JOURNAL OF VIROLOGY, Dec. 1981, p. 800-811
0022-538X/81/120800-12$02.00/0

Vol. 40, No. 3

Characterization of Reticuloendotheliosis Virus Strain T DNA and Isolation of a Novel Variant of Reticuloendotheliosis Virus Strain T by Molecular Cloning

IRVIN S. Y. CHEN,[1] TAK W. MAK,[2] JULIAN J. O'REAR,[1] AND HOWARD M. TEMIN[1]*

McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706,[1] and The Ontario Cancer Institute, Toronto, Ontario, Canada M4X 1K9[2]

Received 17 April 1981/Accepted 10 July 1981

Reticuloendotheliosis virus strain T (REV-T) is a highly oncogenic avian retrovirus which causes a rapid neoplastic disease of the lymphoreticular system. Upon infection, this virus gives rise to two species of unintegrated linear viral DNA, which are 8.3 and 5.5 kilobase pairs long and represent the helper virus (REV-A) and the oncogenic component (REV-T), respectively. Restriction endonuclease cleavage maps of these two DNA components indicate that REV-T DNA has a large portion of the genome deleted with respect to REV-A DNA and a substitution about 0.8 to 1.5 kilobase pairs long that is unrelated to REV-A DNA. These additional sequences comprise the putative transforming region of REV-T (*rel*). A chicken spleen cell line transformed by REV-T produced virus which upon infection gives rise to three species of unintegrated linear viral DNA (8.3, 5.5, and 3.3 kilobase pairs). We isolated the proviruses of the 8.3- and 3.3-kilobase pair species from this cell line by cloning in the phage vector Charon 4A. Restriction enzyme mapping showed that the two proviral clones are proviruses of REV-A and a variant of REV-T, respectively. A subclone of the variant REV-T provirus specific for the *rel* sequences of REV-T was used as a hybridization probe to demonstrate that the *rel* sequences are different from the putative transforming sequences of Schmidt-Ruppin Rous sarcoma virus strain A, avian myelocytomatosis virus, avian myeloblastosis virus, avian erythroblastosis virus, Abelson murine leukemia virus, and Friend erythroleukemia virus. In addition, the *rel*-specific hybridization probe was used to identify a specific set of sequences which are present in uninfected avian DNAs digested with several restriction enzymes. The corresponding cell sequences are not arranged like *rel* in REV-T.

Retroviruses can be grouped into three broad classes on the basis of their oncogenic properties. Viruses which are not oncogenic include most endogenous retroviruses. Most of the natural field isolates (nondefective leukemia viruses) are "weakly oncogenic" and cause leukemia after a latent period of months to years. Other retroviruses are "highly oncogenic" or "strongly transforming." In contrast to the weakly oncogenic retroviruses, these viruses cause neoplastic disease after a latent period of days to weeks.

The highly oncogenic avian retroviruses include the replication-competent avian sarcoma viruses (the avian sarcoma viruses and the Rous sarcoma viruses), the replication-defective avian sarcoma viruses (Fujinami virus and Y73 virus), and the replication-defective avian leukemia viruses (avian myeloblastosis viruses, avian myelocytomatosis viruses, avian erythroblastosis viruses, and avian reticuloendotheliosis virus). Isolates of these highly oncogenic avian retroviruses have been assigned to different subgroups based on the types of neoplasms which they induce and, in some cases, on the differences in the specific nucleotide sequences in their genomes which are associated with the different neoplastic diseases (2, 31; J. M. Coffin, *in* R. A. Weiss, N. M. Teich, H. Varmus, and J. M. Coffin, ed., *Molecular Biology of RNA Tumor Viruses*, part 3, in press).

Avian reticuloendotheliosis virus strain T (REV-T) is a highly oncogenic virus which causes a neoplastic disease of the lymphoreticular system (12, 20, 41). Cell lines established by transformation in vivo and in vitro with REV-T appear to be morphologically like lymphoblasts, and some cell lines display other properties that are characteristic of lymphoblasts (T. Shibuya, I. S. Y. Chen, A. Howatson, and T. W. Mak, manuscript in preparation). REV-T is defective in replication and requires a helper virus. The viral genome is approximately 5.5 kilobases long

(16, 21), and nucleotide sequences that may be associated with the oncogenic properties of this virus have been identified by oligonucleotide fingerprinting (4).

We characterized the genome structure of REV-T by constructing restriction enzyme maps of the linear unintegrated DNAs of REV-T and its associated helper virus, REV-A. In addition, we obtained molecular clones of integrated REV-A DNA and of integrated DNA of a variant of REV-T. These molecular clones enabled us to compare the putative transforming nucleotide sequences of REV-T with those of several other retroviruses. We also determined the possible origin of these REV-T-specific nucleotide sequences by examining the DNAs of several uninfected vertebrate species for the presence of homologous sequences.

We found that the REV-T-specific nucleotide sequences (*rel*) are different than the sequences in Rous sarcoma virus, other defective avian leukemia viruses, Abelson murine leukemia virus, and Friend erythroleukemia virus. These *rel* sequences are present in normal uninfected avian DNA, and sequences that are weakly homologous to these sequences are present in uninfected salmon, mouse, rat, and human DNAs.

MATERIALS AND METHODS

Cells and viruses. The general sources and procedures for obtaining and propagating avian fibroblast cells have been described previously (13).

The BMC cell line (12) was supplied by C. Y. Kang and produced REV-T(REV-A), which was used to generate spleen cell line 1-6 by infection of spleen cells from 3-week-old chickens in vitro (20). The REV-T produced by this spleen cell line was used to generate spleen cell lines 2-4, 2-10, 2-14, 2-16, and 2-20. The transformed cell lines were isolated as individual colonies growing in soft agar. A total of 20 to 50 transformed colonies were obtained from 10^7 chicken spleen cells infected with 1 ml of undiluted virus. Individual colonies were isolated and propagated in RPMI 1640 medium containing 10% fetal calf serum at a density of about 10^6 cells per ml. All cell lines produced REV-A, as determined by the appearance of cytopathic effects and DNA polymerase activity after infection of chicken embryo fibroblast cells (40). All cell lines also produced REV-T, as determined by the in vitro spleen cell colony assay (20) or by transformation of chick bone marrow cells in suspension culture or both.

The 249.521 hepatoma (MC-29) cell line produced MC-29 virus and was obtained from M. Wigler.

Sources of DNAs. The sources of the molecular clones of viral DNA were as follows.

pSRA-2 is a molecular clone of Schmidt-Ruppin Rous sarcoma virus subgroup A DNA in pBR322 (8) and was a generous gift from W. DeLorbe.

λ11A1-1 is a molecular clone of avian myeloblastosis virus strain B proviral DNA in Charon 4A (36) and was a generous gift from L. Souza.

pAEV-11 is a molecular clone of avian erythroblastosis virus strain ES4 DNA in pBR313 (42) and was a generous gift from B. Vennstrom.

pSA-17 is a molecular clone of a fragment of Abelson murine leukemia virus in pSA801 (7a) and was a generous gift from B. Dale and B. Ozanne.

λgtw_{SFFV_P} is a molecular clone of Friend erythroleukemia virus proviral DNA in λgtwes (Mak, unpublished data).

pBR60BS1 is a molecular clone of spleen necrosis virus (SNV) DNA in the *Sal*I site of pBR322 and was a generous gift from K. Shimotohno.

The *Bam*HI and *Bam*HI-*Sal*I DNA fragments of SNV DNA were derived from pBR60BS1 (27a).

p14-44 3′ is a molecular clone of 2.6 kilobase pairs (kbp) of proviral DNA from the 3′ end of the previously described SNV provirus 14-44 (34) and was a generous gift from K. Shimotohno.

70T is a clone that was derived from a molecular clone of a SNV provirus in Charon 4A (27). The only viral sequence which it contains is the long terminal repeat (LTR).

