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NOTES

Genetic Characterization of Human c-*rel* Sequences

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We isolated and sequenced a human genomic-DNA segment that is homologous to a portion of v-*rel*, the transforming gene of reticuloendotheliosis virus (strain T). We also localized the human *rel* sequences to human chromosome 2 by screening a panel of rodent × human somatic-cell hybrids with the newly described human *rel* segment.

Cellular homologs of retroviral transforming genes have been identified in birds and mammals, in which these homologs are thought to perform important roles in regulating cell proliferation and differentiation (for a review, see reference 2). Recent studies suggest, however, that specific alterations in normal cellular oncogene structure and expression may themselves initiate cellular transformation in the absence of retroviral infection (e.g., see references 7, 15, 24, and 30). Such observations have prompted investigators to clone cellular oncogene homologs to study their normal biological properties and to determine whether alterations in these properties are consistently associated with naturally occurring, nonvirally induced malignancies. In this study, we determined the nucleotide sequence and chromosomal localization of a cloned human DNA segment carrying two putative exons homologous to the transforming gene (v-*rel*) of reticuloendotheliosis virus strain T (REV-T). The human c-*rel* sequences may provide a useful tool for studying the structure and function of this cellular oncogene in mammals.

REV-T is a replication-defective (11) type C retrovirus (3, 12, 25, 36) that induces acute leukemia in young chickens and turkeys (32). It is the only known acutely transforming member (9, 10) of a retroviral family that includes spleen necrosis virus, chick syncytial virus, duck infectious anemia virus, and the REV-T helper reticuloendotheliosis-associated virus (REV-A) (11, 14). The REV-T genome carries a genomic substitution of approximately 1.42 kilobases (kb) near its 3′ end (4, 6, 29) that is required for cellular transformation (5). This sequence, termed v-*rel*, is believed to have been derived from several exons of a turkey cellular gene (4, 27, 35), and recent molecular analyses of the turkey c-*rel* locus support this hypothesis (33). We began our investigation by performing Southern transfer and hybridization experiments to determine whether v-*rel* homologies could also be detected in humans and other mammals. The results of one such experiment in which cellular DNAs of hamsters, mice, humans, and chickens were hybridized with *Sst* v-*rel*, a 514-base-pair (bp) subclone of v-*rel*, are shown in Fig. 1e. After a 3-day exposure, one or two *rel*-homologous DNA bands were noted in all of the lanes, including those containing human DNA (see also reference 4).

We next used *Sst* v-*rel* to screen (1) a human partial *Hae*III-*Alu*I genomic-DNA library (18) constructed in the λ vector Charon 4A. Only one of nearly 1.2×10^6 phage recombinants hybridized with *Sst* v-*rel*. Restriction mapping and hybridization analysis of this single phage isolate (HSrel-1) revealed that *Sst* v-*rel* homology was limited to a 530-bp *Pst*I-*Hin*dIII segment at one end of the human DNA insert. This fragment (pPHHSrel-1) was subcloned for further sequence analysis. Additional *Sst* v-*rel* homologies could not be identified, leading us to speculate either that the phage clone carried only a small portion of the human c-*rel* gene or that HSrel-1 and *Sst* v-*rel* were sufficiently divergent to prevent hybridization under the conditions used (1 M Na^+, 35% formamide, 37°C; final wash, 0.02 M Na^+, 0.1% sodium dodecyl sulfate, 37°C), or both. However, HSrel-1 did contain those sequences from the human genome that were most closely related to *Sst* v-*rel* (Fig. 1).

