

## Isolation and Structural Mapping of a Human *c-src* Gene Homologous to the Transforming Gene (*v-src*) of Rous Sarcoma Virus

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We have utilized a lambda Charon 4A human genomic library to isolate recombinant clones harboring a highly conserved *c-src* locus containing nucleotide sequences homologous to the transforming gene of Rous sarcoma virus (*v-src*). Four overlapping clones spanning 24 kilobases of cellular DNA were analyzed by restriction endonuclease mapping. Human *c-src* sequences homologous to the entire *v-src* region are present in a 20-kilobase region that contains 11 exons as determined by restriction mapping studies utilizing hybridization to labeled DNA probes representing various subregions of the *v-src* gene and by preliminary DNA sequencing analyses. A considerable degree of similarity exists between the organization of the human *c-src* gene and that of the corresponding chicken *c-src* gene with respect to exon size and number. However, the human *c-src* locus is larger than the corresponding chicken *c-src* locus, because many human *c-src* introns are larger than those of chicken *c-src*. *alu* family repetitive sequences are present within several human *c-src* introns. This locus represents a highly conserved human *c-src* locus that is detectable in human cellular DNAs from various sources including placenta, HeLa cells, and WI-38 cells.

The Rous sarcoma virus (RSV) oncogene, *v-src*, is capable of inducing tumors in vivo and transforming cells in vitro. It is perhaps the most extensively characterized viral oncogene, and a large body of experimental information exists concerning *v-src* and its gene product, pp60<sup>v-src</sup> (3). Cellular sequences (*c-src*) homologous to *v-src* have been detected in the genomes of a wide variety of metazoan species, ranging from *Drosophila* to higher vertebrates, including humans (32, 35, 36). Of these, the chicken *c-src* gene has been the most extensively characterized and recently has been shown to contain 12 exons, 11 of which harbor coding regions that share strong nucleotide sequence homology with *v-src* (21, 30, 37, 39). It is likely that *v-src* was acquired by an avian retrovirus through recombination with the cellular genome. Plausible mechanisms for acquisition of *v-src* through recombination of a retrovirus with the chicken *c-src* locus have been described (20, 37, 39).

The RSV *v-src* and the chicken *c-src* genes code for phosphoproteins pp60<sup>v-src</sup> and p60<sup>c-src</sup>, respectively, which possess a tyrosine-specific protein kinase activity (6, 7, 19). Although pp60<sup>v-src</sup> and chicken pp60<sup>c-src</sup> are closely related, differences between them have been reported with respect to sites of tyrosine phosphorylation (33) and amino acid sequence and antigenicity of their COOH termini (28, 39). In addition, the level of pp60<sup>v-src</sup> in RSV-transformed cells is approximately 100 times higher than pp60<sup>c-src</sup> levels in normal cells (3). Recent evidence suggests that the oncogenic properties of pp60<sup>v-src</sup> may result from structural and functional differences existing between pp60<sup>v-src</sup> and chicken pp60<sup>c-src</sup> rather than from purely quantitative differences in dosage (12, 20, 22, 29).

Recently, transcriptional activation, gene amplification, or mutational or physical alteration of certain proto-oncogenes has been demonstrated in several animal and human neoplasms (13, 23, 38). These findings point to a general model suggesting that many human neoplasms arise through the involvement of activated or altered proto-oncogenes (2). A comprehensive analysis of the precise mechanisms involved in the causation of a wide variety of human neoplasms would be facilitated by knowledge of the structure, activity, and regulation of various human proto-oncogenes and of the changes affecting these genes which may lead to or accompany neoplasia.

At this time, relatively little is known about human *c-src* loci and their gene products. A human pp60<sup>c-src</sup> phosphoprotein has been detected in cells after immunoprecipitation with antiserum obtained from RSV tumor-bearing rabbits (19, 25, 27). In at least two human cell lines, two distinct forms of pp60<sup>c-src</sup> that differ in apparent molecular weights and sites of phosphorylation have been described (31). It is not known whether these two forms of human pp60<sup>c-src</sup> are synthesized in all human cell types, are products of two structural genes, arise through alternate m-RNA splicing mechanisms, or arise through post-translational modification of a single gene product (31).

In this report, we describe the initial characterization of a major, highly conserved human *c-src* locus that has been isolated from a lambda phage genomic library (16). Restriction mapping, Southern hybridization to labeled probes representing subregions of *v-src*, and preliminary nucleic acid sequencing studies indicated that most human *c-src*-coding sequences are highly homologous to chicken *c-src* and RSV *v-src* sequences and have been strongly conserved throughout evolution. A somewhat lower degree of sequence conservation was observed in certain exons. Also because human *c-src* introns are larger than corresponding chicken *c-src* introns, the human *c-src*-coding region spans a

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distance that is over three times as long (approximately 20 kilobases [kb]) as the corresponding chicken *c-src*-coding region (6 kb) (30, 39).

