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The several truncated cDNA products transcribed from the dihydrolipoamide succinyltransferase gene

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We detected three truncated cDNA products transcribed from the introns 2, 13 and 14 of the dihydrolipoamide succinyltransferase (DLST) gene, respectively and report about the character of the three truncated cDNA products in present study. One truncated cDNA product started from the intron 2 of the DLST gene and contained the region covering from the exon 3 to exon14, but did not contain the exon 15 of the DLST gene. The other one truncated cDNA product was consisted of intron 12, exon 13, intron 13, exons 14 and 15. This unique cDNA product contained the intron 13 between the exons 13 and 14. The another truncated cDNA product was composed of the intron 14 and exon 15 containing two continuous methionines and the termination condon TAG, predicting the existence of a protein consisting of 38 or 39 amino acid residues. The truncated cDNA products were also confirmed using total RNA from rat skeletal muscle by RT-PCR method. Possibly the RNA fragments participate in the control of gene expression of the DLST gene.

α-Ketoglutrate dehydrogenase complex (α-KDHC) is located in mitochondrial matrix, and the catalytic reaction of the complex is the conversion of α-ketoglutarate to succinyl-CoA in the Krebs cycle. The complex is composed of three different component enzymes. One of these component enzymes is dihyrolipoamide succinyltransferase (DLST) forming the structural core of the α-KDHC.

We already isolated and sequenced the cDNA clones for DLST component of the rat and human *(Nakano et al., 1991; 1993)*. In addition, the human and rat DLST genes have been also isolated and sequenced, respectively. The human and rat DLST genes are approximately 23kbp in size with 15 exons and 14 introns *(Nakano et al., 1994, 2002)*. The human DLST gene is located on chromosome 14 at q24.2－q24.3 and the pseudogene is located on chromosome 1 at p31 *(Nakano et al ., 1993)*. We previously reported that a novel truncated mRNA is transcribed starting from intron 7 of the DLST gene *(Kanamori et al., 2003)*. The truncated translation-product (designated MIRTD) is localized to the intermembrance space of mitochondria and contributes to the biogenesis of the mitochondrial respiratory complexes. Thus, the DLST gene produces the two different proteins with different function, suggesting that the DLST gene is bifunctional.

In present study we found several truncated cDNA products transcribed from the other introns of the DLST gene besides the MIRTD starting from the intron 7 of DLST gene. It is unknown whether these truncated cDNA products translate to proteins

with a function as MIRTD. As an alternative explanation in the role of these truncated cDNA products, these cDNA products may control the gene expression of the DLST gene as non-coding RNA without the translation to proteins by the truncated cDNA.

Materials and Methods

Cap site cDNA ™ of rat skeletal muscle (Nippon Gene, Toyama, Japan) were amplified by PCR using the primers designed from the sequences of rat DLST gene *(Nakano et al., 2002).* In the first PCR, the samples were amplified for 35 cycles under the following conditions :95℃ for 20s, 60℃ for 20s, 72℃ for 20s. Aliquots of the first reaction products were used as the template in the second PCR. The second PCR (nested PCR) was performed using each nested primer for 25 cycles. The forward primers were 5'-ggcagatagaaaatttgagaggc-3' (intron 2), 5'-gggtttctctgggatgaatagccca-3' (intron 2), 5'-ggtcg gggaaattaaactatacagag-3' (intron 12), 5'-ggagcagag agccctggtttgactg-3' (intron 13), 5'-ggata caggtctgttg atctctcc- 3' (intron14) and 5'-ctcccctcctccctcagcgg ggcc-3' (exon 14). The reverse primers were 5'-cgtggtttggaagaagcg-3' (exon 4), 5'-atctctgtacaccacc tccttggttgc-3' (exon 12), 5'-cctgatgaggaaccacgacc-3' (exon 13), 5'-ggcagactgaggcgggttga-3' (exon 14), 5'-gcgggttgataatggtgttccgaaaag-3' (exon 14), 5'-gaaagtcacaggctctctgcg-3' (exon 15) and 5'-ctaaa ggtctaggaggaggac-3' (exon 15). The RT-PCR was performed according to the manufacture's protocol of mRNA selective PCR kit (Takara Bio. Co., Japan) using total RNA of rat skeletal muscle (Clontech. Takara Bio. Inc). The sequences of the PCR fragments were determined using an ABI 310 automated sequencer (Applied Biosystems, USA). The Accession numbers of the sequence data used in present study are D 90401, ABO75005 - ABO7 5013 in GenBank, EMBL and DDBJ Data Bank(s).

