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Presence of a novel primary response gene ST2L, encoding a product highly similar to the interleukin 1 receptor type 1

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In the course of studying the ST2 gene, which was initially found to be expressed specifically at the G_0/G_1 transitional state in BALB/c-3T3 cells and was one of the primary response genes, we found another ST2-related mRNA, designated as ST2L, in serum-stimulated BALB/c-3T3 cells in the presence of cycloheximide. Nucleotide sequence analysis of the cloned ST2L cDNA revealed that it had an open reading frame encoding a polypeptide of 567 amino acids. A 5′ region (1,028 nucleotides) of ST2L cDNA was identical with the ST2 cDNA, and a unique 3′ region encoded a putative transmembrane domain of 24 amino acids and a cytoplasmic domain of 201 amino acids. The ST2 gene product is highly similar to the extracellular portion of IL-1 receptors type 1 and type 2, and the ST2L gene product shows a marked similarity with entire IL-1 receptor type 1.

Interleukin 1 receptor; Cell growth; Primary response gene; Immunoglobulin superfamily

1. INTRODUCTION

Growth stimulation induces a number of genes to produce proteins that regulate cell proliferation. We have recently been focusing on the ST2 gene, one of the primary response genes expressed in BALB/c-3T3 cells specifically at the G_0/G_1 transitional state [1,2]. The amino acid sequence deduced from ST2 cDNA is highly similar to the extracellular portion of IL-1 receptors [1,3]. In the course of studying the expression mechanisms of ST2, we found another mRNA of about 5 kb that hybridized with ST2 cDNA [2]. Here we report the presence of a novel ST2-related cDNA (tentatively named ST2L), the deduced amino acid sequence of which has a putative transmembrane and cytoplasmic domain and shows marked similarity with IL-1 receptor type 1 as a whole molecule.

2. MATERIALS AND METHODS

The BALB/c-3T3 cells (clone A31) were gift from Dr. C. Stiles (Harvard Medical School). The multiprime DNA labelling system, the cDNA synthesis system plus kit, [α-^{32}P]dCTP, and [α-^{35}S]dCTP were from Amersham. λZAP II phage was from Stratagene. Taq DNA polymerase was from Perkin-Elmer Cetus Instruments.

Total cytoplasmic RNAs were extracted from BALB/c-3T3 cells 20 h after the stimulation with 10% calf serum in the presence of 10 μg/ml of cycloheximide. Poly(A) RNAs were prepared as described [4]. Complementary DNA synthesis was performed using the cDNA synthesis system plus kit, and the cDNAs longer than approximately 3.5 kb were selected after agarose gel electrophoresis to exclude any contamination by ST2 cDNA (2.7 kb). The size-selected cDNAs were used to construct a cDNA library in λZAP II phage, and then a 1.6-kb *Hin*cII fragment of ST2 cDNA was used as a probe for plaque hybridization [1]. Positive clones were subjected to in vivo excision according to the company's protocol, and the nucleotide sequence of the resultant plasmid (Bluescript II) containing insert of longer than 4-kb was determined as mentioned previously [1,4].

The PCR (94°C for 1 min, 50°C for 2 min, and 72°C for 3 min) was performed in 30 cycles in the presence of Taq polymerase. Northern blot analysis was performed as described [4]. Alignment of predicted amino-acid sequences of ST2L and murine IL-1R1 was carried out by the FASTA program [5].

3. RESULTS AND DISCUSSION

A 1.6-kb *Hin*cII fragment of ST2 cDNA was used to screen a size-selected cDNA library derived from stimulated BALB/c-3T3 cells [1]. Three positive clones obtained out of 110,000 independent clones had a common structure with ST2 on the 5′ side and a unique structure on the 3′ side. Even the longest clone contained only a partial ST2 sequence, starting from the point corresponding to the 23rd amino acid of the predicted amino acid sequence of ST2 protein. To study whether the 5′-flanking region of these clones were identical with that in ST2, we employed PCR. As shown in Fig. 1b, the P1 primer started from T, 42 nucleotides upstream of the initiation codon of ST2 cDNA. The downstream

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The nucleotide sequence of the murine ST2L cDNA presented here have been submitted to the DDBJ, EMBL, and GenBank databases under the accession number D13695.

Abbrevations: IL-1R1, interleukin 1 receptor type 1; dCTP, deoxycytidine 5′-triphosphate; cDNA, complementary DNA; poly(A), polyadenylic acid; PCR, polymerase chain reaction.