The sources of the cellular DNAs used were as follows. Chicken, turkey, pheasant, and quail DNAs were obtained from secondary cultures of embryo fibroblasts. NIH and AKR mouse DNAs were obtained from NIH3T3 cells and AKR mouse embryo fibroblast cells, respectively. The AKR DNA was a generous gift from J. McCubrey. Rat DNA was obtained from Rat-1 cells. Human DNA was obtained from the Epstein-Barr virus-transformed cell line 11/7-4 p159 and was a generous gift from B. Sugden. Salmon DNA was obtained from Calbiochem.

DNA extraction and purification. Unintegrated viral DNA was prepared by the Hirt fractionation procedure, as previously described (5, 19). Cellular DNA was prepared by lysing cells in 0.6% sodium dodecyl sulfate–10 mM EDTA and digesting the lysate with 100 μg of proteinase K per ml at 37°C for 24 h. The DNA was extracted with phenol and chloroform-isoamyl alcohol as previously described (1). The RNA was removed by RNase A digestion, as previously described (1).

Assay of infectious viral DNA. Infectious viral DNA assays were performed as previously described (7).

Gel electrophoresis and DNA transfer to nitrocellulose. Gel electrophoresis was performed as previously described (5). Molecular weights were determined by using restriction enzyme-digested lambda DNA and pBR322 DNA.

DNA was transferred from agarose gels onto nitrocellulose filter paper as described by Southern (35).

Molecular hybridization. The sources and preparation of hybridization probes were as follows.

^{32}P-labeled $cDNA_{REV\text{-}A}$ and ^{32}P-labeled $cDNA_{REV\text{-}T}$ were prepared by using purified virion RNA as a template, as previously described (5) (calculated specific activity, approximately 4×10^8 dpm/μg).

Other hybridization probes were prepared by nick-translation of DNAs (specific activity, approximately 10^8 cpm/μg) essentially as previously described (30), except that DNase I treatment and DNA polymerase I treatment were performed separately for 1 h each at 14°C.

Presoaking and hybridization of DNAs immobilized on nitrocellulose filters were performed as previously described (5). Filters were washed and exposed as previously described (5).

Preparation and growth of molecular clones. The preparation and screening of molecular clones containing viral sequences in the phage vector Charon 4A were performed essentially as previously described (27), with the following modifications. High-molecular-weight DNA from spleen cell line 2-20 was digested partially with restriction enzyme *Eco*RI so that the majority of the DNA was between 8 and 30 kbp long. The DNA between 10 and 30 kbp long was purified after the DNA was fractionated on a 5 to 20% (wt/vol) NaCl gradient (27). This DNA was used for subsequent ligation and packaging steps (27). Approximately 2×10^5 phage were screened by using a ^{32}P-labeled DNA_{SNV} hybridization probe.

Preparation and screening of subclones of cell line 2-20-4 DNA in pBR322 were performed as previously described (34).

Molecular clones of viral DNA in phage vector Charon 4A and plasmid vector pBR322 were grown as previously described (27, 34).

RESULTS

We characterized the genome of REV-T by studying the structure of unintegrated linear viral DNA. Using hybridization with ^{32}P-labeled $cDNA_{REV\text{-}T}$, we detected two species of unintegrated linear viral DNA (8.3 and 5.5 kbp long) in chicken embryo fibroblast cells infected with stocks of REV-T and its helper, REV-A (Fig. 1).

This virus stock was passed in chicken embryo fibroblasts at a low multiplicity of infection, and a cytopathic virus that was not able to transform spleen cells was isolated by endpoint dilution (data not shown). After infection by this virus, we detected only one species of unintegrated linear DNA, which was 8.3 kbp long (Fig. 1). The smaller species of viral DNA (5.5 kbp) was not present. This result confirms previous studies which showed that transforming stocks of REV-T consist of two components, namely, a replication-defective component (REV-T) about 5.5 kbp long, which is necessary for transformation, and a replication-competent, cytopathic, helper virus (REV-A) about 8.3 kbp long, which is nontransforming (20).

To compare the genome structures of REV-A and REV-T, we constructed restriction enzyme cleavage maps of unintegrated linear viral DNAs (Fig. 2). Since both REV-A DNA and REV-T DNA were present in these preparations, digestions performed with REV-A DNA alone enabled us to distinguish the fragments which were derived from REV-A DNA from the fragments which were unique to REV-T DNA. The orientations of the provirus sequences (5′ and 3′ relative to viral RNA) were determined by comparing the restriction enzyme cleavage map of REV-A with the restriction enzyme cleavage map of another reticuloendotheliosis virus, SNV (24, 27). (REV-A and SNV are almost 100% homologous [22].)

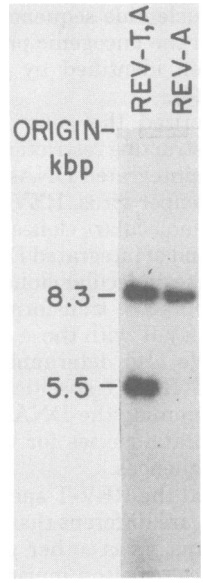

FIG. 1. *Analysis of unintegrated linear REV-T and REV-A DNAs. Chicken embryo fibroblasts grown on 100-mm culture dishes were infected with REV-T(REV-A) (REV-T,A) from spleen cell line 1-6 and REV-A at multiplicities of infection of approximately 1 and 0.1 PFU/cell, respectively. At 3 days after infection, the cells were lysed by the Hirt fractionation procedure (19). DNA was prepared from each Hirt supernatant fraction by phenol and chloroform-isoamyl alcohol extractions, followed by RNase A digestion. Samples of each DNA were subjected to electrophoresis in a 0.8% agarose gel. Virus-specific sequences were detected by hybridization with ^{32}P-labeled $cDNA_{REV\text{-}T}$, as described in the text.*

The restriction enzyme cleavage map of REV-A DNA is identical to the map reported previously for SNV (24), except for additional *Kpn*I and *Hin*dIII sites which are located 1.6 and 7.2 kbp, respectively, from the 5′ end. By analogy with SNV, the two *Sac*I sites at the ends of the DNA probably marked the long terminal repeats (LTRs).

We aligned the restriction enzyme cleavage map of REV-T DNA at the 5′ end with the map of REV-A DNA for comparison (Fig. 2). REV-A and REV-T DNAs are similar at the ends, as shown by the *Sac*I sites marking the LTRs at the 5′ and 3′ ends. In addition, the similar patterns of the *Kpn*I, *Sac*I, and *Sal*I restriction enzyme sites at the 5′ ends indicate that at least

1.6 kbp is the same at the 5′ ends. However, a *Hin*dIII site is located in REV-T DNA 3.0 kbp from the 5′ end, but REV-A DNA does not contain a *Hin*dIII site 3.0 kbp from its 5′ end.

We determined the origin of these REV-T sequences by hybridization to molecular clones of subgenomic fragments of SNV DNA. The internal *Hin*dIII fragment in REV-T DNA hybridized to a *Bam*HI SNV DNA fragment (27a) which was located 3.6 to 5.9 kbp from the 5′ end of SNV DNA and to a 3′ SNV DNA fragment (p1444 3′ [34]) which was located 5.7 to 8.3 kbp from the 5′ end (data not shown). It did not hybridize to SNV DNA fragments to the right of 6.7 kbp from the 5′ end (data not shown). Therefore, the regions that were homologous to SNV DNA in the internal *Hin*dIII fragment of REV-T DNA were derived from regions of REV-A that were between approximately 3.6 and 6.7 kbp from the 5′ end, and the *Hin*dIII site which was located 3.0 kbp from the 5′ end of REV-T DNA was probably derived from the *Hin*dIII site which was located 5.8 kbp from the 5′ end of REV-A DNA. Assuming the identity of these *Hin*dIII sites in REV-T DNA and REV-A DNA, these results indicate that with respect to REV-A there is a deletion about 2.8 kbp long at the 5′ end of REV-T DNA which has joined discontinuous regions of REV-A DNA.