### MATERIALS AND METHODS

**Screening of human libraries.** The *HaeIII-AluI* lambda Charon 4A human genomic library constructed by Maniatis and co-workers (16) was screened for the presence of inserts containing *c-src* sequences by the plaque transfer method of Benton and Davis (1, 18). Phage (80,000 to 250,000 PFU) were plated on *Escherichia coli* DP50supF on NZCY-DT agar (4) in Pyrex baking dishes (6 by 10 in. [ca. 15 by 25 cm] or 8 by 12 in. [ca. 20 by 30 cm]); alternatively 10,000 to 40,000 PFU were plated on 150-mm petri dishes. Replicas were transferred to nitrocellulose filters (Millipore Corp. or Schleicher & Schuell Co.), and DNA was denatured and fixed as described previously (1). Filters were hybridized at 38°C for 24 to 48 h in 100 to 300 ml of solution containing  $3 \times 10^6$  to  $10 \times 10^6$  cpm of  $^{32}\text{P}$ -labeled, nick-translated RSV *v-src* DNA, 50% formamide,  $5 \times \text{SSC}$  ( $1 \times \text{SSC}$  is 0.15 M NaCl plus 0.15 M sodium citrate, pH 7.0),  $1 \times \text{Denhardt}$  buffer (11), 20 mM  $\text{PO}_4$  (pH 6.5), 10% dextran sulfate, 4 to 17  $\mu\text{g}$  of denatured total *E. coli* nucleic acid per ml, and 50 to 200  $\mu\text{g}$  of yeast RNA per ml (solution A). Before hybridization, filters were washed for 5 h at 42°C in solution A without  $^{32}\text{P}$ -labeled DNA. Filters were washed after hybridization for 30 min at 42°C in  $1 \times \text{SSC}$ -0.1% sodium dodecyl sulfate (SDS) and then two times for 30 min each at 42°C in  $0.2 \times \text{SSC}$ -0.1% SDS. Signals were detected with Kodak XAR film and Du Pont Cronex intensifying screens. Clones containing *c-src* sequences were purified by repeated plaque purification cycles.

**Restriction endonuclease mapping.** DNA was digested with restriction endonucleases purchased from New England Biolabs, Bethesda Research Laboratories, or Boehringer-Mannheim with a two- to sixfold excess of enzyme and conditions specified by the supplier. Digested DNAs were electrophoresed on agarose gels (0.7 to 1.6%) and transferred to nitrocellulose sheets (Schleicher & Schuell) as described by Southern (34) with  $20 \times \text{SSC}$  buffer. Most hybridizations were performed with appropriate  $^{32}\text{P}$ -labeled DNAs at 37 to 38°C for 15 to 40 h in solution A (see above). Before hybridization, filters were pre-annealed at 37°C for 4 to 18 h in the same solution without  $^{32}\text{P}$ -labeled DNA. In hybridizations involving digests of human genomic DNA, filters were agitated at 38°C under the hybridization conditions of Wahl et al. (40). Filters were subjected to the following washes (30 min each): three times in  $2 \times \text{SSC}$  at room temperature, once in  $1 \times \text{SSC}$ -0.1% SDS at 37°C, two times in  $0.2 \times \text{SSC}$ -0.1% SDS at 37°C, and once with  $0.2 \times \text{SSC}$ -0.1% SDS at room temperature. Radioactive bands were detected as described above. DNA in agarose gels was stained with ethidium bromide (1  $\mu\text{g}/\text{ml}$ ) and photographed on a UV light box before Southern transfers.

**Preparation of labeled DNAs.** DNA fragments containing the entire *v-src* gene were isolated after restriction enzyme digestion of the RSV SRA-2 pBR322 plasmid provided by W. DeLorbe, H. Varmus, and M. Bishop (10). Fragments containing subregions of *v-src* were isolated from plasmid subclones containing specific restriction enzyme cleavage fragments of the *v-src* gene. Appropriate DNA fragments were electroeluted from gel slices (18) and labeled by nick translation (24). In some cases total plasmid DNAs, rather than DNA fragments, were labeled. The specific activities of the labeled DNAs were  $1 \times 10^7$  to  $50 \times 10^7$  cpm/ $\mu\text{g}$ . *E. coli* DNA polymerase I was purchased from New England Biolabs, and DNase I was from Sigma Chemical Co.

**Growth of lambda phage and preparation of phage DNA.** Lambda phage were grown on *E. coli* DP50 SupF by the PDS method of Blattner et al. (4). Phage were concentrated with polyethylene glycol (41) and purified on CsCl gradients. DNA was prepared by the formamide method (9), treated with proteinase K (10  $\mu\text{g}/\text{ml}$ ) at 37°C for 30 min in STE buffer (0.01 M Tris-hydrochloride [pH 7.4], 0.001 M EDTA, 0.1 M NaCl), extracted twice with phenol, and ethanol precipitated.

