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Conserved chromosomal positions of dual domains of the *ets* protooncogene in cats, mice, and humans

(comparative gene mapping/leukemia)

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ABSTRACT **The mammalian protooncogene homologue of the avian v-*ets* sequence from the E26 retrovirus consists of two sequentially distinct domains located on different chromosomes. Using somatic cell hybrid panels, we have mapped the mammalian homologue of the 5′ v-*ets*-domain to chromosome 11 (*ETS1*) in man, to chromosome 9 (*Ets*-1) in mouse, and to chromosome D1 (*ETS1*) in the domestic cat. The mammalian homologue of the 3′ v-*ets* domain was similarly mapped to human chromosome 21 (*ETS2*), to mouse chromosome 16 (*Ets-2*), and to feline chromosome C2 (*ETS2*). Both protooncogenes fell in syntenic groups of homologous linked loci that were conserved among the three species. The occurrence of two distinct functional protooncogenes and their conservation of linkage positions in the three mammalian orders indicate that these two genes have been separate since before the evolutionary divergence of mammals.**

Transforming genes, or protooncogenes, represent a class of conserved cellular genes that may play an important role in tumorigenesis. They were initially described as transduced RNA segments in transforming retrovirus genomes, and they have also been discovered by focus induction after transfection of mouse 3T3 cells with genomic DNA extracted from human tumors (1–5). The limited number of protooncogenes (≈30) described to date has attracted considerable research emphasis as an experimental opportunity to study neoplastic transformation from both genetic and molecular perspectives. Because of these extensive analyses, there are now at least five documented modes of oncogene activation associated with tumorigenesis. These include the following: (*i*) transduction of portions of protooncogene transcripts by retroviruses, thereby placing the oncogene under regulatory control of strong promoters in the viral long terminal repeats (1–5); (*ii*) chromosomal insertion of an infecting retrovirus adjacent to a protooncogene, similarly altering their control of transcription (5, 6); (*iii*) translocation of cellular oncogenes to chromosomal regions of differential regulation (7, 8); (*iv*) amplification of oncogene-containing segments, thereby increasing the dosage of the oncogene (9–11); and (*v*) point mutation in the cellular coding sequence (2).

The *ets* sequence was identified as a second cellular sequence transduced by the avian replication-defective retrovirus E26. The 5.7-kilobase (kb) RNA genome of E26 contains, in addition to partial retroviral *gag* and *env* genes, a part of the *myb* oncogene originally identified in avian myeloblastosis virus (AMV) and an E26-specific sequence, *ets* (12, 13). The nucleotide sequence of a 2.46-kb DNA region of E26 has revealed a contiguous *gag-myb-ets* open reading frame encoding a 135-kDa protein, p135 (14, 15). E26 induces both myeloblastosis and erythroblastosis *in vivo* and transforms erythroid and myeloid precursors *in vitro*. The myeloid oncogenic properties that E26 and AMV have in common are thought to be encoded by the common *myb* domain, while the unique erythroblastosis of E26 is thought to be encoded by the unique *ets* domain of the tripartite viral *onc* gene (14, 16) or perhaps by a cooperative effect of *myb* and *ets*. We have prepared a series of molecular clones of the human *ets* sequence and discovered that the coding sequence is disrupted in the human genome, where two sequentially distinct, transcriptionally active, and chromosomally separate loci encode homologues of the 5′ and 3′ regions of the avian viral oncogenes (17). We report here the chromosomal localization of the 5′ locus (termed *ets-1*) and the 3′ locus (termed *ets-2*) in three mammals: human, mouse, and cat. Their chromosomal positions in each species reside on homologous (syntenic) chromosomal segments, indicating that the two domains of the *ets* protooncogene have been on separate chromosomes since before the onset of the mammalian radiations.

