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# Cloning of a cDNA for Type II Iodothyronine 5′ Deiodinase in the House Musk Shrew (*Suncus murinus*, Insectivora: Soricidae)

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**Abstract:** Two types of iodothyronine 5′ deiodinase are known to catalyze the conversion of thyroxine to 3,3′,5-triiodothyroine (T3), an active form of thyroid hormone. Our recent study has suggested that type II deiodinase rather than type I is important for the maintenance of plasma T3 as well as the tissue content of T3 in the house musk shrew. The present study aimed to clone cDNA for type II deiodinase and determine the primary structure of DII protein. Total RNA was extracted from the brown adipose tissue in the shrew of Katmandu strain (KAT), where highest DII activity was detected. DII cDNA was cloned in combination by RT-PCR and Rapid Amplification of cDNA Ends (RACE). The alignment of three cloned cDNA fragments includes the entire open reading frame of shrew DII so that the amino acid sequence was deduced. The shrew DII contains two selenocysteins that are essential for the deiodinase activity. The amino acid sequence of the shrew DII showed high homology with that of human (89%), rat (91%) and mouse (92%). The obtained cDNA would be a proper probe to investigate changes in the DII expression when shrews are exposed to various environments and insults.

**Key words:** Type II iodothyronine deiodinase, house musk shrew, cloning, RACE, selenocystein

Deiodination is an important metabolic pathway of thyroid hormone. Three types of deiodinases have been identified; Type I (DI) and type II (DII) convert prohormone, thyroxine (T4) to active 3,5,3′-triiodothyronine (T3) by outer ring deiodination, whereas type III (DIII) converts T4 to inactive 3,3′,5′-triiodothyronine (reverse T3, rT3) by inner ring deiodination. In human, rats and mice, it has been reported that plasma T3 is mainly supplied from the conversion of T4 by DI action, while DII plays a role in tissue T3 maintenance.[1] However, in the house musk shrew, *Suncus murinus* (Insectivora, Soricidae), DII seems to be important for the maintenance of plasma T3 as well as that of tissue T3.[2] Thus, the changes in DII expression could affect serum T3 levels. Previously, we reported a partial nucleotide sequence of complementary DNA (cDNA) for DII of Nagasaki strain (NAG).[3] However, since the 5′ part of cDNA was missing, the amino acid sequence of the shrew DII (sDII) has not been determined yet. By using Rapid Amplification of cDNA Ends (RACE), we succeeded in the cloning of 5′ and 3′ parts of shrew DII cDNA. Because the extended sDII cDNA includes an entire open reading frame of sDII, we could deduce the amino acid sequences of sDII. Here we report a primary structure of sDII and compared the one with other mammalians species.

## Materials and Methods

### 1. Cloning of a sDII cDNA fragment from Katmandu strain (KAT)

Total RNA was extracted from the brown adipose tissue (BAT) an adult shrew of KAT by the acid-guanidium thiocyanate-phenol-chloroform method.[4] Reverse transcription (RT) was performed using an *Adapter Primer* (5′-GGC CAC GCG TCG ACT AGT ACT TTT TTT TTT TTT TTT T-3′) supplied in 3′ RACE System (Life Technologies, Tokyo, Japan). After the RT, the polymerase chain reaction (PCR) was performed using an upstream primer, sD2-1UP (5′-CGG TCA TTC TGC TCA AGC ACG-3′) and a downstream primer, sD2-1DOWN (5′-ACT GAG GAG AAC TCT TCC AC-3′). These primers were designed based on the human DII cDNA. Sequences that were conserved among human,[5] rat[5] and mouse[6] but divergent from DI cDNA sequences[2] of those species were selected. The reaction mixture was prepared according to the instructions of the *TaKaRa Ex Taq* (TaKaRa Bio Inc, Otsu-shi, Japan). The PCR cycles were as follows: 94°C for 5 min; 30 cycles of 94°C for 30 sec, 52°C for 30 sec, 72°C for 1 min; and 72°C for 7 min using TP-3000 thermal cycler (TaKaRa). An amplified cDNA fragment (fragment A in Fig. 1) with 326-bp was cloned using the pGEM-T Easy Vector System I (Promega Corporation, WI. U.S.A.) and was sequenced using

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ABI PRISM 373 DNA sequencer (Applied Biosystems Japan Ltd, Tokyo, Japan).

