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Tissue-Specific Expression of Rat c-*ros-1* Gene and Partial Structural Similarity of Its Predicted Products with *sev* Protein of *Drosophila melanogaster*

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The expression and predicted products of rat c-*ros-1* gene, the proto-oncogene of v-*ros* in UR2 sarcoma virus, were characterized. The c-*ros-1* gene was found to be expressed in a tissue-specific manner, and the sizes of its transcripts were heterogeneous: 8.2 kilobases (kb) long in lung and kidney tissues, 6.9 kb in heart tissue, and 2.4 kb and 1.9 kb in testis tissue. The c-*ros-1* cDNAs were isolated from lung and heart tissues. The predicted product of the c-*ros-1* gene in lung tissue was a receptor-type tyrosine kinase 2,317 amino acids long (including a very large extracellular domain of approximately 1,800 amino acids) which showed a partial but significant structural homology with the *sev* gene product of *Drosophila melanogaster*. An alternatively sliced lung transcript was found to encode a protein with external and transmembrane domains but not a tyrosine kinase catalytic domain. The predicted product in heart tissue was essentially identical to that in lung tissue except for a shorter amino-terminal region and a 21-amino-acid insertion in the extracellular domain. On the basis of these results, the c-*ros-1* gene appears to be active in the lungs and kidneys and probably in the hearts of rats.

About half of the known proto-oncogenes are classified into the receptor-type tyrosine kinase family and are considered to have important functions in signal transduction on the cell surface (7). Among these genes, the v-*erbB* and v-*fms* genes were shown to be derived from the receptor genes for epidermal growth factor and colony-stimulating factor 1, respectively (9, 25, 30, 32). However, the physiological roles of most of the other genes are still unknown.

The transforming gene product, $p68^{gag\text{-}ros}$, encoded by UR2 sarcoma virus genome (1, 26, 31) has tyrosine-specific protein kinase activity (13). Molecular cloning of the v-*ros* gene and a portion of the genomic c-*ros* clones from chickens and humans has revealed that the c-*ros* gene can be classified into the receptor-type tyrosine kinase gene family because of the presence of a transmembrane domain upstream of the kinase domain (19–21). Because of the extremely low expression of the c-*ros* gene in chickens (20, 22, 26), only a portion of the cDNA fragments of the c-*ros* gene has been isolated (22).

In mammalian species, an activated form of human c-*ros-1* gene, *mcf-3*, has recently been isolated from MCF7 cells by using a tumorigenicity assay (5). Since a recombination to a DNA fragment of unknown origin had taken place at the 5′ region of the tyrosine kinase domain, most of the extracellular domain of the human c-*ros-1* gene was lost in the *mcf-3* transforming sequence. With regard to expression, Birchmeier et al. have shown that the c-*ros-1* gene is expressed in glioblastoma multiforme among a variety of human tumors (6). However, the expression of the c-*ros-1* gene in normal tissues has not been extensively studied yet. Thus, the entire structure and the physiological functions of this gene have not yet been characterized in either avian or mammalian species.

In this study, the expression of the c-*ros-1* gene in rats was examined and cDNA fragments containing the whole coding sequence of the c-*ros-1* gene were molecularly cloned. The c-*ros-1* gene was found to be expressed in a tissue-specific manner, and the sizes of the c-*ros-1* transcripts were different in various tissues. The predicted product of the longest transcript expressed in lung and kidney tissues was partially homologous to that of the *sevenless* (*sev*) gene of *Drosophila melanogaster* (3, 8, 16). This might indicate that the c-*ros-1* product in mammals has a function similar to that of the *sev* gene product in a signal transduction such as cell-to-cell communication.

MATERIALS AND METHODS

RNA extraction, blot analysis, and probes. Total cellular RNA was extracted from various tissues of Fischer rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) by the method described by Glisin et al. (14). Poly(A)$^+$ RNA was isolated by oligo(dT) column chromatography.

Poly(A)$^+$ RNA was electrophoresed in a 1% agarose gel containing 2.2 M formaldehyde and 20 mM sodium phosphate buffer (pH 7.2). RNA was transferred to nitrocellulose (Schleicher & Schuell, Inc.) in 20× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.4) (18). Prehybridization was performed overnight at 37°C in a solution containing 50% formamide, 3× SSC, 50 mM Tris hydrochloride (pH 7.5), 20 μg of tRNA (Sigma Chemical Co.) per ml, 20 μg of boiled salmon sperm DNA (Sigma) per ml, 1 mM EDTA, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, and 0.02% Ficoll. Hybridization was performed for about 40 h at 37°C in this solution containing a ^{32}P-labeled DNA probe. Filters were finally washed at 50°C for 30 min in 0.1× SSC and 0.1% sodium dodecyl sulfate.