Alternatively DNA was prepared by adding DNase and RNase (10  $\mu\text{g}/\text{ml}$  each) to phage lysates after clarification at  $8,000 \times g$  for 10 min at 4°C. Phage pellets were prepared by centrifugation at  $30,000 \times g$  for 2 h at 4°C, suspended in 10 mM Tris-hydrochloride-25 mM EDTA (pH 8), and incubated at 68°C for 5 min. SDS (0.2%) and proteinase K (100  $\mu\text{g}/\text{ml}$ ) were then added, and samples were incubated for an additional 30 min at 68°C. DNA was purified by two extractions with phenol-chloroform-isoamyl alcohol (24:23:1) and one extraction with chloroform, followed by ethanol precipitation.

**Preparation of cellular DNA.** Human placental DNA was prepared from placentas that had been frozen at -70°C. Frozen placental material (70 g) was minced with a razor, blended in liquid nitrogen in a steel Waring Blendor, treated for 3 h at 50°C in lysis buffer (100  $\mu\text{g}$  of proteinase K per ml, 0.5% Sarcosyl, 0.5 M EDTA [pH 8]) (5, 18), phenol extracted several times, and dialyzed against 50 mM Tris-hydrochloride (pH 8)-10 mM EDTA-10 mM NaCl. The sample was then treated with 100  $\mu\text{g}$  of RNase per ml at 37°C for 3 h, extracted twice with phenol-chloroform, and dialyzed against TE buffer (10 mM Tris-hydrochloride [pH 7.4], 10 mM EDTA). Wide-bore pipettes were used exclusively to minimize DNA shearing.

DNA was also prepared from the normal human lung fibroblast cell line WI-38 or from HeLa cells by washing cell monolayers with cold (4°C) phosphate-buffered saline and adding cold lysis buffer to the cell monolayers. Cell suspensions were incubated for 3 h at 50°C and extracted as described above.

### RESULTS

**Isolation of recombinant clones containing *c-src* sequences and strategy for analysis.** An *HaeIII-AluI* human genomic library prepared in lambda Charon 4A (16) was screened for phage harboring inserts containing sequences related to *v-src*. A  $^{32}\text{P}$ -labeled, 3.1-kb *EcoRI* fragment of cloned RSV SRA-2 DNA (10), which contains the entire 1.6-kb *v-src*-coding region, was used as a hybridization probe. A total of approximately  $10^6$  PFU of phage were screened, yielding 10 recombinant clones containing human *c-src* sequences. Four clones, designated  $\lambda\text{S3H}$ ,  $\lambda\text{S4H}$ ,  $\lambda\text{S5H}$ , and  $\lambda\text{S11H}$ , were utilized in the mapping studies described here.

We adopted the following general strategy for analysis of the recombinant clones: (i) initial restriction endonuclease mapping analyses of lambda phage inserts combined with hybridization to labeled *v-src* DNA to map the general topography of the regions containing, and those adjacent to, human *c-src* sequences homologous to RSV *v-src*; (ii) the use of a battery of plasmid cloned hybridization probes specific for defined subregions of the *v-src* coding sequence to facilitate fine-structure mapping and the localization of human *c-src* exons.

**Analysis of recombinant clones and localization of *c-src* sequences homologous to *v-src*.** Our initial restriction endonuclease mapping analysis of the lambda clones resulted in the determination of the restriction sites for the enzymes

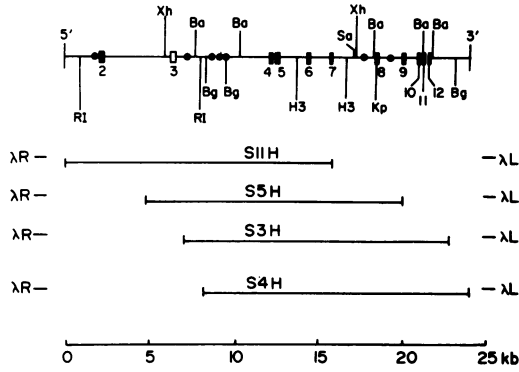


FIG. 1. Restriction map illustrating topography of human *c-src* gene and inserts of lambda phage clones. Abbreviations and symbols: RI, *EcoRI*; Xh, *XhoI*; Ba, *BamHI*; Bg, *BglII*; H3, *HindIII*; Sa, *Sall*; Kp, *KpnI*; ■, exon regions representing human *c-src* coding sequences; □, presumptive exon region (localization is tentative); ●, *Alu* family sequences; λR, lambda Ch4A right arm; λL, lambda Ch4A left arm. Exon regions were determined by restriction mapping, hybridization to *v-src* probes, and by preliminary DNA sequencing studies. Exons are numbered as described in the text.

*EcoRI*, *XhoI*, *BamHI*, *BglII*, *HindIII*, *Sall*, and *KpnI* (Fig. 1). These results indicated that the clones λS3H, λS4H, λS5H, and λS11H contained overlapping inserts that span a 24-kb region. Southern hybridizations using the entire *v-src* gene as a hybridization probe indicated that sequences homologous to *v-src* were located within the 7.2-kb *EcoRI* fragment and the 7.8-, 2.8-, and 0.6-kb *BamHI* fragments (Fig. 1; data not shown).

