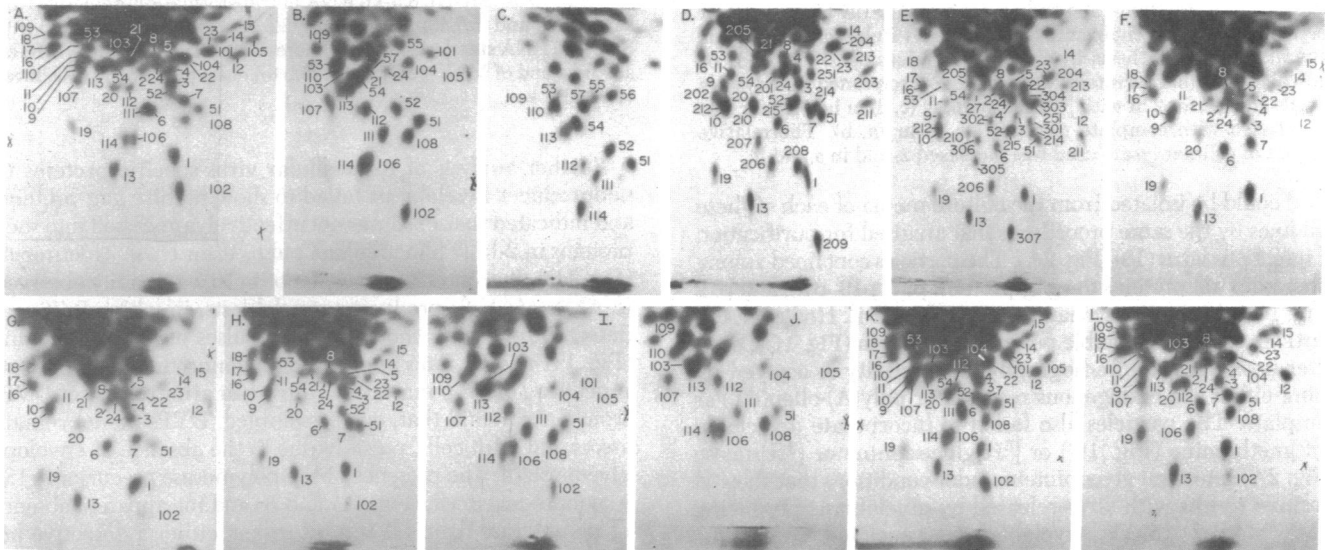


FIG. 3. Two-dimensional electrophoretic (left to right) and chromatographic (bottom to top) fingerprints (17, 23–26) of the RNase T1-resistant ³²P-labeled oligonucleotides of: 7.5-kb DVP RNA (A), a 1-kb (B) and a 2-kb (C) poly(A)-terminating fragment of DVP RNA, MAV-1 RNA (D), MAV-2 RNA (E), poly(A)-selected RNA from DVP produced by the myeloblast line Du 1765 from A. Langlois *et al.* (27) (K), and DVP RNA sequences recovered from hybrids formed with cDNAs from various avian tumor viruses (F–L and J). (D and E) MAV-1 and MAV-2 RNAs were isolated from virus propagated in fibroblasts as described (17, 23–25). (F–J and L) DVP RNA-cDNA hybrids were prepared by incubating $2\text{--}8 \times 10^6$ cpm (0.05–0.2 μ g) of DVP [³²P]RNA with 0.5–3.5 μ g of viral cDNA for 12 hr at 40°C in 10 μ l of 70% (wt/vol) formamide/0.3 M NaCl/0.03 M sodium citrate/0.01 M sodium phosphate, pH 7.0. Removal of unhybridized RNA by digestion with 5 units of RNase T1 in 200 μ l of 0.15 M NaCl/0.015 M sodium citrate, pH 7.0, for 30 min at 40°C followed by chromatography in a Bio-Gel (Bio-Rad) P100 column (15 \times 0.7 cm) as well as subsequent phenol extraction of the hybrid and fingerprinting of hybridized RNA have been described (2, 18). Hybrids were formed with cDNAs of the following viruses: SR RSV (F), MC29(RPV) (G), MAV-1 (H), AMV (MAV-1 and MAV-2) cDNA (2 μ g) prehybridized with 25 μ g of MAV-2 RNA and then hybridized for 2 hr in 20 μ l with 0.2 μ g of DVP [³²P]RNA and subsequently digested with RNase T1 (I) or with RNase T1 and 0.2 μ g of RNase A (J), E26(E26AV) (L).

