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Genetics

Chromosomal mapping of the simian sarcoma virus *onc* gene analogue in human cells

[c-*sis* (human)/somatic cell hybrids/chromosome 22]

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ABSTRACT **The primate cell-derived transforming gene (v-*sis*) of simian sarcoma virus (SSV) is represented as a single copy marker within cellular DNAs of mammalian species including human. The human analogue of v-*sis* can be distinguished from its rodent counterparts by Southern blotting analysis of *Eco*RI-restricted DNAs. By testing for the presence of the human v-*sis*-related fragment, c-*sis* (human), in somatic cell hybrids possessing varying numbers of human chromosomes, as well as in segregants of such hybrids, it was possible to assign c-*sis* to human chromosome 22.**

The study of acute transforming RNA tumor viruses has provided a potentially important approach to elucidating mechanisms involved in human malignancy. These agents generally cause sarcomas and hematopoietic malignancies but, in some cases, also induce carcinomas (for reviews, see refs. 1–4). Convincing evidence has accumulated that sarcoma viruses have arisen by recombination of replication-competent type C RNA viruses with evolutionarily well-conserved cellular genes. These latter sequences, termed *onc* genes, have been found to be required for the induction or maintenance of viral transformation (or both) (5–11). Certain independent sarcoma virus isolates of the same (12–15) and even of different species (16, 17) have been found to contain the same or closely related *onc* genes. These findings suggest that there may exist only a limited set of distinct cellular genes with transforming potential.

The only known transforming retrovirus of primate origin (simian sarcoma virus, SSV) was initially obtained from a naturally occurring tumor of a woolly monkey (18). Recombinant DNA techniques recently have made it possible to clone in biologically active form the integrated genome of this virus (19, 20). The full-length linear 5.1-kilobase-pair (kbp) SSV genome contains a 1.0-kbp segment of helper virus-unrelated information localized toward the 3′ end with respect to SSV RNA. These sequences, designated v-*sis*, have been found to be essential for SSV-transforming functions (unpublished observations). Sequences related to v-*sis* are detectable at low-copy-number within the DNAs of various vertebrates including human (21, 22), and transcripts related to v-*sis* recently have been shown to be present in cells of certain human tumors (23, 24).

Understanding the interactions of the human analogue of v-*sis*, designated c-*sis* (human) (25), with other cellular genetic elements might be greatly facilitated by knowledge of its chromosomal location. Using cloned v-*sis* as a probe to analyze the human/rodent somatic cell hybrids, which segregate human chromosomes, we have determined the chromosomal location of c-*sis* in the human genome.

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MATERIALS AND METHODS

Cell Culture. Human fibroblast lines used in cell fusions included diploid WI 38 (ATCC CCL75), an *hprt*$^-$ simian virus 40-transformed WI 18 line VA2 (26), and a *tk*$^-$ HeLa derivative AV3 (ATCC CCL 21). Rodent cell lines employed were murine *hprt*$^-$ L-A9 (27), murine *tk*$^-$ L-B82 (27) and LMTKC1 1D (28), and an *hprt*$^-$ derivative of the hamster CHV79 line. Cells were grown in monolayer cultures in modified Eagle's minimal essential medium containing 5% or 10% fetal bovine serum. Eagle's spinner medium was used for suspension cultures.

Cell Hybridization. Human and rodent cells were cultivated together (1:1 mixture) in plastic Petri dishes (6- or 10-cm diameter) for 24 hr before induction of cell fusion with 52.5% polyethylene glycol, average M_r 1,000 (29). Selective hypoxanthine/aminopterin/thymidine medium was applied 24 or 48 hr after fusion, and independent colonies were cloned after approximately 2 wk. Hybrid cell lines usually were expanded in nonselective medium and analyzed 1–6 months after isolation.