All DNA and cDNA fragments used as probes were electrophoretically purified from agarose gels and labeled by a method using hexadeoxynucleotide random primers with [α-^{32}P]dCTP (Amersham Corp.) (12). The c-*ros-1* cDNA fragments for probes were a 0.8- and a 0.5-kilobase (kb)

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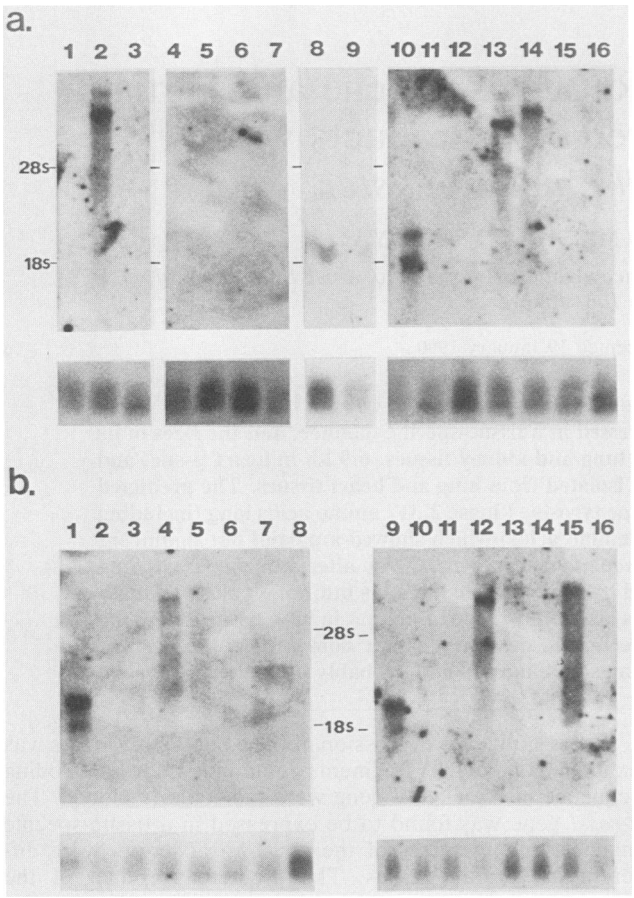


FIG. 1. Expression of rat c-*ros-1* gene in normal tissues. About 4 μg of poly(A)$^+$ RNA from various tissues of rats was analyzed by the Northern blot method with testis-derived 0.8-kb c-*ros-1* cDNA as a probe (see Materials and Methods). RNAs were prepared from embryo (panel a, lanes 1 to 9), 4-week-old (panel a, lanes 10 to 16), 9-week-old (panel b, lanes 1 to 8), and 15-week-old (panel b, lanes 9 to 16) rats. Tissues used were intestine (panel a, lane 1; panel b, lanes 8 and 16), lung (panel a, lanes 2 and 14; panel b, lanes 5 and 13), spleen (panel a, lane 3), brain (panel a, lanes 4 and 12; panel b, lanes 3 and 11), heart (panel a, lanes 5 and 13; panel b, lanes 4 and 12), liver (panel a, lanes 6 and 15; panel b, lanes 6 and 14), muscle (panel a, lane 7), placenta (panel a, lane 8), kidney (panel a, lanes 9 and 16; panel b, lanes 7 and 15), testis (panel a, lane 10; panel b, lanes 1 and 9), and salivary gland (panel a, lane 11; panel b, lanes 2 and 10). As a positive control, the same nitrocellulose filters were hybridized with H-*ras* probe (see Materials and Methods). The H-*ras* mRNA of 1.4 kb is shown in the lower sections of panels a and b.

*Eco*RI fragment (see Fig. 2b). The H-*ras* probe was a 460-base-pair (bp) *Eco*RI fragment from pBS-9 (11).

Genomic DNA and cDNA libraries. Human and rat genomic-DNA libraries were constructed as follows. Human placental DNA partially digested with *Alu*I and *Hae*III was ligated with Charon 4A *Eco*RI arms with *Eco*RI linkers, and rat testis DNA partially digested with *Sau*3AI was ligated with EMBL3 *Bam*HI arms (Promega Biotec), as described by Maniatis et al. (18). Rat c-*ros-1* genomic DNA was isolated from a genomic library by screening with a human c-*ros-1* genomic *Hin*dIII-*Bam*HI DNA fragment 3.2 kb long (including tentative exons 4, 5, and 6 of reference 19). A rat c-*ros-1* genomic *Eco*RI-*Eco*RI DNA fragment 3.8 kb long which corresponded to a region of human c-*ros-1* exons 5 and 6 was subcloned and used for detection of rat testis c-*ros-1* transcripts by Northern (RNA) blot analysis.

TABLE 1. Expression of rat c-*ros-1* gene in normal tissues

Tissues (size of mRNA [kb])	mRNA level[a] at stage:			
	Embryo	4 wk	9 wk	15 wk
Brain	−	−	−	−
Salivary gland	ND	−	−	−
Lung (8.2)	++	+	+/−	+/−
Heart (6.9)	−	++	+	+
Liver	−	−	−	−
Spleen	−	ND	ND	ND
Intestine	−	ND	−	−
Kidney (8.2)	+/−	+/−	+/−	+
Testis (1.9, 2.4)	ND	++	+++	++
Muscle	−	ND	ND	ND

[a] On a scale of − to +++, with − representing no expression. ND, Not determined.

With λgt10, λgt11, or λgtWES · λB bacteriophage as a vector, rat testis, heart, and lung cDNA libraries were constructed as described by Gubler and Hoffman (15) with a slight modification: to generate the blunt ends of cDNA fragments, mung bean nuclease was used instead of T4 DNA polymerase. Genomic-DNA and -cDNA libraries were screened by the method of Benton and Davis (4).

DNA sequencing. DNA fragments were subcloned into pUC118 (Takara Shuzo) and sequentially and unidirectionally deleted with exonuclease III (17). The DNA sequence was determined by using the method of Sanger et al. (24).

