JOURNAL OF VIROLOGY, Feb. 1984, p. 521–529
0022-538X/84/020521-09$02.00/0

Vol. 49, No. 2

Structure and Dimorphism of c-*rel* (Turkey), the Cellular Homolog to the Oncogene of Reticuloendotheliosis Virus Strain T

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Received 27 July 1983/Accepted 12 October 1983

A locus has been identified in turkey DNA that contains nucleotide sequences homologous to the oncogene (v-*rel*) in the avian retrovirus, reticuloendotheliosis virus strain T. This locus, c-*rel*, has been molecularly cloned from an apparently heterozygous turkey. c-*rel* is approximately 23 kilobase pairs in length, with at least seven apparent introns, and contains sequences sufficient to account for all of v-*rel*. Nucleic acid sequence differences exist between v-*rel* and homologous regions of c-*rel*. We examined a population of turkeys to determine whether these sequence differences are the result of polymorphism in the population. Within the turkey population, c-*rel* is dimorphic in apparent introns and 3′ flanking sequences, but polymorphism has not been detected within the regions of the c-*rel* locus that are homologous to v-*rel*. Additionally, no nucleic acid sequence differences have been detected between the regions of c-*rel* in turkeys that are homologous to v-*rel* and the sequences related to v-*rel* of a homologous locus in chickens (Chen et al., J. Virol. **245**:104–113, 1983). The general organization of introns and flanking sequences is conserved for both c-*rel* in turkeys and this locus in chickens, indicating that c-*rel*, like other proto-oncogenes, may have an important developmental or metabolic function.

Retroviruses can be described in relation to their tumor production as nononcogenic, weakly oncogenic, or highly oncogenic. Weakly oncogenic retroviruses are competent for replication and produce tumors in infected animals only after long latent periods and with low efficiency. Highly oncogenic retroviruses are derived from weakly oncogenic retroviruses and contain additional sequences, called oncogenes, that enable them to produce tumors in sensitive animals after a short latent period and with high efficiency. Oncogenes are sequences that have apparently been acquired from cell DNA by weakly oncogenic retroviruses. The sequences that have been acquired from the genomes of cells and give rise to oncogenes are called proto-oncogenes (reviewed in reference 1).

Reticuloendotheliosis virus strain T (Rev-T) is a highly oncogenic avian reticuloendotheliosis virus that contains the oncogene v-*rel* (6, 32) and causes reticuloendotheliosis in animals and transforms spleen cells in cell culture (12, 15, 20, 28). Rev-T was isolated from a tumor-bearing turkey in 1958 (28). Rev-T probably arose when a weakly oncogenic reticuloendotheliosis virus infected a turkey cell, acquired a proto-oncogene homologous to v-*rel*, and then underwent subsequent modifications to form Rev-T. The proto-oncogene sequences homologous to v-*rel* that were acquired by this weakly oncogenic reticuloendotheliosis virus are called c-*rel*.

Rev-T is defective for replication and is associated with nondefective reticuloendotheliosis virus strain A, which supplies helper functions in *trans* for those functions that have been deleted from Rev-T (3, 4, 14–16, 23). Rev-A is probably a reasonable approximation of the weakly oncogenic parental virus that gave rise to Rev-T. v-*rel* DNA in Rev-T is substituted for nucleotide sequences that encode *env* in Rev-A DNA. In addition, Rev-T DNA has relative to Rev-A a deletion of nucleotide sequences that in the parental virus code for much of *gag* and *pol* (4, 15, 16, 23). This deletion of sequences that encode *gag* and *pol* in the parental virus is necessary for the expression of the transforming potential of Rev-T (6).

In this study we investigated the structure of the proto-oncogene c-*rel* in turkeys. The genomic turkey sequences that were detected to have homology to v-*rel* are part of one locus. Two alleles of this locus have been isolated by molecular cloning from an apparently heterozygous turkey. The c-*rel* locus is large and is very similar to a locus previously cloned from chickens (7). Differences exist between the sequences homologous to v-*rel* in the turkey locus and v-*rel*. The sequence differences between v-*rel* and the homologous sequences of c-*rel* cannot be accounted for by polymorphism in the turkey population or by differences between c-*rel* in chickens and turkeys.

MATERIALS AND METHODS

Cells. The procedures for propagating avian fibroblasts have been described previously (13). The turkey embryos used in this study were obtained from Wilmar Poultry, Wilmar, Minn. Embryos 5, 7, and 10 are Orlopp turkeys. Embryo 15 is a bronze turkey.

Virus. Rev-T was obtained from producer cell line 1-6 (4).

Sources of DNA. DNAs were extracted from turkey embryo fibroblasts as previously described (5).

*Eco*RI v-*rel* probe is a subclone in pBR322 of v-*rel*-specific sequences from defective Rev-T proviral clone 2-20-4 (4).

pRev-T 3 is a circularly permuted molecular clone in pBR322 of Rev-T unintegrated circular DNA (6).

pRev-T 3 RI/Sal 3′ is a subclone of pRev-T 3 that contains the v-*rel* sequences 3′ to the *Eco*RI site at 4.3 kilobase pairs (kbp) in Rev-T (6). This subclone is the source of DNA for the 3′ v-*rel* probe.

2-20-6 is an infectious provirus clone of Rev-A in Charon 4A (4).

The clones λ 1059 c-*rel* (chicken 15B) 5, λ Charon 4A c-*rel* (chicken 15B) 1, λ 1059 c-*rel* (chicken 15B) 3, and λ 1059 c-*rel* (chicken 15B) 4 are molecular clones of c-*rel* locus 1 from chicken line 15B (7).