Other internal regions of the two DNAs differed in their patterns of restriction enzyme cleavage sites; for example, one *Cla*I and two *Eco*RI sites are present in REV-T DNA, whereas REV-A DNA has no *Cla*I and *Eco*RI site. These restriction enzyme sites in REV-T DNA indicate the presence of DNA sequences in REV-T which are not present in REV-A. Furthermore, the region between the two *Eco*RI sites in REV-T DNA did not hybridize to a ^{32}P-labeled DNA_{SNV} hybridization probe (data not shown). Since SNV and REV-A are almost 100% homologous (22), this finding indicated that this

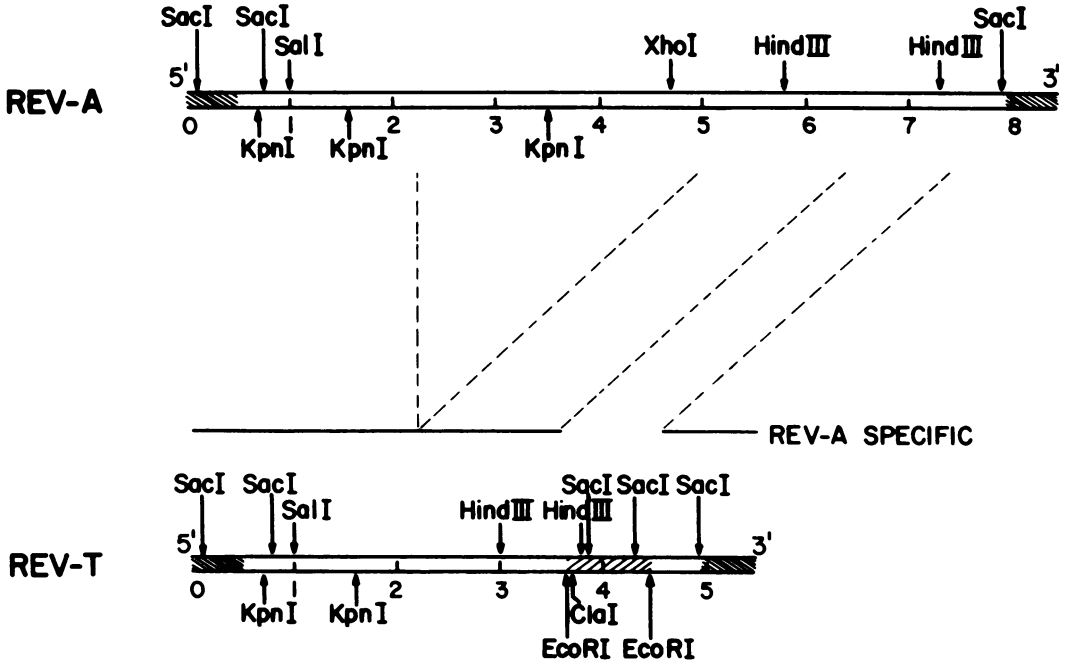

FIG. 2. *Maps of restriction enzyme cleavage sites of REV-A and REV-T unintegrated linear DNAs. REV-T and REV-A DNAs were prepared as described in the legend to Fig. 1, digested by various restriction enzymes, and subjected to gel electrophoresis. Virus-specific sequences were detected by hybridization with* ^{32}*P-labeled* DNA_{SNV} *and* ^{32}*P-labeled* $cDNA_{REV\text{-}T}$ *to identify the viral sequences homologous to REV-A DNA and specific to REV-T DNA, respectively. Maps of restriction enzyme cleavage sites were constructed based on the unique patterns of fragments derived from digestions with various restriction enzymes. 5′ and 3′ refer to the orientation of viral DNA with respect to viral RNA. The numbers indicate the distances (in kilobase pairs) from the 5′ end of viral DNA. The distance between adjacent short vertical hash marks is 1 kbp. The horizontal lines above the map of REV-T DNA indicate the approximate regions of REV-T DNA which are homologous to REV-A DNA. The dashed lines indicate the approximate boundaries of deletions in REV-T DNA compared with REV-A DNA. The hatched region indicates the minimum extent of rel-specific sequences. The shaded regions indicate LTR-specific sequences (see Fig. 5).*

region is not present in REV-A. Since the *Hin*dIII site that is located 7.3 kbp from the 5′ end of REV-A is not present in REV-T, the new sequences in REV-T probably substitute for sequences in REV-A which include this *Hin*dIII site.

This comparison of the restriction enzyme cleavage maps of the two DNAs indicate that the genome of REV-T probably resulted from two deletions of viral sequences from REV-A and the insertion of new DNA sequences. These new DNA sequences in REV-T are referred to as *rel* sequences (Coffin, in press).

Establishment and characterization of REV-T-infected spleen cell lines. We established several spleen cell lines by infecting spleen cells in vitro with REV-T. The virus produced from these cell lines was characterized by examining the intracellular species of viral DNA found after infection of chicken fibroblasts (Fig. 3). As expected, viral DNAs from REV-A (8.3 kbp) and REV-T (5.5 kbp) were detected after infection by virus from all of the cell lines studied. An additional species of viral DNA (3.3 kbp) was detected after infection by virus from cell line 2-20. This unusual variant was not found in the virus produced by any of the other four cell lines studied, including cell line 1-6, the virus of which was used to generate cell line 2-20 (see above). Thus, this variant probably arose during infection of the target cell which gave rise to cell line 2-20.

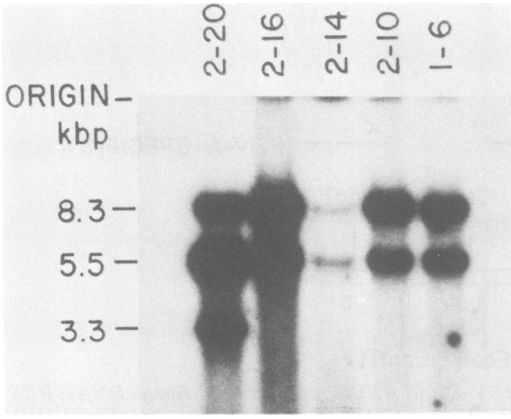

FIG. 3. *Analysis of virus produced by REV-T-infected spleen cell lines. Chicken embryo fibroblasts grown on 100-mm culture dishes were infected with 1-ml portions of virus harvested from different REV-T-infected spleen cell lines (see text). At 3 days after infection, unintegrated linear viral DNA was prepared from the cells in each dish by the Hirt extraction procedure. Samples of each DNA were subjected to electrophoresis in a 0.8% agarose gel. Virus-specific sequences were detected by hybridization with ^{32}P-labeled DNA$_{SNV}$, as described in the text.*

Using hybridization with ^{32}P-labeled DNA$_{SNV}$, we also examined the distributions of REV-A and REV-T proviruses in different spleen cell lines (Fig. 4). Multiple bands were present in the DNAs of all of the cell lines studied. In addition, no DNA fragment of the same size was present in all cell lines. Apparently, the transformed phenotype conferred upon the target cells by infection with REV-T is not dependent upon integration into a specific site, although integration into specific regions of DNA could not be excluded. This observation has been made for integration of highly oncogenic retroviruses in several other systems (7a, 36).

Molecular cloning of an REV-A provirus and a variant REV-T provirus. To facilitate further structural analyses of the genomes of REV-A and REV-T, we obtained molecular clones of integrated viral DNA from spleen cell line 2-20. This spleen cell line produced three species of virus (Fig. 3) and exhibited a relatively simple pattern of viral integration (Fig. 4). We constructed a recombinant DNA phage library from partial *Eco*RI digests of this DNA. By using hybridization with ^{32}P-labeled DNA$_{SNV}$, we isolated two molecular clones. Restriction enzyme cleavage maps were constructed by restriction enzyme digestions and hybridizations with ^{32}P-labeled cDNA$_{REV-A}$ and ^{32}P-labeled cDNA$_{REV-T}$ (Fig. 5).

