Avian Myeloblastosis Virus Transforming Gene Is Related to Unique Chicken DNA Regions Separated by at Least One Intervening Sequence

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Identification of several additional restriction endonuclease sites within the cellular substitution (*amv*) inserted into the avian myeloblastosis virus proviral genome has permitted us to isolate different regions of the *amv* sequence. These subsets of the avian myeloblastosis virus transforming gene have been cloned in the plasmid pBR322 and used as hybridization probes to investigate the topology of homologous (*proto-amv*) normal chicken DNA sequences. The results showed that the cellular *proto-amv* sequences in C/O chicken DNA are interrupted by at least one intervening sequence. A partial arrangement of the *proto-amv* sequences is presented.

Avian myeloblastosis virus (AMV) contains a sequence of approximately 1,000 nucleotides which appears to be responsible for acute myeloblastic leukemia in chickens (25, 26). This sequence, designated *amv*, is homologous to cellular DNA sequences (*proto-amv*) present in all vertebrate species from amphibians to humans (5, 24, 27). This AMV leukemogenic sequence is not present in the helper myeloblastosis-associated virus types 1 and 2 (MAV-1 and MAV-2) or in Rous-associated virus type 0, the endogenous virus produced by some chicken strains (24-26). Previous endonuclease mapping of the λ Charon 4A recombinant clone (λ 11A1-1) carrying the AMV provirus has revealed that the *amv* sequence lies between the *Kpn*I and 3'-proximal *Xba*I sites and contains one *Eco*RI site but no *Bam*HI or *Hind*III site (Fig. 1). The hybridization results obtained earlier with the cloned AMV *Hind*III fragment of 3.9 kilobase pairs (kb) (5) and with other probes (26) (AMV internal *Eco*RI-*Eco*RI and *Eco*RI-3' *Hind*III fragments) indicated that three chicken DNA fragments generated by *Eco*RI digestion contain genetic information homologous to the *amv* sequences. Two of them are adjacent, the 5.4-kb fragment and either the 2.2- or the 8.7-kb fragment, whereas the third one is farther apart or present at a different homologous genetic locus. The first alternative suggests that the normal cellular *proto-amv* DNA may contain an intervening sequence (intron). Most eucaryotic genes contain introns, up to approximately 50 in the α -collagen gene (32), whereas some genes have none, e.g., histone (13) and interferon (15, 18). The cellular analogs of viral transforming se-

quences analyzed so far also seem to fall into two categories with respect to introns. Some have introns, e.g., the cellular sequences homologous to the transforming regions of Abelson murine leukemia virus, feline sarcoma virus, and Rous sarcoma virus (11, 22), whereas some do not, e.g., the Moloney-murine sarcoma virus-related sequences (19). The chicken cellular DNA sequences homologous to the *src* gene of the Prague C strain of Rous sarcoma virus have recently been shown to contain five or six intervening sequences (22).

The probes used in the earlier studies contained some viral sequences common to AMV and MAV in addition to *amv*. Therefore, it could not be ruled out that some *Eco*RI or *Hind*III chicken DNA fragments which hybridized to these probes contained endogenous proviral sequences or that some cellular *proto-amv* fragments had the same size as the endogenous proviral fragments. To carry out a more detailed and accurate analysis of the cellular *proto-amv* sequences, we isolated and purified by subcloning in pBR322 *amv*-containing DNA fragments with fewer or no proviral sequences and used them as hybridization probes with C/O chicken DNA digested with three restriction enzymes.

MATERIALS AND METHODS

Cloning. *Escherichia coli* HB101 (*pro leu thi lac-4 hsdR endA recA rpsL20 ara-14 galK2 xyl-5 mtl-1 svpE44*) was the recipient in transformation experiments (8). The vector used was pBR322. The transformation procedure of Villa-Komaroff et al. (30) was used with minor modifications (S. Horowitz, personal communication). Bacteria were grown in 300 ml of