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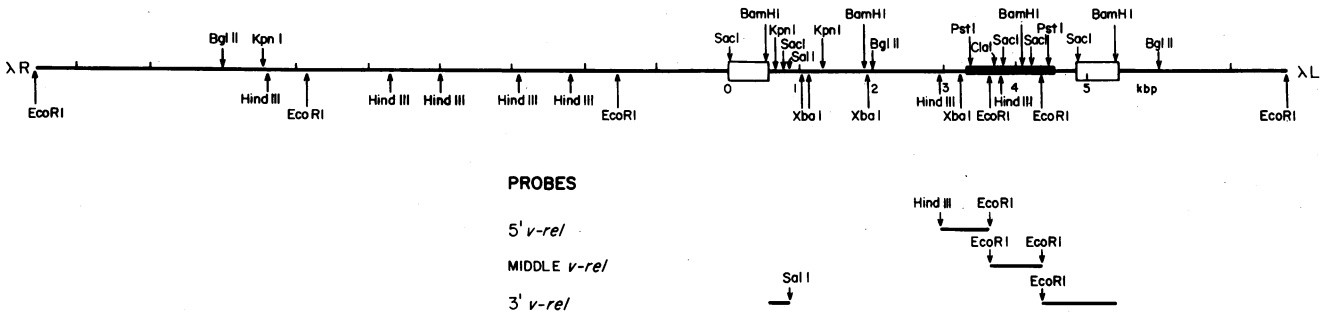

FIG. 1. Restriction endonuclease cleavage site map of insert in λ Charon 4A Rev-T 52B. DNA from recombinant phage λ Charon 4A Rev-T 52B was digested with restriction endonucleases and electrophoresed in an agarose gel. The resulting fragments were visualized with UV light after staining with ethidium bromide. A restriction endonuclease cleavage site map of the insert was made and is shown. The restriction enzyme cleavage sites for *Cla*I, *Sac*I, and *Pst*I were determined only for viral sequences. The regions homologous to v-*rel* sequences were identified by comparison to restriction enzyme cleavage site maps of unintegrated Rev-T DNA and restriction enzyme cleavage site maps of Rev-A (4, 23). The boxes indicate the positions of the long terminal repeats. The v-*rel* sequences shown as a solid bar have been positioned based on heteroduplex electron microscopic analysis of Rev-T and Rev-A (16, 23; Wilhelmsen, unpublished data) and preliminary nucleic acid sequence data of Rev-T and Rev-A (unpublished data). The *Xba*I site at 3.2 kbp is unique to Rev-T and arose by a 1-bp change of Rev-A sequence. Probably a single base change in the long terminal repeats in Rev-T generated *Bam*HI sites not present in Rev-A, or they were present in a parental virus different in this way from Rev-A. Rev-T lost a *Bam*HI site in sequences homologous to Rev-A as a result of a 1-bp change at 3.18 kbp (unpublished data). The right and left lambda arms adjacent to the inserts are indicated with λR and λL, respectively. The sequences which were subcloned (see the text) from Rev-T DNA for use as probes are diagrammed below the restriction map of the insert of λ Charon 4A Rev-T 52B. The 3' v-*rel* probe is a subclone from permuted pREV-T 3 and has only one long terminal repeat.

pc-*rel* *Bam*HI-*Eco*RI 2.5 to 4.5 is a subclone of λ 1059 c-*rel* (chicken 15B) 4 that contains sequences between regions of homology between v-*rel* and c-*rel* 1 and 2 (7).

Phage were grown in *Escherichia coli* strain DP50 (*supF*). Plasmids were grown in *E. coli* strain HB101. HB101 is *dam*$^+$; consequently some *Xba*I sites in c-*rel* may not have been detected as a result of methylation.

Preparation of molecular clones. A recombinant DNA library in vector Charon 4A was constructed with a partial *Eco*RI digest of DNA from Rev-T-infected fibroblasts from turkey embryo 10. The insert DNA was selected on a NaCl velocity gradient to be between 8 and 20 kbp. The library was screened by hybridization to a nick-translated probe made with the *Eco*RI v-*rel* subclone (2). The molecular clone λ Charon 4A Rev-T 52B was obtained. Infectious virus that can transform chicken spleen cells in culture can be recovered from λ Charon 4A Rev-T 52B. Figure 1 shows a restriction enzyme cleavage site map of the insert of λ Charon 4A Rev-T 52B.

5' v-*rel* is a subclone in pBR322 of the *Hin*dIII (2.9 kbp) to *Eco*RI (3.6 kbp) fragment of λ Charon 4A Rev-T 52B. This subclone is the source of DNA for the 5' v-*rel* probe.

Middle v-*rel* is a subclone in pBR322 of the *Eco*RI (3.6 kbp) to *Eco*RI (4.3 kbp) fragment of λ Charon 4A Rev-T 52B. This subclone is the source of DNA for the middle v-*rel* probe.

Two other recombinant DNA libraries were constructed with DNA from turkey embryo 7. The first library was constructed in Charon 4A by using DNA that was partially digested with *Eco*RI and was selected on a NaCl velocity gradient to be between 6 and 20 kbp. This library was screened by hybridization to the subcloned *Eco*RI v-*rel* sequences. λ Charon 4A c-*rel* (turkey 7) 1, 2, and 3 were obtained. When this library was screened by hybridization to pc-*rel* *Bam*HI-*Eco*RI 2.5 to 4.5 sequences, λ Charon 4A c-*rel* (turkey 7) 4 was obtained. λ Charon 4A c-*rel* (turkey 7) 4 contains a 4.7-kbp *Eco*RI fragment that is part of the c-*rel* locus as well as another fragment that is not linked in genomic DNA. A library was constructed in vector λ 1059 with insert DNA from turkey 7 that was completely digested with *Bam*HI and selected on a NaCl velocity gradient to contain inserts of 8 to 24 kbp (19). This library was screened with the pc-*rel* *Bam*HI-*Eco*RI 2.5 to 4.5 sequences. λ 1059 c-*rel* (turkey 7) 5 was obtained. This library was screened by hybridization to the middle v-*rel* probe, and λ 1059 c-*rel* (turkey 7) 6 was obtained. λ 1059 c-*rel* (turkey 7) 7 was obtained when this library was screened by hybridization with the 5' v-*rel* probe. An additional library was constructed by digesting genomic DNA from turkey 7 completely with *Bgl*II and dephosphorylating the ends with calf intestinal alkaline phosphate (New England Nuclear Corp.) before ligation with *Bam*HI-digested λ 1059 vector sequences. This library was screened by hybridization with 5' v-*rel* sequences, and λ 1059 c-*rel* (turkey 7) 8 was obtained.

Electron microscopic heteroduplex analysis. Heteroduplexes were formed by mixing and denaturing CsCl-purified phage (5 μg of DNA each per ml) in 100 mM NaOH–20 mM NaCl–20 mM EDTA at 25°C for 10 min. This solution was then neutralized by adding Tris-hydrochloride (pH 6.0) to a final concentration of 0.2 M. Formamide was then added to a final concentration of 50% (vol/vol). The DNA was allowed to reanneal for 60 min at 25°C. It was then dialyzed at 4°C against 20 mM NaCl–10 mM EDTA (pH 7.0) before spreading (9, 31). The procedure for spreading has been described previously (17). The heteroduplexes were spread onto water from a final concentration of 10 mM Na_2CO_3–5 mM EDTA–1 mM HCl–10 mM NaCl–20 μg of cytochrome *c* per ml–45% formamide. Heteroduplexes were examined with a Hitachi H-500 electron microscope at ×10,000. The lengths measured for heteroduplexes are reported ± their standard errors.