The restriction enzyme cleavage sites in the proviral sequences from clone 2-20-6 are identical to the cleavage sites of the corresponding enzymes in unintegrated linear REV-A DNA (Fig. 2 and 5). In addition, DNA transfection with clone 2-20-6 DNA gave rise to cytopathic virus at an efficiency of about 5×10^4 PFU/μg of DNA. These results indicate that clone 2-20-6 contained a provirus of REV-A.

The proviral DNA in clone 2-20-4 is 3.3 kbp long and consists of regions that are homologous to RNA from the helper virus (REV-A) and other regions that are present only in the RNA from transforming stocks of virus (REV-T). Figure 5 shows the regions of homology.

Sequences at the ends of clone 2-20-4 proviral DNA are the same as sequences at the ends of REV-T DNA and proviral sequences of clone 2-20-6 DNA. In particular, the ends of clone 2-20-4 proviral DNA contain LTR-specific sequences, as shown by hybridization with an LTR-specific probe (data not shown). Regions in the middle of clone 2-20-4 DNA are specific to REV-T; they contain the two *Eco*RI sites and the one *Cla*I site which are present in REV-T DNA but not in REV-A DNA. These results indicate that the molecular clone 2-20-4 contains a provirus with sequences homologous to the *rel* sequences in REV-T DNA.

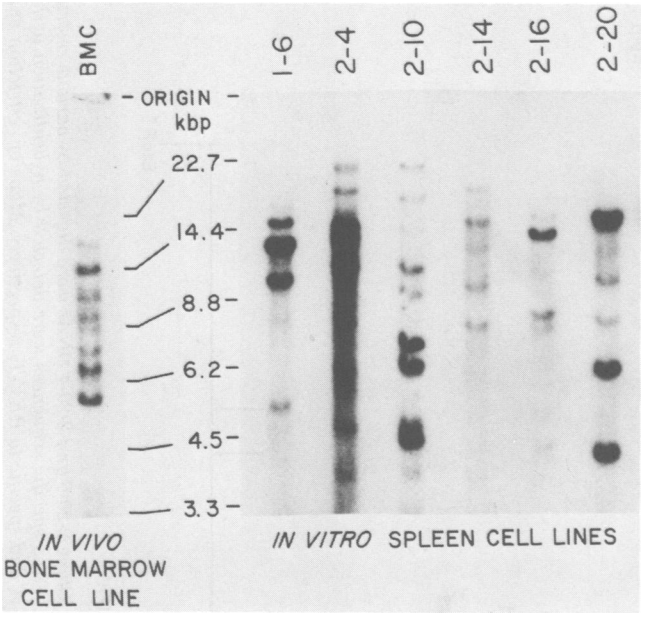

FIG. 4. *Analysis of integrated viral sequences in DNAs from REV-T-infected spleen cell lines. Cellular DNAs were prepared from REV-T-infected cell lines as described in the text. A 10-μg sample of each DNA was digested with restriction enzyme EcoRI and subjected to electrophoresis in a 0.5% agarose gel. Virus-specific sequences were detected by hybridization with ^{32}P-labeled DNA$_{SNV}$, as described in the text. BMC is the cell line established from the bone marrow of a chicken infected with REV-T (12). Cell lines 1-6, 2-4, 2-10, 2-14, 2-16, and 2-20 were derived from in vitro infections of chicken spleen cells (see text).*

To confirm that the region between the two *Eco*RI sites in clone 2-20-4 was *rel* specific, we prepared a subclone of the *Eco*RI fragment (designated *Eco*RI-*rel*) (Fig. 6). When this DNA was used as a hybridization probe to detect unintegrated linear viral DNA, it detected only those species of viral DNA that contained *rel* sequences (data not shown). REV-A DNA was not detected with this probe (data not shown), although REV-A DNA was detected by hybridization with ^{32}P-labeled DNA$_{SNV}$ (Fig. 4). In addition, the small 3.3-kbp viral species derived from virus from cell line 2-20 was detected and, therefore, contains *rel*-specific sequences (data not shown).

Despite the similarities between REV-T DNA and clone 2-20-4 proviral DNA, it is apparent from the restriction enzyme maps that these two DNAs were different. The ends were the same, but about 2.2 kbp of the internal proviral sequences of clone 2-20-4 DNA are deleted compared with REV-T DNA. Most of the region between the *Kpn*I site and the *Eco*RI site at 1.6 and 3.7 kbp, respectively, from the 5′ end of REV-T is deleted (Fig. 5). Therefore, this deletion is about 2.1 kbp long. Another, much smaller deletion is located between the two *Sac*I sites that were within the region subcloned into *Eco*RI-*rel*. This deletion is about 50 base pairs long (data not shown).

The proviral sequences of clone 2-20-4 represented a variant of REV-T DNA that had at least two deletions compared with normal REV-T DNA. The size of the proviral sequences of clone 2-20-4 (3.3 kbp) was the same as the size of the small species of unintegrated linear viral DNA in the virus produced by cell line 2-20. Both clone 2-20-4 DNA and this small DNA contained *rel*-specific sequences (Fig. 5; data not shown), and fragments of a size equivalent to clone 2-20-4 DNA digested with *Eco*RI were detected in the total cell DNA of spleen cell line 2-20 digested with *Eco*RI (data not shown). We conclude that the provirus of clone 2-20-4 gave rise to the smaller viral genome produced by spleen cell line 2-20.

Expression of clone 2-20-4 DNA in DNA transfection assays. Since the variant provirus was expressed in spleen cell line 2-20, we tried to determine whether the DNA of clone 2-20-4 could be expressed in DNA transfection assays and give rise to virus carrying the genome of the variant. Since the provirus of clone 2-20-4 was defective for viral replication functions (data not shown), we performed our DNA transfection assays with clone 2-20-4 DNA in the

