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Exon/intron organization of the genes coding for the δ chains of the human and murine T-cell receptor/T3 complex

(T lymphocytes/antigen receptor/molecular cloning/DNA sequencing)

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ABSTRACT **Genomic DNA clones containing the gene coding for the 20-kDa T3 glycoprotein of the T-cell receptor/T3 complex (T3-δ chain) of human and mouse were isolated and characterized. The human T3-δ gene is ≈4 kilobases (kb) long and contains five exons: a 151-base-pair (bp) exon containing the 5′ untranslated and the coding sequences of the signal peptide, one exon of 219 bp, which contains most of the extracellular segment of the T3-δ chain, one 130-bp-long exon coding mainly for the transmembrane portion of the molecule, and two exons of 44 bp and 156 bp encoding the cytoplasmic domain and 3′ untranslated region of the T3-δ chain, respectively. The murine T3-δ gene, which has a similar organization, contains 5 kb, because the first intron is ≈1 kb larger than in the human gene. Two major mRNA initiation sites within a small area ≈100 nucleotides 5′ of the AUG codon were determined by S1 nuclease analysis and primer-extension studies. The remarkably high level of conservation of nucleotide sequences in this region suggests that this segment may be important for the regulation of T-cell-specific transcription of the T3-δ gene. The T3-δ gene does not contain the "TATA box" found in many eukaryotic promoters.**

Thymus-derived lymphocytes (T lymphocytes) play an important role in the immune defense of higher organisms by eliminating cells that appear foreign. Specific receptors on the surface of human T lymphocytes (the T-cell receptor/T3 complex) are involved in the recognition of foreign antigen and major histocompatibility complex (MHC) products. The T-cell receptor/T3 complex is comprised of at least five different polypeptide chains: the α and β chains, which are the clone-specific T-cell receptor structures involved in the recognition and binding of foreign antigens in the context of polymorphic MHC gene products, and the γ, δ, and ε chains of the T3-complex, which are thought to play a role in signal transduction (refs. 1–10). Human and murine T-cell receptors for antigen are sulfhydryl-linked heterodimers, which consist of two glycosylated polypeptide chains (α and β) ranging in molecular mass from approximately 37 to 50 kDa (11–13). The human T3 antigens are three distinct polypeptide chains: a 25-kDa glycoprotein (T3-γ), a 20-kDa glycoprotein (T3-δ), and a 20-kDa polypeptide that does not contain any detectable oligosaccharides (T3-ε) (1, 14–17).

The genes encoding the α and β chains in human and mouse have been isolated and characterized and have revealed the presence of variable, diversity, joining, and constant gene segments similar in organization and structure to the gene segments of immunoglobulin genes (18–24). Recently, we have isolated and characterized cDNAs coding for the δ chain of the T3/T-cell receptor complex in humans and mice (25, 26). DNA sequence analysis of the T3-δ chain has revealed no homology with members of the T-cell receptor/immunoglobulin/MHC-supergene family. The gene coding for the T3-δ chain of human and mouse is a single copy gene whose expression is restricted to T cells (25, 26). The human T3-δ chain is localized on the long arm of chromosome 11 (11q23-11qter), whereas the murine T3-δ chain maps to chromosome 9 (27).

In this report we describe the isolation and characterization of the genes coding for the human and murine T3-δ chains. DNA sequence analysis revealed that the human and murine T3-δ genes are organized in a very similar fashion. In both species the T3-δ gene lacks a "TATA box" near the putative initiation site for RNA transcription. S1 nuclease analysis and primer-extension studies in combination with a comparison of DNA sequences suggest two major transcription initiation sites about 96 and 106 nucleotides (nt) 5′ of the AUG initiation codon in the human T3-δ gene.

