Proc. Natl. Acad. Sci. USA
Vol. 77, No. 9, pp. 5177–5181, September 1980
Biochemistry

Cellular sequences are present in the presumptive avian myeloblastosis virus genome

(leukemia/Southern blot analysis/electron microscopy)

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Communicated by Renato Dulbecco, June 16, 1980

ABSTRACT *Eco*RI restriction endonuclease fragments from a λ proviral DNA hybrid containing the entire presumptive avian myeloblastosis virus (AMV) provirus, and from a λ proviral hybrid containing a partial myeloblastosis-associated virus type 1 (MAV-1)-like provirus were compared by heteroduplex analysis. The cloned presumptive AMV provirus was also analyzed by electron microscopy, using R-loop formation with purified 35S RNA isolated from virions of the standard AMV complex. The results indicate that the putative AMV genome contains a segment absent in its MAV-1-like helper virus. This segment represents a substitution in the region of the genome that in MAV-1 virus is occupied by the envelope gene and is approximately 900 ± 160 nucleotide pairs in length. Hybridization of specific probes from the presumptive AMV genome to Southern blots of *Eco*RI-digested cellular DNA has revealed that these substituted sequences are homologous to chicken and duck DNA that is not related to chicken endogenous proviral sequences.

In the standard avian myeloblastosis virus (AMV-S) complex, the component (AMV) responsible for acute myeloblastic leukemia (AML) in chickens appears to be defective in replication. The fraction of inoculated chickens that develop AML as a function of the multiplicity of infection does not follow one-hit kinetics (1) and *in vitro* dose–response curves indicate that double infection with AMV and a helper virus is required for an infected cell to produce virions with leukemogenic potential (2). Furthermore, some leukemic cells converted by AMV in chickens or *in vitro* do not produce leukemogenic virus (3, 4). Finally, AML-inducing virions can be rescued from chicken leukemic cells in which there is no detectable virus production, by superinfection with a suitable helper virus (4). In standard AMV stocks the natural helpers, myeloblastosis associated viruses types 1 and 2 (MAV-1 and MAV-2), are present in higher concentrations than AMV, permitting their isolation by limit dilution (5).

Several types of nonconditional replication-defective avian retroviruses have genomic deletions (for review see ref. 6). They may have deletions in the gene coding for the viral envelope protein (*env*) or in the gene for the reverse transcriptase (*pol*), in both genes, or in all three genes (*gag*, *pol*, and *env*) involved in viral replication (7–9). The Bryan strain of Rous sarcoma virus (RSV) is deficient in glycoprotein synthesis (10) and contains a deletion in the *env* gene (8). Its genome is approximately 15% smaller than that of a nondefective RSV, but has the same size as its helper due to the presence of the *src* gene (8). Myelocytomatosis virus 29 (MC29), Mill Hill II (MH II), and avian erythroblastosis virus (AEV) each contain a much greater genomic deletion (1.3–2.1 megadaltons) (MDal) and a large substitution compared with their natural helpers (11, 12). The sequence substitutions in MC29, MH II, and AEV are homologous to avian cellular DNA (13), as is the *src* gene of avian sarcoma viruses (14).

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Recently we have identified and isolated from chicken leukemic myeloblasts a presumptive leukemogenic provirus with a mass of approximately 4.9 MDal (15), i.e., only slightly smaller than that of MAV-1 or MAV-2 (5.3 MDal) (16). A linear viral DNA intermediate of approximately 4.9 MDal has also been extracted from cells infected with AMV-S but not from cells infected with MAV-1 or MAV-2 (16). We have also isolated from the same leukemic myeloblasts 85% of a MAV-1-like genome (unpublished data). Comparison of the presumptive AMV genome with the genome of the MAV-1-like helper by restriction endonuclease mapping has indicated that the presumptive AMV genome contains either a deletion or substitution near the 3′ terminus with respect to viral RNA. In addition, AMV-S RNA used as hybridization probe in Southern blots of *Eco*RI digested DNA from uninfected chicken cells had detected specific fragments not seen with a Rous-associated virus type 0 (RAV-0) (17) or a MAV-2 probe (D. G. Bergmann, personal communication). Using the two λ proviral hybrids for heteroduplex and Southern blot analyses and the presumptive AMV λ proviral hybrid and AMV-S RNA for R-loop analysis, we found that there is a cellular substitution in the *env* region of the presumptive AMV genome.