FIG. 1. Derivation of the hybridization probes. The restriction endonuclease sites in MAV and AMV are mapped as previously described (26). Symbols: ∇ , *HindIII*; $\blacklozenge$, *BamHI*; $\blacktriangledown$, *XhoI*; $\blacksquare$, *BglII*; $\diamond$, *EcoRI*; $\circ$, *XbaI*; $\square$, *KpnI*. The mapping of restriction sites within the *KpnI*-*XbaI* fragment was carried out by digestion of the ^{32}P -labeled purified fragment. DNA fragments bearing *BamHI* linkers at both ends were subcloned after insertion at the *BamHI* site of pBR322. Arrows indicate known restriction sites still available after subcloning.

tryptone-yeast extract medium (10 g of tryptone [Difco] per liter, 5 g of yeast extract per liter, and 5 g of NaCl per liter) until the optical density at 600 nm reached 0.3, spun down, suspended in 30 ml of cold buffer (25 mM sodium acetate buffer [pH 5.6], 70 mM MnCl_2 , 30 mM CaCl_2), and kept for 20 min at 4°C. Competent cells were spun at 4°C and resuspended in 10 ml of the same buffer, and 200 μl of the suspension was mixed with 25 μl of the plasmid solution. After 1 h of incubation at 4°C, 0.1-ml samples were plated on tryptone-yeast extract agar plates containing 100 μg of

ampicillin per ml. Screening of individual transformed clones for their plasmid content was performed as previously described (6, 14).

DNA purification. Purification of plasmid DNA was performed as described by Curtiss et al. (10) after amplification in the presence of chloramphenicol (9). High-molecular-weight cellular DNA (45 kb or larger) was purified from C/O H&N chicken embryos as described earlier (3).

Isolation of λ DNA recombinant clones containing *proto-amv* sequences. A total of 3×10^5 plaques from a

λ Charon 4A library of leukemic chicken DNA partially digested with *EcoRI* (27) were screened by using the in situ plaque hybridization technique of Benton and Davis (4). Nitrocellulose filters were duplicated and hybridized either to the *Kpn-Xba* probe or to the MAV *EcoRI* 2.6 (3.9-kb) probe. Plaques which hybridized with the *Kpn-Xba* DNA fragment but not with the MAV-1 DNA fragment were considered likely to contain *proto-amv* sequences. A total of 18 plaques were picked, and each phage isolate was replaques twice more. Nine *proto-amv*-containing recombinant clones were grown to high-titer stocks in *E. coli* DP50 *supF* (7). The DNA was purified as described (27).

Purification of DNA fragments. Restriction enzyme digestions were performed in the buffers recommended by Bethesda Research Laboratories, Rockville, Md. A 10-fold-excess ratio of enzyme to DNA was generally used. Digestion of cellular DNA and gel electrophoresis procedures have been described (26). The *Kpn-Xba* fragment was isolated from melted sea plaque gel (Seakem low-temperature-melting agarose; Marine Colloids, Rockland, Maine) (26). All other DNA fragments were electroeluted from regular agarose (Sigma Chemical Co., St. Louis, Mo.) gels at 150 mA for at least 90 min in 5 ml of TAE buffer (40 mM Tris-hydrochloride [pH 7.6], 5 mM Na₂C₂H₃O₂ · 3H₂O, 1 mM EDTA). DNA fragments were then extracted twice with a mixture of phenol-chloroform-isoamyl alcohol (1:24:1), twice with chloroform-isoamyl alcohol (24:1), and twice with ether before precipitation with ethanol.

Ligation of *Bam*HI linkers to DNA fragments. *Bam*HI linkers (17, 21) were phosphorylated in reaction volumes of 30 μ l containing 0.8 nM *Bam*HI linkers (Collaborative Research, Inc., Waltham, Mass.), 0.07 M Tris-hydrochloride buffer (pH 7.4), 0.01 M MgCl₂, 0.01 M 2-mercaptoethanol, 0.06 mM ATP, and 5 U of T₄ polynucleotide kinase (Bethesda Research Laboratories). After 2 h of incubation at 37°C, the reaction was stopped by incubation at 70°C for 10 min. Restriction DNA fragments with cohesive ends were incubated in the presence of DNA polymerase I (Bethesda Research Laboratories) to generate blunt ends (1, 2, 12). In the case of enzymes generating 3' protruding ends (*Kpn*I, *Hae*II), DNA fragments (5 μ g) were incubated in the presence of 60 mM Tris buffer (pH 7.5), 10 mM MgCl₂, 10 mM 2-mercaptoethanol, and 1 U of *E. coli* DNA polymerase I for 10 min at 12°C to take advantage of the 3' $\rightarrow$ 5' exonuclease activity of polymerase I. The four deoxynucleotide triphosphates were then added to a final concentration of 0.2 mM, and polymerization was allowed to proceed for 1 h at 12°C. In the case of enzymes generating 5' protruding ends (*Eco*RI, *Xba*I), the restriction sites were filled in by polymerase I. After inactivation of polymerase I for 10 min at 70°C, ATP was added to a final concentration of 1 mM, and 1 U of T₄ DNA ligase (New England Biolabs, Beverly, Mass.) was added to the mixture. Ligation was performed at 12°C for 10 h.

