

c-sis IS TRANSLOCATED FROM CHROMOSOME 22 TO CHROMOSOME 9 IN CHRONIC MYELOCYTIC LEUKEMIA*

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Several relatively specific chromosomal translocations are known to be associated with particular human cancers (1–3). One of these, the Philadelphia translocation, t(9;22) (q34;q11) is observed in over 90% of chronic myelocytic leukemias (CML)¹ (3). Translocation of the q11 to qter segment of chromosome 22 to chromosome 9 results in a deleted form of chromosome 22, referred to as the Philadelphia (Ph') chromosome (4). Recently, we have localized a human oncogene, *c-abl*, on chromosome 9 (q34 to qter) (5) and demonstrated its translocation to chromosome 22q- (the Ph' chromosome) in CML (6). Because of the small size of the segment of chromosome 9 that translocates to chromosome 22 (6) and the localization of immunoglobulin λ light chain sequences on chromosome 22 (7), *c-abl* appears to map in close proximity to λ sequences in the Ph' chromosome.

Another acute transforming retrovirus, the Simian sarcoma virus (SSV), is a genetic recombinant between a nontransforming retrovirus and cellular sequences of woolly monkey origin (8, 9). The SSV transforming gene, *v-sis*, and its human cellular homologue, *c-sis*, have been molecularly cloned (8–10), and *c-sis* has been localized on the q arm of chromosome 22 (11, 12). In the present study, we report the localization of *c-sis* on the q11 to qter segment of chromosome 22 and its translocation from chromosome 22 to chromosome 9 in CML.

Materials and Methods

Cells. Cell lines, propagated in Dulbecco's modified Eagle's medium supplemented with 10% calf serum, included NIH/3T3 mouse cells and a human cell line, A673 (5). Somatic cell hybrids containing full complements of either mouse or Chinese hamster chromosomes and a limited number of human chromosomes were derived by fusion of either mouse or Chinese hamster cells with leukocytes from different CML patients or from normal donors (Table I); details concerning their origin and initial characterization have been previously reported (13, 14).

Preparation of a Human *c-sis* Probe (*c-sis* B_{1,2}). A cosmid clone with a cellular insert of ~30 kb containing *v-sis*-homologous sequences was isolated from a library of human lung

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¹ Abbreviations used in this paper: CML, chronic myelocytic leukemia; Ph', Philadelphia chromosome; SSV, Simian sarcoma virus.

carcinoma DNA (15) using, as a probe, a 1.2 kb PstI *v-sis* restriction fragment in pBR322 (8), generously provided by K. Robbins and S. A. Aaronson. Isolation of a 1.7 kb BamHI *v-sis*-homologous restriction fragment from the cosmid clone was performed according to previously described methods (15).

Molecular Hybridization. Restriction enzymes were purchased from New England Biolabs, Beverly, MA and Bethesda Research Laboratories, Rockville, MD and were used according to the suppliers' specifications. DNA were digested with restriction enzymes, subjected to electrophoresis through 0.75% agarose gels, and transferred to nitrocellulose essentially as described by Southern (16). Nick translation of probes and filter hybridization were as described (15). Specific activity of the probes was $2-5 \times 10^8$ cpm/ μ g. After hybridization, filters were washed under high stringency conditions (10% standard saline citrate, 65°C) and exposed to XAR-2 film (Eastman Kodak Co., Rochester, NY) for up to 5 d at -70°C with Dupont Lightning Plus intensifying screens (Dupont Instruments, Wilmington, DE).

Results

To prepare a probe suitable for identification of somatic cell hybrids containing human *c-sis* sequences, a previously described (15) cosmid library of human lung carcinoma DNA was screened for clones containing sequences homologous to the 1.2 kb PstI *v-sis* probe. As shown in Fig. 1, a single cosmid clone was obtained containing a 30 kb cellular insert with *v-sis*-homologous cellular sequences. By restriction endonuclease analysis this clone was shown to correspond to a previously described *v-sis*-homologous human sequence, designated *c-sis* (12). For generation of a *c-sis*-specific probe, a single 1.7 kb BamHI fragment, possessing strong homology to *v-sis* (*c-sis* B_{1.7}), was isolated from the cosmid cellular DNA insert.

Human and mouse control cellular DNA were digested with Sst-I and analyzed for homology to the above described *c-sis* B_{1.7} probe. As shown in Fig. 1, a single mouse cellular restriction fragment of around 10.0 kb (lane B) is detected, while the only human *c-sis* B_{1.7} cross-reactive Sst-I restriction fragment is 3.6 kb in length (lane C). The size of the latter restriction fragment corresponds to that predicted on the basis of the human *c-sis* restriction map shown in Fig. 1. Cellular DNA from a mouse \times human somatic cell hybrid, PgMe-25NU, previously shown to have chromosome 22 as its only human component (14), contains the 3.6 kb human *c-sis* B_{1.7} cross-reactive Sst-I restriction fragment (lane A), thus confirming the mapping of *c-sis* on chromosome 22. Localization of *c-sis* to the region of chromosome 22 (q11 to qter) which is translocated to chromosome 9 in CML, is established by the absence of *c-sis*-homologous sequences from hybrid WESP-2A (lane D), which contains chromosome 22q- (the Ph' chromosome) but lacks detectable amounts of chromosomes 9, 22, or 9q+ (5).

