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Homology Between Avian Oncornavirus RNAs and DNA from Several Avian Species

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^{3}H-labeled 35S RNA from avian myeloblastosis virus (AMV), Rous associated virus (RAV)-0, RAV-60, RAV-61, RAV-2, or B-77(w) was hybridized with an excess of cellular DNA from different avian species, i.e., normal or leukemic chickens, normal pheasants, turkeys, Japanese quails, or ducks. Approximately two to three copies of endogenous viral DNA were estimated to be present per diploid of normal chicken cell genome. In leukemic chicken myeloblasts induced by AMV, the number of viral sequences appeared to have doubled. The hybrids formed between viral RNA and DNA from leukemic chicken cells melted with a T_m 1 to 6 C higher than that of hybrids formed between viral RNA and normal chicken cell DNA. All of the viral RNAs tested, except RAV-61, hybridized the most with DNA from AMV-infected chicken cells, followed by DNA from normal chicken cells, and then pheasant DNA. RAV-61 RNA hybridized maximally (39%) with pheasant DNA, followed by DNA from leukemic (34%), and then normal (29%) chicken cells. All viral RNAs tested hybridized little with Japanese quail DNA (2 to 5%), turkey DNA (2 to 4%), or duck DNA (1%). DNA from normal chicken cells contained only 60 to 70% of the RAV-60 genetic information, and normal pheasant cells lacked some RAV-61 DNA sequences. RAV-60 and RAV-61 genomes were more homologous to the RAV-0 genome than to the genome of RAV-2, AMV, or B-77(w). RAV-60 and RAV-61 appear to be recombinants between endogenous and exogenous viruses.

The presence in normal chicken cells of RNA tumor virus genetic information in DNA form has been demonstrated by molecular hybridization techniques in several laboratories (2, 5, 30, 31, 34, 35, 36, 38, 43, 50). Cells derived from some normal chicken embryos spontaneously release endogenous avian RNA tumor viruses (10, 11, 18, 44), and certain physical and chemical carcinogens seem to induce endogenous virus from some apparently normal chicken cells (46). Also, activation and/or recombination of endogenous virus genes with exogenous viruses may take place by infection of normal cells with an RNA tumor virus (17, 18, 19). Other chicken embryos express only some viral gene functions, such as group-specific antigen (gs), chicken helper factor (chf), structural protein P-15, or viral RNA (8, 9, 12, 17, 19, 20, 45, 48). Therefore, some normal chickens carry and can express the entire genome of an endogenous avian leukosis virus, whereas others may contain or express only part of the endogenous viral genome. The addition of new oncornavirus-specific DNA sequences in chicken cells infected with exogenous RNA tumor viruses has been reported by other workers and by us (2-5, 34, 35, 36-38, 43).

In our previous studies, we have used 70S or 35S RNA from avian myeloblastosis virus (AMV) as a probe to detect viral DNA in avian cells (2, 3, 5, 36, 38). There are, however, extensive differences in homology among some endogenous and exogenous avian leukosis virus genomes (M. Shoyab and M. A. Baluda, J. Virol., in press). For a better quantitative and qualitative interpretation of the relationship between endogenous and exogenous avian leukosis virus genomes, we have made a survey of viral-specific DNA sequences in various normal avian species, i.e., chickens, ducks, pheasants, turkeys, and Japanese quails, and in AMV-induced leukemic chicken myeloblasts. 35S RNA from different avian oncornaviruses was used as a probe for liquid hybridization experiments in excess of cellular DNA. These viruses are: Rous associated virus (RAV)-0, presumed to be the prototype of induced endogenous avian leukosis virus; RAV-60 and RAV-61, which seem to be recombinants between endogenous and exogenous viruses; and RAV-2, AMV, and B-77(w), which are exogenous viruses with different tumorigenic properties and belong to different subgroups. RAV-0 is a subgroup E virus, spontaneously produced by some normal

embryos of line 7 and line 100 chickens, which appears to be similar to the endogenous chicken virus (44, 46). RAV-60 is also a subgroup E virus produced by the infection of normal C/O chicken cells with an exogenous avian leukosis or sarcoma virus (19). RAV-61 is a subroup F virus produced by the infection of pheasant cells with chicken leukosis or sarcoma viruses (18). RAV-2 belongs to subgroup B of avian leukosis viruses. B-77(w) avian sarcoma virus is a subgroup C virus isolated from a spontaneous sarcoma in the liver of a chicken (42) and passaged only in chicken cells.

In this study we have analyzed the kinetics of RNA-DNA hybridization in DNA excess (7, 14, 15, 29) and the thermal stability of the RNA-DNA hybrids formed to determine the degree of homology that exists between the various avian DNAs and viral RNAs. It was found that: (i) duck cells do not contain DNA that hybridizes with any of the viral RNAs used; (ii) DNA from turkey or Japanese quail cells exhibits little homology with the viral RNAs; (iii) pheasant cell DNA has substantial (39%) homology with RAV-61 (in fact, RAV-61 hybridizes the most with pheasant DNA and has 22% homology with RAV-0); (iv) the degree of hybridization between a given viral RNA and DNA from normal or leukemic chicken cells can be predicted from the extent of homology that exists between the virus tested and RAV-0 or AMV, respectively; and (v) RAV-60 and RAV-61 appear to have arisen by recombination between an exogenous virus and endogenous viral genes in normal chicken cells and pheasant cells, respectively.