Ligation of DNA fragments to pBR322 vector. The pBR322 vector, cleaved with the appropriate restriction endonuclease, was treated with calf intestine alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) to reduce colony background caused by recircularization of vector DNA (29). Six units of calf intestine alkaline phosphatase as ammonium sulfate precipitate were spun in an Eppen-

dorf microfuge tube and suspended with 5 μ g of cleaved pBR322. The reaction mixture (100 μ l) was adjusted to 20 mM Tris-hydrochloride buffer (pH 8.0) and incubated for 1 h at 37°C. The reaction was stopped by the addition of 10 mM Tris-hydrochloride buffer (pH 7.5)–1 mM EDTA (pH 7.5)–0.2 M NaCl–0.5% sodium dodecyl sulfate. The treated vector was then extracted twice with phenol-chloroform-isoamyl alcohol (1:24:1), twice with chloroform-isoamyl alcohol (24:1), and twice with ether before ethanol precipitation. Ligation of DNA fragments to treated pBR322 was performed in 250 mM Tris-hydrochloride (pH 7.4)–50 mM MgCl₂–5 mM ATP–50 mM 2-mercaptoethanol at a DNA concentration of 200 μ g/ml with 1 μ l of T₄ ligase (New England Biolabs).

Nick translation of purified DNA fragments. Nick translation (20) was performed in the presence of 200 μ Ci of [α -³²P]dCTP (Amersham Corp., Arlington Heights, Ill.), 30 μ M each of dATP, dGTP, and dTTP, 5 μ l of nick translation buffer (Bethesda Research Laboratories), 2 μ l of DNA polymerase I (2 U/ μ l), and 2 μ l of DNase I (Worthington Diagnostics, Freehold, N.J.; DPFF grade 0.2 μ g/ml). DNase incubation was started 5 min before the addition of DNA polymerase I. The ³²P-labeled fragments and the remaining [α -³²P]dCTP were separated by chromatography on Sephadex G-75 equilibrated in 0.1 $\times$ SSC plus 0.1% sodium dodecyl sulfate. The specific activity of the ³²P-labeled probes was approximately 2×10^8 cpm/ μ g.

Sizing of the ³²P-labeled DNA fragments obtained after digestion of nick-translated probes with restriction enzymes. The chain length of fragments obtained after single and double digestions of DNAs was determined by electrophoresis in 5% acrylamide gel as described by Maniatis et al. (16). Kodak X-ray films were placed on the top of the gels, covered with Saran Wrap, and exposed for 3 h at 4°C.

Preparation and hybridization of DNA (Southern) blots. The DNA fragments separated by electrophoresis were blotted on nitrocellulose membrane filters (Millipore Corp., Bedford, Mass.) as described by Southern (23). Hybridizations were performed according to Wahl et al. (31). Filters were washed twice in 2 $\times$ SSC plus 0.1% sodium dodecyl sulfate at room temperature and two to three times at 68°C in 2 $\times$ SSC plus 0.1% sodium dodecyl sulfate, rinsed twice in 2 $\times$ SSC, dried, and exposed with Kodak X-ray films (XR5) and a Du Pont intensifying screen at –70°C.

Physical and biological containment. This work was carried out at the P2-EK2 containment levels according to the revised guidelines of the National Institutes of Health.