To independently show the localization of *c-sis* on chromosome 22 (q11 to qter) and demonstrate its translocation to chromosome 9 in CML, a series of Chinese hamster \times human somatic cell hybrids were analyzed for human *c-sis* sequences. As shown in Fig. 1, lanes E and F, the only *c-sis* B_{1.7} cross-hybridizing Sst-I restriction fragment in Chinese hamster cellular DNA is around 2.7 kb in size and thus clearly resolved from the 3.6 kb human Sst-I fragment. Hybrid 1CB-17aNU, which contains chromosome 22q-, lacks detectable human *c-sis* sequences (lane F), while a second hybrid, 14CB-21A, containing chromosome 9q+ but not chromosome 9 or 22 (13), is positive for human *c-sis* sequences (lane

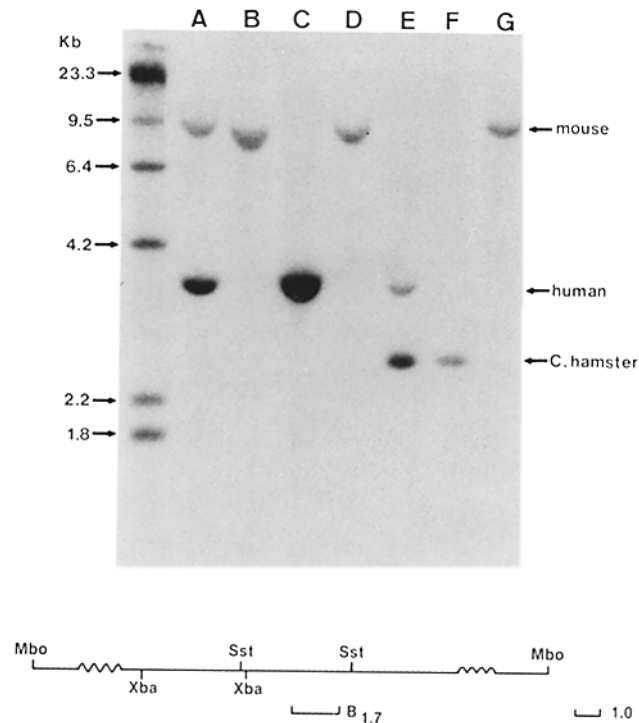


FIGURE 1. Localization of human *c-sis* on chromosome 22 (q11 to qter) and its translocation to chromosome 9 in CML. *Sst*I-digested cellular DNA (10 μ g/lane) were electrophoresed on 0.7% agarose gels, blotted to nitrocellulose, and hybridized to the *c-sis* B_{1.7} probe shown in the lower portion of the figure. Cell lines analyzed are described in Table I and include PgMe25Nu (A), NIH/3T3 (B), A673 (C), WESP 2A (D), 14CB21A (E), 1CB17ANu (F). The positions of single mouse, human, and Chinese hamster *c-sis* B_{1.7} homologous *Sst*I restriction fragments are shown. Hind III-digested DNA, included as a molecular weight marker, is shown on the left side of the figure. In the lower portion of the figure, the restriction map of the 30 kb cellular insert from the *v-sis* cross-reactive cosmid clone of *Mbo*I-digested human lung carcinoma DNA is shown. *Mbo*I (Mbo) sites indicate the ends of the insert; the positions of *Xba*I (Xba) and *Sst*I (Sst) restriction sites are shown for purposes of orientation of this clone with the more detailed previously published restriction maps of *c-sis* (10). The position of a single 3.6 kb human *Sst*I restriction fragment overlapping with the 1.7 kb *Bam*HI restriction fragment (B_{1.7}), used as a *c-sis*-specific probe for analysis of somatic cell hybrids, is also shown.

E). Finally, hybrid 10CB-23B, which contains chromosome 9 in the absence of detectable 22, 9q+, or 21q-, lacks human *c-sis* (Table I). As internal controls, each of the above hybrids were also analyzed for *c-abl*, a marker for the portion of chromosome 9 translocated to chromosome 22 in CML (6), and for AKI, which maps near the breakpoint but within the nontranslocated portion of chromosome 9 (Table I).