MATERIALS AND METHODS

Use of Panels of Somatic Cell Hybrids for Chromosome Mapping of Human, Murine, and Feline Genes. Somatic cell hybrids were derived by PEG-mediated fusion of fresh human lymphocytes to rodent cells (mouse RAG or Chinese hamster E36) lacking the hypoxanthine/guanine phosphoribosyltransferase (*HPRT*) gene, thus allowing selection in hypoxanthine/aminopterin/thymidine medium. A panel of 60 hybrids was selected based on the high frequency of retention of low numbers of human chromosomes in different combinations (18–20). Hybrids were genetically characterized by G-trypsin banding (21), G-11 staining, and enzyme typing of 20–28 isozyme markers previously assigned to human chromosomes at the same passage from which high molecular weight DNA was extracted (20). Genomic DNA was digested with restriction enzymes and subjected to a Southern analysis (22) using the specific v-*ets*, *ets-1*, and *ets-2* probes described below (15, 17). Analogous procedures were used to prepare and characterize the rodent–feline hybrid panel, which segregates cat chromosomes (23), and the Chinese hamster–mouse panel, which segregates mouse chromosomes (24).

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Abbreviation: kb, kilobase(s).

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Identification of Human Cellular *ets*-Related DNA with v-*ets*, c-*ets-1* and c-*ets-2* Molecular Clones as Probes. E.1.28 is a 1.28-kb v-*ets* clone derived by *Bgl* I digestion of pER4, a molecular clone of the E26 provirus (15). E.1.28 contains 95% of the viral *ets* sequence (v-*ets*). Three *human* genomic clones that discriminate between c-*ets-1* and c-*ets-2* were used. The first two, pRD6K and pRD700, are human fragments derived from a single phage clone, RD3. pRD6K is a 5.7-kb *Eco*RI fragment; pRD700 is an 0.83-kb *Eco*RI fragment. Both pRD6K and pRD700 recognize human c-*ets-1*. The third human probe, H33, is a 1.02-kb *Eco*RI fragment that is specific for human c-*ets-2*. The three human clones share sequence homology with different regions of the viral *ets* sequence. From 5′ to 3′, the relative order in *ets* of homologous sequences is pRD6K-pRD700-pH33 (17).

RESULTS

Human Protooncogene *ETS1* Maps to Chromosome 11 and *ETS2* Maps to Chromosome 21. To identify the human protooncogene homologues of v-*ets*, a nearly full-length v-*ets* molecular clone, E.1.28 (Fig. 1*A*), was nick-translated and used as a probe on nitrocellulose filters derived from a Southern analysis (22) of human and rodent DNAs. Fragments of 8.2, 6.2, 3.6, and 0.83 kb were detected following *Eco*RI digestion of human DNA (Fig. 1*A*). The segregation of three of these fragments (8.2, 6.2, and 3.6 kb) was examined using DNAs from a panel of 50 somatic cell hybrids that had been genetically characterized by electrophoretic typing of previously assigned isozyme loci and by karyotype analysis using both G-11 and G-trypsin staining procedures (20, 21, 23). These hybrids, which were prepared by PEG-mediated fusion of normal human lymphocytes with HPRT-resistant mouse (RAG) or Chinese hamster (E36) fibroblasts, retained the entire rodent genome but lost human chromosomes in different combinations.

Two of the human c-*ets* fragments produced by *Eco*RI digestion (6.2 and 8.2 kb) were concordantly retained or lost in the hybrid panel. The 3.6-kb fragment segregated independently of the others (Fig. 1*A*), suggesting that at least two chromosomes contained sequences homologous to v-*ets*. This result has been confirmed by the molecular cloning of two *ets* genes, the characterization and DNA sequences of which are discussed in detail elsewhere (17). These studies have shown that distinct human loci are homologous to the 5′ and 3′ regions of the v-*ets* oncogene sequence. The two genes are both transcriptionally active, since they produce mRNA species of different sizes in several tissues (17).

