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Identification of a proviral genome associated with avian myeloblastic leukemia

(retrovirus/Southern blotting/recombinant DNA)

L. M. SOUZA, M. C. KOMAROMY, AND M. A. BALUDA

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

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ABSTRACT **We have identified and isolated a presumptive leukemogenic provirus from myeloblasts of a chicken in which leukemia had been induced by avian myeloblastosis virus (AMV). Leukemic myeloblasts isolated from peripheral blood or from converted yolk sac cultures of various strains of chickens, regardless of the endogenous proviral content or AMV pseudotype used for infection, contain an *Eco*RI 2.2-megadalton (MDal) and a *Hin*dIII 2.6-MDal proviral fragment. A proviral genome flanked by chicken DNA sequences on either side and containing both the *Eco*RI 2.2-MDal and the *Hin*dIII 2.6-MDal fragments was inserted by molecular recombination into λ phage Charon 4A and then cloned. This presumptive AMV proviral genome has a mass of approximately 4.9 MDal and contains terminal redundancies with respect to 3′ viral RNA sequences.**

The standard avian myeloblastosis virus (AMV-S) complex causes acute myeloblastic leukemia, osteopetrosis, visceral lymphoid leukosis, and nephroblastomas in chickens (1). Two cloned avian myeloblastosis-associated viruses, MAV-1 and MAV-2, subgroups A and B, respectively, cause all the neoplasias noted for AMV-S except acute myeloblastic leukemia (2). Thus, an as-yet-unidentified virus in the AMV-S complex is responsible for myeloblastosis. This leukemogenic component (AMV) is thought to be defective because it can induce formation of leukemic myeloblasts in which there is no detectable virus production but from which AMV can be rescued after superinfection with a suitable helper (3). Recently, preparations of AMV-S unintegrated linear proviruses have been shown to contain a viral DNA genome of approximately 4.9 megadaltons (MDal), which is slightly smaller than that of MAV-1 or MAV-2 (5.3 MDal) and could be the genome of the AMV leukemogenic component (4).

Three approaches have been taken to further identify the AMV genome: (*i*) DNA extracted from leukemic myeloblasts isolated from the peripheral blood of leukemic chickens was analyzed for viral DNA sequences after restriction endonuclease digestion; (*ii*) DNA from cloned myeloblasts converted *in vitro* by AMV infection of yolk sac cell cultures (5) was analyzed in the same manner as the DNA from the myeloblasts isolated from leukemic chickens; and (*iii*) an *Eco*RI partial digest of DNA from leukemic myeloblasts was inserted into λ phage Charon 4A by artificial DNA recombination and analyzed for proviral sequences.

A presumptive leukemogenic provirus has been identified and isolated from leukemic myeloblasts. It has a molecular mass of approximately 4.9 MDal, the same as that of an unintegrated viral DNA present in preparations of AMV-S linear DNA but absent in linear viral DNA preparations of MAV-1 or MAV-2 (4).

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MATERIALS AND METHODS

Chicken Strains 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 (Redmont, WA), C/ABE line 7-2 V^+ from the Regional Poultry Research Laboratory (East Lansing, MI), and Hubbard cross rapid growth broilers from Victor Ryckebosh (Lancaster, CA). AMV-S BAI strain A was kindly provided as frozen leukemic plasma by J. W. Beard (Life Sciences, St. Petersburg, FL). AMV-B (supernate of cultured Spafas C/E leukemic myeloblasts producing AMV of subgroup B only) and AMV-C (AMV-C originated from a clone of nonproducer C/E Spafas leukemic myeloblasts superinfected with tdB77 subgroup C) were kindly provided by C. Moscovici (Veterans Administration Hospital, Gainesville, FL).

Isolation of Leukemic Myeloblasts. Spafas C/E chicken 21710, injected intraperitoneally with 0.1 ml of AMV-B upon hatching, developed acute myeloblastic leukemia and was sacrificed 24 days after injection. All leukemic myeloblasts were cultured in BM II medium (5) supplemented with 10% fetal calf serum and 10% heated chicken serum. Myeloblast clones HL2-500A3 and HL2-5D2 were isolated as foci under agar from cultured yolk sac cells of Hubbard embryo HL2 (1×10^6 cells per 60-mm dish), infected with a 1:5 or a 1:500 dilution of AMV-S. The myeloblast clones isolated *in vitro* were grown on chicken embryonic fibroblast feeder layers irradiated with 10,000 rads (100 grays) until they reached a concentration of approximately 5×10^6 cells per ml. Myeloblast clone 8A61 was isolated from H&N C/O yolk sac cells infected with supernate from cloned L7-2 C/ABE leukemic myeloblasts converted with AMV-C. All embryos used for yolk sac cell cultures were separately frozen for subsequent DNA extraction to analyze their endogenous proviral DNA content.

