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Comparison of nucleotide sequences of the introns 1 and 4 among several mammalian dihydrolipoamide succinyltransferase genes

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Summary

The sizes of introns 4 of the human, Japanese monkey, cat, porcine, mouse and rat DLST genes were 127, 122, 128, 111, 150, 111 and 127bp, respectively. The sizes of introns 1 of the human, Japanese monkey, cat, porcine, mouse and rat DLST genes were 568, 571, 568, 562, 531 and 514bp, respectively. The nucleotide sequences of introns 4 of the DLST genes between human and Japanese monkey of the primate and between mouse and rat of the rodent were 90% and 80% homologous, respectively. The nucleotide sequence of intron 4 of the human DLST gene was 83% homologous to that of the rat DLST gene. The sequence of intron 4 of the human DLST gene was 83%, 66% and 66% homologous to the sequences of introns 4 of the rat, cat, and porcine DLST genes, respectively. The homology between the nucleotide sequences of introns 4 of the primate and rodent DLST genes was higher than that among the primate, cat and porcine. The sequence of intron 1 of the human DLST gene was 84%, 70% and 75% homologous to those of the introns 1 of the Japanese monkey, cat and porcine DLST genes, respectively. Thus, the nucleotide sequences of the introns 1 and 4 were significantly homologous among the six mammalian DLST genes, but no homologous sequence was found in the other introns. Possibly, the introns 1 and 4 contribute to the selective splicing of the DLST gene in the physiological role.

Introduction

Dihydrolipoamide succinyltransferase (DLST) is a subunit enzyme of the α-ketoglutarate dehydrogenase complex (α-KDHC) of Krebs cycle in the mitochondrial matrix. The complex is composed of three different enzymes, α-ketoglutarate dehydrogenase (E1), dihydrolipoamide succinyltransferase (DLST) and dihydrolipoamide dehydrogenase (E3). The DLST forms the structural core of the α-KDHC and contains α-lipoate covalently bound to ε-amino group of a lysine residue. The complex converts α-ketoglutarate to succinyl-CoA in the Krebs cycle. We isolated and sequenced the cDNA clones for DLST components of the rat and human. In addition, the DLST genes have been also isolated from the rat and human and sequenced. The human and rat DLST genes are approximately 23kbp in size with 15 exons and

14 introns (*Nakano et al ., 1994, 2002*).

Recent studies suggest that the numbers of the protein-coding genes in human are about 25000 and that even in the non-coding regions for protein the most regions are transcribed to RNA. Usually, it is known that the nucleotide sequences of introns are not homologous among species because of the introns are not coded for protein and that only the two percentage of whole genome encode for the protein and the ninety eight percentage of the remainder is non-coding region. In the present study we compared the nucleotide sequences of introns among several mammalian DLST genes. However, we found that the sequences of the introns 1 and 4 of the DLST genes were significantly homologous among the mammals. We previously reported that a novel truncated mRNA is transcribed starting from intron 7 of the DLST gene and the truncated gene product is designated as MIRTD (*Kanamori et al., 2003*). Possibly, it is explained that the conserved sequences of the introns 1 and 4 contribute to the selective alternative splicing of the DLST gene in the physiological role.

Materials and Methods

Monkey gemonic DNA was isolated from the blood of Japanese monkey (*Macaca fuscate*) using Gene trapping by liquid extraction (Takara com., Japan). The cat, mouse and porcine genomic DNAs were purchased from NoVagen Com. (Germany). The introns 1 and 4 of the several mammalian DLST genes were amplified by PCR using the primers designed from the sequences of the human and rat DLST cDNAs (*Nakano et al., 1991, 1993*). The primers sets were 5'-TGCGTGTCCCGGGCGTTCAGC-3' and 5'-CAGG GAACGTCTCCTAGAGG-3' (rat), 5'-GTGTTCGCTTCTTCCAAACCACG-3' and 5'-TGTGCCATTTGC TGGTGATGG-3' (rat), 5'-GCATTAACAACAGTGTC TTCAGTG-3' and 5'-CCTTGTCAGTTTCAATC TCACAAACC-3' (human). The PCR conditions were 94°C for 30s, 60°C for 30s, and 72°C for 2 min for 30 cycles. The sequences of the PCR fragments were determined using an ABI310 automated sequencer (Applied Biosystems, USA). The accession numbers of the sequence data used in this study were ABO75005 - ABO75013, D26535, D29970, D90401 and D16373 in Gen Bank, EMBL and DDBJ bank Libraries.