Subclones of human DNA sequences homologous to different portions of the v-*ets* gene were used in the mapping experiments. Clone H33, which is homologous to the 3′ portion of v-*ets*, hybridized to the 3.6-kb human *Eco*RI fragment but not to the 0.83-, 6.2-, or 8.2-kb fragments (Fig. 1*B*). Two additional cloned human sequences, pRD6K and pRD700, were shown to be homologous to contiguous 5′ portions of v-*ets*. These probes recognized the *Eco*RI fragments of 6.2 and 0.83 kb, respectively, but did not cross-hybridize with clone H33. The human DNA segment homologous to the 5′ regions of v-*ets*, which is characterized by the 0.83/6.2-kb *Eco*RI pattern, is referred to as c-*ets-1*. The human locus homologous to the 3′ region of v-*ets*, for which the 3.6-kb *Eco*RI fragment is diagnostic, is termed c-*ets-2*.

The chromosomal positions of *ets-1* and *ets-2* in the human genome were determined by correlating the presence of the diagnostic *Eco*RI fragments in the hybrid panels using both clone E.1.28 (v-*ets*) and clone H33 (*ets-2*-specific) as probes. The presence of the *ets-1* locus was 92–97% concordant with human chromosome 11 and the chromosome 11 isozyme markers *LDHA* and *ACP2* but was highly discordant (33–58%) with each of the other human chromosomes (Fig. 2). The presence of *ets-2* was 100% concordant with the presence of human chromosome 21 and its isozyme marker, *SOD1* (Fig. 2*B*). These data document that the *ets-1* protooncogene is located on chromosome 11 and that the *ets-2* locus is on chromosome 21. The assignment of *ets-1* to chromosome 11 is consistent with the recent study of de Taisne *et al.* (25), who reported the assignment of an *ets* protooncogene to 11q23-24.

In situ hybridization of clone H33 (*ets-2* specific) clone to normal human chromosome preparations confirmed the as-

