Expression of Endogenous Avian Myeloblastosis Virus Information in Different Chicken Cells

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Uninfected chicken cells were found to contain endogenous avian myeloblastosis virus (AMV)-specific information. Different tissues from chicken embryos and chickens expressed different amounts of the AMV-specific information. The endogenous AMV-related RNA was most abundant in bone marrow cells, which contained about 20 copies per cell. About 5 to 10 copies of AMV endogenous RNA per cell were found in embryonic yolk sac cells and bursa cells. The spleen, muscle, liver, and kidney cells of chickens and the fibroblasts of chicken embryos contained about two copies per cell. The amounts of AMV endogenous RNA in bone marrow, yolk sac, and bursa varied with age. From 19-day-old embryos to 2-week-old chickens, the bone marrow contained 20 copies of AMV RNA per cell. Bone marrow cells from 2-year-old chickens contained five copies per cell. Yolk sac cells of 10-day-old embryos and 1-day-old chickens were found to contain two copies per cell, whereas in 15- to 17-day-old embryos, these cells contained 5 to 10 copies. These results indicate that the level of endogenous AMV expression correlates with the development of granulopoiesis of the chicken hemopoietic system. The results of experiments on the thermostability of RNA-DNA hybrids indicated that the endogenous AMV RNA is closely related to viral AMV RNA. The expression of endogenous AMV information is independent of the activity of the chick helper factor. This endogenous AMV information is expressed as 20 to 21S RNA in both bone marrow and yolk sac cells.

Avian retroviruses comprise three main groups: (i) sarcoma virus; (ii) lymphoid leukemia virus; and (iii) acute (or defective) leukemia viruses (ALV or DLV) (9, 11; in this communication, DLV will be used in the description of this group of viruses). The endogenous viral sequences of sarcoma and leukemia viruses have been extensively investigated (1, 22, 27, 31, 36). Expression of these endogenous virus genes has been recognized either in the form of protein (2, 3, 24, 32, 33) or in the form of RNA (3, 14). Recently, the existence of endogenous viral information of DLV has also been recognized in uninfected chicken cells (4, 28, 30). Evidence indicating the presence of endogenous DLV sequences was provided by the detection of uninfected chicken cell DNA and RNA hybridizable to the different complementary DNA (cDNA) probes for different members of DLV (4, 28, 30).

Studies described in a previous report showed that although cellular DNA from different tissues of uninfected chickens contained the same amount of DNA sequences related to avian myeloblastosis virus (AMV) cDNA (cDNA_{AMV}), the amount of AMV-related RNA varied (4). About 20 copies of AMV-related RNA per cell were demonstrated in bone marrow cells of uninfected chickens, whereas only one to two copies of

AMV-related RNA per cell were found in chicken embryo fibroblasts (CEF). These data indicate that the expression of endogenous AMV sequences is probably regulated at the transcriptional level and may be related to the tissue or target cell specificity analogous to that found in murine leukemia virus.

To extend these observations, the expression of the endogenous AMV-related sequence was examined in different tissues from uninfected chickens and chicken embryos and during the development of chicken embryos to young hatched chickens. The size distribution of AMV-related RNA in the hematopoietic cells of uninfected chickens and chicken embryos was also investigated.

MATERIALS AND METHODS

Materials. Oligodeoxythymidine [oligo(dT)]-cellulose was purchased from Collaborative Research (Waltham, Mass.), DNase I from Worthington Biochemicals Corp. (Freehold, N.J.), proteinase K from Beckman Instruments, Inc. (Palo Alto, Calif.), [³H]TTP from New England Nuclear Corp. (Boston, Mass.), and RNase-free sucrose from Schwarz/Mann (Orangeburg, N.Y.). Phenol was redistilled to remove impurities and stored at -20°C. TEN buffer consisted of 0.01 M Tris-hydrochloride (pH 7.4), 0.1 M NaCl, and 0.001

M EDTA. ETS buffer contained 0.01 M Tris-hydrochloride (pH 7.4), 0.01 M EDTA, and 0.2% sodium dodecyl sulfate (SDS). All solutions were autoclaved. All glassware was autoclaved and baked at 150°C overnight.