MATERIALS AND METHODS

Viruses. AMV strain A of the Bureau of Animal Industries belonging to subgroup B (Vogt classification) was used. RAV-0-producing chicken embryonic fibroblasts (CEF) from line 100 or line 7 chickens, RAV-60-infected Japanese quail embryonic fibroblasts, and RAV-2-infected CEF negative for group-specific (gs) antigens and chicken helper factor (chf) were kindly supplied by H. Hanafusa of Rockefeller University, New York, N.Y. H. Hanafusa also kindly provided B-77(w) virus and RAV-61 obtained directly from pheasant cells infected with RAV-2. RAV-61 was grown by the infection of duck embryonic fibroblasts (first passage), and B-77(w) was grown in CEF.

Embryonated eggs and adult birds. Chickens of the White Leghorn strain K-137 were obtained from Kimber Farms, Pomona, Calif. Chicken embryonated eggs from parents homozygous for the absence of gs antigens were kindly provided to us by R. E. Luginbuhl, University of Connecticut College of Agriculture and Natural Resources, Storr, Conn. Peking duck fertile eggs and two adult birds were bought from Ward Duck Farms, LaPuente, Calif. Ring-necked pheasant fertile eggs and four adult birds were purchased from Marsh Farms, Garden Grove, Calif. Fertile Japanese quail eggs were supplied by Life Sciences Inc., St. Petersburg, Fla., under contract with the National Cancer Institute, Special Virus Cancer Program. White Holland turkey fertile eggs and two adult birds were purchased from Curd Caviary of LaPuente, Calif. Adult birds were bled by cardiac puncture, and the erythrocytes were isolated by centrifugation and used for DNA extraction. Embryos (47) were used for DNA isolation after 16 days of incubation.

Leukemic chicken myeloblasts. Leukemic myeloblasts were obtained from the peripheral blood of acutely leukemic chickens (33). Many were provided by J. Beard of Life Sciences Inc.

Cell culture and propagation of virus. The general descriptions of the methods for culturing cells and propagating viruses have been published (1, 33, 37). All cultured cells were tested for the absence of congenital viremia before use (4).

^{3}H-labeled 35S viral RNA. The preparation of ^{3}H-labeled 70S RNA from purified virions and the sucrose velocity gradient isolation of 35S subunits obtained from ^{3}H-labeled 70S RNA after melting at 80 C for 3 min in 10^{-2} M Tris-hydrochloride (pH 7.4) and 10^{-3} M EDTA have been published (36).

Cellular DNA and RNA. Extraction and purification of cellular RNA and DNA have been described previously (5).

Virus production tests. Virus production tests were carried out as described earlier (4).

Preparation of DNA for liquid hybridization. The DNA for liquid hybridization was isolated and processed as described earlier (36). All DNAs were sheared by sonic treatment to a size of 6–8S, as determined by alkaline sucrose velocity sedimentation.

DNA-RNA hybridization in DNA excess. This liquid hybridization procedure has been described (36–39).

Thermal denaturation of DNA-RNA hybrids. Hybrids were obtained by hybridization at 65 C to a C_0t of 350 or 15,000 mol·s per liter of a reaction mixture consisting of 16 mg of sonically treated avian DNA and 8,000 counts/min of ^{3}H-labeled 35S viral RNA in 4 ml of 0.4 M phosphate buffer, pH 6.8, containing 0.1% sodium dodecyl sulfate. In those cases where we studied the thermal stability of RNA-DNA hybrids formed between C_0ts of 350 and 15,000, the reaction mixture without viral RNA was hybridized up to a C_0t of 350, and then viral RNA was added and hybridized to the desired C_0t. After hybridization, 4 ml of cold water and 32 ml of cold 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.1) were added. Duplicate 2-ml samples were removed, heated for 10 min at the indicated temperature, and immersed in ice-cold water. The samples were further diluted with 2× SSC to lower the DNA concentration to 50 μg/ml. One-half of each sample was treated with RNases A and T_1 for 1 h at 37 C, and the other half served as control. All samples were then precipitated with 5% trichloroacetic acid (final concentration), filtered through nitrocellulose, dried, and counted as described earlier (36).

The fraction of RNA-DNA hybrids not dissociated after incubation at different temperatures was plotted against temperature (Celsius). The temperature at which 50% of the hybrids were dissociated (T_m) was estimated from the melting profiles.