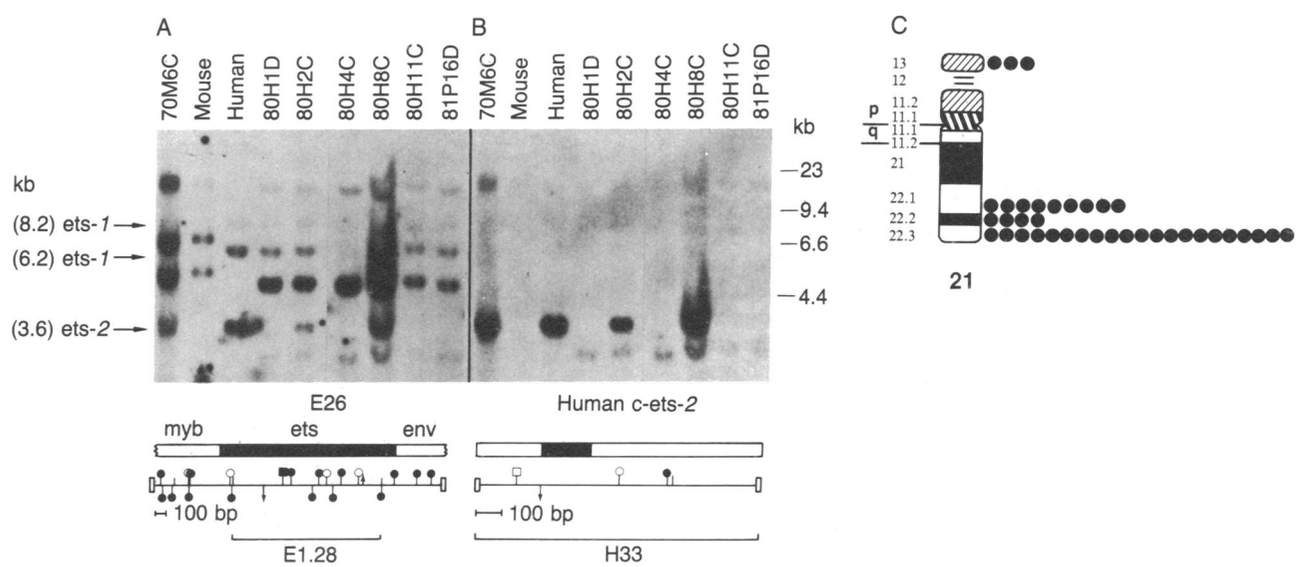

FIG. 1. Analysis of human *ets-1* and *ets-2*. Restriction maps of the avian provirus and of clone H33 (c-*ets-2* specific) are presented below the corresponding autoradiographs. ▬, *ets*-specific sequences (14, 17). (*A*) DNAs from mouse–human (70 series) and Chinese hamster–human (80 and 81 series) somatic cell hybrids were digested with *Eco*RI and probed with a molecular clone of the avian v-*ets* gene (E.1.28). (*B*) The gels were then washed and probed with H33, a molecular clone of the human c-*ets-2* homologue that recognizes the 3.6-kb fragment but not the 0.83-, 6.2-, and 8.2-kb fragments that are diagnostic for c-*ets-1*. ○, *Pvu* II; ↑, *Hin*dIII; |, *Bgl* I; ■, *Bgl* II; ◆, *Pst* I; ↓, *Hph* I; □, *Eco*RI; □, *Xba* I; ◆, *Taq* I. bp, Base pairs. (*C*) Distribution of 37 grains that fell on human chromosome 21 in 89 metaphase spreads after *in situ* hybridization using H33 (*ets-2* specific) as a probe.