Cells and viruses. Avian myeloblasts were derived from single foci of AMV-transformed yolk sac cells or single colonies of AMV-transformed bone marrow cells in soft agar cultures, using the method previously described (6, 20). Different tissues from chicken embryos and chickens at various ages were trypsinized to obtain single-cell suspensions before nucleic acid extraction. Since the quantity of bone marrow cells obtained from individual chicken embryos or young chickens was very small, bone marrow cells from several chickens or embryos were pooled for this study regardless of their chick helper activity. Preparation of yolk sac cells followed the procedure described previously (21).

Viruses used in this study, AMV-A, AMV-B, AMV (td B77), myeloblastosis-associated virus-1 (MAV-1), MAV-2, and Rous-associated virus-60, have been described previously (4, 12, 19, 20). In AMV preparations obtained from cultured myeloblasts, the ratio of transforming virus to helper virus is generally low. Much effort was required to obtain an adequate amount of AMV from tissue cultures for the synthesis and selection of cDNA_{AMV}. To obtain AMV preparations with a high ratio of transforming virus to helper virus, the following alternative procedure was adapted. Forty to 60 chick helper factor-negative (chf⁻) 1-day-old chickens were injected intravenously with AMV-A, AMV-B, or AMV (td B77). Plasma samples from each leukemic chicken were collected. AMV plasmas from chickens containing 3×10^8 to 5×10^8 myeloblasts per ml of blood were pooled and used for virus purification and viral RNA extraction. Some of the AMV plasma samples which contained predominantly AMV-A were kindly provided by J. W. Beard of this institute.

Preparations of cDNA probes. Synthesis of cDNA_{MAV-2} and selection of cDNA_{AMV} have been described (4). Polyadenylate [poly(A)]-containing 70S RNA derived from AMV-A or AMV-B plasma was used for the isolation of cDNA_{AMV}.

Nucleic acid extraction. Viral RNA and cellular DNA and RNA were extracted according to the procedure described elsewhere (13). To minimize the degradation of high-molecular-weight RNA, freshly trypsinized cells from tissues were lysed in TEN containing 0.5% SDS and 1 to 1.5 mg of proteinase K per ml at room temperature for 45 min. The lysate was passed three times through a 20-gauge needle to reduce the viscosity, extracted four times with a mixture of equal volumes of phenol containing 0.1% 8-hydroxyquinoline and chloroform containing 1% isoamyl alcohol and twice with chloroform containing 1% isoamyl alcohol. The aqueous phase was adjusted to 0.4 M sodium acetate and precipitated with 2 volumes of ice-cold ethanol. For hybridization kinetics experiments, cellular RNA was treated with DNase I (50 µg/ml) for 30 min at 37°C in a buffer containing 0.01 M Tris (pH 7.0) and 0.005 M MgCl₂ for the removal of cellular DNA and then reextracted as described above. Cellular DNA was extracted from different tissues by the procedure of Schincariol and Joklik (29). DNA was

depurinated according to the procedure described elsewhere (13).

Oligo(dT) cellular chromatography of cell RNA. To fractionate cellular nucleic acid, total cell nucleic acid was dissolved in 0.01 M Tris-hydrochloride (pH 7.4), 0.5 M NaCl, and 0.2% SDS. The nucleic acid solution was then loaded onto an oligo(dT)-cellulose column that had been equilibrated with the same buffer. Chromatography was carried out at room temperature. The column was washed extensively until the optical density at 260 nm was less than 0.01. DNA and the majority of cellular RNA were removed at this step. The poly(A)-containing RNA was then eluted with a buffer containing 0.01 M Tris-hydrochloride (pH 7.4) and 0.2% SDS. The poly(A)-negative and poly(A)-positive RNAs were pooled separately, adjusted to 0.4 M sodium acetate (pH 7.4), and precipitated with 2 volumes of ice-cold ethanol.