MATERIALS AND METHODS

Fowl and Viruses. The strains and sources of our fertile chicken eggs were: C/E Spafas negative for group-specific antigen, chicken helper factor, and virus production (gs^- chf^- V^-) from Spafas (Roanoke, IL); C/O H & N gs^- chf^- V^- from H & N Farms (Redmond, WA); C/E $L6_3$ gs^+ chf^+ V^- from the Regional Poultry Research Laboratory (East Lansing, MI); and C/E Spafas gs^- chf^- V^- from Life Sciences (St. Petersburg, FL).

Extraction and Restriction Endonuclease Digestions of Cellular DNA. High molecular weight cellular DNA was extracted from decapitated 13-day-old chicken and 19-day-old duck embryos as described (18) after 1 min of homogenization in a Waring Blendor. Restriction endonuclease digestion of cellular DNA has also been described (17).

Phage λ Proviral Hybrids. Phage λ proviral DNA recombinants were constructed and characterized as reported (ref. 15; unpublished data). λ proviral hybrid 11A1-1 contains the entire presumptive AMV provirus and hybrid 10A2-1 contains 85% of a MAV-1-like provirus. Both propagation of λ hybrids and DNA preparation have been described (15).

Isolation of DNA Fragments for Nick Translation. DNA from λ proviral hybrids λ11A1-1 (AMV) and λ10A2-1 (MAV-1-like) was treated with the appropriate restriction en-

Abbreviations: AMV, avian myeloblastosis virus; AML, acute myeloblastic leukemia; MAV, myeloblastosis-associated virus; MC29, myelocytomatosis virus 29; MH II, Mill Hill II; AEV, avian erythroblastosis virus; ASV, avian sarcoma virus; nt, nucleotide(s); ntp, nucleotide pair(s); MDal, megadaltons; RSV, Rous sarcoma virus; RAV, Rous-associated virus.

donuclease and fractionated electrophoretically in a TAE (0.4 M Tris·HCl/50 mM sodium acetate/10 mM EDTA; pH 7.6) 0.7% Seaplaque gel (Seakem low temperature melting agarose) (Marine Colloids, Rockland, ME). The *Eco*RI/*Hin*dIII 0.85-MDal fragment from the AMV hybrid was prepared by digesting purified *Hin*dIII 2.6-MDal fragments with *Eco*RI followed by electrophoretic fractionation in a Seaplaque gel. DNA fragments were eluted from the gel by adding 5 vol of buffer C (50 mM Tris·HCl/pH 8.0/10 mM EDTA/10 mM NaCl) and melting the agarose at 65°C for 10 min, followed by two phenol extractions at 37°C, two chloroform extractions at room temperature, and two ether extractions. The DNA was then precipitated with ethanol and resuspended in H_2O. The DNA was labeled with [α-^{32}P]dCTP by a modification of the nick translation procedure described by Maniatis *et al.* (19). Radioactively labeled DNA fragments had a specific activity of approximately 8×10^7 cpm/μg.

Hybridization with ^{32}P-Labeled DNA. ^{32}P-Labeled DNA (0.1–0.2 μg in 10 ml of hybridization solution) was hybridized to DNA in Southern blots as described by Wahl *et al.* (20). Filters were washed three times in 0.30 M NaCl/0.03 M sodium citrate with 0.1% sodium dodecyl sulfate at room temperature and twice in the same solution at 68°C for 30 min each. Filters were exposed for autoradiography as described (17).

