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Characterization of Avian Myeloblastosis-Associated Virus DNA Intermediates

D. G. BERGMANN, L. M. SOUZA, AND M. A. BALUDA*

UCLA School of Medicine and Molecular Biology Institute, Los Angeles, California 90024

The major species of unintegrated linear viral DNA identified in chicken embryonic fibroblasts infected with either the avian myeloblastosis-associated viruses (MAV-1, MAV-2) or the standard avian myeloblastosis virus complex (AMV-S) has a mass of 5.3×10^6 daltons. An additional minor DNA component observed only in AMV-S-infected cells has a mass of 4.9×10^6 daltons. The unintegrated linear viral DNAs and integrated proviruses of MAV-1 and MAV-2 have been analyzed by digestion with the restriction endonucleases *Eco*RI and *Hin*dIII. MAV-2 lacks a *Hin*dIII site present in MAV-1. These fragments have been compared to those generated by *Eco*RI and *Hin*dIII digestion of linear viral DNAs of AMV-S. Restriction enzyme digestion of AMV-S viral DNA produced unique fragments not found with either MAV-1 or MAV-2 viral DNAs. The major viral component present in AMV-S stocks has the *Hin*dIII restriction pattern of MAV-1. Restriction enzyme analysis of the 5.3×10^6-dalton unintegrated MAV viral DNAs and their integrated proviruses suggests that the DNAs have a direct terminal redundancy of approximately 0.3 megadaltons and integrate colinearly with respect to the unintegrated linear DNA.

The standard avian myeloblastosis virus complex (AMV-S) causes four major types of neoplasias in the chicken: acute myeloblastic leukemia, nephroblastoma, visceral lymphoid leukosis, and osteogenic osteoblastoma (5). AMV-S preparations contain at least three viruses: the leukemogenic agent that is responsible for myeloblastosis and converts hematopoietic cells in vitro and two myeloblastosis-associated viruses (MAV-1 and MAV-2) (14). MAV-1 and MAV-2 (subgroups A and B viruses, respectively) cannot induce myeloblastic leukemia or convert hematopoietic cells in vitro. However, under appropriate conditions MAV-2 can induce nephroblastomas, osteoblastomas, and visceral leukosis in chickens (19). MAV-1 can also induce nephroblastomas, but the full tumorigenic potential of this virus is unknown (19; unpublished data). A leukemogenic agent free of any MAV has yet to be isolated from AMV stocks, and it has been suggested that it is defective and may require a helper virus (e.g., MAV) for its replication (14).

Three different forms of viral DNA have been identified in avian retrovirus-infected avian cells: linear DNA molecules of approximately 6.2 megadaltons (Md), circular DNA molecules of approximately the same mass (1, 9, 18), and proviral DNA integrated into the host genome (1, 13, 23, 24). The length of cytoplasmic linear proviral DNA synthesized in avian sarcoma virus-infected Japanese quail cells approximates a complete linear copy of one subunit of the viral RNA (18). Restriction enzyme analysis of avian sarcoma virus DNA has shown that the termini of the linear viral DNA bear a sequence redundancy of approximately 300 nucleotides (9, 18) and that the integrated proviral DNA is colinear with respect to the unintegrated linear DNA (10, 16). In addition, it has been shown that the DNA of various avian sarcoma virus strains can be differentiated on the basis of their restriction enzyme cleavage sites (18, 22).

In this study we have determined the size of unintegrated linear viral DNA genomes produced in chicken embryonic fibroblasts (CEF) infected by either MAV-1, MAV-2, or AMV-S as well as the location of *Eco*RI and *Hin*dIII restriction sites within the MAV DNAs. Our data indicate that the MAV DNAs are terminally redundant and integrate colinearly with respect to the unintegrated viral DNA. Also, we have identified in AMV-S-infected cells a viral DNA species that is not found in cells infected with either MAV-1 or MAV-2.

MATERIALS AND METHODS

Cells and viruses. CEF were prepared from individual 11-day-old C/E chicken embryos (Spafas, Inc.) negative for group-specific antigen (gs^-) and for chicken helper factor (chf^-). Avian myeloblastosis virus, BAI strain A (AMV-S), was obtained from plasma

of leukemic chickens (J. W. Beard, Life Sciences, St. Petersburg, Fla.). Avian MAV-1 and MAV-2 were a gift from C. Moscovici (Veterans Administration Hospital, Gainesville, Fla.) and were propagated in C/E gs^-chf^- CEF.