RESULTS

Expression of rat c-*ros-1* gene in normal tissues. As an initial step for the screening of gene expression, we examined the transcripts of human c-*ros-1* gene in cultured cells by using a genomic DNA fragment as a probe (19). However, no clear bands were detected in poly(A)$^+$ RNAs obtained from more than 20 human tumor cell lines (data not shown). Since murine species are convenient for surveying gene expression in a variety of normal tissues, rat c-*ros-1* genomic DNA was isolated (see Materials and Methods) and hybridized with poly(A)$^+$ RNA prepared from various tissues of embryonic and 4-, 8-, and 15-week-old rats. Very faint bands 2.4 and 1.9 kb long were detected only in testis tissue. Although these mRNAs seem to be too short to encode a receptor-type tyrosine kinase, molecular cloning and sequencing of these cDNAs from the testis library revealed that at least the entire tyrosine kinase domain of rat c-*ros-1* gene was present in these molecules (see below).

Since a cDNA fragment is more sensitive than a genomic-DNA fragment as a probe in Northern blot analysis, the expression of rat c-*ros-1* gene was further examined with these testis-derived c-*ros-1* cDNA fragments. The results obtained with a 0.8-kb *Eco*RI cDNA fragment which encoded the carboxyl-terminal two-thirds of the kinase domain are shown in Fig. 1. Unexpectedly, clear bands of c-*ros-1* mRNA in not only testis tissue but also lung, heart, and kidney tissues were observed. Moreover, the sizes of the detected bands were heterogeneous: the bands in lung and kidney tissues were 8.2 kb long, and that in heart tissue was 6.9 kb long, whereas those in testis tissue were much shorter, as described above (2.4 and 1.9 kb).

To rule out the possibility that the bands shown in Fig. 1 were generated by cross hybridization with the transcripts of other protein kinase genes, a 0.5-kb *Eco*RI testis c-*ros-1*

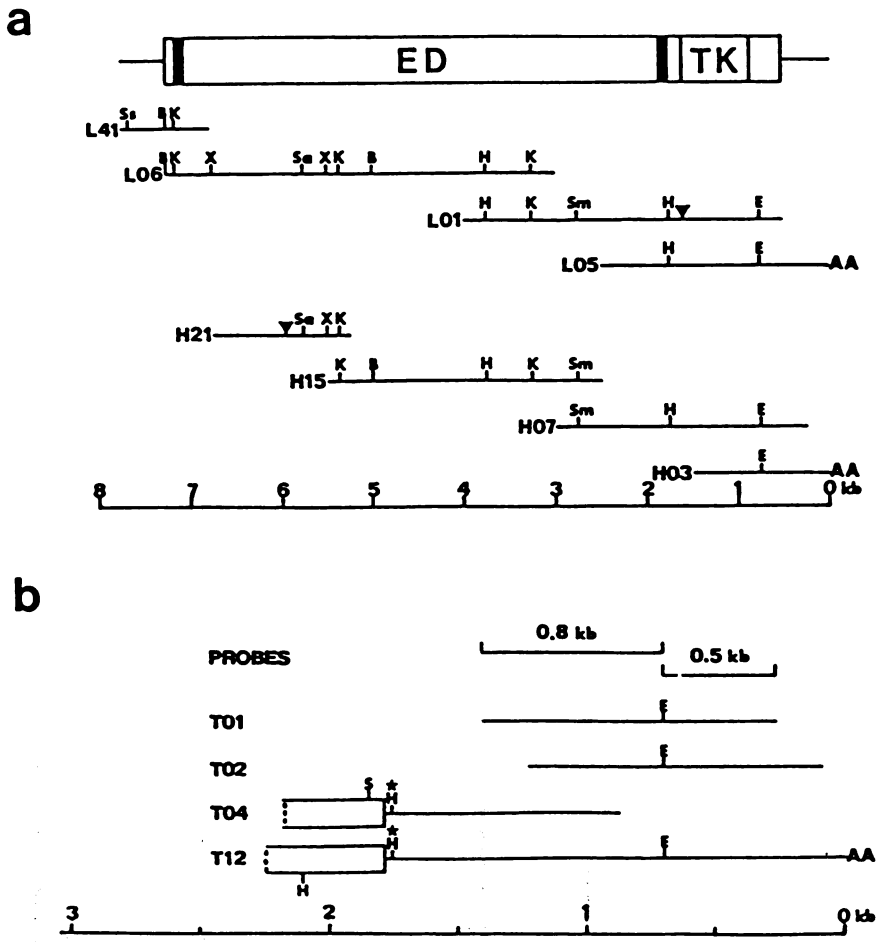


FIG. 2. Structure and restriction map of rat c-*ros-1* cDNAs. (a) Structures of four c-*ros-1* cDNA clones from the lung library (L01, L05, L06, and L41) and four clones from the heart library (H03, H07, H15, and H21). A predicted product of lung c-*ros-1* mRNA is shown by the large box. Abbreviations: ED, extracellular domain; TK, tyrosine kinase domain; Ss, *Sst*I; B, *Bam*HI; K, *Kpn*I; X, *Xho*I; Sa, *Sal*I; Sm, *Sma*I; E, *Eco*RI; H, *Hin*dIII. Symbols: ■, hydrophobic stretches; ▼, insertion sequences shown in Fig. 3b and c on L01 and H21 clones, respectively. (b) Structures of four cDNA clones (T01, T02, T04, and T12) isolated from the rat testis cDNA library. The 5′ sequences of T04 and T12 (□) are different from each other. Some *Hin*dIII sites (★) are identical. S, *Sal*I. AA, Poly(A) tail.