RESULTS

Our previous analysis of c-*rel* in chickens indicated that it is a large, complex locus. DNA from the c-*rel* locus in chickens was completely cloned in five overlapping recombinant DNA clones and contains at least seven regions that are homologous to v-*rel*. To determine whether these features are similar in chickens and turkeys and to determine the

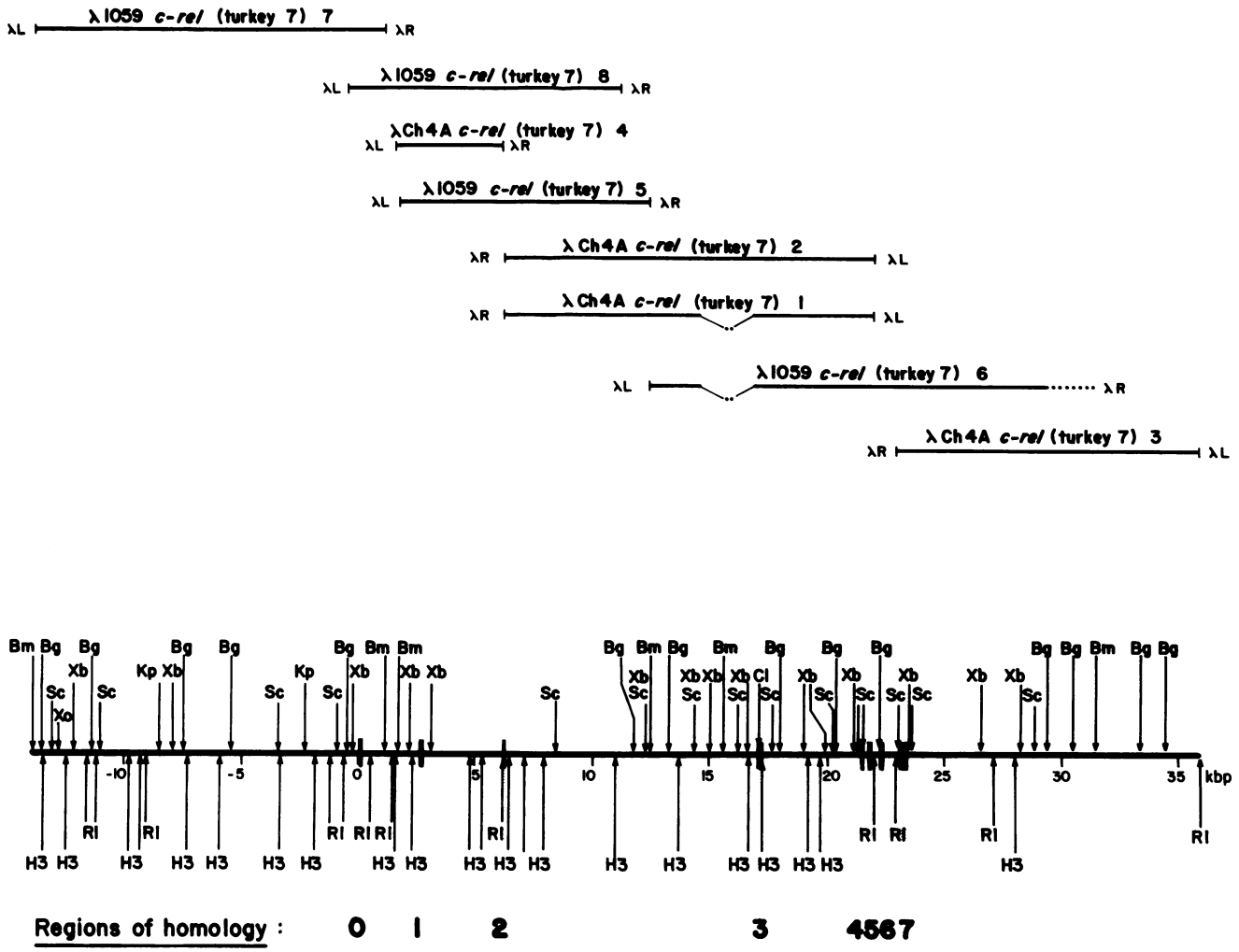

FIG. 2. Restriction endonuclease cleavage site map of the c-*rel* (turkey 7) locus. Molecular clones of c-*rel* sequences were obtained from recombinant phage libraries that were constructed with DNA from turkey embryo 7 (see the text). Fragments containing sequences homologous to v-*rel* were identified by Southern blot analysis with v-*rel* probes (data not shown). Restriction endonuclease cleavage site maps were made for each of the clones obtained. (Symbols used: RI, *Eco*RI; Bg, *Bgl*II, X, *Xba*I; H3, *Hin*dIII; Sc, *Sac*I; Cl, *Cla*I; Xh, *Xho*I; Kp, for *Kpn*I). Overlapping clones were identified by comparison of their restriction endonuclease cleavage site maps. The molecular clones that have been obtained for this allele are shown above the restriction map. λ Charon 4A c-*rel* (turkey 7) 1 and λ 1059 c-*rel* (turkey 7) 6 are from an alternative allele of turkey embryo 7 compared with the other clones shown in this figure. The thick dashed lines indicate sequences of λ Charon 4A c-*rel* (turkey 7) 1 and λ 1059 c-*rel* (turkey 7) 6, which show allelic differences from the other molecular clones in this figure. To obtain a more detailed restriction endonuclease cleavage map of these sequences, smaller subclones of these sequences were made by using pBR322 as a vector. These subclones are from *Hin*dIII at −1.9 kbp to *Bam*HI at 1.0 kbp, *Eco*RI at 1.5 kbp to *Eco*RI at 6.0 kbp, *Eco*RI at 6.0 kbp to *Bam*HI at 12.4 kbp, *Bam*HI at 12.5 kbp to *Eco*RI at 22.0 kbp, *Cla*I at 17.0 kbp to *Eco*RI at 22.0 kbp, and *Eco*RI at 22.9 kbp to *Eco*RI at 27.6 kbp. There are additional *Hin*dIII cleavage sites between −7.3 to −5.9 kbp and 13.6 to 15.7 kbp as well as an additional *Bgl*II cleavage site between −7.5 and −5.4 kbp. The regions of homology between v-*rel* and c-*rel* are numbered 0 to 7 and are shown as solid boxes. Electron microscopic heteroduplex analysis has allowed a more precise estimation of the size and position of the regions of homology (Fig. 3). The left and right lambda arms adjacent to the inserts are indicated with λL and λR, respectively.

