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5′ Viral and Human Cellular Sequences Corresponding to the Transforming Gene of Simian Sarcoma Virus

Abstract. *The 5′ nucleotide sequences of the transforming gene of simian sarcoma virus (v-*sis*) and its human cellular homolog (c-*sis*) were compared. A short homology was found between helper virus and cellular DNA sequences at the junction of v-*sis *and c-*sis*, which may have had a role in the original recombination event leading to the generation of simian sarcoma virus.*

Retroviruses are etiological agents in naturally occurring leukemias and lymphomas in a number of animal species (*1*). Most of these viruses cause disease after long latency periods, and their genomes do not contain a definable transforming gene. In contrast, the rapidly transforming retroviruses contain transforming genes (v-*onc* genes) as a result of recombination with normal cellular (c-*onc*) genes (*2*). The latter are highly conserved and may be important for cell growth or differentiation (or both). The simian sarcoma virus (SSV), a transforming virus isolated from a spontaneous fibrosarcoma of a pet woolly monkey (*3*) contains a 1.0-kilobase (kb) transforming gene (v-*sis*) that is derived from woolly monkey cellular DNA sequences (*4–7*). The helper virus (SSAV) associated with SSV is highly homologous to the gibbon ape leukemia viruses (GaLV's) (*8*). Thus, an infection of the woolly monkey by a virus of the GaLV group probably preceded the generation of SSV.

In the human genome, *sis*-related sequences are present at a single locus, which is interrupted by at least four stretches of nonhomologous cellular sequences (*9, 10*). A 4.2-kb c-*sis* transcript is detected in some human tumor tissues (*11, 12*), suggesting that the functional c-*sis* gene contains coding sequences additional to the 1.0-kb v-*sis* gene. The exact relation of the viral to the cellular gene homolog and the molecular basis of the mechanism by which SSV and other *onc*-containing retroviruses are generated are not known, although several possibilities have been proposed (*13, 14*). As a step toward understanding such mechanisms, we have compared the nucleotide sequences at the 5′ recombination site of SSV to the 5′ analogous region of the related human cellular homolog, c-*sis*. We found a 6-base-pair (bp) sequence homology between helper virus and cellular DNA sequences immediately preceding the v-*sis* and c-*sis* regions of homology. This 6-bp homology may have played an important role in the recombination process that led to the generation of SSV.

The complete nucleotide sequence of v-*sis* has been determined [(*15*) and our unpublished data]. The results showed that the v-*sis* gene potentially codes for a 27,000-dalton protein that is initiated within the helper-derived sequences and is terminated at a site two-thirds within the v-*sis* substitution. To understand better the 5′ boundary of the cellular *sis* gene, we determined a portion of the nucleotide sequence of a human c-*sis* gene. The structure of a recombinant DNA, L33, containing 12 kb of human DNA, including the entire 1.0 kb of v-*sis* homologous sequences, is shown in Fig. 1. The five noncontiguous regions of homology, designated as black boxes numbered 1 to 5, are colinear with v-*sis*, as determined by heteroduplex analyses and restriction enzyme mapping (*10*). The 5′ Eco RI–Pst I fragment was subcloned into the plasmid pBR322 (*16*), and a 596-nucleotide sequence, including the first region of homology and surrounding sequences, was determined by the Maxam-Gilbert procedure (*17*) (Fig. 1). This nucleotide sequence (Fig. 2A) re-