Isoenzyme Analyses. Washed cell pellets were simultaneously prepared for DNA isolation and isoenzyme analyses from hybrid cell lines that had been expanded to 2–10 × 10^8 cells. These pellets were stored frozen at −80°C until used. The human chromosomes present in each hybrid cell line were determined from starch gel electrophoretic analyses (30, 31) of isoenzyme markers which have been previously assigned to each of the human chromosomes (32). The isozyme markers used were enolase 1 (ENO-1; EC 4.2.1.11), peptidase C (pepC; EC 3.4.11.-), phosphoglucomutase 1 (PGM-1; EC 2.7.5.1), and phosphogluconate dehydrogenase (6-PGD; EC 1.1.1.44) [chromosome (chr) 1]; soluble malate dehydrogenase (MDH-S; EC 1.1.1.37) and acid phosphatase 1 (ACP-1; EC 3.1.3.2) (chr 2p); soluble isocitrate dehydrogenase (IDH-S; EC 1.1.1.42) (chr 2q); aminoacylase 1 (ACY-1; EC 3.5.1.14) and sometimes glutathione peroxidase 1 (GPX-1; EC 1.11.1.9) (chr 3); peptidase S (PepS; EC 3.4.11.-) and phosphoglucomutase 2 (PGM-2; EC 2.7.5.1) (chr 4); hexosamidase B (Hex B; EC 3.2.1.30) (chr 5); soluble malic enzyme (ME-1; EC 1.1.1.40), phosphoglucomutase 3 (PGM-3; EC 2.7.5.1), and mitochondrial superoxide dismutase (SOD2; EC 1.15.1.1) (chr 6); beta glucuronidase (GUS; EC 3.2.1.31) and uridine phosphorylase (UP; EC 2.4.2.3) (chr 7); glutathione reductase (GSR; EC 1.6.4.2) (chr 8); adenylate kinase 1 (AK-1; EC 2.7.4.3) and soluble aconitase (ACON-S; EC 4.2.1.3) (chr 9); soluble glutamate oxaloacetate transaminase (GOT-S; EC 2.6.1.1) and adenosine kinase (ADK; EC 2.7.1.20) (chr 10); lactate dehydrogenase A (LDH-A; EC 1.1.1.27) and esterase A4 (EsA-4; EC 3.1.1.1) (chr 11); lactate dehydrogenase B (LDH-B; EC 1.1.1.27) and peptidase B (Pep B; EC 3.4.11.-) (chr 12); esterase D (EsD; EC 3.1.1.1) (chr 13);

Abbreviations: SSV, simian sarcoma virus; kbp, kilobase pair(s); bp, base pair(s).

purine nucleoside phosphorylase (NP; EC 2.4.2.1) (chr 14); mannose phosphate isomerase (MPI; EC 5.4.1.8), pyruvate kinase 3 (PK-3; EC 2.7.1.40), and hexosaminidase A (Hex A; EC 3.2.1.30) (chr 15); adenine phosphoribosyltransferase (APRT; EC 2.4.2.7) and NADH diaphorase 4 (DIA-4; EC 1.6.2.2) (chr 16); galactokinase (GALK; EC 2.7.1.6) (chr 17); peptidase A (Pep A; EC 3.4.11.-) (chr 18); glucosephosphate isomerase (GP I; EC 5.3.1.9) and peptidase D (Pep D; EC 3.4.13.9) (chr 19); adenosine deaminase (ADA; EC 3.5.4.4) (chr 20); soluble superoxide dismutase (SOD-1; EC 1.15.1.1) (chr 21); mitochondrial aconitase (ACON-M; EC 4.2.1.3) and NADH diaphorase 1 (DIA-1; EC 1.6.2.2) (chr 22); hypoxanthine phosphoribosyltransferase (HPRT; EC 2.4.2.8) and glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) (X chromosome).

The presence of the short arm of human chromosome 6 (chr 6p) was also determined by blot hybridization with a molecularly cloned probe of DNA for the HLA histocompatibility antigen (33). Human–mouse hybrid cell lines were also tested for sensitivity to diphtheria toxin, the toxin receptor having been mapped to human chromosome 5 (32). The human chromosome content of some hybrids was confirmed by karyotypic analysis of samples of these same cell populations by using alkaline Giemsa or Giemsa-banding procedures (34, 35).