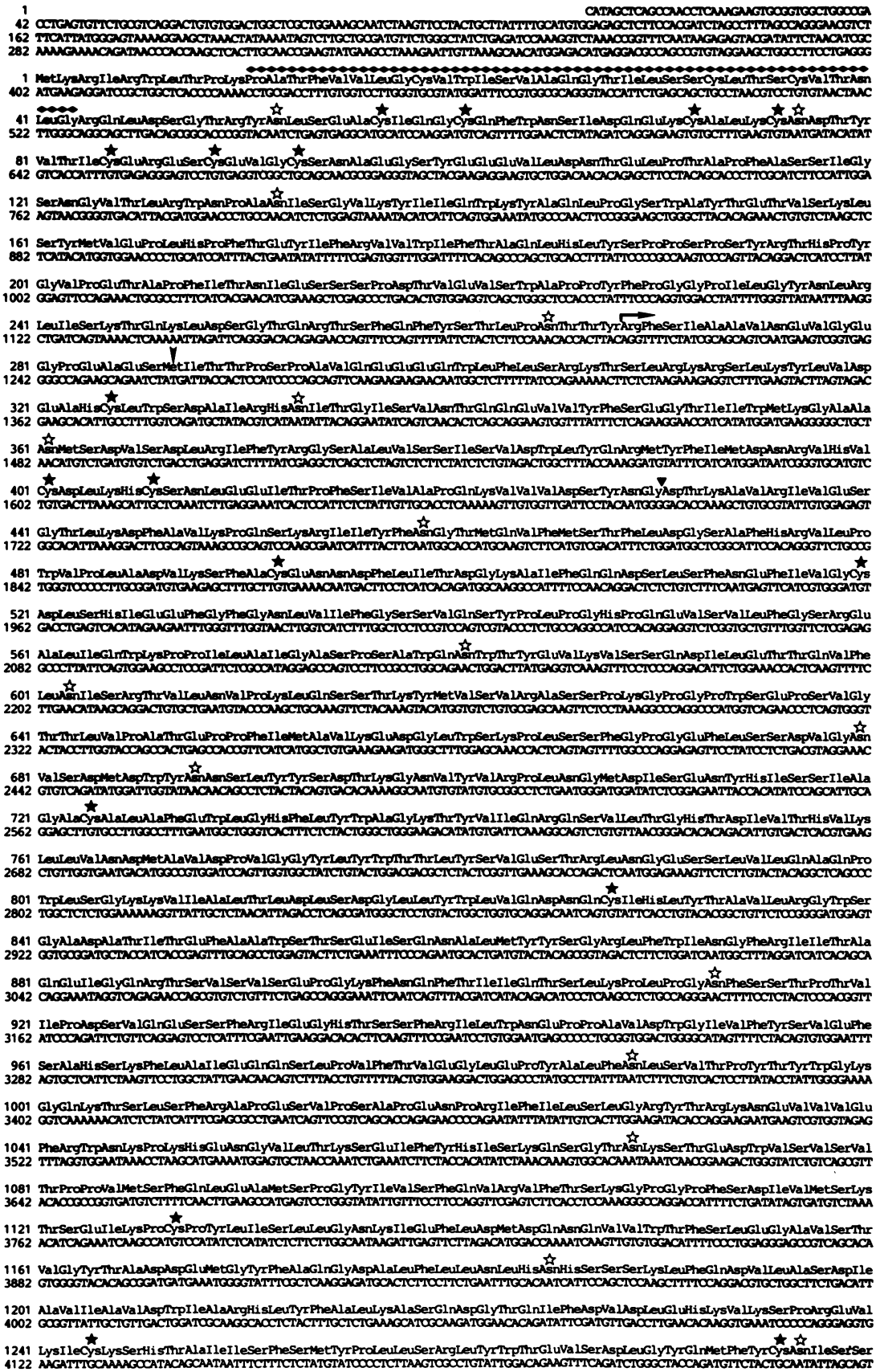
cDNA fragment which coded for a region downstream from the c-*ros-1* kinase domain was used as a probe in Northern blot analysis. Essentially the same pattern as that shown in Fig. 1 was obtained (data not shown), indicating that the c-*ros-1* gene is expressed as different sizes of mRNA in different tissues. The levels of c-*ros-1* transcripts in these tissues changed during the development and aging of the rats: in lung tissue the level of c-*ros-1* mRNA was higher in embryos than after birth, but in heart and kidney tissues this mRNA level was elevated after birth (Table 1).

Structure of lung-type c-*ros-1* cDNA. To examine the possible products encoded in these heterogenous transcripts, c-*ros-1* cDNA molecules were isolated from lung and heart cDNA libraries (see Materials and Methods). The structures of four overlapping clones from lung tissue are shown in Fig. 2a. The nucleotide sequencing analysis of the cDNA fragments revealed that the predicted product of the c-*ros-1* gene consisted of 2,317 amino acids initiating from the ATG at nucleotide residues 402 to 404 in Fig. 3a. Since a stop codon was found 6 bp upstream from this ATG codon in the same reading frame (residues 396 to 398 in Fig. 3a) in both the cDNA and genomic DNA sequences and since other open reading frames are very short, this ATG is most likely the translation initiation codon of the c-*ros-1* gene.