RESULTS

Kinetics of hybridization of 35S AMV RNA with avian DNAs. The hybridization between 35S AMV RNA and an excess of sonically treated single-stranded DNA from different avian species is shown in Fig. 1. The data are plotted as fractions of RNA made RNase resistant as a function of C_0t (nucleotide concentration in moles per liter × time in seconds) (36). Of the input RNA, 72% was rendered RNase resistant by leukemic DNA, whereas only 37% was rendered RNase resistant by normal chicken DNA. If we assume that the entire curve represents the hybridization of only one type of homologous viral DNA sequences in chicken cells, the frequency of these viral DNA sequences in normal and leukemic chicken cells can be estimated approximately by procedures detailed in an earlier paper (36). The $C_0t_{1/2}$ of curves in Fig. 1 was compared to the $C_0t_{1/2}$ of *Escherichia coli* complementary RNA hybridized with *E. coli* DNA and, after giving allowance for the different complexities of the *E. coli* and chicken genomes, approximately four and two copies of the AMV genome were estimated to be present in leukemic and normal chicken cells, respectively.

Pheasant DNA hybridized 10% of 35S AMV

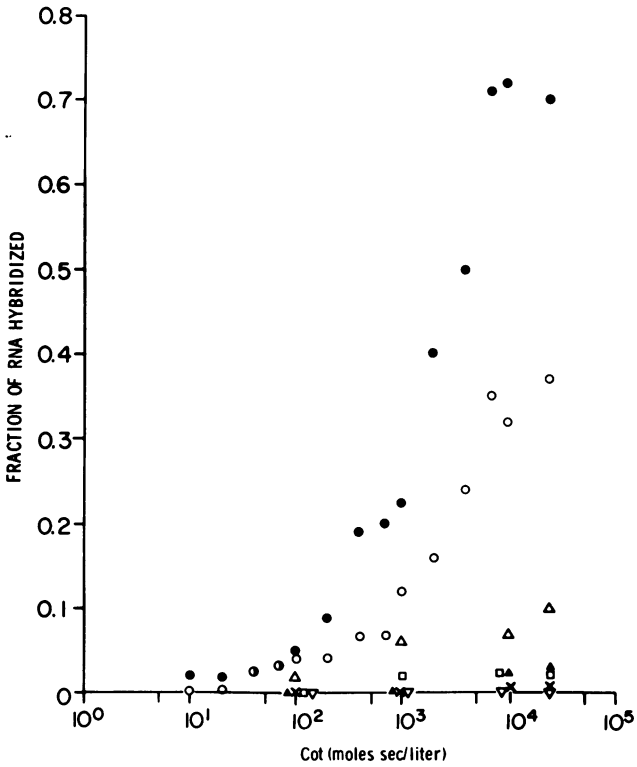

FIG. 1. *Hybridization kinetics of 35S AMV RNA with different avian DNAs. The hybridization mixture (4 ml total) contained (per ml) 4 mg of cellular DNA sheared to a fragment size of 6-8S, 1,600 counts/min of sonically treated ^{3}H-labeled 35S AMV RNA (size 8 to 10S, specific activity 10^6 counts/min per μg), and 0.1% sodium dodecyl sulfate in 0.4 M phosphate buffer, pH 6.8. The hybridization was carried out in tightly silicone-stoppered tubes. After boiling for 3 min in a water-ethylene glycol bath, the mixture was quickly transferred to a bath at 65 C. For the C_0ts of 10^0 and 10^1 mol·s per liter, a lower salt concentration (0.1 M phosphate) was used, and an appropriate correction to 0.12 M phosphate was made (49). Samples of 0.25 ml were taken at different time intervals and diluted with cold water in an ice-water bath. One-half of each sample was then treated with pancreatic and T_1 ribonucleases to determine the fraction of viral RNA rendered ribonuclease resistant as described earlier (36). A background of 2% obtained at a C_0t of 0 was deducted from all experimental values. Symbols: ○, gs-negative normal chicken embryos; ●, leukemic chicken myeloblasts; ×, normal duck; □, normal Japanese quail; △, normal pheasant; ▲, normal turkey; ▽, normal mouse embryos.*

RNA at a C_0t of 25,000 mol·s per liter, and maximum hybridization obtained with Japanese quail DNA and turkey DNA was 2 and 3%, respectively. Only 0.5% of AMV RNA formed hybrids with normal duck DNA. As reported earlier, mouse DNA did not hybridize with AMV RNA or with other avian oncornaviral RNAs tested.