Nucleic acid hybridization. To determine hybridization kinetics, unselected total cell RNA was used at 10 to 14 mg/ml as indicated in the figure legends. The 5-µl hybridization mixture in a sealed capillary tube contained, in addition to cellular RNA, 30% formamide, 0.45 M NaCl, 0.045 M sodium citrate (pH 7.2), 0.1% SDS, 0.005 M EDTA, and about 500 cpm of cDNA_{AMV}. The reaction mixtures were incubated at 50°C for various intervals. The degree of hybridization was determined by the resistance of the hybrid to S1 nuclease treatment (14). All data were corrected from 0.7 to 1.8% of S1-resistant cDNA probes and 20 cpm of the background of the scintillation counter.

DNA-DNA annealing. Cellular DNA from various tissues was depurinated as described to yield the average length of 150 nucleotides (13). Annealing was carried out in 0.6 M NaCl-0.06 M sodium citrate, using cellular DNA at 12 mg/ml and cDNA_{AMV} at 2.5 ng/ml (500 cpm per reaction) in a 10-µl volume in a sealed capillary tube. Samples were boiled for 3 min and incubated at 68°C for various periods of time as indicated by the C₀t value. The degree of annealing was monitored by S1 nuclease treatment as for DNA-RNA hybrids.

Size analysis of endogenous AMV RNA in uninfected cells. The method was adapted from the procedures used for the size analysis of endogenous virus RNAs in CEF described by Wang et al. (37). Briefly, poly(A)-containing RNA was dissolved in 0.2 to 0.5 ml of ETS, heated at 85°C for 90 s or 90°C for 1 min, and quickly chilled in ice and loaded on a 15 to 30% sucrose gradient prepared in 0.01 M Tris-hydrochloride (pH 7.4), 0.02 M LiCl, 0.001 M EDTA, and 0.2% SDS. The gradient was then centrifuged at 25°C in a Beckman SW41 rotor at 40,000 rpm for 7.5 h. The gradient was fractionated from the bottom of the tube into siliconized tubes with a peristaltic pump. Generally, 30 to 32 fractions were fractionated from a gradient. Fifty micrograms of tRNA was then added to each fraction as a carrier, adjusted to 0.4 M sodium acetate, and precipitated with 2 volumes of ice-cold ethanol. The precipitate was collected by centrifugation in a Beckman J21 centrifuge and dried in a vacuum. The RNA was dissolved in 15 µl of hybridization buffer. For size determination of poly(A)-containing virus-specific RNA, 3 µl of each RNA fraction was mixed with 3 µl of hybridization buffer containing

300 to 500 cpm of cDNA_{AMV} and incubated at 50°C in a sealed capillary tube. The reaction was carried out to a 30 to 40% hybridization level. The RNA excess was maintained in most of these experiments under conditions in which the extent of hybridization was proportional to RNA concentration. [³²P]MAV-2 35S RNA and rRNA were used as markers in parallel sucrose gradients for each poly(A)-containing cellular RNA sample for size analysis.

Thermostability of DNA-RNA hybrids. Hybridization was carried out as described (15) with the modification that poly(A)-containing viral and cellular RNA was used to form hybrids with cDNA_{AMV}. Samples were incubated to a C_tt value higher than that required to achieve the saturation level [C_tt of 1×10^1 for poly(A)-containing 70S viral RNA and 5×10^3 for poly(A)-containing cellular RNA] and precipitated with ethanol. The precipitated hybrids were dissolved in a buffer containing 0.01 M Tris-hydrochloride (pH 7.4), 0.15 M NaCl, and 0.001 M EDTA. Five-microliter samples containing 500 cpm of cDNA_{AMV}-RNA hybrid in sealed capillaries were incubated for 15 min at the indicated temperature, quickly chilled in an ethanol-dry ice bath, and stored at -70°C. The temperature was increased at 2°C intervals. The amount of DNA in each sample that remained hybridized after heating was determined by its resistance to S1 nuclease treatment. Each thermostability curve was constructed from an average of three determinations.