The predicted product of c-*ros-1* in lung tissue could be separated into three regions: an about 1,830-amino-acid extracellular domain, a 24-amino-acid transmembrane domain, and a 463-amino-acid cytoplasmic region including the tyrosine kinase domain. Amino acid residues in the tyrosine kinase domain of rat c-*ros-1* gene are 92% identical to those of human c-*ros-1* gene and 75% identical to those of chicken c-*ros* gene (19, 20). The hydrophobic amino acid stretch near the amino terminus was located 10 amino acids downstream from the first methionine residue, and this position seems unusual for a signal peptide of membrane proteins. At this moment it is not clear whether this stretch is cleaved off by a signal peptidase or remains in the c-*ros-1* molecule, as in the hypothetical model of the *sev* gene product of *D. melanogaster* (3, 8). Other structural similarities between the products of c-*ros-1* and *sev* genes will be described in the Discussion.

In a previous study, homology at the amino acid level between the tyrosine kinase domains of c-*ros* and insulin receptor genes was pointed out (10, 21, 29). However, the extracellular domain in the c-*ros-1* product had no cysteine cluster region and no stretch with basic amino acids, indicating that this domain is not related to the ligand-binding

a.



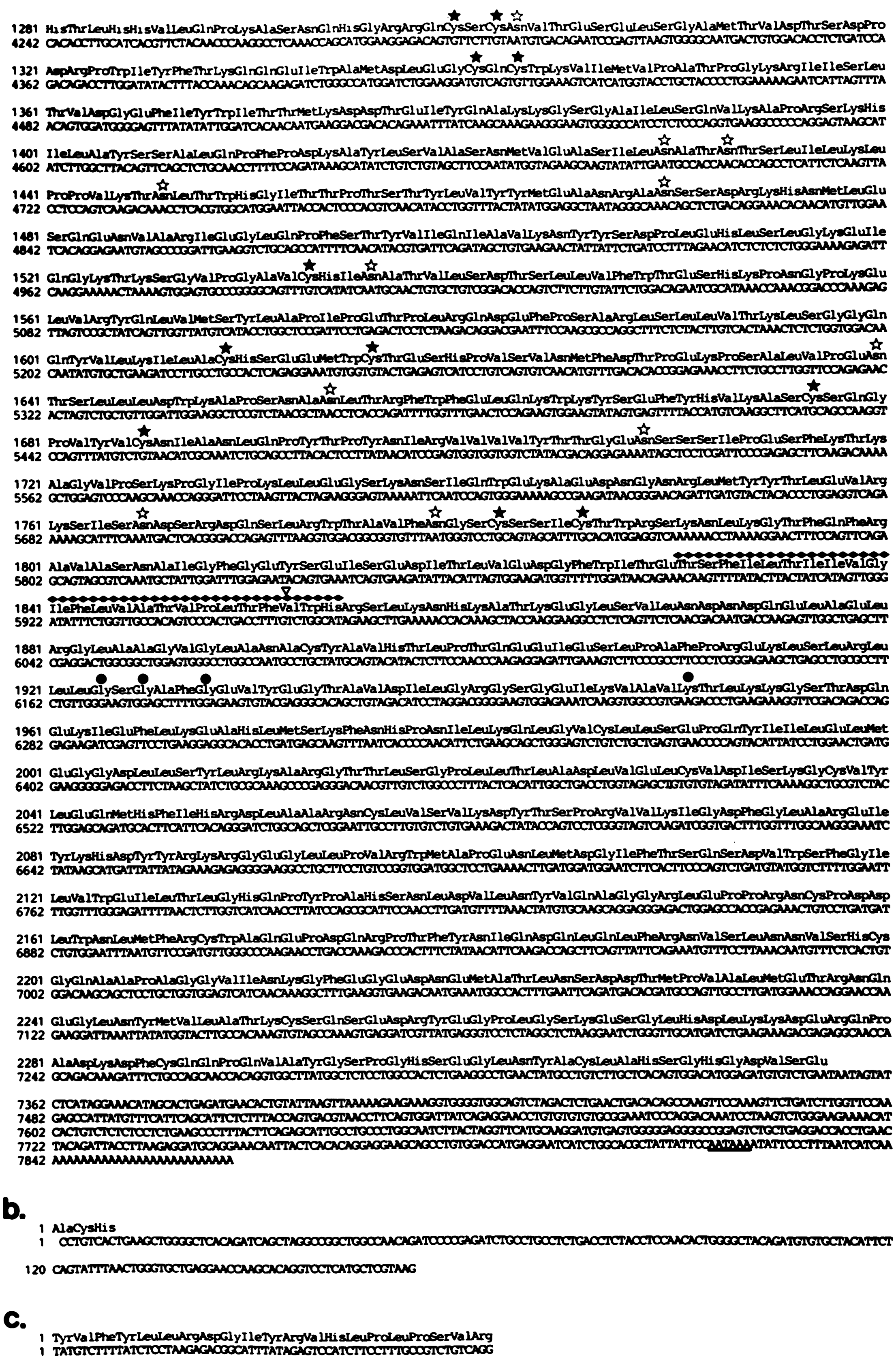


