

CLONING AND SEQUENCING OF MURINE T₃ γ cDNA
FROM A SUBTRACTIVE cDNA LIBRARY

BY WAYNE G. HASER,* HARUO SAITO,^{†,§} TAKUMI KOYAMA,^{†,§} AND
SUSUMU TONEGAWA*

*From the *Center for Cancer Research and the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; the †Division of Tumor Immunology, Dana-Farber Cancer Institute; and the §Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115*

The T cell receptor complex consists in part of the clonally diverse disulfide-linked heterodimer containing a glycosylated α and β chain (molecular weight 40,000–45,000 and 42,000–44,000, respectively) (1, 2) that seems to recognize both antigen and MHC gene product determinants. The human α/β heterodimer is noncovalently associated with the clonally invariant T₃ complex consisting of two glycoproteins of 25,000 and 20,000 (γ and δ) and two nonglycosylated proteins of 20,000 and 16,000 (ϵ and ξ) (3–6). Comparison of the amino acid sequences deduced from the cDNA clones of γ (7), δ (8), and ϵ (9) revealed significant overall homology between the first two chains, while no homology was observed between ϵ and γ or ϵ and δ . The primary structure of the ξ chain cDNA has yet to be determined. Studies of the murine antigen receptor complex revealed a structure containing seven distinct polypeptides (10–12), of which two were identified as the antigen recognizing the α/β heterodimer. The remaining five polypeptides comprise the murine T₃ complex, which consists of two glycoproteins of 28 and 21 kD, and three nonglycosylated polypeptides of 25,000, 17,000, and 14,000. The murine δ chain has been identified as the 28,000 polypeptide (12), and a cDNA clone coding for this polypeptide has been isolated (13). As of yet, no other cDNA clones of the murine T₃ complex have been isolated.

The T₃ complex may be involved in signal transduction leading to T cell activation that is triggered by a conformational change in the α/β heterodimer occurring upon antigen binding (14). The T₃ γ chain is suspected to play a particularly crucial role in this scheme because the association between T₃ and the α/β heterodimer is mediated through a direct interaction of the β subunit with the T₃ γ subunit (15).

We report here the isolation and characterization of cDNA clones encoding the murine analogue of the human T₃ γ glycoprotein.

Materials and Methods

Cells, RNA, and Library Construction. The alloreactive CTL clones 2C and G4 of BALB.B origin have been described previously (16). The mRNAs from mouse brain, 3T3

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fibroblast, and prekeratinocyte cells were the kind gift of H. Greene. The mouse B cell lymphomas A20-2J and CH1 have been described elsewhere (17, 18). The subtraction-cloning method of Davis (19) was applied to the murine CTL clone, 2C, as previously described (20). In brief, the cDNA synthesized on the poly(A)⁺ RNA from 2C was subtracted twice by hybridization with poly(A)⁺ RNA from the B cell lymphoma, A20-2J, and a library was constructed from this subtracted cDNA using the vector pBR322 and G-C tailing. Differential screening of the resulting clones was performed using two hybridization probes. The first was 2C cDNA prepared by subtracting poly(A)⁺ RNA of polysomes with poly(A)⁺ RNA of the B cell lymphoma, CH1. The second was cDNA prepared from poly(A)⁺ RNA from A20-2J.

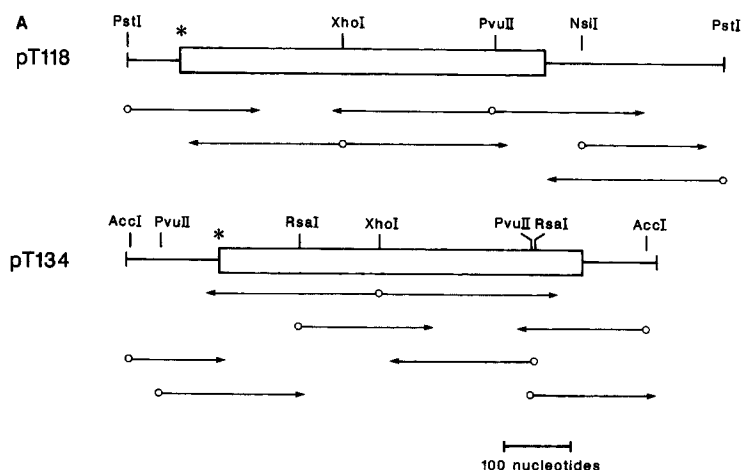
Analysis of T₃ γ mRNA Level. Total cellular RNA was isolated by the guanidinium/CsCl method as described by Chirgwin et al. (21). For Northern analysis, ~1.5 μg of poly(A)⁺ RNA were denatured with glyoxal and electrophoresed through 1% agarose in 10 mM sodium phosphate buffer, pH 6.5. RNA was transferred to a nylon membrane, and the hybridization reaction contained 5 × 10⁶ cpm/ml of nick-translated clone pT134.