RESULTS

Derivation of the *amv*-specific probes. The preparation of specific *amv* probes started with the isolation of the *KpnI-XbaI* (3'-proximal) fragment from the AMV *Hind*III 2.6 fragment (2.6×10^6 daltons [4 kb]) (Fig. 2) already cloned in pBR322 (5). The *Kpn-Xba* DNA fragment was isolated by gel electrophoresis after double digestion of AMV *Hind*III 2.6/pBR322 recombinant DNA with *Kpn*I and *Xba*I, ligated to *Bam*HI linkers, and cloned in pBR322 (pBR322/KX162). This probe still contains ap-

FIG. 2. Hybridization of restriction enzyme-generated DNA fragments with *amv*-containing probes. C/O chicken DNA was digested with *Bam*HI (a), *Eco*RI (b), or *Hind*III (c), electrophoresed in 0.7% agarose gel, and transferred to nitrocellulose filters (Southern blots). The three different restriction enzyme digests were run in parallel in the same gel but were blotted in four separate sets. A sample of λ DNA (0.5 μ g) digested with *Hind*III endonuclease and run in a parallel lane was used as molecular size markers expressed in kilobase pairs. The hybridization probes shown in Fig. 1 were used after labeling with [α - 32 P]-dCTP by the nick translation procedure.

proximately 200 base pairs of viral sequences located at the 5' terminus, as estimated from previous electron microscopic studies (27). Therefore, a search was made for a restriction enzyme which could remove about 250 base pairs from the 5' end of the *Kpn*-*Xba* fragment.

The 32 P nick-translated *Kpn*-*Xba* fragment was treated with several restriction enzymes: *Alu*I, *Msp*I, *Sau*3A1, *Cfo*I, *Hae*III, *Taq*I, *Hae*II, *Pvu*II, *Hin*FI, *Hpa*I, *Sal*I, *Eco*RI, and *Sma*I. Among the few enzymes which gave a single cleavage in the *Kpn*-*Xba* fragment, *Sal*I cleaved at 450 base pairs from the *Xba*I site, and *Hae*II cleaved at 290 base pairs from the *Kpn*I site (Fig. 1). A *Hae*II (*Xba*I-*Bam*HI)-*Hae*II fragment was isolated after digestion of KX162 by *Hae*II endonuclease. After ligation of *Bam*HI linkers to the *Hae*II sites and digestion with *Bam*HI endonuclease, a *Hae*II-*Xba* fragment having *Bam* linkers at both ends was cloned into pBR322.

One of the resulting clones (HAX4) was hybridized with the 32 P nick-translated MAV *Eco*RI 2.6 fragment (also 2.6×10^6 daltons [4 kb]), to determine whether helper viral sequences remained at the 5' end of the *Hae*II-*Xba* fragment. The results (data not shown) indicated that helper viral sequences were absent at the 5' end of the *amv* insert in HAX 4. HAX 4 was also digested with *Sal*I to establish the orientation of the insert in pBR322. Clones bearing the *Hae*II-*Xba*I DNA fragment in the two possible orientations within the plasmid DNA were identified.

The presence of a single *Eco*RI site in the *Hae*II-*Xba*I fragment and in pBR322 DNA (28) was exploited to generate an *Eco*RI-*Xba*I DNA fragment from pBR322/HAX4 after digestion with *Eco*RI. This fragment was cloned into pBR322 after treatment with DNA *P*oII, ligation of *Bam*HI linkers, and digestion with *Bam*HI endonuclease. The orientation of the *Eco*RI-*Xba*I DNA fragment with respect to plasmid DNA in one (pBR322/EXII) of the clones obtained was determined by digestion with *Sal*I and *Eco*RI endonucleases. This clone was then used to isolate two fragments carrying the *amv* sequences located on the 3' side of the *Eco*RI site: an *Eco*RI-*Sal*I (ES2) and a *Sal*I-*Xba*I fragment (SX1).

The sizes and derivations of the different hybridization probes are shown in Fig. 1.

Hybridization of chicken DNA fragment to *amv*-containing probes. High-molecular-weight (larger than 45 kb) DNA from C/O chicken embryos was digested with either *Eco*RI, *Hind*III, or *Bam*HI restriction endonuclease, electrophoresed in agarose gels, and transferred to nitrocellulose. The blots were hybridized with the 32 P-labeled probes described above (Fig. 2 and Table 1).