FIG. 3. Nucleotide sequence of rat c-*ros-1* cDNAs. (a) Lung-type c-*ros-1* cDNA of 7,839 bp. Symbols: ◆, hydrophobic amino acid stretches; ★ and ☆, cysteine residues and possible N-linked glycosylation sites between two hydrophobic stretches on extracellular domain, respectively; ↼, the 5′ end of heart H21 cDNA; ▼, candidate initiation methionine of the heart-type c-*ros-1* product; ●, G-X-G-X-X-G stretch and ATP binding site, lysine, in the tyrosine kinase domain; ▽ and ▼, sites of sequences shown in panels b and c, respectively. A stretch of AATAAA at residues 7816 to 7821 is a possible polyadenylation site. (b) Insertion sequence detected in a lung-derived L01 c-*ros-1* cDNA clone between nucleotide residues 5955 to 5956. This position is the same as that at which the 5′ divergence occurs in testis cDNA T04 and T12. (c) Insertion sequence detected in a heart-derived H21 c-*ros-1* cDNA clone between nucleotide residues 1691 to 1692.

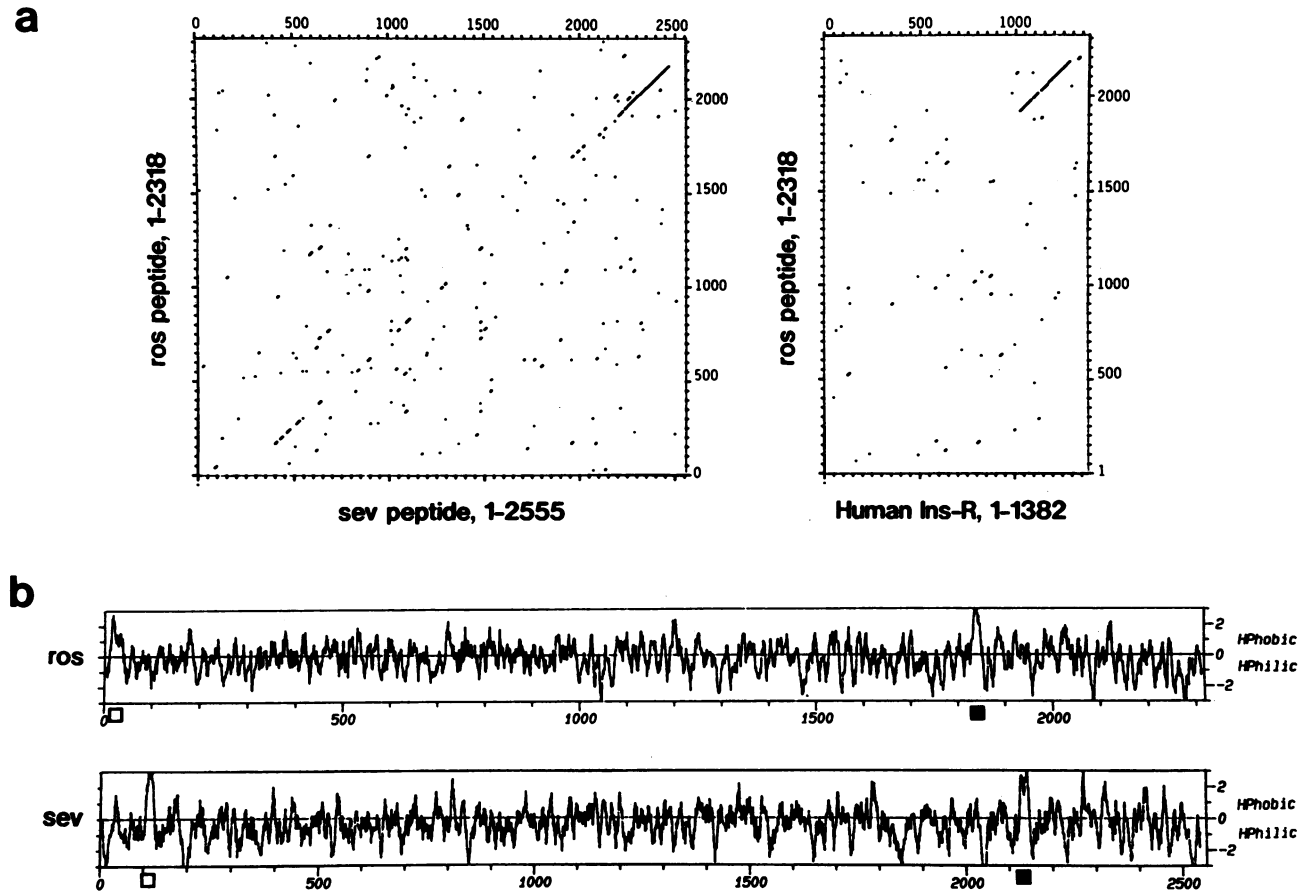


FIG. 4. Comparison of predicted c-*ros-1* and *sev* gene products. (a) DIAGON type plots between c-*ros-1* and *sev* gene products and between c-*ros-1* gene product and human insulin receptor (Ins-R). Pair, 0.6; window, 20; stringency, 13.0 (UWGCG Program). (b) Hydrophobicity profiles of c-*ros-1* and *sev* gene products. Symbols: □ and ■, possible signal peptide and transmembrane domain, respectively. Abbreviations: HPhobic, hydrophobic; HPhilic, hydrophilic. Units are amino acids.

domain of the insulin receptor/insulinlike growth factor-1 receptor family (Fig. 4a).