Heteroduplex and R-Loop Mapping. Heteroduplexes of the 5′-proximal or internal *Eco*RI fragments were formed between the AMV hybrid (λ11A1-1) and the MAV-1-like hybrid (λ10A2-1) and prepared for visualization by the procedure of Davis *et al.* (21). R-loops were formed between the AMV hybrid (λ11A1-1) and poly(A)-containing 35S AMV-S RNA purified on sucrose gradients (17). For R-loop formation, duplex DNA was crosslinked at an average of four sites per λ DNA molecule by the method of Kaback *et al.* (22). R-loops were then formed in sealed capillary tubes at 56°C for 16–24 hr in 0.1 M 1,4-piperazinediethanesulfonic acid (Pipes; Sigma) pH 7.8/0.4 M NaCl/10 mM EDTA/70% recrystallized formamide (MCB) with DNA at 4 μg/ml and RNA at 2 μg/ml. Immediately before spreading, approximately 4 μl of incubation mixture was diluted to 50 μl in 0.1 M Tris·HCl, pH 8.5/10 mM EDTA/70% formamide. Subsequent steps were similar to those used for the visualization of heteroduplexes. Grids were examined in a JEOL 100B transmission electron microscope and molecular lengths were measured in relation to the length of phage ϕX174 replicative form DNA, using a Hewlett–Packard 9825A computer and 9864A digitizer. All duplexed regions in both the heteroduplexes and the R-loops were approximately 10% shorter than lengths determined by restriction endonuclease mapping. All single-stranded regions measured in heteroduplexes were approximately 20% shorter than their lengths measured in gels. All measurements have been corrected to match those measured in gels.

Physical and Biological Containment. This work was carried out at the P2–EK2 containment levels as specified in the revised guidelines of the National Institutes of Health (1978).

RESULTS

Heteroduplex Analyses of *Eco*RI Fragments from the Presumptive AMV and MAV-1-like Proviruses. The λ proviral hybrids λ11A1-1 (containing the entire presumptive AMV genome) (15) and the hybrid λ10A2-1 (containing 85% of a MAV-1-like genome) (unpublished data) were derived from the DNA of AMV-B-induced leukemic myeloblasts after partial digestion with *Eco*RI. Maps obtained with seven restriction endonucleases presented in Fig. 1 show that the presumptive AMV genome and the MAV-1-like genome contain the same enzyme sites from the 5′ end (with respect to viral RNA) to the

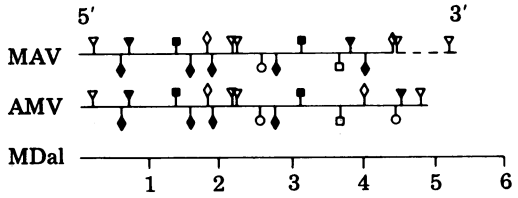

FIG. 1. Restriction enzyme maps of the presumptive AMV and MAV-1-like genomes. Restriction endonuclease sites are localized for the proviral DNA of the presumptive AMV provirus in λ hybrid 11A1-1 and the partial MAV-1-like provirus in λ hybrid 10A2-1. The broken line in the MAV map indicates that part of the MAV-1-like genome not present in the λ10A2-1 hybrid. The location of the 3′-terminal *Hin*dIII site in MAV-1 was determined with linear viral DNA (16). Enzyme sites: ▽, *Hin*dIII; ◇, *Eco*RI; ○, *Xba* I; □, *Kpn* I; ♦, *Bam*HI; ■, *Bgl* II; and ▼, *Xho* I.

Kpn I site. From the *Kpn* I site to the 3′-proximal *Eco*RI site, the MAV-1-like genome is 0.4 MDal larger than the presumptive AMV genome. This difference equals the total difference in mass between the two genomes (16). Two restriction endonuclease sites, for *Xho* I and *Bam*HI, present in the MAV-1-like genome between the *Kpn* I and the 3′ proviral *Eco*RI sites, are absent in the putative AMV provirus.

Heteroduplex analysis of the *Eco*RI fragments containing the 5′ terminus of the two proviral DNAs showed them to be duplexed over the entire length of the proviral portion [2800 ± 55 (± SD) nucleotide pairs (ntp)] (Fig. 2*A*). The single-stranded regions correspond to cellular DNA sequences adjacent to the AMV [4460 ± 540 nucleotides (nt)] or the MAV-1-like (1630 ± 190 nt) provirus.

Heteroduplex analysis of the internal *Eco*RI fragments from the putative AMV and MAV-1-like proviruses showed a duplex region of 2940 ± 110 ntp and two single-stranded arms measuring 1160 ± 80 nt and 455 ± 60 nt as measured for 12 molecules (Fig. 2*B*). The long single-stranded arm belongs to the MAV-1-like fragment and the short arm belongs to the AMV fragment, because the former is the larger (as deduced from the rates of migration in gels). The single-stranded arms also orient the duplexed molecule, because the restriction enzyme maps of the two proviruses appear identical to each other up to the *Kpn* I site. Hence, the single-stranded fork is at the 3′ end of the duplex with respect to viral RNA.