Kinetics of hybridization of 35S RAV-0 RNA with avian DNAs. The kinetics of hybridization between 35S RAV-0 RNA and different avian DNAs are presented in Fig. 2. The rate of hybridization of the RAV-0 RNA was faster with DNA from leukemic chicken myeloblasts than with DNA from normal chicken embryos. Also, at a C_0t of 25,000 mol·s per liter 71% 35S RAV-0 RNA was converted into RNA-DNA hybrids with leukemic chicken myeloblast DNA, but only 60% of the RNA formed hybrids with DNA from uninfected normal chicken embryos. By using the same method of reiteration frequency calculation as that described earlier for AMV RNA, the number of RAV-0 genome copies was estimated to be approximately 4 and 2.5 per leukemic and normal cell, respectively. Therefore, under our reaction conditions, the endogenous viral sequences in normal chicken DNA should have been at least in 10-fold excess over the viral RNA sequences, and with leukemic cell DNA there should have been a 20-fold excess of viral DNA (6; M. Shoyab, P. D. Markham, R. M. Evans, and M. A. Baluda, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, V87, p. 215). The lesser hybridization of RAV-0 to normal chicken DNA than to leukemic chicken myeloblast DNA may be a result of either experimental limitations caused by a lesser DNA:RNA ratio and a rate of DNA reassociation faster than DNA-RNA hybridization or to incomplete copies of the endogenous viral genome in some chicken cells. Similar hybridization kinetics were also obtained with RAV-0 RNA if the gs-positive K-137 chicken embryonic DNA was replaced by gs-negative chicken DNA.

With DNA from ring-necked pheasants, 21.5% of RAV-0 35S RNA formed hybrids. RAV-0 hybridized 2% with Japanese quail DNA, 3% with turkey DNA, and not detectably with duck or mouse DNA.

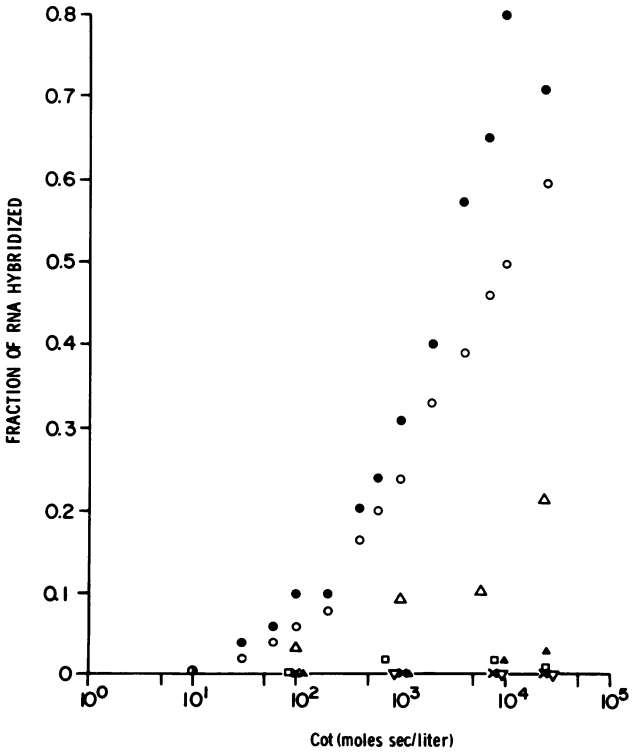

FIG. 2. *Hybridization kinetics of 35S RAV-0 RNA with avian DNAs. The conditions of hybridization in DNA excess were identical to those of Fig. 1. Symbols:* ○, *gs-negative chicken;* ●, *leukemic chicken myeloblasts;* ×, *normal duck,* □, *normal Japanese quail;* △, *normal pheasant;* ▲, *normal turkey;* ▽, *normal mouse embryos.*

Kinetics of hybridization of 35S RAV-60 RNA with avian DNAs. The hybridization kinetics between 35S RAV-60 RNA and different avian DNAs are presented in Fig. 3. At a C_0t of 25,000 mol·s per liter, the maximum level of hybridization between RAV-60 RNA and DNA from leukemic or normal chicken cells was very close, 29 and 27%, respectively. The kinetics of hybridization between RAV-60 RNA and leukemic or normal chicken DNA were similar to the kinetics of hybrid formation between AMV RNA and DNA from normal chicken cells (Fig. 1). Under similar conditions, 60% of RAV-0 35S RNA formed hybrids with the same normal chicken DNA (Fig. 2). The data indicate that normal chicken cells contain only about half of the sequences present in RAV-60 RNA. The slightly faster hybridization of RAV-60 RNA with DNA from leukemic myeloblasts might be a result of the presence of approximately twice as many viral DNA sequences in leukemic cells as in normal chicken cells.

Like RAV-0 and AMV RNAs, RAV-60 35S RNA hybridized to a limited extent (8%) with pheasant DNA and little with Japanese quail DNA (2%), turkey DNA (2 to 3%), and duck DNA (1 to 2%). Again, there was no hybridization with mouse DNA.

Kinetics of hybridization of RAV-61 35S RNA with different avian DNAs. The kinetics of hybrid formation between 35S RAV-61 RNA and avian DNAs are presented in Fig. 4. The shape of the hybridization curves obtained either with normal or leukemic chicken DNA is similar to that obtained between RAV-60 RNA and the same DNAs. Normal and leukemic chicken DNA hybridized 29 and 34% with RAV-61 RNA, respectively. Again, the hybridization rate was approximately two times faster with leukemic DNA than with normal chicken DNA, indicating twice as many viral-specific DNA sequences in leukemic cells as in normal cells.

