Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 6140–6144, July 1993
Cell Biology

The protooncogene c-*sea* encodes a transmembrane protein-tyrosine kinase related to the Met/hepatocyte growth factor/scatter factor receptor

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Communicated by Peter K. Vogt, February 22, 1993

ABSTRACT **c-*sea* is the cellular homologue of the avian erythroblastosis virus S13-encoded oncogene v-*sea*. We have isolated and determined the nucleotide sequence of overlapping chicken cDNAs that encode the putative c-*sea* protooncogene product. The predicted reading frame encoded a 1404-aa polypeptide that had the structure of a receptor-like protein-tyrosine kinase and exhibited the highest degree of sequence similarity with the Met/hepatocyte growth factor/scatter factor receptor. Analysis of steady-state RNA expression revealed that c-*sea* mRNA levels were elevated ≈5-fold in chicken embryo cells transformed by activated variants of the *src* nonreceptor protein-tyrosine kinase gene but not in cells transformed by the nuclear oncogenes v-*myc* or v-*rel*. A survey of c-*sea* expression in a variety of chicken tissues indicated that the highest levels of mRNA were located in peripheral white blood cell populations and in the intestine.**

The avian erythroblastosis virus S13 is an oncogenic retrovirus that transforms both chicken and quail embryo fibroblasts and chicken erythroid cultures *in vitro* (1, 2). The oncogenic potential of the S13 virus results from the acquisition of cellular DNA sequences encoding a tyrosine kinase termed *sea* (for *s*arcoma, *e*rythroblastosis, and *a*nemia) (3). The transforming oncogene of S13 (v-*sea*) encodes a 155-kDa transmembrane glycoprotein that contains extracellular and transmembrane regions derived from the viral envelope gene and a *sea*-derived intracellular domain possessing tyrosine-specific protein-kinase activity (4). The v-*sea*-encoded protein shares amino acid homology with the kinase domain of the Met protein, a receptor tyrosine kinase (5).

The cellular *met* locus encodes a receptor protein-tyrosine kinase with a distinctive heterodimeric structure (6–8). The protein is synthesized as a 190-kDa precursor that is proteolytically processed into an amino-terminal 50-kDa α chain and a carboxyl-terminal 145-kDa β chain. Recently, Met was identified as the receptor for hepatocyte growth factor (HGF) and scatter factor (SF), two identical proteins with distinct biological activities (9, 10).

In this report we describe the cloning and characterization of cDNAs encoding the cellular homologue of the *sea* oncogene, c-*sea*.‡ c-*sea* was found to encode a receptor tyrosine kinase that exhibited structural similarity to Met in both the extracellular ligand-binding domain and the cytoplasmic tyrosine kinase domain. c-*sea* exhibited a highly restricted pattern of expression in cells and tissues. The highest levels of c-*sea* expression were observed in peripheral white blood cell populations, whereas most tissues (muscle, liver, heart, brain, kidney, spleen, and thymus) contained low or undetectable levels of c-*sea* RNA. We also show that c-*sea* mRNA levels were significantly elevated in chicken embryo (CE) cells transformed by oncogenic forms of the cytoplasmic protein-tyrosine kinase pp60^{src}, but not in cells transformed by the avian oncogene *myc* or *rel*. The potential role of the Sea receptor during cell growth and/or differentiation is discussed.