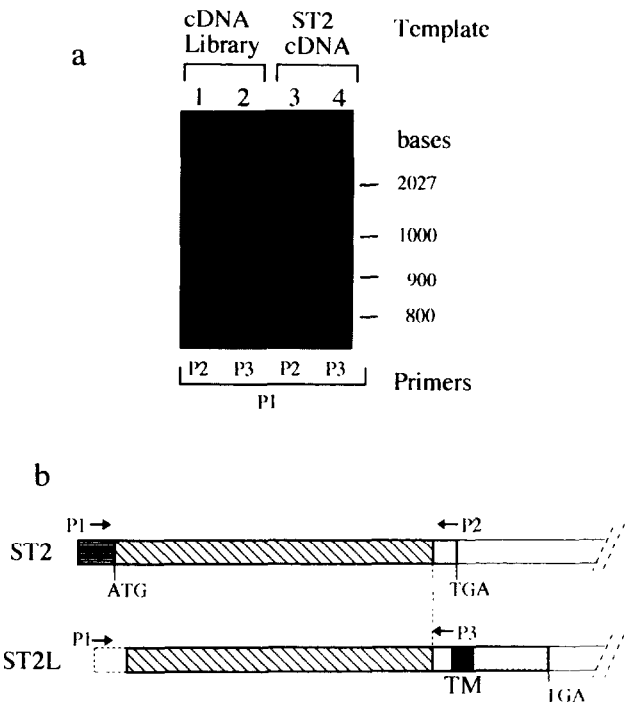

Fig. 1. Identification of the 5′-flanking region of ST2L cDNA by PCR. Panel a: PCR products were analyzed by 5% polyacrylamide gel electrophoresis and visualized by ethidium bromide staining. A size-selected cDNA library (lanes 1 and 2) or ST2 cDNA (lanes 3 and 4) was used as a template. Primers are described in Panel b. Panel b: arrows indicate the positions of P1 (5′-TGCCATTGCCATAGAGAGAC-3′), P2 (5′-TCAAGCAATGTGTGAGGGAC-3′), and P3 (5′-GTAGATGCTTCGGTGATCAA-3′) primers. Cross-hatched regions represent the common sequence found in ST2 and ST2L cDNAs. The area surrounded by the dotted line corresponds to the portion verified by PCR. The 5′ non-coding region of ST2 cDNA (striped area), a putative transmembrane domain of ST2L cDNA (TM), and a putative cytoplasmic domain of ST2L cDNA (dotted area) are shown.

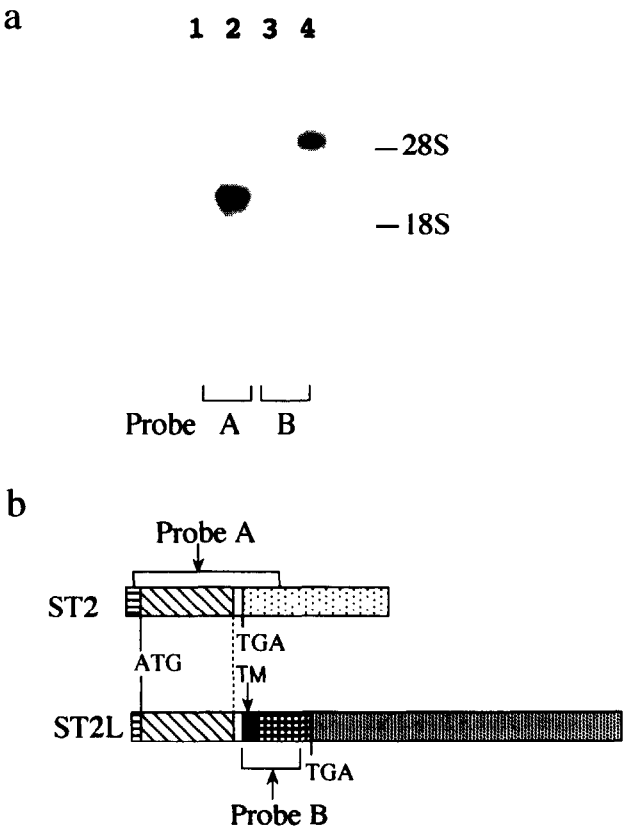

Fig. 3. The transcripts detected by the probes derived from ST2 and ST2L cDNAs. Panel a: four micrograms of total RNAs extracted from quiescent BALB/c-3T3 cells (lanes 1 and 3) or from cells 20 h after serum stimulation (lanes 2 and 4) were analyzed by Northern blotting. Panel b: schematic representation of the probes for Northern hybridization. Probe A corresponds to the 1.6-kb *Hin*cII fragment of ST2 cDNA and probe B is a fragment (nucleotide number of 1,057 to 1,827) of ST2L cDNA. Cross-hatched regions are common in ST2 and ST2L cDNAs. TM denotes the transmembrane domain.

