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Avian Reticuloendotheliosis Virus: Characterization of Genome Structure by Heteroduplex Mapping†

SYLVIA S. F. HU,[1] MICHAEL M. C. LAI,[2]* TIMOTHY C. WONG,[2] ROBERT S. COHEN,[2] AND MARTIN SEVOIAN[3]

Department of Pediatrics, City of Hope Medical Center, Duarte, California 91010[1]; Department of Microbiology, University of Southern California School of Medicine, Los Angeles, California 90033[2]; and Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, Massachusetts 01003[3]

The genome structure of defective, oncogenic avian reticuloendotheliosis virus (REV) was studied by heteroduplex mapping between the full-length complementary DNA of the helper virus REV-T1 and the 30S REV RNA. The REV genome (5.5 kilobases) had a deletion of 3.69 kilobases in the *gag-pol* region, confirming the genetic defectiveness of REV. In addition, REV lacked the sequences corresponding to the *env* gene but contained, instead, a contiguous stretch (1.6 to 1.9 kilobases) of the specific sequences presumably related to viral oncogenicity. Unlike those of other avian acute leukemia viruses, the transformation-specific sequences of REV were not contiguous with the *gag-pol* deletion. Thus, REV has a genome structure similar to that of a defective mink cell focus-inducing virus or a defective murine sarcoma virus. An additional class of heteroduplex molecules containing the *gag-pol* deletion and two other smaller deletion loops was observed. These molecules probably represented recombinants between the oncogenic REV and its helper virus.

Avian reticuloendotheliosis virus (REV) causes visceral reticuloendotheliosis and peripheral nerve lesion in various species of fowl (31, 32, 36, 38, 42). It transforms fibroblasts as well as hematopoietic cells derived from bone marrow and spleen in tissue culture (4, 10, 11, 15, 16). REV is unrelated to other avian leukosis and sarcoma viruses (13, 22, 29). It is also defective in replication and requires as helper virus other members of the reticuloendotheliosis group of retroviruses for the synthesis of viral progeny (16). The defectiveness of the REV genome is consistent with the absence of virion-related proteins in REV-transformed nonproducer cells (16). Recent studies further showed that REV contained a 28S RNA genome, as compared with the 35S RNA for the helper virus (4, 16). Oligonucleotide fingerprinting and nucleic acid hybridization studies further suggested that at least 30% of the REV genomic sequences are specific for REV and are presumably the oncogenic sequences which are responsible for its acute leukemogenic potential in vivo and its transforming activity in vitro.

To determine the genetic structure of REV and to localize the transformation-specific sequences in the REV genome, we compared the genomes of REV and its helper virus REV-A by an electron microscopic heteroduplex method. We found that REV had an extensive deletion in the *gap-pol* region but contained a stretch of transformation-specific sequences in the *env* region which was not contiguous with the *gag-pol* deletion. This genetic structure distinguishes REV from other avian acute leukemia viruses.

MATERIALS AND METHODS

Cells and viruses. The bone marrow cell (BMC) subline of REV-transformed chicken BMC came originally from H. Bose of the University of Texas at Austin (11) and was obtained through the courtesy of C. Moscovici, Gainesville, Fla. The cells were maintained in F-10 medium supplemented with 10% tryptose phosphate broth, 10% calf serum, and 5% chicken serum. The REV-transformed lymphoblastoid cell lines TV-1 and TV-2 were isolated from the spleens of moribund SPAFAS and line 6 chicken, respectively, infected with REV. These cells carry B-cell determinants on their surfaces (23). The TV-1 and TV-2 cells were maintained in RPMI 1640 medium supplemented with 10% calf serum. REV-T1, an attenuated preparation of REV, has been described previously (41). This strain does not cause proliferation of reticuloendothelial cells but induces nerve lesions in vivo (33, 42) and does not transform fibroblasts or BMC in vitro. REV-T1 is genetically very similar to the helper virus, REV-A, isolated from the viruses released from the BMC line (4).

Preparation of viral RNA. The RNA used for heteroduplex mapping was prepared from the viruses released over a 12-h period from the infected culture.

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Viruses were purified from the media according to a modification of published procedures (25). Briefly, virus was pelleted from the media in an SW27 rotor at 25,000 rpm for 90 min and then sedimented on a 25 to 60% (wt/vol) sucrose gradient in an SW50.1 rotor at 50,000 rpm for 150 min. The 70S RNA was extracted from the virion and prepared according to published procedures (28).

