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ANALYSIS OF HUMAN TERMINAL DEOXYNUCLEOTIDYLTRANSFERASE cDNA EXPRESSIBLE IN MAMMALIAN CELLS

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SUMMARY: Human Molt3 cDNA library was constructed using pcD vector system which permits the expression of cDNA inserts in mammalian cells. Nearly full-length human terminal deoxynucleotidyltransferase (TdT) cDNA was cloned using a fragment of bovine TdT cDNA as a probe. The human TdT cDNA contains an open reading frame of 1,557 bp coding for 519 amino acids, including 31 bp and 341 bp from 5' and 3' untranslated regions, respectively. The TdT cDNA was transfected into COS7 monkey fibroblasts directed the synthesis of enzymatically active protein of Mr 59,495. The cloned TdT cDNA hybridized with poly A+ RNAs of 2,100 b and 3,300 b from stable T-cell leukemia Molt3 and Molt4 cells.

Terminal deoxynucleotidyltransferase (TdT) catalyzes the polymerization of deoxyribonucleotides in the absence of DNA template (1). TdT activity is detected only in thymocyte and bone marrow cells in higher vertebrates having immune system. Recent investigations suggest that TdT plays an important role of genetic rearrangement of immunoglobulin genes, namely, TdT inserts the extra nucleotides at either the V-D or D-J junctions (2).

To study structure of TdT and the regulation of TdT expression, we have isolated cDNA clone for human TdT. Recently cDNA clone encoding human TdT lacking 5' and 3' end regions has been reported, using λgt expression

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vector system (3). We have constructed cDNA library in a pcD vector which expresses the cDNA inserts in mammalian cells (4) using mRNA prepared from Molt3 cells which express TdT. In this paper, we describe the isolation of nearly-full length human TdT cDNA clone and the nucleotide sequences for 5' and 3' regions of the cDNA. We also show that human cDNA clone is able to promote the transient expression of TdT activity in COS7 monkey cells and that this TdT cDNA hybridized with poly A+ RNAs of 2,100 b and 3,300 b from T-cell leukemia Molt3 and Molt4 cells.

MATERIALS AND METHODS

Construction and screening of cDNA library. 1 g of Molt3 cells was homogenized and the total RNA was extracted by guanidinium-thiocyanate (5). About 100 μg of polyA+ RNA were purified by oligo-dT cellulose column chromatography. A pcD cDNA library was constructed according to the method of Okayama-Berg (4). We cloned the TdT cDNA by screening our library with bovine TdT cDNA (6). Colonies were transfered to nitrocellulose filters and the filters were hybridized with the probe at 50° C in 6xSSPE (5) containing 20 % formamide. The filters were washed with 2xSSPE at 50° C. Nucleotide sequences were determined by the M13 dideoxy chain termination method (7).

Northern blotting. Electrophoresis of 10 μg of poly A+ RNA was in a 1 % agarose gel containing formaldehyde (5). After blotting to a nitrocellulose sheet, the RNA was hybridyzed with a nicktranslated EcoR1 fragment (1,100 bp) of human TdT cDNA as a probe.

Transfection of TdT cDNA clone. The procedures for DNA transfection into COS7 cells are essentially the same as described (6,8). 40 μg of TdT cDNA was transfected into 1×10^7 COS7 cells. The electrophoretic transfer of protein to nitrocellulose (Western blotting) was performed to detect the human TdT expressed in COS7 cells. 20 μg of anti-TdT (kind gift from Dr.H. Nakamura; Aichi Cancer center) was reacted with the transfered protein.

Assay of TdT. TdT activity was assayed in Tris-Mn assay system (6). One unit is defined as the incorporation of 1 nmol of dGTP into an acid-precipitatable form in one hour. The amount of TdT expressed in COS7 cells were determined with enzyme immunoassay system (9).

