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GENOMIC ORGANIZATION OF A MOUSE TYPE I ACTIVIN RECEPTOR

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We have characterized the genomic organization of a mouse type I activin receptor. Using the mouse tsk7L cDNA, 4 overlapping λ clones containing the activin receptor IA (ActRIA) gene were isolated from a mouse 129 Sv genomic library. The mouse ActRIA gene is encoded by 10 exons and spans approximately 40 kb. The size of the introns was determined and the intron/exon boundaries were sequenced. Primer extension analysis of the 5' non-translated region using RNA from different organs or tissues revealed a strong transcription start site 68 nucleotides upstream of the ATG. Knowledge of the structure of the ActRIA gene is essential for the production of ActRIA deficient mice by homologous recombination.

Activins are members of the transforming growth factor ß (TGF-ß) superfamily which regulate growth, proliferation and differentiation of many cell types (1). Their biological effects are mediated by transmembrane receptors characterized by a serine (Ser)/threonine (Thr) kinase activity. Signaling requires the heterodimerization of receptors from two distinct classes (2, 3). Class I receptors are necessary for signal transduction and class II receptors are required for ligand binding. Two activin receptors from class I (ActRIA and ActRIB) and two receptors of class II (ActRIIA and ActRIIB) have been cloned at present (4). These receptors are composed of an extracellular ligand binding domain, a single transmembrane domain and a cytoplasmic Ser/Thr kinase domain (5). The receptor heterogeneity is highest in the extracellular domain and might underlie the different responses that ligands can elicit in a dose and

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cell-type specific manner (6). Type I receptors have an additional intracellular motif of repeated serines and glycines termed SG-box. Phosphorylation by type II kinases of Ser in the SG-box is believed to mediate signal transduction (7). Activins are believed to have an important function during embryonic development. Their involvement in the cascade of signals leading to the production of mesoderm is suggested from Xenopus animal cap assays (8). The expression of a dominant-negative activin receptor IIA in Xenopus embryos inhibits mesoderm induction and formation of axial structures (9). In order to analyze the role of activins and their receptors in mouse development, it is possible to produce loss of function mutations. Therefore it is necessary to determine the structure of the corresponding gene. The genomic structure of mouse ActRIIA and bone morphogenetic protein 2/4 receptor (Bmpr) have at present been reported (10, 11). Here we present the genomic structure of mouse ActRIA, an activin receptor which was originally identified as tsk7L.

MATERIALS AND METHODS

Library screening: A 129 SvJ mouse genomic library was constructed using the Lambda Fix II vector (Stratagene). $2x10^6$ recombinant λ plaques were transferred in duplicates onto Hybond N nylon membranes (Amersham) and screened with a 2 kb Xho I fragment comprising the entire tsk7L open reading frame (12). The probe was labeled using a random prime labeling kit (Pharmacia) and [α^{32}P] dCTP (Amersham). Filters were prehybridized for several hours in 5x Denhardt's (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% BSA), 0.5% SDS and 5x SSC (0.75 M NaCl, 75 mM Na citrate) at 65°C. Hybridizations were performed overnight at 65°C using $6x10^7$ cpm probe in 40 ml prehybridization solution. The filters were washed twice 30 minutes in 0.1% SSC / 0.1% SDS at 65°C prior to autoradiography.

λ genomic clones: Positive clones were purified to homogeneity by secondary and tertiary screening using the tsk7L cDNA as a probe. DNA from these clones was digested with Not I, Eco RI, Sal I and Xba I restriction endonucleases and analyzed by gel electrophoresis. DNA was transferred to Hybond N membranes and probed with portions of the cDNA to generate a physical map. Restriction mapping from 4 overlapping clones was also done by performing a partial digestion of the Not I insert and probing a Southern blot of the resulting fragments with T3 and T7 oligonucleotide probes. Exon containing fragments were subcloned into pBluescript II SK (Stratagene).

DNA sequencing: Sequence analysis was performed using the Sequenase T7 DNA polymerase kit (United States Biochemicals). Computational analysis of the sequences was performed using the GCG programs (Genetics Computer Group, Madison, WI).

Primer extension: The transcriptional start sites were determined by primer extension analysis according to a previously described method (13). We used the following oligonucleotide 5'-TGGGCTTCTCATCTTCCACACTCGG -3' corresponding to positions 550 to 574 of the tsk7L cDNA (12). For the annealing, we mixed 10 ng end-labeled oligonucleotide with 2.5 μg of poly(A)$^+$ RNA.