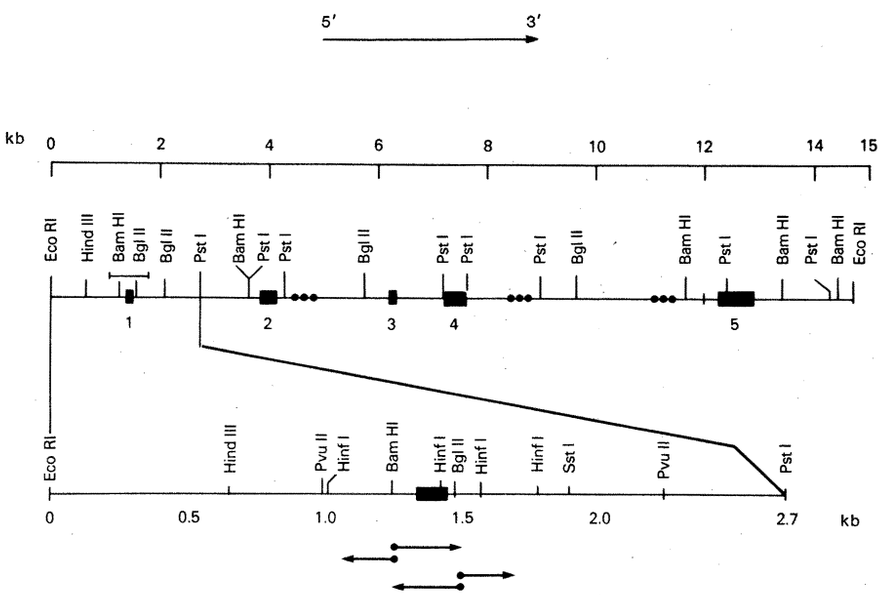


Fig. 1. Genetic structure of a human DNA clone (L33) containing sequences homologous to v-*sis*. The black boxes numbered 1 to 5 represent the noncontiguous regions of homology with v-*sis*. The dots show the location of the Alu family of repeated sequences (*10*). Arrows under the expanded map of the 5′ Eco RI–Pst I fragment indicate the directions for DNA sequencing by the Maxam-Gilbert procedure (*17*).

vealed several interesting features when aligned with SSV.

1) The first region of homology is now clearly defined. This comprises a ten-nucleotide stretch that contains ten base mismatches with v-*sis*. A single open reading frame in this region is in phase with the v-*sis* reading frame (Fig. 2B). The region encodes 33 amino acids, four of which differ from those encoded by v-*sis* because of single base substitutions. Six other base changes were silent. These changes probably reflect both the divergence of the human and woolly monkey c-*sis* sequences and that of woolly monkey c-*sis* and v-*sis*. A comparison of viral and mouse cellular counterparts of a mouse-derived *onc* gene, *mos*, also revealed some silent as well as sense alterations (*18*). Thus, even direct parental and viral *onc* counterparts have undergone divergence by as much as 1.6 percent.

2) There is no candidate initiator ATG codon, no promoter sequence analogous to TATAAAA (*19*), and no associated CCAT sequence (*20*) in the 247 nucleotides upstream from or within the first region of homology. This suggests that the functional c-*sis* gene contains additional coding sequences (exons) 5′ to the start of homology with v-*sis*.

3) A donor splice site (*21*) located at the 3′ end of the region of homology (Fig. 2B) may mark the beginning of an intron of c-*sis*. However, there are three potential acceptor splice sites in the 5′ half of the sequence. One, located at +5 within the region of homology at position 252, has the sequence CCTGGCAG. This is conserved in v-*sis* (see Fig. 2B and below). Two additional splice sites, with the sequences ACTCCCAG and CTCACTCAG, are located at −27 and −18 bases, respectively, upstream from the region of homology. The open reading frame extends through these two points. Because of the high degree of