RESULTS

AMV-specific sequences in DNA from different uninfected chicken cells. Results of previous reports showed that AMV-related RNA was detected in uninfected CEF (28) and bone marrow cells (4), thus indicating the presence of an endogenous AMV sequence in chicken cells. To determine whether there is any quantitative difference among endogenous AMV sequences in different chicken cells, DNAs were isolated from different cells of chickens or chicken embryos and tested for nucleotide sequences homologous to cDNA_{AMV}. The same amount of AMV DNA was detected in all types of chicken cells tested (Fig. 1). However, the maximum level of hybridization reached only about 60%, probably because of the competition of negative strands of cellular DNA with the cDNA_{AMV} probe. There were about two copies of AMV DNA per cell.

Expression of endogenous AMV RNA in different tissues of chickens and chicken embryos. It had been shown previously that uninfected chicken bone marrow cells and CEF contained different amounts of RNA which hybridized to cDNA_{AMV} (4). To investigate these observations further, total cell RNA was extracted from different tissues from 1- to 3-day-old chickens and 15- to 19-day-old chicken embryos and hybridized to DNA_{AMV}. The conditions for hybridization were maintained so that

FIG. 1. AMV-specific DNA in different tissues of uninfected 1-day-old chickens. [³H]cDNA_{AMV} was annealed with depurinated DNA isolated from bone marrow cells (●), erythrocytes (○), and fibroblasts (△). Depurinated salmon sperm DNA (x) was used as a heterologous control. The cell DNA concentration was 12 mg/ml, and cDNA_{AMV} was used at 500 cpm/reaction. C_{ot} values shown are not corrected for standard conditions of salt concentration.

the possible presence of endogenous virus RNA in total cell RNA preparations was in excess (usually 400 to 500 cpm per point, i.e., about 0.02 to 0.03 ng of cDNA per hybrid, was used).

The fastest rate of hybridization between cellular RNA and cDNA was observed in RNA extracted from AMV-transformed myeloblasts (Fig. 2). The hybridization rate between cDNA and RNA extracted from bone marrow cells of 1- to 3-day-old chickens was about 200- to 300-fold less than that between cDNA and AMV-transformed myeloblasts.

Bone marrow cells contained more endogenous AMV RNA than other tissues at this age (Fig. 2). The rate of hybridization between the RNA from bursa and cDNA was about one-half to one-third that between bone marrow RNA and cDNA. The RNAs from the spleen, liver, and kidney cells hybridized to cDNA at $\frac{1}{10}$ the hybridization rate of bone marrow RNA and cDNA. Among the tissues of 15- to 19-day-old chicken embryos, the endogenous AMV RNA was most abundant in bone marrow cells of 19-day-old embryos. Yolk sac cells from 15-day-old embryos and bursa cells from 19-day-old embryos contained about one-half to one-third as

much endogenous AMV RNA as bone marrow cells. As in the hatched young chickens, the spleen, liver, and kidneys of 15- to 19-day-old embryos exhibited the same level of endogenous AMV RNA as did CEF (Fig. 3).