Preparation of linear unintegrated viral DNA. Approximately 10 roller bottle cultures of C/E gs^-chf^- fibroblasts were infected at a multiplicity of 1 to 5 infectious units per cell with either AMV-S plasma, MAV-1, or MAV-2. At 20 h postinfection, the cells were harvested and separated into nuclear and cytoplasmic fractions by a modified method of Guntaka et al. (7). Viral DNA was isolated from the cytoplasm by modified methods of Shank et al. (17 , 18). Briefly, washed cells were resuspended at 4°C to approximately 2×10^7 cells per ml in lysis buffer (10 mM Tris, pH 8.5; 1.5 mM $MgCl_2$; 0.14 M NaCl), and Nonidet P-40 (Bethesda Research Laboratories) was added to a concentration of 0.5%. After vigorous pipetting, the nuclei were pelleted at 2,000 × *g* for 10 min at 4°C. The supernatant (cytoplasmic fraction) was harvested and treated for 2 h at 37°C with 250 μg of proteinase K per ml (EM Biochemicals) in the presence of 0.5% sodium dodecyl sulfate. The cytoplasmic fraction was then extracted with phenol-chloroform, precipitated with ethanol, redissolved in TE buffer (10 mM Tris, pH 7.4; 1 mM EDTA) and treated with RNase A (25 μg/ml) and RNase T_1 (20 U/ml) for 30 min at 37°C. The cytoplasmic fraction was again treated with proteinase K, extracted, and ethanol precipitated. The precipitated DNA was resuspended in NTE buffer (0.1 M NaCl; 10 mM Tris, pH 7.4; 1 mM EDTA) and centrifuged (15 h, 30,000 rpm, 4°C) through 5 to 20% sucrose gradients (NTE, pH 7.4) in a SW-40 rotor. rRNA served as an external marker.

Preparation of cellular DNA. High-molecular-weight (i.e., 3×10^7 or larger) cellular DNA from either MAV-1- or MAV-2-infected CEF was extracted and purified by a modification of methods previously described (6). Briefly, the nuclei of virus-producing CEF were isolated (7) and fractionated by the method of Hirt (8). The Hirt pellets were redissolved in TE buffer (pH 7.4) and digested for 12 to 16 h with 500 μg of proteinase K per ml at 37°C. The digests were then extracted with phenol-chloroform (1:1), and the high-molecular-weight DNA was spooled from an ethanol interface. The spooled DNA was stored in water at 4°C until used.

Restriction endonuclease digestion. Restriction endonuclease *Eco*RI was a gift of M. Komaromy (UCLA), and *Hin*dIII was prepared in our laboratory. Digestions with *Eco*RI or *Hin*dIII were carried out for 90 min at 37°C with a threefold excess of enzyme (21). Phage λ DNA (Miles Laboratories) digested with *Hin*dIII served as molecular weight markers (21).

Southern blotting. DNA was applied to 0.7 or 1.2% horizontal agarose gels and electrophoresed as described previously (21). After gel electrophoresis, the DNA was transferred to nitrocellulose paper by the method of Southern (20). The viral DNA sequences were detected by hybridization with ^{125}I-labeled 70S viral RNA (specific activity of approximately 2×10^8 cpm/μg) (21).

Viral RNAs. AMV-S was purified from the plasma of leukemic chickens. MAV-2 was purified from the supernatant of MAV-2-infected CEFs. Viral 70S RNA was extracted, purified (3), and iodinated with ^{125}I as described previously (15, 21). In some experiments, a hybridization probe complementary to the 3′ end of the viral RNA was selected by retention of polyadenylic acid-containing ^{125}I-labeled AMV-S RNA (12 to 16S) in an oligodeoxythymidylic acid-cellulose column (Collaborative Research, Inc.) as previously described (4, 21).

RESULTS

Size of unintegrated linear double-stranded viral DNA of MAV-1, MAV-2, and AMV-S. Unintegrated linear viral DNA was isolated from the cytoplasmic fraction of CEF infected with either MAV-1 or MAV-2 as described in Materials and Methods. After sucrose velocity gradient sedimentation, fractions of 0.7 ml were collected from the bottom of gradients, and 10 μg of sonicated calf thymus DNA (10S) was added to each fraction. The DNA fractions were electrophoresed in 0.7% agarose gels and transferred to nitrocellulose paper (20). After hybridization with a ^{125}I-labeled 70S AMV-S RNA probe, the only viral DNA detected was from the 18S region of the sucrose gradients. Southern blots of MAV-1 or MAV-2 viral DNA pooled from the 15 to 20S fractions of sucrose velocity gradients and electrophoresed in parallel in 0.7% agarose gels showed a single band corresponding to a mass of 5.3 Md. If equimolar amounts of MAV-1 and MAV-2 viral DNA were mixed and electrophoresed, the only detectable hybridized band also had a mass of 5.3 Md (Fig. 1, lane 2). Thus, both MAV-1 and MAV-2 linear viral DNAs have the same mass.