The heteroduplexes confirm that the presumptive AMV genome and the MAV-1-like genome are homologous for approximately 3.6 MDal starting from the 5′ terminus as suggested by the restriction endonuclease maps. The two genomes appear to lack homology beyond the *Kpn* I site as estimated from the lengths of the single-stranded arms in Fig. 2*B*.

The 3′-proximal *Eco*RI fragments could not be compared by this method because the available MAV-1-like hybrid lacks that fragment. In order to complete the comparison of the two genomes we carried out the following R-loop analyses.

R-Loops of the Presumptive AMV Provirus with 35S AMV-S RNA. R-loops of the putative AMV λ hybrid with 35S AMV-S RNA from purified AMV-S virions, which contain AMV and its helper, were studied with the following objectives: (*i*) to substantiate that the AMV provirus contains a substitution *vis-à-vis* the helper genome, (*ii*) to estimate the approximate size of the substitution, (*iii*) to determine whether the substitution consists of a single continuous sequence, and (*iv*) to determine whether sequences near the 3′ end of the genome are homologous in both the presumptive AMV and the MAV-1-like helper virus. Poly(A)-containing 35S AMV-S RNA contains two species—2.6 MDal (the RNA of the helper) and 2.4 MDal—separable by methylmercury gel electrophoresis (unpublished findings). The 2.6-MDal species is in greater abundance and is the only species detectable in 35S RNA isolated from MAV-1

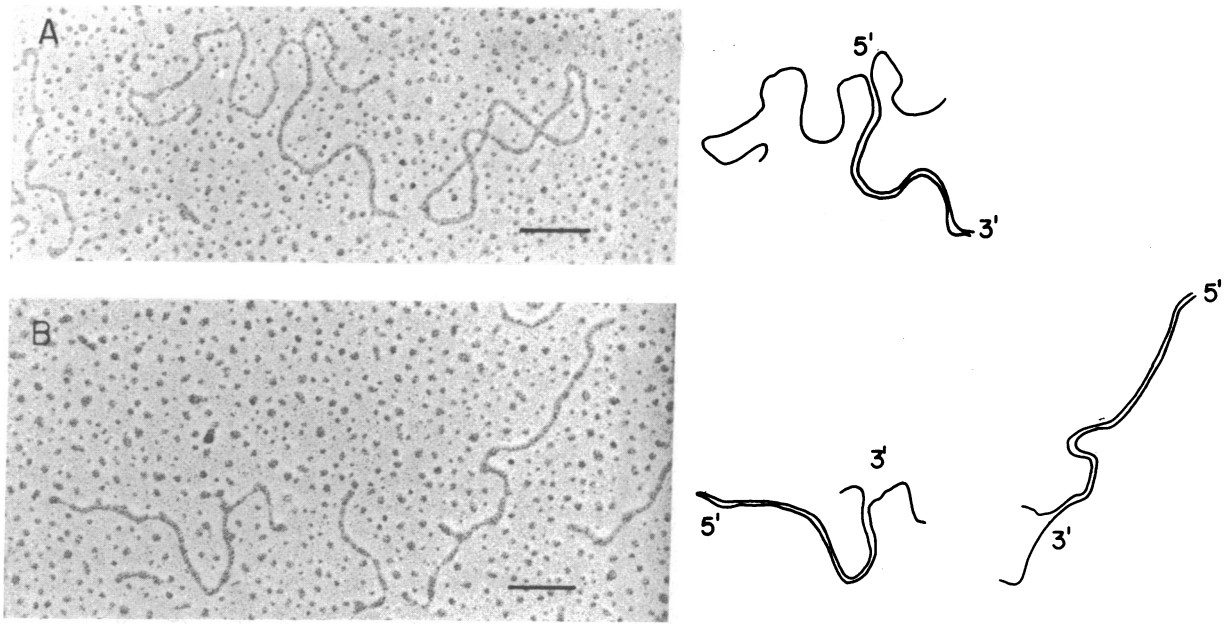

FIG. 2. Electron micrographs of heteroduplexed *Eco*RI fragments. (*A*) 5′-Terminal *Eco*RI proviral juncture fragments from λ hybrids 11A1-1 (AMV) and 10A2-1 (MAV). (*B*) Internal *Eco*RI proviral fragments from the same two hybrids. Bar represents 0.2 μm.

or MAV-2 (unpublished findings). This viral RNA mixture was R-looped with DNA from the λ11A1-1 AMV hybrid as described in *Materials and Methods*.