structural relationship of the v-*rel* progenitor sequences to v-*rel*, we have molecularly cloned c-*rel* sequences from turkeys.

c-*rel* (turkey). To clone the c-*rel* proto-oncogene sequences, we constructed recombinant DNA bacteriophage libraries from DNA prepared from fibroblasts of a turkey embryo (turkey 7). Recombinant phage were screened for clones that contain c-*rel* sequences by in situ hybridization with v-*rel* probes and with subclones of c-*rel*-containing sequences (see above). A total of eight types of recombinant DNA clones were obtained from turkey 7. Restriction enzyme cleavage site maps of these clones were made. These clones have overlapping restriction enzyme cleavage site maps. λ Charon 4A c-*rel* (turkey 7) 1 and λ 1059 c-*rel* (turkey 7) 6 have some insert sequences that overlap with λ Charon 4A c-*rel* (turkey 7) 2 or 3 (or with both) and some insert sequences that are not homologous to these clones. The substitution and 3′ sequence deletions are allelic differences (see below). A total of 50 kbp of contiguous turkey DNA was cloned in these eight recombinant lambda clones. Each of these clones contains inserts that comigrate with fragments detected in genomic DNA when they are digested with the

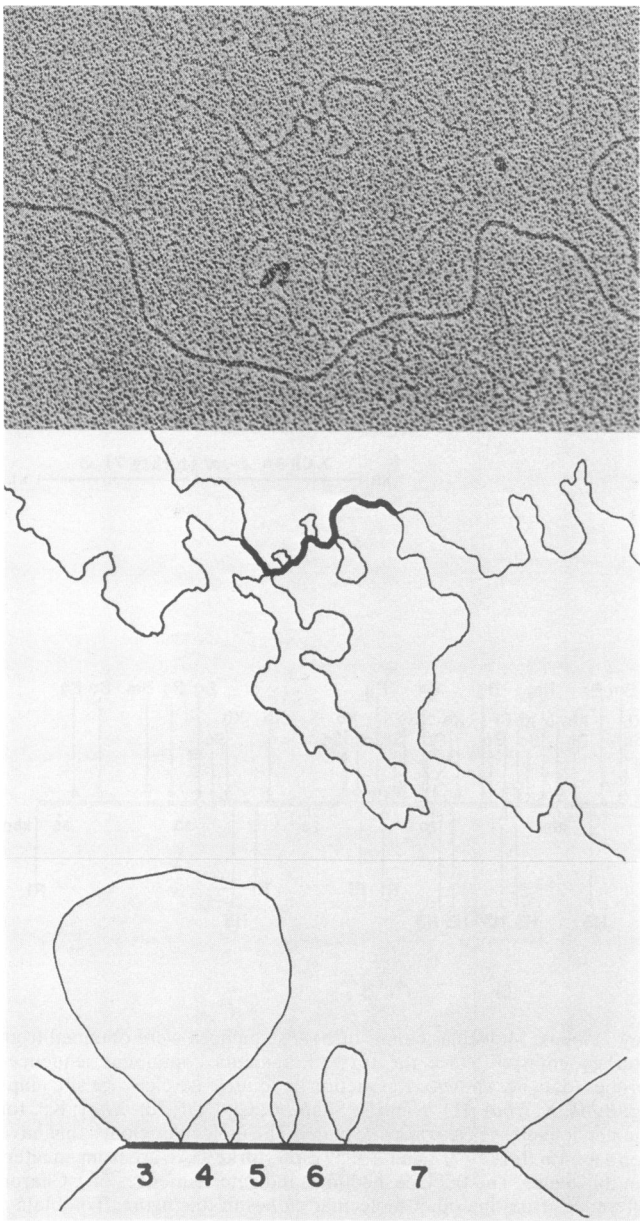

FIG. 3. Regions of homology between v-*rel* and c-*rel* (turkey 7) locus 1 allele 1. Electron microscopic analysis was made of heteroduplexes between λ Charon 4A Rev-T-52B and λ 1059 c-*rel* (turkey 7) 6. A representative electron micrographs is shown above. The diagrams below the electron micrograph identify the regions of interest in the heteroduplexes. The sizes of regions of homology between v-*rel* and c-*rel* as well as the sizes of presumed introns are in the text.

same enzyme (data not shown). A restriction endonuclease cleavage map of these c-*rel* sequences from turkey 7 is shown in Fig. 2.