The specific orientation and localization of the v-*rel*-related region were determined by nucleotide sequencing (19) of appropriate subcloned segments of the phage insert (Fig. 1c) by using reactions for G, G+A, C+T, C, and A>C. The nucleotide homology was divided into two discontinuous open reading frames (Fig. 2a), each of which was bounded by consensus splice acceptor and splice donor signals (21). In v-*rel*, the two regions of homology were contiguous (see Fig. 1). These two open reading frames in the human clone probably represented exons because nucleotide and predicted amino acid homologies ended abruptly 5′ to the putative splice acceptors and 3′ to the putative splice donors. Only two of the three reading frames in the presumptive 98-bp intron contained termination signals however; reading frame 2 began near the middle of the first predicted exon and extended 164 bp to a termination codon immediately after the start of the second predicted exon (see Fig. 2a). Sequence information from the corresponding chicken and turkey c-*rel* introns would have helped to clarify whether this observation was biologically meaningful. We did note, however, that on the basis of length and sequence, the two apparent human exons were homologous to exons 4 and 5 of the turkey c-*rel* gene (33, 34) (see Fig. 1c and d). Homologs of turkey c-*rel* exons 6a, 6b, and 7 could not be identified in predicted regions of HSrel-1, either by sequencing (Fig. 1c) or by hybridization with *Sst* v-*rel*.

A comparison of the predicted amino acid sequences of exons 4 and 5 of turkey c-*rel* (33), v-*rel* (29), and the *rel*-related human clone is presented in Fig. 2b. An immediate conclusion from this comparison is that exon 4 was

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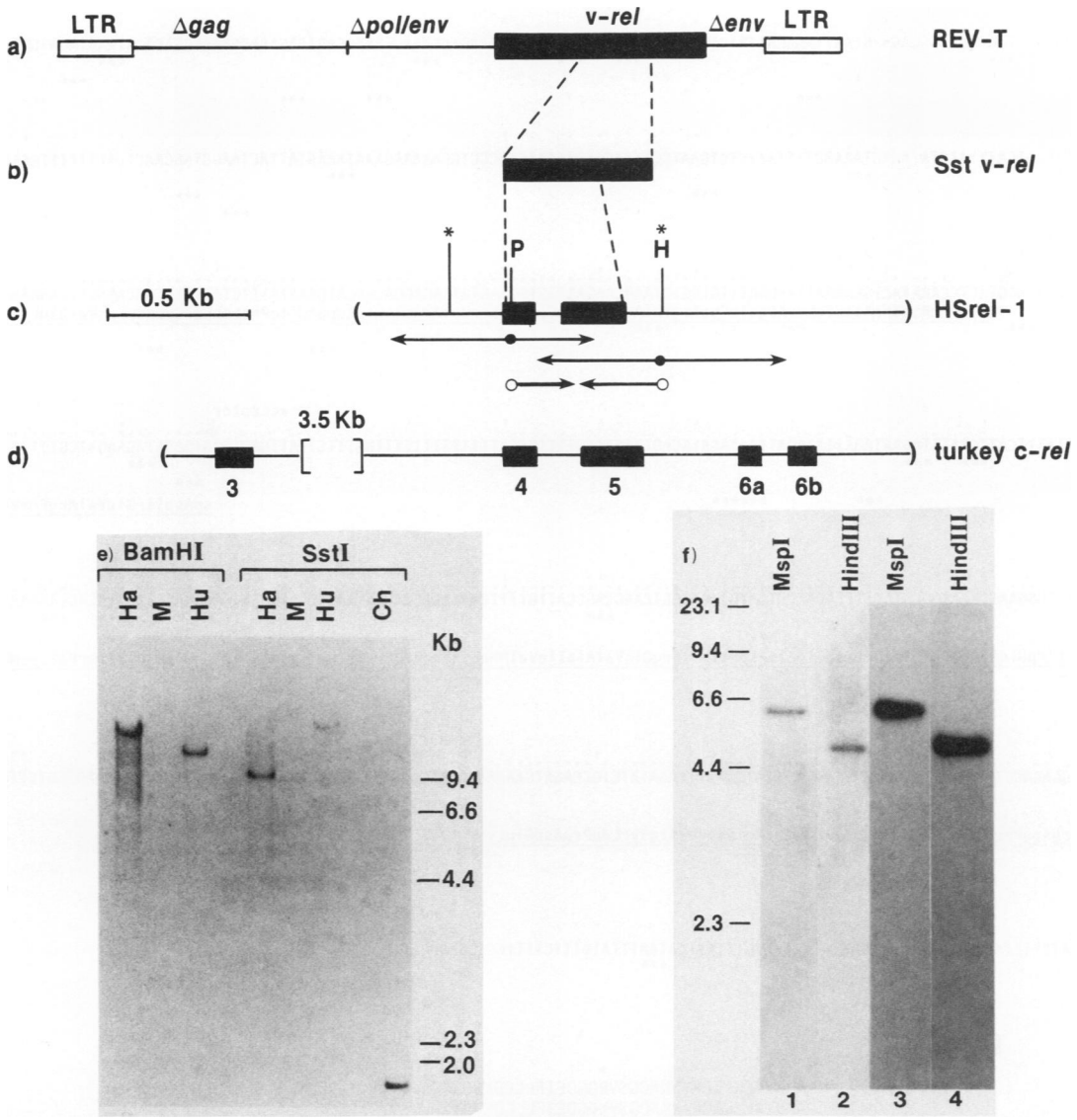