Table 1 lists the number of copies of endoge-

nous AMV RNA in different tissues from 1- to 3-day-old chickens and 15- to 19-day-old chicken embryos. Bone marrow cells from 19-day-old embryos or 3-day-old chicks contained about 20 copies of endogenous AMV RNA per cell. Yolk sac cells from 15-day-old embryos and bursa

FIG. 2. AMV-specific RNA in different tissues of uninfected 1- to 3-day old chickens. [^3H]cDNA_{AMV} was hybridized to total cell RNA extracted from bone marrow, bursa, spleen, liver, and kidney of newly hatched chickens. The reaction mixture contained 0.45 M NaCl, 0.045 M sodium citrate, 30% formamide, 5 mM EDTA, 0.2% SDS, and 500 cpm of cDNA and cell RNA, in a volume of 10 μl . The hybrids were incubated at 50°C for various times. The degree of hybridization was monitored by S1 nuclease digestion. RNA concentrations were: AMV myeloblasts ($\circ$), 4 mg/ml; bone marrow ($\bullet$), 11 mg/ml; bursa ($\times$), 10 mg/ml; spleen (Δ), 12 mg/ml; liver ($\square$), 14 mg/ml; and kidney (∇), 12 mg/ml.

FIG. 3. AMV-specific RNA in different tissues of chicken embryos. [^3H]cDNA_{AMV} was hybridized to total cell RNA extracted from bone marrow cells of 19-day-old embryos, 15-day-old embryonic yolk sac cells, and liver of 19-day-old embryos. The reaction condition was the same as that described in Fig. 2. RNA concentrations were: AMV myeloblasts ($\circ$), 2 mg/ml; bone marrow ($\bullet$), 14 mg/ml; yolk sac ($\times$), 10 mg/ml; and liver (Δ), 15 mg/ml.

cells from 19-day-old embryos and 3-day-old chickens contained about 5 to 10 copies of endogenous AMV RNA per cell. Spleen, muscle, kidney, and liver cells and fibroblasts from embryos of different ages or from young hatched chickens contained about two copies of endogenous AMV RNA per cell. The estimated amounts of RNA per cell in bone marrow and yolk sac cells reflected only the minimal estimation, since cells obtained from these tissues

still contained some cells of CEF origin. Nevertheless, these results indicate that the expression of endogenous AMV sequences is correlated with the presence of cells involved in granulopoiesis of the chicken hematopoietic system.

Expression of endogenous AMV sequences in the same tissues at different ages. Since expression of the endogenous AMV sequences correlated with the presence of myeloid cells of the chicken hematopoietic system and the location of active hematopoietic changes during the development from chicken embryo to adult chicken (26), the expression of endogenous AMV sequences was examined in the same tissues at different ages in both chicken embryos and adult chickens. For this, bone marrow, bursa, spleen, and yolk sac cells of chicken embryos and chickens at different ages were studied.

Chicken bone marrow cells maintained high levels (about 20 copies per cell) of endogenous AMV sequence expression in 19-day-old embryos and 4-week-old chickens (Fig. 4a). The only bone marrow sample that showed a lower level of endogenous AMV RNA (five copies per cells) was from a 2-year-old bird.

Bursa cells of 1-day-old chicks and 2-week-old chickens contained 5 to 10 copies of endogenous RNA per cell, which decreased to 2 copies per cell at 2 years of age (Fig. 4c; only the data of 1-day-old chicks are shown). The expression of

TABLE 1. Content of AMV RNA in uninfected chicken cells

Tissue	RNA content (copies/cell) ^a	
	Chicken (1-3 days old)	Embryo (15-19 days old)
Bone marrow	20.6	21.3 ^b
Bursa	6.7	9.1 ^b
Kidney	1.2	1.3
Liver	1.2	1.7
Spleen	1.5	2.0
Yolk sac	1.3 ^c	9.6
CEF		1.5
Muscle	1.4	1.1

^a AMV RNA content was calculated from the hybridization kinetics between cDNA_{AMV} and total cell RNA as described in the text. The values listed were average of three experiments for each cell sample.

^b Only 19-day-old embryos were examined.

^c Only hatched chickens were examined.