Electron microscopic examination revealed two distinct types of R-looped molecules (Fig. 3). The RNA in the R-loop shown in Fig. 3*A* was hybridized to one DNA strand along the entire length of the RNA. Four molecules of the type shown in Fig. 3*A* were measured; they had an average duplex length of 7160 ± 150 ntp, which is close to that of the 2.4-MDal RNA. These findings clearly demonstrate the existence of viral RNA complementary to the presumptive AMV provirus in RNA isolated from purified virions of the AMV-S complex. The second type of R-looped molecules (Fig. 3 *B* and *C*) conforms to the structure predicted by restriction enzyme and heteroduplex analyses for MAV RNA hybridized to the presumptive AMV DNA. These two electron micrographs best illustrate the three regions of RNA·DNA and DNA·DNA hybridization. From the measurement of nine structures of this type the 5′-proximal RNA·DNA hybrid has an average length of 5500 ± 50 ntp. The next region is a DNA·DNA duplex with a small "bubble" in most of the molecules, and is 900 ± 160 ntp. This DNA·DNA duplex region presumably represents the substituted sequences in the presumptive AMV genome and corresponds to the region in the MAV and AMV genomes where restriction enzyme mapping and heteroduplex analyses have also located a lack of homology. The small bubble probably represents a region of the DNA duplex that is unstable under the temperature and buffer conditions used for hybridization. The MAV RNA then enters into a second R-loop of 660 ± 260 ntp that continues up to the 3′ terminus (Fig. 3*C*). The relatively high variation in the lengths of the DNA·DNA duplex and of the 3′-proximal RNA·DNA loop probably reflects branch migrations in the switch-over between an RNA·DNA hybrid and a DNA·DNA duplex.

Southern Blot Analysis of Uninfected Chicken and Duck DNA with Specific AMV DNA Probes. Hybridization of 35S or 70S AMV-S ^{125}I-labeled RNA to Southern blots (23) of *Eco*RI-digested DNA from uninfected chickens of various strains had revealed homology to two cellular fragments that were not detectable with a RAV-0 probe (17). If AMV RNA contains sequences homologous to uninfected chicken DNA, then our cloned presumptive AMV DNA should also contain these sequences. To test this possibility, the AMV *Hin*dIII 2.6-MDal DNA fragment was labeled with [α-^{32}P]dCTP by the nick translation procedure and used as hybridization probe. The AMV *Hin*dIII 2.6-MDal fragment includes the region where AMV and MAV lack homology. Hybridization of this ^{32}P-labeled probe to Southern blots of *Eco*RI-digested DNA from uninfected chickens of four different strains and from one Peking duck is shown in Fig. 4. Duck DNA was chosen as a control because it does not contain sequences homologous to chicken endogenous proviruses (24). Two *Eco*RI fragments of 3.7 and 1.5 MDal previously detected with ^{125}I-labeled AMV-S RNA in uninfected chicken DNA hybridized to the *Hin*dIII 2.6-MDal fragment from the putative AMV provirus (Fig. 4, lanes a–d). The other bands represent endogenous proviral DNA also detectable with RAV-0 (17, 25) or MAV hybridization probes (D. G. Bergmann, personal communication). In an earlier publication (17) we had assigned masses of 3.1 and 1.4 MDal to the two cellular fragments that hybridized specifically with AMV-S RNA. The disparity in molecular mass reflects the current use of better molecular standards. The AMV *Hin*dIII 2.6-MDal probe revealed four homologous fragments in *Eco*RI-digested duck DNA (Fig. 4, lane e).