Nucleotide Sequence Analysis. Restriction maps of the cDNA clones were constructed by standard procedures and the DNA sequences were determined by the method of Maxam and Gilbert (22).

Results and Discussion

Previously, a subtracted cDNA library was constructed by hybridizing cDNA prepared from the alloreactive CTL clone 2C with poly(A)⁺ RNA from a mouse B cell lymphoma, A20-2J (20). T cell-specific cDNA clones were isolated and classified into 16 distinct groups on the basis of mutual hybridizability, Southern gel blot patterns of genomic DNA, and the size of the corresponding poly(A)⁺ RNA present in T cells. Three groups were judged to represent the rearranging T cell receptor genes, α, β, and γ. The γ sequence does not seem to have a function as a receptor in this CTL clone since no γ protein can be detected on its cell surface (23). Several clones from the remaining groups were sequenced in an attempt to assign them to known T cell-specific proteins. This led to the identification of one group that showed a remarkable homology to the human T₃ γ polypeptide (7). Two representative clones of this group, clones pT134 and pT118, were sequenced. Fig. 1 shows the sequencing strategy and the resulting composite nucleotide sequence of 933 bp. As shown in Fig. 1B the longest open reading frame found in this sequence was 546 bp long and starts and ends at positions 140 and 685, respectively. The translation initiation codon is in alignment with the previously assumed initiation codon of the human T₃ γ. Two overlapping polyadenylation signals of AATAAAATAAA were found between positions 850 and 860, which occurs at a slightly shorter distance from the translation termination codon than that found in the human sequence.

Comparison of the deduced amino acid sequences of the murine and human T₃ γ polypeptide revealed a considerable homology (Fig. 2). Alignment of these two sequences indicated that the murine and human protein consists of a 22-amino-acid leader followed by a 160-amino-acid polypeptide. Both contain a 22-amino-acid leader (68% homology), an 89-amino-acid extracellular domain (61% homology), a 27-amino-acid transmembrane domain (70% homology), and a 44-amino-acid cytosolic domain (89% homology). The position of four cysteines (residues 24, 65, 82, and 85) are conserved, as is the unusual acidic amino acid, glutamic acid (residue 100), within the transmembrane domain. This acidic residue could potentially interact with the arginine residue in the transmembrane sequence of the β chain of the α/β receptor. Previously it was reported that the



