Expression of the Chicken *c-src* Gene in COS Cells

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The cellular homolog of the Rous sarcoma virus transforming gene (*v-src*) was cloned into a plasmid containing the simian virus 40 origin of replication and transcriptional signals. This recombinant plasmid, designated pSVOHCS11, directs the synthesis of relatively high levels of *c-src* mRNA and *c-src* protein (pp60^{*c-src*}), when the plasmid is studied 48 to 72 h after calcium phosphate-mediated DNA transfection of COS (monkey) cells. The level of *c-src* mRNA synthesis is 50-fold higher than the amount of *c-src* RNA produced in uninfected chicken embryo fibroblasts. Furthermore, the level of pp60^{*c-src*} expressed in pSVOHCS11-transfected COS cells is approximately the same as that of pp60^{*v-src*} in Rous sarcoma virus-transformed cells. Using this recombinant plasmid, we demonstrated that *c-src* mRNA contains sequences which map 3' to the previously identified *c-src-v-src* regions of homology. In view of the small amount of *c-src* mRNA and protein that can be isolated from uninfected cells, this transient expression system offers a convenient source of material for further analyses of the *c-src* gene product.

Cellular sequences related to the Rous sarcoma virus (RSV) transforming gene have been identified in a wide variety of vertebrate species as well as in *Drosophila melanogaster* (34, 37). The products of the cellular and viral sequences are phosphoproteins of 60,000 daltons each, designated pp60^{*c-src*} and pp60^{*v-src*}, respectively (2, 5, 20, 30). Both classes of proteins have tyrosine-specific kinase activity (7-9, 12, 19, 20, 24, 30). The evolutionary conservation of pp60^{*c-src*} suggests that the protein serves an important function in normal cells. Studies of pp60^{*c-src*} have been hindered by the small amount of protein expressed in uninfected cells, 10- to 100-fold lower than that of pp60^{*v-src*} in RSV-transformed cells (5, 20, 30).

Molecular clones containing the chicken *c-src* locus have been isolated, and the regions of the *c-src* gene which contain homologies to *v-src* have been identified (31, 33, 38-40). In contrast to the *v-src* sequences, the *c-src* gene is separated by multiple introns. The RNA that is needed to code for pp60^{*c-src*} is ca. 1.7 kilobases (kb); however, the *c-src* mRNA that has been identified by sucrose sedimentation analyses and denaturing agarose gel electrophoresis is ca. 4 to 6 kb (31, 36). Utilizing two of the previously characterized molecular clones (40), we reconstructed 14 kilobase pairs (kbp) of cloned chicken *c-src* DNA in a simian virus 40 (SV40)-derived vector. When introduced into COS cells (15), the *c-src*-containing recombinant DNA is amplified and serves as the template for large amounts of *c-src* mRNA. This 4.5-kb mRNA is translated into authentic avian pp60^{*c-src*} which has tyrosine-specific kinase activity. From Northern blot analyses, we found that *c-src* mRNA contains sequences which map 3' to the regions of homology with *v-src*.

MATERIALS AND METHODS

Cells and DNA transfections. Primary chicken embryo fibroblasts (CEF) were prepared from 12-day-old *gs⁻ chf⁻* Marek⁻ embryos (Spafas Inc., Norwich, Conn.) and cul-

tured in Dulbecco modified medium (DME) supplemented with 10% tryptose phosphate and 5% fetal calf serum.

COS7 cells (CV-1 monkey cells transformed with an origin-defective mutant of SV40) were obtained originally from Y. Gluzman, Cold Spring Harbor Laboratory, N.Y., and cultured as previously described (15). DNA transfections were carried out as described by Mellon et al. (28). One day before transfection, COS cells were subcultured 1:4. One milliliter of plasmid DNA (10 µg/ml) as a calcium phosphate coprecipitate (16) was added to cells in 10-cm dishes. After 5 h at 37°C, the cells were washed with DME, and 1 ml of 25% glycerol in DME was added for 1 min at room temperature. The cells were washed with medium, 10 ml of DME containing 10% fetal calf serum was added, and the cells were incubated at 37°C for 48 to 72 h.