### 2. 3′ RACE

In the next, 3′ RACE was performed using two primers, sD2-RACE-4 (5′-CGA GAA GAC CAT TGA TGG AGC C-3′) and sD2-RACE-5 (5′-CTG GTG GTC AAC TTC GGA TCG G-3′). These primers were designed based on the sequence of the fragment A. The first reaction was carried out using sD2-RACE-4 primer and *Abridged Universal Amplification Primer* (AUAP) (5′-GGC CAC GCG TCG ACT AGT AC-3′) supplied in the 3′-RACE kit. Nested PCR was carried out using sD2-RACE-5 and AUAP primers. The PCR amplification cycles was as follows: 94°C for 5 min; 10 cycles of 94°C for 30 sec, 58°C for 30 sec, 72°C for 6 min; 20 cycles of 94°C for 30 sec, 58°C for 30 sec, 72°C for 6 min with additional extension time (5 sec × cycle number); and 72°C for 7 min using TP-3000 thermal cycler (TaKaRa). An amplified fragment with 1376-bp (fragment B) was cloned using the pGEM-T Easy Vector System I (Promega). The sequence was determined by ABI PRISM 373 DNA sequencer. Since fragment B was too long to be sequenced with T7 and SP6 promoters, we digested fragment B with NsiI and subcloned into the vector. A partial sequence of fragment B was sequenced using two primers, sD2-Seq-f1 (5′-TGA ATC CAC AAT TTC AAG ACA G-3′) and sD2-Seq-r1 (5′-TTA GAT TTC AAA TGA TCA TTC-3′).

### 3. 5′ RACE

5′ RACE was performed using a specific primer, sD2-7 down (5′-TTC ATT AGA AAC ATG TAC C-3′) and 5′ RACE system, Version 2.0 (Life Technologies). The first strand product was purified by GENECLEAN II Kit (Bio101 Inc.) and poly-C tailing was performed according to the instruction by the supplier. The poly-C tailed cDNA was amplified using sD2-3.5 down (5′-TCC CTC TGA GGT CAG CAT ACG C-3′) nested gene specific primer and a poly-G contained primer, *5′ RACE Abridged Anchor Primer* (5′-GGC CAC GCG TGC ACT AGT ACG GGI IGG GII GGG IIG-3′) of the kit. The reaction mixture was prepared according to the instructions by the supplier of the *TaKaRa Ex Taq* (TaKaRa). The PCR condition was as a follows: 94°C for 5 min; 30 cycle of 94°C for 30 sec, 52°C for 30 sec, 72°C for 1 min; 72°C for 7 min with TP-3000 thermal cycler (TaKaRa). An amplified product with 208bp (fragment C) was cloned using the pGEM-T Easy Vector System I (Promega), and sequenced by an ABI PRISM 373 DNA sequencer with primers, T7 and SP6.

## Results and Discussion

By the RT-PCR using a set of primers, sD2-1UP and sD2-1DOWN, fragment A shown in Fig. 1 was amplified. When sequences for human, rat and mouse D2 cDNA were referred, the fragment A was supposed to include a middle part of the open reading frame (ORF) of sDII (Fig. 1). After 3′ and 5′ RACE, fragment B and C had been cloned, respectively, then these three fragments (A, B, C) were aligned and the nucleotide sequence of sDII cDNA was shown in Fig. 2. As shown in Fig. 2, the cDNA includes the entire ORF so that the amino acid sequence of sDII was deduced. The ORF contains two in-frame TGA codons at positions 444-446 and 844-846 that are possibly translated to selenocycteine (Sec). The amino acid sequence of sDII was compared with those of other mammalian species. As shown in Fig. 3, sDII showed high homology with those of human[5] (89%), rat[5] (91%) and mouse[6] (92%).

It has been reported that a selenocysteine insertion sequence (SECIS) element that exists in 3′-untranlated region of the mRNA is essential for the translation of TGA codon to a selenocystein.[7,8] Unfortunately, the cloned sequence in the present study does not include SECIS element. Since the ele-