(i) **Hybridization with the *Kpn*-*Xba*I probe.** The *Kpn*-*Xba* DNA fragment obtained by *Bam*HI digestion of pBR322/KX162 was purified by agarose gel electrophoresis, electroeluted, and 32 P nick translated. The chicken DNA *Eco*RI fragments hybridizing with the *Kpn*-*Xba* probe are 8.7, 5.4, 3.9, and 2.2 kb. The low intensity of hybridization obtained for the *Eco*RI 3.9-kb fragment suggests that this hybridization results from the 200 base pairs of viral sequences present at the 5' end of the KX162 probe. However, this probe no longer detects the *Eco*RI 20.8-kb fragment which hybridized to the *Hind*III 2.6 probe (5). The chicken DNA *Hind*III fragments detected are 5.4, 3.3, and 1.4 kb. Hybridization was not detected with the 2.6-kb fragment which hybridized with the *Hind*III 2.6 probe. The intensity of the *Hind*III 3.3-kb fragment relative to the other two is greatly reduced, suggesting that this may also be due to the presence of viral sequences remaining in the probe. The detection of the 5.4-kb fragment might be due either to the viral sequences remaining in the KX162 probe or to the existence of an *amv* fragment which has the same molecular weight as the endogenous proviral DNA fragment which hybridized to the MAV *Eco*RI 2.6 probe described earlier (5). The patterns obtained with the C/O DNA *Bam*HI digest showed that four fragments hybridize with the AMV *Kpn*-*Xba* probe (19.6, 12.4, 5.5, and 4.8 kb) and four fragments hybridized with the MAV probe (12.4, 5.8, 4.8, and 1.9 kb). There-

TABLE 1. Hybridization of C/O chicken DNA fragments with different *amv*-containing probes^a

DNA probes	Cellular fragments (kb) generated with:		
	<i>Eco</i> RI	<i>Hind</i> III	<i>Bam</i> HI
MAV-1 (<i>Eco</i> RI 2.6)	3.9	5.4, 3.3, 2.6, 1.9	12.4, 5.8, 4.8, 1.9
AMV			
<i>Hind</i> III 2.6	20.8, 8.7, 5.4, 3.9, 2.2	5.4, 3.3, 2.6, 1.4	ND ^b
<i>Kpn</i> I- <i>Xba</i> I	8.7, 5.4, 3.9, 2.2	5.4, 3.3, 1.4	19.6, 12.4, 5.5, 4.8
<i>Hae</i> II- <i>Xba</i> I	8.7, 5.4, 2.2	5.4, 1.4	19.6, 5.5
<i>Eco</i> RI- <i>Sal</i> I	2.2	5.0	19.6
<i>Sal</i> I- <i>Xba</i> I	2.2, 8.7	5.4, 1.4	19.6, 5.5

^a The MAV-1 (*Eco*RI 2.6) and AMV (*Hind*III 2.6) probes were obtained from pBR322 clones previously described (5). The sizes of the fragments were determined by comparison with those of λ DNA markers.

^b ND, Not determined.

fore, it appears that the 19.6- and 5.5-kb fragments are *amv* specific and that the 12.4- and 4.8-kb fragments are detected by KX162 owing to the presence of viral sequences in this probe.

(ii) **Hybridization with the *Hae*II-*Xba*I probe.** The *Hae*II-*Xba*I DNA fragment obtained by *Bam*HI digestion of pBR322/HAX4 was isolated by agarose gel electrophoresis, electroeluted, and ³²P nick translated. Three bands were obtained after hybridization with C/O chicken DNA digested with *Eco*RI: 8.7, 5.4, and 2.2 kb. Thus, there is no *Eco*RI 3.9-kb band containing *amv*-related sequences. Hybridization of this probe to C/O chicken DNA digested with *Hind*III revealed the presence of only two fragments complementary to the *amv* sequences, the 5.4-kb band being much darker than the 1.4-kb band. Therefore, the detection of the *Hind*III 3.3-kb fragment by the *Kpn*-*Xba* probe resulted from the presence of endogenous proviral sequences. Hybridization of the *Hae*II-*Xba*I probe to chicken DNA digested with *Bam*HI confirms the conclusions reached above from the comparison of the band patterns detected with the MAV *Eco*RI 3.9-kb and the AMV *Kpn*-*Xba* fragments. Only two *amv*-related *Bam*HI bands were detected: 19.6 and 5.5 kb, the latter being relatively darker than the 19.6-kb band.