From the R-loop analysis it appears that the 3′-proximal *Eco*RI site in the presumptive AMV genome is located within the cellular substitution. If that is the case, then a probe consisting of AMV DNA from the 5′ side of this *Eco*RI site should detect either the 3.7-MDal or the 1.5-MDal *Eco*RI chicken DNA fragment. Conversely, an AMV DNA probe from the 3′ side of this *Eco*RI site should detect the other cellular DNA fragment. To test this possibility, the internal AMV *Eco*RI 2.2-MDal fragment and an AMV 0.85-MDal fragment located between the 3′-proximal *Eco*RI site and the 3′ *Hin*dIII site were used as probes representing the 5′ side and the 3′ side, respectively, of the 3′-proximal *Eco*RI site. The *Eco*RI 2.2-MDal probe detected the *Eco*RI cellular fragment of 3.7 MDal in addition to the 2.6-MDal endogenous proviral fragment in chicken DNA (Fig. 4, lanes f and g) and only two fragments in duck DNA (Fig. 4, lane h). The *Eco*RI/*Hin*dIII 0.85-MDal probe detected the *Eco*RI cellular fragment of 1.5 MDal and an endogenous proviral fragment in chicken DNA (Fig. 4, lanes

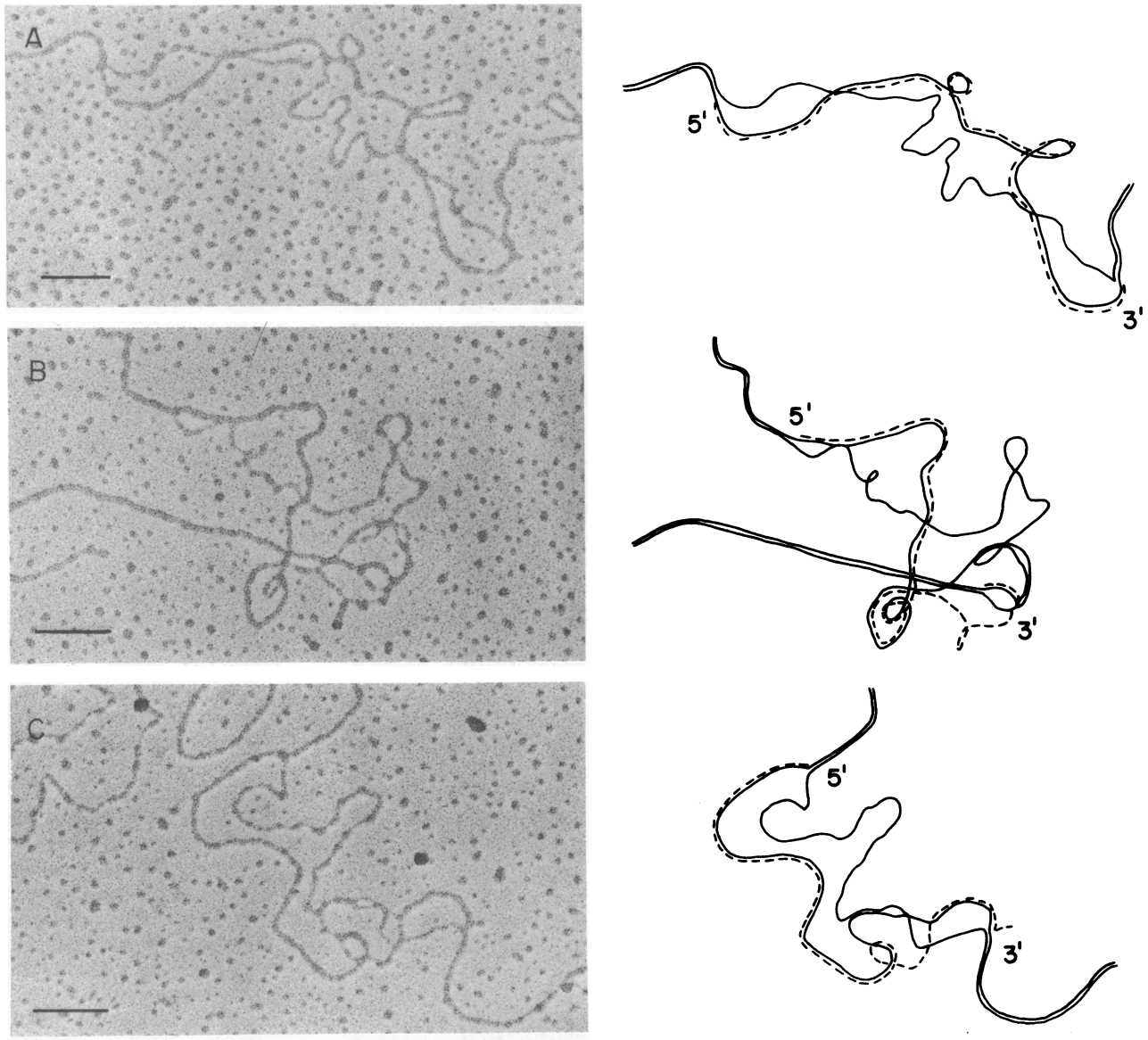

FIG. 3. Electron micrographs of R-loops formed between the presumptive AMV hybrid, λ11A1-1, and 35S AMV-S RNA. (*A*) Type 1 R-loops (AMV–AMV). (*B* and *C*) Type 2 R-loops (MAV–AMV). The broken line represents viral RNA. The R-loops are oriented 5′ to 3′ from our knowledge that the 5′ end of the provirus is adjacent to the short arm of the λ proviral hybrid DNA. Bar represents 0.2 μm. We point out that in *B* the 3′-terminal RNA·DNA hybrid and in *C* the 5′-terminal RNA·DNA hybrid are shorter than the average lengths, probably as a result of RNA degradation during hybridization.

i and j), and the other two fragments in duck DNA (Fig. 4, lane k). This demonstrates unequivocally that the 3′-proximal *Eco*RI site in the presumptive AMV genome resides in the inserted cellular DNA.