MATERIALS AND METHODS

Construction and Screening of a Human Genomic DNA Library in Bacteriophage λ Charon 35. To isolate the human T3-δ gene, a genomic library of DNA isolated from human peripheral leukocytes was constructed by ligating *Sau*3A partial digests [average size, 10–20 kilobases (kb)] into bacteriophage Charon 35 arms generated by digestion with *Bam*HI (28). Following amplification through *Escherichia coli* LE392, T3-δ-specific sequences in the library were identified by screening ≈4 × 10^5 plaques with the cDNA insert of pPGBC#9, which codes for the human T3-δ chain after labeling by nick-translation (25). One recombinant bacteriophage containing human T3-δ chain sequences was isolated after plaque purification and rescreening (λ KR-δ). λ KR-δ contained ≈20 kb of genomic DNA. From this recombinant bacteriophage an 8.5-kb *Eco*RI fragment was isolated that contained all of the coding exons. This fragment was subcloned into the *Eco*RI site of pUC8, resulting in pKR-1 (see Fig. 1 *Upper*).

Construction and Screening of a Mouse Genomic Library Prepared in the pUC8 Vector. The mouse T3-δ gene was isolated from DNA of mouse B8C3 cells (an anti-porcine insulin T-T hybridoma) (29, 30). DNA was enriched for the 2.7-kb T3-δ *Eco*RI and 8-kb T3-δ *Pst* I fragments by preparative gel electrophoresis. The purified material was cloned into pUC8 to yield two size-selected libraries of ≈3 × 10^4 colonies (*Eco*RI) and ≈4 × 10^4 colonies (*Pst* I) in MC1061 (31, 32). T3-δ-specific sequences were identified by screening with the cDNA insert of pPEM-T3δ, which codes for the murine T3-δ chain, ^{32}P-labeled by nick-translation (26). Plasmids pMT-1 and pMT-2 containing a 2.7-kb *Eco*RI

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Abbreviations: MHC, major histocompatibility complex; kb, kilobase(s); U, unit(s); nt, nucleotide(s).

fragment and an 8-kb *Pst* I fragment of genomic DNA, respectively, were isolated from these libraries and used for subsequent restriction enzyme and nucleotide sequence analysis (see Fig. 1).

DNA Sequence Analysis. Individual DNA fragments derived from purified inserts of pKR-1, pMT-1, and pMT-2 were subcloned into bacteriophage M13 mp8 or mp9 and M13 mp18 or mp19 (31, 33) using standard procedures. Single-stranded templates were prepared from these subclones and sequencing was performed employing the dideoxy chain-termination procedure of Sanger *et al.* (34). Sequence data were analyzed using the IntelliGenetics Gel program.

S1 Nuclease Analysis. S1 nuclease analysis with human (HPB-ALL) and mouse (KD13) RNA was performed essentially as described (28). The ^{32}P-labeled (uniformly labeled) single-stranded probes were generated by priming M13 clones containing DNA fragments that comprise the 5′ untranslated and leader exon regions with a 20-mer oligonucleotide (human 5′ AGCCAGGTCACCGAACTATC 3′; mouse 5′ GCAAGCCACAGGATGATCAG 3′) derived from this sequence. Following purification through denaturing polyacrylamide gels, $\approx 5 \times 10^4$ cpm of radiolabeled single-stranded fragments was used for the S1 nuclease analysis. The size of each protected fragment was compared with the dideoxynucleotide-derived sequencing ladder obtained with the same primer and the M13 template used to generate the probe.

Primer Extension. One microgram of the synthetic oligonucleotide was 5′ end-labeled with 200 μCi (1 Ci = 37 GBq) of [γ-^{32}P]ATP (5000 Ci/mmol, Amersham) and 20 units (U) of T4 polynucleotide kinase (New England Biolabs). Following phenol extraction and ethanol precipitations, $\approx$20 ng of the 5′ labeled oligonucleotide was added to 5 μg of poly(A)$^+$ RNA derived from the human T-leukemic cell line HPB-ALL and HeLa cells, and hybridization to the mRNA was initiated by heating for 5 min at 80°C, followed by 2 hr at 42°C and slowly cooling to room temperature in the presence of 100 mM Tris·HCl, pH 8.2/100 mM KCl/12 mM $MgCl_2$. After hybridization, the nucleic acids were recovered by ethanol precipitation and resuspended in a mixture of 50 mM Tris·HCl, pH 8.2/50 mM KCl/6 mM $MgCl_2$/500 μM (each) dNTPs/10 mM dithiothreitol/20 U of RNAsin/10 U of reverse transcriptase. Synthesis was performed for 45 min at 42°C. The extended products were recovered by ethanol precipitation following phenol extraction and were analyzed on an 8% denaturing polyacrylamide gel.