Preparation of AMV-S ^{125}I-Labeled 70S RNA and Hybridization Conditions. The 70S viral RNA isolated from AMV-S (6) was labeled with ^{125}I to a specific activity of 2.0×10^8 cpm/μg as described (7). Hybridization of iodinated 70S AMV RNA at 0.045 μg/ml to Southern blots was carried out as before (7). Poly(A)-containing AMV ^{125}I-RNA of 6–14 S was selected as described (7) and used as 3′ end probe. Hybridization with poly(A)-selected AMV ^{125}I-RNA at 0.01 μg/ml to Southern blots was carried out as with total AMV ^{125}I-RNA.

Isolation and Restriction Endonuclease Cleavage of Cellular DNA. High molecular weight ($\geq 3.0 \times 10^7$) DNA was isolated from leukemic myeloblasts by the Hirt procedure. Restriction endonucleases *Eco*RI and *Hin*dIII were prepared in our laboratory. Cellular DNA (20 μg) was digested with either *Eco*RI or *Hin*dIII as described (7). Phage λ DNA frag-

Abbreviations: AMV, avian myeloblastosis virus; AMV-S, standard AMV; MAV, myeloblastosis-associated virus; MDal, megadaltons, moi, multiplicity of infection.

ments generated with *Hin*dIII under the same conditions as the cellular DNA fragments served as molecular weight markers.

λ Hybrid Phage Construction and Isolation of Hybrids Containing Proviral DNA Sequences. DNA from leukemic myeloblasts of chicken 21710 was partially digested with *Eco*RI (0.5 mg of cellular DNA plus 500 units of *Eco*RI in a reaction volume of 2.5 ml for 5 min at 37°C), then extracted twice with an equal volume of phenol/$CHCl_3$ (1:1, vol/vol) and once with $CHCl_3$ only, and finally precipitated with ethanol. The DNA was resuspended in 1 ml of buffer E (10 mM Tris·HCl, pH 8.0/10 mM EDTA), held at 68°C for 20 min, layered on a sucrose gradient, and sedimented for 43 hr at 20,000 rpm (8) in an SW 27 rotor of a Beckman centrifuge. Fractions containing DNA molecules ranging in size from 16,000 to 22,000 base pairs were pooled and precipitated with ethanol. The DNA was resuspended in H_2O at 2 mg/ml. The leukemic DNA fragments were then ligated to the *Eco*RI-generated arms of λ Charon 4A (8) and packaged *in vitro* (9). The yield was 1.5×10^6 plaque-forming units per μg of inserted DNA. Screening of 250,000 plaques (10) on *Escherichia coli* DP-50 *supF* (9) by hybridization with AMV-S 70S ^{125}I-RNA yielded 6 positive plaques. One clone, λ11A1-1, was used to prepare high-titer stocks in *E. coli* DP-50 *supF* for subsequent DNA purification and restriction endonuclease analysis.

Purification of λ Hybrid Phage DNA. Crude phage lysates were concentrated by precipitation with 10% polyethylene glycol, resuspended in buffer F (50 mM, Tris·HCl, pH 7.4/10 mM $MgSO_4$), and treated with 20 μg of DNase I (Sigma, DN-100) per ml at 37°C for 30 min. The phage suspension was extracted once with an equal volume of $CHCl_3$, then solid CsCl was added to a density of 1.5 g/ml. The λ hybrid phage was purified by CsCl density equilibrium centrifugation in a Beckman SW 50 rotor at 35,000 rpm for 40 hr. The phage were dialyzed against buffer G (10 mM, Tris·HCl, pH 7.4/5 mM $MgSO_4$), then mixed with 1/5 vol of 5 × buffer C (250 mM Tris·HCl, pH 7.4/50 mM NaCl/50 mM EDTA). Sodium dodecyl sulfate was added to a final concentration of 0.2% and proteinase K to 250 μg/ml. The mixture was incubated at 37°C for 1 hr, extracted twice with phenol/$CHCl_3$ and once with $CHCl_3$ alone, then precipitated with ethanol.