Finally, when the internal *Eco*RI 2.6-MDal fragment from the cloned MAV-1-like proviral DNA was ^{32}P-labeled and used as hybridization probe, only the *Eco*RI endogenous proviral fragment of 2.6 MDal was detected in uninfected chicken DNA (Fig. 4, lanes l and m) and there was no hybridization with duck DNA (Fig. 4, lane n). These findings are those expected with a probe that contains only sequences homologous to chicken endogenous proviral DNA.

DISCUSSION

Several independent findings have shown that the presumptive AMV genome contains a cellular substitution in the *env* gene. The substitution was identified as normal cellular DNA sequences by hybridization to specific chicken or duck DNA fragments that do not contain endogenous proviral sequences. By restriction endonuclease mapping and heteroduplex and R-loop analyses, the substitution has been shown to originate within 150 nt of the *Kpn* I site and to extend 900 ± 160 nt in the 3′ direction with respect to viral RNA. The R-loop and Southern blot analyses indicate that the substitution consists of a single continuous cellular DNA sequence. A probe containing over 1000 ntp of DNA sequence located on the 3′ side of the *Kpn* I site in the MAV-1-like helper provirus did not reveal any homology to cellular sequences other than to the *Eco*RI 2.6-MDal endogenous proviral fragment. We were unable to probe with MAV-1-like sequences nearer the 3′ genomic terminus because the MAV-1-like λ hybrid (10A2-1) lacks 15% of the viral genome located beyond the 3′-proximal *Eco*RI site. However, when MAV-2 RNA was hybridized to uninfected chicken DNA, the only detectable sequences were the endogenous proviral sequences, which hybridize also to RAV-0 RNA (D. G. Bergmann, personal communication). In addition, the R-loop data showing complete homology near the 3′ genomic termini of AMV and MAV make it unlikely that the 0.9 MDal of sequences located in that region of the MAV genomes has any homology to normal cellular sequences.