RESULTS

Isolation of human TdT cDNA clone. A pcD cDNA library contained a 1.3×10^5 independent colonies per 5μg of poly A+ RNA. Six clones hybridized to a bovine probe. Restriction analysis of the cDNA inserts by Pst1 and EcoR1 showed that the longest cDNA (H10) was about 2,000 bp. Entire sequence of H10 clone was determined by M13 dideoxy method. The insert of human TdT cDNA (H10) contains 1,928 bp with an open reading frame comprising 1,557 bp, Mr 59,495. Figure 1 shows the DNA sequences of the 5' and 3' regions for human, bovine and mouse TdT cDNA. The first initiation codon at the position 32 in the human H10 clone is in frame as the bovine TdT clone. Because the molecular weight of TdT on acrylamide gel is about 60,000, this first ATG codon may be the translation initiation site. There is only one consensus

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                  10          20                Met Gly Arg Pro Asp Gly Arg
1  CAGTCTCCCTCCCTTCTGGAGACACCACCAG ATG GGC CAG CCA GAC GGC AGG
2              CTCTTCTGGAGATACCACTTG ATG GCA CAG CAG AGG CAG CAT
3                ATTCTGGAGACACCACCTG ATG GTA CAG ACA GAG CTA GAC

   Gln Pro Leu Pro Met Asp          1800
   CAG CCT CTT CCC ATG GAT..... GAATTCACAATGCATTTTTCAAGAGAAA
   CAG CGT CTT CCC ATG GAT..... GAACTCGCAATGCATTTTCCATAGAAAC
   TGT CTG CTT CC  ATG GAT..... GAATTCATAGTCTTTTGTCTGCTTTGTTT

   TGATGTTGTCACTGGTGGCTCATTCAGGGAAGCTCATCAAAGCCCACTTTGTTCGC
   AGTGTTGGAATTGGTGGCTCATTTCCAGGGAAGTTCATCAAAGCCCACTTTGCCCA
   GAATAAAAATGGTGTTGTCATTGCAGCATCATCTAGAGAAGCGCATCAATGCCCA
                        1900
   AGTGTAGCTGAAATACTGTCTATCTCTAATAAAAACAGGAGGAAAC
   CAGTGTAGCTGAAATACTGTATACTTGCCAATAAAAATAGGAAAC
   CCTGACTCCTGTGTGTCTGAATCTCTGAGTATTGCCAATAAAAATAG GAAAC
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Fig. 1. DNA sequences of 5' and 3' regions for human TdT cDNA. The DNA sequences of bovine and mouse TdT cDNA are shown for comparison with the human's. Amino acids corresponding to human TdT sequence are also shown. Lane 1,2,3 are human, bovine and mouse TdT cDNA sequences, respectively. Under lines and wavy lines show Eco R1 restriction sites and consensus sequences for polyadenylation,respectively.