Molecular cloning. The recombinant phage were grown on *Escherichia coli* LE392, and phage DNA was purified as described previously (26, 42). λRCS3 and λRCS14 DNAs were digested with *Hind*III, and the 12-kbp fragment from λRCS3 and the 4.7-kbp fragment from λRCS14 were isolated from an agarose gel by electroelution (27). The 12-kbp fragment from λRCS3 was ligated to *Hind*III-cut pBR322 with T4 DNA ligase and transformed into HB101. Recombinant plasmids were isolated from minilysates (18) and subjected to partial *Hind*III digestion. The purified 4.7-kbp (λRCS14) fragment was added with T4 DNA ligase, and the mixture was transformed into HB101. Recombinants were screened by restriction endonuclease mapping, and DNA from one recombinant pCS25 with the desired orientation was prepared (4). After digestion of pCS25 with *Bgl*III, a 14-kbp fragment was isolated from an agarose gel by electroelution and inserted into the *Bam*HI site of pSVOH with T4 DNA ligase. Plasmid pSVOH contains the SV40 *Hind*III C fragment (nucleotide 5171 to nucleotide 1042 on the SV40 map [11]) which includes the SV40 origin of replication. It does not, however, contain the pBR322 sequences inhibitory to replication in monkey cells (25). After transformation of HB101, a plasmid, pSVOHCS11, was isolated which had the desired orientation.

DNA and RNA purification. Cells were washed with phosphate-buffered saline and suspended in lysis buffer (200 mM Tris-hydrochloride [pH 8.5], 140 mM NaCl, 2 mM MgCl) 48

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to 72 h after transfection, and Nonidet P-40 was added to a final concentration of 0.5%. After 4 min on ice, the nuclei were pelleted by centrifugation at $500 \times g$ for 5 min (4°C), and the supernatant was reserved for RNA isolation. The nuclei were lysed by addition of 1.25% sodium dodecyl sulfate (SDS), and unintegrated DNA was isolated by the method of Hirt (17). DNA was subjected to electrophoresis in 0.75% agarose gels and transferred to nitrocellulose filters (35).

Cytoplasmic supernatants were diluted with 2 volumes of TSE buffer (10 mM Tris-hydrochloride [pH 8.5], 5 mM EDTA, 0.5% SDS) and extracted three times with TSE-saturated phenol-chloroform-isoamyl alcohol (20:20:1 [vol/vol]) and twice with chloroform-isoamyl alcohol. RNA was precipitated with ethanol and collected by centrifugation. RNA was enriched for polyadenylated [poly(A)⁺] sequences by oligodeoxythymidylate-cellulose chromatography (1). After fractionation by formaldehyde agarose gel electrophoresis (23), the RNA was transferred to nitrocellulose filters (41).

Hybridization. An *src*-specific probe was prepared from a clone containing the 3.0-kbp *EcoRI* *v-src* fragment in pBR325 (14). This plasmid was digested with *XhoI* and *EcoRI*, and the *v-src*-containing fragment was purified by agarose gel electrophoresis and was recovered by electroelution (27). *c-src* DNA fragments used as probes in Fig. 5 were isolated as described above, and each purified fragment was labeled by nick translation to a specific activity of 1×10^8 to 3×10^8 cpm/ μ g and used for hybridization of filters. The specificity of each *c-src* probe was validated by monitoring hybridization to digested *c-src* DNA. Filters were prehybridized overnight at 42°C in a solution of 50% (vol/vol) formamide, $5\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate), $5\times$ Denhardt solution (10), 50 mM KP_i (pH 6.5), 0.25% SDS, and 250 μ g of yeast RNA per ml. Hybridizations were carried out for 48 h at 42°C in the same buffer, except that the Denhardt solution was $1\times$. The filters were washed with two changes of $2\times$ SSC-0.1% SDS for 15 min each at room temperature and then washed with four changes of $0.1\times$ SSC-0.1% SDS for 30 min each at 50°C. The filters were exposed to Kodak XAR film or Dupont Cronex film with or without Dupont Cronex intensifying screens. *c-src*-specific RNA was quantitated by scanning the autoradiograph with an LKB 2202 Ultrascan laser densitometer and correcting the values for the different amounts of RNA loaded in each lane.