Synthesis of genome-length cDNA from REV-T1. The genome-length complementary DNA (cDNA) was synthesized from REV-T1 by endogenous reverse transcription according to a published procedure (26). This procedure was established for several strains of avian and mammalian oncoviruses. We found that it was also applicable to the REV-T1 strain. The endogenous reverse transcription was performed in a reaction mixture containing 1 mM Tris-hydrochloride (pH 8.0), 30 mM dithiothreitol, 3 mM magnesium acetate, 1 mM each dATP, dCTP, and dGTP, 0.5 mM [^{3}H]-TTP (200 mCi/mmol), 1 mg of purified virus per ml, and 0.02 to 0.03% Triton X-100 at 37°C for 18 h. The reaction products were extracted with sodium dodecyl sulfate-phenol and then digested with RNase A (50 μg/ml) at 37°C for 1 h. The full-length DNA was separated by sedimentation through an alkaline sucrose gradient in an SW40 rotor at 40,000 rpm for 11 h (26). Most of the full-length DNA was 9 kilobases (kb) long. The authenticity of this in vitro-synthesized genome-length cDNA was confirmed by restriction enzyme mapping after it was converted into double-stranded DNA in vitro (37; K. Steele, J. M. Taylor, and M. M. C. Lai, unpublished data) and by RNA-DNA hybridization (see Results).

Heteroduplex mapping. REV-T1 cDNA (20 μg/ml) was hybridized to REV DNA (10 μg/ml) in 20 μl of a solution containing 80% formamide, 0.4 M NaCl, and 0.01 M piperazine-*N*,*N*′-bis-(2-ethanesulfonic acid) (PIPES) buffer (pH 6.4) at 46°C for 30 min. The hybrids were then dialyzed against 1 M glyoxal in 0.01 M sodium phosphate buffer (pH 7.0) for 1 h at 37°C. Excess glyoxal was removed by dialysis against 0.01 M Tris-hydrochloride containing 0.001 M EDTA (pH 7.5) at 4°C overnight. Portions of the hybrids were then annealed to simian virus 40 (SV40)-polybromodeoxyuridine [poly(BUdR)] marker molecules at room temperature for 20 min in the presence of 0.5 M *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), and 0.05 M EDTA (pH 7.5) (17). The final product was then adjusted to 55% formamide and spread onto 18% formamide. Spreading with 66% urea plus formamide was performed according to the procedure described by Kung et al. (24).

The SV40 DNA (5.224 kb) was used as the internal double-stranded nucleic acid-length standard. The length of single-stranded DNA or RNA was taken to be 0.714 times the length of a corresponding duplex molecule as determined by spreading denatured SV40 DNA under the same conditions (S. Hu, unpublished data).

Electrophoretic analysis of RNA. Polyacrylamide gel electrophoresis was carried out according to a modification of the method of Duesberg (7). The RNA was electrophoresed in 2% polyacrylamide gels cross-linked with bisacrylamide (7 by 0.6 cm) at 50 V for 5 h. After electrophoresis, the gels were frozen and sliced into 1-mm fractions which were incubated in toluene-based scintillation fluid containing 10% NCS (Amersham/Searle) and 1% water at 50°C for 5 h before counting.

RESULTS

Electrophoretic analysis of the RNA genome of REV. We have previously shown that the virus released from an REV-transformed BMC line (11) contains a 35S RNA, which corresponds to the RNA of the helper virus REV-A, and a 28S RNA, which represents the genome of the defective transforming component (4, 16). This cell line was used as one of the virus sources for heteroduplex mapping in this study. In addition, two different cell lines, TV-1 and TV-2, established from the spleen cells of REV-infected chickens were also used (23). These two cell lines carry B-cell determinants, and the virus released from these cells is capable of causing reticuloendotheliosis (23). To identify the RNA species of viruses released by these cell lines, we analyzed the 50 to 70S RNA genome of the viruses by polyacrylamide gel electrophoresis. The viruses from both TV-1 and TV-2 contained a 35S RNA and a smaller RNA species which migrated slower than the 28S rRNA marker (Fig. 1). In analogy to the REV released from the BMC line (Fig. 1c), the 35S RNA probably represented helper REV-A and the smaller RNA species represented the REV genome which is responsible for oncogenicity in vivo and transforming capacity in vitro (4). We henceforth designate this small RNA as 30S REV RNA. However, in contrast to the BMC line, the virus released from TV-1 and TV-2 contained about equal ratios of the 35S helper RNA and the 30S transforming component. The virus released from BMC occasionally contained, in addition to 35S and 30S RNA, a smaller RNA species which migrated almost together with the 28S rRNA in polyacrylamide gel electrophoresis (Fig. 1c). This RNA species has been noted before (4). From heteroduplex studies (see below), we suggest that this small RNA species might represent a defective form of the transforming RNA component.