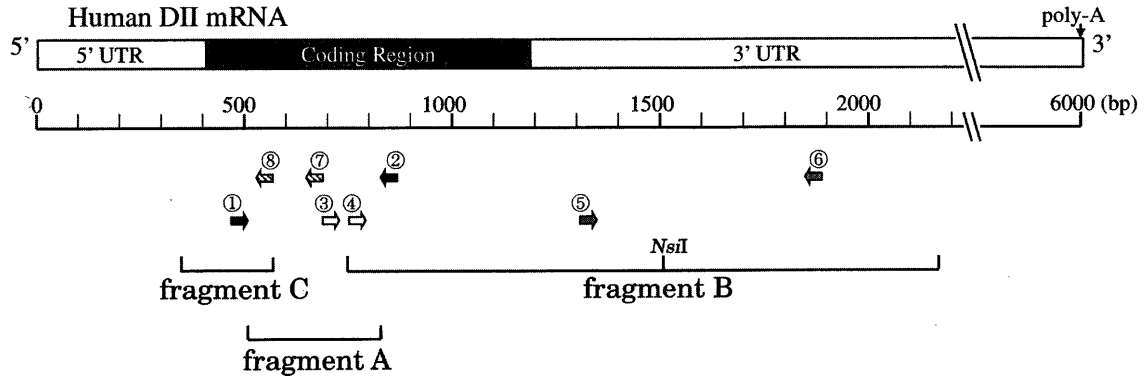


Fig. 1 Cloning strategy for DII cDNA in the house musk shrew.
A structure of human DII mRNA is used as a reference. Primers used for RT-PCR are shown in filled arrows (① and ②). Primers (③ and ④) indicated in open arrows are used for 3′ RACE, ⑤ and ⑥ are for sequencing, and ⑦ and ⑧ are for 5′ RACE. These primers are named as follows: ①; sD2-1 UP, ②; sD2-1 DOWN, ③; sD2-RACE-4, ④; sD2-RACE-5, ⑤; sD2-Seq-f1, ⑥; sD2-Seq-r1, ⑦; sD2-7down and ⑧; sD2-3.5down. The *Nsi*I site in fragment B was used for subcloning.

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GGTGAAGGGGAACCAGAAGCCACAAGGGGACTGACACAGGAGGCAGAGAAGATGGGCATCCTCAGCGTACACTTGCTGATCACGCTGCAA 90
                                                    M  G  I  L  S  V  H  L  L  I  T  L  Q  13

ATTCTGCCAGTTTTTTTCTCCAACTGCCTTTTCCTGGCGCTCTATGACTCGGTCATTCTCCTCAAGCACGTGGTGCTACTGTTGAGCCGC 180
 I  L  P  V  F  F  S  N  C  L  F  L  A  L  Y  D  S  V  I  L  L  K  H  V  V  L  L  L  S  R  43

TCCAAGTCCACTCGCGGGGAGTGGAGGCGTATGCTGACCTCAGAGGGAATGCGTTGCATCTGGAAGAGCTTCCTCCTGGATGCCTACAAA 270
 S  K  S  T  R  G  E  W  R  R  M  L  T  S  E  G  M  R  C  I  W  K  S  F  L  L  D  A  Y  K  73

CAGGTAAAATTGGGTGAAGATGCACCCAATTCCAGTGTGGTACATGTTTCTAATTCCGAAAGTGACAGTGGCAGAAATGGTGTCCCCGAG 360
 Q  V  K  L  G  E  D  A  P  N  S  S  V  V  H  V  S  N  S  E  S  D  S  G  R  N  G  V  P  E  103

AAGACCATTGATGGAGCCGAATGCCACCTTCTTGACTTTGCCAGCGCTGAGCGCCCACTGGTGGTCAACTTCGGATCGGCCACTTGACCT 450
 K  T  I  D  G  A  E  C  H  L  L  D  F  A  S  A  E  R  P  L  V  V  N  F  G  S  A  T  Se P  133

CCTTTTACAAGCCAGCTGCCAGCCTTCAGCAAACTTGTGGAAGAGTTCTCATCAGTGGCTGACTTCCTGTTGGTCTACATAGATGAAGCT 540
 P  F  T  S  Q  L  P  A  F  S  K  L  V  E  E  F  S  S  V  A  D  F  L  L  V  Y  I  D  E  A  163

CATCCTTCAGATGGTTGGGCAGTACCTGGGGATTCTTCTTTGTCGTTTGAGGTGAAGAAGCACCGGAGCCAAGAAGACAGATGTGCAGCA 630
 H  P  S  D  G  W  A  V  P  G  D  S  S  L  S  F  E  V  K  K  H  R  S  Q  E  D  R  C  A  A  193

GCCCTTCAGCTCCTGGAGCGTTTCTCCTTGCCGCCCCAGTGCCAAGTTGTGGCTGACCGCATGGACAATAATGCCAACGTAGCGTATGGT 720
 A  L  Q  L  L  E  R  F  S  L  P  P  Q  C  Q  V  V  A  D  R  M  D  N  N  A  N  V  A  Y  G  223

GTAGCTTTTGAACGTGTGTGCATTGTGCAGAGACAGAAAATTGCTTATCTGGGAGGAAAGGGTCCTTTTTGCTACAATCTTCAGGAAGTC 810
 V  A  F  E  R  V  C  I  V  Q  R  Q  K  I  A  Y  L  G  G  K  G  P  F  C  Y  N  L  Q  E  V  253

CGGGATTGGCTGGAGAAAAATTTCAGCAAGAGATGAATTCTAACTTAGTTGTTCAAAGGTATGACTCTACTAGAATTTTATTTTAATTAT 900
 R  D  W  L  E  K  N  F  S  K  R  Se I  L  T  *                                              268