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MATERIALS AND METHODS

Cell Culture, Tissue Isolation, and Preparation and Analysis of RNA. Primary CE cells were prepared and cultured as described (11). Cells were transformed with retroviral vectors containing *src* or *myc* as described (12, 13). Total cellular RNA was extracted from cells and tissues by lysis in guanidinium isothiocyanate followed by ultracentrifugation through a cesium trifluoroacetate cushion (Pharmacia) (14, 15). Poly(A)-containing RNA was isolated by two rounds of chromatography on oligo(dT)-cellulose (type 3; Collaborative Research). White blood cell populations were purified by centrifugation through Ficoll-Paque (Pharmacia LKB) as described (16). Northern analysis was performed according to established procedures (17). RNA loading and transfer were monitored by hybridization to the cDNA encoding the stably transcribed chicken glyceraldehyde phosphate dehydrogenase (*gad*) gene (18). For RNase protection assays, antisense c-*sea*-specific and *gad*-specific RNA probes were synthesized from pBluescript vectors (Stratagene) that contained either a 210-bp *Pst* I restriction enzyme fragment from the 5′ end of c-*sea* clone 1.2 or a 180-bp *Hin*dIII fragment from chicken *gad* cDNA. Twenty-microgram samples of cellular RNA were hybridized to 1×10^6 cpm of ^{32}P-labeled antisense RNA in a solution consisting of 80% formamide, 40 mM Pipes (pH 6.7), 0.4 M NaCl, and 1 mM EDTA for 12–18 hr at 45°C. Samples were subjected to digestion with RNase A (80 μg/ml) in 10 mM Tris·HCl (pH 7.4), 1 mM EDTA, 200 mM NaCl, and 100 mM LiCl for 30–60 min at 37°C. Protected fragments were resolved on a 6% denaturing polyacrylamide gel (19).

Library Construction, Screening, and cDNA Sequence Analysis. λgt10 cDNA libraries, which contained $\approx 5 \times 10^5$ independent recombinant phage, were constructed by using poly(A)-containing RNA isolated from either normal CE cells or from CE cells transformed by c-*src*^{527F}, an activated c-*src* variant (12). For isolation of 5′ c-*sea* cDNAs, a cDNA library was constructed (Clontech) by using RNA prepared from 6-hr serum-stimulated CE cells. First-strand cDNA synthesis was primed using random hexamers in addition to a c-*sea*-specific primer with the sequence 5′-GCCACCGTCCACATTCTG-3′

Abbreviations: CE, chicken embryo; HGF, hepatocyte growth factor; SF, scatter factor.

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‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. L12024).

(complementary to bp 166–183 of clone 1.2). Genomic c-*sea* clones were obtained from an adult liver genomic library made in EMBL3 (Clontech). DNA and amino acid sequences were compiled and analyzed using the MBIR software package of Baylor College of Medicine and FASTA (20).

RESULTS AND DISCUSSION

Identification and Characterization of cDNA Clones Encoding the c-*sea* Protooncogene. Fig. 1 summarizes the structures of overlapping cDNA clones that make up the full-length coding sequence for c-*sea*. The initial c-*sea*-specific cDNA clone was isolated serendipitously from a λgt10 cDNA library constructed from normal CE cell RNA. A low-stringency screen of this library with a kinase domain probe derived from the v-*src* gene yielded a 750-bp cDNA, clone tjl1. The predicted amino acid sequence of clone tjl1 showed only partial amino acid identity with pp60^{src} (37%); however, this cDNA exhibited ≈98% identity with the tyrosine kinase oncogene v-*sea*. To isolate additional cDNA clones encoding c-*sea*, clone tjl1 was used to screen a second λgt10 cDNA library prepared from c-*src*^{527F}-transformed CE cell RNA. Seven additional clones were isolated. The largest, clone 1.2, was 3.3-kbp in length and encoded a predicted polypeptide of 1001 amino acids with a 300-bp 3′ untranslated region (Fig. 1). To identify clones containing the remaining 5′ coding sequences, an additional cDNA library was constructed by using a c-*sea*-specific primer (see *Materials and Methods*). This library yielded two additional clones (25 and 62) that extended 1100 bp 5′ to clone 1.2 (Fig. 1). To obtain 5′-most coding sequences, a chicken genomic library was screened using an 800-bp fragment derived from clone 62 (Fig. 1, fragment C). A phage containing an insert of ≈14 kb was isolated. DNA sequence analysis of a 400-bp *Pst* I–*Sma* I subfragment (clone 8C8; Fig. 1) revealed an ATG codon followed by an uninterrupted open reading frame that directly overlapped sequences contained in the cDNA clone 62. Alignment of sequences from cDNA clones 1.2, 25, 62, and the genomic fragment 8C8 yielded a 4673-bp sequence encoding a theoretical protein of 1404 residues (Fig. 2). The predicted start of translation is defined by an ATG codon embedded within a preferred translational initiation sequence (21) and is followed by a 22-aa sequence conforming to the rules for a signal peptide sequence (22). The region directly 5′ of the ATG start codon is interrupted by stop codons in all three reading frames (Fig. 2).