Relationship between c-*rel* and v-*rel*. The regions of homology between the eight c-*rel* (turkey 7) clones in Fig. 2 and v-*rel* have been determined by restriction enzyme cleavage site mapping and nucleic acid hybridization of c-*rel* clones with v-*rel* subclones. The size and positions of some regions of homology between c-*rel* and v-*rel* have been determined more precisely by electron microscopic heteroduplex analysis of the c-*rel* (turkey 7) recombinant DNA clones and an Rev-T proviral recombinant clone (Ch4A Rev-T 52B) (see below). There are at least eight regions of homology between v-*rel* and c-*rel* (turkey 7) spread over approximately 23 kbp of cell DNA. These regions are designated 0 through 7 in Fig. 2. Regions 0 and 1 hybridize to sequences at the 5′ end of v-*rel*. Region 7 hybridizes to sequences at the 3′ end of v-*rel*. Electron microscopic heteroduplex analysis and nucleic acid hybridization data indicate that each portion of v-*rel* is homologous to one and only one of the eight regions found in c-*rel* (Fig. 3). Restriction endonuclease cleavage site mapping localizes regions 0 and 1 between the *Xba*I site at −0.3 kbp and the *Eco*RI site at 0.5 kbp and the *Hin*dIII site at 2.3 kbp and the *Xba*I site at 3.1 kbp, respectively (Fig. 2). The measured length of region 0 has not been made by electron microscopic heteroduplex analysis. It is estimated to be approximately 200 base pairs (bp) by filter hybridization analysis. The length of region 1 by electron microscopic heteroduplex analysis is very small (less than 100 bp). However, the measurement of region 1 is at the limit of detection of the method and may not be a precise measure of the size. A more precise measure will be made by nucleotide sequence analysis. Region 2 has been detected only rarely in heteroduplexes and also appears to be less than 100 bp. Restriction endonuclease cleavage site mapping and nucleic acid hybridization data indicate that this region begins at or near the *Eco*RI site at 6.0 kbp in Fig. 2. Regions 3, 4, 5, 6, and 7 are 0.19 ± 0.03 kbp, 0.14 ± 0.04 kbp, 0.21 ± 0.07 kbp, 0.19 ± 0.09 kbp, and 0.54 ± 0.03 kbp, respectively (Fig. 3). Regions 3 and 4 are separated by 3.8 ± 0.4 kbp in c-*rel*, but they are adjacent in v-*rel*. Similarly regions 4 and 5 appear to be separated by 0.23 ± 0.05 kbp in c-*rel*, but they are not separated in v-*rel* (Fig. 3). It is possible however, that the 0.23 ± 0.05-kbp single strand deletion loop seen in Fig. 3 is part of v-*rel*, and regions 4 and 5 are a contiguous segment of DNA in c-*rel*. This ambiguity will be resolved when nucleotide sequence data are available for this region. Region 5 is separated from the *Eco*RI site at the 3′ boundary of the insert of λ Charon 4A c-*rel* (turkey 7) 2 by 0.12 ± 0.04 kbp (data not shown). Region 5 is separated from region 6 by 0.44 ± 0.12 kbp, and region 6 is separated from region 7 by 0.84 ± 0.17 kbp. (The sizes indicated above are based on measurements of eight heteroduplexes.) Region 7, which hybridizes to v-*rel* sequences both 3′ and 5′ to the *Eco*RI site at 4.3 kbp in Rev-T, is 0.15 ± 0.06 kbp 3′ to the *Eco*RI site at 22.2 kbp in c-*rel* by electron microscopic heteroduplex analysis.

To estimate crudely the frequency of nucleic acid sequence differences between v-*rel* and the regions of c-*rel* homologous to v-*rel*, we have looked for restriction endonuclease cleavage site differences. This type of analysis is limited since only a small portion of possible sites of sequence difference are examined. The absence of a restriction endonuclease cleavage site is evidence that sequence differences exist between v-*rel* and c-*rel*. Without additional nucleic acid sequence data it is not possible to conclude that the presence of corresponding restriction sites are from homologous regions. Figure 4 shows a comparison of restriction enzyme cleavage site maps of v-*rel* and the homologous regions of c-*rel*. There are numerous restriction endonuclease cleavage sites which are apparently conserved. There are also restriction enzyme cleavage sites that are absent from c-*rel* sequences and are found in the homologous portion of v-*rel*, *Bam*HI at 0.9 kbp, *Eco*RI at 1.33 kbp, and *Pst*I at 1.44 kbp (Fig. 4).

Polymorphism in c-*rel* (turkey). To decide whether the difference in *Eco*RI cleavage sites detected between c-*rel*