Discussion

Several of the human cellular homologues of viral oncogenes studied to date including *c-abl* (5, 6), *c-sis* (11, 12), *c-fes* (5, 17), and *c-mos* (18, 19) have been localized on human chromosomes frequently involved in translocations associated with specific human cancers. One of these, *c-myc*, is translocated from chromo-

TABLE I
Translocation of *c-sis* from Chromosome 22 to Chromosome 9 in Chronic Myelocytic Leukemia

Cell line	AK1	Human chromosomes				Oncogenes	
		9	22	9q+	22q-	<i>c-abl</i>	<i>c-sis</i>
Mouse NIH/3T3	NT	-	-	-	-	-	-
Human A673	NT	+	+	+	+	+	+
Mouse × human hybrid							
PgMe-25NU	-	-	+	-	-	-	+
WESP-2A	-	-	-	-	+	+	-
Chinese hamster × human hybrid							
10CB-23B	+	+	-	-	-	+	-
14CB-21A	+	-	-	+	-	-	+
1CB-17a NU	-	-	-	-	+	+	-

PgMe-25NU cells contain chromosome 22 as their only human component, while each of the other five hybrid clones contain a few human chromosomes in addition to those relevant to the t(9;22) (q34;q11) translocation (13, 14). Identification of hybrid clones containing *c-abl* sequences (6) and analysis of human adenylate kinase (AK1) enzymatic activity (6) have been previously reported. Cells were analyzed for human *c-sis*-specific sequences as described in Fig. 1.

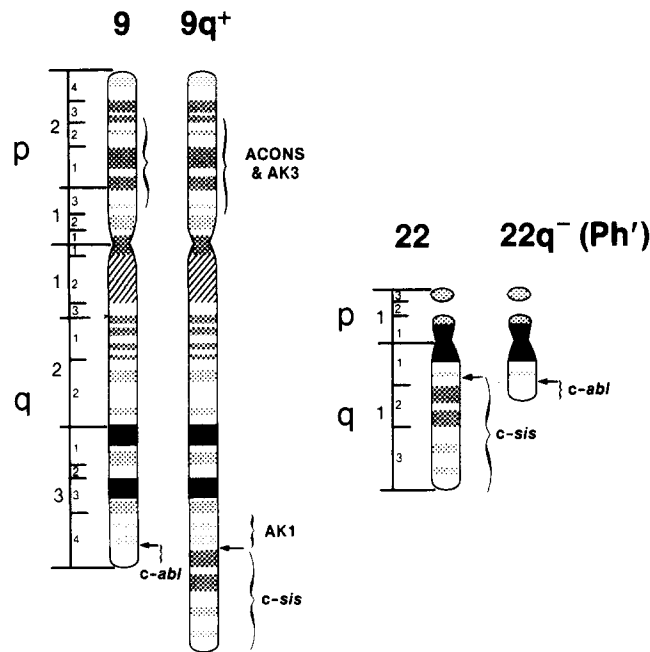


FIGURE 2. Diagrammatic representation of the involvement of *c-abl* and *c-sis* in the Ph' translocation. Chromosome banding patterns are as previously shown by Yunis (3); map positions of ACONS, AK3, and AK1 are as previously reported (24, 25). Localization of *c-abl* within the terminal portion of chromosome 9 (q34), which is translocated to chromosome 22 in CML, is as described by de Klein et al. (6) while localization of *c-sis* in the region of chromosome 22 (q11 to qter) translocated to chromosome 9 is based on the results of the present study.

some 8 to chromosomes 14, 2, or 22, each of which contain immunoglobulin sequences, in Burkitt's lymphoma (1, 19-21). Similarly, *c-abl* maps on the region of chromosome 9 which translocates to chromosome 22 in CML (5, 6). Other translocations, such as the t(15;17) reciprocal translocation associated with acute promyelocytic leukemia (3), involve regions to which human cellular oncogenes (in this case *c-fes*) have been mapped (22), but appear to be independent of immunoglobulin sequences. The present demonstration that *c-sis* is translocated from chromosome 22 to chromosome 9 in CML raises the possibility that *c-sis* rather than *c-abl* may be involved in CML. Resolution of these alternatives will require a determination of the proximity of these genes to the breakpoints in chromosomes 22 and 9, respectively, and analysis of the expression of their transcriptional and translational products in CML cells.

In addition to possible implications regarding the cause of CML and the significance of the associated t(9;22) (q34;q11) translocation, the localization of *c-sis* within the translocated region of chromosome 22 (Fig. 2) provides a unique molecular marker for studies of the more complex translocations associated with minority populations of CML patients. These can involve translocation of the q11 to qter region of chromosome 22 to chromosomes other than chromosome 9, or can in some instances involve more complex rearrangements including three or occasionally even four or five chromosomes (23). Analysis of these translocations using *c-abl* and *c-sis* probes should allow a determination of the critical translocation event resulting in the generation of CML.

Summary

By analysis of a series of somatic cell hybrids derived by fusion of either mouse or Chinese hamster cells with leukocytes from different chronic myelocytic leukemia (CML) patients or from normal donors, we have localized the human oncogene, *c-sis*, on the q11 to qter segment of chromosome 22 and demonstrated its translocation from chromosome 22 to chromosome 9 (q34) in CML.

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