FIG. 1. (a) Genetic map of the 5.5-kb REV-T genome (26, 29); (b) origin of *Sst* v-*rel*, a 514-bp *Sst*I-*Sst*I segment of the viral oncogene v-*rel* (29); (c) diagram of a small portion of human genomic clone HSrel-1 showing regions of homology with *Sst* v-*rel*. (▬), apparent exons. Examples of the sequencing strategy are shown underneath the line (●, segments labeled at the 3′ end; ○, segments labeled at the 5′ end). *——*, Sequenced region shown in Fig. 2a. Abbreviations: H, *Hin*dIII; P, *Pst*I. (d) Portion of the turkey c-*rel* locus (33, 34). Exons 4 and 5 are homologous to the two putative exons contained in HSrel-1. (e) Genomic digests of Chinese hamster (Ha), mouse (M), human (Hu), and chicken (Ch) DNAs probed with an *Sst* v-*rel* insert. The sizes of the marker bands are given in kilobases. Faint bands at ≈8 and 9.5 kb appear in the lanes containing mouse genomic DNA restricted with *Bam*HI and *Sst*I, respectively. (f) Restriction digests of human genomic DNA (lanes 1 and 2) and HSrel-1 phage clone DNA (lanes 3 and 4) hybridized with an *Sst* v-*rel* insert. For the experiments in panels e and f, 20 μg of mammalian and 10 μg of chicken DNA were hybridized at 37°C in buffer containing 5× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate), 35% formamide, and 10% dextran sulfate. The filters were then washed at a final stringency of 0.1× SSC–0.1% sodium dodecyl sulfate at 37°C.

considerably more conserved, in an evolutionary sense, than was exon 5. Exon 4 showed only one conservative amino acid difference (lysine v-*rel* → arginine HSrel-1) in the entire coding region. There were 16 nucleotide differences between v-*rel* and human *rel* (15% of the entire exon), but 14 of these were degenerate third base changes. Exon 5 of human *rel* differed at 54 nucleotides (25%) from v-*rel*, and 35 of these substitutions were in the third codon position. In amino acid sequence, this region of human c-*rel* showed 13 substitutions relative to v-*rel* or to turkey c-*rel*, about half of which appeared to be nonconservative replacements. There were only two amino acid differences (and three nucleotide differences) between v-*rel* and turkey c-*rel*, and both of these amino acids were also different from v-*rel* in the human homolog. In the first of the two positions, turkey c-*rel* and HSrel-1 contained positively charged amino acids instead of glycine. At the second position, both c-*rel* sequences contained negatively charged residues instead of alanine. It is notable that a 12-amino acid sequence which contains a possible serine phosphorylation site (Arg-Arg-Pro-*Ser*-Asp) (17) is conserved in the three *rel* sequences.

