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Short sequence-paper

Characterization of the human *SDHD* gene encoding the small subunit of cytochrome *b* (cybS) in mitochondrial succinate–ubiquinone oxidoreductase

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Abstract

We have mapped large (cybL) and small (cybS) subunits of cytochrome *b* in the succinate–ubiquinone oxidoreductase (complex II) of human mitochondria to chromosome 1q21 and 11q23, respectively (H. Hirawake et al., Cytogenet. Cell Genet. 79 (1997) 132–138). In the present study, the human *SDHD* gene encoding cybS was cloned and characterized. The gene comprises four exons and three introns extending over 19 kb. Sequence analysis of the 5′ promoter region showed several motifs for the binding of transcription factors including nuclear respiratory factors NRF-1 and NRF-2 at positions −137 and −104, respectively. In addition to this gene, six pseudogenes of cybS were isolated and mapped on the chromosome.

Keywords: Mitochondria; Succinate dehydrogenase; Cytochrome *b*; Gene structure; *SDHD*; Nuclear respiratory factor (NRF); (Human)

Succinate–ubiquinone oxidoreductase (complex II) is an important enzyme complex in both the tricarboxylic acid cycle and the aerobic respiratory chains of eukaryotic cell mitochondria and prokaryotic cells. Complex II catalyzes the oxidation of succinate to fumarate (succinate dehydrogenase: SDH) and transfers its reducing equivalent to ubiquinone [1,2]. Complex II also catalyzes the reduction of fumarate (fumarate reductase: FRD), which is the reverse of the reaction catalyzed by SDH, in the respiratory chain of anaerobic bacteria and in the mitochondria of facultative anaerobic animals such as adult *Ascaris suum* [3,4]. Complex II is generally composed of four polypeptides with apparent molecular masses of 70, 30, 15, and 13 kDa, and contains five prosthetic groups, one covalently linked FAD, three iron–sulfur clusters (2Fe–2S, 4Fe–4S, and 3Fe–4S), and heme *b*. The larger two subunits, the flavoprotein subunit (Fp) and the iron–sulfur protein subunit (Ip), comprise the catalytic portion of the enzyme complex and catalyze electron transfer from succinate to artificial electron acceptors such as phenazine methosulfate (PMS). The amino acid sequence of Fp and Ip are highly conserved and cDNAs and genes have

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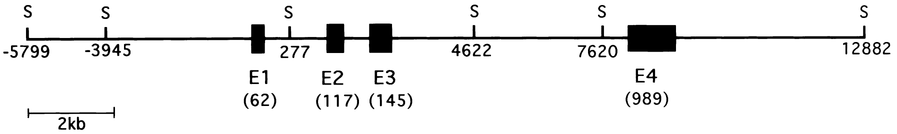

Fig. 1. Genomic structure of the human *SDHD* gene. The four exons of the gene (E1–4) are boxed. Restriction sites for *Sac*I are shown. Numbers indicate positions from the possible transcription start site (+1) determined by 5′-end analysis of the cDNA.

been cloned from various species using homology probing strategies (see for reviews [1,2]). The presence of a two-subunit cytochrome *b* composed of large (cybL, also referred to as QPs-1 or C_{II-3}) and small (cybS, also referred to as QPs-3 or C_{II-4}) subunits acting as hydrophobic membrane anchor peptides is a general feature of mitochondrial complex II [5,6]. In addition, the cytochrome *b* in complex II is essential for the interaction between the complex and quinone species. The ubiquinone-binding domains in cybL and cybS of bovine heart complex II have been suggested to be residues 113–140 and 20–37, respectively, by matching the sequences of azide–ubiquinone linked peptides to their respective protein sequences [7,8]. However, the function and structure of cytochrome *b* in complex II is poorly understood as compared with the well-characterized Fp and Ip because of a lack of sequence conservation at the nucleotide and/or amino acid sequence level.

To elucidate the molecular basis of a mitochondrial disease caused by a deficiency in the SDH activity of complex II ([9] and see [10] for review), we cloned the cDNAs for all four subunits of human liver complex II and found their unique features [11–13]. All the genes for human complex II are encoded on nuclear DNA, and the genes for Fp (*SDHA*) and Ip (*SDHB*) have been mapped on chromosomes 5 [14] and 1 [15]. Recently, we mapped the genes for cybL and cybS, *SDHC* and *SDHD*, to chromosomes 1q21 and 11q23, respectively [13]. Among these genes, the structures of *SDHB* and *SDHC* have been reported[15,16]. In this study, the entire nucleotide sequence of human *SDHD* was determined and characteristics of the promoter region were analyzed.