Restriction Endonuclease Cleavage of DNA from λ Hybrids. λ hybrid DNA (0.5 μg) was digested with a 3-fold excess of either *Eco*RI or *Hin*dIII as described (7). The digested DNA was fractioned in 0.7 or 1.2% agarose gel electropherograms and blotted onto nitrocellulose paper. Hybridization conditions with a 70S AMV-S ^{125}I-RNA probe were identical to those used for cellular DNA blots.

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

RESULTS

***Eco*RI or *Hin*dIII Restriction Endonuclease Analysis of Proviral DNA in Chicken Peripheral Blood Leukemic Myeloblasts.** To determine what additional proviral sequences appear in leukemic cells induced by AMV-B, the endogenous proviral DNA background of each experimental chicken must be determined, because the endogenous pattern varies from strain to strain and even within a particular strain (7, 11–13). Southern blots of *Eco*RI- or *Hin*dIII-generated DNA fragments from uninfected chicken embryo 13-3 (Spafas C/E) hybridized with ^{125}I-labeled AMV-S RNA are shown in Fig. 1, lanes a and c, respectively. The banding patterns observed for the uninfected 13-3 embryonic DNA are representative of the endogenous background for all the experimental birds that were injected with AMV-B. DNA from virus-producing leukemic myeloblasts isolated from leukemic chicken 21710 injected with AMV-B was analyzed in the same manner. *Eco*RI-digested leukemic myeloblast DNA (Fig. 1, lane b) contains one band (2.2 MDal) in addition to the endogenous sequences. *Hin*dIII-digested leukemic myeloblast DNA (Fig. 1, lane d) contains three new bands (2.6, 1.95, and 0.8 MDal) in addition to the endogenous bands. Both the *Eco*RI 2.2-MDal fragment and the *Hin*dIII 2.6-MDal fragment have also been observed in identically treated unintegrated linear viral DNA of AMV-S, but not in that of either MAV-1 or MAV-2 (4).

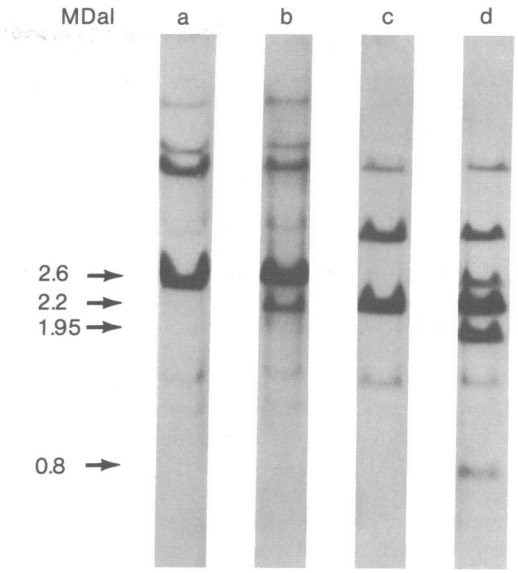

FIG. 1. Proviral Southern blots of: lane a, *Eco*RI-digested embryo 13-3 DNA; lane b, *Eco*RI-digested leukemic myeloblast 21710 DNA; lane c, *Hin*dIII-digested embryo 13-3 DNA; lane d, *Hin*dIII-digested leukemic myeloblast 21710 DNA. All blots were hybridized with AMV-S ^{125}I-RNA.

*Hin*dIII treatment of linear MAV-1 DNA, either unintegrated or integrated, generates internal fragments of 2.3, 1.95, and 0.8 MDal, and *Hin*dIII treatment of MAV-2 viral DNA generates fragments of 3.1 and 1.95 MDal (4). Two additional MAV-1 and MAV-2 *Hin*dIII fragments of 0.2 MDal or less, representing the 5′ and 3′ termini of linear viral DNA, are attached to cellular sequences in the integrated state (juncture fragments) (4). Such juncture fragments are not normally seen in uncloned infected chicken cell cultures (unpublished).