FIG. 4. AMV-specific RNA in bone marrow, bursa, and yolk sac cells of chicken embryos and chickens of different ages. [³H]cDNA_{AMV} was hybridized to total cell RNA extracted from bone marrow, bursa, and yolk sac cells of chicks or chicken embryos of different ages. The hybridization condition was the same as that in Fig. 2. (a) Bone marrow. Symbols: (○) 19-day-old embryo, RNA concentration of 12 mg/ml; (△) 4-week-old chickens, RNA concentration of 13 mg/ml; (●) 2-year-old chickens, RNA concentration of 14 mg/ml. (b) Yolk sac. Symbols: (□) 15-day-old embryo, RNA concentration of 12 mg/ml; (○) 19-day-old embryo, RNA concentration of 15 mg/ml. (c) Bursa. Symbols: (○) 1-day-old chick, RNA concentration of 10 mg/ml; (●) 2-year-old chicken, RNA concentration of 14 mg/ml.

endogenous AMV RNA in the spleen was maintained at two copies per cell throughout life, from 19-day-old embryos to 2-year-old chickens (data not shown).

Yolk sac cells of 10-day-old embryos contained two copies of endogenous AMV RNA per cell. This increased to 5 to 10 copies per cell in 15-day-old embryos. Yolk sac cells of 19-day-old embryos or 1-day-old chickens contained two copies per cell (Fig. 4b). These results indicate that the expression of endogenous AMV sequences correlates also with the site of development of myeloid cells of the avian hematopoietic system.

Thermostability of endogenous AMV RNA-DNA hybrids. It has been shown that in a nucleic acid duplex an incorrect base pairing reduces the melting temperature (T_m) by about 1.5°C per 1% mismatch in nucleic acid sequences (35). Therefore, the T_m was measured to determine the degree of mismatch between endogenous AMV RNA and cDNA hybrids. To circumvent the instability of hybrids between endogenous AMV RNA and cDNA due to long incubation times in order to carry hybridization to the maximal level, poly(A)-containing cell RNA was used to prepare hybrids for thermostability experiments. Most, if not all, endogenous AMV RNA contained poly(A) tracts (see below). Use of the poly(A)-containing RNA fraction of total cell RNA in these experiments will concentrate virus-related RNA sequences in poly(A) fractions and thus shorten the time for hybridization to reach its maximal level. The melting profiles from the hybrids of AMV viral RNA and cDNA and that of cell RNA and DNA were very similar (Fig. 5). The T_m from viral RNA-cDNA was estimated as 81°C, whereas that for cell RNA-cDNA was estimated as 78°C. This fact indicates that endogenous AMV RNA is closely related to exogenous AMV RNA and suggests the cellular origin of exogenous AMV sequences.

Size of endogenous AMV RNA. In comparison with AMV-transformed myeloblasts, uninfected cells contained a much smaller amount of AMV-related RNA. To determine the size of endogenous AMV RNA, poly(A)-containing cellular RNA was separated from non-poly(A)-containing cellular RNA by oligo(dT)-cellulose chromatography. Since most of the viral RNA contained poly(A) (Fig. 6a), this approach enriches the virus-specific RNA in the cell for the examination of endogenous viral RNA. Because they contain comparatively higher levels of endogenous AMV RNA than other tissues, bone marrow and yolk sac cells were used to examine the size of endogenous AMV RNA. Since only small amounts of bone marrow were obtainable from the tibias of individual baby chicks, a pool

FIG. 5. Thermostability of DNA-RNA hybrids. [3 H]cDNA_{AMV} was hybridized to poly(A)-containing AMV RNA (○) or poly(A)-containing bone marrow cell RNA (△). Portions of each hybrid sample were incubated at the indicated temperature for 15 min and analyzed by S1 nuclease digestion to determine the percentage of hybridized cDNA that was dissociated.

of bone marrow cells from several chicks was used without regard to the activity of chick helper factor in each individual. In yolk sac cells, the primary yolk sac from both chf^+ and chf^- 15-day-old chicken embryos was used.