B

MU	CTTGCACTACCTGTAGGGTTGAAAACTCTCTACTGGGTGGAGCCAGGCCAGCTGCTGCACAGGCT	68
MU	GTCTGGGTGGCTGGGTGGCTGGGTGGCTAAGGGACTCTCAATACTTCTACTGGAGAAGCAAAGAGACT	136
HU	GGGCTGC-C-ACGCTT-TGCCGGAGAC-----	34
MU	GAC ATG GAG CAG AGG AAG GGT CTG GCT GGC CTC TTC CTG GTG ATC TCT CTT	187
HU	--- --- -A --- G-- --- -C --- --- -T- --- A-- --- -CT --- AT- ---	85
MU	CTT CAA GGC ACT GTA GCC CAG ACA AAT AAA GCA AAG AAT TTG GTA CAA GTG	238
HU	--- --- -T --- T-G --- --- T-- -TC --- -G- -C C-C --- -T A-G ---	136
MU	GAT GGC AGC CGA GGA GAC GGT TCT GTA CTT CTG ACT TGT GGC TTG ACT GAC	289
HU	T-- -A- TAT -A- -A- -T --- -G --- --- --- -AT GCA GAA -C-	187
MU	AAG ACT ATC AAG TGG CTT AAA GAC GGG AGC ATA ATA AGT CCT CTA AAT GCA	340
HU	--A -A- --- -CA --- T-- --- -T --- -AG --G --C G-C TTC --- -C- -A-	238
MU	ACT AAA AAC ACA TGG AAT CTG GGC AAC AAT GCC AAA GAC CCT CGA GGC ACG	391
HU	GA- --- -A -A- --- --- --- -A -GT --- --- -G --- --- -G -T-	289
MU	TAT CAG TGT CAA GGA GCA AAG GAG ACG TCA AAC CCC CTG CAA GTG TAT TAC	442
HU	--- --- --- A-- --- T-- C-- A-C -A- --- --A --A -C --- --- ---	340
MU	AGA ATG TGT GAA AAC TGC ATT GAG CTA AAC ATA GGC ACC ATA TCC GGC TTT	493
HU	--- --- --- C-G --- --- --A --- -T GC- -C- --- --- ---	391
MU	ATC TTC GCT GAG GTC ATC AGC ATC TTC TTC CTT GCT CTT GGT GTA TAT CTC	544
HU	C-- -T --- -A A-- G-- --- -T --- G-- --- G-- -G --C --C T--	442
MU	ATT GCG GGA CAG GAT GGA GTT CGC CAG TCA AGA GCT TCA GAC AAG CAG ACT	595
HU	--- -T --- --- --- --- --- --- -G --- --- --- --- ---	493
MU	CTG TTG CAA AAT GAA CAG CTG TAC CAG CCC CTC AAG GAC CGG GAA TAT GAC	646
HU	--- --- -CC --- -C --- -C --- --- --- --- -T --A --- G-- ---	544
MU	CAG TAC AGC CAT CTC CAA GGA AAC CAA CTG AGG AAG AAG TGA ACTCAGCAGGA	699
HU	--- --- --- -C --T --- --- --- -G T-- --- -G- --T --- -----G-CTC	597
MU	CTCAGGGTGTCCTCCCTTCTATCCAGCACCCAGAATCAAACAATGCATTTTGGAGAGAGCCAGTA	766
HU	AGAGTA--CCAGGTGT-CTCC---TATT-AGTTCCAG--T---A---A-GCATT---GAAAGCTCC	664
MU	GAGAGATTTTCAACCTACAGGTAGACTGCAAGCAGAGAGGAAGAAGTGTCAAAGAAATTTTGGTCT	833
HU	T-GCAGAGAGACTTT-AG-CCTA-AT--AG-CT--AG-TTCCAG-GATGAC--ATGGAGAAGAAAG	731
MU	TTTTTTTTTTTTTCAAATAAAATAAAAGCITGGAGGAGCCAGTGGTATGACTCAGAAGTGGAGCAC	900
HU	GCCA-CAGAGCAAATTTGGGGT-TCTCAA- <u>AA-AT-AAA-TAAA-CA-A-ACTGTG-TTCAG-A</u>	798
MU	TTGTCAACCTTGTGGGGTTCTCAGCACCCCA	933
HU	GC-CC-C-TA-TGGG-AAAA-TGT-AA-AAAA-	831

FIGURE 1. (A) Depicts the restriction maps and sequencing strategy of clones pT118 and pT134. Open circles represent restriction sites as indicated; arrows represent direction and degree of sequencing. Coding regions are shown as the boxed area and untranslated regions are shown as a solid line. The asterisk represents the beginning of each coding region. (B) Comparison of the composite nucleotide sequence of clones pT134 and pT118 (MU) with the human T3 γ nucleotide sequence (HU). Nucleotide positions are indicated in the right margin. The translation initiation codon is indicated by an asterisk and the translation stop codon is indicated by three asterisks. The potential polyadenylation signals are underlined. These sequence data have been submitted to the EMBL/Gen Bank Data Libraries under the accession number Y00635.