AAAGGGAAGGGGATAAAGAAATGAATCCACAATTTCAAGACAGTCCCAATATCTCACAGAAAAAAAGGGATTTATGTGCCAGAAGAAGAA 990
GAACCCCTAACATCTCAATGCGTCTTTCTTCATTCAAATGGCATTTGGCTAAGAAGAAGCCAGATTACATCTTTTTCTACTGAGAAATCC 1080
TGGGTGAAAGATCCCAATGTGCAGAGGAGGAAATCAGCATGTGTGCTCATCTGTCTTGAGAAAGAAGTGATGCATCTGATGTGCTTGCAG 1170
ACAAGAAGAAATATCAGAAATATCTAATTTACCTGAATCACGACTGAGTTAGGGAAGCTTCTGTCTATGTTAATATATGGGATATTGACT 1260
TGATGTTTCTCAGCATGGACAGAGTCCAAATGGAAATTTCCCCCATTAACTTCTTTATAATGAGATCACAGATCAATTGGTTAATAACGT 1350
GGTGTTTAATATGTGAGAAATAGTTTTTGATGTTCAAGTTTCTAAGTATGCCAGTCTGAATGAGCCAGAATTCACAGGTACCTTTGAAGA 1440
TACCAAGAAAGATTCATTATCATGTAGAGTTAGAATGATCATTTGAAATCTAAGGGAAACTTTATAATCAGGTGGGGAAATTACTAAAAC 1530
AAAACTCGAATTGAAGAGATAAGAAAAAGGAGGTAAATATATTAGGAAACTTCATTGGATGTTACCTTAAAGCTACATAGAACTGGACTG 1620
ATGTGTTAGACATAGACACCTGGGAACATATGCAAGGCCAGGAAAAAAAAAGAGTCGTGACAATAGGATCGAAAGAATAAGTATAAAAATG 1710
CTATATTTATGATCCAGTCCTTTATTGAGAGTGAAAAGAGGGATTAAATGGAAAAGCACACTTCAATATTTAAGGGATTGTGTGTACATG 1800
TGTGTTAAATTAATT                                                                              1815
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Fig. 2 Nucleotide sequence of sDII and its primary structure.
Two in-frame TGA codons are designated as coding for selenocysteine (Se). The TAG stop codon is marked by an *asterisk*.

ment usually exits at the 3′ end of the mRNA and this part of sDII mRNA still remains to be determined, a further effort to clone the rest of the cDNA would reveal the presence of SECIS element in sDII mRNA.

DII has been suggested to be important in the maintenance of T3 not only in tissues but also in circulating plasma in shrews.[2] Thyroid hormones are known to play a crucial role for the maintenance of homeostasis such as energy expenditure and nutritional economy. Therefore, changes in DII expression would be important for adaptation mechanisms of shrews, when they are exposed to various environments and insults. The obtained sDII cDNA in this study would be a suitable probe to investigate the changes in the expression of DII in house musk shrew.

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Shrew  MGILSVHLLITLQILPVFFSNCLFLALYDSVILLKHVVLLLSRSKSTRGEWRRMLT
Human  ......D.....................................................
Mouse  ..L...D.....................................A...............
Rat    ..L...D.....................................A...............

Shrew  SEGMRCIWKSFLLDAYKQVKLGEDAPNSSVVHVSNSESD-SGRNGVPEKTIDGAEC
Human  ...L..V.....................................ST.GGDNSG..TQ..IAE..T.
Mouse  ...L..V.N...................................P..---.N.YAS...A.....
Rat    ...L..V.N...................................P.A---.N.CAS...A.....

Shrew  HLLDFASAERPLVVNFGSATSePPFTSQLPAFSKLVEEFSSVADFLLVYIDEAHPS
Human  .......P.....  .....R.....................
Mouse  ..............  .....R.....RQ..............
Rat    ..............  .....R.....RQ..............

Shrew  DGWAVPGDSSLSFEVKKHRSQEDRCAAALQLLERFSLPPQCQVVADRMDNNANVAY
Human  ....I.............QN........Q............R...........I..
Mouse  ..................N........H.....................................
Rat    ..........M........N........H.....................................

Shrew  GVAFERVCIVQRQKIAYLGGKGPFCYNLQEVRDWLEKNFSKRSeILT*
Human  ..........................S.......H.........  .KK.RLAG*
Mouse  ............R...........S.......S.........  ...D*
Rat    ............R...........S.......S.........  ...D*
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Fig. 3 Comparison of the DII amino acid sequences among the house musk shrew, human, mouse and rat. The stop codons are indicated by *asterisks*.

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