(iii) **Hybridization with *Sal*I-*Xba*I and *Eco*RI-*Sal*I probes.** These probes were generated by *Sal*I digestion of pBR322/EX11, purified by agarose gel electrophoresis, electroeluted, and ³²P nick translated. The *Sal*I-*Xba*I probe contains *amv* sequences complementary to the chicken DNA *Eco*RI 8.7- and 2.2-kb fragments, to the *Hind*III 5.4- and 1.4-kb fragments, and to the *Bam*HI 19.6- and 5.5-kb fragments. The hybridization intensity of the *Eco*RI 8.7-kb, *Hind*III 1.4-kb, and *Bam*HI 5.5-kb bands was greatly increased when the *Sal*I-*Xba*I probe was used instead of the *Hae*II-*Xba*I probe, reflecting the increased concentration of *amv* sequences in the former probe. Only one chicken DNA *Eco*RI

fragment (2.2 kb), one *Hind*III fragment (5.4 kb), and one *Bam*HI fragment (19.6 kb) were found to contain sequences homologous to the *Eco*RI-*Sal*I probe.

Analysis of chicken- λ DNA recombinants carrying *proto-amv* sequences. Nine DNA recombinant clones carrying cellular *proto-amv* sequences identified as described in Materials and Methods were digested with *Eco*RI and fractionated by electrophoresis. They fall into three different categories whose prototypes are λ 111, λ 533, and λ 1041 (Fig. 3A). In addition to the 19.8- and 10.9-kb λ DNA fragments (7), clone λ 111 contains three *Eco*RI fragments (8.7, 2.2, and 1.8 kb), clone λ 533 contains three *Eco*RI fragments (8.7, 4.1, and 1.8 kb), and clone λ 1041 contains four *Eco*RI fragments (15.1, 8.7, 5.4, and 2.6 kb) (Table 2). The internal *Eco*RI frag-

FIG. 3. Analysis of chicken- λ recombinant DNA clones containing *proto-amv* sequences. Chicken- λ Charon 4A recombinants carrying *proto-amv* sequences were isolated and their DNA was digested with *Eco*RI as described in the text. (A) a, λ +; b, λ 1041; c, λ 533; d, λ 111. Parallel digests were blotted on nitrocellulose and hybridized with the ³²P-labeled *Kpn*-*Xba* probe. (B) a, λ 1041; b, λ 533; c, λ 111. The marker λ DNA fragments with sizes in kilobase pairs were run in the same gel.

TABLE 2. Size of the DNA fragments generated by *EcoRI* digestion of chicken- λ DNA recombinants

Recombinant clone	Fragment size (kb)
λ 111	19.8, ^a 10.9, ^a 8.7, ^b 2.2, ^b 1.8
λ 533	19.8, ^a 10.9, ^a 8.7, ^b 4.1, 1.8
λ 1041	19.8, ^a 15.1, 10.9, ^a 8.6, 5.4, ^b 2.6

^a λ Charon 4A *EcoRI* fragments.^b Fragments which hybridize with the AMV (*KpnI-XbaI* or *HaeII-XbaI*) probes.

ments of λ Charon 4A can be replaced by foreign DNA inserts in the size range of 8.2 to 22.2 kb (7). Also, the leukemic chicken DNA fragments used to prepare our chicken- λ library ranged in

size between 16 and 22 kb (27). Consequently, from the summation (approximately 32 kb) of the fragment sizes obtained with λ 1041, it appears that the 15.1-kb *EcoRI* fragment might represent an intramolecular recombinant, e.g., from looping out during amplification of the DNA stock.