In the previous study of mapping the *SDHD* gene, we obtained, two independent genomic clones from a human EMBL-3 library (Clontech, Lot 1125), S-3 and S-36, and used both in the chromosome assignment by FISH [13]. We determined the complete nucleotide sequence of the DNA fragment from clone S-36, since restriction enzyme analysis and Southern hybridization using probes specific to the cDNA for liver cybS showed that the S-3 clone (11.7 kb) contains three exons from the 5′-terminal, and the S-36 clone (18.6 kb) contains the entire coding sequence of four exons, the entire 3′-untranslated region, and at least part of the 5′-untranslated region. Fig. 1 shows a schematic representation of the genomic structure of the human *SDHD* gene,

Table 1
Potential sites for transcription factors in 5′ upstream region of the *SDH D* gene

Transcription factor	Sequence	Position
CdxA	AATAATAATA	−5560, −4813, −4490, −2915, −1043
GATA-1	GCCAATCAGA	−1881
GATA-2	ACCTATCCCC	−1857
Lyf-1	TTTGGGAGG	−5512
Nkx-2	CACTTGA	−5662, −5354, −2830, −2800
NRF-1	TTGCGCATGCGCG	−137
NRF-2	GACTTCCGGT	−428, −104
Sox-5	TTAACAATAC	−4949
SRY	AAACAAA	−4597, −3995, −3916
USF	GCACGTGG	−1130, −596
YY1	GATAGTAAATGGTTT	−3263, −3227, −1857, −945

Consensus sequences as targets for transcription factors were analyzed by TFSEARCH (Y. Akiyama, Kyoto University).