Immunoprecipitation and protein analysis. Labeling of cells with [³⁵S]methionine or [³²P]phosphate was performed as described previously (2). Cells were lysed with 150 mM NaCl-1% Triton X-100-1% sodium deoxycholate-0.1% SDS-10 mM Tris-hydrochloride (pH 7.2)-1 mM EDTA and centrifuged at $100,000 \times g$ for 30 min at 4°C. Extracts were immunoprecipitated, and the proteins were eluted and electrophoresed as described previously (21). One-dimensional peptide mapping by limited proteolysis was carried out as described by Cleveland et al. (3).

RESULTS

Construction of plasmids. Two *HindIII* restriction fragments were isolated from overlapping recombinant phages provided by Hidesaburo Hanafusa (40; Fig. 1). These two fragments span the *c-src* region of the chicken genome. A 12-kbp *HindIII* fragment (map coordinates, -2.2 to 9.8) containing the entire coding region of *c-src* (39, 40) was isolated from λ RCS3 and ligated into pBR322. A 4.7-kbp *HindIII* fragment (map coordinates, -6.9 to -2.2) containing a

noncoding exon of *c-src* and 5' upstream sequences was isolated from λ RCS14 and ligated into the previous construction which had been linearized by partial *HindIII* digestion. Recombinants were screened by restriction endonuclease mapping. One plasmid, designated pCS25, includes both *HindIII* fragments in an orientation which reconstructs 16.7 kbp of chicken DNA. The intact *c-src* coding region and its 5' and 3' flanking sequences spanning 14 kbp were removed from pCS25 by *BglIII* digestion and inserted into the *BamHI* site of pSVOH (provided by Chi-Bom Chae). This vector contains the *HindIII* C fragment of SV40 (11) which includes the SV40 origin of replication, two complete copies of the 72-base pair (bp) repeat required for enhancer activity (29), and the SV40 early promoter and late transcription and splice donor sites in a pBR322 derivative (25). The resulting recombinant plasmid pSVOHCS11 has the orientation shown in Fig. 1.

Replication of the vector in COS cells. To test the construction for expression of the *c-src* gene, pSVOHCS11 DNA was introduced into COS cells (15) by transfection using the calcium phosphate precipitate technique (16) followed by glycerol shock treatment to enhance DNA uptake (28). In all experiments COS cells that had been transfected with the vector plasmid pSVOH or COS cells that had been subjected to glycerol shock treatment alone were used as controls.

Circular DNAs containing the SV40 origin of replication that are introduced into COS cells are amplified due to the presence of the SV40 T antigen in these cells (15). Replication of pSVOHCS11 DNA in COS cells was examined at 72 h after transfection. The nuclei of treated cells were isolated, and unintegrated DNA was prepared by the method of Hirt (17). The extrachromosomal DNA was separated by agarose gel electrophoresis, transferred to nitrocellulose filters, and hybridized with a ³²P-labeled probe prepared from cloned *v-src* DNA (14). Lane 3 of Fig. 2A illustrates the presence of amplified pSVOHCS11 DNA in the transfected cells. No *src*-specific unintegrated DNA was detected in nuclei of either control COS cells (lane 2) or pSVOHCS11-transfected cells 5 h after the addition of DNA (data not shown).