Results and Discussion

The human and rat DLST genes are approximately 23kbp in size with 15 exons and 14 introns (*Nakano et al., 1994, 2002*). The size of each intron of the human and rat DLST genes is shown in Table 1. The each intron size of the rat and human DLST gene varies widely from 127bp to the about 4kbp. However, in the comparison of each intron size they were roughly in agreement between rat and human. In particular, the sizes of the intorns 1 and 4 of the rat DLST gene substantially coincided with the sizes of the intorns 1 and 4 of the human DLST gene. In addition we previously demonstrated the significant homology of the introns 1 and 4 between the rat and human DLST genes (*Nakano et al., 2002*).

Table 1 Comparison of intron size between rat and human DLST genes

Intron number	Intron size (bp)	
	rat	human
1	514	568
2	*3500	2954
3	*3000	3456
4	127	127
5	201	548
6	380	1111
7	*3000	1661
8	635	362
9	*1000	886
10	*4000	3961
11	*1300	1425
12	*1100	292
13	370	691
14	*1700	962

* The symbols indicate intron size estimated from restriction enzyme anaylsis

Table 2 Comparison of sizes of introns 1 and 4 among several eukarytic DLST genes

Eukaryote	Intron size (bp)	
	intron 1	intron 4
Human	568	127
Monkey	571	122
Porcine	562	150
Cat	568	128
Rat	514	127
Mouse	531	111

Therefore, in the present study we further compared the nucleotide sequences and sizes of the introns 1 and 4 of the Japanese monkey, porcine, cat and mouse DLST genes (Fig. 1 and Table 2). As shown in Fig. 1-A, the nucleotide sequence of intron 4 of the human DLST gene was 90% homologous to that of intron 4 of the Japanese monkey DLST gene. Also, the nucleotide sequence of intron 4 of DLST gene was 80% homologous between mouse and rat of the rodent. However, as reported previously, the nucleotide sequence of intron 4 of human DLST gene was 83% homologous to that of rat DLST gene (*Nakano et al., 2002*). As shown in Fig. 1-B, the nucleotide sequence of intron 4 of the human DLST gene was 66% homologous to those of introns 4 of the cat and porcine DLST genes. Also, the homology in the nucleotide sequences of the introns 4 was 64% between cat and porcine. Thus, the homology of the nucleotide sequences of introns 4 between the primate and rodent DLST genes was higher than that among primate, cat and porcine. Although it is difficult to explain why the nucleotide sequences of introns 4 between the primate and rodent DLST genes were better conserved those that among the primate, cat and porcine DLST genes, each intron may be significant on the molecular evolution.

Next, we compared the nucleotide sequences of introns 1 among the six mammalian DLST genes. As shown in Fig. 2-A and -B, the homology in the sequences of introns 1 of the DLST genes was 84% between human and Japanese monkey of the primate and was 82% between mouse and rat of the rodent, respectively. Also, the nucleotide sequence of the human DLST gene-intron 1 was 70% and 75% homologous to the nucleotide sequences of introns 1 of the cat and porcine DLST genes, respectively. Furthermore, we have previously reported that several homologous sequences existed sporadically between introns 1 of the rat and human DLST genes and the lengths of the homologous sequences were 40-100 bases (*Nakano et al., 2002*). Thus, the homology of the sequences of the introns 1 among primate, cat and porcine was higher than that