The presence of the 0.8-MDal *Hin*dIII fragment in the 21710 leukemic DNA (Fig. 1, lane d) indicates that the virus-producing 21710 myeloblasts may contain a MAV-1-like helper virus in addition to the AMV genome. The 2.3-MDal *Hin*dIII fragment of the MAV-1-like helper would comigrate with an endogenous fragment that has the same size, and the 1.95-MDal band is common to MAV-1 and MAV-2. By interference assay, it was found that the AMV-B preparation used in this study contained only subgroup B virus despite the fact that its *Hin*dIII-generated banding pattern is identical to that of MAV-1 (unpublished), which is a subgroup A virus (14).

The presence of the 2.2-MDal *Eco*RI and 2.6-MDal *Hin*dIII fragments either in leukemic myeloblast DNA (proviral DNA) or in AMV-S linear viral DNA indicates that both of these fragments are internal in a proviral genome. Both the *Eco*RI 2.2-MDal and the *Hin*dIII 2.6-MDal bands have been detected in AMV-induced leukemic myeloblasts from several leukemic chickens regardless of their endogenous background or of the pseudotype of AMV used for infection (unpublished).

EcoRI or HindIII Analysis of Proviral DNA in Cloned Myeloblasts from AMV-Infected Yolk Sac Cultures. Two additional approaches with cloned myeloblasts converted *in vitro* were used to further characterize as AMV specific the *Eco*RI 2.2-MDal and the *Hin*dIII 2.6-MDal fragments seen in peripheral blood leukemic myeloblasts. First, cultured yolk sac cells from Hubbard cross embryo HL2 were infected with AMV-S at two different dilutions. Leukemic myeloblasts arising in cultures inoculated with a low input multiplicity of infection (moi) are more likely to contain only the AMV genome, whereas at high moi it is more likely that both the helper genome and the AMV genome will be present in the same cell. Two leukemic myeloblast clones were isolated, HL2-5D2 (low moi) and HL2-500A3 (high moi), and their DNA was analyzed with *Eco*RI or *Hin*dIII.

Again, as in the *Eco*RI-treated 21710 leukemic myeloblast DNA, a band of 2.2 MDal is apparent in the DNA of both myeloblast clones (Fig. 2, lane b and Fig. 3, lane c), but is absent in the uninfected HL2 embryonic DNA (Fig. 2, lane a). Other bands present in addition to the endogenous bands and the 2.2-MDal band presumably represent juncture bands between cellular DNA and DNA from either AMV or the helper virus. The masses of the two juncture bands, approximately 1.9 and 1.0 MDal, detected in HL2-5D2 (Fig. 2, lane b) appear to be close to those of the *Eco*RI fragments expected from unintegrated viral DNA. However, digestion of this clone DNA with *Kpn* I, which cleaves AMV and MAV DNAs only once, does not reveal any free viral DNA (unpublished).

In addition to the endogenous band pattern (Fig. 2, lane d), *Hin*dIII-digested DNA from HL2-5D2 myeloblasts contains 2.6 and 1.95-MDal fragments (Fig. 2, lane e), whereas HL2-500A3 myeloblasts contain 2.6, 1.95, and 0.8-MDal fragments (Fig. 2, lane f). Because of the presence of a faint endogenous 1.95-MDal fragment, great care was taken to use identical amounts of DNA from the control embryo and from leukemic myeloblasts to demonstrate a quantitative difference. This difference was reproduced in each of two additional experiments. The absence of the *Hin*dIII 0.8-MDal band in clone HL2-5D2 induced at low moi of AMV indicates that this clone lacks the MAV-1-like helper genome. Clone HL2-500A3, however, appears to contain the MAV-1-like helper, because it contains the 0.8-MDal band. The apparent absence of the helper genome in HL2-5D2 allows the tentative conclusion that the AMV genome contains both 2.6 and 1.95-MDal *Hin*dIII fragments.