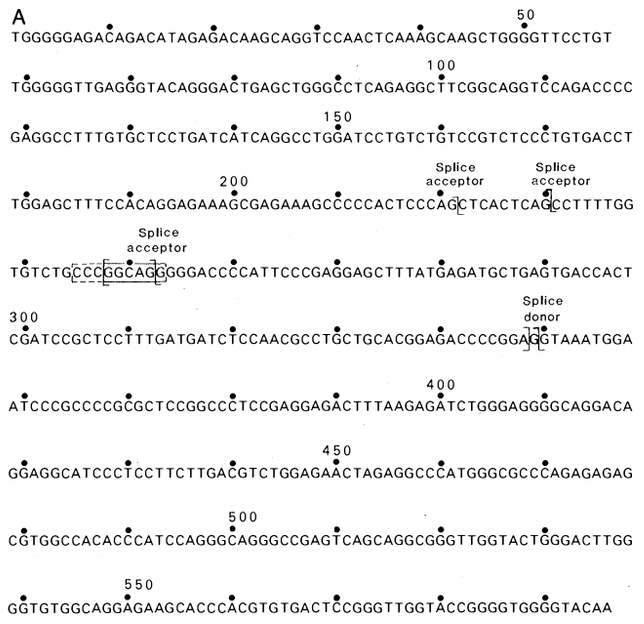
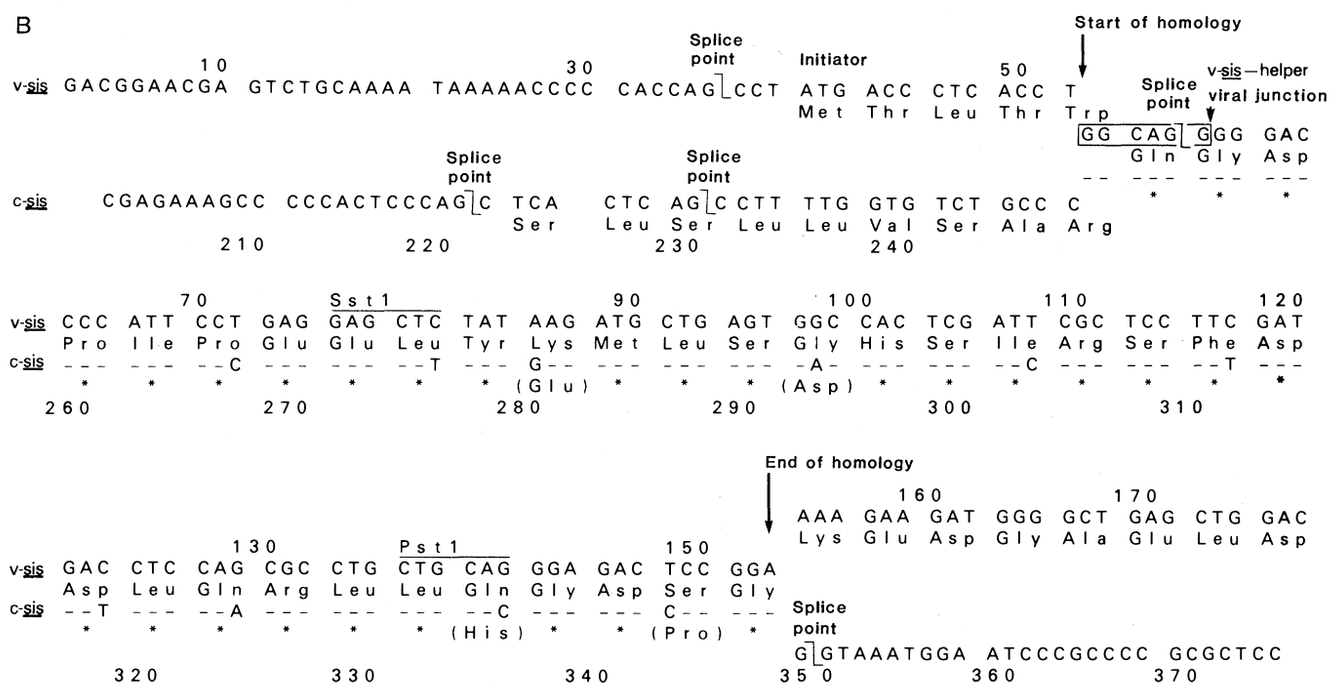


Fig. 2. (A) Partial nucleotide sequence of human c-*sis*. The brackets denote the boundaries of the first region of homology to SSV, which extends from positions 248 to 348. The boxed six bases are also present in SSAV. Potential donor and acceptor splice points are indicated. (B) Comparison of SSV and c-*sis* sequences. A, adenine; C, cytosine; G, guanine; and T, thymine. The six-base sequence GGCAGG is present in SSV, SSAV (not shown), and c-*sis*. The DNA sequence homology of c-*sis* and v-*sis* is shown by the dashed lines from c-*sis* positions 248 to 348 with the nonhomologous bases inserted. The potential acceptor and donor splice sites of c-*sis* are at positions 221, 230, 252, and 349. A potential acceptor splice site in v-*sis* is located at position 36. The restriction sites for Sst I and Pst I on v-*sis* are overlined. The predicted translation products are shown with the homology in c-*sis* indicated by asterisks, and the different amino acids are indicated in parentheses.



homology between human c-*sis* and v-*sis*, we would expect the woolly monkey c-*sis* locus from which v-*sis* has been derived to be similar to human c-*sis*. If so, the cellular c-*sis* exon could extend to either of two possible splice sites and begin upstream from the region of homology. This allows the possibility that the recombination event that led to v-*sis* formation may have occurred within a cellular exon.