Results and Discussion

Since the DLST is a component enzyme of the α-KGDC in mitochondrial matrix, so far DLST has been found only in mitochondria among intercellular organelle. However, we found that the MIRTD is located in the intermembrance space of mitochondria as an alternative product of the DLST gene (Fig. 1). The MIRTD protein plays a physiological role in the assembly of the subunits of complexes Ⅰ and Ⅳ of the mitochondrial respiratory chain. In this study, we tried to find the truncated cDNA products transcribed from the other introns of the DLST gene besides the MIRTD from intron 7 of DLST gene. We performed PCR using rat Cap site cDNA (skeletal muscle) and various sets of primers, one of which corresponds to sequence of an intron and the other to that of an exon as described in Materials and Methods.

As shown in Figs. 2-5, we found three truncated cDNA products transcribed from the introns 2, 12, and 14 of DLST gene. As the first step, we tried the detection of truncated cDNA product starting from the intron 2 of the DLST gene. A fragment was clearly amplified using a pair of the primers corresponding to intron 2 and exon 4 (Fig. 2). The amplified product was sequenced, and the results showed that the fragment consisted of the intron 2, exon3 and exon4 of the DLST gene. Thus, since it was found that the truncated cDNA product starting from intron 2 exists, next, we tried to know the nucleotide sequence of the 3'-terminal side of the truncated cDNA. Namely, we performed PCR coupled with reverse transcription using the total RNA from rat skeletal muscle and each primers set of intron 2 and exon 13, intron 2 and exon 14 and of intron 2 and exon 15. The amplified RT-PCR products were covered in the two primers sets of the intron 2 and exon 13 and of the intron 2 and exon