DNA Isolation and Restriction Endonuclease Digestion. DNA samples were prepared from hybrid cell lines by the method of Blin and Stafford (36). Control DNA samples were similarly prepared from mouse and Chinese hamster livers and human placenta. Enzymatic digestions were carried out with *Eco*RI according to standard procedures.

Filter Hybridization. Digested DNA samples were size-fractionated by 1% agarose gel electrophoresis and transferred to nitrocellulose or diazobenzyloxymethyl-derivatized paper. Hybridization was carried out in 50% formamide/0.75 M NaCl/0.075 M sodium citrate/20 mM sodium phosphate, pH 6.5/0.1% Ficoll/0.1% polyvinylpyrrolidone/0.1% bovine serum albumin/sheared, denatured salmon sperm DNA at 100 μg/ml/10% dextran sulfate/^{32}P-labeled v-*sis* DNA at 20 ng/ml. The v-*sis* probe was prepared by nick-translation of the *Sac* I/*Xba* I fragment (Fig. 1*A*) derived from λ-SSV-11 cl 1 (19).

RESULTS

The structure of the SSV genome has been determined by analysis of DNA clones containing the integrated form of SSV (19, 20). As shown on the restriction map of SSV DNA (Fig. 1), v-*sis* is located near the 3′ end of the viral genome. A 970-base pair (bp) subgenomic *Sac* I/*Xba* I fragment composed entirely of v-*sis* sequences was subcloned in pBR322 and used as a molecular probe for the detection of c-*sis* (human). Because *sis* sequences are known to be conserved within the DNAs of mammalian species, it was necessary to select a restriction enzyme that would make it possible to distinguish the c-*sis* (human) sequence from corresponding sequences present in rodent DNAs. The results of analysis of DNAs digested with *Eco*RI are shown in Fig. 1*B*. A single 20-kbp fragment of v-*sis*-related DNA was detected in human DNA. This 20-kbp fragment was readily distinguished from a *sis*-related 17-kbp fragment present in Chinese hamster DNA and three fragments (17, 10, and 3 kbp) in mouse DNA (Fig. 1*B*).

Somatic cell hybrids formed between human and either mouse or Chinese hamster cells are known to segregate human chromosomes (37). As shown in Fig. 2, hybridization of v-*sis* to *Eco*RI-restricted DNAs from 24 such hybrids detected the homologous human sequence in only 6 of the lines (lines 1, 2, 3,