Characterization of the full-length REV-T1 cDNA. To characterize the size and location of the genetic deletion as well as the transformation-specific sequences in the REV genome, we performed electron microscopic heteroduplex mapping between the full-length cDNA of the helper virus REV-T1 and the 30S REV RNA. The full-length REV-T1 cDNA was synthesized by the endogenous reverse transcription from REV-T1 according to published methods (26).

Since it has been reported that the endogenous reverse transcription of reticuloendotheli-

osis viruses produces unfaithful cDNA (30), the authenticity of the full-length REV-T1 cDNA used for heteroduplex mapping was first studied. Three pieces of evidence suggest that the full-length (9.0 kb) REV-T1 cDNA synthesized under our conditions was authentic. (i) RNA-DNA hybridization showed that this cDNA species could anneal to REV-T1 70S RNA to 85 to 100%, depending on the hybridization conditions (Table 1). It did not hybridize to any other viral RNA tested. These results suggest that this full-length cDNA represents the entire genomic sequences of REV-T1 and also that the cDNA is a faithful copy of REV-T1 genome. (ii) Electron microscopic homoduplex mapping between REV-T1 full-length cDNA and 70S REV-T1 RNA showed that they were completely homologous (not shown). (iii) The full-length (9.0 kb) REV-T1 cDNA was converted into double-stranded DNA, using DNA polymerase I, and then analyzed by restriction enzyme mapping as described previously (37). The restriction patterns suggest that this cDNA species is a homogeneous species of DNA and that it represents an authentic copy of the REV-T1 RNA genome (Steele et al., unpublished data).

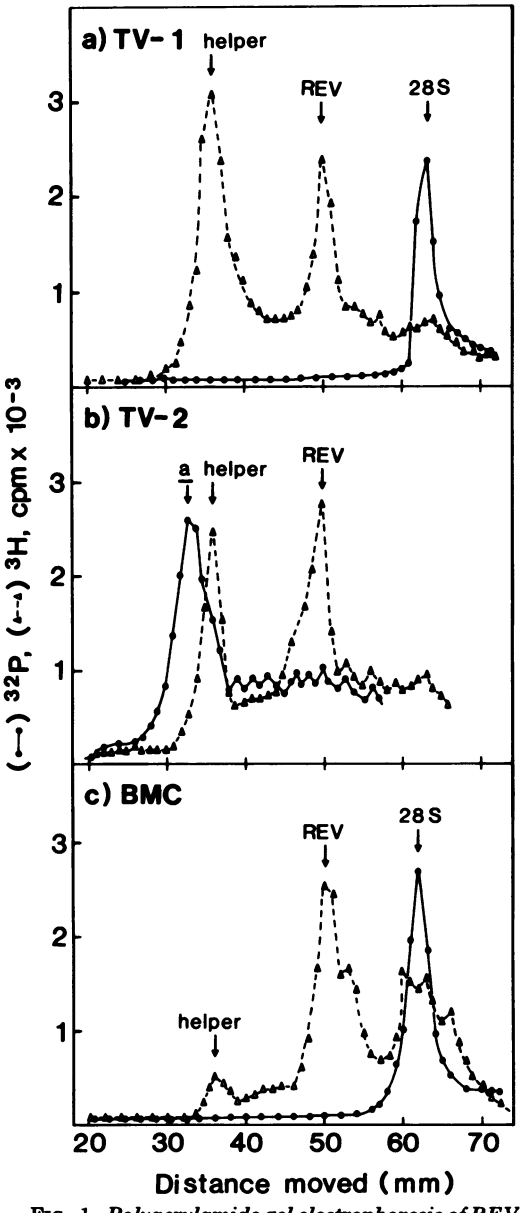


FIG. 1. *Polyacrylamide gel electrophoresis of REV RNA. The [^{3}H]uridine-labeled 50 to 70S virion RNAs released from (a) TV-1, (b), TV-2, and (c) BMC cell lines were electrophoresed in 2% polyacrylamide gels at 50 V for 5 h. The markers used were ^{32}P-labeled 28S rRNA and 35S class a RNA of the Prague strain of Rous sarcoma virus (9).*