This distribution of *v-src* related sequences over a large region of genomic DNA suggested that the human *c-src*-coding sequences were organized into several exons separated by relatively large introns. To more precisely localize the human *c-src* exons, we combined detailed restriction enzyme mapping with hybridizations to specific *v-src* subgenomic probes. To facilitate the mapping, appropriate restriction fragments of human DNA were subcloned from the lambda phage inserts into plasmid vectors (pAT153, pBR322, or pBR328). Restriction digests of these plasmid subclones, as well as of lambda phage clones, were analyzed by hybridization to a battery of region-specific *v-src* probes (Fig. 2).

Typical hybridization results illustrating the overlapping nature of the λS3H and λS11H inserts are shown in Fig. 3. In this experiment, DNAs of λS3H and λS11H were digested in triplicate with various restriction endonucleases and analyzed by Southern blotting. Filter replicas were hybridized to one of three different DNA probes. Probe I, which contains 5' sequences of *v-src* (Fig. 2), hybridized to restriction fragments derived from λS11H, but not to fragments derived from λS3H (Fig. 3B). Conversely, probe V, which contains 3' sequences of *v-src*, hybridized to λS3H fragments, but not to λS11H fragments (Fig. 3D). However probe II, derived from an internal region of *v-src*, hybridized to several fragments arising from the central region of the human *c-src* locus that were common to both the λS3H and λS11H inserts (Fig. 3C). These results illustrated the overlapping nature of the inserts and established the 5' to 3' polarity of the human *c-src*-coding sequences (Fig. 1). Similar analyses were utilized to map the boundaries, restriction sites, and polarity of the λS4H and λS5H inserts.

Results of several hybridization analyses similar to these were used to construct a more extensive restriction map

(Fig. 4), which includes several additional enzymes that cleave the human *c-src* locus relatively frequently. These studies also facilitated the identification and localization of exon regions containing human *c-src*-coding sequences homologous to *v-src* sequences (Fig. 1 and 2). The localization of 10 possible exons containing sequences homologous to the *v-src* gene was confirmed and further refined by preliminary nucleic acid sequencing studies. These exons correspond to the chicken *c-src* exons 2 and 4 through 12, as numbered by Takeya and Hanafusa (39; unpublished results) and span the entire coding region of pp60<sup>c-src</sup>. Therefore, we have adopted the same exon numbering system for the human *c-src* locus described here. However, we have not mapped exon 3 precisely at this time because of difficulties in clearly detecting human exon 3 sequences by hybridization to *v-src* probes, which apparently arise from a relatively low degree of nucleotide homology.

In addition, several regions containing sequences homologous to the human *Alu* repetitive family (14) were localized within some human *c-src* introns by hybridization to a BLUR-8 probe (15) (Fig. 1). Although the function of *Alu* family sequences in human DNA has not been clearly elucidated, such sequences also have been reported in intron regions of at least three other human proto-oncogenes (8).

***c-src* sequences in human cellular DNA.** To verify that the human *c-src* gene described here represents an authentic locus present in human genomic DNAs, we hybridized <sup>32</sup>P-labeled *v-src* DNA to restriction enzyme digests of human placental DNA. DNA was digested with various enzymes and hybridized to probe II (Fig. 5). Probe II contains sequences representing an internal segment of *v-src* (Fig. 2) which are homologous mainly to sequences present in exons 4 to 6 of the chicken *c-src* gene (39). Lane A (Fig. 5) clearly reveals hybridization to the characteristic human 7.8-kb *BamHI* fragment, lane B shows strong hybridization to the expected 2.6-kb *HindIII* fragment, and lane C shows the expected hybridization to the 2.4-kb *PvuII* fragment. These results indicate that the human *c-src* gene represented in these clones corresponds to the most highly conserved locus present in normal human cellular DNA; also no obvious rearrangements have occurred during the propagation of these clones. Under the hybridization conditions

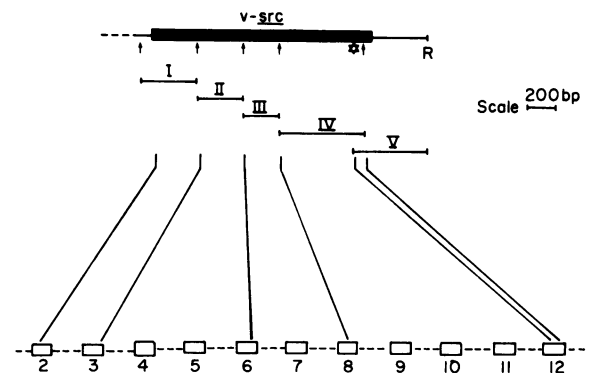


FIG. 2. Subregions of *v-src* DNA originating from the 3.1-kb *EcoRI* fragment of RSV DNA (10) utilized as hybridization probes to detect homologous human *c-src* sequences. Restriction sites utilized to generate these fragments are indicated by the following symbols: ↑, *PstI*; \*, *AvaI*; R, *EcoRI*. Fragments I through IV were subcloned into pBR322 to facilitate purification as described in the text. The *v-src*-coding region is indicated by the solid bar. Human *c-src* exons (□) and introns (---) are not drawn to scale.