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cactaccggt caccagggacaggagagccataactttgtctttcgtgaggggaatgggatgcagccgggatcgagcaccagtgag    -344
   NRF-2
ccgccagtgtacagacctccgagcgtgcccaggaccaccaaggaaggtgaaacttcctttcccttcaccctccccgtccccgcac    -259
ctgtgcagtaaactgcgccttctgctgctcggcggccaccaggcgctgcaactccgcttcatcggcttcgcccagctccgccatt    -174
gttcgcctcaggctcgccaccttccgacagctgtgtttgcgcatgcgcg acgggtgtgcaccgcctctcgacttccggt tcacc   -90
                                          NRF-1                             NRF-2
cagcatttcctcttccctgttttctttcgtcgtcgtgggtgggaattgtcgcctaagtggttccgggttggtggatgaccttgagc   -3
cctCAGGAACGAGATGGCGGTTCTCTGGAGGCTGAGTGCCGTTTGCGGTGCCCTAGGAGGCCGAGgt gaggggtcttcccaccct   +82
  +1          M  A  V  L  W  R  L  S  A  V  C  G  A  L  G  G  R  A
gaggtgcttagcgtagcctccagccagggaaggggatggaagtgaggactcatctgccgggtgggagatctcttgaggagaagaa    +167
aataccgaaatcacagcaatgaccactgtagtctaggggtccagatgtttacccgaaggtatatttcacttgctgtgagctgacg    +252
agttgagggaataatcagaaagagagctccctctggaagtcgcagtcctgatgaggctaatccacatagcagttctgttttctcc    +337
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aagatgttatcccctatttattgttaagtagcttacctatggtcatttagaaagtttgtcagtcctgttaaaggagaggttctta    +932
tgatcatcctaatgactctttcctcag CTCTGTTGCTTCGAACTCCAGTGGTCAGACCTGCTCATATCTCAGCATTTCTTCAGGA   +1017
                               L  L  L  R  T  P  V  V  R  P  A  H  I  S  A  F  L  Q  D
CCGACCTATCCCAGAATGGTGTGGAGTGCAGCACATACACTTGTCACCGAGCCACCATTgt atgttctctccatcgctgctgctt  +1102
  R  P  I  P  E  W  C  G  V  Q  H  I  H  L  S  P  S  H  H  S
tctgggctctagccatctttaccttcactaatggtcatgcctttagcaggacttcctacctgtagggggggactcttgtgtccaac   +1187
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tctagcctaagtctttacaaatttttttcttttgtttcttttttttttgagacagagtctcgctctgttatgcaggctggagtgc    +1357
agtggtgcgatctcggctcactgcaacctccaccttctgagttcaagcaattctcctgcccctgcctcccgactagctgggatta    +1442
caggcgcccaccaccatgcccagctaagttttgtactttagtagagacgggtttcaccgtgttggccgggctggtctcaaactc    +1527
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tatgtacactgcctgtcagtttgggttactgtgtggcatatgttgaacatgaaagatgtgtgtttctcacatcaacttttatgaa    +1952
tctggtccttttgtag CTGGCTCCAAGGCTGCATCTCTCCACTGGACTAGCGAGAGGGTTGTCAGTGTTTTGCTCCTGGGTCTG   +2037
                    G  S  K  A  A  S  L  H  W  T  S  E  R  V  V  S  V  L  L  L  G  L
CTTCCGGCTGCTTATTTGAATCCTTGCTCTGCGATGGACTATTCCCTGGCTGCAGCCCTCACTCTTCATGGTCACTGgc aagtat  +2122
 L  P  A  A  Y  L  N  P  C  S  A  M  D  Y  S  L  A  A  A  L  T  L  H  G  H  W
agcaattcca ...intron 3...atttttctttttctttag GGGCCTTGGACAAGTTGTTACTGACTATGTTCATGGGGATG   +7952
                                             G  L  G  Q  V  V  T  D  Y  V  H  G  D  A
CCTTGCAGAAAGCTGCCAAGGCAGGGCTTTTGGCACTTTCAGCTTTAACCTTTGCTGGGCTTTGCTATTTCAACTATCACGATGT  +8037
   L  Q  K  A  A  K  A  G  L  L  A  L  S  A  L  T  F  A  G  L  C  Y  F  N  Y  H  D  V
GGGCATCTGCAAAGCTGTTGCCATGCTGTGGAAGCTCTGACCTTTTTGACTTCATACTTTGAAGAATTGATGTATGCCTCTTTGC  +8122
  G  I  C  K  A  V  A  M  L  W  K  L  ***      ***
CTCTGCTTTGTCATGCCATTAAGCTCACAATAAGGAAGAAATAACAGATAAGTCCATTGGTGGACAGCCTTCTTCTCTTAATCAC  +8207
AAGATTATTTTCAGAATTTAATCTTTGAGGAAAAGGTTTGAGAGGAATTATATCTAAGTTGTGAGACTGAGTTCTATATTCTGGT  +8292
GAGTTAATGGGGTTGCCTCCCAGCTTCTTATAAGACTCACAGTATAACTAAACATGATATATCAGCTTTTGCCTTTCAATTTATC  +8377
AATCTCTTAAAGAGAATCCAACTTTATTACGATTAGTATATGATCAAACTTCCATATTTGCCTTGGGAATAATGGACAAAGGGAA  +8462
ATACTCTTAATTCATGAATAAAAACTTTGCAGAAAATTAGACAGTGTTTAATTTTCGAAAACTTCCCTCTCTAGACAGTAGATAC  +8547
CACCTACTGATGGTTACATATACTAGGGAAATTTTAAAATTAGGAAATGCTGATAGCTCATATTATAAATTTCTAAATCCTAGGA  +8632
AGAAACGCTTGGAGTGCTTCTGAATATACAGAAGTTCCATTTAAGGGCAAGTTTCCCCGTAGATGTATCAAAATACTACCAACTG  +8717
TAAATTGAGATTTAATTCCCAAATGTATTCTACTTGTTCTAAAACAATCTGTCCACAAATATAAAACTATAAGTAATAAATTGTT  +8802
ATTTTCGCACAATGGGAATCTCTAATGTGAAAATGTATTCTATGAAAATAATTTTTTTAAATAAAATGTTATATAATAAA AGTG   +8886
TCTTCTATGCTTTT                                                                           +8900
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Fig. 2. Nucleotide sequence of the human *SDHD* gene. Uppercase letters indicate exon sequences and lowercase letters indicate promoter and intron sequences. Amino acid sequences of the coding region are also shown. Splice donor and acceptor sites are underlined for each intron. Consensus sequences as targets for transcription factors were analyzed by TFSEARCH (Y. Akiyama, Kyoto University). Potential nuclear respiratory factor binding sites, NRF-1 and NRF-2, are also underlined. This sequence is available in the DDBJ, EMBL, and NCBI databases under accession number AB026906.