Heteroduplex mapping between REV-T1 cDNA and REV (REV-A) RNA from the transformed spleen cell lines TV-1 and TV-2. The heteroduplex molecules were studied by published procedures (18, 24). The 50 to 70S RNA from TV-1 and TV-2 cell lines was first used. The 3′ ends of the RNA molecules were identified by binding a poly(BUdR)-tailed cir-

TABLE 1. *Hybridization of full-length (9.0 kb) REV-T1 cDNA with REV-T1 RNA*[a]

cDNA	RNA	% cDNA or RNA hybridized
^{3}H-REV-T1	REV-T1	100
	PR-B	6
	None	4
REV-T1	^{32}P-REV-T1	85
	^{32}P-PR-B	3
	^{32}P-Rauscher MLV	2
	None	1.5

[a] Hybridization was performed in a solution containing 0.01 M Tris-hydrochloride, pH 7.4, 0.6 M NaCl, 0.05% sodium dodecyl sulfate, 25 μg of tRNA, and appropriate amounts of cDNA and RNA at 66°C for 12 h as previously described (4). In the first experiment, 1,000 cpm (specific activity, 20,000 cpm/μg) of full-length (9.0 kb) ^{3}H-labeled REV-T1 cDNA was hybridized with 0.2 μg of 70S RNA from different viruses. After hybridization, the hybridization mixture was digested with nuclease S1, and the percentage of [^{3}H]DNA hybridized was determined by precipitation with trichloroacetic acid. In the second experiment, 1,000 cpm of ^{3}H-labeled REV-T1 cDNA was hybridized with 1,000 cpm of ^{32}P-labeled 70S RNA (specific activity, 10^6 cpm/μg). After hybridization, the hybridization mixture was digested with RNase A (10 μg/ml), and the percentage of [^{32}P]RNA hybridized was determined by trichloroacetic acid precipitation. MLV, Murine leukemia virus.

cular SV40 DNA to the 3′ polyadenylic acid [poly(A)] (1, 18, 27). Two types of molecules were observed. (i) The first type included the heteroduplex molecules formed between full-length REV-T1 cDNA and the helper virus, REV-A RNA (not shown). These molecules were circular or linear with complete homology. This result confirmed our oligonucleotide mapping showing that REV-T1 and REV-A are almost completely identical (4). (ii) The second type included the heteroduplex formed between REV-T1 cDNA and 30S REV RNA (Fig. 2). This type of heteroduplex molecule had the following structural features. (i) Starting from the 3′ end of the genome and immediately adjacent to the poly(A) sequence was a stretch of homologous sequences of 0.73 ± 0.07 kb. This region ("a" in Fig. 2) corresponded to the C region which is constant in the RNA genome of the avian leukosis-sarcoma virus complex (5). This stretch of homologous sequences might also represent the sequences of similar nature in the reticuloendotheliosis group of viruses. (ii) Next to the homologous sequences at the 3′ end was a large substitution loop, with the two arms measuring 1.92 ± 0.21 ("b" in Fig. 2) and 1.64 ± 0.20 kb ("c" in Fig. 2), respectively. The difference in length between the two arms might not be significant, and the assignment of the strands to either virus is arbitrary. This nonhomologous region suggests that REV lacks 1.6 to 1.9 kb of the REV-A sequences and contains some REV-specific sequences of similar size which are distinct from REV-A sequences. This region (1.6 to 1.9 kb), therefore, represented the REV-specific sequences which might be related to the oncogenic activity of REV. In its location, it corresponded to the *env* gene of the avian leukosis-sarcoma virus complex. (iii) A large deletion loop ("e" in Fig. 2) of 3.64 ± 0.25 kb was located at 1.64 ± 0.22 kb from the 5′ end. This deletion loop represented the second stretch of the sequences missing in REV and corresponded to the 3′ half of the *gag* gene and 5′ half of the *pol*