The second experimental approach was to change the helper virus by using a different AMV pseudotype. AMV-C—i.e., AMV(tdB77-C)—was used for this purpose because the restriction endonuclease map for B77 viral DNA is known (15, 16), and tdB77 differs from B77 only in the deletion of the *src* gene from its genome (17). DNA from one leukemic myeloblast clone (8A61) isolated from an AMV-C-infected yolk sac culture of H&N C/O embryo 8 was analyzed. The 2.2-MDal *Eco*RI fragment again is present (Fig. 3, lane b), but not in the uninfected embryonic DNA of H&N 8 (Fig. 3, lane a). Also, there are three other *Eco*RI bands (3.1, 1.7, and 0.9 MDal) in the 8A61 DNA that are not present in the uninfected embryonic DNA. Because B77 contains *Eco*RI sites in the terminal genomic repeats and at two other internal sites (neither of which are in the *src* gene) (15, 16), tdB77 should also contain these sites, generating three internal viral DNA fragments of 2.6, 1.7, and 0.9 MDal. All three of these bands are present in the 8A61 clone, but the 2.6-MDal fragment comigrates with an endogenous 2.6-MDal fragment. The *Eco*RI band of 3.1 MDal should represent an AMV juncture band because the terminal *Eco*RI sites in tdB77 viral DNA do not leave enough of the viral genome attached to cellular DNA to be detected. A second AMV juncture band is not seen, perhaps because it is of the same size as one of the other fragments and comigrates with it.

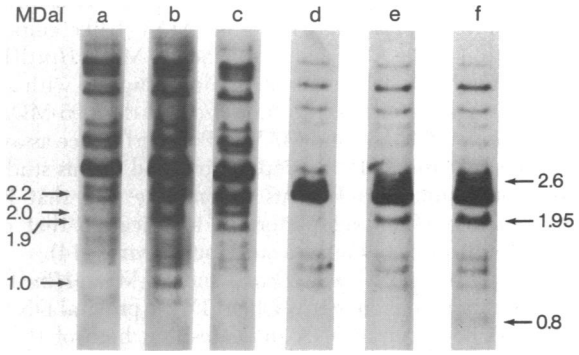

FIG. 2. Proviral Southern blots of *Eco*RI-cleaved DNA from: lane a, HL2 embryo; lane b, HL2-5D2 leukemic myeloblast; lane c, HL2-500A3 leukemic myeloblast. Proviral Southern blots of *Hin*dIII-cleaved DNA from: lane d, HL2 embryo; lane e, HL2-5D2; lane f, HL2-500A3. All blots were hybridized with AMV-S ^{125}I-RNA.

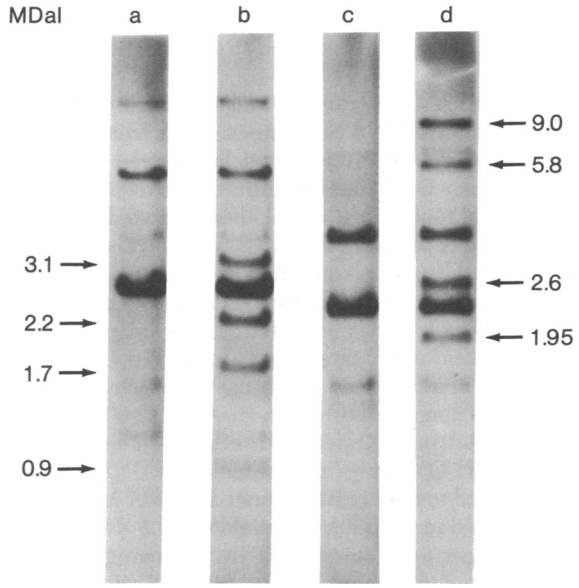

FIG. 3. Proviral Southern blots of: lane a, *Eco*RI-cleaved H&N 8 embryo DNA; lane b, *Eco*RI-cleaved 8A61 leukemic myeloblast DNA; lane c, *Hin*dIII-cleaved H&N 8 embryo DNA; lane d, *Hin*dIII-cleaved 8A61 leukemic myeloblast DNA. All blots were hybridized with AMV-S ^{125}I-RNA.

After *Hin*dIII digestion, DNA from clone 8A61 (Fig. 3, lane d) shows four fragments not seen in the control DNA (Fig. 3, lane c). One fragment of 2.6 MDal and another one of 1.95 MDal are the same bands present in clone HL2-5D2, which appears to contain only the AMV genome. Linear B77 DNA contains two *Hin*dIII sites approximately 0.1 MDal apart that are located approximately 2.1 MDal from the 5′ end with respect to the viral RNA (15, 16). These two sites should also be present in tdB77 provirus and, if cleaved, should yield two juncture bands and an internal fragment of approximately 0.1 MDal. The two juncture bands are present in *Hin*dIII-digested clone 8A61 DNA (9.0 and 5.8 MDal), but the 0.1 MDal fragment would migrate off these gels.