RESULTS AND DISCUSSION

Isolation of Genomic DNA Fragments Containing the Human and Mouse T3-δ Genes. The human T3-δ gene was isolated from a phage λ library, as described in *Materials and Methods*, resulting in the construction of pKR-1, which

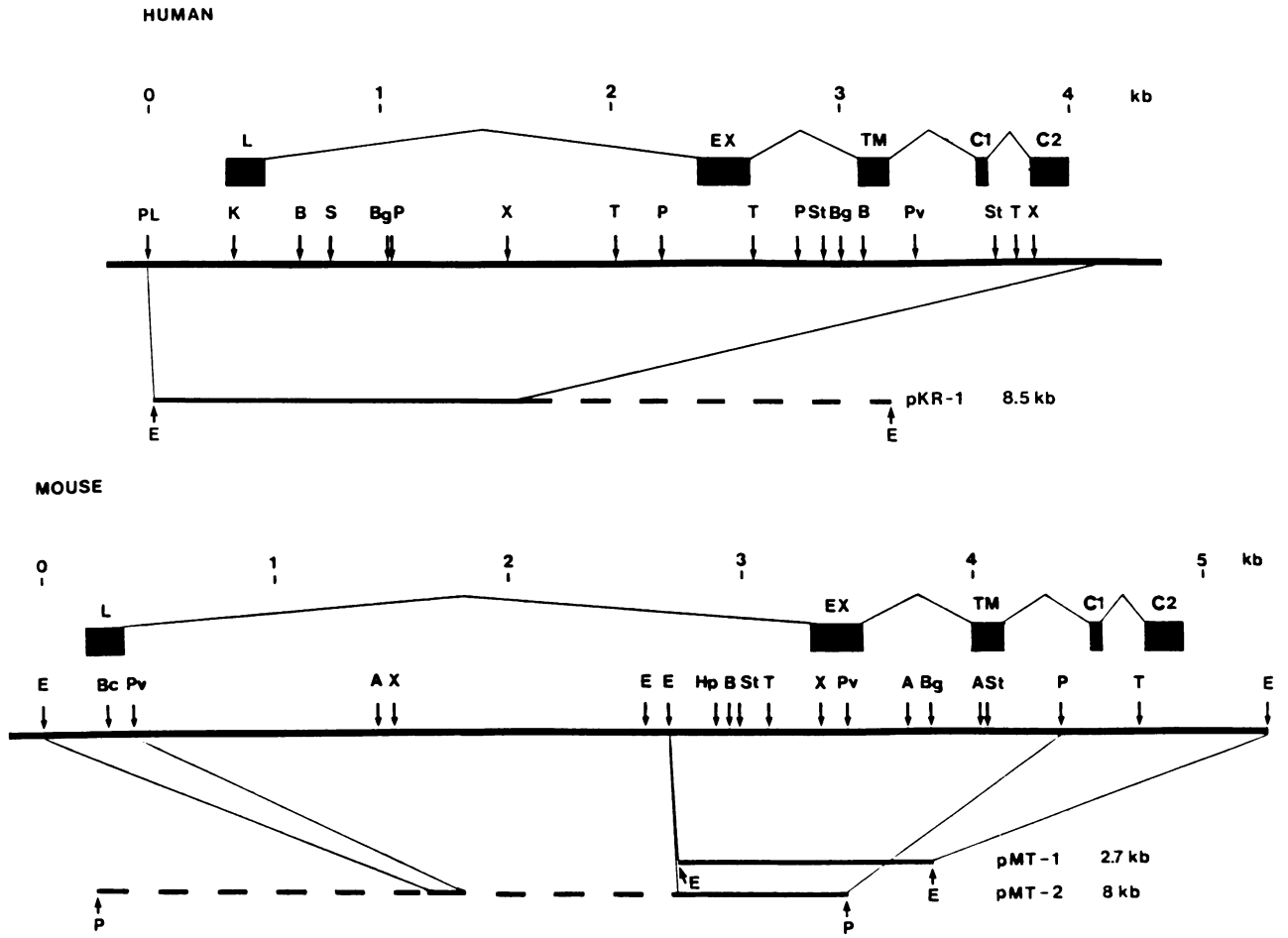

FIG. 1. Partial restriction maps of the human (*Upper*) and murine (*Lower*) T3-δ chain. The boxes above the restriction maps represent exons. Cleavage sites of restriction enzymes are in a one-letter code. A, *Acc* I; B, *Bam*HI; Bg, *Bgl* II; Bc, *Bcl* I; E, *Eco*RI; Hp, *Hpa* I; K, *Kpn*HI; P, *Pst* I; R, *Pvu* II; S, *Sma* I; St, *Stu* I; T, *Taq* I; X, *Xba* I. PL represents the polylinker cloning site present in Charon 35 (*Eco*RI, *Sst* I, *Sma* I, *Xba* I, *Sal* I, *Pst* I, *Hin*dIII, and *Bam*HI). The solid bars of the genomic DNA clones represent the portions that have been sequenced. These sequences code for the following: L, leader peptide; EX, extracellular domain; TM, transmembrane domain; C1 and C2, cytoplasmic domain.

contained an 8.5-kb *Eco*RI fragment comprising all of the coding exons (see Fig. 1 *Upper*).

Southern blotting experiments using the cDNA insert of pPEM-T3δ as hybridization probe (26) had suggested that the gene coding for the murine T3-δ chain is contained within a 2.7-kb *Eco*RI fragment of genomic DNA. Therefore, we constructed a partial library that was enriched for the 2.7-kb T3-δ *Eco*RI fragment. Nucleotide sequence analysis of pMT-1 derived from this library (see below) revealed that the exon coding for the 5′ untranslated sequences and the leader peptide was not present on this 2.7-kb T3-δ *Eco*RI fragment.