Linear viral DNA from the cytoplasmic fraction of AMV-S-infected CEF was centrifuged through a 5 to 20% sucrose gradient, and fractions were collected and treated as described for MAV-1 and MAV-2. After hybridization with an ^{125}I-labeled 70S AMV-S RNA probe, again the only viral DNA detected was from the 18S region of the sucrose gradients. The majority of the AMV-S linear viral DNA molecules had an apparent mass of 5.3 Md, but there was also a minor species with a slightly smaller mass of 4.9 Md (Fig. 1, lane 1). The 4.9-Md viral DNA species was reproducibly seen in five independent preparations of AMV-S linear viral DNA. Therefore, it appears that in addition to viral DNA of 5.3 Md, AMV-S gives rise to an unidentified 4.9-Md component which is not present in MAV-1 or MAV-2 viral DNA.

Cleavage of unintegrated viral DNA with restriction endonuclease *Eco*RI or *Hin*dIII. MAV-1, MAV-2, or AMV-S linear viral DNA was treated with the restriction enzyme *Eco*RI or *Hin*dIII. The resulting DNA fragments were

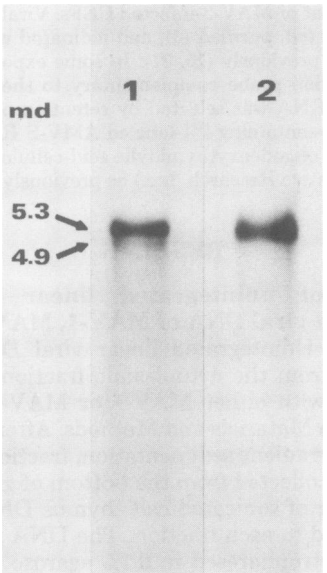

FIG. 1. *Size of linear viral DNAs of MAV-1, MAV-2, and AMV-S. Linear viral DNA was isolated from the cytoplasm of infected CEF, electrophoresed in 0.7% agarose gels, transferred to nitrocellulose paper, and hybridized with an ^{125}I-labeled AMV-S RNA probe. AMV-S linear viral DNA (lane 1); mixture of MAV-1 and MAV-2 viral DNA in equimolar concentrations (lane 2).*

electrophoresed in either 0.7 or 1.2% agarose gels, transferred to nitrocellulose paper, and hybridized with an ^{125}I-labeled AMV-S 70S RNA probe. Preliminary experiments had shown that ^{125}I-labeled 70S RNA from AMV-S has sufficient homology to MAV-1 or MAV-2 proviral DNA to be used as a hybridization probe for these viral DNAs. MAV-2 viral DNA treated with *Eco*RI or *Hin*dIII showed the same band pattern whether hybridized with ^{125}I-labeled 70S RNA from MAV-2 (Fig. 2A, lanes 3 and 7) or from AMV-S (Fig. 2A, lanes 2 and 6). Also, the MAV-2 probe efficiently detects all AMV-S viral DNA fragments generated by restriction endonuclease digestion (data not shown).

Digestion of either MAV-1 DNA (Fig. 2A, lane 1) or MAV-2 DNA (Fig. 2A, lane 2) with *Eco*RI produced fragments of 4.4, 2.6, 1.8, and 0.9 Md. The intensity of the 4.4-Md *Eco*RI fragment relative to the other fragments has varied from one preparation to another. An analogous fragment has been detected in *Eco*RI-treated viral DNA from avian sarcoma viruses (18). The current interpretation is that this 4.4-Md band probably represents the cleavage product of some partially single-stranded viral DNA intermediate (18). The remaining MAV-1 or MAV-2 DNA fragments collectively make a viral DNA of 5.3 Md.