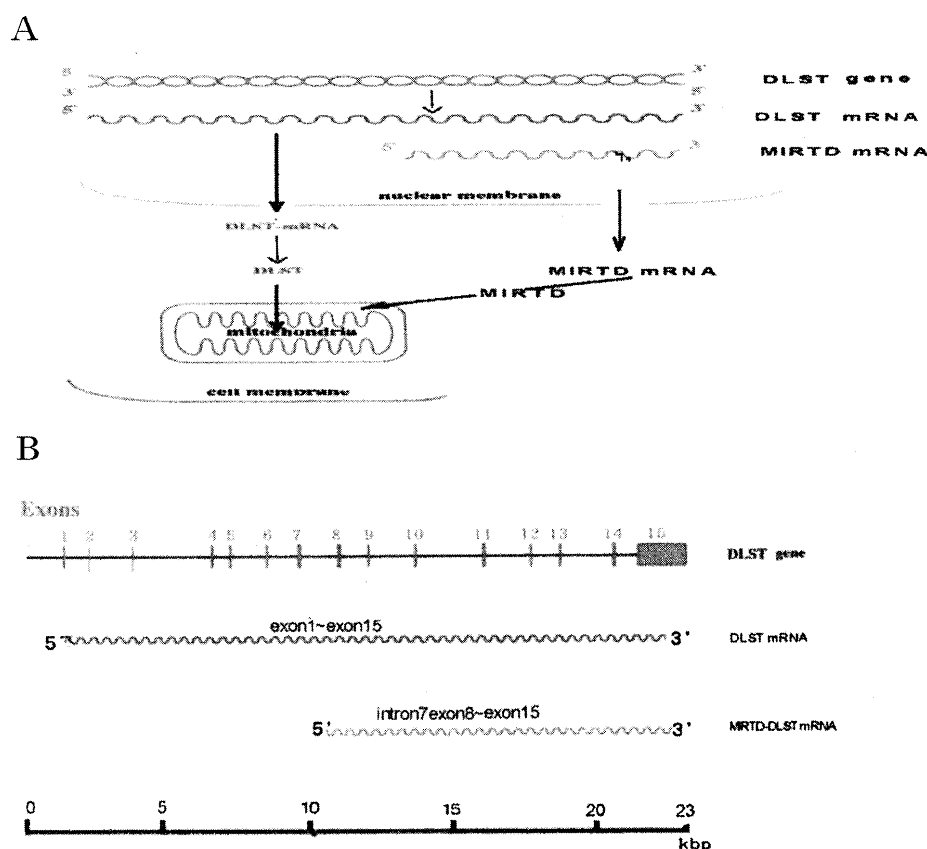

Fig. 1. Schematic diagrams of the multifunction of the DLST gene (A), and the structural organization of DLST gene and a truncated mRNA transcribed from intron 7 (MIRTD mRNA) (B).

14. However, the RT-PCR product was not obtained in the primer set of the intron 2 and exon 15 and in the primer set of the introns 2 and 13 as shown in Fig. 3. Next, the nucleotide sequences of the amplified RT-PCR products were analyzed. The results exhibited the existence of a mRNA containing the intron 2 to the exon 14 of the rat DLST gene (Fig. 3).

As the second step, we carried out PCR using a pair of primers corresponding to intron 12 and exon 15. A PCR product amplified was obtained and sequenced. The result showed that the product was consisted of intron 12, exon 13, intron 13, exon 14 and exon 15, suggesting that the truncated cDNA product contains the intron 13 between the exons 13 and 14 and therefore, the cDNA product is unique. An initiation codon ATG coding for methionine is contained in the exon 13, and the 5'-teminal nucleotide sequence in the intron 13 is gtacagtag. The 5'-teminal nucleotide sequences of gta and cag correspond to valine, glutamic acid, respectively, and the sequence tag is terminal codon. Therefore, if this cDNA is translated to protein, the peptide of 18 amino acid residues translated from the methionine in the exon 13 may be biosynthesized. The 5'-teminal sequence of gatcagtag in the intorn 13 of the rat DLST gene was identical with the sequence of intron 13 of human DLST gene. It is unclear as yet whether this peptide consisting of 18 amino acid residues exists in skeletal muscle.

Next, as the third step, rat skeletal Cap site cDNA was amplified by PCR using a pair of primers corresponding to intron 14 and exon 15 containing terminal codon TAG.

As shown in Fig. 5, a fragment was clearly amplified by the PCR and the fragment agreed with the

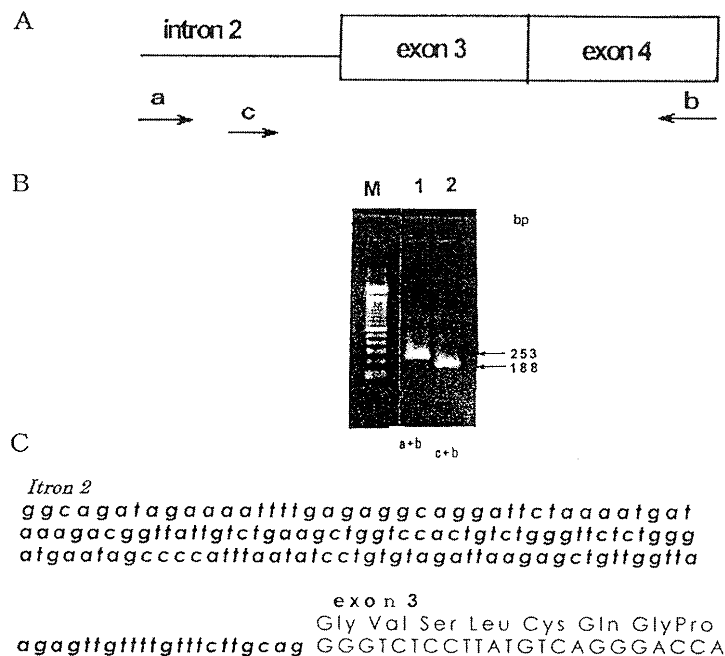

Fig. 2. Detection of cDNA containing the intron 2 of rat DLST gene.

(A) Schematic diagram of the primers (a, b, c) used in this study. The primers a and c correspond the sequence of the intron 2 of DLST gene.