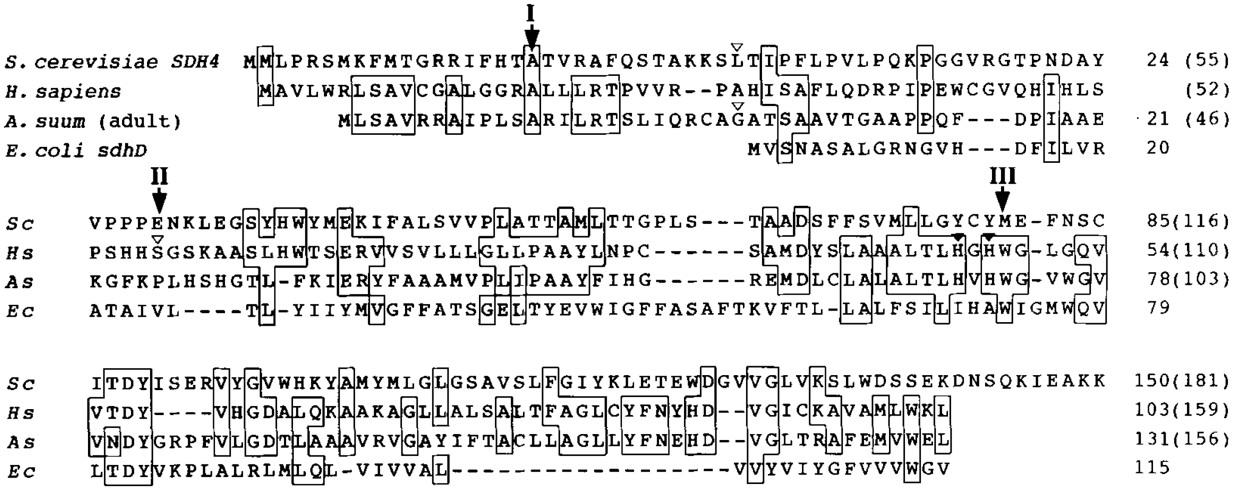

Fig. 3. Localization of introns in the human *SDHD* gene. Sites are indicated by arrows. ▼ indicates conserved histidine residues. ▽ indicates the amino termini of mature peptides. Amino acids identical to those in human cybS are boxed. *S. cerevisiae SDH4* [26]; *Homo sapiens* [13]; *A. suum* [27]; *Escherichia coli sdhD* [28].

and the sequence, including the entire coding region, is shown in Fig. 2. The nucleotide sequence of the human *SDHD* gene appears in the DDBJ, EMBL, and Gen Bank nucleotide sequence databases with the accession number AB026906. All exons are quite small, 52, 117, 145, and 163 nucleotides in exons 1, 2, 3, and 4, respectively, and the nucleotide sequence of the coding regions is completely identical to that of the liver cDNA, which we reported previously [13]. Donor and acceptor sites for RNA splicing were confirmed except 'gc' in the third intron. The localization of introns in the amino acid sequence of liver cybS is shown in Fig. 3. The first intron was found in the mitochondrial presequence that is essential for the import of mitochondrial proteins encoded by nuclear DNA [17]. The second intron is located near the cleavage site of the mitochondria presequence. This site corresponds to the putative amino-terminal of bovine heart cybS (Ser–Asp–Ser–Lys–Ala–Ala–Ser) determined from the peptide [18]. The third intron divides the region containing two conserved histidine residues in the cybS peptide of mitochondria from multicellular organisms. These two histidine residues are found in the putative second transmembrane segment and appear to be axial ligands of heme *b* in complex II [19–21].

An analysis of the approximately 6 kb upstream of exon 1 was carried out to identify potential consensus sequences as targets for transcription factors. The *SDHD* gene is typical of mammalian housekeeping genes with TATA-less promoters. Interestingly, there are two distinct potential nuclear respiratory factor binding sites, NRF-1 and NRF-2 [22], at positions −137 and −104, respectively (Figs. 2 and 4). These sites have been found upstream of *SDHB* [23] and *SDHC* [16], indicating that two factors and sites are required for the coordinated control of gene expression for the stoichiometric assembly of a functional complex II. In addition to these sites, several potential sites for other transcription factors were found (Table 1). Many experiments, including gel shift assay, will be necessary to determine the requirements for these factors and their binding sites in the expression of *SDHD*.

Genetic heterogeneity and the presence of tissue specific isoforms of human complex II have been suggested from the study of the variable clinical expression of complex II deficiency including tissue-to-tissue variation [24]. Recently, we found two different stage-specific isoforms of complex II containing distinct cytochrome *b*s in *A. suum* mitochondria [25]. In this regard, it is of interest to note the high positive background obtained during the screening of the

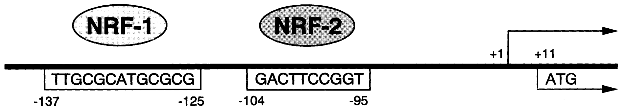

Fig. 4. Schematic representation of the putative promoter region of human *SDHD*.

Table 2
Chromosomal localization of pseudogenes for human *SDHD*

Clone	Localization	Mutation	Comment
#6	1p36.1	No	poly A, 6 amino acid substitutions
#8	2q32.1	Stop, deletion	
#11	3p21.3	Stop, frame shift	
#12	3q26.3	Deletion, frame shift	
#10	7q32.1	Stop	
#5	18q11.2	Stop, frame shift	

No introns were found in these pseudogenes.

SDHD gene using a cDNA probe for cybS. Eleven clones in addition to S-3 and S-36 were isolated, sequenced, and mapped. These clones were divided into six groups and appear to be for pseudogenes of *SDHD* because they contain stop codons, deletions, and frame shifts in their sequences (Table 2). One exception is clone S-1, which was mapped to chromosome 1p36 by FISH. This clone has an open reading frame almost identical to the liver cDNA with only six amino acid substitutions, L4P, L14Q, L77P, M91T, L120S, and L131F. Since it would be of interest to prove the presence of two or more active genes of cybS, the expression of the clone S-1 gene and screening of another candidate for a second gene are now in progress.

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