Finally, the human c-*rel* sequences were chromosomally

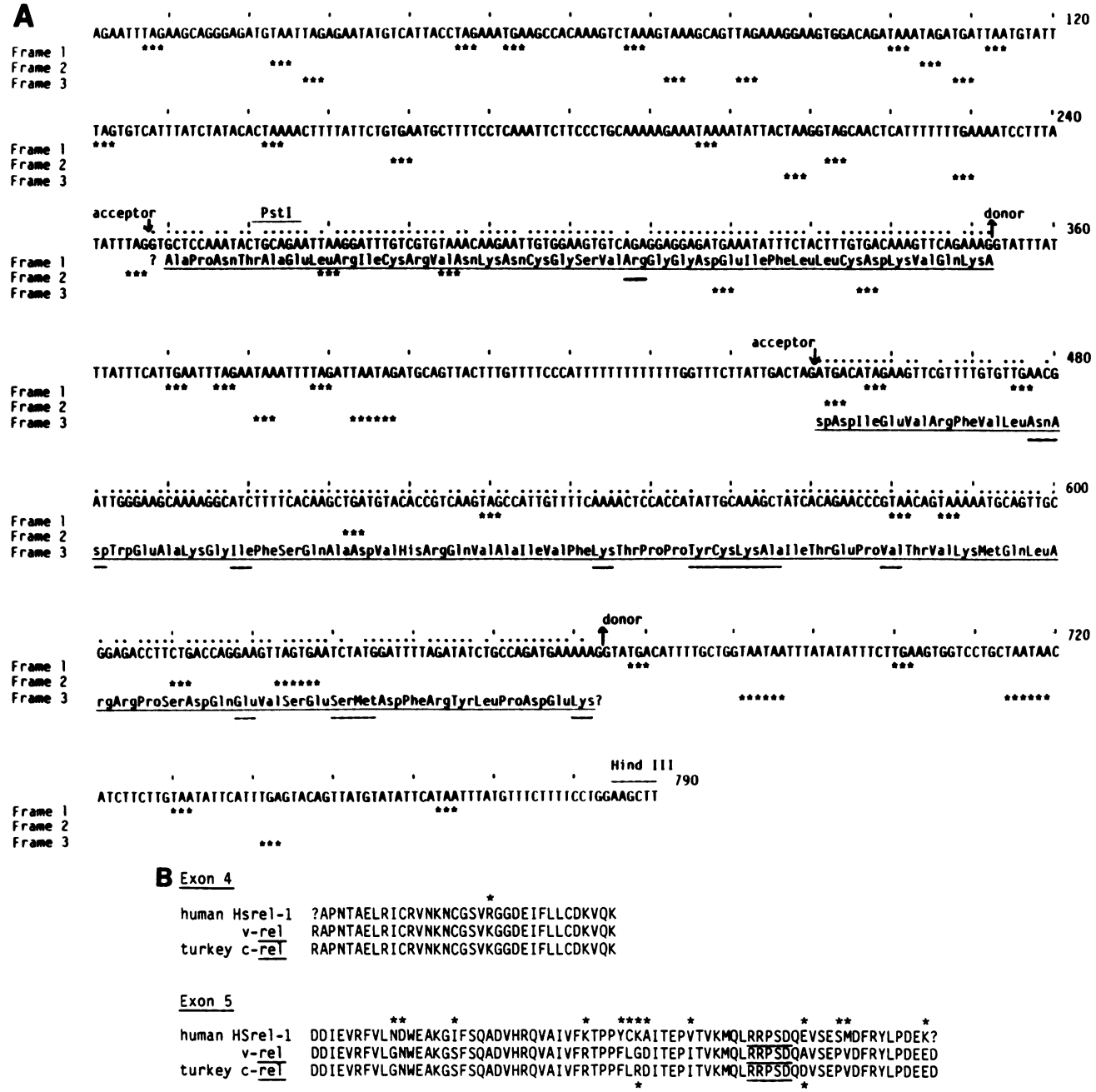


FIG. 2. (A) Nucleotide sequence of a 790-bp portion of HSrel-1. ...., Nucleotide homology between HSrel-1 and v-*rel* (noted above the two extended open reading frames); (↑ ↓) potential splice acceptor and splice donor sites; *, termination codon. Predicted amino acid homologies between HSrel-1 and v-*rel* are underlined once, whereas differences are shown by double underlines. Relevant restriction sites are overlined. This sequenced region is indicated by asterisks in Fig. 1c. (B) Comparison of the predicted amino acid sequences of homologous portions of HSrel-1, v-*rel* (29), and exons 4 and 5 of turley c-*rel* (33). Differences between v-*rel* and the two c-*rel* sequences are noted. ?, amino acid position that spans a probable splice junction in