(B) PCR was carried out with primers a and b (lane 1), or with primers c and d (lane 2) using Cap site cDNA™ library of rat skeletal muscle. M; molecular marker of DNA, bp; base pairs.

(C) Nucleotide sequence of the intron 2 in the cDNA containing the intron 2 of DLST gene.

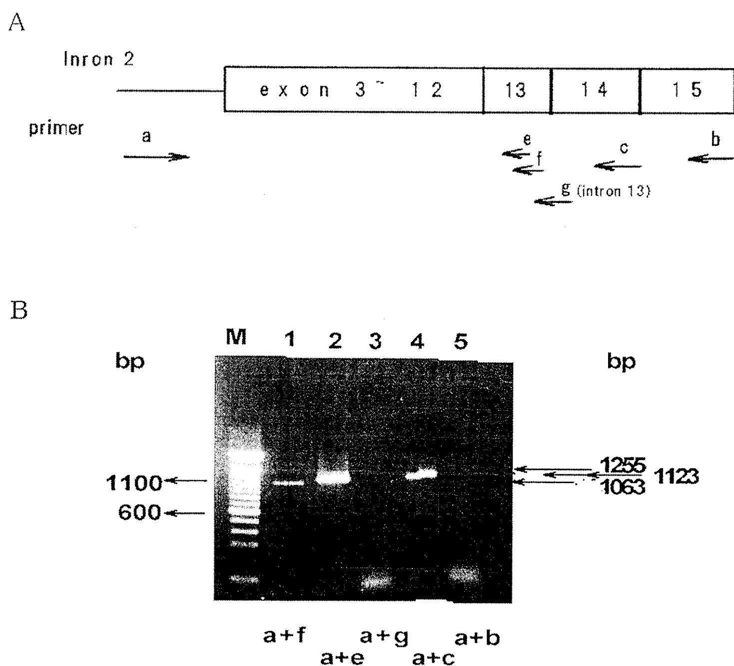

Fig. 3. Detection of a truncated mRNA transcribed from intron 2.

(A) Schematic diagram of the primers in this study. The primer a and g represents the introns 2 and 13 of the DLST gene, respectively. The primers e and f represent the exons 13, and primers c and b represent exons 14 and 15 of the DLST gene, respectively.

(B) Poly(A)-RNA was isolated from rat skeletal muscle. The primers sets used were primers a and f (lane1), or primers a and e (lane 2), or primers a and g (lane 3), or primers a and c (lane 4), or primers a and b (lane 5). M shows a marker of 100bp ladder DNA. bp; base pairs.