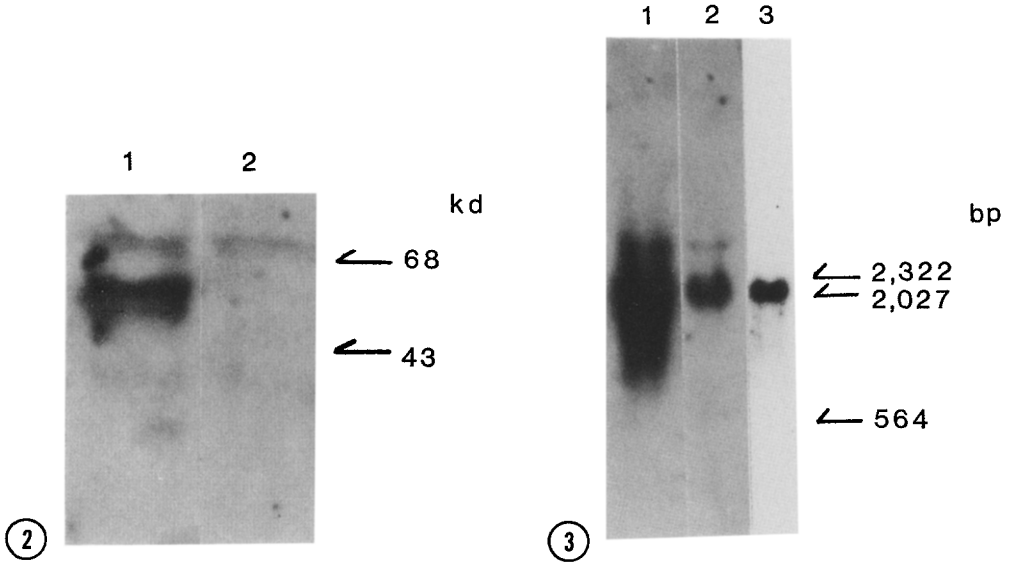

Fig. 2. Expression of human TdT cDNA in COS7 cells. COS7 cells transfected with H10 clone was analyzed by Western blotting as described in "MATERIALS AND METHODS". Lane 1, H10 clone. Lane 2, control (no DNA was transfected).

Fig. 3. Northern blotting of human and bovine poly A+ RNA. Lane 1, human molt3 polyA+ RNA. Lane 2, molt4 poly A+ RNA. Lane 3, calf thymus poly A+ RNA.

sequence for polyadenylation for human and bovine TdT cDNAs contrasting to the mouse cDNA sequence with two consensus sequences. The DNA sequence between the position 65 and 1,804 was the same as reported by Peterson *et al* (3).

Expression of human TdT cDNA clone in COS7 cells. The plasmid H10 was transfected into COS7 monkey fibroblast cells. The expression of TdT in COS7 cells was analyzed using polyclonal rabbit antibody against calf TdT. Figure 2 shows the expressed TdT band corresponding to about Mr 60,000. Protein bands of Mr 70,000 and Mr 38,000 were due to the background cross-reaction with the antibody used. TdT activity expressed in COS7 cells was assayed using extract prepared from $1x10^7$ cells. The total activity per $1x10^7$

cells was 0.5 units at the level of normal small thymocytes. The amount of TdT expressed in the cells was about 10 ng by enzyme immunoassay (9).

Analysis of TdT mRNA. Figure 3 shows the Northern blotting pattern of human Molt3, Molt4 and calf thymus polyA+ RNA's. Two bands of 2,100 b and 3,300 b for Molt3 and Molt4 polyA+ RNA's were hybridized with the human TdT cDNA probe (EcoR1 fragment), whereas a single mRNA of about 2,100 b was hybridized with the same TdT cDNA probe for calf. The amount hybridizing to 2,100 b is about 10 times greater than that of 3,300 b.

DISCUSSION

TdT was originally reported as a complex of Mr 26,000 and Mr 8,000 polypeptides from calf thymus (10). Recently, the structures of mouse and calf TdT were determined as Mr 60,000 by the purification with the anti-TdT column chromatography (11), and by the analyses of TdT cDNA sequences and the expression of TdT cDNA clone in COS7 cells (6). Structure of human TdT has been reported as Mr 62,000 (12) or Mr 58,000 (13). We have cloned the nearly full-length human TdT cDNA with a pcD vector system. DNA sequence analysis predicts that the molecular weight of human TdT is 59,495 which is consistent with the value Mr 60,000 estimated on an acrylamide gel.

TdT activity is normally detected in thymocytes and bone marrow cells for a very short period during lymphocyte development (14). TdT activity several times greater than normal is detected in certain malignant leukemia cells (ex. acute lymphoblastic leukemia; ALL). The control mechanism of TdT expression may be altered in these cells. In fact, we detected poly A+ RNA

of 33,00 b hybridizing with human TdT cDNA probe other than 2,100 b in stable T-cell leukemia Molt3 and Molt4 cells. The long mRNA might be specific for certain leukemia cells (ex. ALL), otherwise we could detect this TdT mRNA of 3,300 b in normal calf thymus cells. Now we are underway to investigate the regulation of TdT expression in malignant leukemia cells.

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