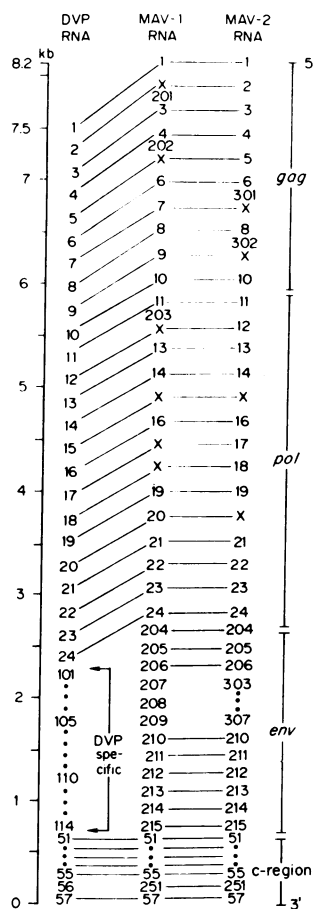


FIG. 4. Oligonucleotide maps of 7.5-kb DVP RNA and 8.2-kb MAV-1 and MAV-2 RNAs. Oligonucleotides are numbered as in Fig. 3 and Table 1. Identical oligonucleotides from different viral RNAs have the same numbers. The numbers reflect map order in the 5'-to-3' direction within distinct map segments (compare also Table 1). Dots replace sequential numbers within map segments in which the density of oligonucleotides per unit length RNA was too high to give numbers. Oligonucleotides shared by viral RNAs are connected horizontally. X denotes the presence in MAV-1 or MAV-2 [also in SR RSV, MC29(RPV), and E26] RNAs of unidentified oligonucleotides equivalent to the respective DVP oligonucleotide. The presence of such oligonucleotides was deduced from the hybrids in Fig. 3 F, G, H, and L.

DVP RNA Is Present in AMV and in DVP from Other Laboratories and Is Related to the RNA of E26, an AMV-Like Virus. The following experiments were done to determine whether DVP RNA was present in AMV stocks from this and other laboratories. First, it was asked whether DVP-specific RNA could be detected in AMV(MAV-1 and MAV-2) obtained from J. W. Beard. For this purpose AMV(MAV-1 and MAV-2) cDNA was made and hybridized with an excess of MAV-2 RNA, in order to base-pair all MAV-2-related DNA sequences. The cDNA was then hybridized to DVP [³²P]RNA. The RNA of the resulting RNase T1-resistant hybrid contained all 14 DVP-specific oligonucleotides (Fig. 3I) and that of the RNase A and T1-resistant hybrid contained all but the two 5'-terminal DVP-specific oligonucleotides, 101 and 102 (Fig. 3J). The loss of these two oligonucleotides upon digestion with RNase A could signal a sequence variation between our DVP RNA and its equivalent in Beard's AMV stock, or it could signal a partial sequence relationship between DVP and MAV-2 resulting in displacement of DVP sequences from AMV cDNA hybrid. The latter interpretation is consistent with the above result that DVP oligonucleotide 102 was partially hybridized by the MAV cDNAs (Fig. 3H). The finding of the 5'-most c-region oligonucleotide 51 in the DVP RNA-AMV-specific cDNA hybrids may reflect displacement of MAV-2 RNA by DVP [³²P]RNA by strand migration from the adjacent DVP-specific region of cDNA (see Fig. 4). Moreover, all DVP-specific oligonucleotides that are not overlapped by MAV-2 oligonucleotides—i.e., nos. 102, 103, 108, 111, and 113—could also be detected as minor oligonucleotides in AMV(MAV-2) from myeloblasts prepared by us or by M. Baluda (not shown).