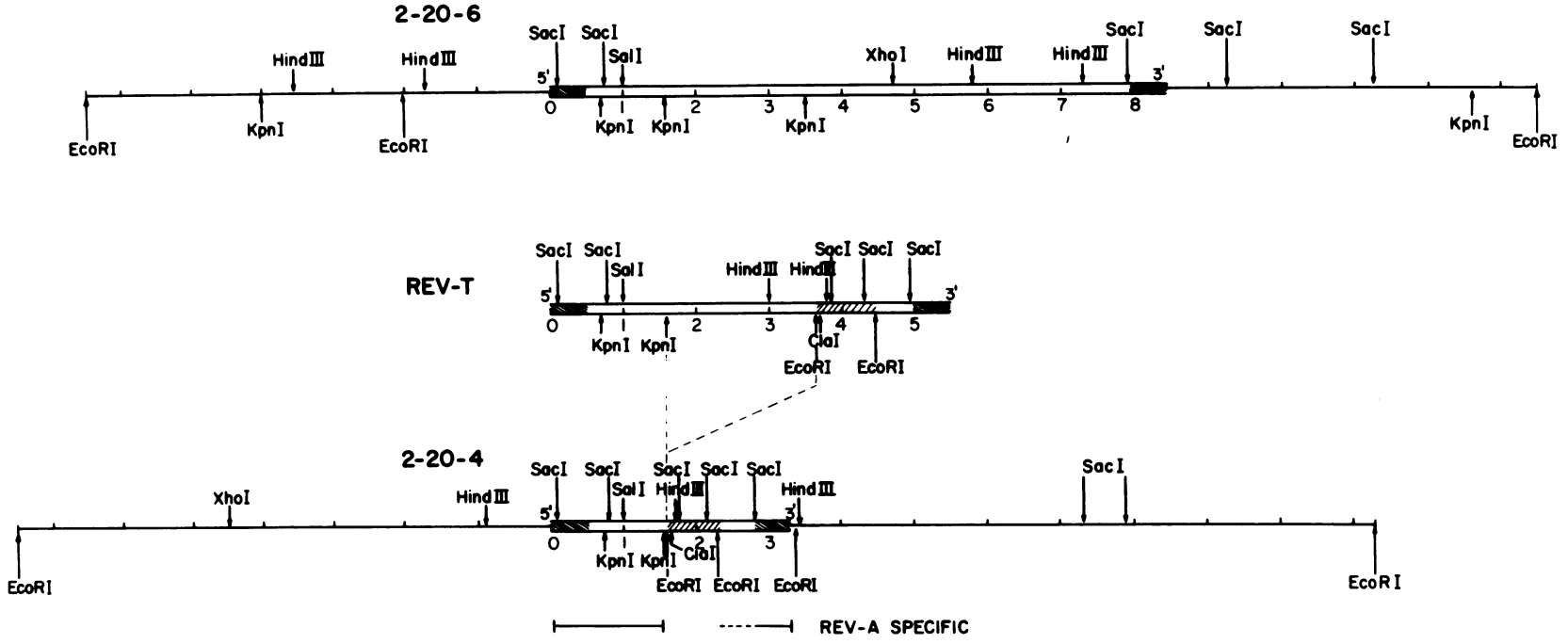

FIG. 5. *Maps of restriction enzyme cleavage sites of insertions in molecular clones 2-20-6 and 2-20-4. Clone 2-20-6 and 2-20-4 DNAs were digested by various restriction enzymes and then subjected to gel electrophoresis. The DNA was visualized as described in the text. Virus-specific sequences were detected by hybridization with* ^{32}*P-labeled* $cDNA_{REV\text{-}A}$ *and* ^{32}*P-labeled* $cDNA_{REV\text{-}T}$ *to identify the proviral sequences homologous to REV-A and specific to REV-T, respectively. Maps of restriction enzyme cleavage sites were constructed based on the unique patterns of fragments derived from digestions with various restriction enzymes. The restriction enzyme cleavage map of REV-T DNA is the same map shown in Fig. 2. Viral sequences are indicated by double horizontal lines. Cellular sequences are indicated by single horizontal lines. 5' and 3' refer to the orientation of viral DNA with respect to viral RNA. The numbers indicate the distances (in kilobase pairs) from the 5' end of viral DNA. The distance between adjacent short vertical hash marks is 1 kbp. The vertical and oblique dashed lines indicate the approximate extent of the deletion in clone 2-20-4 proviral DNA compared with REV-T DNA. The hatched regions indicate rel-specific sequences. The shaded regions indicate LTR-specific sequences, as determined by hybridization with* ^{32}*P-labeled* DNA_{70T} *and by analogy with SNV (27, 27a). The horizontal lines below the clone 2-20-4 map indicate regions in clone 2-20-4 that are homologous to REV-A; the dashed part of these lines indicates that the exact extent of the REV-A-specific sequences is unknown.*

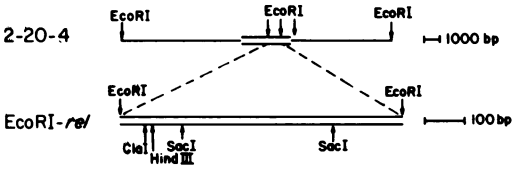

FIG. 6. *Map of the restriction enzyme cleavage sites of a rel-specific DNA fragment. Clone 2-20-4 DNA was digested with EcoRI, and subclones were constructed by using pBR322, as described in the text. The subclone containing rel-specific sequences was designated EcoRI-rel. Maps of restriction enzyme cleavage sites were constructed based on the unique patterns of fragments derived from digestions with various restriction enzymes. The rel fragment contains a deletion of about 50 base pairs (bp) compared with REV-T, which is located between the two SacI sites. The viral sequences are indicated by double horizontal lines. The cellular sequences flanking the provirus in clone 2-20-4 are indicated by single horizontal lines.*

presence of DNA from the REV-A proviral clone 2-20-6. The virus recovered from this cotransfection gave rise to several species of viral DNA that were homologous to SNV DNA (Fig. 7). The major species of viral DNA detected was REV-A DNA, which resulted from expression of clone 2-20-6 DNA. Several smaller species of viral DNA were detected. However, only one DNA species, which was the same size as the viral DNA of clone 2-20-4 (3.3 kbp), hybridized with a *rel*-specific probe (Fig. 7). The nature of the other DNA species detected is not known.

This result shows that clone 2-20-4 DNA was expressed in DNA transfection assays and could be recovered as virus in the presence of *trans*-acting viral replication functions. Preliminary experiments indicate that this variant of REV-T was not capable of transforming chicken spleen and bone marrow cells in vitro (data not shown).

Lack of homology between the transforming sequences of REV-T and the transforming sequences of other strongly transforming retroviruses. Each of the strongly transforming retroviruses which have been studied contains nucleic acid sequences which presumably are responsible for the distinct neoplastic diseases that are specific for each type of virus (Coffin, in press). Using the nucleic acid hybridization technique, we compared the putative transforming sequences of REV-T with the unique sequences of an avian sarcoma virus, three defective avian leukemia viruses, and two defective murine retroviruses. Either clone 2-20-4 DNA or *Eco*RI-*rel* DNA was used as a hybridization probe. Hybridization was not observed with any of the DNAs tested (Table 1). Parallel control hybridizations to clone 2-20-4 DNA, *Eco*RI-*rel* DNA, and REV-T DNA were consistently positive. These results indicate that the *rel* sequences in REV-T are different from the putative transforming sequences in other strongly transforming retroviruses.

DNA sequences in uninfected vertebrate cells are related to the putative transforming sequences of REV-T. Sequences homologous to the putative transforming sequences of several highly oncogenic retroviruses are present in uninfected vertebrate DNAs and are conserved as a function of the phylogenetic distance from the species in which the virus was isolated (7a, 11, 14, 28, 31, 32, 37, 38, 46; Coffin, in press). Using the nucleic acid hybridization technique, we determined whether sequences related to the putative transforming sequences of REV-T were present in the genomes of several vertebrate species.

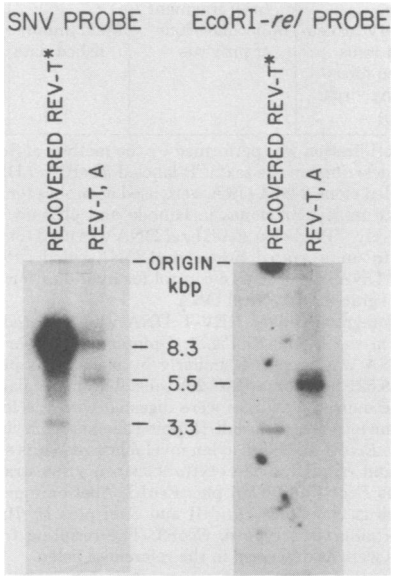

FIG. 7. *Analysis of viruses recovered from cotransfection of clone 2-20-6 and 2-20-4 DNAs. Samples (0.1 μg) of clone 2-20-6 and 2-20-4 DNAs were used for cotransfection of chicken embryo fibroblasts in 60-mm culture dishes as described in the text. Virus was harvested from the cells 8 days after the application of DNA. The recovered virus (RECOVERED REV-T*) was then used undiluted to infect chicken embryo fibroblasts. At 3 days after infection, unintegrated linear viral DNA was prepared by the Hirt extraction procedure. Duplicate samples of the DNA were subjected to electrophoresis in a 0.8% agarose gel. REV-T(REV-A) (REV-T,A) DNA prepared as described in the legend to Fig. 1 was subjected to electrophoresis in parallel. Virus-specific sequences were detected by hybridization with ^{32}P-labeled DNA$_{SNV}$ and ^{32}P-labeled DNA$_{EcoRI\text{-}rel}$, as described in the text.*