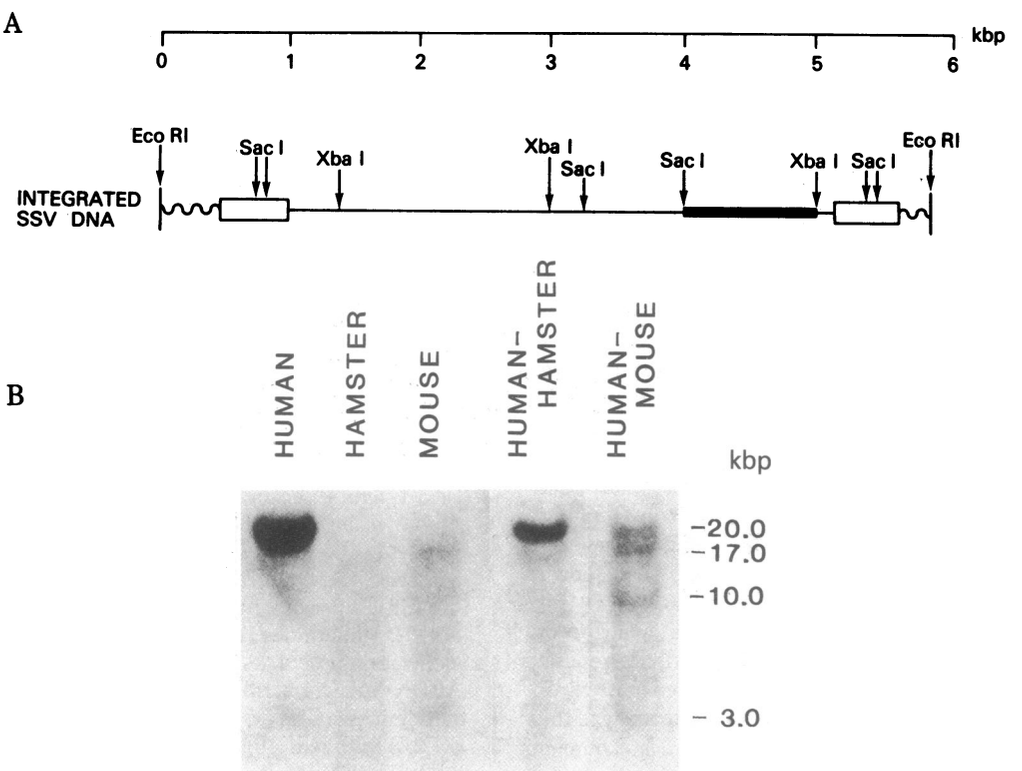

FIG. 1. (*A*) Map of the cloned SSV genome. □, The large terminal repeat (LTR) sequences; ■, the v-*sis* transforming region. (*B*) Hybridization of v-*sis* to *Eco*RI-digested genomic DNAs. Human placenta, Chinese hamster kidney, and mouse liver DNAs were digested with *Eco*RI. The digests of these DNAs were run individually in the first three lanes starting at the left. Mixtures (50:50) of human–Chinese hamster DNA and human–mouse DNA are shown in the remaining lanes at the right. Size separation and detection of the v-*sis*-related fragments were performed as described.