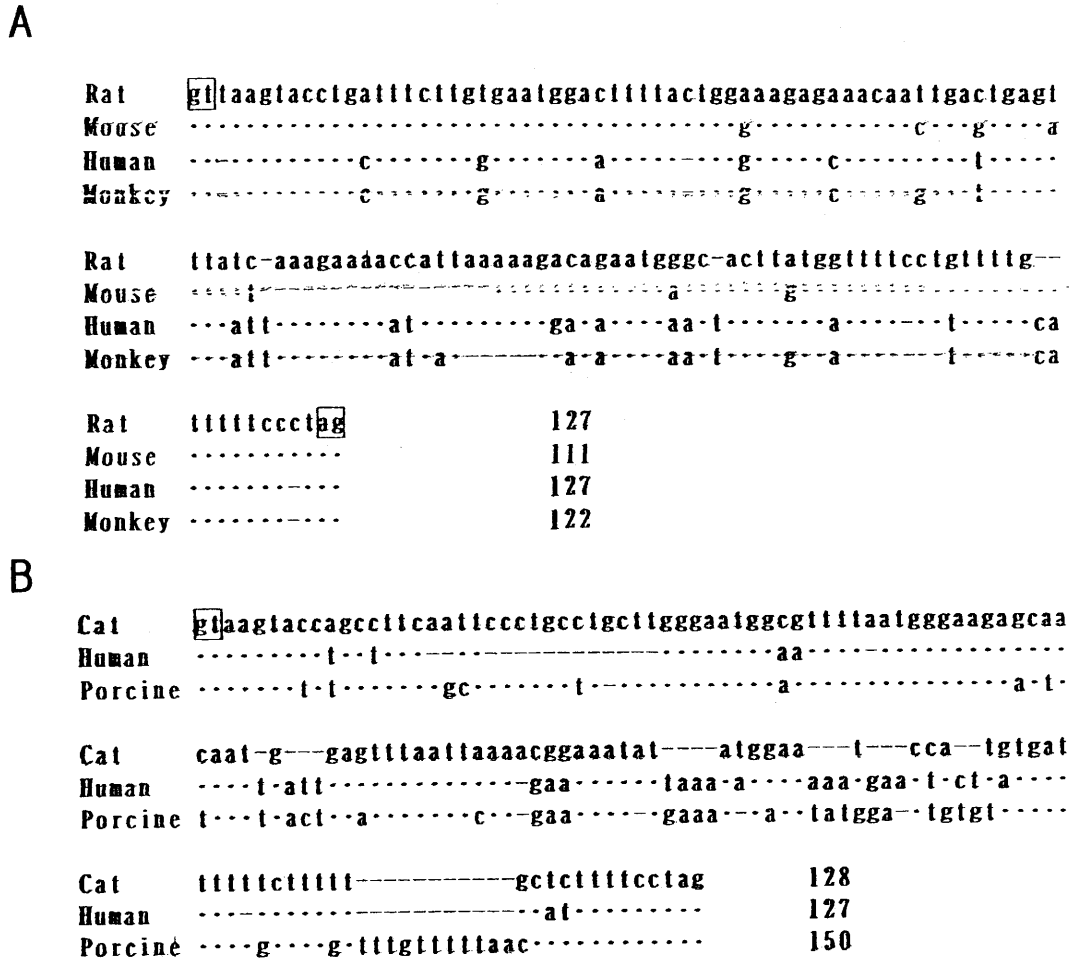

Fig. 1. Nucleotide sequence of intron 4 of the rat DLST gene and comparison of the sequence of introns 4 among rat, mouse, Japanese monkey and human DLST genes (A). Nucleotide sequence of intron 4 of the cat DLST gene and comparison of the sequence of introns 4 among cat, human and porcine DLST genes (B).

between human and rat in contrast with the results that in the intron 4 the homology between primate and rodent was higher than that among primate, cat and porcine.

Thus, the nucleotide sequences of introns 1 were sporadically consered to each other in small range among the six mammalian DLST genes. The sizes of introns 4 in the six mammalian DLST genes were from 110 to 150 bases and relatively small, and the nucleotide sequences were significantly homologous to each other. We previously reported that three pairs of direct repeats are present in intron 1 of the human DLST gene (*Nakano et al ., 1994*). In the current study, we also compared the sequences of the three pairs of direct repeats among the six mammalian DLST genes. As a result, we found the two pair of the direct repeats of CCGGGGGCC (DR-1) and GGCGCGGC (DR-2) in introns 1 of the Japanese monkey and cat DLST genes, but not in introns 1 of the rat and mouse DLST genes. Especially, the nucleotide sequence of the DR-1 was completely conserved among the human, Japanese monkey, porcine and cat DLST genes. However, the third one pair CCGCGCGGGG (DR-3) of the three pairs of the direct repeats was not found in the introns 1 of the other mammals without human.