Hybridization of Southern blots of the *EcoRI*-digested DNA with the ³²P-labeled *Kpn-Xba* probe (Fig. 3B) shows that λ 111 contains two DNA fragments (8.7 and 2.2 kb) carrying *proto-amv* sequences, and λ 533 and λ 1041 contain only one each, 8.7 and 5.4 kb, respectively. The same results were obtained with the HAX4 probe. Thus, the *proto-amv*-containing *EcoRI*

FIG. 4. Partial arrangement of the *proto-amv* sequences in C/O chicken DNA. (A) The *proto-amv* sequences are interrupted by at least one intervening sequence (IS) in C/O chicken DNA. (B) The relative position of the *EcoRI*, *HindIII*, and *BamHI* fragments carrying *proto-amv* sequences was established from hybridization patterns obtained with the probes described in the legend to Fig. 1. It is not known whether the 2.2- and 8.7-kb *EcoRI* fragments are contiguous. The same uncertainty applies to the two *HindIII* and *BamHI* fragments; i.e., there may be one or more restriction enzyme sites within the intron shown. Also, as discussed in the text, there may be a second intron between the 5.4- and 2.2-kb *EcoRI* fragments.

DNA fragments from these chicken- λ recombinant clones have the same sizes as those detected in hybridization studies between the *Kpn*-*Xba* probe and normal chicken DNA digested with *Eco*RI.

DISCUSSION

Previous studies performed with different AMV internal restriction fragments as hybridization probes indicated that the *amv* sequence present in the AMV genome was homologous to a set of normal chicken DNA sequences (*proto-amv*) from which it might have originated (27). A complex organization of the *proto-amv* sequences in chicken DNA was suggested by the existence of several *Hind*III and *Eco*RI fragments complementary to the *amv* sequence (5, 27).

In this study, DNA fragments representing different regions of the *amv* sequence having little or no homology with MAV helper sequences were used as hybridization probes to map the *proto-amv* sequences in chicken DNA. Three *Eco*RI fragments (8.7, 5.4, and 2.2 kb), two *Hind*III fragments (5.4 and 1.4 kb) and two *Bam*HI fragments (19.6 and 5.4 kb) in chicken DNA are homologous to *amv*. Since the *amv* sequence contains only one *Eco*RI site but no *Hind*III or *Bam*HI site (26), these results indicate that *proto-amv* information might not be contained in a single set of DNA sequences.

Hybridization with the AMV *Eco*RI 3.3-kb fragment and with the AMV 1.3-kb fragment located between the 3'-proximal *Eco*RI and *Hind*III sites had shown (27) that the 5.4-kb *Eco*RI fragment was adjacent to either the 8.7- or the 2.2-kb fragment. The hybridization patterns obtained with the *Sall*-*Xba*I and *Eco*RI-*Sall* probes now place the 2.2-kb *Eco*RI fragment adjacent to the *Eco*RI 5.4-kb fragment and indicate that approximately one half of the *amv* sequences located between the *Sall* and *Xba*I sites originate from the *Eco*RI 2.2-kb fragment. Therefore, it appears that the 3' region of the *proto-amv* chicken sequences are interrupted by at least one intervening sequence.

The isolation of a λ Charon 4A recombinant (λ 111) DNA carrying the two *Eco*RI 8.7- and 2.2-kb fragments in the same insert indicates that these two fragments are relatively close to each other and certainly are not located on different chromosomes. Comparison of the inserts of λ 111 and λ 533 reveals that the *Eco*RI 8.7- and 2.2-kb fragments are either contiguous or separated by 1.8 kb of chicken DNA at the most.

Since we did not find a chicken- λ recombinant carrying both the *Eco*RI 5.4- and 2.2-kb fragments among the 18 clones obtained so far, the possibility exists that another intervening se-

quence might be present between these two *proto-amv* fragments.

These results, along with those obtained previously (5, 27), permit a preliminary arrangement of the *proto-amv* sequences in H&N C/O chicken DNA (Fig. 4). Two different domains of *proto-amv* sequences (L1 and L2) are separated in chicken DNA by an intervening sequence which contains at least one *Bam*HI site, one *Eco*RI site, and one *Hind*III site. The relative positions of the *Eco*RI, *Bam*HI, and *Hind*III sites with respect to each other within the intron, and the size of this intron, are being investigated.

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