Digestion of AMV-S viral DNA with *Eco*RI

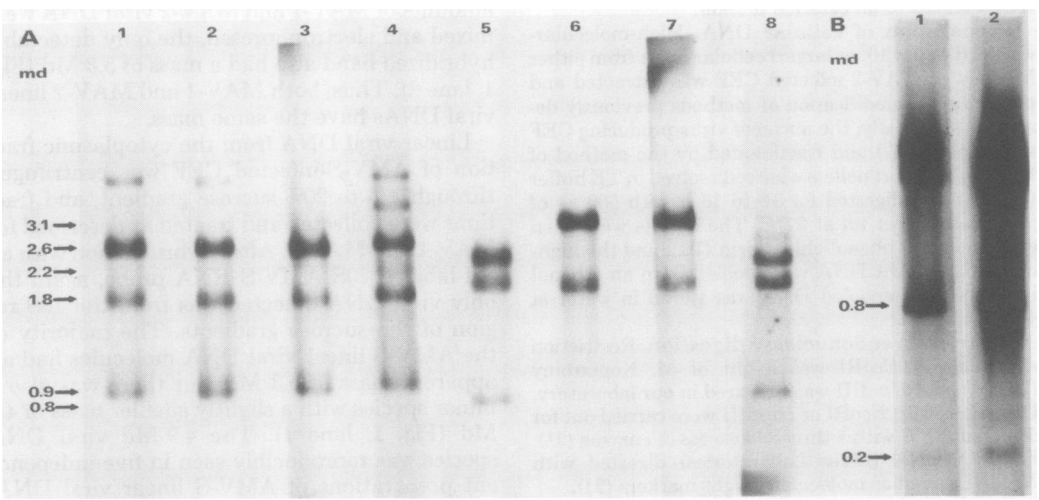

FIG. 2. *Autoradiographs of EcoRI and HindIII digests of MAV-1, MAV-2, and AMV-S linear viral DNAs. The viral DNAs were isolated, treated with restriction endonucleases, electrophoresed in agarose gels, Southern blotted, and hybridized with ^{125}I viral RNA as described in the text. (A) 0.7% gels: EcoRI-digested MAV-1 DNA (lane 1), MAV-2 DNA (lane 2), and AMV-S DNA (lane 4) hybridized to ^{125}I-labeled AMV-S RNA; a separate MAV-2 DNA preparation was hybridized to ^{125}I-MAV-2 RNA (lane 3). HindIII-digested MAV-1 DNA (lane 5), MAV-2 DNA (lane 6), and AMV-S DNA (lane 8) hybridized to ^{125}I-AMV-S RNA; a separate MAV-2 DNA preparation was hybridized to ^{125}I-MAV-2 RNA (lane 7). (B) 1.2% gels: HindIII-digested MAV-1 DNA (lane 1) and AMV-S DNA (lane 2) hybridized to an ^{125}I-labeled AMV-S RNA probe.*

produced three fragments (5.3, 3.5, and 2.2 Md) in addition to those observed in MAV-1 and MAV-2 DNA digests (Fig. 2A, lane 4). These fragments did not result from incomplete enzymatic digestion since the λ control was properly cleaved and an increase in enzyme concentration or incubation period did not eliminate these bands. The small amount of apparently uncut DNA (5.3-Md band) could represent an as yet unidentified MAV. The 3.5-Md fragment could represent a partially single-stranded replicative intermediate, or, alternatively, it could be part of an unknown virus present in AMV-S stocks in which the *Eco*RI site between the 2.6- and 0.9-Md fragments is missing. The addition of the 1.8- and 3.5-Md fragments would produce a MAV-sized (5.3 Md) viral DNA.

After *Hin*dIII digestion of MAV-1 DNA, fragments of 2.3, 1.9, and 0.8 Md were detected in Southern blots of 0.7% gels (Fig. 2A, lane 5). Long exposure of Southern blots of 1.2% gels revealed the presence of an additional *Hin*dIII fragment of 0.2 Md (Fig. 2B, lane 1). *Hin*dIII digestion of MAV-2 DNA yielded fragments of 3.1 and 1.9 Md in 0.7% gels (Fig. 2A, lanes 6 and 7), and an additional fragment of 0.2 Md in 1.2% gels. Thus, MAV-1 DNA contains an additional *Hin*dIII site not present in MAV-2 DNA.

Digestion of AMV-S viral DNA with *Hin*dIII produced fragments of 3.1, 2.6, 2.3, 1.9, 0.8, and 0.2 Md (Fig. 2A, lane 8, and Fig. 2B, lane 2).

Mapping of *Eco*RI and *Hin*dIII sites in the linear MAV DNA genomes. (i) Cleavage of integrated MAV-1 and MAV-2 proviruses with *Eco*RI or *Hin*dIII. DNA from virus-producing CEF infected with either MAV-1 or MAV-2 was digested with *Eco*RI and electrophoresed in 0.7% agarose gels. The viral DNA band pattern which resulted was identical to that generated from the DNA of uninfected CEF, i.e., only the endogenous viral DNA fragments appeared to be present (Fig. 3, lanes 1 and 2). The 1.8- and 0.9-Md *Eco*RI end fragments of linear viral DNA are missing. This indicates that unintegrated viral DNA is absent and that integration has involved both of these fragments. Therefore, the 2.6-Md fragment of either MAV-1 or MAV-2 DNA must be located between the 1.8- and 0.9-Md fragments, and it would have comigrated with an endogenous fragment of the same size. The absence of juncture bands between integrated viral DNA and cellular DNA suggests that the MAV proviruses are integrated at multiple sites in the chicken DNA, as has been found for several other retroviruses (2, 10, 11, 16). Such juncture bands have been detected after *Eco*RI cleavage of DNA from cell colonies derived from individual