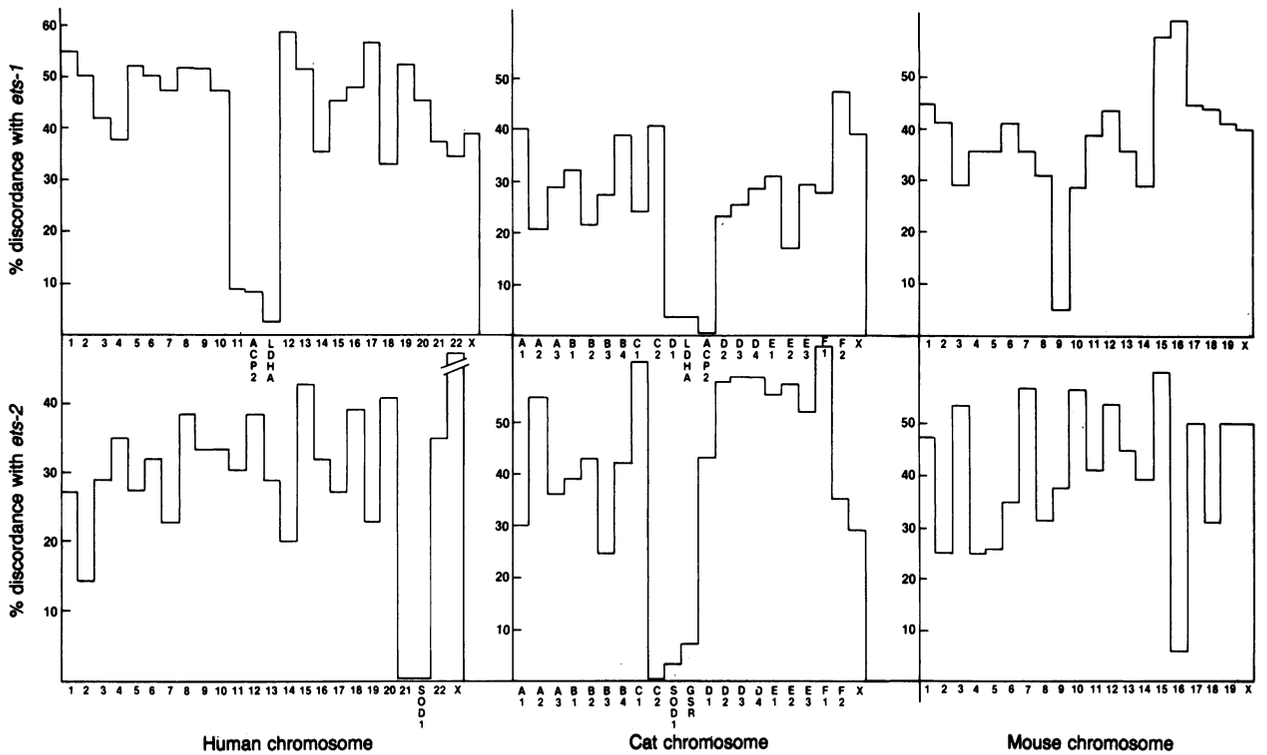

FIG. 2. Analysis of the association of *ets-1* and *ets-2* diagnostic fragments with segregant chromosomes in three panels of somatic cell hybrids. The human panel consists of 40 hybrids (18, 19); the murine panel, 16 hybrids (24); and the feline panel, 38 hybrids (21, 23). Chromosome scores represent the consensus result of karyotyping (G-banding) and isozyme scores. Thirty-six isozyme systems diagnostic for human chromosomes and 24 systems diagnostic for feline chromosomes were tested for each appropriate hybrid. Assignment of feline *LDHA* (*lactate dehydrogenase-1*) and *ACP2* (*acid phosphatase-2*) to feline chromosome D1 is a corrected assignment (S.J.O'B. *et al.*, unpublished data).

signment of *ets-2* to chromosome 21 and further localized the gene to the long arm (Fig. 1*C*). In an analysis of 89 metaphase spreads from normal human peripheral blood cells, 37 grains were found located on chromosome 21; 34 of these were located on the terminal portion. These labeled sites, each consisting of 1–3 grains, represented 20% (20 out of 100) of all labeled sites distributed throughout the 89 metaphase spreads. Compilation of grain positions from multiple ($n = 50$) labeled chromosomes 21 revealed a clustering of grains on segments 22.1-22.3. On the basis of a significant labeling of this region on the long arm of chromosome 21, we conclude that the *ets-2* gene is regionally located on human chromosome 21q22.1-22.3.

Assignment of Mouse *Ets-1* to Chromosome 9 and *Ets-2* to Chromosome 16. The murine homologues of v-*ets* were visualized as three fragments with *Eco*RI (5.1, 6.8, and 15 kb; Fig. 1*A*) and with *Pst* I (2.9, 7.8, and 9.8 kb, Fig. 3) using both the v-*ets* (E.1.28) and the *ets-2* (H33) clones as probes. The *ets-1*-specific pRD700 probe described above recognized only the 3.2-kb *Hin*dIII fragment (data not shown). The *ets-2* specific human probe (H33) recognized both *ets-1* and *ets-2* murine homologues for reasons that were not immediately apparent. Nonetheless, the homology of H33 to the *ets-1* locus in the mouse permitted the use of this probe in tracking the gene segregation in somatic cell hybrids. A previously characterized panel of mouse–Chinese hamster (E36) hybrids was used to study chromosome association of the murine *ets* loci (24). These hybrids retain the entire Chinese hamster chromosomal complement but lose mouse chromosomes in different combinations. DNA was extracted from each of 16 hybrids concomitant with isozyme and karyologic analyses to determine the murine chromosome complement.

The murine DNA fragments segregated as two distinct loci in the hybrid panel (Fig. 3). The *Pst* I fragment that is diagnostic for *Ets-1* (7.8 kb) was 94% concordant with mouse chromosome 9 but discordant with the *Ets-2* (2.9 and 9.8 kb) segments and with each of the 19 other mouse chromosomes (30–70%; Fig. 2). The 2.9-kb segment diagnostic for *Ets-2* was