Among four c-*ros-1* cDNA clones in lung tissue which covered both the transmembrane and tyrosine kinase domains, one clone (L01) had a 171-bp insertion just downstream of the transmembrane domain (Fig. 2a and 3b). Since the insertion point of this sequence on the cDNA was identical to the junction between two exons in human and chicken c-*ros-1* genes (19, 20), an alternative splicing seems most likely to account for this insertion. Interestingly, a stop codon was present in this inserted sequence (Fig. 3b). Thus, if this type of transcript is efficiently translated, a c-*ros-1* product in lung tissue may be a receptorlike molecule lacking the entire cytoplasmic domain.

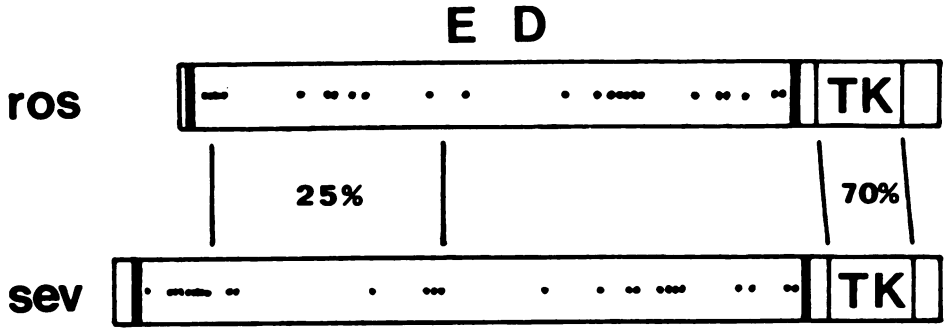
Structure of heart-type c-*ros-1* cDNA. The nucleotide sequence of the cDNA fragments obtained from the heart library was identical to the 3′ 6,636 bp of lung-type cDNA except for a 63-bp insertion (Fig. 3c) in the extracellular domain (between residues 1691 and 1692 in Fig. 3a). The longest open reading frame starting from the ATG sequence in the H21 clone (residues 1260 to 1262 in Fig. 3a) could encode 2,052 amino acids. Since the c-*ros-1* transcript is about 6.9 kb (shown by Northern blot analysis) (Fig. 1) and we have obtained 6.6-kb cDNA without a poly(A) tail, we believe that the major portion of the heart-type c-*ros-1* cDNA has been characterized in this study. Furthermore, two other cDNA clones obtained from the heart cDNA library showed 5′ boundaries of 10 and 30 nucleotides downstream from the 5′ end of the H21 cDNA clone. The clustering of the 5′ boundaries of these three clones within 30 nucleotides supports the hypothesis that the start site of heart c-*ros-1* transcription is located in this region. However, we cannot completely rule out the possibility that a very short cDNA fragment (0.1 to 0.2 kb) bearing the initiation codon for the heart-type c-*ros-1* product has not yet been isolated.

Structure of testis-type c-*ros-1* cDNA. Four c-*ros-1* cDNA clones were isolated from the rat testis cDNA library. Among these, only two clones carried 5′ sequences upstream from the tyrosine kinase domains (Fig. 2b). These 5′ sequences were found to be different from each other and unrelated to lung- and heart-type cDNAs. The junction points between the unique 5′ sequences and the kinase domains in these clones were identical to the point at which an insertion was found in the cDNA clone L01 in lung tissue. Since testis c-*ros-1* transcripts 2.4 and 1.9 kb long are too short to encode a receptor-type tyrosine kinase, in this study we did not characterize them further.

DISCUSSION

We have demonstrated that the proto-oncogene c-*ros-1* in rats is expressed in at least four normal tissues: lung, heart, kidney, and testis. The sizes of the transcripts, 6.9 to 8.2 kb

a.



b.

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c-ros-1  1922  LGSGAFGEVYEGTAVDILGRGSGEIKVAVKTLKKGSTDQEKIEFLKEAHLMSKFNHPNILKQLGVC
               :::::::::::::  +          +::+:+:+:: ++    : : :: ::: : : ::+  +:+:
sev      2215  LGSGAFGEVYEG-QLKTEDSEEPQ-RVAIKSLRKGASEFA--ELLQEAQLMSNFKHENIVCLVGIC

c-ros-1  1988  LLSEPQYIILELMEGGDLLSYLRKARGT-T-LSGPL--LTLADLVELCVDISKGCVYLEQMHFIHR
                 +:   +: : :: ::::::::: :: : :    :   :+: +:+  :+:+  :: ::: :::+::
sev      2277  FDTESISLIMEHMEAGDLLSYLRAARATSTQEPQPTAGLSLSELLAMCIDVANGCSYLEDMHFVHR