HSrel-1. The underlined region denotes a potential recognition site for serine phosphorylation by cAMP-dependent protein kinase (17). Abbreviations: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

mapped by a Southern transfer analysis of cellular DNAs derived from a panel of 43 rodent × human somatic-cell hybrids (22, 23). Briefly, parental and somatic-cell hybrid DNAs (approximately 50-μg samples) were digested with *Hin*dIII, transferred to nitrocellulose filters (28), and hybridized with a nick-translated probe derived from pPHHSrel-1 (Fig. 3a). For these experiments, the hybridization stringency was increased to 40% formamide–1 M Na^+ at 42°C; the final wash contained 0.08 M Na^+ and 0.1% sodium dodecyl sulfate and was performed at 68°C. By this strategy,

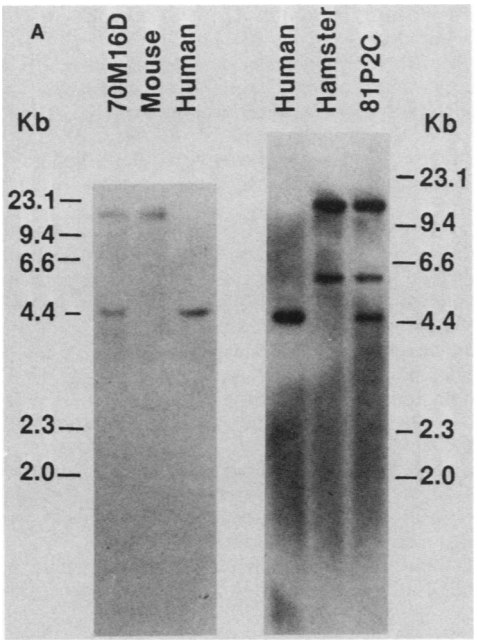
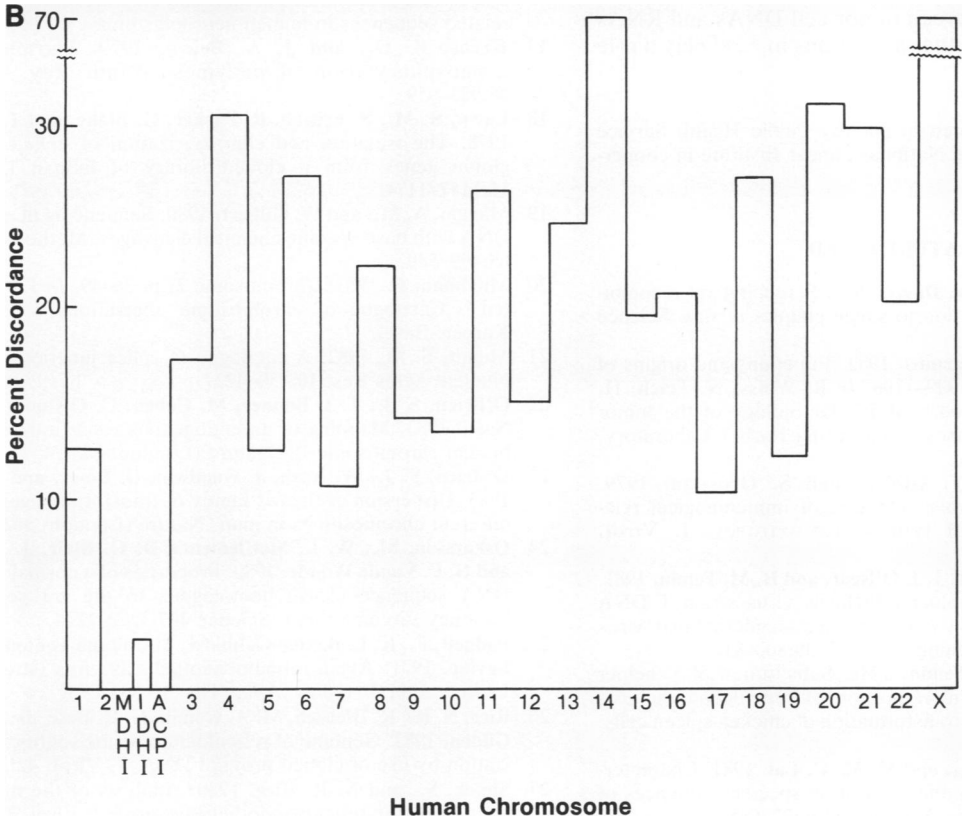