primers P2 and P3 were specific for ST2 and ST2L cDNA, respectively. With P1 and P3, a band of appropriate length was found on the gel (Fig. 1a, lane 2). Nucleotide sequence analysis after cloning the DNA fragment revealed that ST2 and ST2L had the same sequence around the initiation codon (Fig. 2, oblique letters). Whole nucleotide sequence analysis of the longest clone together with the result of the PCR experiment revealed that ST2L cDNA consisted of 4,989 bases and shared an identical 5′ region of 1,028 nucleotides with ST2 until just before the stop codon of ST2 cDNA (the boxed region in Fig. 2), and then started a unique nucleotide sequence thereafter (Fig. 2). ST2L cDNA coded for a protein consisting of 567 amino acids that has a putative transmembrane domain of 24 amino acids (Fig. 2, underlined) and a cytoplasmic domain of 201 amino acids. Three AUUUA mRNA destabilizing sequences were found in the 3′-untranslated region of ST2L cDNA (Fig. 2) [6].

Northern blotting analysis was performed with two probes (Fig. 3). With probe A, which could hybridize to both ST2 and ST2L, two bands appeared in the case of serum stimulated cells (Fig. 3a, lane 2) as previously described [2]. On the other hand, ST2L specific probe B hybridized only to the upper band (Fig. 3a, lane 4), confirming that the cloned ST2L cDNA corresponded to the 5 kb mRNA which was expressed in growth stimulated BALB/c-3T3 cells.

A computer search of the GenBank database identified significant similarities of ST2L over 500 amino acids with murine, human, and chicken IL-1R1. The sequence identity between ST2L and murine IL-1R1 was 28% over the whole molecules (Fig. 4a). We pointed out that the ST2 gene product corresponding to the putative extracellular portion of ST2L had a 25% amino acid identity with the extracellular portion of murine IL-1R1 [1]; and subsequently, murine IL-1R2 was also found to share 23% amino acid identity with ST2 [3]. As for the cytoplasmic portion, ST2L and murine IL-1R1 had a higher similarity of 38%, while ST2L showed no

similarity with the cytoplasmic region of IL-1R2 (Fig. 4b).

Among a battery of IL-1R related proteins reported, a product of vaccinia virus has recently been shown to be a soluble IL-1R [7,8]. Other soluble IL-1 binding proteins remain to be studied [9,10]. ST2 was initially found to be a G_0/G_1-specific gene coding for a protein highly similar to the extracellular portion of IL-1R [1]. An identical cDNA was reported as T1, which was induced by serum and oncoproteins [11,12]. We further characterized the genomic DNA of murine ST2 and revealed that the *St2* locus is tightly linked to the *Il-1r1* and *Il-1r2* loci on mouse chromosome one, suggesting the strong relationship between ST2 and IL-1Rs [3,13].