RESULTS AND DISCUSSION

Isolation of the mouse ActRIA gene

A mouse 129 Sv genomic library was screened using the tsk7L cDNA (12). 15 recombinant λ clones were isolated and 4 overlapping genomic fragments having an approximate size of 15 kb each were further analyzed. They allowed us to determine the structure of the ActRIA gene as shown in Figure 1. The gene consists of 10 exons spanning over 40 kb approximately.

Analysis of the exons, introns and introns/exons boundaries

The position of introns compared to the cDNA coding sequence is shown in Figure 2. The first exon is not translated. The signal peptide is entirely encoded by the second exon of 67 bp. Exon 3 encodes for the major part of the extracellular domain. This differs from ActRIIA and Bmpr where the ligand binding domain is encoded by 2 exons (number 2 and 3) and 3 exons (number 2, 3 and 4), respectively (10, 11). The entire transmembrane domain is encoded by exon 4. The SG-box is located in exon 5 and exons 6 to 10 encode for the intracellular kinase domain. Exon 10, like a

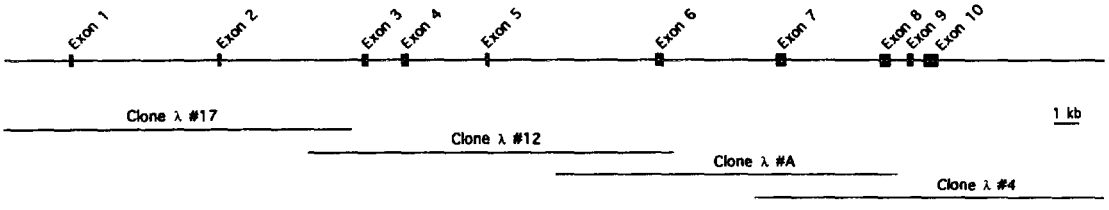

Figure 1. Structure of the mouse ActRIA gene. Exons are indicated by numbered boxes. The DNA fragments isolated from 4 overlapping λ clones are represented in the lower part of the figure.

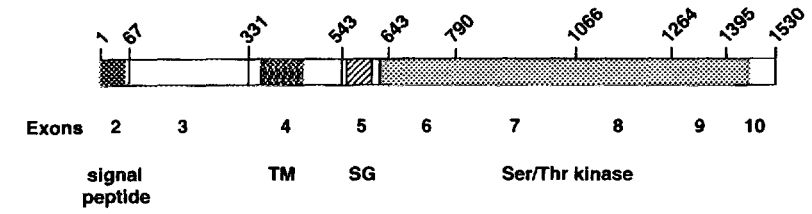

Figure 2. Location of introns in the mouse ActRIA coding sequence. The nucleotide preceding the intron is shown. Nucleotides 1 and 1530 are the start and the end of the translated protein, respectively. The positions of the signal peptide, the transmembrane domain (TM), the Ser/Gly repeat (SG) and the kinase domain are indicated.

typical last exon in mammalian genes, has more than 520 nucleotides whereas exons 3 to 9 have a size comprised between 100 and 276 bp.

As shown in Figure 3, the introns have a size comprised between 0.6 and 6.5 kb. ActRIA does not have a very large first intron as found in ActRIIA (10). All intron/exon boundaries satisfy the consensus splice donor/acceptor sites found in rodents (14). The position of the last 2 intron/exon boundaries is well conserved between ActRIA, ActRIIA and Bmpr (not shown). The structural analogies of these genes suggest that they could originate from a

Exon n°	(size)	Exon 3'			Intron n°	(size)		Exon 5'	
Exon 1	(>61 bp)				Intron 1	(>5 kb)	ttttttccttccacag	TTATAC	untranslated
Exon 2	(74 bp)	GAA Glu	G A	gtgcctgagacgcag	Intron 2	(~6.5 kb)	acctctcctttgcag	AT sp	GAG Glu
Exon 3	(255 bp)	AAA Lys	G G	gtccttctattacta	Intron 3	(~1.8 kb)	cttcccattctccag	GG ly	AAG Lys
Exon 4	(212 bp)	CTA Leu	GCG Ala	gtgagtttgtgaccc	Intron 4	(~3.5 kb)	tgttttacatcacag	GAA Glu	CTA Leu
Exon 5	(99 bp)	GTC Val	G G	gtaattctatgtttc	Intron 5	(>4 kb)	gtttgctgtccacag	GG ly	AAG Lys
Exon 6	(147 bp)	TTA Leu	G G	gtgagtaccaggtga	Intron 6	(~5 kb)	ctcctccctctccag	GT ly	TTC Phe
Exon 7	(274 bp)	TTG Leu	G G	gtctggcagtcatgc	Intron 7	(~3.8 kb)	actttctccccttag	GC ly	CTG Leu
Exon 8	(201 bp)	AAT Gly	G I	gtaagtgcctgacgt	Intron 8	(~0.6 kb)	tcatcttgtacacag	GT le	ATA Val
Exon 9	(130 bp)	GAC Asp	CCG Pro	gtaagaagaacacca	Intron 9	(~0.8 kb)	ctctttcctctccag	ACA Thr	TTA Leu
Exon 10	(>520 bp)								