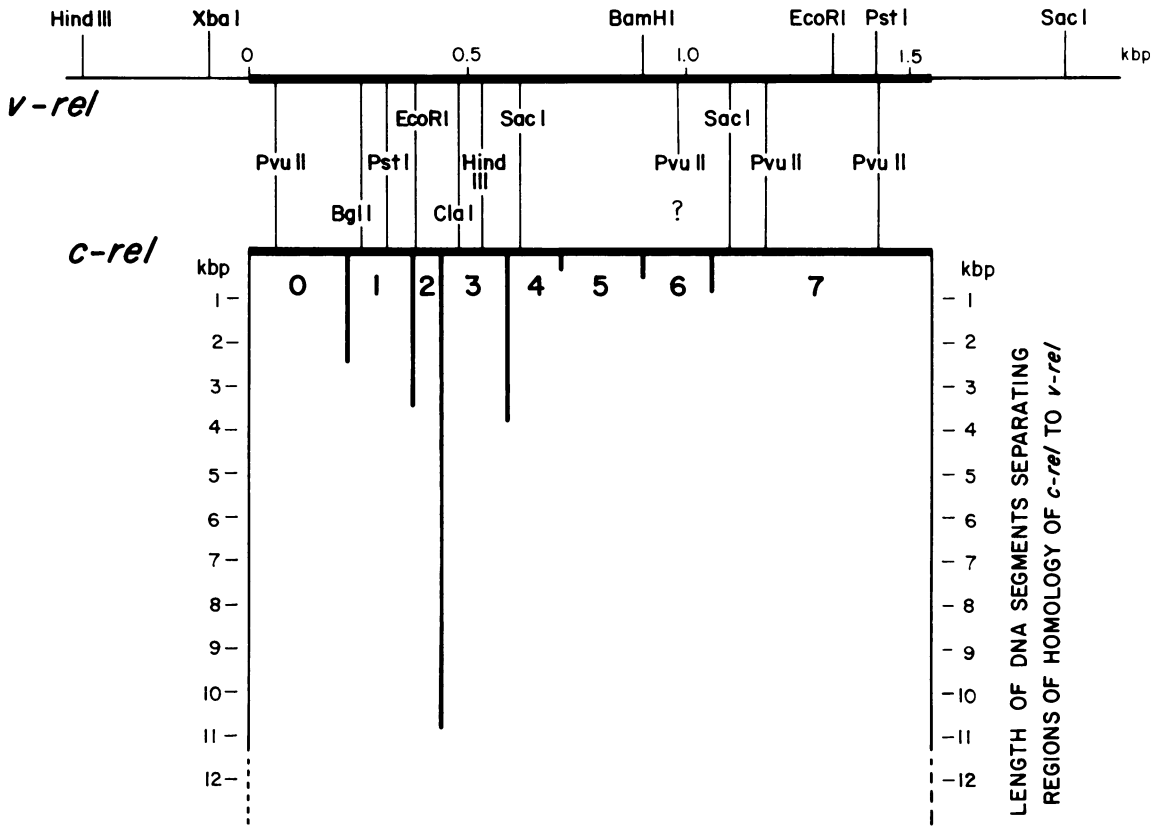

FIG. 4. Comparison of the restriction endonuclease cleavage site maps of c-*rel* and v-*rel*. A fine structure restriction endonuclease cleavage site map of v-*rel* was made by digesting each of the 5', middle, and 3' v-*rel* subclones with the enzymes indicated. The sizes of the resulting fragments were determined by electrophoresis through 1.5% agarose and 3 or 12% polyacrylamide gels and comparing the resulting fragments with the sizes of fragments of pBR322 digested with *Alu*I. This map of v-*rel* is more precise than the map of Rev-T in Fig. 1 since the sizes of small fragments were determined directly. The recombination site between v-*rel* and parental virus sequences was determined by sequencing the 5' and 3' ends of v-*rel* and corresponding regions of Rev-A (data not shown). The restriction enzyme cleavage pattern of v-*rel* by *Pvu*II and *Bgl*II corresponds well to the map of Rev-T made by Rice et al. (23). λ phage recombinant c-*rel* clones and plasmid subclones were digested with the restriction endonucleases that were expected to digest based on the restriction endonuclease cleavage site mapping of v-*rel*. The identified restriction enzyme cleavage sites presumed to be shared in common by v-*rel* and c-*rel* and estimates of the size of regions of homology between v-*rel* and c-*rel* based on nucleic acid filter hybridization data and electron microscopic heteroduplex analysis were used to determine the relationship between c-*rel* and v-*rel* shown above. The regions of homology between v-*rel* and c-*rel* are labeled 0 through 7 as in Fig. 2. Restriction endonuclease cleavage sites presumed to be in common to v-*rel* and c-*rel* are indicated between the restriction endonuclease cleavage maps of v-*rel* and c-*rel*, and those sites where no candidate cleavage site was found in c-*rel* for a corresponding site in v-*rel* are identified above the restriction endonuclease cleavage map of v-*rel*. The *Pvu*II cleavage site at 0.98 kbp may or may not be conserved and is indicated with a question mark. Where more than one restriction endonuclease cleavage site was found in a single region of c-*rel*, the distance between cleavage sites was determined to be as close to the corresponding distance in v-*rel* as could be determined without nucleic acid sequencing. For reference, lengths and position of segments of DNA which separate regions of c-*rel* that are homologous to v-*rel* are shown below the v-*rel* restriction endonuclease cleavage map.

and v-*rel* was due to population differences or mutation of sequences acquired by the virus, we have examined the incidence and nature of polymorphism in a turkey population. To determine the incidence and nature of polymorphism in c-*rel*, DNAs of a small collection of unrelated turkey embryos were examined (Fig. 5). The DNAs were cleaved with *Eco*RI, separated as to size by electrophoresis in an agarose gel, transferred to nitrocellulose, and hybridized to v-*rel*-containing probes. The probes used in this study were three subclones of Rev-T that divide v-*rel* into three nonoverlapping regions. The junctions between these subclones are the *Eco*RI sites at 3.6 and 4.3 kbp (Fig. 1).

Except for the large *Eco*RI fragments that hybridize to the middle v-*rel* probe, each of the embryos studied has the same pattern of cleavage of c-*rel* sequences by *Eco*RI. Embryos 5, 10, and 15 do not have an additional *Eco*RI cleavage site that could account for the *Eco*RI cleavage site found at 4.3 kbp in the v-*rel* sequences of Rev-T and not in the corresponding sequences of c-*rel* from turkey embryo 7 (discussed above).

Each of the embryos analyzed (Fig. 5) has one or two large *Eco*RI fragments that hybridize to the middle v-*rel* probe. The simplest model to explain this observation is that they represent two possible alleles of the c-*rel* locus.

Molecular clones were obtained of two allelic *Eco*RI fragments from an apparently heterozygous turkey embryo (turkey 7 in Fig. 5). Electron microscopic heteroduplex analysis of these two clones shows that they differ by a substitution. The larger *Eco*RI fragment (part of allele 1) contains 2.4 ± 0.2 kbp not present in the smaller *Eco*RI fragment (Fig. 6), whereas the smaller *Eco*RI fragment (part of allele 2) contains 0.17 ± 0.03 kbp not found in the larger

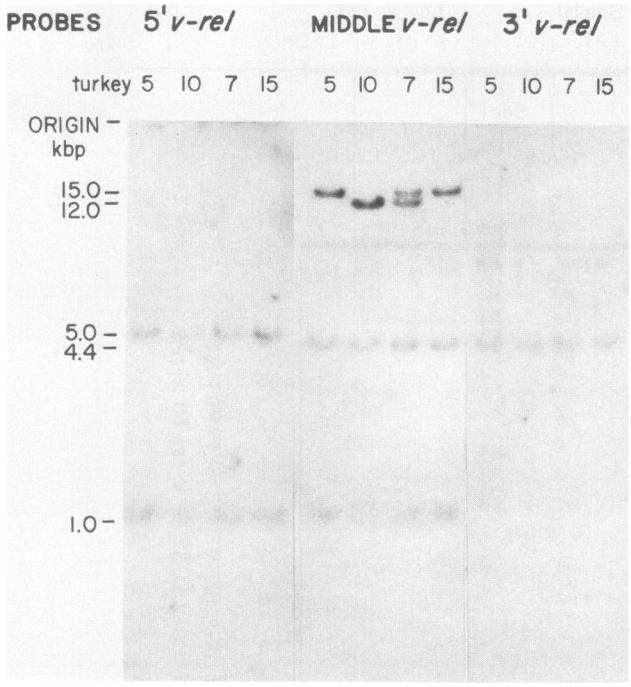

FIG. 5. c-*rel* polymorphism in a turkey population. DNA was isolated from embryo fibroblasts from four individual turkeys (numbers 5, 7, 10, and 15). DNA (60 μg) from each individual was digested with a 20-fold excess of *Eco*RI. The samples were then divided into three equal portions and electrophoresed in a 0.5% agarose gel. The DNA was denatured and transferred to nitrocellulose filters for hybridization with 5′ v-*rel*, middle v-*rel*, and 3′ v-*rel* probes (Fig. 1). Note that the 5.0-kbp fragment detected with the 5′ v-*rel* probe is a different size than the 4.4-kbp fragment that hybridizes to the 3′ and middle v-*rel* probes and that there are two different 1.0-kbp *Eco*RI fragments, one that hybridizes to the 5′ v-*rel* probe and one that hybridizes to the middle v-*rel* probe.