c-*sea* Is Structurally Related to the Met/HGF/SF Receptor. The Met and Sea receptors exhibited an overall 35% amino acid identity. The highest degree of homology was observed in the cytoplasmic tyrosine kinase domain (70% identity). Both Met and Sea possess a cysteine residue in the ATP-binding domain, which is generally a valine residue in most other kinases studied (6, 7, 23). Two tyrosine residues [Tyr-1245 and Tyr-1246 in Sea and Tyr-1252 and Tyr-1253 in Met (ref. 6; Figs. 2 and 3)] are located in a position analogous to the autoregulatory site of tyrosine phosphorylation, Tyr-416, of pp60^{src} (23). In the Met protein, Tyr-1253 is the major site of receptor autophosphorylation (24). Like Met, the cytoplasmic kinase domain of Sea is preceded by a large juxtamembrane domain (101 residues) (6, 7) and a 23-aa membrane-spanning domain (Fig. 2). Carboxyl terminal to the kinase domain, the amino acid similarity between Sea and Met diminishes. However, two tyrosine residues (Tyr-1360 and Tyr-1367 in Sea) are conserved and may play a role in receptor regulation and receptor–substrate interactions (25, 26). The extreme carboxyl-terminal region of Sea contained 14 out of 19 acidic amino acid residues, which were preceded by a proline-rich stretch (residues 1374–1380) (Fig. 2). This structural motif is not shared with Met and may reflect a distinctive regulatory region that may be required for Sea-mediated signaling.

The 963-aa extracellular domain of Sea exhibited ≈28% amino acid identity with Met. Sea was found to contain 36 cysteine residues within the extracellular domain, with two clusters residing between amino acids 94 and 171 and between residues 522 and 585 (Figs. 2 and 3). Twenty-seven cysteine residues were conserved positionally in the human Met protein. Sea also contained a proteolytic processing site (K^{297}RRRRR) that aligned with a similar site in the Met protein. The Sea protein appears to be similarly processed (unpublished observation). The conservation of cysteine residues and the presence of a putative cleavage site suggested that the overall topology of the extracellular domain of Sea may be similar to that of Met and may possibly reflect the

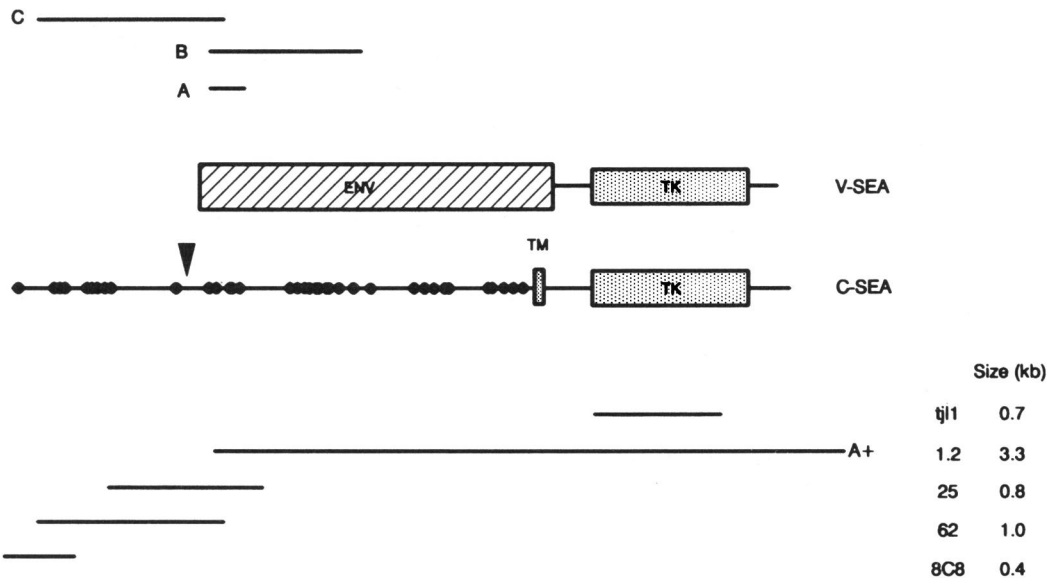

FIG. 1. Schematic representation of c-Sea protein and *sea*-specific cDNA clones. Circles indicate the positions of cysteine residues in the extracellular domain, and the arrowhead indicates a potential proteolytic processing site. TM, transmembrane region; TK, tyrosine kinase domain; ENV, viral envelope sequences. Fragments B and C were used as hybridization probes in library screening, B was used in Northern analysis, and A was used in RNase protection assays.