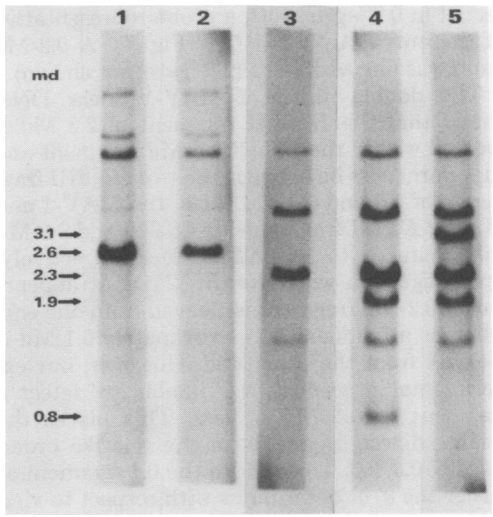

FIG. 3. *Digestion of uninfected and MAV-1- or MAV-2-infected CEF with EcoRI and HindIII. High-molecular-weight cellular DNA was isolated, digested with either EcoRI or HindIII, electrophoresed in 0.7% agarose gels, blotted, and hybridized with an ^{125}I-labeled AMV-S RNA. EcoRI-digested DNA from uninfected CEF (lane 1) and from MAV-1-infected CEF (lane 2). HindIII-digested DNA from uninfected CEF (lane 3), MAV-1-infected CEF (lane 4), and MAV-2-infected CEF (lane 5). A 20-μg amount of DNA was run in each lane.*

CEF infected with MAV-2 (Bergmann et al., submitted for publication).

If DNA from CEF productively infected with either MAV-1 or MAV-2 was digested with *Hin*dIII, two viral DNA bands appeared in addition to those arising from endogenous proviruses (Fig. 3, lanes 3, 4, and 5). These exogenous viral DNA bands were 1.9 and 0.8 Md for MAV-1-infected CEF and 3.1 and 1.9 Md for MAV-2-infected CEF. These two *Hin*dIII fragments generated from integrated MAV-1 and MAV-2 proviruses are identical to those generated from their respective unintegrated linear viral DNAs. Therefore, a 2.3-Md band must also have been generated from digests of integrated MAV-1 DNA and would have comigrated with the endogenous fragment of the same size. This indicates there are *Hin*dIII sites near both termini in linear viral DNA and that integration must occur outside these sites. Therefore, MAV-1 and MAV-2 proviruses are integrated colinearly with respect to their linear viral DNA.

(ii) Sequential digestion of unintegrated linear viral DNAs with *Hin*dIII and *Eco*RI. If MAV-1 or MAV-2 linear viral DNA was sequentially digested with *Hin*dIII and *Eco*RI, fragments of 2.3, 1.6, 0.8, and 0.3 Md were de-

tected in 0.7% gels, with a probe representative of the entire AMV-S RNA (Fig. 4). A 0.2-Md band was also visible in 1.2% gels (not shown).

The double digest of MAV-1 linear DNA shows that the *Hin*dIII fragment of 2.3 Md is located within the *Eco*RI 2.6-Md fragment and therefore must be between the two *Hin*dIII fragments of 1.9 and 0.8 Md. Also, the MAV-1 and MAV-2 *Eco*RI fragments of 1.8 Md and 0.9 Md were reduced to 1.6 and 0.8 Md, respectively, upon digestion with *Hin*dIII. Thus, it appears that a 0.2-Md fragment is cleaved from one end, whereas a fragment of approximately 0.1 Md is cleaved from the other end. However, our experimental procedure was unable to detect a fragment of 0.1 Md or less. This places the double digest fragments in the relative order: 0.2, 1.6, 0.3, 2.3, 0.8, 0.1 with the 0.1 fragment at either the 3′ or 5′ terminus with respect to viral RNA.

(iii) Hybridization of *Eco*RI or *Hin*dIII cleaved linear viral DNA with a 3′-specific RNA probe. The viral DNA fragments were oriented with respect to the 3′ terminus of the viral RNA by hybridization with 12 to 16S polyadenylic acid-containing ^{125}I-labeled AMV-S RNA (3′ probe). If *Eco*RI-digested MAV-1 or MAV-2 DNA was hybridized with the 3′ AMV-S probe, the 0.9-Md band appeared as intense as with a total RNA probe, and the 1.8-Md band hybridized faintly (Fig. 5, lanes 1 and 2). This indicates that the 0.9-Md fragment corresponds to the 3′ end of the viral RNA. The limited homology of the 1.8-Md fragment with the 3′ probe despite its location at the opposite end of the linear genome indicates that the MAV viral DNA contains a terminal redundancy, as does the DNA from other retroviruses examined so far (9, 11, 18).