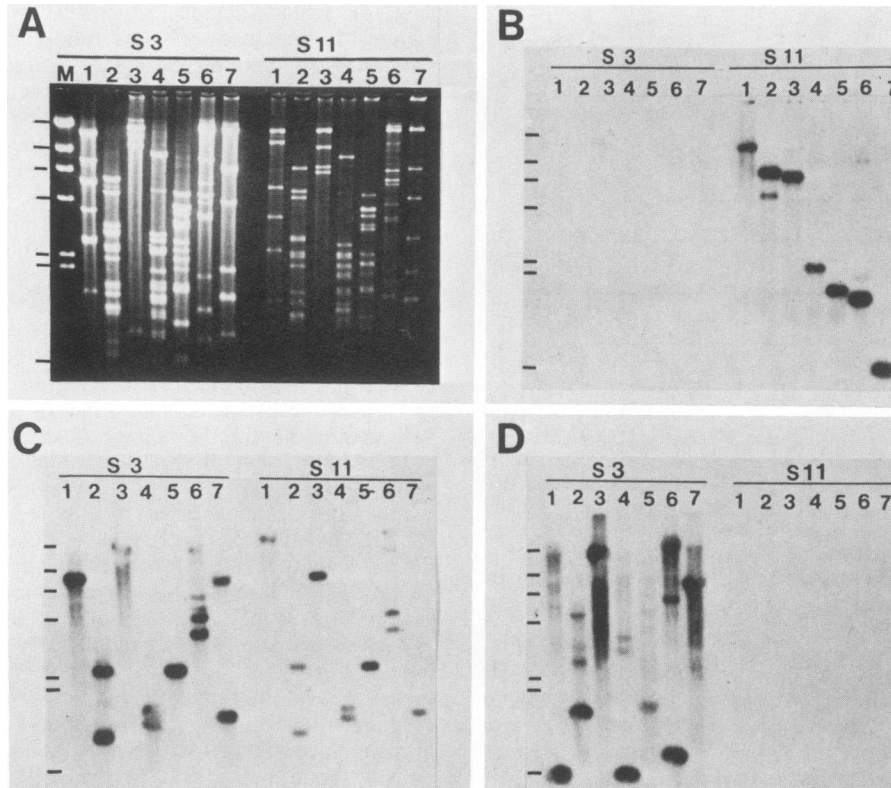


FIG. 3. Hybridization of  $\lambda$ S3H and  $\lambda$ S11H DNAs to  $^{32}$ P-labeled *v-src* probes. DNAs of  $\lambda$ S3H and  $\lambda$ S11H were digested and subjected to agarose gel electrophoresis followed by Southern blotting. (A) Ethidium bromide-stained gel. (B) Hybridization to probe I (Fig. 2). (C) Hybridization to probe II. (D) Hybridization to probe V. Lanes: 1, *Bam*HI; 2, *Bgl*II; 3, *Eco*RI; 4, *Pst*I; 5, *Pvu*II; 6, *Sac*I; 7, *Sma*I; M, *Hind*III-cleaved lambda DNA markers. The position of the markers (23, 9.5, 6.7, 4.3, 2.3, 2.0, and 0.6 kb) is indicated by the bars on the left of the figures.

used ( $5\times$  SSC, 50% formamide, 10% dextran sulfate, 38°C), the major bands observed correspond to bands predicted by our clones. We presume that one of the two weakly hybridizing, high-molecular-weight bands of approximately 25 and 17 kb in lane B (Fig. 5) represents a large *Hind*III fragment that results from cleavage at the upstream *Hind*III site found in these clones and at a site upstream of the cloned region. The other weakly hybridizing band may possibly represent hybridization to a second human *src*-related locus (R. Parker,

G. Mardon, H. Varmus, and J. M. Bishop, personal communication; see below).

Similar results, in which hybridization to DNA fragments predicted by the restriction maps of the lambda clones was

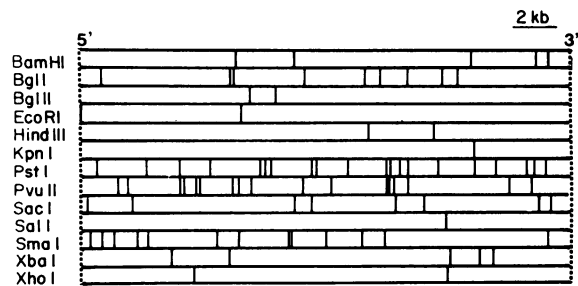


FIG. 4. Fine-structure restriction map of human *c-src*. The map was constructed from restriction and Southern blotting analyses of  $\lambda$ S3H and  $\lambda$ S11H inserts and of subfragments that had been cloned into plasmid vectors. The left border of the map represents the *Eco*RI site near the 5' end of the  $\lambda$ S11H insert; the right border of the map represents the 3' end of the  $\lambda$ S3H insert (Fig. 1). The relative order of *Pvu*II fragments of  $\leq 0.4$  kb is not known clearly. The order of the *Sma*I fragments of 0.9 and 0.5 kb generated by the third, fourth, and fifth *Sma*I sites from the 5' end is not certain.