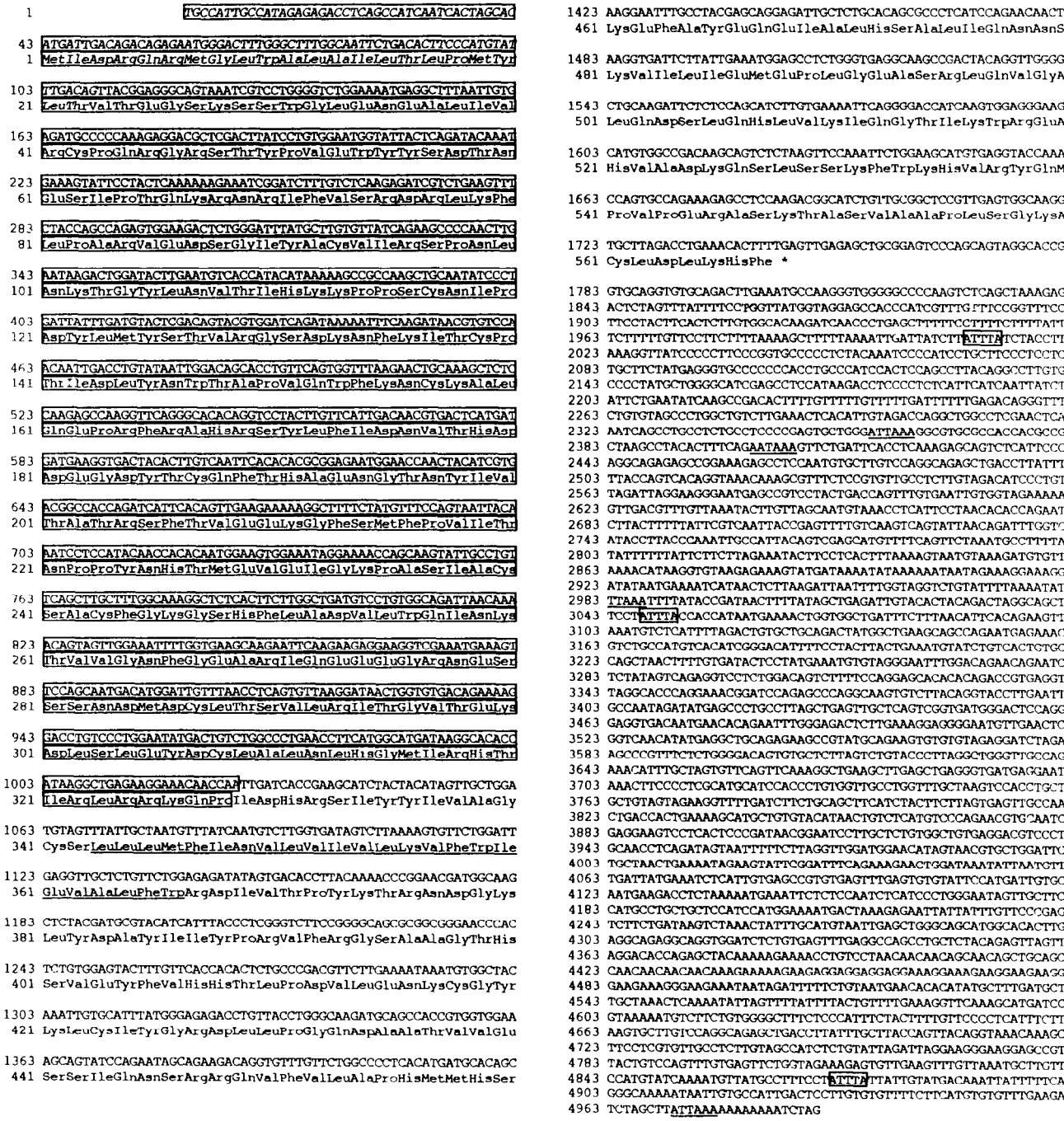

Fig. 2. Complementary DNA sequence and deduced amino acid sequence for ST2L. The boxed region indicates the common sequence of ST2 and ST2L. The nucleotide sequence shown in oblique letters corresponds to the portion determined by PCR and subsequent sequencing. The putative transmembrane domain is underlined. The stop codon is indicated by an asterisk. The poly(A) signals (underlined) and three mRNA destabilizing sequences (AUUUA) (boxed) are shown in the 3′ non-coding region.