FIG. 3. (A) *Hin*dIII restriction digests of mouse, hamster, human, and rodent × human somatic-cell hybrid DNAs probed with pPHHSrel-1. 70M16D is a mouse × human hybrid somatic-cell line, and 81P2C is a Chinese hamster × human hybrid somatic-cell line. The human band at ca. 4.5 kb is found in both of the hybrid cell line DNAs, but not in the DNAs of the rodent parents. (B) Plot of the frequency of discordancy between human *rel* sequences and each of 23 human chromosomes in panels of somatic-cell hybrids which are segregating human chromosomes.



the human probe recognized a 4.5-kb fragment in human DNA, a high-molecular-weight band (≥18 kb) in mouse DNA, and two bands in hamster DNA (ca. 12 and 6 kb) (see Fig. 3a). Thus, by *Hin*dIII digestion of DNA from the hybrid panel, it was possible to determine which hybrids retained the 4.5-kb human c-*rel* segment and the chromosome on which it resided. The presence of the c-*rel* fragment was 100% concordant with human chromosome 2 (HSA2) and two of its included isozymes, MDH1 and ACP1 (Fig. 3b). Two of our panel hybrids were discordant for a third marker, *IDH1*, and had the following phenotypes: hybrid 80H12C:HSA2$^-$, MDH1$^-$ IDH$^+$ ACP1$^-$ REL$^-$; hybrid

81P8C:HSA2$^+$, MDH1$^+$ IDH1$^-$ ACP1$^+$ REL$^+$. One of these, 80H12C, retained a chromosome 2 homolog which had lost the short arm, but retained the long arm (2q1.1-qter). The concordant loss of ACP1 and MDH1 (both of which mapped to 2p) and REL in this hybrid, which lacked 2p but retained 2q, permitted the provisional regional assignment of *rel* to HSA 2pl.1-pter.

Recent studies show that some human tumor cells carry specific chromosomal rearrangements that alter the structure and expression of cellular genes that normally reside on chromosome 2p. For example, in one variant form of Burkitt lymphoma, a t(2;8) translocation results in movement of part of the Igk locus from chromosome 2 to a position ≥20 kb 3′ to the c-*myc* locus on chromosome 8 (8). Structural and regulatory alterations in the c-*myc* gene are also evident in this lymphoma, although it is not clear whether they result from the specific translocation (31). In several human neuroblastoma cell lines, furthermore, a chromosome 2-derived (13) cellular oncogene (N-*myc*) is amplified and rearranged to form homogeneously staining regions or double minutes (16). (N-*myc* exhibits no significant nucleic acid sequence homology with v-*rel*, turkey c-*rel*, or the human *rel* sequences described here.) Many nonspecific rearrangements of chromosome 2 have also been noted in other human tumors (20). Future experiments with the newly isolated human *rel* sequences to screen tumor cell DNAs and RNAs will help to determine whether alterations in c-*rel* play a role in human carcinogenesis.

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