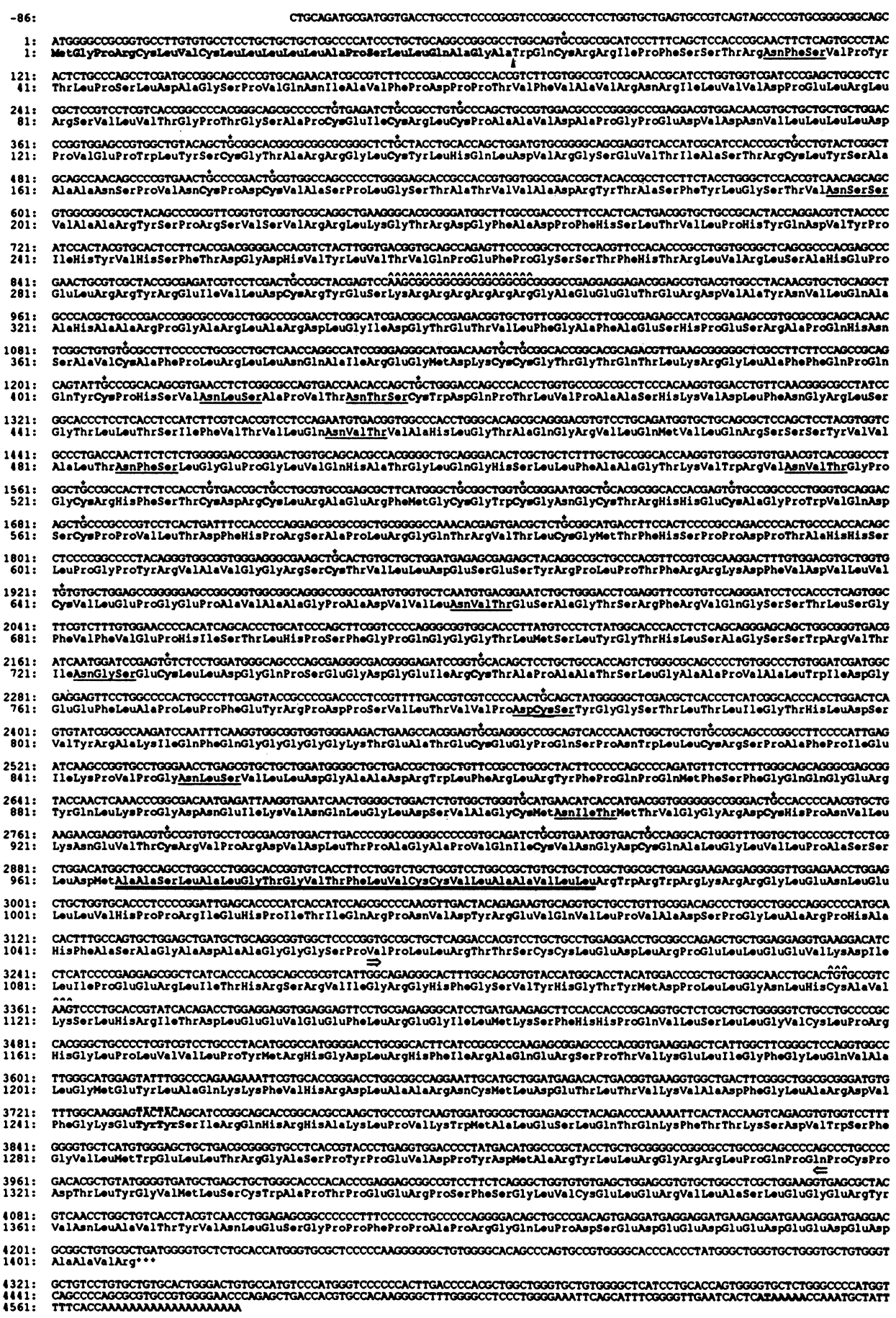

FIG. 2. (*Legend appears at the bottom of the opposite page.*)