Transcription of chicken *c-src* sequences in COS cells. Poly(A)⁺ RNA was prepared from the cytoplasm of COS cells transfected with pSVOHCS11 and from the cytoplasm of CEF and was electrophoresed in adjacent lanes in a formaldehyde agarose gel (23). RNA in the gel was transferred to nitrocellulose paper and hybridized to a ³²P-labeled *v-src* probe. A predominant band was detected by hybridization of the probe to mRNA prepared from pSVOHCS11-transfected COS cells (Fig. 2B, lane 3). The apparent size of this band of ~4.5 kb is consistent with previous size estimates of *c-src* mRNA (31, 36) and corresponded to the single band hybridizing with mRNA from CEF (Fig. 2B, lane 1). However, the *HindIII* C fragment in the vector pSVOH contains the 5' terminal leader sequences of late mRNAs of SV40 which are ca. 200 nucleotides in length (22). The *c-src* mRNA produced in this system hybridized with the *HindIII* C fragment, indicating that at least some of the mRNA is initiated from the SV40 late promoter (data not shown). Longer exposure of lane 3, Fig. 2B, revealed two additional hybridizing bands of about 6 kb and >10 kb which hybridized with the *src* probe and the SV40 probe. These bands probably represent minor readthrough transcripts which are initiated from the SV40 late or early promoters.

The amount of *c-src* mRNA produced in the pSVOHCS11-transfected cells was ca. 50-fold higher than that made in CEF, as determined by a densitometer scan of the autoradiogram. Under the conditions used, no hybridization was

FIG. 1. Construction of a plasmid expressing pp60^{c-src}. Maps A and B are from reference 40. (A) Restriction endonuclease maps of the chicken *c-src* locus. The 12 identified *c-src* exons are designated by the closed boxes as determined by electron microscopy (33, 40) and DNA sequencing (39). The open box indicates a portion of the carboxy-terminal region of *v-src* DNA which maps about 900 bp downstream from exon 12. The precise exon-intron structure of *c-src* is not clear in this region. The arrows designate the direction of transcription. Restriction endonucleases are abbreviated as follows: H, *Hind*III; Bg, *Bgl*II; RI, *Eco*RI; B, *Bam*HI. (B) Overlapping *c-src* region inserts of λ RCS3 and λ RCS14 and their respective restriction maps. The *c-src* boundaries of the two clones are designated by thin lines. Heavy lines indicate the *c-src* *Hind*III restriction fragments isolated from each insert and used in the cloning procedures. (C) Restriction map of pCS25, a recombinant clone containing the two *Hind*III *c-src* restriction fragments inserted in the *Hind*III *c-src* site of pBR322. Thin lines denote the pBR322 sequences. (D) Restriction map of pSVOHCS11. The open box represents the SV40 *Hind*III C fragment. The small closed boxes in the SV40 insert indicate the 72-bp repeat sequences. The *c-src* *Bgl*II fragment designated by the heavy lines was inserted in the *Bam*HI site of pSVOH.

detected between the *v-src* probe and RNA prepared from control COS cells (Fig. 2B, lane 2).

Immunoprecipitation and characterization of the *c-src* protein in pSVOHCS11-transfected COS cells. Cellular extracts of [³⁵S]methionine-labeled or [³²P]phosphate-labeled pSVOHCS11-transfected COS cells were immunoprecipitated with anti-p60 serum (antiserum made against bacterium-produced *v-src* [13] which is cross-reactive with avian pp60^{c-src}). A 60,000-*M_w* protein was specifically immunoprecipitated with anti-p60 serum (Fig. 3A) and not with normal rabbit serum (data not shown). The protein was not immunoprecipitated from control COS cells. To confirm that the 60,000-*M_w* protein precipitated from pSVOHCS11-transfected COS cells was pp60^{c-src}, the partial proteolytic cleavage map of this protein was compared with pp60^{c-src} immunoprecipitated from CEF. The two cleavage maps with two concentrations of *Staphylococcus aureus* V8 protease are identical (Fig. 3B). This shows that the 60,000-*M_w* protein is pp60^{c-src} and not pp60^{v-src} since the carboxy-terminal peptides of the two proteins can be distinguished by V8 mapping (5, 30). The amount of pp60^{c-src} produced in pSVOHCS11 COS cells 48 to 60 h after transfection is ca. 0.025% of the total labeled soluble protein from these cells and is consistent with the amount of pp60^{v-src} produced in RSV-transformed cells (5, 20; unpublished data).