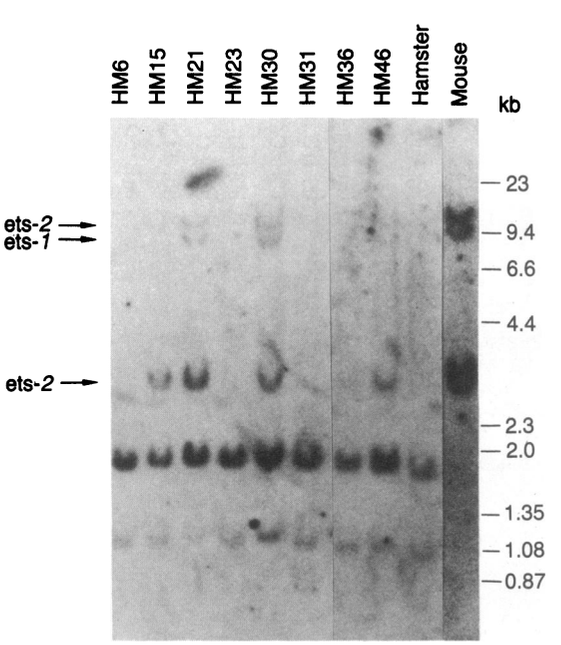

FIG. 3. Analysis of murine *Ets-1* and *Ets-2* in mouse–Chinese hamster somatic cell hybrids. Hybrid DNA was digested with *Pst* I and hybridized with a probe prepared for the human cellular clone H33, which is specific for *ets-2* in man but hybridizes to *ets-1* and *ets-2* fragments in mouse. Hybrid DNA was also digested with *Hin*dIII and hybridized with the human genomic pRD700 molecular clone, specific for *ets-1*.

94% concordant with mouse chromosome 16 and highly discordant (Fig. 2) with *Ets-1* (37%) and the 19 other mouse chromosomes (25–60%). In addition, one of the hybrids positive for *Ets-2* contained no other mouse chromosomes except for chromosome 16. These data permit assignment of *Ets-1* and *Ets-2* to murine chromosomes 9 and 16, respectively.

The assignment of *ets-2* to chromosome 21 in man and chromosome 16 in mouse represents the fifth homologous locus assigned to both of these chromosomes in the two species (26). The map distance involved in this homologous syntenic group was determined by a linkage analysis between the *Sod-1* and *ets-2* loci using restriction fragment length polymorphisms between inbred mouse strains. Briefly, DNA from several inbred mouse strains was examined with 14 restriction enzymes and two molecular probes: (*i*) an *ets-2*-specific 1.27-kb *Pst* I fragment that was homologous to H33 (Fig. 1*A*) derived from a murine genomic library and (*ii*) a 4.1-kb cloned fragment containing the fifth exon of the human *SOD1* gene plus part of the 5′ intron and 3′ flanking DNA (kindly provided by Yoram Groner). The murine *ets-2* probe detected fragments of 9.4 kb in BALB/cJ and 17 kb in CBA/J DNAs after digestion with *Bam*HI. The CBA mouse strain displays a 4.4-kb *Bst*EII fragment when probed with the human *SOD1* clone, while BALB/c has a 2.1/2.3-kb doublet with this restriction enzyme. F_1 hybrid females (CBA × BALB/c) were mated to BALB/c males and DNAs from 40 offspring were analyzed with the two probes. Thirty-eight of the offspring received the parental combination of *Ets-2* and *Sod-1* alleles from their F_1 parent (20 were BALB/c type, 18 were CBA type) and two were recombinant. These results confirm the assignment of *Ets-2* to mouse chromosome 16 and establish the linkage distance between *Ets-2* and *Sod-1* as 5.0 ± 1.4 centimorgan units.

Assignment of Feline *ETS1* to Chromosome D1 and *ETS2* to Chromosome C2. The domestic cat homologues of the viral *ets* sequence were resolved following digestion of genomic DNA with *Xba* I and hybridization with one of two probes: (*i*) H33, specific for c-*ets-2*, and (*ii*) pRD700, specific for c-*ets-1*. The somatic cell hybrid panel used for the cat mapping was expanded and analyzed for chromosomal complement by the same strategies used in the human analysis (21, 23).