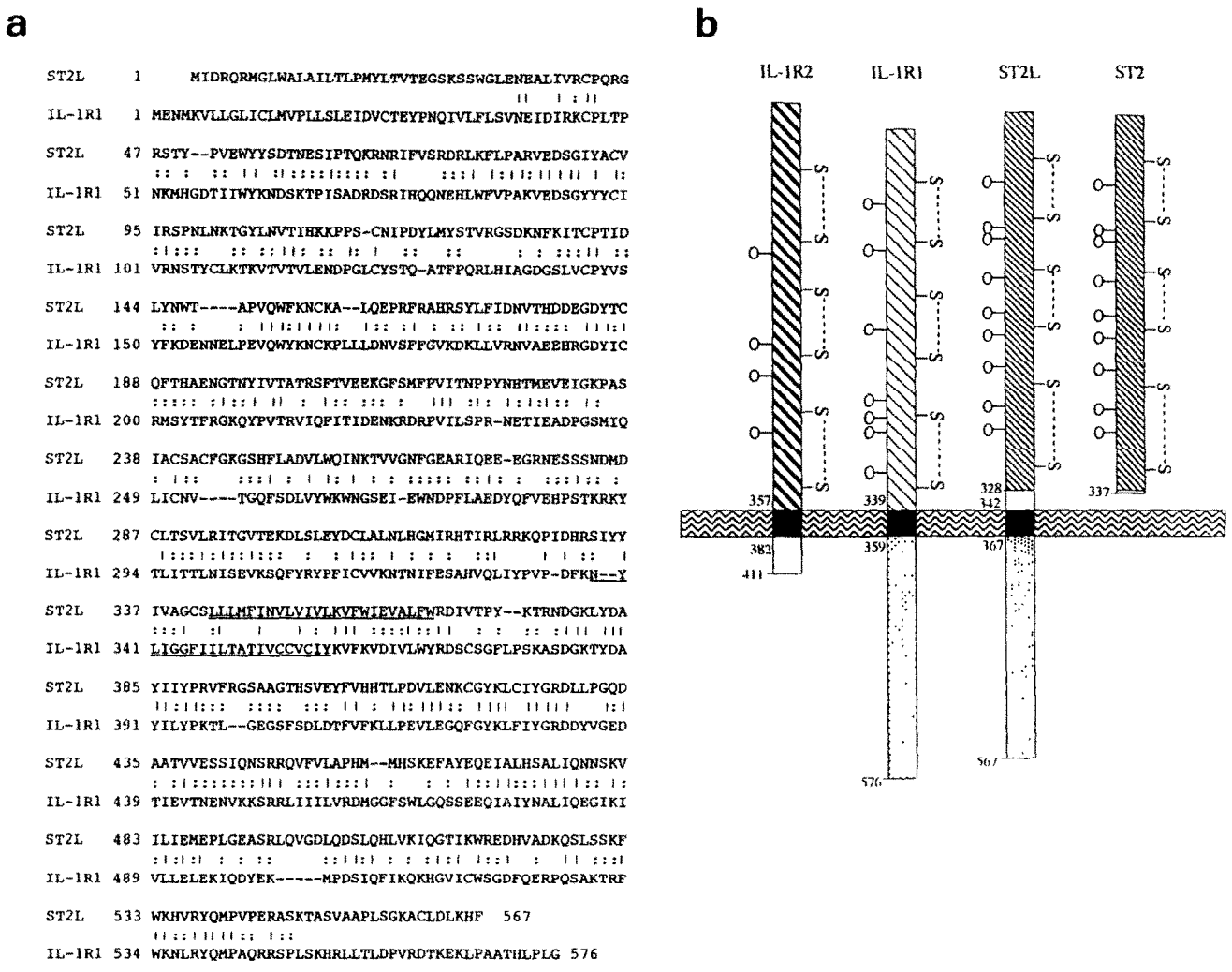

Fig. 4. Comparison of the structures of ST2L to closely related molecules. Panel a: amino-acid residues are shown in single-letter code. Vertical lines represent the identical amino acid residues, and double dots indicate high similarity. The transmembrane domains of each protein are underlined. Panel b: schematic representation of ST2L-related proteins. Cross-hatched regions of the proteins indicate the similarity of extracellular domains of IL-1R1, IL-1R2, and ST2L. ST2 is also almost identical with the extracellular domain of ST2L. Striped areas of IL-1R1 (solid line) and ST2L (dotted line) represent a high similarity of cytoplasmic domains.

The IL-1 binding activity of ST2 and ST2L is now being investigated; at present, ST2L remains as a possible 'orphan' receptor.

Several soluble receptors and binding proteins for cytokines and lymphokines have been identified. The secreted receptors for human G-CSF, GM-CSF, IL-5, and IL-7 are derived from an internal deletion of mRNAs [14–17]. Based on the sequence data of ST2 genomic DNA, the soluble form (ST2) cannot be generated by deletion [13]. Further analysis of the exon–intron organization of ST2 and ST2L is necessary to elucidate any possible involvement of alternative splicing.

ST2 and ST2L mRNA expression turned out to be a primary response independent of de novo protein synthesis to growth stimulation, suggesting the involvement of ST2 and ST2L gene products in the signal transduction of cell proliferation [2]. Physiological roles of ST2 and ST2L are now under investigation with 'overexpression' and 'gene targeting' procedures.

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