Pheasant DNA hybridized the most with RAV-61 35S RNA, seemingly in a biphasic manner. The slower sequences were estimated to be present in two to four copies per cell. If all the sequences of RAV-61 RNA had been present in normal pheasant cells, 60 to 70% hybridization should have been reached between viral RNA and pheasant DNA under our hybridization conditions since 35S AMV RNA hybridizes that much with an excess of DNA from leukemic chicken myeloblasts and we know that the entire AMV genome is present in these AMV-producing cells. Also, RAV-0 RNA hybridizes 60% with an excess of normal chicken DNA. Since only 39% of the viral RNA was converted into RNA-DNA hybrids, RAV-61 does not seem to be an endogenous pheasant virus. However, it is possible that the RAV-61 RNA preparation is a mixture of two or more viruses consisting of

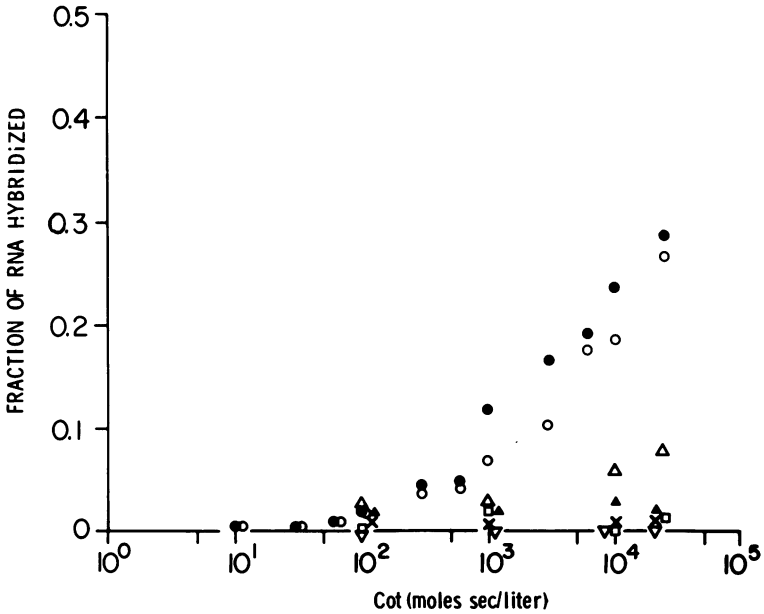

FIG. 3. *Hybridization kinetics of 35S RAV-60 RNA with avian DNAs. The conditions of hybridization in DNA excess were identical to those of Fig. 1. Symbols: ○, gs-negative chicken; ●, leukemic chicken myeloblasts; ×, duck; □, Japanese quail; △, pheasant; ▲, turkey; ▽, mouse.*

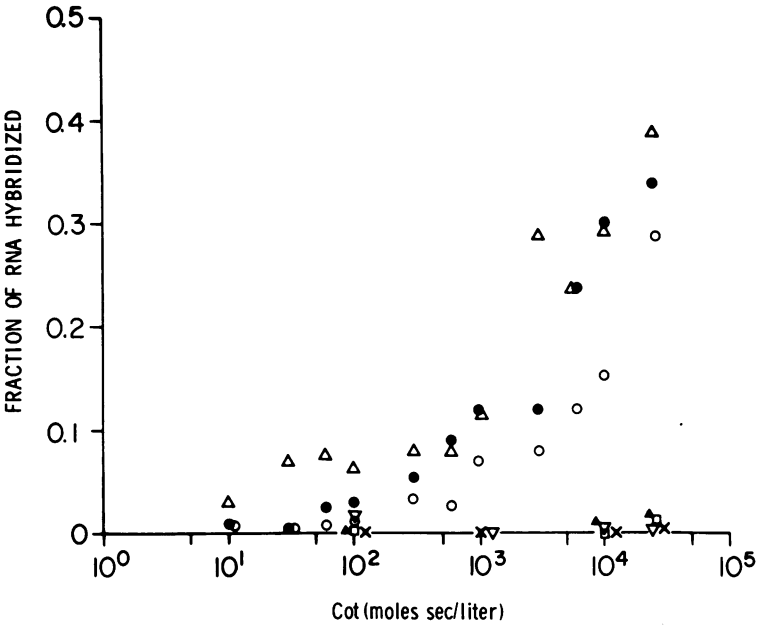

FIG. 4. *Hybridization kinetics of 35S RAV-61 RNA with avian DNAs. The conditions of hybridization in DNA excess were identical to those of Fig. 1. Symbols: ○, gs-negative chicken; ●, leukemic chicken myeloblasts; ×, duck; □, Japanese quail; △, pheasant; ▲, turkey; ▽, mouse.*

50% endogenous pheasant virus.

RAV-61 RNA hybridized 2% or less with duck, turkey, or mouse DNA.