As shown previously (7, 30), when extracts of CEF are immunoprecipitated with tumor-bearing rabbit serum cross-reactive with pp60^{c-src} and incubated with [³²P]ATP, the *src* protein catalyzes the transfer of phosphate to a tyrosine residue in the heavy chain of the tumor-bearing rabbit immunoglobulin. In addition, when partially purified pp60^{c-src} is incubated with [³²P]ATP, the protein undergoes autophosphorylation (32). We therefore examined the pp60^{c-src} precipitated from pSVOHCS11-transfected COS cells for protein kinase activity. Cellular lysates from control COS cells or pSVOHCS11-transfected COS cells were immunoprecipitated with tumor-bearing rabbit serum cross-reactive with avian or mammalian pp60^{c-src} (a gift of J. S. Brugge), and the complex was incubated with [³²P]ATP in a phosphotransferase assay as described previously (8). Lysates from pSVOHCS11-transfected cells exhibited greater levels of pp60^{c-src} kinase activity than lysates from control COS cells (Fig. 4, lanes 1 and 2). No phosphorylation of immunoglobulin G occurred when normal rabbit serum was used to immunoprecipitate extracts of pSVOHCS11-transfected COS cells (lane 3). Some preparations of anti-p60 sera made against bacterium-produced *v-src* recognize primarily pp60^{c-src} from avian cells and allow autophosphorylation of the *src* protein in protein kinase assays, but the immunoglobulin G does not become phosphorylated (13). One such

FIG. 2. (A) Replication of pSVOHCS11 DNA in COS cells. COS cells were transfected with pSVOHCS11 DNA as described in the text. Seventy-two hours later, nuclei were isolated and low-molecular-weight DNA was prepared as described by Hirt (17). One-fourth of the DNA sample was fractionated by electrophoresis on a 0.75% agarose gel, transferred to nitrocellulose filters, hybridized with a ^{32}P -labeled *src*-specific probe, and autoradiographed. Lane 1, 10 ng of pSVOHCS11 DNA; lane 2, DNA from nuclear lysates of control COS cells; lane 3, DNA from nuclear lysates of COS cells transfected with pSVOHCS11 DNA. The positions of form I and form II DNA are indicated. (B) Transcription of pSVOHCS11 DNA in COS cells. Poly(A)⁺ RNA was extracted from the cytoplasm of cells and electrophoresed in adjacent lanes through a 1.2% formaldehyde agarose gel. The RNA was transferred to nitrocellulose, hybridized with a ^{32}P -labeled *src*-specific probe, and autoradiographed. Lane 1, 20 μg of poly(A)⁺ RNA from uninfected CEF cells; lanes 2 and 3, 1 μg of poly(A)⁺ RNA from control COS cells (lane 2) or pSVOHCS11-transfected COS cells (lane 3). The positions of rRNA size markers are indicated.

FIG. 3. (A) Immunoprecipitation of pp60^{c-src} from COS cells transfected with pSVOHCS11 DNA. Control COS cells or COS cells that had been transfected with pSVOHCS11 DNA were labeled with [^{35}S]methionine or [^{32}P]phosphate. After immunoprecipitation of extracts with anti-p60 serum, the samples were electrophoresed in SDS-polyacrylamide gels and autoradiographed. (B) Partial proteolytic cleavage of pp60^{c-src}. The [^{32}P]phosphate-labeled *c-src* protein was immunoprecipitated from pSVOHCS11-transfected COS cells (lane a) and from uninfected CEF (lane b) and was excised from preparative SDS-polyacrylamide gels. The protein was subjected to limited proteolysis during reelectrophoresis as previously described (3). Digestion was with 5 and 50 ng of *S. aureus* V8 protease. The two large fragments of pp60^{c-src} which are the amino and carboxy termini, are designated NH₂ and COOH, respectively (6). The [^{35}S]methionine protein was not subjected to partial proteolytic cleavage analysis.