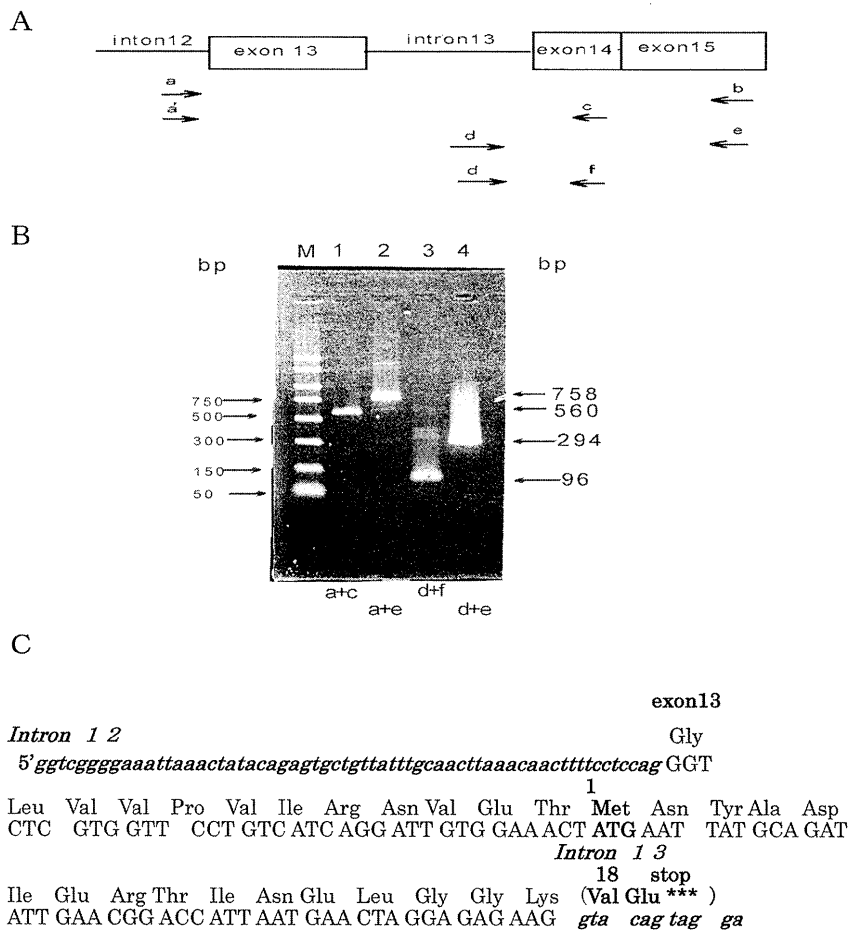

Fig. 4. Detection of cDNA containing the intron 12 of the rat DLST gene.
(A) Schematic diagram of the primers (a, a', b ,c, d, e and f) used in this study. The primers a and a' represent the intron 12 of DLST gene. The primer d represents the intron 13 of DLST gene.
(B) PCR was carried out with primers a and c (lane 1), or primers a and e (lane 2), or primers d and f (lane 3), or primers d and (lane 4) using Cap site cDNA™ library of rat skeletal muscle. M shows a maker of PCR. The marker mixture consists of 8 double stranded linear DNA bands of 2000, 1500, 1000, 750, 300, 150 and 50 bp.
(C) Nucleotide sequences of the introns 12 and 13 and exon 13 in the cDNA containing the introns 12 and 13 of the DLST gene. *** : stop codon.

length covering the intron 14 to exon 15 of the rat DLST gene. The PCR product was sequenced. As expected, it was found that the fragment was composed of the intron 14 and exon 15 of the rat DLST gene. The exon 15 possesses two continuous methionines. Therefore, if this cDNA is translated to protein, the protein will be consisted of 38 amino acid residues or 39 amino acid residues.

Computer analysis on the protein of 38 or 39 amino acid residues was carried out using TANGO program for prediction of aggregation of protein *(Fernandez-Escamilla et al., 2004).* The result revealed that the protein consisting of 39 amino acid residues possesses a property of strong aggregation like amyloid β protein that is considered a main cause of neuronal cell death in Alzheimer's disease (Fig. 6). We already reported the DLST genotype contributes to pathogenesis of Alzheimer's disease *(Nakano et al, 1997).* This result suggests that the small protein of 39 amino acid residues may relate with Alzheimer's disease during the accumulation of the protein in neuronal cell. However, it is