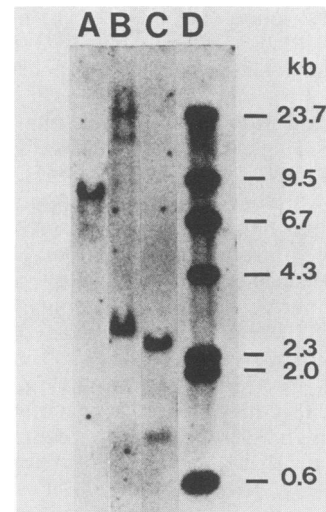


FIG. 5. Hybridization of *v-src* sequences to human cellular DNA. Human placental DNA (25  $\mu$ g per lane) was digested and analyzed by hybridization to  $^{32}$ P-labeled *v-src* probe II DNA after gel electrophoresis and Southern transfer. Lanes: A, *Bam*HI; B, *Hind*III; C, *Pvu*II; D,  $^{32}$ P end-labeled, *Hind*III-cleaved lambda DNA markers.

observed, were obtained with DNAs from a different placenta and from HeLa and WI-38 cells. Also, a variety of other *v-src* DNA probes (probe I, probe V, and the entire 3.1-kb *Eco*RI fragment) hybridized to the expected DNA fragments in cellular DNA (data not shown). In addition, other weakly hybridizing bands, which presumably represent hybridization to one or more other *src*-related loci, were sometimes observed with these probes.

#### DISCUSSION

We have isolated and analyzed by restriction endonuclease mapping a major human locus containing nucleotide sequences homologous to the *v-src* gene of RSV. Previous studies have demonstrated that the RSV *v-src* gene was probably acquired from the chicken genome through a recombinational process involving an avian retrovirus and a chicken *c-src* gene (37, 39). The chicken *c-src* gene is highly homologous to RSV *v-src* over most of its coding region, except for a short region at the 3' extreme end (39). The human *src* gene locus described here is highly conserved and exhibits a strong structural and organizational similarity to the chicken *c-src* gene. It will be referred to as human *c-src*-1, because molecular clones representing two human *src*-related loci have been isolated by others (R. Parker, G. Mardon, H. E. Varmus, and J. M. Bishop, personal communication). Recently a human *c-src* gene has been mapped to chromosome 20 (26). It seems highly probable that this gene corresponds to human *c-src*-1, because it contains *Eco*RI fragments (28 kb and  $\approx$ 7 kb) that are consistent with our map of *c-src*-1.

Hybridization to  $^{32}$ P-labeled DNAs specific for different subregions of *v-src* has indicated that there are at least nine discrete coding regions, separated by introns, in human *c-src*-1. Nucleotide sequencing studies suggest that there are 11 discrete exons that contain the entire human *c-src*-coding sequence homologous to RSV *v-src*, as was previously described for chicken *c-src* (unpublished results; 39). Furthermore, most of the human *c-src* exons are the same size as the corresponding chicken *c-src* exons. Because of this, we have adopted the exon numbering system used for chicken *c-src* exons by Takeya and Hanafusa (39), in which the protein-coding exons are numbered 2 through 12. These human *c-src* exon assignments are supported by preliminary nucleic acid sequencing evidence indicating that the AUG codon specifying the N-terminal methionine residue of human pp60<sup>src</sup> is located in exon 2, and the coding sequences that specify the COOH-terminal region are located in exon 12 (unpublished results). In contrast, the most apparent difference between the human *c-src*-1 locus and the chicken *c-src* locus is the larger overall size of human *c-src*-1. Some of the human *c-src*-1 introns are much larger than those of the chicken *c-src* gene, and consequently human *c-src*-coding sequences are dispersed over a distance of about 20 kb, whereas the coding sequences of chicken *c-src* are dispersed over a distance of 6 kb (30, 37, 39).