TABLE 1. *Hybridization of rel-specific sequences to DNAs of strongly transforming retroviruses*[a]

Virus	Source of DNA[b]	Reference	Hybridization
Reticuloendotheliosis virus strain T	Unintegrated linear viral DNA		+
Schmidt-Ruppin Rous sarcoma virus subgroup A	Molecular clone of circular DNA	8	–
Avian myeloblastosis virus strain B	Molecular clone of provirus	36	–
Avian myelocytomatosis virus strain MC-29	Unintegrated linear viral DNA		–
Avian erythroblastosis virus strain ES4	Molecular clone of circular DNA	42	–
Abelson murine leukemia virus	Molecular clone of Abelson murine leukemia virus-specific (*abl*) fragment	7a	–
Friend erythroleukemia virus (spleen focus-forming virus type p)	Molecular clone of provirus	Mak, unpublished data	–

[a] Hybridization was performed by the method of Southern (35), as described in the text. ^{32}P-labeled *Eco*RI-*rel* DNA and ^{32}P-labeled clone 2-20-4 DNA were used as probes for hybridization to molecular clones in lambda and plasmid vectors, respectively. ^{32}P-labeled *Eco*RI-*rel* DNA was used for hybridization to unintegrated linear viral DNAs, and ^{32}P-labeled pSRA-2 DNA was used as a control for hybridization to MC-29 unintegrated linear viral DNA.

[b] Unintegrated linear REV-T DNA was prepared as described in the legend to Fig. 1. Unintegrated linear MC-29 viral DNA was prepared similarly by using virus produced from 249.521 hepatoma (MC-29) cells. The molecular clones from the indicated sources were digested with the following restriction enzymes: Schmidt-Ruppin Rous sarcoma virus subgroup A, *Eco*RI and *Sal*I; avian myeloblastosis virus strain B, *Eco*RI and *Hin*dIII; avian erythroblastosis virus strain ES4, *Sal*I plus *Eco*RI and *Xho*I plus *Eco*RI; Abelson murine leukemia virus, *Sac*I plus *Hin*dIII and *Sac*I plus *Bgl*II; spleen focus-forming virus type p, *Eco*RI. The resulting fragment patterns were as described in the references listed.

Cellular DNAs from several different species were digested with restriction endonuclease *Hin*dIII and hybridized with an *rel*-specific hybridization probe. All of the avian species tested showed hybridization in several specific bands (Fig. 8). Under these standard hybridization conditions, we also detected much weaker hybridization to the DNAs of all of the other vertebrate species tested, including fish, rodents, and humans (Fig. 8). In contrast, we detected no homology between helper virus sequences (^{32}P-labeled DNA_{SNV}) and normal cell DNA (24, 25; data not shown). These results indicate that sequences related to the putative transforming sequences of REV-T are present in normal avian cells. This result is consistent with the observation that REV-T originated from a tumor in an adult turkey (41).

Since there was one *Hin*dIII site in *rel*, we expected at most two bands if the cellular sequences were unique and arranged like *rel*. However, four or more bands were observed in each avian DNA. This discrepancy was analyzed by digesting avian cell DNAs with *Eco*RI. If the cell sequences were the direct progenitors of the sequences in REV-T, we should have found a fragment in *Eco*RI-digested cell DNA which corresponded in size to the *rel*-specific *Eco*RI fragment in REV-T. Of the four avian species studied, turkey and pheasant DNAs contained sequences which were the same size (0.8 kbp) as *Eco*RI-digested REV-T DNA (Fig. 9). Since

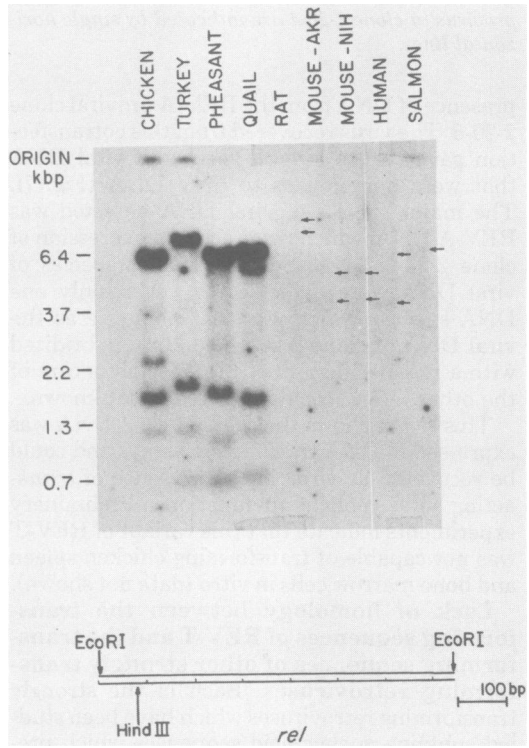

FIG. 8. *Analysis of rel-specific sequences present in the DNAs of different vertebrate species. The preparation and sources of the cellular DNAs are described in the text. A 10-μg sample of each type of avian DNA and 20-μg samples of all other DNAs were digested with HindIII and subjected to electrophoresis in a 1.0% agarose gel. Virus-specific sequences were detected by hybridization with ^{32}P-labeled $DNA_{EcoRI\text{-}rel}$, as described in the text. The arrows indicate faint bands of hybridization. The rel-specific region between the two EcoRI sites in REV-T is shown below the autoradiograph. The single HindIII site in the rel region is indicated. bp, Base pairs.*

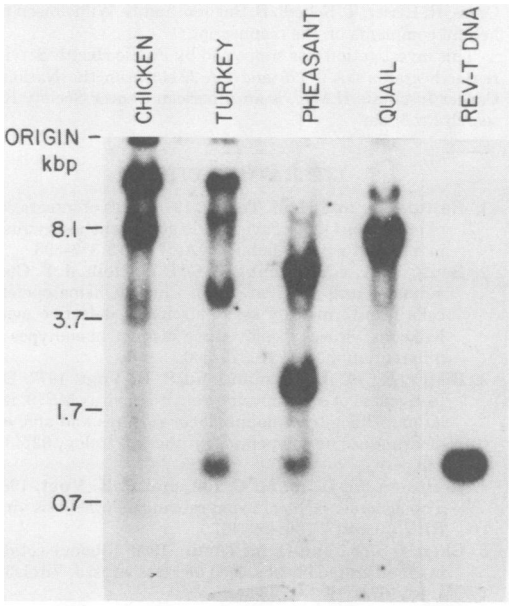

FIG. 9. *Analysis of rel-specific sequences present in the DNAs of different avian species. The preparation and sources of the cellular DNAs are described in the text. Samples (10 μg) of avian DNAs and a sample of REV-T DNA prepared as described in the legend to Fig. 1 were digested with EcoRI and subjected to electrophoresis in a 1.0% agarose gel. Virus-specific sequences were detected by hybridization with ^{32}P-labeled DNA$_{EcoRI\text{-}rel}$, as described in the text.*

REV-T was first reported to have been isolated from a turkey, this fragment in turkey DNA may have been part of the progenitor sequence which gave rise to the *rel* sequences in REV-T.

In addition, other much larger species of DNA were detected in turkey DNA digested with *Eco*RI (Fig. 9). The presence of these multiple bands in turkey and pheasant DNAs probably indicates that multiple sequences homologous to REV-T are present. Alternatively, the presence of a 0.8-kbp fragment in both turkey and REV-T DNAs may be a coincidence. In such a case, the multiple bands may reflect a discontinuous arrangement of cell sequences relative to *rel*.