Poly(A)-containing RNA from bone marrow cells revealed only one size of RNA which hybridized to cDNA_{AMV} (Fig. 6a). This RNA sedimented at the 20 to 21S region of the gradient. 32 P-labeled MAV-2 35S RNA was used as a marker. The same RNA sample in the gradient was also used to hybridize with cDNA_{MAV-2}. The bone marrow RNA contained two species of RNA hybridizable to cDNA_{MAV-2}, one at 31S and another at 21S. This is similar to the endogenous retrovirus RNA in chf^+ gs^+ CEF reported elsewhere (37).

The poly(A)-negative bone marrow RNA did not hybridize to either cDNA_{AMV} or cDNA_{MAV-2}. The poly(A)-containing cellular RNA from yolk sac cells also contained one size of RNA hybridizable to cDNA_{AMV} regardless of whether yolk sac cells were derived from chf^+ gs^+ or chf^- gs^- embryos (Fig. 6b, c). This RNA was also sedimented as 20 to 21S. Occasionally a small peak of 17 to 18S was observed. Whether this represents a distinct size of endogenous viral RNA or

FIG. 6. Size distribution analysis of poly(A)-containing AMV-specific RNA from uninfected chicken cells on a 15 to 30% sucrose gradient. (a) 100 μ g of poly(A)-containing RNA from bone marrow cells. Equal portions of each fraction were hybridized to $cDNA_{MAV-2}$ (▲) for 10 h and to $cDNA_{AMV}$ (○) for 45 h. (x) Poly(A)-negative RNA; (●) 32 P-labeled 35S MAV-2 RNA. $cDNA_{MAV-2}$ was used at 510 cpm per hybrid; $cDNA_{AMV}$ was used at 450 cpm per hybrid. (b) Poly(A)-containing RNA (150 μ g) from chf^+ yolk sac cells and hybridization to $cDNA_{AMV}$ for 65 h. $cDNA_{AMV}$ was used at 420 cpm per hybrid. (c) Poly(A)-containing RNA (170 μ g) from chf^+ yolk sac cells and hybridization to $cDNA_{AMV}$ for 60 h. $cDNA_{AMV}$ was used at 450 cpm per hybrid. (d) Poly(A)-containing RNA from 10 mg of chf^+ CEF total nucleic acid and hybridization to $cDNA_{AMV}$ for 100 h. $cDNA_{AMV}$ was used at 380 cpm per hybrid.

a slight degradation of RNA remains to be determined (Fig. 6). The size of RNA from CEF was poorly resolved; however, it seemed to have a peak at 21S (Fig. 6d). These results showed that (i) endogenous AMV-related RNA contained one size of RNA at 20-21S; (ii) this endogenous AMV RNA contained a poly(A) tract; and (iii) the expression of endogenous AMV RNA in chicken bone marrow and embryonic yolk sac cells was independent of the activity of chick helper factor.

DISCUSSION

Cellular differentiation plays an important role in the interaction between cells and retrovirus and in the expression of the integrated viral genome. In DLV, at least two levels of virus cell interaction can be distinguished: (i) tissue specificity of the expression of endogenous virus (25) and (ii) target cell specificity of the transformation induced by DLV (7, 8, 10). The work described in this report was concerned with examination of the tissue and cell specificity of endogenous AMV expression. The results confirm those of previous reports showing that endogenous AMV sequences are present in the DNA of uninfected chicken cells (4, 28). However, the degree of expression of the endogenous AMV sequence in different tissues of chicken embryos and chickens varied. The endogenous AMV sequence was preferentially expressed in tissues of hematopoietic systems of chicken embryos and chickens, such as the bone marrow of chickens and older chicken embryos and the yolk sac in 14- to 17-day-old chicken embryos. The only tissue other than that of myeloid cell lineage of the chicken hematopoietic system that showed preferential expression of endogenous AMV sequence was the bursa, where B lymphocytes are produced. This was probably due to the existence of active granulopoiesis in the bursa of 19- to 20-day-old embryos or newly hatched chickens. Since this development of the chicken hematopoietic system starts in the yolk sac of 14- to 17-day-old embryos and then shifts to the bone marrow in 17-day-old embryos and hatched chicks (26), the preferential expression of endogenous AMV sequences clearly correlated with the development of granulopoiesis of the chick hematopoietic system. The tissue specificity of endogenous viral genome expression has also been observed with murine leukemia virus (18, 34). However, endogenous murine leukemia virus expression is for the replication genes. The tissue specificity of endogenous AMV expression is similar to the expression of Abelson antigen on murine hematopoietic cells (25). These observations also indicate that the process of cellular differentiation influences the