Next, we isolated pMT-2 from a second size-selected library, which was used for further analysis. pMT-2 contained an 8-kb *Pst* I fragment, comprising 5′ adjacent se-

FIG. 2. Alignment of part of the human (h) and mouse (m) T3-δ gene sequences. The putative transcription initiation sites are indicated (▼ from S1 nuclease analysis; ○ from primer extension). Arrows (→) indicate pentameric repeats. Dashed lines in the sequences represent gaps as a result of the alignment. Dashed lines underneath the sequences represent interesting features. The poly(A) sequence AATAAA is underlined. LE (L), EX, TM, and C1 and C2, as in Fig. 1 legendA.

Table 1. Exon/intron organization of the human and murine T3-δ chain

Exon number	Source	Exon size, bp	Sequence of exon/intron boundaries: 5′ Boundary	Sequence of exon/intron boundaries: 3′ Boundary	Intron length, kb	Amino acid interrupted	% nucleic acid homology in exons
1	H	151	TCG CAA G/GTAAGG	TTTCCCCTTTAG/TG AGC C	≈2.1	Valine	69.7
	M	153	CCC CAA G/GTAAGG	CTTCCACCTCAG/GG AGC C	≈3.0	Glycine	
2	H	219	TAT CGA A/GTACGT	CTTCCCCCACAG/TG TGC C	0.468	Methionine	69.3
	M	219	TAC CGA A/GTATGT	TCTCCCCACCAG/TG TGC C	0.413	Methionine	
3	H	130	TCT GGG G/GTTAGT	TGGTTCTTCTAG/CT GCC G	0.280	Alanine	83.8
	M	130	TCT GGG G/GTTAGT	CCTTGCTTCTAG/CT GCT G	0.322	Alanine	
4	H	44	TAT CAG/GTGAGC	CTCCTCTCTTAG/CCC CTC	0.221	None	70.4
	M	44	TAT CAG/GTAAGC	CTTGCCTCTCAG/CCT CTT	0.171	None	
5	H	156					65.8
	M	151					
			Consensus: AG/GTAAGT	$CCCCCC_NC_{AG/exon}$ TTTTTT T			