DISCUSSION

Including REV-T, seven different strongly transforming avian retroviruses have been described (Coffin, in press). These viruses can be distinguished on the basis of the distinct neoplastic diseases which they cause (17), the phenotypes of transformed cells in vitro (2), and, most directly, the differences in the nucleic acid sequences of their genomes (31; Coffin, in press). We have shown that the putative transforming sequences of REV-T (*rel*) are different from those of avian sarcoma viruses and defective avian leukemia viruses. Although we have not shown that the *rel* sequences are different from the transforming sequences of Fujinami and Y73 viruses, it is likely that REV-T is distinct from these viruses since Fujinami virus and Y73 virus cause predominantly sarcomas (10, 46; Coffin, in press), whereas REV-T causes reticuloendotheliosis (41). We have also shown that the *rel* sequences are distinct from the putative transforming sequences of Abelson murine leukemia virus (*abl*) and Friend erythroleukemia virus. The comparison of REV-T with Abelson murine leukemia virus was particularly interesting since some cell lines established by infection with both viruses appear to be lymphoid in character (Shibuya et al., manuscript in preparation). It will be of interest to determine whether *rel* and *abl* are distantly related by using less stringent conditions of hybridization.

Protein products which are encoded by some defective, strongly transforming viruses have been identified (3, 10, 18, 29, 39; Coffin, in press). In most cases, these proteins are fusion products between products of the *gag* gene and the products of the putative transforming sequences and are encoded by genomic size mRNA's. Protein products have not been identified in vivo for avian myeloblastosis virus and Moloney murine sarcoma virus, probably because the protein products are not fusion products and consequently are not antigenically related to viral structural proteins. This hypothesis is consistent with the finding of subgenomic size mRNA's for avian myeloblastosis virus and Moloney murine sarcoma virus which contain the putative transforming sequences of these viruses (9, 16; Coffin, in press).

No gene product specific for REV-T has been identified. We found that the 5′ end of REV-T to the left of the *rel* sequences is derived from discontinuous regions of REV-A. The REV-A-specific sequence directly adjoining the *rel* sequences appears to be derived from the 3′ end of REV-A between 5 and 7 kbp from the 5′ end (Fig. 2) (21). Therefore, REV-T resembles avian myeloblastosis virus and Moloney murine sarcoma virus (Coffin, in press) more closely than it resembles the other strongly transforming retroviruses. In that case, REV-T might not encode a *gag*-related fusion protein from genomic size RNA, but probably encodes a protein product from a subgenomic species of mRNA.

A common feature of the strongly transforming viruses appears to be that the putative transforming sequences have homologous counterparts in normal cellular DNAs. In the case of Moloney murine sarcoma virus, these cellular sequences appear to be arranged in the same

way as the sequences in the virus (28). In fact, if joined to LTR sequences, these cellular sequences appear to have transforming properties similar to those of the homologous viral counterpart (28). In the case of most other strongly transforming retroviruses, the cellular sequences appear to be arranged discontinuously, with intervening sequences between virus-specific sequences (11). Our results with REV-T show that the cellular sequences homologous to *rel* are different than the *rel* sequences. A *rel*-specific sequence in the DNA of turkeys, from which REV-T originated, appears to be the same as part of *rel* in REV-T. In addition, several other *rel*-specific regions were found in turkey DNA. These results may indicate that a small family of three or four *rel*-specific sequences exists in turkey DNA, one member of which is part of the genome of REV-T. Alternatively, the multiple *rel*-specific sequences may reflect a discontinuous arrangement of small regions of *rel* sequences separated by large intervening sequences. If this hypothesis were true, the coding sequences of the cellular counterpart of *rel* would span about 20 to 30 kbp in chicken and turkey DNAs (Fig. 9). Such a structural arrangement of coding sequences is unusual, but genes spanning such large DNA sequences have been reported (26, 43, 44). Further experiments with different restriction enzymes to digest cellular DNA and molecular cloning of cellular sequences will distinguish between these two possibilities. (A preliminary characterization of molecular clones of cellular *rel*-specific sequences indicates the presence of at least one large [>10-kbp] intervening sequence.)

Variant of REV-T. The spleen cell line 2-20 produces three species of virus. In addition to REV-A and REV-T, another virus that has a genome about 3 kbp long is produced. Other variants of REV-T have been reported (15, 21). However, the sizes of these other variants and the nature of the alterations are different from those in the variant which we isolated.

It is interesting that such a small molecule (less than one-half the size of the genome of REV-A) can be propagated as a virus and transcribed into viral DNA. Apparently, the variant genome suffers no disadvantage because of its small size. The LTRs and about 1.0 kbp of additional sequences at the 5′ end are conserved in the variant provirus compared with REV-A. Further studies on the nature of the deletions and the conserved sequences of the REV-T variant may provide further insight into what structural features are required to be a retrovirus.

ACKNOWLEDGMENTS

We thank Francis Bandettini, Virginia Goiffon, and Susan Hellenbrand for excellent technical assistance and R. Fitts, C. Gross, R. Risser, T. Schedl, B. Sugden, and K. Wilhelmson for helpful comments on the manuscript.

This investigation was supported by Public Health Service research grants CA-07175 and CA-22443 from the National Cancer Institute. H.M.T. is an American Cancer Society Research Professor.

LITERATURE CITED

1. **Battula, N., and H. M. Temin.** 1978. Sites of integration of infectious DNA of avian reticuloendotheliosis viruses in different avian cellular DNAs. Cell **13**:387–398.
2. **Beug, H., A. von Kirchbach, G. Döderlein, J. F. Conscience, and T. Graf.** 1979. Chicken hematopoietic cells transformed by seven strains of defective avian leukemia viruses display three distinct phenotypes of differentiation. Cell **18**:375–390.
3. **Bister, K., M. J. Hayman, and P. K. Vogt.** 1977. Defectiveness of avian myelocytomatosis virus MC29: isolation of long-term nonproducer cultures and analysis of virus-specific polypeptide synthesis. Virology **82**:431–448.
4. **Breitman, M. L., M. M. C. Lai, and P. K. Vogt.** 1980. The genomic RNA of avian reticuloendotheliosis virus REV. Virology **100**:450–461.
5. **Chen, I. S. Y., and H. M. Temin.** 1980. Ribonucleotides in unintegrated linear spleen necrosis virus. J. Virol. **33**: 1058–1073.
6. **Chen, J. H.** 1980. Expression of endogenous avian myeloblastosis virus information in different chicken cells. J. Virol. **36**:162–170.
7. **Cooper, G. M., and H. M. Temin.** 1974. Infectious Rous sarcoma virus and reticuloendotheliosis virus DNAs. J. Virol. **14**:1132–1141.

7a. **Dale, B., and B. Ozanne.** 1981. Characterization of mouse cellular deoxyribonucleic acid homologous to Abelson murine leukemia virus-specific sequences. Mol. Cell. Biol. **1**:731–742.