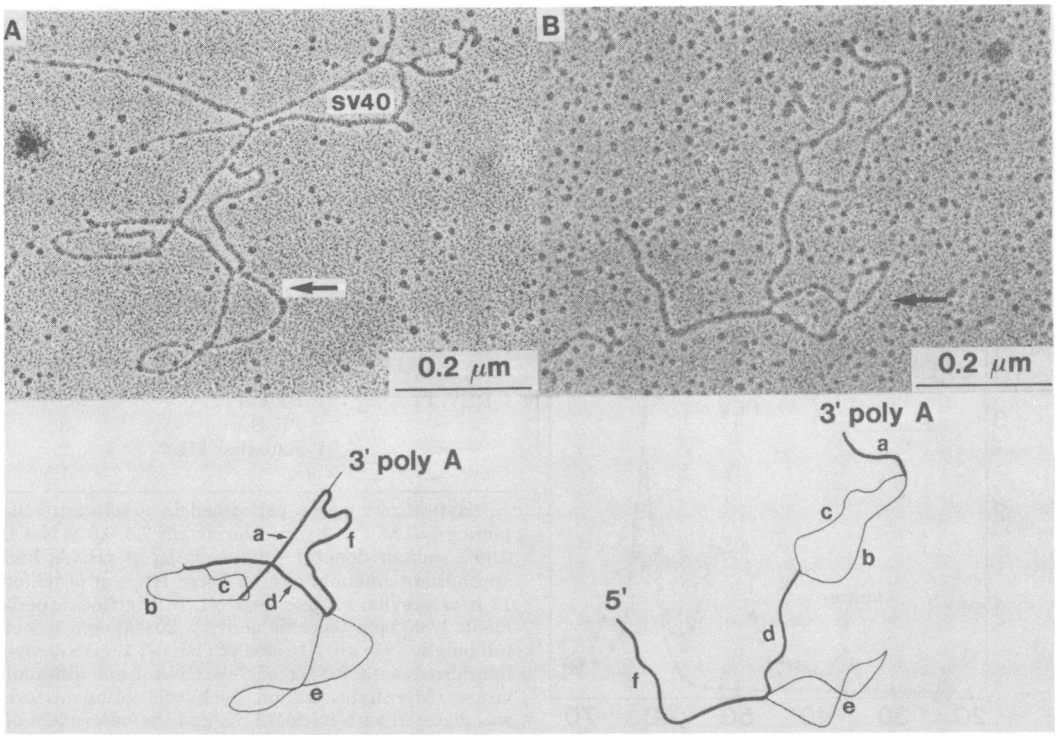


FIG. 2. *Electron micrographs of REV-T1·REV heteroduplex molecules. (A) Circular heteroduplex; (B) linear heteroduplex. About 15% of the heteroduplex molecules was circular because of the noncolinearity between the cDNA and the RNA (21). In the circular heteroduplex, the 3′ poly(A) was linked to an SV40-poly(BUdR) marker. There were a few other linear RNA strands attached to the same marker molecule. In the linear heteroduplex molecule shown (B), no SV40 marker DNA was attached to the 3′ end. The orientation of 3′ and 5′ ends was determined from other linear heteroduplexes (not shown). The larger gag-pol deletion loop (shown by the arrows) was frequently seen with a figure eight structure, suggesting the presence of a short stretch of self-complementary sequences within the loop. See also Fig. 4.*

gene, if we assume that REV-A has the same gene order as do other oncoviruses. This structure confirms the genetic defectiveness of REV (15, 16). Between the 3'-half substitution loop and the 5'-half deletion loop was a stretch of homologous sequences of 1.04 ± 0.08 kb ("d" in Fig. 2). This region should correspond to the partial sequence of the *pol* gene and possibly also part of the *env* gene. The presence of this stretch of homologous sequences between the 5'-end deletion loop and the 3'-half REV-specific sequences distinguishes REV from other avian acute leukemia viruses, such as MC29 or avian erythroblastosis virus, in which the *gag-pol* deletion is contiguous with the leukemia virus-specific sequences (19, 28).

Depending on the strand assignment of the substitution loop, the genome size of REV-A will be (1.64 + 3.64 + 1.04 + 1.64 + 0.73 + 0.20) = 8.89 ± 0.82 kb or 9.17 ± 0.83 kb, and the genome size of REV will be (1.64 + 1.04 + 1.92 + 0.73 + 0.20) = 5.53 ± 0.58 kb or 5.25 ± 0.57 kb. These estimates are in agreement with the values obtained by electrophoresis in denaturing gels, namely, 8.7 kb for REV-A and 5.9 kb for REV (16).