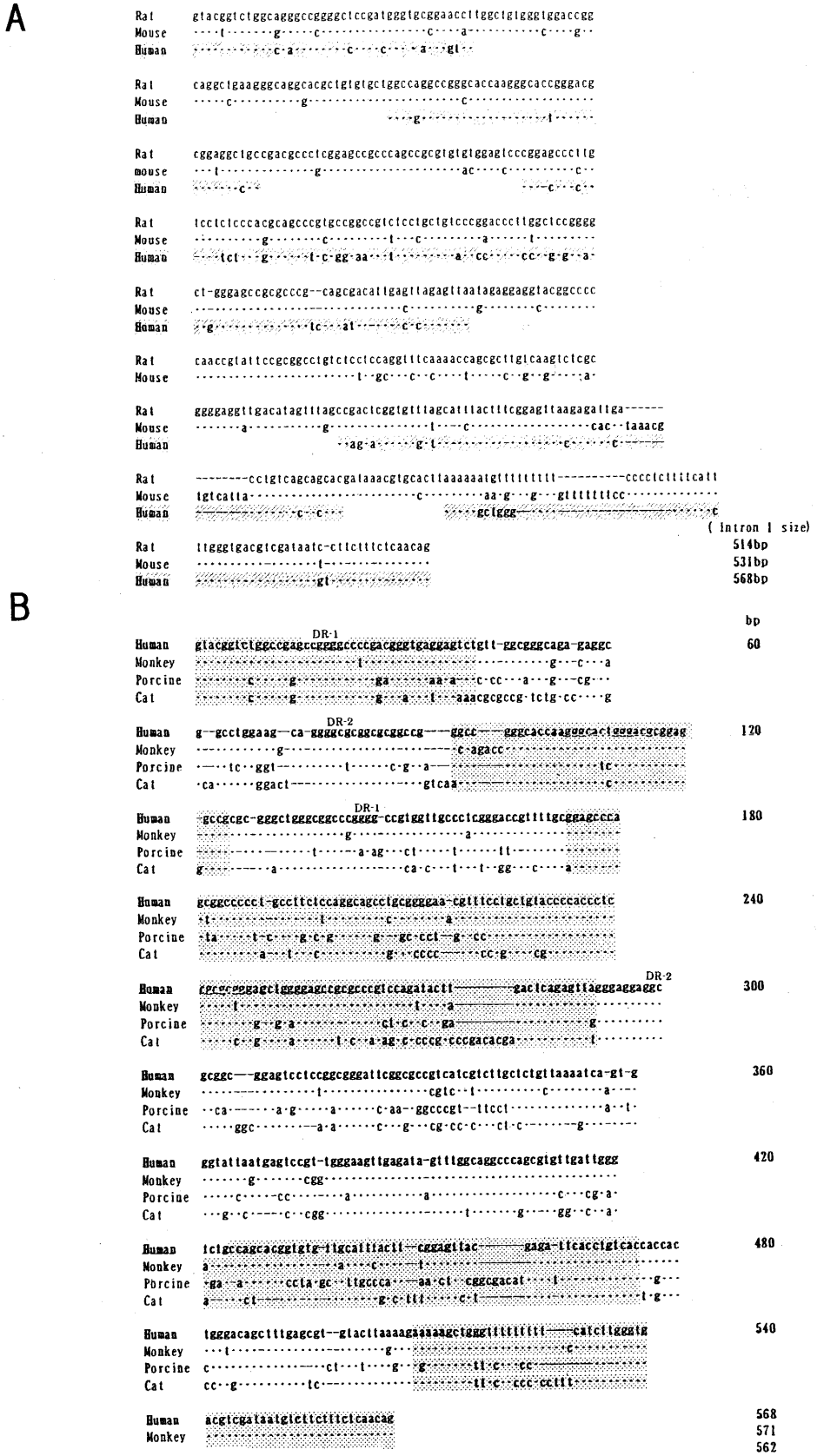

Fig. 2. Nucleotide sequence of intron 1 of the rat DLST gene and comparison of the sequence of intron 1 among rat, mouse and human DLST genes (A). Nucleotide sequence of introns 1 of the human DLST gene and comparison of the sequence of intron 1 among human, Japanese monkey, cat and porcine DLST genes (B).