Figure 3. Intron/exon boundaries of the ActRIA gene. Nucleotide sequence of each intron/exon boundary, size of each intron and exon are shown. The consensus splice donor sequence in rodents is (C/A)AG/GTUAGT and the consensus acceptor sequence is YYYYYYYYYYNCAG/G (Y, pyrimidine; U, purine; N, any nucleotide), (14).

common ancestor. It is remarkable that the introns are often positioned between the first and the second nucleotide of the codon (Figure 3). Alternative splicing events leading to exon skipping would preserve the open reading frame. Deletion of exons 6, 7 and/or 8 in the kinase domain would then produce receptors which vary in kinase activity. The occurence of such receptors has been reported for ActRIB/SKR2 in human HepG2 cells (15).

Analysis of the transcription start site

In order to determine the transcriptional start site, primer extension experiments were carried out. Our results indicate that in

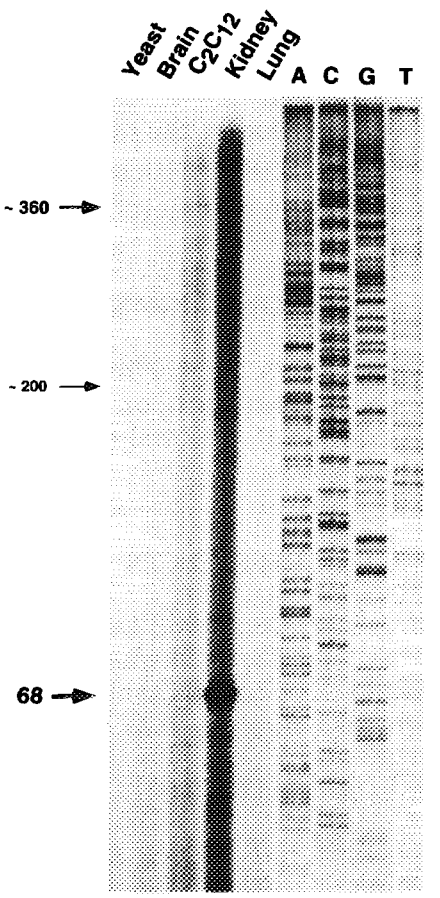

Figure 4. Determination of the transcription start sites of the ActRIA mRNA through primer extension. Poly $(A)^+$ RNA from mouse brain, kidney and lung, from the myogenic cell line C_2C_{12} and from yeast RNA, as control, were used. The size of extension products, shown by arrows, is indicated relative to the translational start site. As a size marker, DNA sequencing of the corresponding genomic region was realized using the same oligonucleotide as for primer extensions.

kidney the major pool of mRNA encoding ActRIA extends only 68 nucleotides upstream of the translation start codon (Figure 4). In lung, two weak extension products up to 59 and 200 nucleotides upstream of the ATG are observed. Several products of approximately 59, 230, 280 and 360 nucleotides are obtained with RNA from the myogenic cell line C_2C_{12} (16). Only a weak product of 59 nucleotides is obtained using brain RNA. No extension products are detected when total yeast RNA is used as a control. Moreover the sequence of the tsk7L cDNA, isolated from mouse mammary gland epithelial cells (NMS90) bears an extension of 495 bp upstream of the initiation codon (12). Together, these results indicate that alternative transcription start sites might be used for expression of the mouse ActRIA gene resulting in a variable size of the 5' untranslated region. This could be due to the presence of multiple promoters which might have a tissue restricted activity.
In order to analyze the function of the mouse ActRIA gene product, it is possible to produce ActRIA deficient mice. Our results will contribute to the construction of a targeting construct.

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