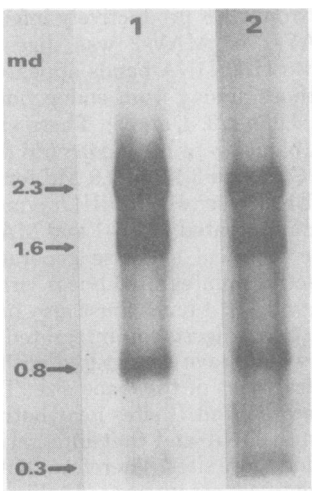

FIG. 4. *Sequential digestion of MAV-1 and MAV-2 viral DNA with HindIII and EcoRI. MAV-1 or MAV-2 DNA sequentially digested with HindIII and then EcoRI was electrophoresed in separate 0.7% agarose gels, blotted, and hybridized with an ^{125}I-labeled AMV-S RNA probe. MAV-1 (lane 1) and MAV-2 (lane 2) viral DNA.*

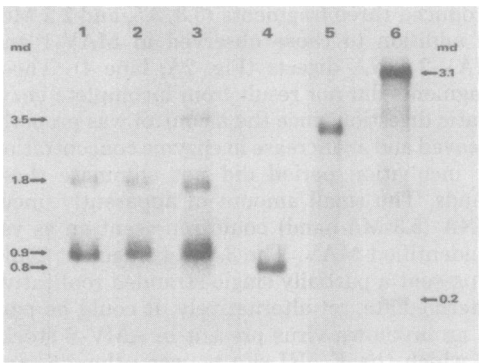

FIG. 5. *Hybridization of EcoRI- and HindIII-generated viral DNA fragments with a 3′ terminal RNA probe. Linear viral DNA was digested with either EcoRI or HindIII, electrophoresed in either 0.7 or 1.2% agarose gels, blotted, and hybridized with a 12 to 16S polyadenylic acid-containing ^{125}I-labeled AMV-S RNA (3′ probe). EcoRI-digested MAV-1 DNA (lane 1); EcoRI-digested MAV-2 DNA (lane 2); EcoRI-digested AMV-S DNA (lane 3); HindIII-digested MAV-1 DNA (lane 4); and HindIII-digested MAV-2 DNA (lane 5) electrophoresed in 0.7% agarose gels. HindIII-digested MAV-2 DNA electrophoresed in a 1.2% gel (lane 6). The mass scale at right applies only to lane 6.*

If *Hin*dIII-digested MAV-1 or MAV-2 DNA was hybridized with the 3′ AMV-S probe, the only detectable bands in 0.7% gels were the MAV-1 0.8-Md or the MAV-2 3.1-Md fragments (Fig. 5, lanes 4 and 5). After similar digests were run in 1.2% gels, the 0.2-Md fragments could also be detected as depicted for MAV-2 DNA (Fig. 5, lane 6). This indicates that the MAV-1 0.8-Md fragment and the MAV-2 3.1-Md fragment are located near the 3′ end and that the 0.2-Md fragment which comes from the 5′ terminus contains sequences complementary to the 3′ terminus of the viral RNA.

If *Eco*RI-digested AMV-S viral DNA was hybridized to the 3′ AMV-S probe, fragments of 3.5, 1.8, and 0.9 Md were detected (Fig. 5, lane 3). Therefore, the 3.5-Md fragment includes 3′ sequences and may represent a MAV-like viral DNA with an *Eco*RI site missing at 0.9 Md from the 3′ terminus. The loss of the *Eco*RI site between the 2.6- and 0.9-Md fragments of either MAV-1 or MAV-2 would generate an *Eco*RI fragment of 3.5 Md.

The data from integrated proviral DNA, double digests, and 3′ end analysis of free viral DNA have been combined to assemble an *Eco*RI and *Hin*dIII cleavage map of MAV-1 and MAV-2 linear viral DNAs (Fig. 6). From the data, the *Hin*dIII site present in MAV-1 but absent in MAV-2 cannot be exactly located relative to the *Eco*RI site near the 3′ end. However, this *Hin*dIII site must lie very near the *Eco*RI site as all the fragments generated by a double digest of MAV-2 appear identical to those generated from MAV-1.

DISCUSSION

The major species of unintegrated linear viral DNA identified in CEF infected with either MAV-1, MAV-2, or AMV-S has a mass of 5.3 Md. A minor viral DNA component observed only in AMV-S-infected CEF has a mass of 4.9 Md. RNA from purified AMV-S virions analyzed by methyl mercury-agarose gel electrophoresis contains two viral RNA species of 2.6 and 2.4 Md (Chiswell, Souza, and Baluda, unpublished data; J. Meyers, personal communication). RNA from MAV-1 and MAV-2 virions analyzed in the same manner showed only a 2.6-Md component. Thus, the 4.9-Md DNA made from AMV-S could be the reverse transcription product of the 2.4-Md viral RNA component.