*Eco*RI fragment. This substitution is in an apparent intron just 5′ to region 3 (Fig. 2). λ 1059 c-*rel* (turkey 7) 6 (Fig. 2) is from allele 2.

In addition to this substitution detected in turkey c-*rel* alleles, we found another form of polymorphism. Molecular clones of the two alleles of embryo 7 were obtained which contain exon number 7 and 3′ flanking sequences. Restriction enzyme cleavage site mapping of λ Charon 4A c-*rel* (turkey 7) 3 and λ 1059 c-*rel* (turkey 7) 6 indicates that there is an increased incidence of discordant restriction enzyme cleavage sites with increasing distance 3′ to region 7 (data not shown).

Comparison of c-*rel* in chickens and turkeys. As previously stated, there is one major c-*rel* locus in chickens. Sequences from this locus have been isolated by molecular cloning (7). Electron microscopic heteroduplex analysis of chicken and turkey recombinant clones of c-*rel* indicates that the presumed coding sequences of c-*rel* are conserved, that presumed intervening sequences contain unequal length substitutions, and that there are regions of increased mismatching in presumed intervening sequences. Figure 7 shows a composite comparison diagram of the c-*rel* loci of chickens and turkey DNA (allele 1).

DISCUSSION

Rev-T is a defective highly oncogenic avian retrovirus that contains the oncogene v-*rel*. We have obtained molecular clones of Rev-T and have made subclones of v-*rel* sequences to examine the cellular sequences that are homologous to v-*rel*.

c-*rel* (turkey). We have molecularly cloned DNA of two alleles of locus 1 of c-*rel* from a heterozygous turkey. c-*rel* in turkeys has eight regions of homology to v-*rel* (Fig. 2). Region 0 is approximately 23 kbp from the 3′ end of region 7. Regions 2 and 3 are separated by 11 kbp. The sum of the lengths of the regions of c-*rel* that are homologous to v-*rel* (assuming regions 0, 1, and 2 are together 400 bp) is 1.65 kbp. The length of v-*rel* is approximately 1.55 kbp. The sum of the lengths of regions of homology to v-*rel* in c-*rel* locus is consistent with this locus being the source of all of the v-*rel* sequences. A more precise accounting of v-*rel* sequences relative to c-*rel* locus 1 will be made when nucleic acid sequence data are available.

Since the c-*rel* locus from turkey is probably the progenitor sequence for v-*rel*, sequences in c-*rel* between the regions in c-*rel* homologous to v-*rel* were lost. The structural relationship between oncogenes and proto-oncogenes has been studied for other oncogenes (8, 10, 11, 21, 22, 24, 27, 29, 30). Some proto-oncogenes do not have segments of DNA sequence that interrupt their regions of homology with their respective oncogenes, others have a few segments of DNA sequence that interrupt their regions of homology with their respective oncogenes, and others have many segments of DNA sequence that interrupt the regions of homology to their respective oncogenes. c-*rel* is similar to the last. It is believed that segments of DNA in proto-oncogenes that interrupt the regions of homology with oncogenes are introns (18). Shimotohno and Temin (25) and Sorge and Hughes (26) have shown that if cellular genes with introns are inserted in a retrovirus vector, the introns are lost after a few cycles of virus replication.

Nucleotide sequence differences between c-*rel* and v-*rel*. Restriction endonuclease cleavage sites have been detected in v-*rel* for which corresponding restriction endonuclease cleavage sites cannot be found in the c-*rel* locus sequences that are homologous to v-*rel*. The significance of these differences is hard to assess. These restriction endonuclease cleavage site differences could have been generated by the splicing together of regions of c-*rel* during the formation of v-*rel*. Alternatively, the sequence differences could have arisen by mutation during the process of acquisition or after the sequences were acquired by the virus. Additionally, it cannot be determined whether the proto-oncogene studied is the allele from which an oncogene was acquired. A polymorphic allele of c-*rel* may exist that has identical coding sequences with v-*rel*. The differences between such an allele and the allele in turkey 7 would then have been generated either by somatic mutation or by evolutionary divergence of germline sequences or by both.

c-*rel* locus dimorphism. We have examined the c-*rel* locus within a turkey population to see whether an allele could be found which had the sequence changes found in v-*rel*. Within the turkey population we found dimorphism of the c-*rel* locus, but only in apparent noncoding sequences. We found substitutions in an apparent intron of a turkey heterozygous at the c-*rel* locus. We also found that 3′ to the regions of homology to v-*rel*, c-*rel* locus 1 has a high frequency of base changes when two turkey alleles are compared. We have found no evidence for c-*rel* coding sequence polymorphism in the turkey population we have studied.

Comparison of c-*rel* in chickens and turkeys. To determine what types of changes occurred in c-*rel* over a greater evolutionary time span, we compared c-*rel* sequences from