Two *Xba* I fragments (6.8 and 11.0 kb) were detected in cat DNA and these segregated independently in the hybrid panel (Fig. 4). As with the mouse homologues, both fragments were resolved with the H33 (human *ets-2* specific) probe. The pRD700 probe, which is specific for human *ets-1*, preferentially detected the 4.0-kb band. This fragment was 96–100% concordant with feline chromosome D1 and its included markers *LDHA*, and *ACP2* (Fig. 2), but highly discordant (18–47%) with the 18 additional feline chromosomes. The appearance of the 11.0-kb *Xba* I feline fragment in the panel was 93–100% concordant with the presence of chromosome C2 and its isozyme markers, *SOD1* and *GSR* (glutathione reductase) but highly discordant (25–70%) with other cat chromosomes. These data permit assignment of the *ets-1* cat homologue to chromosome D1 and of the *ets-2* cat homologue to chromosome C2.

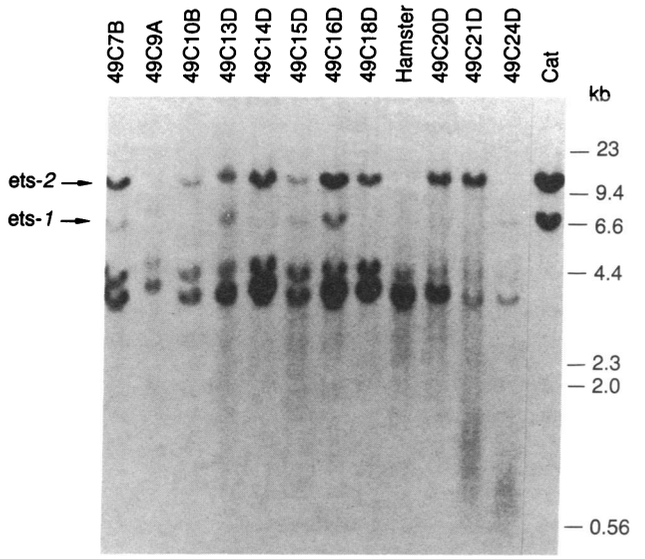

FIG. 4. Analysis of feline *ets-1* and *ets-2* in hamster–cat somatic cell hybrids. Hybrid DNAs were digested with *Xba* I and hybridized with the human H33 probe, which is specific for *ets-2* in man but hybridizes to both *ets-1* and *ets-2* fragments in cat and mouse. Hamster–feline hybrids were also digested with *Xba* I and hybridized with the human pRD700 probe, which is specific for *ets-1* in man, mouse, and cat.

DISCUSSION

At least three different prototype transforming retroviruses have been described that contain two distinct oncogenes encompassed by helper viral sequences. These include the avian carcinoma virus MH2, which contains v-*myc* and v-*mht* (also called *mil; raf*); avian erythroblastosis virus, which contains v-*erbA* and v-*erbB*; and the E26 virus, which contains v-*myb* and v-*ets*. We have reported here that the v-*ets* segment of E26 is derived from two domains that are themselves encoded on different chromosomes in mammals. In addition to E26, two oncogenic viruses, Gardner–Rasheed feline sarcoma virus (GR-FeSV) and FBR murine sarcoma virus (FBR-MuSV), contain hybrid *onc* genes that include genetic elements from two different cellular genes and from essential retrovirus genes (27–29). Diagnostic genomic clone probes for the two domains (termed *ets-1* and *ets-2*) have been used to demonstrate transcriptional activity of both loci (17) and to chromosomally map the homologues in this study. The genes overlap in a stretch of 14 amino acids in man (17) and have been assigned to chromosomes 11 and 21, respectively.

This dispersal of mammalian proto-*ets* sequences is in apparent contrast to organization in the chicken, from which the E26 virus was originally isolated. Avian c-*ets* has a genomic complex of 40–50 kb (15) that encodes a 7.5-kb *ets* mRNA (13, 17). This single RNA transcript is resolved in RNA blots with both *ets-1*- and *ets-2*-specific probes, suggesting that the two domains are contiguous in the chicken genome. The biologically active viral *ets* mRNA is a truncated 1.5-kb version of the chicken message. Since v-*ets* is a rather small subset of the chicken proto-*ets* locus, as well as of the two mammalian c-*ets* loci, the relative contributions of the *myb*, *ets-1*, and *ets-2* domains to viral transformation are not obvious.