Thus, the nucleotide sequences of the introns 1 and 4 were significantly homologous among the mammalian DLST genes, but the homologous sequences could not be found in the other introns. Taken together, possibly, the conserved small range sequences of the introns 1 and 4 play an important role in the expression of DLST gene. Recently, by reports, larger numbers of non-coding RNA transcript are revealed by cDNA cloning and genome tiling array studies in animals. *Mattick and Makunin* have reported that many transcripts including some non-coding transcripts are alternatively spliced and both exons and introns may transmit information. They have reported that many miRNAs and all small nucleolar RNAs in animals are sourced from introns (*Mattick and Makunin, 2005*).

Here, we suggest that the conserved small range sequences of the introns 1 and 4 of mammalian DLST genes may have a function of gene expression like miRNAs and small RNAs. Small RNAs are 20- to 26-nucleotide non-coding RNAs that regulate transcript and protein abundance via multiple mechanism (*Baulcombe, 2005, Zamore and Haley, 2005*). We previously found an unusual mRNA transcribed from intron 7 of the human DLST gene in addition to the full-length form (designated MIRTD) (*Kanamori et al., 2003*). The MIRTD transcribed with alternative splicing manner may be regulated by these consensus sequences conserved in the introns 1 and 4 of the DLST gene.

References

Baulcombe D. (2005) RNA silencing. *Trends Biochem. Sci.*, 30, 290-293.

Kanamori K., Nishimaki K., Asoh S., Yoshitomo I., Takata I., Kuwabara T., Taira K., Yamaguchi H., Sugihara S., Yamazaki H., Ihara Y., Nakano K., Matuda S. and Ohta S. (2003) Truncated product of the bifunctional DLST gene involved in biogenesis of the respiratory chain. *The EMBO Journal*, 22, 2913-2923.

Mattick J. S. (2005) The functional genomics of noncoding RNA. *Sci.*, 309, 1527-1528.

Mattick J. S. and Makunin J. V. (2005) Small regulatory RNAs in mammals. *Hum. Mol. Genet.* 14, R 121- R132

Nakano K., Matuda S., Yamanaka T., Tsubouch H., Nakagawa S., Titai K., Ohta S. and Miyata T. (1991) Purification and molecular cloning of succinyltransferase of the rat α-ketoglutarate dehydrogenase complex. Absence of a sequence motif of the putative E3 and/or E1 binding site. *J. Biol. Chem.*, 266, 19013-19017.

Nakano K., Matuda S., Sakamoto T., Takase C., Nakagawa S., Arima T., Inazawa J, Abe T. and Miyata T. (1993) Human dihydrolipoamide succinyltrasferase cDNA cloning and localization on chromosome 14q24.2-24.3. *Biochim. Biophys. Acta*, 1216, 360-368.

Nakano K., Takase C., Sakamoto T., Nakagawa S., Inazawa J., Ohta S. and Matuda S. (1994) Isolation, characterization and structural organization of the gene and pseudogene for the dihydrolipoamide succinyltransferase component of the human 2-oxoglutarate dehydrogenase complex. *Eur. J. Biochem.*, 224, 179-189.

Nakano K., Tanabe M., Nakagawa S., Ohta S., Suzuki S., Shimura M. and Matuda S. (2002) Isolation and sequence analysis of the rat dihydrolipoamide succinyltransferase gene. *DNA sequence*, 13, 363-367.

Zamore P. D. and Haley B. (2005) Ribo-gnome: The big world of small RNAs. *Sci.*, 309, 1519-1524.

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