We have observed that, although the exon regions encoding the C-terminal two-thirds of human pp60<sup>src</sup> (exons 6 through 12) exhibited strong hybridization to *v-src* DNA, the exon regions coding for the N-terminal one-third of pp60<sup>src</sup> exhibited weaker hybridization to *v-src* DNA. In fact hybridization of exon 3 to *v-src* probes has been too weak to detect clearly and unambiguously under our hybridization conditions. These results suggest that the 3' two-thirds of human *c-src*-1-coding sequence, which includes the coding region for the tyrosine kinase domain of pp60<sup>src</sup> (17), has been highly conserved over a wide evolutionary distance. How-

ever the 5' one-third of the human *c-src*-coding region has been somewhat less highly conserved. This would suggest that nucleotide changes in the 5' one-third of *c-src* have been tolerated throughout vertebrate evolution to a higher degree than in other regions of *c-src*. The question of whether such changes in this region have functionally important consequences must await the results of further experiments on pp60<sup>src</sup> structure and function.

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#### LITERATURE CITED

- Benton, W. D., and R. W. Davis. 1977. Screening lambda gt recombinant clones by hybridization to single plaques in situ. *Science* **196**:180-182.
- Bishop, J. M. 1981. Enemies within: the genesis of retrovirus oncogenes. *Cell* **23**:5-6.
- Bishop, J. M., and H. E. Varmus. 1982. Functions and origins of retroviral transforming genes, p. 999-1108. In R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), *RNA tumor viruses*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Blattner, R. F., B. Williams, A. Blechl, K. Denniston-Thompson, H. Faber, L.-A. Furlong, D. Grunwald, D. Kiefer, D. Moore, J. Schumm, E. Sheldon, and O. Smithes. 1977. Charon phages: safer derivatives of bacteriophage lambda for DNA cloning. *Science* **196**:161-169.
- Blin, N., and D. W. Stafford. 1976. Isolation of high molecular-weight DNA. *Nucleic Acids Res.* **3**:2303-2309.
- Collett, M. S., J. S. Brugge, and R. L. Erikson. 1978. Characterization of a normal avian cell protein related to the avian sarcoma virus transforming gene product. *Cell* **15**:1363-1369.
- Collett, M. S., and R. L. Erikson. 1979. Protein kinase activity associated with the avian sarcoma virus *src* gene product. *Proc. Natl. Acad. Sci. U.S.A.* **75**:2021-2024.
- Dalla Favera, R., E. P. Gelmann, R. C. Gallo, and F. Wong-Staal. 1981. A human *onc* gene homologous to the transforming gene (*v-sis*) of simian sarcoma virus. *Nature (London)* **292**:31-35.
- Davis, R. W., D. Botstein, and J. R. Roth. 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- DeLorbe, W. J., P. A. Luciw, H. M. Goodman, H. E. Varmus, and J. M. Bishop. 1980. Molecular cloning and characterization of avian sarcoma virus circular DNA molecules. *J. Virol.* **36**:50-61.
- Denhardt, D. T. 1966. A membrane filter technique for the detection of complementary DNA. *Biochem. Biophys. Res. Commun.* **23**:741-746.
- Hanafusa, H., H. Iba, T. Takeya, and F. R. Cross. 1984. Transforming activity of the *c-src* gene, p. 1-8. In G. F. Vande Woude, A. J. Levine, W. C. Topp, and J. D. Watson (ed.), *Cold Spring Harbor Conference on Cell Proliferation and Cancer*, vol. 2: the cancer cell. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Hayward, W. S., B. G. Neel, and S. M. Astrin. 1981. Activation of a cellular *onc* gene by promoter insertion in ALV-induced lymphoid leukemia. *Nature (London)* **290**:475-480.