Heteroduplex mapping between REV-T1 cDNA and REV (REV-A) RNA released from the BMC line. To further confirm the genetic structure of REV, we performed heteroduplex mapping between REV-T1 and the virion released from another REV-transformed cell line, the BMC line (11). This cell line released a higher proportion of the transforming component, REV, than did the REV-transformed spleen cell lines TV-1 and TV-2 (Fig. 1) (4). As with the virus released from the TV cell lines, these heteroduplexes contained the same two types of heteroduplex molecules: (i) the heteroduplex between REV-T1 cDNA and RNA of the helper virus present in the REV (REV-A) virus population (these molecules were completely homologous [not shown]) and (ii) the heteroduplexes between REV-T1 cDNA and 30S REV RNA, which had a structure identical to that described in Fig. 2. Thus, this RNA species likely represented the REV RNA which was responsible for the oncogenic activity of the virus.

In addition to these two kinds of hybrid molecules, a third kind was consistently observed in all of the virus preparations released from the BMC line (Fig. 3). This new type of heteroduplex is henceforth termed type II, and the first type is termed type I. The type II heteroduplex molecules (Fig. 3) had the following structural features. Starting at 0.90 ± 0.12 kb from the 3' end, there were three deletion loops, with sizes of 0.60 ± 0.07 (c_1), 1.09 ± 0.12 (b_2), and 3.69 ± 0.25 kb (e), in the middle of the heteroduplex molecules.

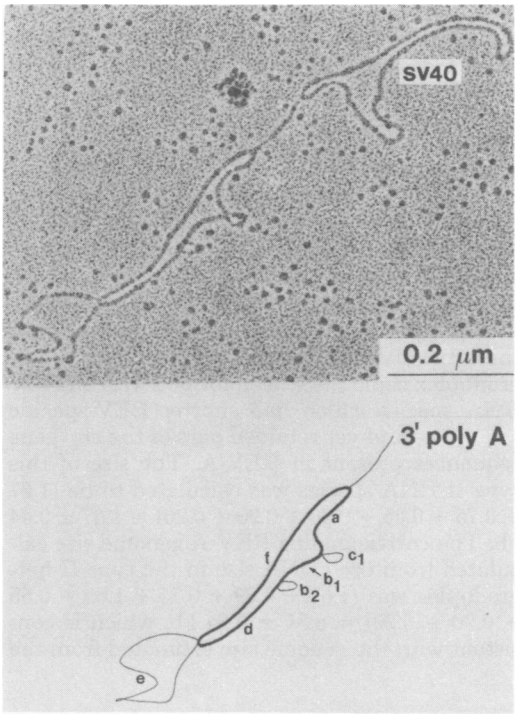


FIG. 3. *Electron micrograph of a type II heteroduplex molecule between REV-T1 and REV released from a BMC line. An SV40-poly(BUdR) marker molecule was attached to the 3' poly(A) sequences of the hybrid. The molecule was circular as explained in the legend of Fig. 2. The orientation of 3' and 5' ends was determined from the linear heteroduplex molecules with the attached SV40 DNA (not shown).*

The third deletion loop was located at 1.67 ± 0.10 kb from the 5' end and was, therefore, similar in both size and location to the deletion loop in the type I heteroduplex. Thus, this loop represented the deletion in the *gag-pol* genes. The other two smaller deletion loops fell in the region roughly corresponding to the substitution loop in the type I molecules. This suggests that the type I and type II molecules might differ in the extent of deletion and the size of the REV-specific sequences in this region.

To determine the origin and nature of these two small deletion loops, we further examined the type II heteroduplexes under a spreading condition (hyperphase, 50% formamide; hypophase, 15% formamide) under which single-stranded DNA was well extended but single-stranded RNA tended to form a collapsed configuration. The two larger deletion loops (3.69 and 1.09 kb) were always well extended (Fig. 4), suggesting that they represented DNA strands and, therefore, REV-A sequences which were missing in REV. In contrast, the 0.60-kb loop

(denoted by an arrow in Fig. 4) was condensed into a hairpin-like structure under this condition, suggesting that the 0.60-kb loop represented an RNA strand, and, therefore, REV-specific sequences which were missing in REV-A. This stretch of REV-specific sequences was much smaller than that in type I molecules. Likewise, the 1.09-kb deletion loop representing the REV-A-specific, and possibly *env*-related, sequences which were missing in REV was also smaller than the 1.6- to 1.9-kb deletion observed in the type I molecules. Furthermore, there were homologous sequences (0.55 kb) (b_1) between these two deletion loops. Thus, the type II heteroduplex could have been formed with an REV RNA species which had shorter REV-specific sequences and yet retained part of the *env* gene sequences present in REV-A. The size of this type II RNA species was calculated to be (1.67 + 0.75 + 0.55 + 0.60 + 0.90 + 0.20) = 4.67 ± 0.44 kb. The corresponding REV-A genome size calculated from the cDNA size in the type II heteroduplex was (1.67 + 3.69 + 0.75 + 1.09 + 0.55 + 0.90 + 0.20) = 8.85 ± 0.74 kb, which is consistent with the genome size estimated from the type I heteroduplex and from electrophoresis (16). Measurements of type I and type II heteroduplexes are summarized in Fig. 5.