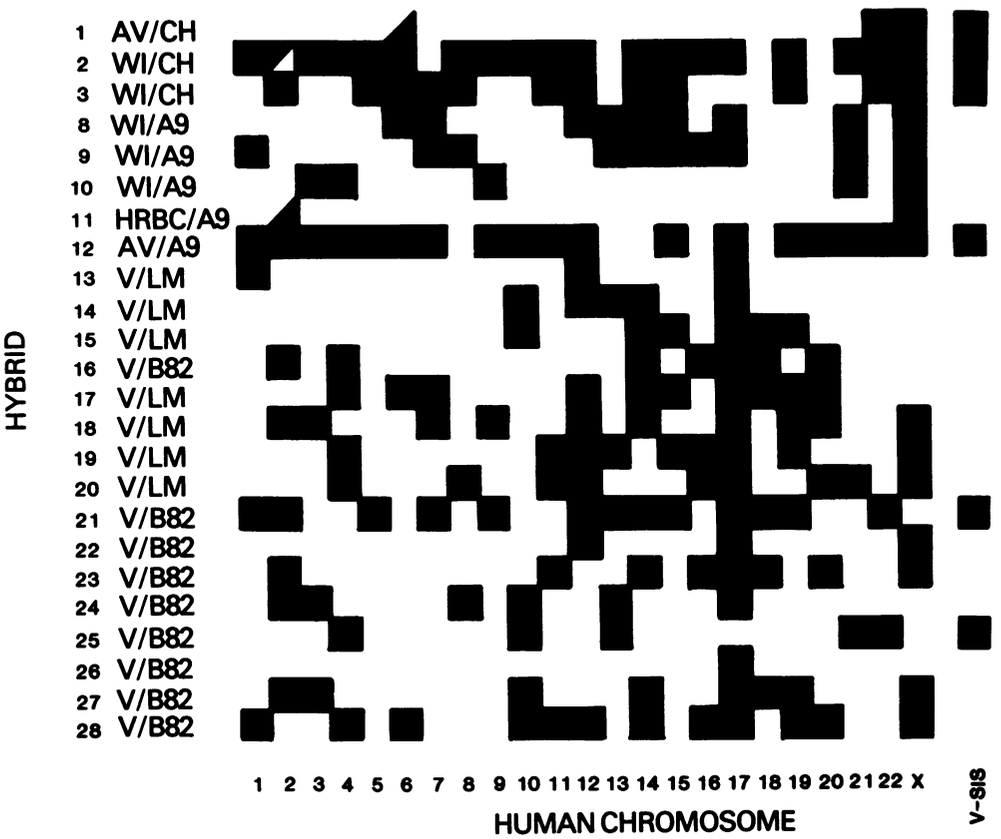

FIG. 2. Human chromosome and c-*sis* (human) distribution in somatic cell hybrids. Individual hybrid cell lines are represented on the ordinate and specific human chromosomes on the abscissa. Solid squares indicate the presence of a particular chromosome in a particular cell line; ◭ and ◤ indicate the presence of only the short arm or long arm, respectively; solid squares in the last column indicate the presence of c-*sis* (human).

12, 21, and 25). Analysis of the human chromosome content of these hybrids revealed concordance only between the presence of c-*sis* (human) and chromosome 22 (Fig. 2).

As a confirmation of the assignment of c-*sis* (human) to chromosome 22, three of the hybrid cell lines containing chromosome 22 were subcloned. This procedure served to permit further human chromosome segregation. Spontaneous loss of chromosome 22 was observed in many of the subclones. As shown in Fig. 3, detection of c-*sis* (human) by molecular hybridization was completely concordant in each series of subclones with the presence of chromosome 22.

We utilized two additional DNA probes to test the chromosomal assignment of c-*sis*. The human c-*sis* locus including flanking sequences has recently been molecularly cloned (ref. 22; unpublished observations). A DNA probe comprised of human unique sequences 9 kbp upstream of c-*sis* detected a 5-kbp *Hin*dIII fragment specifically in DNAs of those cell lines that scored as positive with the v-*sis* probe (data not shown). The human λ immunoglobulin light chain locus has recently been mapped on chromosome 22 (38, 39). When this gene was used as a molecular probe in the analysis of the same somatic cell hybrids, complete concordance of its presence with that of c-*sis* was observed (data not shown). All of the above results make it possible to assign the c-*sis* gene to human chromosome 22.

DISCUSSION

In an attempt to examine possible interactions of the human c-*sis* locus with other cellular genetic elements, we undertook its chromosomal mapping. The analytical procedures involved in mapping c-*sis* (human) utilized digestion of DNA from somatic cell hybrids with various restriction endonucleases, size fractionation of DNA fragments by electrophoresis, and Southern blotting analysis utilizing molecularly cloned v-*sis* as a probe. By selection of an appropriate restriction enzyme, this method permitted analysis despite background hybridization of the v-*sis* probe to homologous rodent DNA sequences. By analysis of a large number of hybrid cell lines containing different numbers of human chromosomes, as well as segregant subclones, we were able to assign c-*sis* (human) to human chromosome 22.

Similar molecular approaches have been utilized successfully in the chromosomal mapping of a variety of eukaryotic genes (38–43). All such studies are dependent upon the accuracy with which human chromosomes can be identified. Chromosome 22 has several biochemical markers (31, 38) which facilitate its detection in hybrid cells. However, it is possible that unanticipated translocations might cause erroneous gene assignment. In an attempt to preclude this possibility, we utilized more than one human parental cell line in the construction of the somatic cell hybrids analyzed in the present studies.

Chromosome 22 is a small acrocentric chromosome to which no cancer-associated genes have been previously assigned. However, this human chromosome is one of several in which translocations are associated with specific types of tumors. The so-called Philadelphia chromosome, found in almost all cases of chronic myelogenous leukemia, involves the reciprocal translocation of the distal portion of chromosome 22 (22q11) onto the long arm of chromosome 9 (44). The Philadelphia chromosome also has been identified in some cases of multiple myeloma as well as in other B cell neoplasms (45). A different translocation of chromosome 22 is found in many instances of Burkitt's lymphoma. The same region of chromosome 22 (22q11) is translocated but in this case onto chromosome 8 (46).