The data from AMV-C-induced clone 8A61 further demonstrate that the putative genome of AMV contains 2.6- and 1.95-MDal *Hin*dIII fragments as previously indicated by data from clone HL2-5D2.

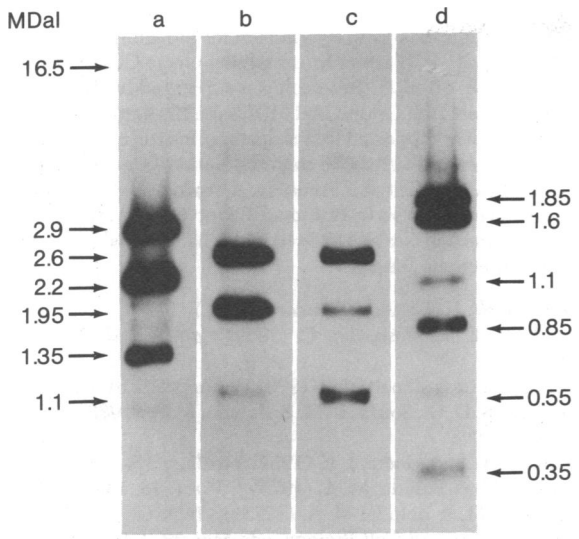

FIG. 4. Southern blots of λ11A1-1 DNA hybridized with AMV-S ^{125}I-RNA: lane a, *Eco*RI-digested; lane b, *Hin*dIII-digested. Lane c, Southern blot of *Hin*dIII-digested λ11A1-1 DNA hybridized with 6–14S poly(A)-selected AMV-S ^{125}I-RNA. Lane d, Southern blot of λ11A1-1 DNA sequentially digested with *Eco*RI and *Hin*dIII run in a 1.2% gel and hybridized with AMV-S ^{125}I-RNA. The molecular masses on the left are for lanes a–c, while those on the right are for lane d.

Characterization of a Leukemic DNA–λ Hybrid Containing the Presumptive AMV Provirus. Provirus–λ hybrid clones isolated from a chicken–λ hybrid library could contain endogenous, MAV-1-like, or AMV sequences, which can be distinguished from each other by analysis of their *Eco*RI and *Hin*dIII Southern blots. One leukemic chicken–λ hybrid clone, λ11A1-1, appears to contain the entire presumptive AMV provirus. After digestion with *Eco*RI, Southern blotting, and hybridization with AMV-S ^{125}I-RNA, three viral DNA bands are detected: 2.9, 2.2, and 1.35 MDal (Fig. 4, lane a). After digestion with *Hin*dIII, four viral DNA bands are detected: 16.5, 2.6, 1.95, and 1.1 MDal (Fig. 4, lane b). The appearance of the *Eco*RI 2.2- and *Hin*dIII 2.6- and 1.95-MDal fragments demonstrates compellingly that λ clone 11A1-1 contains the presumptive AMV genome. The other two *Hin*dIII fragments (16.5 and 1.1 MDal) and *Eco*RI fragments (2.9 and 1.35 MDal) should then represent juncture fragments, because they are not detected in their respective digests of 21710 leukemic myeloblast DNA.

To map the *Eco*RI and *Hin*dIII sites within the proviral sequences of the putative AMV clone, we double digested the clonal DNA with both enzymes (Fig. 4, lane d). As dictated by the data from both the double and single digestions, these fragments can be ordered only in the following manner: 0.55, 0.85, 1.85, 0.35, 1.6, 1.1 MDal (details of the mapping will be presented elsewhere). The 1.1 and 0.55-MDal fragments represent the juncture fragments. To approximate the amount of proviral sequences in these two fragments, we scanned the autoradiograph with a Joyce–Loebl densitometer. Relative to the 0.35-MDal internal proviral fragment, the 1.1-MDal fragment contained 0.2-MDal of proviral sequence and the 0.55 MDal-fragment contained 0.07-MDal of proviral sequence. Summation of all the internal proviral fragments and 0.27 MDal representing the amount of provirus in the two juncture bands yields a proviral genome of approximately 4.9 MDal.