The type I and type II molecules were present in a 30:70 ratio in all of the RNA preparations obtained from the BMC line, regardless of the passage history of the cells. To rule out the possibility that the type II heteroduplex might be an artifact of the spreading condition, resulting from partial homology in the two arms of the substitution loop observed in type I heteroduplex molecules, we further studied the heteroduplex under various spreading conditions. We compared the heteroduplexes after treatment with glyoxal followed by formamide spread or without glyoxal treatment and spread directly with 65% urea plus formamide. The latter method has been shown to cause less random denaturation and allow for detection of partially homologous sequences (18). If the type I and type II molecules are derived from the same RNA species, the ratio of these two heteroduplexes will vary with the stringency of the spreading method (18). However, no difference in the ratio of type I and type II heteroduplex molecules was observed under different conditions. We therefore conclude that these two structures are heteroduplexes formed with dif-

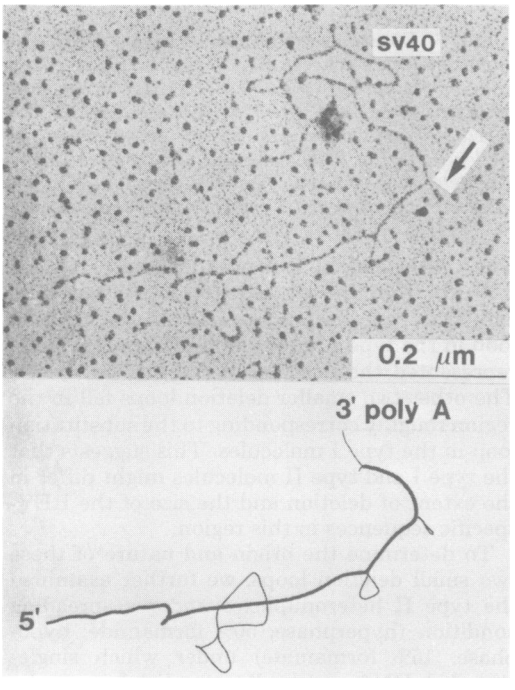
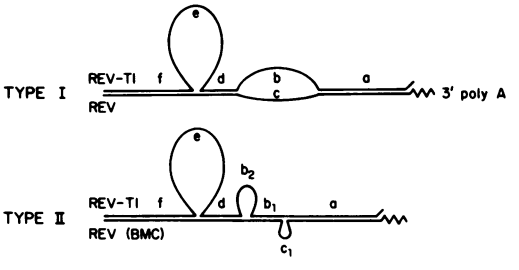


FIG. 4. *Electron micrograph of a type II REV-T1 REV heteroduplex when spread under 50% formamide without prior glyoxal treatment. The deletion loop closest to the 3' end (arrow) is now seen collapsed, whereas the other two deletion loops are still well extended.*



LENGTHS OF SEGMENTS OF HETERODUPLEX (IN KILOBASES)

	TYPE I	TYPE II
a	0.73 ± 0.07	0.90 ± 0.12
b	1.92 ± 0.21	- -
b_1	- -	0.55 ± 0.05
b_2	- -	1.09 ± 0.12
c	1.64 ± 0.20	- -
c_1	- -	0.60 ± 0.07
d	1.04 ± 0.08	0.75 ± 0.10
e	3.64 ± 0.25	3.69 ± 0.25
f	1.64 ± 0.22	1.67 ± 0.10

FIG. 5. *Schematic drawing and measurements of type I and type II heteroduplex molecules formed with virion RNA released from a BMC line. A total of 78 type I heteroduplex molecules and 144 type II heteroduplex molecules were observed. Out of these, 21 type I molecules and 35 type II molecules were used for length measurements.*

ferent RNA species and that the type II molecules are not the products of partial denaturation of type I molecules. Thus, type II molecules represent a new species of RNA with a smaller size and smaller REV-specific and possibly oncogenic sequences. These kinds of molecules have so far only been found in virus released from the BMC cell line.