FIG. 3. Alignment of c-Sea and human Met protein sequences. Conserved cysteine residues are shown in boldface type and are indicated by stars. The predicted proteolytic cleavage site is indicated by carets, the transmembrane domains are shown in boldface type, the amino acids encompassing the tyrosine kinase domain are indicated by open arrows, the location of the cysteine residue and the conserved lysine residue in the ATP-binding domain are indicated by tildes, and the conserved tyrosine residues are shown in boldface type and are indicated by dashes.

binding of a structurally similar or related ligand. Both Sea and Met constitute members of a distinctive family of receptor protein-tyrosine kinases (8) as suggested by earlier chromosomal mapping studies of Williams *et al.* (27).

c-*sea* mRNA Expression and Tissue Distribution. Both normal and transformed CE cell RNAs contained a major c-*sea*-specific mRNA of 5.3–5.5 kb and less abundant mRNAs of 2.8 kb and 3.0–4.4 kb (Fig. 4). The relative levels of c-*sea* mRNAs were elevated ≈5-fold in *src*-transformed cells as compared to normal cells. The increase in c-*sea* expression was not observed in CE cells transformed by v-*myc* (13) or in cells infected by a nontransforming deletion variant of *src* (data not shown). The $pp60^{src}$-mediated increase in expression of Sea receptor mRNA is unusual and contrasts with previous experiments showing the Src-induced down-regulation of epidermal growth factor receptors in transformed fibroblasts (28). Although it has not been shown that the increased level of c-*sea* mRNA is mirrored by an increase in Sea receptor number, it is possible that the increased expression contributes to the mechanism of Src-mediated oncogenesis and may indicate that Sea is functioning to potentiate Src-induced transformation by either an autocrine or a paracrine mechanism.

An analysis of the tissue distribution of c-*sea* mRNA was carried out by using an RNase protection assay (Fig. 5). Low levels of c-*sea* expression, comparable to that observed in normal CE cells (lane 15), were observed in 17-day embryo skeletal muscle (lane 1), bone (lane 5), brain (lane 6), 1-day chicken bursa (lane 7), spleen (lane 8), thymus (lane 9), adult ovary (lane 13), and tongue (lane 14). Increased c-*sea* mRNA expression was detected in RNA from 1-day chicken intestine (lane 11), while the highest levels of c-*sea* expression were detected in RNA isolated from a peripheral blood-derived white cell population (lane 10). It is unclear at present which particular lymphoid cell population exhibited elevated c-*sea* expression.

c-*sea* and c-*met* appear to be quite distinct in their tissue expression patterns. In humans, *met* is abundantly expressed in a restricted subset of tissues, including stomach, liver, thyroid, and kidney, with no expression detected in white blood cells (29, 30). In contrast c-*sea* expression appeared to be even more highly restricted in tissue distribution; it is confined to an as yet undefined population of white blood cells. The narrow tissue distribution suggested that c-*sea* expression may be limited to a particular cell lineage or to a specific developmental stage.

Normal Function of the Sea Receptor. The similarity exhibited by Sea to the Met receptor in the extracellular domain, including the conservation of cysteine residues and the presence of a potential proteolytic processing site, indicated that these two proteins may possess similar structural features. The ligand of the Met receptor, HGF/SF, exhibits uniquely diverse properties, inducing mitogenesis in hepatocytes and alterations in cell–cell interactions in epithelial cells. Recently an HGF-like factor that contains a similar domain structure and exhibits ≈50% amino acid identity to HGF has been identified (31, 32). This factor fails to bind to cells expressing the Met/HGF/SF receptor, indicating that other receptors may mediate the activity of this protein. Identification of Sea as a second member of the Met/

FIG. 2. Nucleotide and deduced amino acid sequence for c-*sea*. The predicted signal peptide cleavage is indicated by an arrowhead. Cysteine residues in the extracellular domain are shown in boldface type and are denoted by stars. Potential sites of N-linked glycosylation (Asn-Xaa-Ser/Thr) are underlined. The putative amino-terminal proteolytic processing site is indicated by carets (∧), and the transmembrane domain is indicated by a double underline. The tyrosine kinase domain is indicated by open arrows. The triple carets indicate the location of the cysteine residue and the conserved lysine residue in the ATP-binding domain. The potential sites of autoregulatory tyrosine phosphorylation are shown in boldface type and are indicated by dashes.