H, human; M, mouse; bp, base pairs.

quences to the 2.7-kb *Eco*RI fragment (see Fig. 1 *Lower*).

Nucleotide Sequence Analysis. Previous studies have shown that the primary structure of the T3-δ chain includes a 21 amino acid long signal peptide, a 79 amino acid long extracellular segment, one transmembrane domain of 27 amino acids, and a cytoplasmic portion of 44 (human) or 46 (mouse) amino acid residues (25, 26). To examine the exon/intron organization of the T3-δ gene, the nucleotide sequence of the genomic DNA clones was determined by the dideoxy chain-termination method using bacteriophage M13. The partial restriction maps of the human and murine T3-δ genes are shown in Fig. 1 *Upper* and *Lower*. In Fig. 2 the alignment of part of the derived sequences of the human and mouse T3-δ genes is presented. Comparison of the obtained sequence data with the nucleotide sequences of the respective cDNA clones revealed that the T3-δ gene in humans and mice contained five exons.

Similar analyses with the murine T3-δ gene revealed an identical genomic structure. A summary of the exon/intron organization of the human and murine T3-δ genes is given in Table 1. Comparison of the human and murine T3-δ gene sequences showed a high level of exon sequence homology, with less homology in the introns (see Table 1). The analysis of the genomic DNA clones showed that most of the proposed domains in the T3-δ chain were coded for by separate exons with the exception of the cytoplasmic domain, which is coded for by two exons. Previous studies from our laboratory, however, suggested that part of the COOH terminus of the T3-δ chain might be removed by proteolytic processing (25, 35). The existence of two cytoplasmic exons could indicate that each exon may code for a cytoplasmic domain with a unique function.

By computer analysis the presence of an *Alu*-like sequence (36) was identified in the intron separating the leader and extracellular exon in the human DNA (data not shown). In general, the intron sequences separating the coding exons showed a low level of nucleotide sequence homology (see Fig. 2). However, in the intron separating the transmembrane and the first cytoplasmic exon a higher level of conservation of nucleotide sequences was observed. Furthermore, human and mouse both shared a stretch of alternating G/T residues in this intron (as indicated in Fig. 2).

Mapping of the mRNA Initiation Sites. To determine the initiation site of mRNA transcription of the T3-δ chain S1 nuclease and primer-extension analyses were performed.

The uniformly labeled single-stranded DNAs used in the S1 nuclease protection assay were derived by priming M13 clones containing a specific T3-δ gene fragment with a synthetic oligonucleotide as described in *Materials and Methods*. Following hybridization to mRNA from the human T-leukemic cell line HPB-ALL, treatment with S1 nuclease and gel analysis of the product resulted in the identification of two S1 nuclease-resistant fragments (Fig. 3). Similar results were obtained in the S1 nuclease protection assays with a murine single-stranded DNA probe and poly(A)$^+$ RNA from the cell line KD13 (data not shown).

Comparison with the M13 sequencing ladder indicated that in the human T3-δ gene the two major putative initiation sites are located about 96 and 106 nt 5′ of the AUG initiation codon. In the murine T3-δ gene two major clusters of putative initiation sites are observed located about 89 and 95 nt 5′ of the AUG initiation codon (data not shown, indicated in Fig. 2). In the human the deduced mRNA initiation site at about 96 nt 5′ of the AUG corresponds to the end of a full-length cDNA clone (pPGBC#9) (25) isolated from a cDNA library made according to Okayama and Berg (37). Therefore, we conclude that this is the major initiation site of transcription.