4) A six-base sequence (GGCAGG) is present in SSV, SSAV (*15*), and c-*sis* at the 5′ junction of the v-*sis* substitution (boxed in Fig. 2, A and B). The location of such a homology between helper virus and cellular DNA at the exact site of recombination is not likely to be due to chance (probability $< 3 \times 10^{-4}$). Furthermore, this is a minimal estimate for the homology that may exist between SSV and c-*sis* of the woolly monkey, the host of origin (*6, 7*). For example, the homology of SSAV and c-*sis* may be extended to nine bases (CCTGGCAGG) by allowing a possible change of C to T for the corresponding woolly monkey DNA sequence. This short homology between helper virus and cellular DNA may have played a role in the initial recombination event.

The following sequence of events could have occurred. An SSAV provirus was integrated upstream from c-*sis* in the woolly monkey tumor DNA. Recombination occurred between the homologous regions, deleting out the intermediary sequences. This brought into proximity the defective helper virus genome and c-*sis*. Goldfarb and Weinberg (*14*) proposed that the first step in generation of defective transforming viruses is integration of the helper virus genome adjacent to a cellular *onc* gene followed by read-through transcription. Our result suggests that a legitimate or homologous recombination between the helper virus and the cellular *sis* gene at the DNA level may have occurred first, deleting out a part of the helper virus genome including the transcriptional termination signals. Consequently, the initial read-through transcript was a hybrid molecule of defective helper viral sequences and cellular sequences that was then processed to remove the cellular introns. Further events, which must have occurred to account for the 3′ SSAV sequences of the transforming virus genome, might have taken place at the level of reverse transcription (*13, 14*).

Our studies suggest that v-*sis* may contain only the 3′ portion of the functional c-*sis* gene and that a short sequence homology between SSAV and c-*sis* may have played a role in the initial recombination event leading to the generation of SSV. Although the mechanism of recombination proposed here may not apply to all retroviral *onc* genes, it has been reported that c-*mos* and v-*mos* also share a 4-bp homology at the junction of recombination (*18*). That result would suggest that the homology need not be as extensive as the one we found.

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22. R. Dalla Favera is a Special Fellow of the Leukemia Society of America.

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Leaf Dimorphism in Aquatic Angiosperms: Significance of Turgor Pressure and Cell Expansion

Abstract. *Depending on environmental conditions, the aquatic angiosperm* Callitriche heterophylla *can develop two different leaf types with distinctive morphological characteristics. Cellular turgor pressure seems to act as the biophysical mechanism responsible for the selection of leaf form in control conditions designed to mimic the natural habitat of this plant. The experimental induction of leaf form involves the ability of various treatments to mediate cell expansion through their effects on turgor pressure or wall extensibility.*

Botanists have long been intrigued by the problem of leaf development in aquatic angiosperms (*1*). In the natural environment the leaves of these plants can develop into two alternative forms depending on the position of the shoot meristem relative to the water surface. The elongate, often dissected leaves that originate on submerged apices are called water forms and the shortened, broad leaves from emergent apices are designated land forms. It is possible to force immature leaves to develop into the form atypical for a given environment by applying a wide variety of treatments, including different light exposures, growth regulators, osmotica, and temperature extremes (*2*). This marked sensitivity to environmental conditions and cultural manipulations has made aquatic angiosperms favored experimental organisms in the search to identify the basic mechanisms that control leaf development in all plants. In the present study we attempted to determine whether cellular water relations play a causal role in the developmental choice between alternative leaf forms in aquatic angiosperms.

Clonal specimens of the aquatic *Callitriche heterophylla* Pursh. were grown at 23° ± 2°C in aquariums (submerged apices) or on wet soil (emergent apices) in a growth chamber with a photoperiodic cycle having 16 hours of white light (12 W/m^2) and 8 hours of darkness. Representative shoots grown under the two cultural conditions are shown in Fig. 1, A and B. Water-form leaves on submerged apices exhibit higher ratios of length to width, lower stomatal densities, and fewer vascular bundles than do land forms on emergent apices.

Repeated sprayings with $10^{-5}M$ gibberellic acid caused the growth of typical water-form leaves on emergent shoots, whereas the inclusion of $10^{-5}M$ abscisic