FIG. 4. Analysis of pp60^{c-src}-specific kinase activities in lysates from pSVOHCS11-transfected COS cells. After immunoprecipitation of equal amounts of protein from control COS cells (lanes 1 and 4) or pSVOHCS11-transfected COS cells (lanes 2, 3, and 5), the immune complexes were incubated at room temperature for 10 min with 10 mM Tris-hydrochloride (pH 7.5)–5 mM MgCl₂–1 μM [γ - ^{32}P]ATP (2900 Ci/mmol; New England Nuclear Corp. Boston, Mass.). Extracts were immunoprecipitated with tumor-bearing rabbit serum (lanes 1 and 2) normal rabbit serum (lane 3), or anti-p60 serum (lanes 4 and 5). Proteins were then analyzed by polyacrylamide gel electrophoresis and autoradiography. IgG, Immunoglobulin G.

preparation of rabbit anti-p60 serum (a gift of J. T. Parsons) was used to demonstrate autophosphorylation of pp60^{c-src} precipitated from pSVOHCS11-transfected COS cells (Fig. 4, lane 5). Phosphoamino acid analysis of these reactions revealed that the major amino acid phosphorylated was tyrosine (data not shown).

Mapping of the 3' end of the *c-src* mRNA. Since the amount of *c-src* mRNA produced in pSVOHCS11-transfected COS cells was at least 50-fold greater than that made in CEF, *c-src* DNA regions that were transcribed into mRNA were examined. Three adjacent restriction fragments (Fig. 5) spanning the 3' flanking region of the *c-src* gene were isolated, and probes were prepared and hybridized to poly(A)⁺ RNA from pSVOHCS11-transfected COS cells (Fig. 5). Probe A contains sequences from a 1.1-kbp *SacI*-*Bam*HI fragment (map coordinates, 4.9 to 6.0). The *SacI* site is located 10 bp downstream from the *c-src* termination codon described by Takeya and Hanafusa (39). Probe B is the adjacent 500-bp *Bam*HI fragment to the right (map coordinates, 6.0 to 6.5), whereas probe C is the 2.3-kbp *Bam*HI fragment to the right of and adjacent to the fragment used as probe B (map coordinates, 6.5 to 8.8). All three probes hybridized with the 4.5-kb *c-src* mRNA produced in pSVOHCS11-transfected COS cells (Fig. 5A, B, and C). There was no detectable hybridization, under the conditions used, with RNA prepared from control COS cells. In addition, a 3.4-kbp *SacI*-*SacI* fragment (map coordinates, 1.5 to 4.9) which contains most of the *c-src* coding region also hybridized to the 4.5-kb mRNA (data not shown). Thus, *c-src* mRNA is transcribed from regions as far as 500 bp downstream from the rightward-most region of the *c-src* locus having homology with *v-src*. However, the precise exon-intron structure of the 3' flanking sequences cannot be determined from this analysis.

DISCUSSION

We have demonstrated that a plasmid carrying the SV40 origin of DNA replication, the 72-bp repeat sequences, the SV40 early and late promoters, and the 14-kbp region of the

FIG. 5. Northern blot analysis of RNA from pSVOHCS11-transfected COS cells. Poly(A)⁺ RNA (1 μ g per lane) from cytoplasmic extracts of control COS cells or pSVOHCS11-transfected COS cells was electrophoresed in adjacent lanes through a 1.2% formaldehyde agarose gel. The RNA was transferred to a nitrocellulose filter, and the filter was cut into strips and hybridized with probes A, B, and C as indicated above the lanes. The positions of rRNA size markers are indicated. The *c-src* restriction map is the 10-kbp *EcoRI*-*HindIII* fragment (map coordinates, 0 to 10) as shown in Fig. 1A. Exons 4 to 12 are designated by the closed boxes. The open box designates the *v-src* carboxy-terminal region. Restriction endonucleases are abbreviated as follows: H, *HindIII*; Bg, *BglII*; RI, *EcoRI*; B, *BamHI*; Sc, *SacI*.

chicken genome encoding the *c-src* gene with its 5' and 3' flanking regions is capable of expressing high levels of *c-src* mRNA and pp60^{*c-src*}.