DISCUSSION

Biological data suggested that REV is defective and requires helper virus for its replication (11, 16). Electrophoresis and heteroduplex mapping showed that it has a 5.5 to 5.9-kb or 30S RNA genome (4, 16) and that 30% of its sequences are REV specific and presumably are responsible for the oncogenic activity of the virus (4). Our heteroduplex studies further demonstrated that REV has a 3.64-kb deletion in the *gag-pol* genes, if one assumes that REV has the same gene order as other oncoviruses. Thus, REV is similar to other avian acute leukemia viruses in that it has an extensive deletion in the *gag-pol* genes (19, 28). The REV-specific, and presumably oncogenic, sequences in the REV genome which are responsible for visceral reticuloendotheliosis in vivo and transformation of fibroblasts and bone marrow cells in vitro constitute a contiguous stretch of 1.6 to 1.9 kb in length. These sequences account for 30 to 35% of the genetic sequences of REV, which is in good agreement with the published data obtained by cDNA-RNA hybridization (4). Our study showed that these REV-specific sequences were localized in the region corresponding to the *env* gene and were not contiguous with the *gag-pol* deletion in the REV genome. This genetic structure is in contrast to that of other avian acute leukemia viruses (19, 28) and is rather similar to that of the defective Friend strain of spleen focus-forming virus (39), defective murine sarcoma virus (17), and defective mink cell focus-inducing viruses, which are recombinants in the *env* gene between ecotropic and xenotropic viruses (3, 40). Recently, we have also shown that the transformation-specific sequences of REV are not related to those of other known avian or murine acute leukemia viruses such as MC29, MH2, avian erythroblastosis virus, avian myeloblastosis virus, or Abelson murine leukemia viruses (T. C. Wong and M. M. C. Lai, Virology, in press). Thus, REV represents a new class of avian acute leukemia virus.

All of the avian acute leukemia viruses studied so far code for a polyprotein which is a fusion protein between part of the *gag* sequences and the transformation-specific sequences (2, 14, 20). Since the *gag-pol* gene and the presumably transformation-specific sequences in REV are not contiguous, we would not expect REV to code for such a fusion polyprotein. However, it is still possible that the REV-specific sequences localized in the *env* region extend beyond the termination point of the *pol* gene. If this is the case, a polyprotein containing part of the *gag-pol* and the transformation-specific sequences might exist. It has been reported that, in some of the REV-transformed nonproducer cells, a protein of 130,000 molecular weight could be detected (16). However, this protein was not observed in all of the REV-transformed cell lines and it was not certain whether it was a cellular protein or a virus-coded protein. Despite repeated attempts, we have not been able to demonstrate such a polyprotein in various REV-transformed cell lines. Alternatively, the REV-specific sequences located in the region corresponding to the *env* gene of REV-A might code for a transformation-related glycoprotein, in analogy to Friend spleen focus-forming virus (6, 34, 35). Further experiments are required to resolve this issue.

The type II RNA observed in the virus populations released from the BMC line is particularly interesting. This RNA might correspond to the RNA species which migrated nearly together with the 28S rRNA marker in 2% polyacrylamide gel electrophoresis (Fig. 1c) (4). Gonda et al. have also detected such a small RNA species (12). The type II RNA molecules contain smaller REV-specific sequences and a smaller deletion in the *env* region than do type I RNA molecules, but it retains some of the *env* gene sequences homologous to those of REV-A. As a result, two smaller deletion loops, in place of the big substitution loop in the type I molecules, are present in type II heteroduplexes. These two deletion loops represent REV-specific sequences and deleted sequences, respectively, in the *env* gene of REV-A. Since we could not detect such an RNA species in REV-A populations, we suggest that the type II RNA was probably a recombination product of an unequal crossover between the helper virus, REV-A, and the transforming component, REV. It is not clear whether this RNA has any biological significance. Nevertheless, it is interesting to note that an RNA species of similar genetic structure has also been detected in avian myelocytomatosis virus MC29 released from a transformed quail cell line (Q10) (19). This RNA was termed ΔMC29. Both the REV-transformed BMC line and MC29-transformed Q10 line, in which this small RNA species was discovered, contain unusually low titers of the helper viruses (4, 8). This fact suggests that this small RNA species might function as defective

interfering particles and interfere with the replication of the helper viruses. Further studies are required to determine the biological significance of this small RNA species.

ACKNOWLEDGMENTS

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