Kinetics of hybridization of 35S RAV-2 RNA with different avian DNAs. The RAV-60 and RAV-61 used in this study were obtained by the infection of cultured chicken and pheasant cells, respectively, with RAV-2. RAV-2 was chosen as representative of a nontransforming leukosis exogenous virus, and the kinetics of hybrid formation between 35S RAV-2 RNA and different avian DNAs are shown in Fig. 5. RAV-2 RNA hybridized faster with DNA from leukemic chicken myeloblasts than with normal chicken DNA up to a C_0t value of 1,000 mol·s per liter. At higher C_0t values, the rate of hybrid formation with either DNA became similar. At a C_0t of 25,000, 52% of the viral RNA was rendered RNase resistant by leukemic DNA, whereas only 40% was converted into RNA-DNA hybrids by normal DNA. Under similar conditions, RAV-0 RNA hybridized approximately 60% with the same normal chicken DNA (Fig. 2). This suggests that 67% of the base sequences in RAV-0 and RAV-2 are homologous. Also, RAV-2 RNA seems to contain some nucleotide sequences that are present in AMV RNA but not in the endogenous-like RAV-0 genome.

The maximum hybridizations observed at a C_0t of 25,000 were: pheasant DNA, 11%; turkey DNA, 4%; Japanese quail DNA, 3%; and duck DNA, 1%. No hybridization was detected with mouse DNA.

Kinetics of hybridization of 35S B-77(w) avian sarcoma virus RNA with different avian DNAs. Figure 6 shows the kinetics of hybridization between 35S B-77(w) RNA and various avian DNAs. The rate of hybridization is approximately twice as fast with leukemic chicken DNA as it is with normal chicken DNA throughout the reaction, suggesting that there was a doubling of B-77-specific DNA sequences after the infection of chicken cells with AMV. B-77 RNA hybridized 41% with leukemic cell DNA, whereas only 31% hybridized with normal cell DNA, thereby indicating more sequence homology between the B-77 and AMV genomes than between the B-77 and RAV-0 RNAs.

Eleven percent of B-77(w) RNA formed hybrids with pheasant DNA at a C_0t of 25,000 mol·s per liter, as did RAV-2 RNA. The maximum hybridizations found between B-77(w) RNA and Japanese quail, turkey, or duck DNAs were 5, 3, and 0.5%, respectively, at a C_0t of 25,000. Mouse DNA hybridized about 0.5% of the B-77(w) RNA under similar conditions.

Thermal stability of RNA-DNA hybrids formed between viral RNAs and avian DNAs. The thermal stability of RNA-DNA hybrids is a function of the proportion of comple-

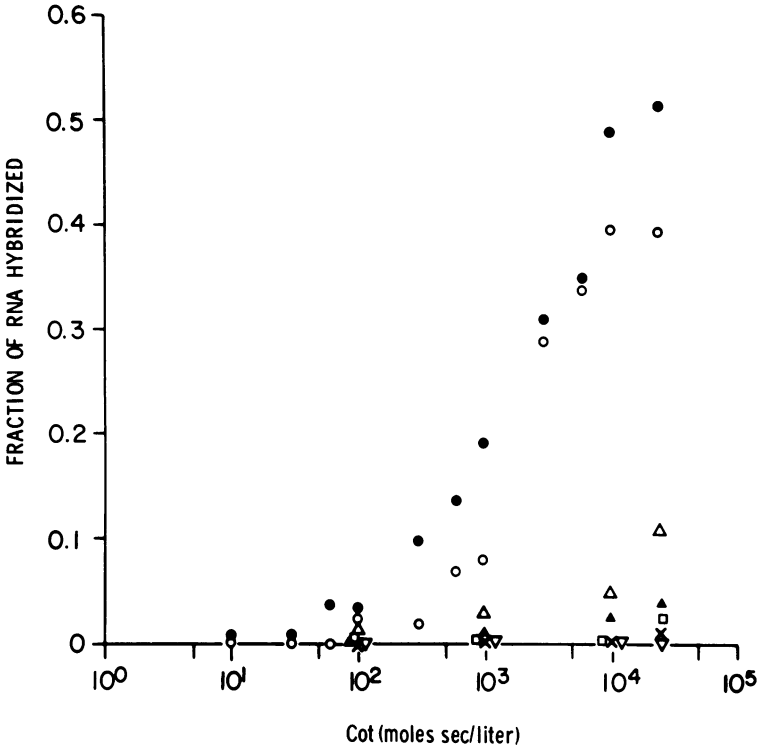

FIG. 5. *Hybridization kinetics of 35S RAV-2 RNA with avian DNAs. The conditions of hybridization in DNA excess were identical to those of Fig. 1. Symbols: ○, gs-negative chicken; ●, leukemic chicken myeloblasts; ×, duck; □, Japanese quail; △, pheasant; ▲, turkey; ▽, mouse.*