In addition, an AMV-transformed myeloblast line derived by Langlois *et al.* (27), which produces no or very little infectious virus, was found to release DVP with an RNA indistinguishable from ours (Fig. 3K), although the yield of DVP was lower than with our cell lines.

The oncogenic spectrum of AMV is closely related to that of E26, a defective avian acute leukemia virus that was isolated in Bulgaria in 1962 (31, 32). In particular, both viruses lack the ability to transform fibroblasts (refs. 31 and 32; unpublished data). AMV has also been reported to share with E26 some helper virus-unrelated sequences as measured by RNA-cDNA hybridization (7). To test whether DVP RNA is related to E26, the RNA of an RNase T1-resistant DVP [³²P]RNA-E26(E26-associated virus) cDNA hybrid was fingerprinted. All but 4 (nos.

In contrast, all oligonucleotides of the 5' 5.3-kb DVP map segment (nos. 1-24, Fig. 4) were hybridized by each of the above cDNAs [Fig. 3 F, G, H (MAV-2 not shown), and L; see below], indicating that these DVP oligonucleotides have related or identical counterparts in each of these viruses. However the c-region oligonucleotides of DVP RNA were not hybridized by SR RSV and MC29(RPV) cDNAs, except no. 51 (Fig. 3 F and G). This extends the oligonucleotide analyses and indicates that the particular c-region of DVP and the MAVs is little related to those of other avian tumor viruses.

Table 1. RNase A-resistant sequences of DVP, MAV-1, and MAV-2 T1 oligonucleotides numbered to reflect map positions within certain RNA regions

Common gag/pol-related			
1 5U, 7C, G, 3AC, 3AU, GpppGmpC	19 C, G, AC, A ₄ C, A ₅ C	104 3U, AC, AU, AG, A ₂ U	
2 4U, 5C, G, 2AC, AU	20 U, 2C, G, 2AC, AU, A ₅ C	105 4U, 2C, 2AU, AG	
3* 4U, 6C, G, AU	21 U, 4C, G, AC, 2AU, A ₂ C	106 4U, 4C, G, 4AC, AU	
4 1-2U, 4C, G, 2AU, A ₂ U, A ₃ U	22 4U, 4C, G, AC, AU	107 U, 6C, G, 2AC, A ₂ C	
5 3U, C, AC, 2AU, AG, A ₃ U	23* 5U, 2C, G, 4AU, A ₂ U	108 U, 2C, G, 4AU, A ₂ U, A ₃ U	
6* 5U, 6C, A ₂ G, A ₄ C	24 3U, 4C, G, AC, A ₂ U	109 2C, 2AC, AG, A ₂ C	
7 4U, 3C, 2AC, 3AU, AG	Common c-region		
8 4U, 3C, AC, AG, A ₂ C	51 3U, 2-3C, G, AU, 3A ₂ U	111 4U, 3C, G, 1-2AC, AU, A ₃ C	
9* 2U, 5C, 3AC, A ₂ G	52 4U, 3C, AC, 2AU, A ₂ G, A ₃ U	112 3U, 6C, 2AC, AU, AG	
10 U, 3C, AC, AG, A ₂ U	53 4C, AC, A ₂ G, A ₃ U	113 2U, 4C, 2AC, AU, AG, A ₂ C	
11* 3C, AU, AG, A ₅ C	54 U, 3C, A ₂ C, A ₂ U, A ₃ G	114 3U, 8C, 4AC, AU, A ₂ G	
12 5U, 4C, G, AU	55 2U, G, AC, 2AU, A ₂ C	MAV-2-specific	
13† 2-3U, 8C, 2AC, AU, A ₂ C, A ₂ G	56 2U, 5C, G, 2AU	301 5U, 4C, G, 2AC, 3AU	
14 3U, 3C, G, 2AU	57 2U, 5C, G, AC, 2AU	302 2U, C, G, AC, 2AU, A ₅ C	
15 4U, 2C, AU, AG	DVP-specific		
16 6C, 4AC, AG	101 4U, G, AC, AU, A ₂ U	305a, b 10U, 4C, 2G, 3AC, AU, A ₂ C, A ₂ U	
17 6C, G, 5AC, A ₃ C	102 8U, 6C, 2AC, 3AU, A ₂ U, A ₃ G	304 3U, 4C, G, AC, AU	
18 2C, 1-2AC, AG, A ₂ C	103 2U, 3C, G, 2AC, A ₂ C	305 3U, 6C, 3AC, AU, AG	
		306† 3U, 6C, 2AC, AU, A ₂ C	
		307 5U, 6C, G, AC, 2AU, 2A ₂ C, A ₂ U	