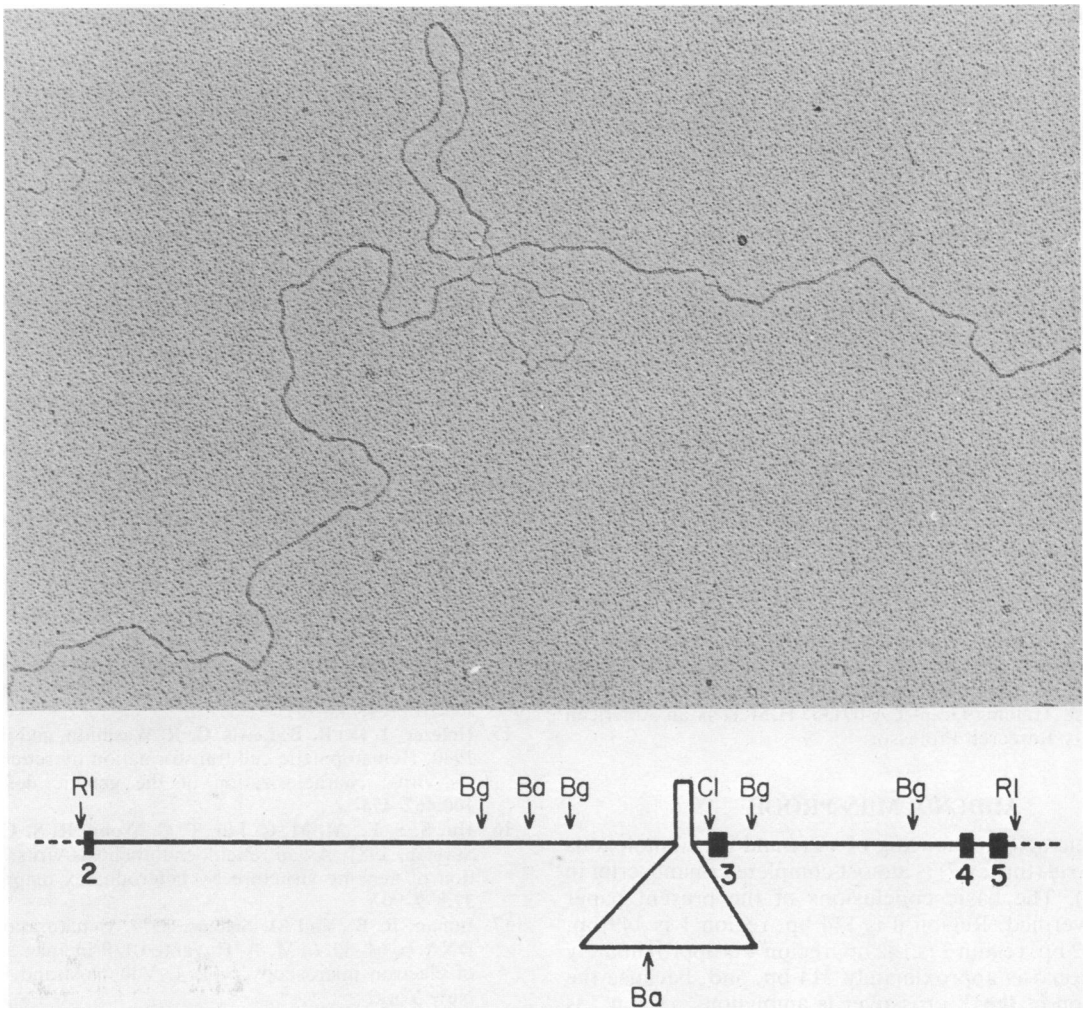

FIG. 6. Substitution in c-*rel* locus 1 in a heterozygous turkey. Two large *Eco*RI fragments of approximately 12 and 15 kbp that hybridize to the middle v-*rel* probe were molecularly cloned from turkey embryo 7 in the vector Charon 4A. A map of one of these clones, λ Charon 4A c-*rel* (turkey 7) 2, is presented in Fig. 2. A restriction endonuclease cleavage site map was also made for the other clone, λ Charon 4A c-*rel* (turkey 7) 1 (at the bottom of the above figure). These maps are colinear at both 5′ and 3′ ends. A representative electron micrograph of a heteroduplex of these two clones is shown above. There is a substitution which by restriction endonuclease cleavage site mapping and electron microscopic heteroduplex analysis is close to, but probably 5′ to, region 3. The larger single strand of the substitution is 2.4 ± 0.2 kbp in length. The smaller single strand of the substitution is 0.17 ± 0.03 kbp (based on 10 micrographs).

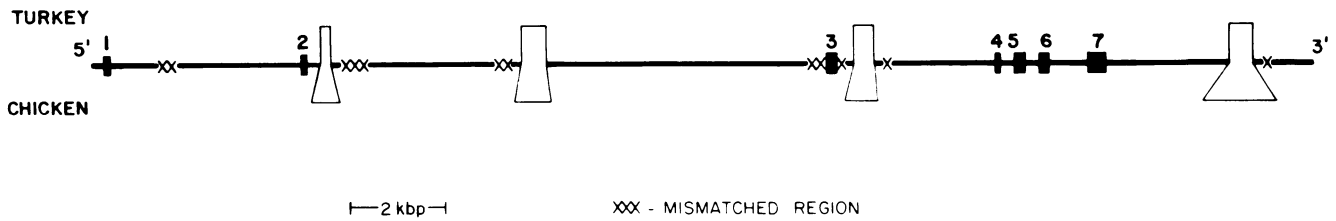

FIG. 7. Differences between c-*rel* locus 1 in chicken and in turkey DNA. Regions of homology between chicken and turkey DNAs are drawn as a single line. Regions of homology between chicken and turkey DNA in which there is a high frequency of mismatches are marked ×. Regions with no homology are shown with lines above the center line for turkey DNA and below the center line for chicken DNA. The regions of homology between v-*rel* and c-*rel* are shown as boxes and numbered as in Fig. 2. The turkey allele that is diagrammed in this figure is allele 1 as shown in Fig. 2. The substitutions between regions 2 and 5 are drawn such that the larger strand of the substitution is present in chicken DNA. This decision is based on the length of the distance between regions 2 and 5 in chicken and turkey DNAs. The substitution 3′ to region 7 could be longer in turkey or in chicken DNA. More than eight heteroduplexes were used for each of the comparisons made.

chickens and turkeys. The c-*rel* loci of chickens and of turkeys are very similar with respect to the size and distribution of the regions homologous to v-*rel*. However, there are differences between chickens and turkeys in restriction endonuclease cleavage sites, increased base pair changes in certain regions, and unequal length substitutions in presumed introns of c-*rel*.

Since specific germline changes in apparent coding sequences of the c-*rel* locus are rare within a turkey population and have not been detected in comparison of chickens and turkeys, it is less likely that the sequence changes between v-*rel* and c-*rel* were the result of transduction of a rare germline mutant. The conservation of c-*rel* locus 1 between chickens and turkey indicates that this locus has an important function in normal cells.

ACKNOWLEDGMENTS

We thank Jamie Lewis and Susan Hellenbrand for excellent technical assistance, B. E. Funnell, M. Schnös, and R. B. Inman for advice and instruction in electron microscopic heteroduplex analysis, and I. Chen, M. Emerman, C. Miller, N. Panganiban, R. Risser, R. Schedl, B. Sugden, and G. Tarpley 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. K.C.W. is a predoctoral trainee supported by Public Health Service Training Grant CA-09135. H.M.T. is an American Cancer Society Research Professor.

ADDENDUM IN PROOF

The nucleic acid sequencing of v-*rel* and the homologous regions of c-*rel* (turkey 7) is almost completed (manuscript in preparation). The basic conclusions of the present paper have been verified. Region 0 is 144 bp, region 1 is 149 bp, region 2 is 92 bp, region 3 is 142 bp, region 4 is approximately 104 bp, region 5 is approximately 214 bp, and, because the exact position of the 3′ crossover is ambiguous, region 7 is between 370 and 377 bp.

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