With the recent rapid expansion of the human, murine, and feline gene maps, a more complete picture of chromosomal homologies between these species is emerging (30). More than 100 homologous genes have been mapped in both mouse and man, and comparison of the two gene maps has revealed that considerable rearrangement has occurred since these species shared a common ancestor (26, 30, 31). The cat has fewer mapped loci than the mouse, but the extent of retention of homologous syntenic groups to man seems to be two to three times greater than between man and rodents (23, 30). The *ets-1* locus is mapped here to chromosome 11q in man, to the chromosome 11 homologue in the cat (D1), and to the murine homologue of 11q (chromosome 9). Fig. 5 shows the homologous loci mapped to the respective linkage groups in the three species. The *ets-2* locus mapped to human chromosome 21, to the murine counterpart chromosome 16, and

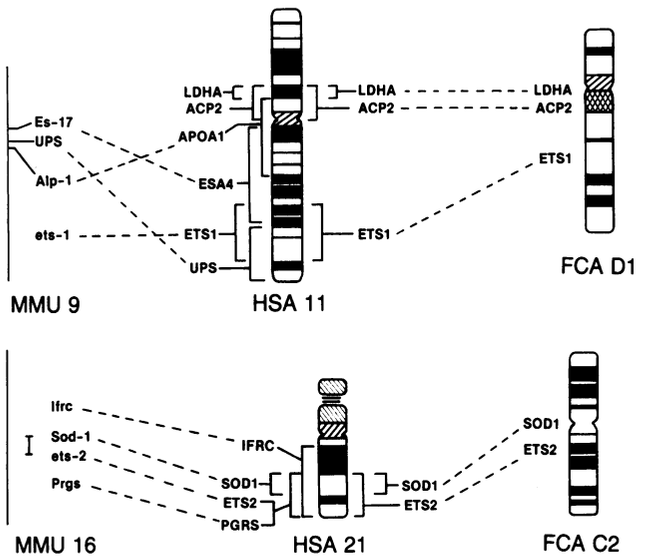

FIG. 5. Diagram of regional positions of homologous loci on chromosomes to which *ets-1* and *ets-2* have been assigned in man, mouse, and cat. All of the loci have been regionally assigned in the human map and several have been positioned in mouse. The figure is modified from genetic maps of man (32), mouse (33), and cat (34). The basis for homology of the genes included is discussed in refs. 26 and 30.

to the feline homologue, chromosome C2. The conservation of the linkage position of the two protooncogene domains in the same chromosomal positions in the three species indicates that their duality has persisted since the divergence of mammals began more than 100 million years ago.

Because the occurrence of specific translocations in human tumorigenesis has been shown to alter the *cis* regulation of adjacent protooncogene loci, the specific positions of such loci may be important. The same chromosome region that contains human *ets-1* (11q23-24) has been reported to be involved in nonspecific translocations in acute myelogenous leukemia, as well as in Ewing sarcoma (25, 35, 36). The localization of *ets-2* represents the first potential *onc* sequence mapped to chromosome 21. It may be noteworthy that trisomy 21 or Down syndrome patients have an elevated incidence of acute leukemias. Since the region implicated (37, 38) as operative in Down syndrome (21q22) includes the *ets-2* sequence (21q22.1-22.3), dosage aneuploidy of this gene should be considered as a candidate for a physiological role in the development of Down syndrome. Furthermore, this same region of chromosome is involved in a specific translocation (8;21)(q22;q22) commonly found in acute myelogenous leukemia with morphology M2 (39, 40). Preliminary results from our laboratory show that certain human acute myeloid leukemias with translocated chromosomes 11 or 21 show altered transcriptional expression of *ets-1* and *ets-2*, respectively (41). (For consistency with the nomenclature for other human and feline genes, the sequences referred to here as c-*ets-1* and c-*ets-2* should be identified as *ETS1* and *ETS2*, respectively).

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