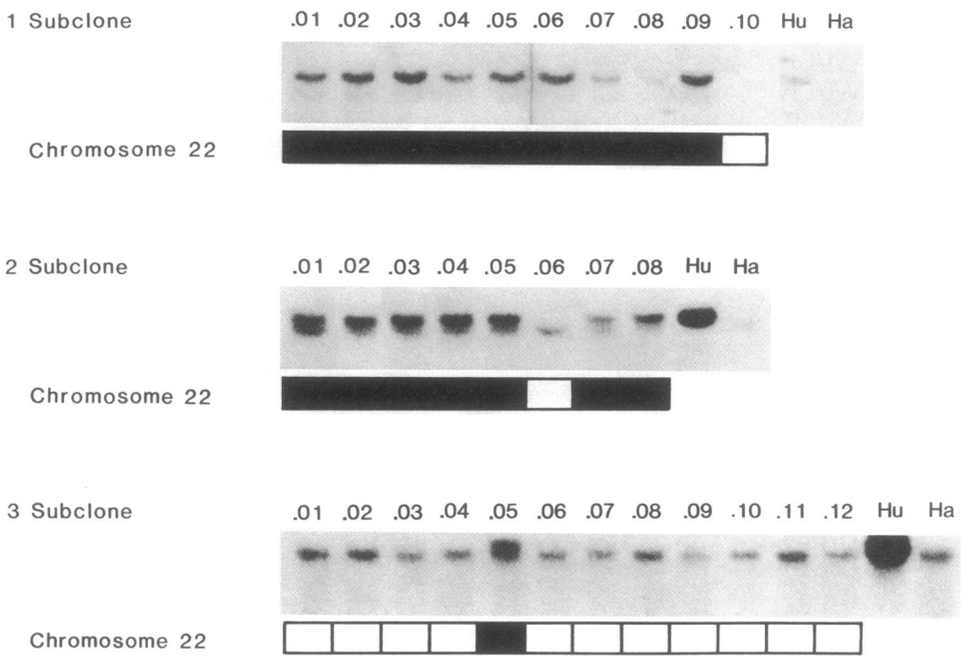

FIG. 3. Concordance of c-*sis* (human) with chromosome 22 in subclones. Three c-*sis* (human)-positive cell lines were subcloned and then analyzed for retention of the c-*sis* (human) gene. The complete karyotype of each subclone was determined, although only the presence of human chromosome 22 is indicated here (solid square). DNA from each subclone was restricted with *Eco*RI and hybridized to nick-translated v-*sis* after transfer to nitrocellulose filters. In each series, human placenta (Hu) and Chinese hamster (Ha) DNA samples were included as controls.

It has been suggested that the specific translocations associated with certain tumors may cause activation of genes involved in the transformation process (47). The evidence for gene activation is strengthened when one considers the translocations most commonly seen in certain B cell malignancies. These involve translocations of chromosomes 2, 14, and 22 into chromosome 8. The human immunoglobulin genes for the κ and λ light chains and the heavy chains are known to be on chromosomes 2, 22, and 14, respectively (38, 48). Analogous, nonrandom chromosome translocations in mouse plasmacytomas also involve chromosomes containing immunoglobulin genes (49). Translocations involving such chromosomes may be related to the DNA rearrangements that are known to take place in the course of B cell differentiation (50). It must be noted that specific chromosomal rearrangements associated with other cancers, such as acute myelogenous leukemia and acute nonlymphocytic leukemia, involve other chromosomes as well (44). Although the translocations associated with those malignancies cannot be dependent upon immunoglobulin gene rearrangements, it is possible that other DNA rearrangements are involved.

In efforts to explore the possible role of c-*sis* (human) in malignancy, we have sought to study a variety of human tumors for the presence of transcripts related to this retroviral *onc* gene (23, 24). In a limited number of tumors and tumor cell lines derived from B cell lymphomas or chronic myelogenous leukemias so far analyzed, *sis*-related transcripts have not been detected. These findings argue that translocations involving chromosome 22 in those tumors have not resulted in *sis* gene activation. However, *sis*-related transcripts have been detected in tumor cell lines derived from certain human sarcomas and glioblastomas (23). It will be of interest to determine whether translocations or other detectable aberrations affecting chromosome 22 can be detected in these tumor cells.

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