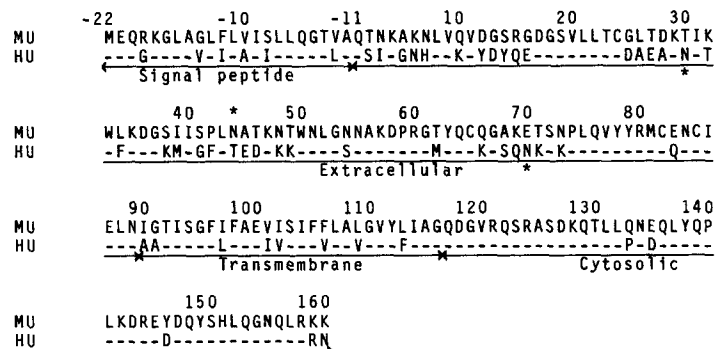


FIGURE 2. Comparison of the deduced amino acid sequence of the murine (MU) and human (HU) T₃ γ polypeptide chains. Regions of sequence spanning the signal peptide, extracellular, transmembrane, and cytosolic domains are indicated by horizontal arrows beneath the sequences. The position of potential N-linked glycosylation sites are marked by an asterisk above the murine sequence and below the human sequence.

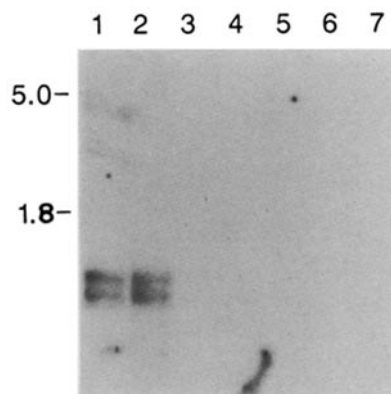


FIGURE 3. Northern analysis of murine T₃ γ transcripts in mRNA isolated from the alloreactive CTL cloned lines 2C and G4 (lanes 1 and 2, respectively), the B lymphoma lines A20-2J and CH1 (lanes 3 and 4, respectively), murine brain tissue (lane 5), 3T3 fibroblasts (lane 6), and prekeratinocytes (lane 7). Arrows indicate the positions of the 28 S and 18 S ribosomal RNA bands represented as 5.0 and 1.8 kb, respectively.

human T₃ γ chain is phosphorylated on serine after activation of protein kinase C (24). A region of basic amino acids followed by alanine-serine are potential sites for phosphorylation by protein kinase C (25). As was described for human T₃ γ, such a stretch of residues was observed at position 123 and is conserved in the murine T₃ γ. The human γ polypeptide contains two regions for potential N-linked glycosylation (asparagine-x-serine, asparagine-x-threonine) at residues 30 and 70, while there appears to be only one potential site for N-linked glycosylation for the murine γ at residue 44.

Two transcripts of 3.5 and 0.8 kb were reported for human T₃ γ. The murine T₃ γ also has the potential to be expressed as two transcripts (Fig. 3). In the two CTL clones, 2C and G4, transcripts of 1.0 and 0.8 kb are visualized. In two T helper hybridomas, both transcripts are observed, although the 1.0 kb transcript is expressed at a lower level (data not shown). Finally, no expression of γ mRNA is seen in non-T lymphocytic mRNA (Fig. 3), indicating that the murine T₃ γ chain is tissue specific for thymus-derived lymphocytes.

Summary

The coding sequences of the murine and human T₃ γ chains are of identical length (182 amino acids) and contain a remarkable conservation of residues. The

most striking observation is the high degree of homology between the murine and human cytosolic domains (89%), suggesting that the effector function of the T₃ complex may be extremely similar or identical within human and murine lymphocytes. Both murine and human T lymphocytes can express two T₃ γ mRNA transcripts, suggesting that a second polyadenylation signal is present downstream. A poly(A) tail is not found in the 3' untranslated region of the murine γ presented here, indicating that the murine clones analyzed represent mRNA generated by reading through the overlapping poly(A) signals at position 850–860 and possibly terminating at a position that would produce the 1.0 kb transcript.

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