After cleavage with *Eco*RI or *Hin*dIII, unintegrated linear AMV-S viral DNA yields unique bands not detectable in similarly treated MAV-1 or MAV-2 viral DNAs. Two fragments in particular, an *Eco*RI 2.2-Md fragment and a *Hin*dIII 2.6-Md fragment, have been associated with leukemogenesis (Souza et. al., Proc. Natl. Acad. Sci. U.S.A., in press). They have been detected in all leukemic myeloblasts regardless of the nature, or even the presence, of the helper virus used in the infection. Furthermore, these two fragments have been associated with a proviral genome of 4.9 Md isolated from leukemic myeloblasts (Souza et al., in press). Therefore, the 4.9-Md viral DNA present in AMV-S viral DNA preparations may represent the unintegrated form of the putative AMV proviral genome.

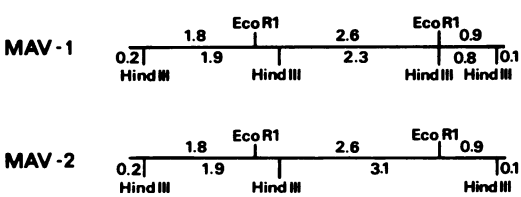

FIG. 6. *EcoRI and HindIII cleavage sites in the linear viral DNAs of MAV-1 and MAV-2. Right side of map corresponds to 3′ side of viral RNA. Numbers represent the size of the fragments (Md). The location of the EcoRI and HindIII cleavage sites at 0.9 md from the 3′ end of MAV-1 DNA could not be determined unambiguously, and both sites have been arbitrarily positioned at the same place.*

The absence in MAV-2 DNA of one *Hin*dIII site that is present in MAV-1 DNA permits the two viruses to be distinguished biochemically as well as biologically (e.g., by lack of interference based on their different envelope glycoproteins). AMV-S consists predominantly of subgroup B viruses with subgroup A viruses as a minor component. Unexpectedly, MAV-2 is present in low concentration (approximately 10%) relative to MAV-1-like virus in AMV-S viral DNA preparations as estimated from the proportion of the 3.1-Md fragment in *Hin*dIII digests of AMV-S viral DNA. This suggests that the major component of AMV-S is either MAV-1 or a MAV with *Hin*dIII sites identical to those of MAV-1. The possibility that MAV-2 replication is selectively inhibited, whereas MAV-1 is not, in our CEF can be excluded since MAV-2 replicates as well as MAV-1 in appropriately infected cells (unpublished data). Our studies also suggest the existence of a MAV-like viral DNA in AMV-S which has an *Eco*RI cleavage pattern different from that of either MAV-1 or MAV-2. It remains to be determined whether this represents a partially single-stranded replication intermediate or another MAV.

The hybridization of a probe specific for the 3′ viral RNA terminus to both the 3′ and 5′ termini of the linear MAV DNA shows that these DNAs have terminal sequence redundancies. *Hin*dIII appears to cleave MAV-1 and MAV-2 DNA at 0.2 Md from the 5′ terminus and at approximately 0.1 Md from the 3′ terminus, as evidenced by *Eco*RI and *Hin*dIII double digestion. *Pvu*I, which cleaves asymmetrically within the terminal repeats of ASV linear viral DNA, generates two end fragments of different size (9, 18). Thus, our data are consistent with the presence of *Hin*dIII sites within terminal redundancies of approximately 0.3 Md in MAV-1 and MAV-2 DNAs.

The internal viral DNA fragments generated by restriction enzyme digestion of either MAV-1 or MAV-2 DNA are present in similar digests of their respective integrated proviruses. Conversely, the terminal fragments of the *Eco*RI- or *Hin*dIII-digested MAV viral DNAs are not detected in these proviral DNA digestions. These end fragments are presumably attached to cellular DNA at many sites and would not be detectable. These results indicate that the MAV proviral DNAs are integrated colinearly with respect to their linear viral DNAs.