To orient the proviral genome with respect to the viral RNA, a poly(A)-containing 3′ end probe of 6–14S ^{125}I-labeled AMV-S RNA was hybridized to a *Hin*dIII blot (Fig. 4, lane c) and an

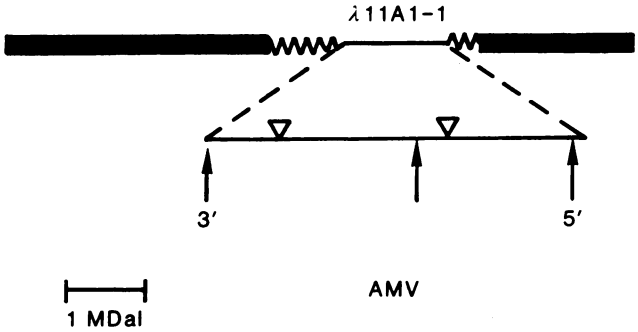

FIG. 5. Map of the presumptive AMV proviral λ clone 11A1-1. The map is divided into domains representing Charon 4A phage λ sequences (■), chicken cellular sequences (〰), and proviral sequences (—). That section of the clone representing the presumptive AMV sequences is enlarged below the clone to show *Eco*RI sites (▽), and *Hin*dIII sites (↑). The genome is also shown oriented with respect to viral RNA as indicated by 3′ and 5′. The scale shown applies only to the presumptive AMV genome, but the total clonal DNA is drawn according to another smaller scale.

*Eco*RI blot (data not shown). The *Hin*dIII 2.6-MDal and *Eco*RI 1.35-MDal fragments contained the most homology to the 3′ probe and as such represent the 3′ end with respect to the viral RNA. There is also considerable homology to the 3′ probe in the *Eco*RI 2.9-MDal and *Hin*dIII 1.1-MDal fragments. These two fragments are derived from the opposite end of the provirus (5′ end), indicating a terminal redundancy with respect to 3′ RNA sequences. The *Hin*dIII 1.95-MDal and *Eco*RI 2.2-MDal fragments have little homology to the 3′ probe and might be detected as a result of contaminating RNA sequences from other than the 3′ end. The *Hin*dIII 16.5-MDal fragment, which contains only 0.07 MDal of proviral sequences and is derived from the 3′ end of the provirus, is only faintly detected after long exposure.

All the mapping data are summarized in Fig. 5.

DISCUSSION

A proviral genome associated with leukemogenesis has been identified and isolated from chicken leukemic myeloblasts induced by AMV. The isolation of λ hybrid clone 11A1-1 directly associates the *Eco*RI 2.2-MDal and *Hin*dIII 2.6-MDal leukemia-specific fragments within a proviral genome. These two fragments have been detected in leukemic myeloblasts from various strains of chickens regardless of their endogenous proviral DNA complement. The same two fragments appear in cloned leukemic cells independently of the nature of the helper virus used in the infection—e.g., myeloblast clone 8A61—or even in the absence of the helper genome—e.g., myeloblast clone HL2-5D2. In contrast, the red blood cells from some leukemic birds lack these specific fragments and appear to contain only the helper genome (unpublished data). The putative AMV proviral genome, by virtue of the presence of the *Eco*RI 2.2-MDal and *Hin*dIII 2.6-MDal fragments, is different from both MAV-1 and MAV-2, which do not cause myeloblastic leukemia. A λ clone containing MAV-1-like proviral sequences has also been isolated from the DNA of 21710 leukemic myeloblasts (unpublished). Thus, as suggested by the *Eco*RI and *Hin*dIII analysis of total DNA from 21710 leukemic myeloblasts, both the presumptive AMV and the MAV-1-like proviruses are present although the helper is not necessarily present in all leukemic cells.

The size of the presumptive AMV genome (4.9 MDal) is only slightly smaller than that of its natural helper(s) (5.3 MDal), and is the same as the size of the minor species of unintegrated linear viral DNA generated by AMV-S, but not by either MAV (4).

In methylmercury gels, 70S RNA from AMV-S shows two components of approximately 2.6 and 2.4 MDal (our unpublished results; J. Myers, and J. M. Bishop, personal communications), whereas MAV contains only one RNA species of 2.6 MDal (our unpublished results). Thus, the AMV-S linear viral DNA of 4.9 MDal may be the reverse transcription product of the 2.4-MDal RNA. This makes the AMV DNA genome larger than that of other defective acute leukemia viruses—e.g., MC29, which is much smaller (3.8 MDal) than its helper (5.6 MDal) (18).