                       ●●●●●●●
c-ros-1  2050  DLAARNCLVSVKDYTSPR--VVKIGDFGLAREIYKHDYYRKRGEGLLPVRWMAPENLMDGIFTSQS
               ::: ::::::+    ++ :   ::::::::::+::: ::::: :::::::::: :: : ::+::+::
sev      2343  DLACRNCLVTESTGSTDRRRTVKIGDFGLARDIYKSDYYRKEGEGLLPVRWMSPESLVDGLFTTQS

c-ros-1  2114  DVWSFGILVWEILTLGHQPYPAHSNLDVLNYVQAGGRLEPPRNCPDDLWNLMFRCWAQEPDQRPTF
               ::: ::+: :::::::: ::: :  : +::  :  :::: :  : + :  :   :: +:  ::+:
sev      2409  DVWAFGVLCWEILTLGQQPYAARNNFEVLAHVKEGGRLQQPPMCTEKLYSLLLLCWRTDPWERPSF
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FIG. 5. Comparison of predicted products of the rat c-*ros-1* gene and *Drosophila sev* gene. (a) Percentages indicate the amino acid homology (including related amino acids) between these two genes. Symbols: ▮, hydrophobic amino acid stretches; ●, cysteine residues within the extracellular domains. Abbreviations: ED, extracellular domain; TK, tyrosine kinase domain. (b) Amino acid homology in tyrosine kinase domain between c-*ros-1* and *sev* genes. Symbols: : and +, identical and related amino acids, respectively; ▼ and △, junction points of exons; ●, short amino acid inserts found in both c-*ros-1* and *sev* genes.

in the first three tissues, appear to be enough to encode a receptor-type tyrosine kinase with a ligand-binding domain, whereas the transcripts in testis tissue were very short. Most of the heterogeneity in these transcripts may be due to alternative splicing mechanisms.

We and others previously showed that the c-*ros* gene in chickens is expressed at a very low level in kidney tissue (20, 22, 26). The size (8.2 kb) of the c-*ros-1* transcript in rat kidneys (Fig. 1) is consistent with that in chickens (8.3 kb). In addition, Birchmeier et al. found an 8.3-kb c-*ros-1* transcript in human glioblastoma cells (6). Thus, 8.2-kb c-*ros-1* mRNAs in lung and kidney tissues of rats appear to be representative transcripts of this gene. However, in heart and lung tissues of chickens, the c-*ros* gene was not expressed at detectable levels (20, 26). The reason for this discrepancy in the tissue specificity of expression of the c-*ros-1* gene between avian and mammalian species is not clear.

The structure of the predicted product of lung-type c-*ros-1* cDNA exhibited a partial but significant homology with that of the *sev* gene of *D. melanogaster* (3, 8). (i) Both products carry extremely large extracellular domains of 1,830 to 2,124 amino acids with a similarity to each other (Fig. 4a), although the distribution patterns of cysteine residues and the hydrophobicity profiles in the domains are not quite the same (Fig. 4b and 5a). (ii) Within the tyrosine kinase domain, a short insert 5 to 7 amino acids long exists at the same position in both the c-*ros-1* and *sev* genes (from amino acid residues 2063 to 2067 in rat lung c-*ros-1* product; Fig. 5b). This insert is not present in any other member of the tyrosine kinase gene family. (iii) At the level of amino acids, the c-*ros-1* and *sev* gene products are about 25% homologous (including related amino acids) in a region of the extracellular domain and 70% homologous in the tyrosine kinase domain (Fig. 4 and 5). The homology between the c-*ros-1* and *sev* gene products is higher than those between the c-*ros-1* product and other kinases. (iv) Several exon-intron junction points in the tyrosine kinase domain are identical in the c-*ros* and *sev* genes (Fig. 5b) (3, 8, 19, 20). (v) The lengths of the peptides between the transmembrane domain and tyrosine kinase domain are the same in these two gene products. These results suggest that the c-*ros-1* gene in vertebrates and the *sev* gene in *D. melanogaster* belong to a supergene family.

A stretch of about 20 hydrophobic amino acids near the amino terminus in the *sev* gene product is located about 50 amino acids downstream from the initiation methionine residue (3, 8). On the basis of the unusual position of this signal peptide-like sequence, a model of a loop shape for the extracellular domain of the *sev* gene product has been

proposed (3, 8). A possible signal peptide sequence in the c-*ros-1* gene product is also located slightly downstream from the amino terminus. It will be of interest to see whether the c-*ros-1* protein is anchored to the cell membrane with this hydrophobic stretch near the amino terminus as well as with its transmembrane domain.

In addition, the population of c-*ros-1* transcripts in lung tissue has an insertion immediately downstream of the transmembrane domain which carries a termination codon in the same frame. This suggests that two types of c-*ros-1* products, one carrying a tyrosine kinase domain and the other lacking it, might be present in lung tissue. The physiological significance of this unique molecule without a tyrosine kinase domain is unknown, but it could modulate the signal transduction pathway through the normal c-*ros-1* receptor kinase because it contains an intact ligand-binding domain.

The *sev* gene belonging to the receptor tyrosine kinase category in *D. melanogaster* is known to be essential for the differentiation of the photoreceptor cell R7 and to transduce the position information from another photoreceptor cell, R8, to R7 (2, 23, 27, 28). Thus, the ligand for the *sev* gene product appears to be either expressed on the cell surface of the R8 cell or secreted from this cell (23). The structural homology between the c-*ros-1* and *sev* genes suggests that the c-*ros-1* gene might act as a signal transducer for cell-to-cell communication in tissues such as lung and kidney. In *D. melanogaster*, the *sev* gene is mainly expressed in the eye imaginal disk of third-instar larvae (16), but the expression of the c-*ros-1* gene in eyes has not been detected so far in poly(A)$^+$ RNA prepared from 14-day-old chick embryos or 7-day-old chicks (20). The expression of the c-*ros-1* gene in mammalian eyes has not yet been examined because of limited availability of the materials. An antibody specific to the c-*ros-1* gene product would be useful for histological localization of this protein.

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