The *c-src* mRNA transcript encoded by this plasmid migrates to the same position in denaturing formaldehyde agarose gels as does *c-src* mRNA prepared from CEF and is present in 50-fold-greater amounts. Hybridization experiments indicate that at least some of the *c-src* mRNA is initiated from the SV40 late promoter.

The large amount of pp60^{*c-src*} produced in this system is equal to the amount of pp60^{*v-src*} produced in RSV-transformed cells. In addition, the pp60^{*c-src*} synthesized in pSVOHCS11-transfected COS cells has the same protease cleavage map as pp60^{*c-src*} isolated from CEF and possesses an intrinsic protein kinase activity. This system has been useful in identifying monoclonal antibodies prepared against pp60^{*v-src*} that are cross-reactive with pp60^{*c-src*} (S. A. Parsons, J. T. Parsons, and T. M. Gilmer, unpublished data).

The mRNA for *c-src* is known to be ca. 4 to 4.5 kb by denaturing agarose gel electrophoresis (31; Fig. 2B). This amount of information is considerably greater than the 1.7 kbp of *c-src* information that was transduced to make the RSV genome (38, 39). Hybridization analysis of Northern blots with DNA restriction fragments from the *c-src* locus indicates that at least some of the additional information is derived from sequences mapping 3' to the *v-src/c-src* regions of homology. One of the fragments which hybridizes with *c-src* mRNA produced in pSVOHCS11-transfected COS cells is a 1.1-kbp *SacI*-*BamHI* fragment (map coordinates, 4.9 to 6.0) that contains homology with the 3' terminal region of *v-src*. Furthermore, the *SacI*-*BamHI* fragment and a *NcoI*-

BamHI fragment (map coordinates, 5.2 to 6.0) hybridize to *c-src* mRNA from CEF (Shinji Iijima and Lu-Hai Wang, personal communication). In addition, two *BamHI* fragments (map coordinates, 6.0 to 6.5 and 6.5 to 8.8) mapping downstream from this region hybridize to *c-src* mRNA produced in pSVOHCS11-transfected COS cells. However, the *c-src* gene is flanked on the 3' side by a 4.7-kbp *HindIII* fragment (map coordinates, 9.6 to 14.2) which hybridized with a 2.0-kb mRNA species (31).

DNA sequence analysis of the 12 exons of *c-src* by Takeya and Hanafusa (39) revealed a tentative carboxy-terminal region of *c-src*, including a termination codon, which was different than that of *v-src*. The carboxy-terminal *v-src* coding region (excluding the last five nucleotides and termination codon) was located 0.9 kbp downstream of exon 12 (39, Fig. 1 and 5). This region is contained within the *SacI*-*BamHI* fragment described above.

An alternative possibility is that this 3'-most region of homology between *v-src* and *c-src* represents a 13th exon which contains the coding region for the carboxy terminus of *c-src* (38). A polyadenylation site for RNA has not been identified in either *c-src* region by DNA sequence analysis.

The data presented in this paper suggest that *c-src* DNA sequences containing homologies to the *v-src* carboxy terminus (exon 13) may be transcribed into *c-src* mRNA. Additional sequences within 3' of this region are also transcribed. The precise structure of the *c-src* mRNA in this 3' terminal region and the determination of the *c-src* 3' carboxy-terminal coding region can be determined most directly by analysis of cDNA clones of *c-src* mRNA. COS cells transiently expressing high levels of *c-src* mRNA should provide a convenient source of RNA for this purpose.

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