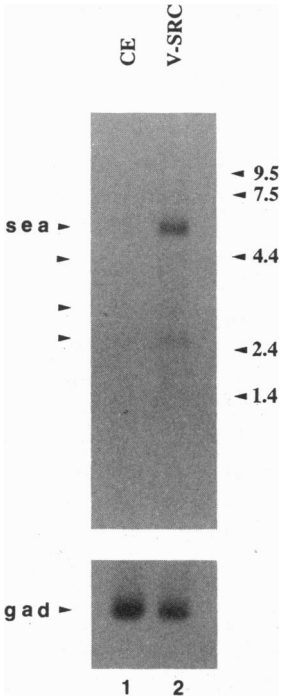

FIG. 4. Northern blot analysis of c-*sea* expression in normal and v-*src*-transformed CE cells. Duplicate nylon filters containing 5.0-μg samples of poly(A)-RNA from both normal CE cells and RSV-transformed CE cells were hybridized with either ^{32}P-labeled DNA from clone tjl1 or from chicken *gad*. Arrowheads on the left indicate the position of c-*sea* mRNAs. Arrowheads on the right indicate the position of RNA standards run in parallel.

HGF/SF receptor family suggests the possibility that Sea may be the receptor for such a factor or for another as yet unidentified HGF-like protein. The elevated level of c-*sea* expression in Src-transformed cells also suggests the possibility that HGF/SF-like activities may be induced in response to transformation and therefore may contribute to the tumorigenic properties of such cells *in vitro* and possibly *in vivo*.

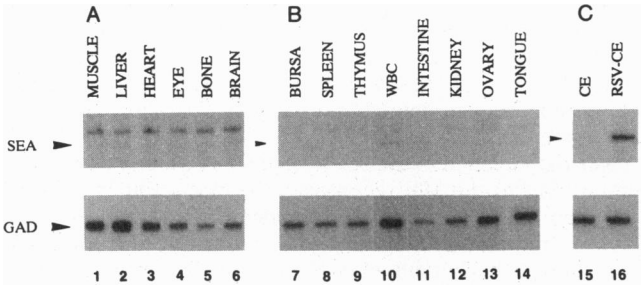

FIG. 5. c-*sea* expression in normal chicken tissues. RNA samples were hybridized to c-*sea*- and *gad*-specific RNA probes. Arrowheads indicate the position of the protected fragments. (*A*) RNA from 17-day-old embryo skeletal muscle (lane 1), liver (lane 2), heart (lane 3), eye (lane 4), bone (lane 5), and brain (lane 6). (*B*) RNA from bursa (lane 7), spleen (lane 8), thymus (lane 9), intestine (lane 11), and kidney (lane 12) of 1-day-old chickens and from white blood cell populations (WBC; lane 10), ovary (lane 13), and tongue (lane 14) of an adult hen. (*C*) RNA from normal (lane 15) and RSV-transformed CE (lane 16) cells. The band appearing above the *sea*-specific band results from incomplete digestion of the *gad* probe. Exposure times were 30 min for *gad* and 2 days for *sea*. RSV, Rous sarcoma virus.

We acknowledge the late Betty Creasy for preparation of chicken embryo cell cultures. We are indebted to T. Bender, T. Jamieson, and J. Hildebrand for assistance in the preparation of chicken tissue RNA. We thank A. Bouton, M. Schaller, M. Weber, and T. Bender for helpful discussions during the course of this work and in the preparation of this manuscript. This research was supported by National Institutes of Health Grant CA27578 and American Cancer Society Grant MV430.

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