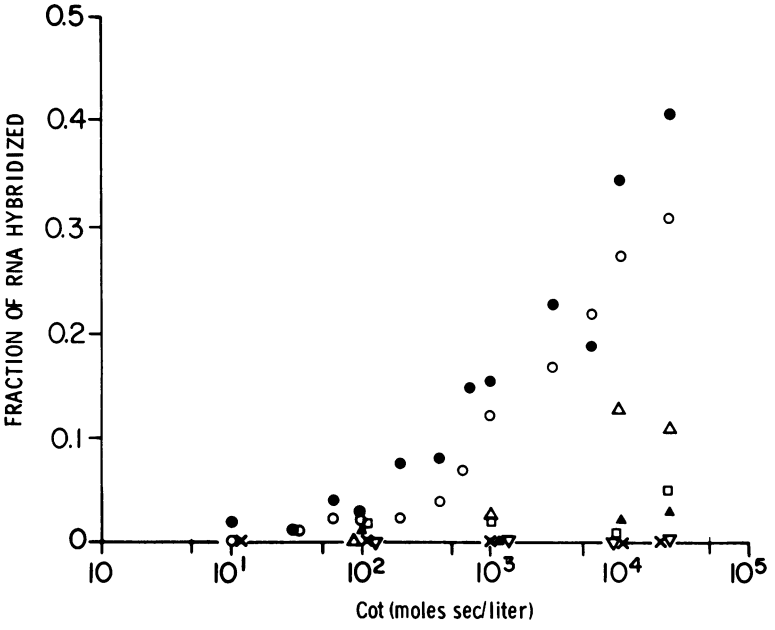

FIG. 6. *Hybridization kinetics of 35S B-77(w) RNA with avian DNAs. The conditions of hybridization in DNA excess were identical to those of Fig. 1. Symbols: ○, gs-negative chicken; ●, leukemic chicken myeloblasts; ×, duck; □, Japanese quail; △, pheasant; ▲, turkey; ▽, mouse.*

mentary base pairs in the hybrids and of the size and base composition of the two reacting nucleic acid strands (23, 26). Therefore, we analyzed the thermal denaturation of hybrids formed with nucleic acid fragments of similar size between normal or leukemic chicken DNA and 35S RNA from various avian oncornaviruses. Hybrids formed with AMV or RAV-0 RNAs at C_0ts of 0 to 350 mol·s per liter and C_0ts of 350 to 15,000 were heat denatured separately to examine whether there was a difference in the thermal stability of hybrids formed during either the early or the late phase of hybridization. Hybrids formed between RAV-0 RNA and normal chicken DNA either at C_0ts of 0 to 350 or at C_0ts of 350 to 15,000 had the same heat stability and T_m (88 C), whereas hybrids formed between AMV RNA and normal cellular DNA at the lower C_0t values melted with a T_m (86 C) 2 C lower than similar hybrids formed at the higher C_0t values (T_m 88 C). However, with leukemic cell DNA, both viral RNAs formed more stable hybrids that had T_m's 2 C higher than with normal DNA. Surprisingly, RAV-0 RNA formed more stable hybrids (T_m 90 to 92 C) with leukemic DNA than with normal DNA (T_m 88 C). This was unexpected since RAV-0 should have more homology with the endogenous chicken virus than with AMV. Also, it is not clear why hybrids formed during the early C_0ts are less heat stable by approximately 2 C. Although small, these 2 C T_m differences have been consistently observed (5, 36).

T_m values from the thermal dissociation profiles of hybrids formed between the other viral RNAs and normal or leukemic chicken DNA are summarized in Table 1, which also includes hybrids formed between RAV-61 and normal pheasant DNA. All of the hybrids formed between the viral RNAs and excess chicken DNA exhibited a high thermal stability in 2× SSC (84 to 92 C), indicating long complementary sequences and a high fidelity of base pairing (24–28, 32). All of the viruses used in this study, except RAV-61, formed hybrids with leukemic chicken DNA, which had a T_m 1 to 6 C higher than hybrids formed with normal chicken DNA. In the case of RAV-61 RNA, hybrids formed with either normal or leukemic chicken DNA had a similar T_m of 89 C. Also, RAV-61 formed slightly less stable hybrids with pheasant DNA (T_m 88 C) than with the chicken DNAs. However, this difference may not be significant. B-77(w) RNA formed the least stable hybrids with normal chicken DNA (T_m 84 C).

TABLE 1. T_m (Celsius) of hybrids formed between 35S viral RNAs and avian DNAs[a]

Viral RNA	DNA		
	Leukemic chicken myeloblasts	Normal chicken embryos	Normal pheasant
AMV	90[b]	88[b]	ND[c]
RAV-0	92[b]	88[b]	ND
RAV-60	90	87	ND
RAV-61	89	89	88
RAV-2	91	90	ND
B-77 (w)	90	84	ND

[a] The thermal dissociation of RNA-DNA hybrids formed between 35S viral RNAs and avian DNAs and the T_m determination were carried out as described in the text.

[b] Hybrids formed between C_0ts of 350 and 15,000 mol·s per liter.

[c] ND, Not done.