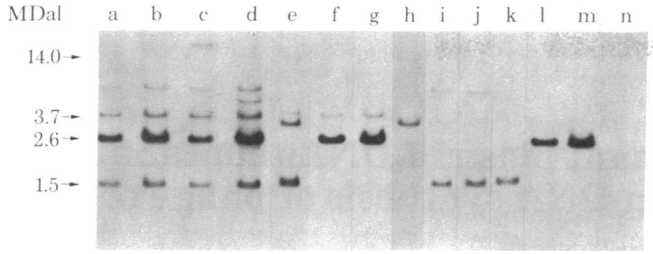

FIG. 4. Autoradiographs of Southern blots of *Eco*RI-digested chicken or duck embryonic DNA hybridized to specific ^{32}P-labeled proviral fragments from the AMV and MAV λ hybrids. Lanes a–e were hybridized to the *Hin*dIII AMV 2.6-MDal fragment: a, H & N C/O chicken; b, Spafas C/E chicken (from Spafas); c, $L6_3$ C/E chicken; d, Spafas C/E chicken (from Life Sciences); and e, Peking duck. Lanes f–h were hybridized to the *Eco*RI AMV 2.2-MDal fragment: f, H & N; g, Spafas C/E (Spafas); and h, Peking duck. Lanes i–k were hybridized to the *Eco*RI/*Hin*dIII AMV 0.85-MDal fragment: i, H & N; j, Spafas C/E (Spafas); and k, Peking duck. Lanes l–n were hybridized to the *Eco*RI MAV-1-like 2.6-MDal fragment: l, H & N; m, Spafas C/E (Spafas); and n, Peking duck. The duck DNA autoradiographs were exposed 1.5 times longer than the chicken DNA autoradiographs.

Many of the restriction endonuclease sites located on the 5′ side of the *Kpn* I site in linear viral DNA and on the 3′ side of the 3′-proximal *Eco*RI site are apparently conserved among the viruses of the AMV-S complex and the various strains of avian sarcoma viruses (ASV). In ASV, by comparing the restriction enzyme maps of mutants with a deletion of the *env* to the map of wild-type virus, *env* has been positioned between the *Kpn* I and the 3′-proximal *Xba* I sites of linear viral DNA (26). In the presumptive AMV provirus the location of the cellular substitution between the *Kpn* I and the 3′-proximal *Xba* I sites suggests that the inserted cellular sequences have replaced most, if not all, of the *env* gene. This would be consistent with the apparent defective nature of the leukemogenic component of AMV-S and its rescue with helper viruses belonging to various *env* subgroups (4).

The observation of homology along the entire length of the viral RNA in R-loops formed between the presumptive AMV λ hybrid and 35S AMV-S RNA indicates that an RNA genome homologous to the cloned provirus is present in AMV-S virions. RNA of the appropriate size (2.4 MDal) has been detected in methylmercury gels of AMV-S RNA, along with helper virus RNA of 2.6 MDal (unpublished findings).

The cellular substitution in the presumptive AMV genome is analogous to the *src* gene in ASV in that both types of sequences are present in various avian species. The *src* gene also has homology to cellular DNA sequences from diverse vertebrate species (27). The evolutionary conservation of the cellular sequences in AMV has not been tested, but it is suspected in view of their presence in duck DNA. The substitution in the putative AMV genome is approximately two-thirds the size of the *src* gene and one-half to one-third the size of the substitution in MC29, MH2, or AEV (11, 12). The substitution in MC29 and MH2 extends from within the *gag* gene and replaces all of the *pol* gene (12). The substitution in AEV also extends from within the *gag* gene, through the *pol* gene, and into the *env* gene (11). Adding the mass of the substituted cellular sequences (approximately 0.6 MDal) and that of the remaining deletion (0.4 MDal) indicates that the presumptive AMV genome appears to lack the entire *env* gene. A large unprocessed protein with *gag*-related antigens like that found in cells infected with MC29, MH2, or AEV (28–30) has not been detected in AMV-induced leukemic myeloblasts (unpublished results). Therefore, AMV appears to resemble the Bryan strain of RSV rather than the other avian transforming retroviruses. If the cellular sequences present in the AMV genome code for a specific leukemogenic protein, the latter's identification may require the use of techniques similar to those used to identify the *src* gene product. By analogy with the sarcoma-inducing *src* gene of ASV, the cellular insertion in AMV may code for the unique leukemogenic potential of this virus and may become known as the "*luk*" gene.

We thank Dr. J. W. Beard for providing AMV-S and Dr. L. B. Crittenden for providing Line 6_3 fertile eggs. Research was supported by Research Grant CA-10197 from the National Cancer Institute. L.M.S. was supported by predoctoral trainee awards from Institutional National Research Service Grant CA-09056 from the National Cancer Institute and Institutional Grant IN-131 from the American Cancer Society. J.N.S. was supported by Institutional National Research Service Grant CA-9030 from the National Cancer Institute. M.C.K. was supported by Research Grant AI-13410 from the National Institute of Allergy and Infectious Disease.

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