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LITERATURE CITED

1. **Ali, M., and M. A. Baluda.** 1974. Synthesis of avian oncornavirus DNA in infected chicken cells. J. Virol. **13**:1005–1013.
2. **Bacheler, L. T., and H. Fan.** 1979. Multiple integration sites for Moloney murine leukemia virus in productively infected mouse fibroblasts. J. Virol. **30**:657–667.
3. **Baluda, M. A., and D. P. Nayak.** 1970. DNA complementary to viral RNA in leukemic cells induced by avian myeloblastosis virus. Proc. Natl. Acad. Sci. U.S.A. **66**:329–336.
4. **Bantle, J. A., I. H. Maxwell, and W. E. Hahn.** 1976. Specificity of oligo(dT)-cellulose chromatography in the isolation of polyadenylated RNA. Anal. Biochem. **72**: 413–427.
5. **Beard, J. W.** 1963. Avian virus growths and their etiological agents. Adv. Cancer Res. **7**:1–127.
6. **Gross-Bellard, M., P. Oudet, and P. Chambon.** 1973. Isolation of high-molecular-weight DNA from mammalian cells. Eur. J. Biochem. **36**:32–38.
7. **Guntaka, R. V., O. C. Richard, P. R. Shank, H. J. Kung, N. Davidson, E. Fritsch, J. M. Bishop, and H. E. Varmus.** 1976. Covalently closed circular DNA of avian sarcoma virus: purification from nuclei of infected quail tumor cells and measurement by electron microscopy and gel electrophoresis. J. Mol. Biol. **106**: 337–357.
8. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
9. **Hsu, T. W., J. L. Sabran, G. E. Mark, R. V. Guntaka, and J. M. Taylor.** 1978. Analysis of unintegrated avian RNA tumor virus double-stranded DNA intermediates. J. Virol. **28**:810–818.
10. **Hughes, S. H., P. R. Shank, D. H. Spector, H. J. Kung, J. M. Bishop, H. E. Varmus, P. K. Vogt, and M. L. Breitman.** 1978. Proviruses of avian sarcoma virus are terminally redundant, co-extensive with unintegrated linear DNA and integrated at many sites. Cell **15**:1397–1410.
11. **Keshet, E., J. O'Rear, and H. M. Temin.** 1979. DNA of noninfectious and infectious integrated spleen necrosis virus (SNV) is colinear with unintegrated SNV DNA and not grossly abnormal. Cell **16**:51–61.
12. **Keshet, E., and H. Temin.** 1978. Sites of integration of reticuloendotheliosis virus DNA. Proc. Natl. Acad. Sci. U.S.A. **75**:3372–3376.
13. **Markham, P. D., and M. A. Baluda.** 1973. Integrated state of oncornavirus DNA in normal chicken cells and in cells transformed by avian myeloblastosis virus. J. Virol. **12**:721–732.
14. **Moscovici, C., and P. K. Vogt.** 1968. Effects of genetic cellular resistance on cell transformation and virus replication in chicken hematopoietic cell cultures infected with avian myeloblastosis virus (BAI-A). Virology **35**: 487–497.
15. **Prensky, W., D. M. Steffensen, and W. L. Hughes.** 1973. The use of iodinated RNA for gene localization. Proc. Natl. Acad. Sci. U.S.A. **70**:1860–1864.
16. **Sabran, J. L., T. W. Hsu, C. Yeater, A. Kaji, W. S. Mason, and J. M. Taylor.** 1979. Analysis of integrated avian RNA tumor virus DNA in transformed chicken, duck, and quail fibroblasts. J. Virol. **29**:170–178.
17. **Shank, P. R., J. C. Cohen, H. E. Varmus, K. R. Yamamoto, and C. 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 circulization. Proc. Natl. Acad. Sci. U.S.A. **75**: 2112–2116.
18. **Shank, P. R., S. H. Hughes, H. J. Kung, J. E. Majors, W. Quintrell, R. V. Guntaka, J. M. Bishop, and H. E. Varmus.** 1978. Mapping unintegrated avian sarcoma virus DNA: termini of linear DNA bear 300 nucleotides present once or twice in two species of circular DNA. Cell **15**:1383–1395.
19. **Smith, R. E., and C. Moscovici.** 1969. The oncogenic effects of nontransforming viruses from avian myeloblastosis virus. Cancer Res. **29**:1356–1366.
20. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
21. **Souza, L. M., and M. A. Baluda.** 1978. Qualitative studies of the endogenous provirus in the chicken genome, p. 217–229. *In* J. Stevens, G. J. Todaro, and C. F. Fox (ed.), Persistent viruses. Academic Press Inc., New York.
22. **Taylor, J. M., T. W. Hsu, and M. M. C. Lai.** 1978. Restriction enzyme sites on the avian RNA tumor virus genome. J. Virol. **26**:479–484.
23. **Temin, H.** 1972. The RNA tumor viruses—background and foreground. Proc. Natl. Acad. Sci. U.S.A. **69**:1016–1020.
24. **Varmus, H. E., P. K. Vogt, and J. M. Bishop.** 1973. Integration of deoxyribonucleic acid specific for Rous sarcoma virus after infection of permissive and nonpermissive hosts. Proc. Natl. Acad. Sci. U.S.A. **70**:3067–3071.