DISCUSSION

AMV-RNA hybridizes less with normal chicken DNA (30 to 40%) than with leukemic DNA (70 to 80%), presumably because of the difference in base sequence homology between AMV and the endogenous viral RNA. As determined by competition hybridization (Shoyab and Baluda, in press), the AMV and RAV-0 genomes have 60 to 70% homology. Also, presumably the level of hybridization that takes place between 35S RNA from other avian oncornaviruses and DNA from various avian species is determined by the degree of homology that exists between the endogenous virus of that avian species and the viral RNA used as probe. As expected, among the normal avian DNAs tested, normal chicken DNA has the maximum degree of homology with all viruses tested, except RAV-61 (Table 2). The avian RNA tumor viruses that had replicated in chicken cells [AMV, RAV-0, RAV-2, B-77(w)] or Japanese quail cells (RAV-60) or that had been passaged only once through duck cells (B-77, RAV-61) also show little homology with DNA from ducks, Japanese quails, or turkeys (Table 2). This suggests that either these avian species do not harbor endogenous virus or the genome of the endogenous viruses, if they exist, shares little or no homology with the viral RNAs used. To the best of our knowledge, endogenous viruses have not been detected in ducks, turkeys, or Japanese quails. Pheasant DNA hybridizes substantially with RNA from all viruses tested. Maximum hybridization was obtained with the duck-grown RAV-61, followed by the chicken-grown RAV-0, which hybridizes approximately half as much as RAV-61 does. The other viral RNAs hybridize approximately one-fourth as much with pheasant DNA as does RAV-61. Since RAV-61 was initially obtained by the in-

TABLE 2. *Summary of hybridization data between ^{3}H-labeled 35S RNA from various avian oncornaviruses and DNA from different avian species*[a]

^{3}H-labeled 35S RNA			Fraction of RNA maximally hybridized with DNA from:						
Cells used for virus production[b]	Virus	Sp act (counts/min per μg)	Leukemic chicken myeloblasts	Chicken	Pheasant	Japanese quail	Turkey	Duck	Mouse
Chicken leukemic myeloblasts	AMV	1.00×10^6	72	37	10	2	3	0.5	0
Line 100 CEF	RAV-0	1.07×10^6	71	60	22	2	3	0	0
QEF	RAV-60	9.10×10^5	29	27	8	2	3	1	0
DEF	RAV-61	1.59×10^6	34	29	39	2	2	0.5	0.5
gs$^-$chf$^-$ CEF	RAV-2	1.05×10^6	52	40	11	3	4	1	0.5
gs$^-$chf$^-$ CEF	B-77 (w)	1.12×10^6	41	31	12	5	3	0.5	0.5

[a] Data are taken from Fig. 1 to 6.

[b] QEF, Japanese quail embryonic fibroblasts; DEF, duck embryonic fibroblasts.

fection of pheasant cells with RAV-2, it is not surprising that it hybridizes the most with pheasant DNA. These results on the presence of oncornavirus sequences in different avian species are in total or partial agreement with those of other workers (22, 31). Neiman (31) has reported that RAV-0 RNA hybridized 70, 10, and 4% with DNA from normal chickens, pheasants, and Japanese quails, respectively. Kang and Temin (22) found hybridization values of 55, 20, 15, 10, and less than 1% with RAV-0 and DNA from normal chickens, pheasants, Japanese quails, turkeys, and ducks, respectively.

The complete genetic information of RAV-61 is not contained in normal pheasant DNA because RAV-61 RNA hybridizes 39% at the most with pheasant DNA. A hybridization of 60 to 70% would be expected if RAV-61 were an endogenous pheasant virus. Nevertheless, after appropriate corrections for the hybridization limitations, 60 to 70% of the RAV-61 genetic information seems to be present in pheasant DNA. The rest might be contributed by the exogenous virus RAV-2 since RAV-2 infection of pheasant cells resulted in the production of RAV-61, probably by recombination between RAV-2 and some pheasant endogenous virus. Recombination between endogenous and exogenous viruses has been previously demonstrated (16, 18, 48).

RAV-60 is different from RAV-0 in that RAV-60 RNA hybridizes less than half as much as RAV-0 RNA with DNA from normal or leukemic chicken cells or from pheasant cells. However, T_m's of the hybrids are approximately the same. Also, RAV-60 appears to contain very few AMV-specific sequences not shared by AMV and RAV-0. Therefore, RAV-60 does not seem to be another endogenous chicken virus (16, 17). Both RAV-0 and RAV-60 belong to subgroup E, a property determined by the envelope glycoproteins, and both viruses appear to share approximately two-thirds of their genetic information (Fig. 2 and 3; Shoyab and Baluda, in press).

DNA from turkeys, ducks, or Japanese quails has 30 to 40% homology with chicken DNA (22). Even if these different species have their own endogenous virus(es), the latter has little homology with the chicken endogenous virus RAV-0. Therefore, it appears that either avian endogenous virus genes evolve at a faster rate than total avian DNA or that the so-called endogenous viruses infected different avian species after their evolutionary divergence.

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