*, †, and ‡ indicate gag-, pol-, and env oligonucleotides, respectively, of PR RSV (24-26).

101, 105, 111, and 114) of the 14 DVP-specific oligonucleotides were recovered (Fig. 3L). The partial recovery of DVP oligonucleotide no. 102 may reflect homology with E26 or with E26AV, as has been observed above with DVP RNA and MAV or MC29(RPV) cDNA hybrids. The failure of E26 cDNA to hybridize more than one—i.e., no. 51—of the c-region oligonucleotides of DVP [which was also hybridized by MC29(RPV) cDNA] indicates that E26 does not contain the particular c-region of DVP and the MAVs.

DISCUSSION

The following arguments favor the view that the 7.5-kb DVP RNA is the genome of AMV.

(i) The genetic structure of the DVP fits the model of a defective transforming virus, containing an internal specific sequence, which we term *amv*. It is nonallelic with helper viruses and flanked by helper-virus-related terminal sequences (1, 2). The finding that the allelic sequences of DVP and MAV RNAs are almost isogenic is also typical of other defective virus-helper virus complexes (1, 2, 11, 18, 29). Because the 1.5-kb DVP-specific sequence takes the place of a 2-kb *env* gene of nondefective MAVs, it may be argued that it represents a unique and probably defective *env* gene. This appears unlikely because the sequence was not hybridized by cDNAs of avian tumor viruses of four different envelope subgroups and because no *env*-related protein was serologically detectable in infected cells, although *env* genes from different subgroups are known to share group-specific sequences (19, 24–26). Therefore, the specific sequence of DVP may be analogous to the sequences of fibroblast-transforming viruses and may encode a nonstructural transforming protein. Because no nonstructural *gag*- (1, 2, 10–12), *pol*-, or *env*- related DVP-specific protein was detected in transformed myeloblasts, the protein possibly encoded by the DVP-specific sequence may be translated as is the *src* gene product of RSV, which is unrelated to virion proteins and coded for by a subgenomic messenger RNA (33, 34). The genetic structure of DVP RNA is then similar to that of the *env*-defective RSV(–) analyzed earlier (1, 29) and, as in the case of RSV(–) (35), it allows for the formation of a DVP. It is not yet known whether the unique c-region of the DVP and the MAVs, which specifically cause osteopetrosis and nephroblastomas (5), also plays a role in oncogenicity.

(ii) The correlation between leukemogenicity and the presence of the DVP RNA in transformed myeloblasts and in all infectious AMV stocks tested directly supports the view that DVP RNA is the genome of AMV. It is plausible that the 7.8-kb RNA (7) and proviral DNA (10, 11) components found recently in AMV stocks belong to the DVP described here. The argument that DVP RNA is the genome of AMV is independently supported by the presence of a DVP-related nucleotide sequence in E26, a distinct, AMV-related virus (7, 31, 32).

(iii) Several preliminary experiments showed that DVP fused with susceptible cells infected with helper virus caused myelocytic leukemia in chickens (unpublished).

We conclude that rapidly transforming avian tumor viruses fall into at least five RNA subgroups (1) based on contiguous helper-virus-unrelated sequences: the RSV-subgroup (1), the Fujinami sarcoma virus subgroup (12), the MC29 virus subgroup (2, 11), the avian erythroblastosis virus subgroup (2, 18), and the AMV subgroup defined here.

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