14. Jelinek, W. R., and C. W. Schmid. 1982. Repetitive sequences in eukaryotic DNA and their expression. *Annu. Rev. Biochem.* **51**:813–844.
15. Jelinek, W. R., T. P. Toomey, L. Leinwand, C. H. Duncan, P. A. Biro, P. V. Choudary, S. M. Weissman, C. M. Rubin, C. M. Houck, P. L. Deininger, and C. W. Schmid. 1980. Ubiquitous, interspersed repeated sequences in mammalian genomes. *Proc. Natl. Acad. Sci. U.S.A.* **77**:1398–1402.
16. Lawn, R. M., E. F. Fritsch, R. C. Parker, G. Blake, and T. Maniatis. 1978. The isolation and characterization of a linked  $\delta$ - and  $\beta$ -globin gene from a cloned library of human DNA. *Cell* **15**:1157–1174.
17. Levinson, A. D., S. A. Courtneidge, and J. M. Bishop. 1981. Structural and functional domains of the Rous sarcoma virus transforming protein (pp60<sup>src</sup>). *Proc. Natl. Acad. Sci. U.S.A.* **78**:1624–1628.
18. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. Opperman, H., A. D. Levinson, H. E. Varmus, L. Levintow, and J. M. Bishop. 1979. Uninfected vertebrate cells contain a protein that is closely related to the product of the avian sarcoma virus transforming gene. *Proc. Natl. Acad. Sci. U.S.A.* **76**:1804–1808.
20. Parker, R. C., R. Swanstrom, H. E. Varmus, and J. M. Bishop. 1984. Transduction and alteration of a cellular gene (*c-src*) created an RNA tumor virus: the genesis of Rous sarcoma virus, p. 19–25. *In* Cold Spring Harbor Conference on Cell Proliferation and Cancer, vol. 2: the cancer cell. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. Parker, R. C., H. E. Varmus, and J. M. Bishop. 1981. Cellular homologue (*c-src*) of the transforming gene of Rous sarcoma virus: isolation, mapping, and transcriptional analysis of *c-src* and flanking regions. *Proc. Natl. Acad. Sci. U.S.A.* **78**:5842–5846.
22. Parker, R. C., H. E. Varmus, and J. M. Bishop. 1984. Expression of *v-src* and *c-src* in rat cells demonstrates qualitative differences between pp60<sup>v-src</sup> and pp60<sup>c-src</sup>. *Cell* **37**:131–139.
23. Reddy, E. P., R. K. Reynolds, E. Santos, and M. Barbacid. 1982. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. *Nature (London)* **300**:149–152.
24. Rigby, P. W., M. Dieckmann, C. Rhodes, and P. Berg. 1977. Labelling of deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase. *J. Mol. Biol.* **113**:237–251.
25. Rohrschneider, L. R., R. N. Eisenman, and C. R. Leitch. 1979. Identification of a Rous sarcoma virus transformation-related protein in normal avian and mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* **76**:4479–4483.
26. Sakaguchi, A. Y., S. L. Naylor, and T. B. Shows. 1983. A sequence homologous to Rous sarcoma virus *v-src* is on human chromosome 20, p. 279–283. *In* W. E. Cohn (ed.), *Progress in nucleic acids research*, vol. 29. Academic Press, Inc., New York.
27. Sefton, B. M., T. Hunter, and K. Beemon. 1980. Relationship of polypeptide products of the transforming gene of Rous sarcoma virus and the homologous gene of vertebrates. *Proc. Natl. Acad. Sci. U.S.A.* **77**:2059–2063.
28. Sefton, B. M., and G. Walter. 1982. An antiserum specific for the carboxy terminus of the transforming protein of Rous sarcoma virus. *J. Virol.* **44**:467–474.
29. Shalloway, D., P. M. Coussens, and P. Yaciuk. 1984. Effects of *c-src* and *v-src* homolog overexpression in mouse cells, p. 9–17. *In* Cold Spring Harbor Conference on Cell Proliferation and Cancer, vol. 2: the cancer cell. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. Shalloway, D., A. Zelentz, and G. M. Cooper. 1981. Molecular cloning and characterization of the chicken gene homologous to the transforming gene of Rous sarcoma virus. *Cell* **24**:531–541.
31. Shealy, D. J., and R. L. Erikson. 1981. Human cells contain two forms of pp60<sup>c-src</sup>. *Nature (London)* **293**:666–669.
32. Shilo, B.-Z., and R. A. Weinberg. 1981. DNA sequences homologous to vertebrate oncogenes are conserved in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* **78**:6789–6792.
33. Smart, J. E., H. Oppermann, A. P. Czernilofsky, A. F. Purchio, R. L. Erikson, and J. M. Bishop. 1981. Characterization of sites for tyrosine phosphorylation in the transforming protein of Rous sarcoma virus (pp60<sup>v-src</sup>) and its normal cellular homologue (pp60<sup>c-src</sup>). *Proc. Natl. Acad. Sci. U.S.A.* **78**:6013–6017.
34. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**:503–517.
35. Spector, D. H., H. E. Varmus, and J. M. Bishop. 1978. Nucleotide sequences related to the transforming gene of avian sarcoma virus are present in the DNA of uninfected vertebrates. *Proc. Natl. Acad. Sci. U.S.A.* **75**:4102–4106.
36. Stehelin, D., H. E. Varmus, J. M. Bishop, and P. K. Vogt. 1976. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature (London)* **260**:170–173.
37. Swanstrom, R., R. Parker, H. E. Varmus, and J. M. Bishop. 1983. Transduction of a cellular oncogene: the genesis of Rous sarcoma virus. *Proc. Natl. Acad. Sci. U.S.A.* **80**:2519–2523.
38. Tabin, C. J., S. M. Bradley, C. I. Bargmann, R. A. Weinberg, A. G. Papageorge, E. M. Scolnick, R. Dhar, D. R. Lowy, and E. H. Chang. 1982. Mechanism of activation of a human oncogene. *Nature (London)* **300**:143–149.
39. Takeya, T., and H. Hanafusa. 1983. Structure and sequence of the cellular gene homologous to the RSV *src* gene and the mechanism for generating the transforming virus. *Cell* **32**:881–890.
40. Wahl, G. M., M. Stern, and G. R. Stark. 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzoyloxy-methyl-paper and rapid hybridization by using dextran sulfate. *Proc. Natl. Acad. Sci. U.S.A.* **76**:3683–3787.
41. Yamamoto, K. R., B. M. Alberts, R. Benzinger, L. Lawhorne, and G. Treiber. 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale purification. *Virology* **40**:734–741.