*Hin*dIII digestion of clone HL2-500A3 indicates that both the AMV and MAV-1-like genomes are present, but only one juncture band is apparent after *Eco*RI digestion. One explanation could be that the other three bands comigrated with endogenous bands, although that seems unlikely. A more likely explanation is that the helper viral DNA did not become integrated until after the myeloblasts had divided one or more times, effectively making the cells nonclonal with respect to the helper virus.

The presumptive AMV provirus, like that of MAV-1 or MAV-2, contains at both termini *Hin*dIII sites and redundancies complementary to the 3′-terminal viral RNA sequences. Avian sarcoma virus (ASV) linear DNA, unintegrated or integrated, also contains large terminal repeats that consist of 3′-RNA-related sequences followed by 5′-RNA-related sequences (15, 16). The ASV 3′ sequence is approximately twice the size of the 5′ sequence, and *Pvu* I cuts within these large terminal repeats to yield two end fragments of different sizes. If we assume that AMV and the MAV proviruses contain similarly structured large terminal repeats, the *Hin*dIII sites should be at the border between the 3′ and 5′ sequences in the large terminal repeats. This would explain the paradox that the *Hin*dIII 16.5-MDal juncture fragment that originates from the 3′ end lacks homology to the 3′ viral RNA probe. The presumptive proviral AMV *Eco*RI juncture fragments contain approximately 1.8 and 0.9 MDal of viral DNA as determined by *Eco*RI/*Hin*dIII double digestion. The size of these fragments is apparently identical to that of the *Eco*RI end fragments of MAV-1 or MAV-2 viral DNA (4). However, the internal 2.2-MDal fragment of the putative AMV genome is 0.4 MDal shorter than its corresponding fragment in the helper and may contain the alteration that makes it defective and leukemogenic.

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1. Beard, J. W. (1963) *Adv. Cancer Res.* **7,** 1–127.
2. Smith, R. E. & Moscovici, C. (1969) *Cancer Res.* **29,** 1356–1366.
3. Moscovici, C. & Zanetti, M. (1970) *Virology* **42,** 61–67.
4. Bergmann, D. G., Souza, L. M. & Baluda, M. A. (1980) *J. Virol.*, in press.
5. Baluda, M. A. & Goetz, I. E. (1961) *Virology* **15,** 185–199.
6. Shoyab, M. & Baluda, M. A. (1975) *J. Virol.* **16,** 1492–1502.
7. Souza, L. M. & Baluda, M. A. (1978) in *ICN-UCLA Symposia on Molecular and Cell Biology*, eds. Stevens, J. G., Todaro, G. J. & Fox, C. F. (Academic, New York), Vol. 11, pp. 217–229.
8. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K. & Efstratiadis, A. (1978) *Cell* **15,** 687–701.
9. Blattner, F. R., Blechl, A. E., Thompson, K. D., Faber, H. E., Richards, J. E., Slightom, J. L., Tucker, P. W. & Smithies, O. (1978) *Science* **202,** 1279–1284.
10. Benton, W. D. & Davis, R. W. (1977) *Science* **196,** 180–182.
11. Astrin, S. M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5941–5945.
12. Hughes, S. H., Payvar, F., Spectro, D., Schimke, R. T., Robinson, H. L., Payne, G. S., Bishop, J. M. & Varmus, H. E. (1979) *Cell* **18,** 347–359.
13. McClements, W., Hanafusa, H., Tilghman, S. & Skalka, A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2165–2169.
14. Moscovici, E. & Vogt, P. (1968) *Virology* **35,** 487–497.
15. Shank, P. R., Hughes, S. H., Kung, H. J., Major, J. E., Quintrell, W., Guntaka, R. V., Bishop, J. M. & Varmus, H. E. (1978) *Cell* **15,** 1383–1395.
16. Hsu, T. W., Sabrane, J. L., Mark, G. E., Guntaka, R. V. & Taylor, J. M. (1978) *J. Virol.* **28,** 810–818.
17. Vogt, P. K. (1971) *Virology* **46,** 939–946.
18. Duesberg, P. H., Bister, K. & Vogt, P. K. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4320–4324.