expression of endogenous virus as suggested by Jaenisch and Bern (16). Since AMV is the only member of DLV which transforms exclusively hematopoietic cells (9, 19), the preferential expression of the endogenous AMV sequence in hematopoietic tissue in chicken embryos and chickens may also reflect the target cell specificity of AMV.

Like the RNA of endogenous provirus in uninfected chicken cells, endogenous AMV RNA is polyadenylated. However, the size distribution of endogenous AMV RNA is slightly different from that in AMV-transformed myeloblasts. In such myeloblasts, two size classes of RNA, 33 to 34S and 20 to 21S, have been detected (J. H. Chen, W. S. Hayward, and C. Moscovici, manuscript in preparation). In uninfected bone marrow or yolk sac cells, only 20 to 21S RNA has been found. The 20 to 21S RNA found in transformed cells contains leader sequences, whereas the endogenous AMV RNA does not (Chen, unpublished data). This indicates that the mechanisms of generating subgenomic AMV RNA in transformed cells and in uninfected cells are different. The size of endogenous AMV RNA is very similar to the sizes of *src* and *env* mRNA in other avian sarcoma-leukosis virus-infected cells (13, 39) and the size of *env* messenger of endogenous viruses (37). However, it seems unlikely that endogenous AMV RNA also represents the transcript of *env* or *src* genomes for the following reasons. First, it has been demonstrated previously that AMV genomes do not contain related sequences homologous to the *src* sequences of Rous sarcoma virus (4). Second, the cDNA_{AMV} probe used for the detection of endogenous AMV RNA in uninfected bone marrow and yolk sac cells is highly specific. The probe has been selected by genomic RNAs from different helper viruses, indicating that the cDNA_{AMV} probe is not due to the accumulated sequences of different subgroups of helper viruses (4). Furthermore, genomic 33 to 34S AMV and subgenomic 20 to 21S RNAs detected in the transformed myeloblasts do not contain *env* sequences (Chen et al., in preparation). Therefore, the 20 to 21S endogenous AMV RNA may represent the mRNA coded for the AMV-specific protein in uninfected cells, which may be analogous to the endogenous *sarc* gene coded for PP60^{sarc} proteins found in uninfected cells (5, 17, 23). It will be extremely interesting to study the translational product of this 20 to 21S endogenous AMV RNA.

The small differences in T_m between hybrids of endogenous AMV cDNA and exogenous AMV RNA-cDNA showed a very close sequence homology between endogenous and exogenous

AMV RNAs. This suggests that the unique exogenous AMV sequence has a cellular origin, in agreement with the report indicating that the unique AMV sequences are well conserved in the evolutionary process (28). It has been suggested that viral RNA instead of integrated provirus DNA is involved in the recombination process (38). Furthermore, it has been shown that recombinant Rous-associated virus-60 can only be produced in *chf*⁺ and not in *chf*⁻ CEF (12). It is conceivable that exogenous AMV may have been generated via recombination between the helper virus and endogenous AMV RNA in the cells or tissues where endogenous AMV sequences are preferentially expressed, i.e., bone marrow and yolk sac cells.

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