8. **DeLorbe, W. J., P. A. Luciw, H. M. Goodman, H. E. Varmus, and J. M. Bishop.** 1980. Molecular cloning and characterization of avian sarcoma virus circular DNA molecules. J. Virol. **36**:50–61.
9. **Donoghue, D. J., P. A. Sharp, and R. A. Weinberg.** 1979. An MSV-specific subgenomic mRNA in MSV-transformed GI-124 cells. Cell **17**:53–63.
10. **Feldman, R. A., T. Hanafusa, and H. Hanafusa.** 1980. Characterization of protein kinase activity associated with the transforming gene product of Fujinami sarcoma virus. Cell **22**:757–765.
11. **Franchini, G., J. Even, C. J. Sherr, and F. Wong-Staal.** 1981. *onc* sequences (v-*fes*) of Synder-Theilen feline sarcoma virus are derived from noncontiguous regions of a cat cellular gene (c-*fes*). Nature (London) **290**:154–157.
12. **Franklin, R. B., R. L. Maldonado, and H. R. Bose.** 1974. Isolation and characterization of reticuloendotheliosis virus transformed bone marrow cells. Intervirology **3**:342–352.
13. **Fritsch, E., and H. M. Temin.** 1977. Formation and structure of infectious DNA of spleen necrosis virus. J. Virol. **21**:119–130.
14. **Goff, S. P., E. Gilboa, O. N. Witte, and D. Baltimore.** 1980. Structure of the Abelson murine leukemia virus genome and homologous cellular gene: studies with cloned viral DNA. Cell **22**:777–785.
15. **Gonda, M. A., N. R. Rice, and R. V. Gilden.** 1980. Avian reticuloendotheliosis virus: characterization of the high-molecular-weight viral RNA in transforming and helper virus populations. J. Virol. **34**:743–751.
16. **Gonda, T. J., D. K. Sheiness, L. Fanshier, J. M. Bishop, C. Moscovici, and M. G. Moscovici.** 1981. The genome and the intracellular RNAs of avian myeloblastosis virus. Cell **23**:279–290.
17. **Graf, T., and H. Beug.** 1978. Avian leukemia viruses:

interaction with their target cells *in vivo* and *in vitro*. Biochim. Biophys. Acta **516**:269-299.
18. **Hayman, M. J., B. Royer-Pokora, and T. Graf.** 1979. Defectiveness of avian erythroblastosis virus: synthesis of a 75K gag-related protein. Virology **92**:31-45.
19. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365-369.
20. **Hoelzer, J. D., R. B. Lewis, C. R. Wasmuth, and H. R. Bose.** 1980. Hematopoietic cell transformation by reticuloendothelosis virus: characterization of the genetic defect. Virology **100**:462-474.
21. **Hu, S. S. F., M. M. C. Lai, T. C. Wong, R. S. Cohen, and M. Sevoian.** 1981. Avian reticuloendotheliosis virus: characterization of genome structure by heteroduplex mapping. J. Virol. **37**:899-907.
22. **Kang, C.-Y., and H. M. Temin.** 1973. Lack of sequence homology among RNAs of avian leukosis-sarcoma viruses, reticuloendotheliosis viruses, and chicken endogenous RNA-directed DNA polymerase activity. J. Virol. **12**:1314-1324.
23. **Kang, C.-Y., and H. M. Temin.** 1974. Reticuloendotheliosis virus nucleic acid sequences in cellular DNA. J. Virol. **14**:1179-1181.
24. **Keshet, E., J. J. O'Rear, and H. M. Temin.** 1979. DNA of noninfectious and infectious integrated spleen necrosis virus (SNV) is colinear with unintegrated SNV DNA and not grossly abnormal. Cell **16**:51-61.
25. **Keshet, E., and H. M. Temin.** 1978. Sites of integration of reticuloendotheliosis virus DNA in chicken DNA. Proc. Natl. Acad. Sci. U.S.A. **75**:3372-3376.
26. **Nunberg, J. H., R. J. Kaufman, A. C. Y. Chang, S. N. Cohen, and R. T. Schimke.** 1980. Structure and genomic organization of the mouse dihydrofolate reductase gene. Cell **19**:355-364.
27. **O'Rear, J. J., S. Mizutani, G. Hoffman, M. Fiandt, and H. M. Temin.** 1980. Infectious and non-infectious recombinant clones of the provirus of SNV differ in cellular DNA and are apparently the same in viral DNA. Cell **20**:423-430.
27a. **O'Rear, J. J., and H. M. Temin.** 1981. Mapping of alterations in noninfectious proviruses of spleen necrosis virus. J. Virol. **39**:138-149.
28. **Oskarsson, M., W. L. McClements, D. G. Blair, J. V. Maizel, and G. F. vande Woude.** 1980. Properties of a normal mouse cell DNA sequence (*sarc*) homologous to the *src* sequence of Moloney sarcoma virus. Science **207**:1222-1224.
29. **Reynolds, F. H., T. L. Sacks, D. N. Deobagkar, and J. R. Stephenson.** 1978. Cells nonproductively transformed by Abelson murine leukemia virus express a high molecular weight polyprotein containing structural and nonstructural components. Proc. Natl. Acad. Sci. U.S.A. **75**:3974-3978.
30. **Rigby, D. W. J., M. Dieckman, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237-252.
31. **Roussel, M., S. Saule, C. Lagrou, C. Rommens, H. Beug, T. Graf, and D. Stehelin.** 1979. Three new types of viral oncogene of cellular origin specific for haematopoietic cell transformation. Nature (London) **281**:451-455.
32. **Sheiness, D., and J. M. Bishop.** 1979. DNA and RNA from uninfected vertebrate cells contain nucleotide sequences related to the putative transforming gene of avian myelocytomatosis virus. J. Virol. **31**:514-521.
33. **Sheiness, D., B. Vennstrom, and J. M. Bishop.** 1981. Virus-specific RNAs in cells infected by avian myelocytomatosis virus and avian erythroblastosis virus: modes of oncogene expression. Cell **23**:291-300.
34. **Shimotohno, K., S. Mizutani, and H. M. Temin.** 1980. Sequence of retrovirus provirus resembles that of bacterial transposable elements. Nature (London) **285**: 550-554.
35. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503-517.
36. **Souza, L. M., M. C. Komaromy, and M. A. Baluda.** 1980. Identification of a proviral genome associated with avian myeloblastic leukemia. Proc. Natl. Acad. Sci. U.S.A. **77**:3004-3008.
37. **Spector, D. H., H. E. Varmus, and J. M. Bishop.** 1978. Nucleotide sequences related to the transforming gene of avian sarcoma virus are present in DNA of uninfected vertebrates. Proc. Natl. Acad. Sci. U.S.A. **75**:4102-4106.
38. **Stehelin, D., H. E. Varmus, and J. M. Bishop.** 1976. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. Nature (London) **260**:170-173.
39. **Stephenson, J. R., A. S. Khan, A. H. Sliski, and M. Essex.** 1977. Feline oncornavirus-associated cell membrane antigen: evidence for an immunologically cross-reactive feline sarcoma virus-coded protein. Proc. Natl. Acad. Sci. U.S.A. **74**:5608-5612.
40. **Temin, H. M., and V. K. Kassner.** 1974. Replication of reticuloendotheliosis viruses in cell culture: acute infection. J. Virol. **13**:291-297.
41. **Theilen, G. H., R. F. Zeigel, and M. J. Twiehaus.** 1966. Biological studies with RE virus (strain T) that induces reticuloendotheliosis in turkeys, chickens, and Japanese quail. J. Natl. Cancer Inst. **37**:731-743.
42. **Vennström, B., L. Fanshier, C. Moscovici, and J. M. Bishop.** 1980. Molecular cloning of the avian erythroblastosis virus genome and recovery of oncogenic virus by transfection of chicken cells. J. Virol. **36**:575-585.
43. **Wahli, W., I. B. David, T. Wylen, R. Weber, and G. U. Ryffel.** 1980. Comparative analysis of the structural organization of two closely related vitellogenin genes in *X. laevis*. Cell **20**:107-117.
44. **Wozney, J., O. Hanahan, R. Morimoto, H. Boedtker, and P. Doty.** 1981. Fine structural analysis of the chicken pro α 2 collagen gene. Proc. Natl. Acad. Sci. U.S.A. **78**:712-716.
45. **Witte, O. N., N. Rosenberg, M. Paskind, A. Shields, and D. Baltimore.** 1978. Identification of an Abelson murine leukemia virus-encoded protein present in transformed fibroblast and lymphoid cells. Proc. Natl. Acad. Sci. U.S.A. **75**:2488-2492.
46. **Yoshida, M., S. Kawai, and K. Toyoshima.** 1980. Uninfected avian cells contain structurally unrelated progenitors of viral sarcoma genes. Nature (London) **287**: 653-655.