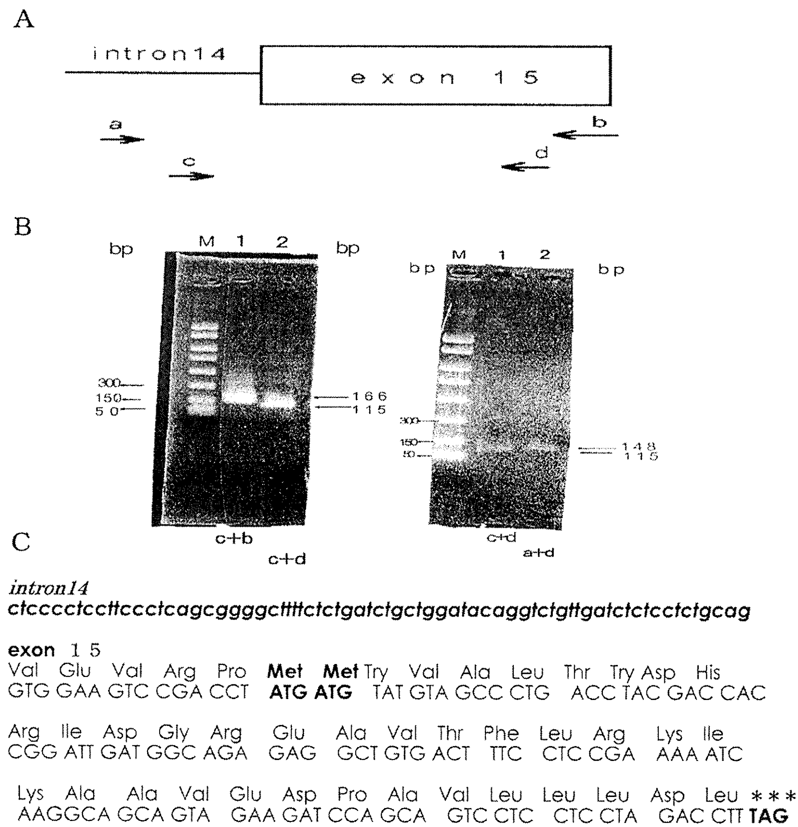

Fig. 5. Detection of cDNA containing the intron 14 of the rat DLST gene.

(A) Schematic diagram of the primers (a, b ,c, and d) used in this study. The primers a and c represent the intron 14 of DLST gene. The primers b, and d represent the exon 15 of DLST gene.

(B) PCR was carried out with primers c and b (lane 1 in left panel), or primers c and d (lane 2 in left panel), or primers c and d (lane 1 in right panel), or primers a and d (lane 2 in right panel) using Cap site cDNA™ library of rat skeletal muscle. M shows a maker of PCR.

(C) Nucleotide sequences of the intron 14 and exon 15 in the cDNA containing the intron 14 and exon 15 of the DLST gene.

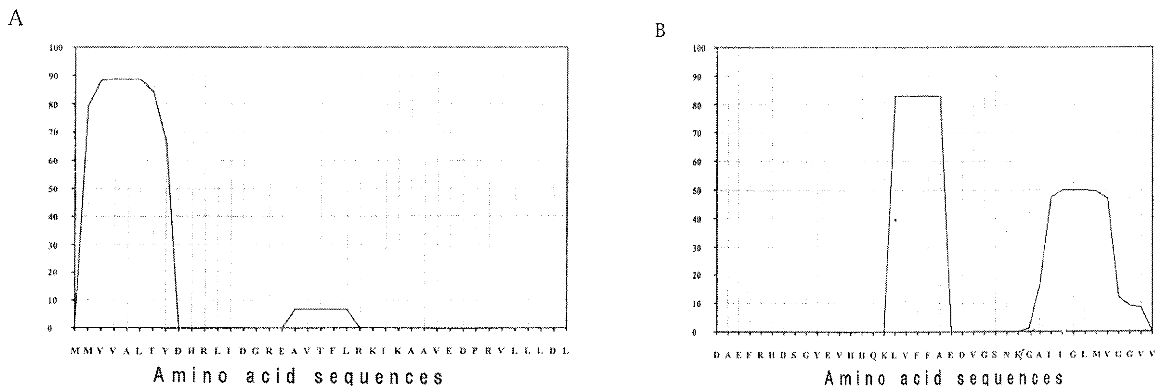

Fig. 6. Computer analysis for prediction of aggregation of the protein consisting of 39 amino acid residues.
The computer analysis was performed using the TANGO program for prediction of aggregation of protein. (A); protein of 39 amino acid residues of MMYVALTYDHRLIDGREAVTFL RKIKAAVEDPR VLLLDL as shown in Fig. 5(C). (B); amyloid β protein (Aβ-40: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGG VVIA). Each amino acid is abbreviated as a single letter.

unknown whether the protein of 39 amino acid residues actually exists in living cell.

In living cell the regulation of gene expression is a very important system. However, it is not well known about the gene expression. Recently, it is reported frequently that the non-coding RNA controls the gene expression through the transcriptional interference time- or space-specifically *(Petruk et al., 2006)*. This present study revealed the existence of three RNA fragments starting from introns of DLST gene. The three RNA fragments may be not translated to proteins and may act as non-coding RNA. Although the physiological role of the three RNA fragment should be resolved in future, the three RNA fragments may participate in gene expression of the DLST gene.

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