To confirm that the S1 nuclease-protected fragments represent transcription initiation sites, primer-extension studies

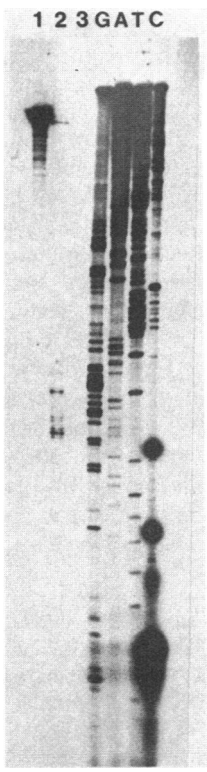

FIG. 3. S1 nuclease analysis of the 5′ end of the T3-δ mRNA. An internally labeled single-stranded fragment of human DNA was hybridized to human HPB-ALL mRNA, digested with S1 nuclease, and fractionated along side an M13 sequencing ladder (GATC) obtained by priming M13 clones with the oligonucleotide used for synthesis of the single-stranded probes. Lane 1, human probe; lane 2, S1 nuclease analysis with human HPB-ALL mRNA; lane 3, S1 nuclease control experiment with tRNA.

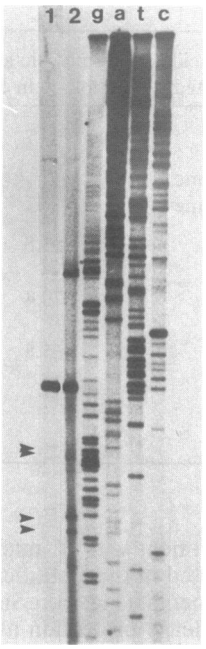

FIG. 4. The synthetic oligonucleotide 5′ AGCCAGGTCACCGAACTATC 3′ was hybridized to RNA from the human T-leukemic cell line HPB-ALL and HeLa cells and used in a primer-extension experiment. The extended products were analyzed on a denaturing gel along side an M13 sequencing ladder obtained by priming M13 clones containing specific fragments with the same primer. Lane 1, HeLa mRNA; lane 2, HPB-ALL mRNA. Arrowheads indicate the positions of specific primer-extension products.

were performed. A synthetic oligonucleotide (described in *Materials and Methods*) was hybridized to mRNA from the human T-leukemic cell line HPB-ALL and HeLa cells. Following reverse transcription and analysis on a denaturing gel, several major extended products were observed whose lengths corresponded to the putative transcription initiation sites as determined by S1 nuclease analysis (Fig. 4). Two additional stronger bands, which do not correspond with the results of the S1 nuclease analysis, were found. Since these bands were also detected when HeLa cell mRNA was used, they represent artifacts caused by nonspecific hybridization of the oligonucleotide probe (Fig. 4).

Alignment of the DNA sequences showed that the 5′ flanking of the major transcription initiation site exhibited a remarkably high degree of conservation: the human and murine T3-δ genes shared a stretch of 56 nt that are 89.3% homologous (indicated in Fig. 2). Of interest is the presence of three pentameric repeats (GCAGA) in this region. Upstream of this region is a second highly homologous segment. Nineteen of 22 nt are identical (86.4%). Comparison of the T3-δ sequences with other promoter regions has not revealed striking similarities. The conserved TATA and CAAT boxes found in many eukaryotic promoters appear to be absent in the human and mouse T3-δ genes. Recently, a number of other genes has been described that also lack the so-called TATA and CAAT boxes. They include the genes coding for adenosine deaminase (38), human β-tubulin (39), hypoxanthine phosphoribosyl transferase (40), 3-hydroxy-3-methylglutaryl coenzyme A (41), dihydrofolate reductase (42), some viral promoters (43, 44), and Thy-1 (45). For most of these genes multiple mRNA initiation sites have been described that were also detected in the human and mouse T3-δ genes. Determination of which sequences in the 5′ upstream region are involved in the tissue-specific expression of the T3-δ gene requires further investigation.

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