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Structure, sequence and location of the *UQCRFS1* gene for the human Rieske Fe-S protein

(Recombinant DNA; nuclear gene; respiratory chain; electron transport; mitochondrial protein; myopathy; nucleotide sequencing; chromosome 19q12; *Homo sapiens*)

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SUMMARY

We have identified and studied the chromosomal location of the human Rieske Fe-S protein-encoding gene *UQCRFS1*. Mapping by hybridization to a panel of monochromosomal hybrid cell lines indicated that a *UQCRFS1* partial cDNA was derived from either chromosome 19 or 22. By screening a human chromosome 19 specific genomic cosmid library with a probe from this cDNA sequence, we identified a corresponding cosmid. Portions of this cosmid were sequenced directly. The exon, exon:intron junction and flanking sequences verified that this cosmid contains the genomic locus. Fluorescent in situ hybridization (FISH) was performed to localize this cosmid to chromosome band 19q12.

INTRODUCTION

Iron-sulfur (Fe-S) proteins have long been known to be key enzymes in the electron transport pathway. The ubiquinol cytochrome-*c* reductase UQCRFS1 (as it is officially designated), otherwise known as the Rieske Fe-S protein, is a key subunit of the cytochrome bc_1 complex in the mitochondria (Rieske, 1967; 1976; Trumpower and Edwards, 1979). The Rieske Fe-S protein is important in redox reactions in these organelles as it is involved in electron transport and photosynthesis (Rieske, 1976). Although this protein is used primarily in chloroplasts and mitochondria, the Rieske gene itself is located in the nuclear genome of eukaryotes (Glick et al., 1992; Hartl and Neupert, 1990). After being transcribed, this protein's message is translated on cytoplasmic ribosomes and transported through the mt membrane to the mt cytochrome bc_1 complex (Glick et al., 1992; Hartl et al., 1990). Brandt et al. (1993) have proposed that after a single post-translational modification this protein inserts into the cytochrome bc_1 complex.

The mt myopathies are a group of disorders characterized by electron transport deficiencies within the mitochondria (DiMauro et al., 1989). Recently, others have suggested that the Rieske Fe-S protein may be implicated in mt myopathies (Slipetz et al., 1991). Schapira et al. (1990) suggest that a defect limiting the protein's ability to transverse the mt membrane may underlie at least one particular myopathy. These mt myopathy patients, in

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Abbreviations: aa, amino acid(s); bp, base pair(s); cDNA, DNA complementary to mRNA; COX, cytochrome-*c* oxidase; COXVIIa, COX subunit VIIa; COXVIb, COX subunit VIb; DAPI, 4,6-diamidino-2-phenylindole; Fe-S, iron-sulfur; FISH, fluorescent in situ hybridization; kb, kilobase(s) or 1000 bp; LLNL, Lawrence Livermore National Laboratory; mt, mitochondrial; nt, nucleotide(s); PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; SSC, 0.15 M NaCl/0.015 M Na_3· citrate pH 7.6; UQCRCI, ubiquinol cytochrome-*c* reductase subunit I; UQCRCII, ubiquinol cytochrome-*c* reductase subunit II; UQCRFS1, ubiquinol cytochrome-*c* reductase Rieske Fe-S protein; *UQCRFS1* (Rieske locus), gene encoding UQCRFS1.

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which the disease is inherited as an autosomal recessive, may have mutant Fe-S protein leader sequences preventing entry into the mitochondria. Others have hypothesized a possible iron uptake deficiency within these patients (Hall et al., 1993). In 1988, Yanamura et al. found tissue-specific differences between heart and liver cytochrome-*c* oxidase, providing the first model for isoenzyme forms of the respiratory chain complexes. Furthermore, they refered to numerous other mt myopathies where defects are present in only one to a few tissues. In order to provide data to complement genetic linkage studies, as well as to provide a genomic source for mutation scanning within patients with various mt myopathies, we have isolated, mapped and characterized the genomic *UQCRFS1* locus.

EXPERIMENTAL AND DISCUSSION

(a) Chromosomal assignment of the Rieske Fe-S protein-encoding gene (UQCRFS1)

As part of a sequencing project focusing on cDNA 3′ termini (Okubo et al., 1992), a partial cDNA clone, known as *hm02g01*, was mapped by Southern hybridization to DNA prepared from human monochromosomal mouse somatic cells (Fukushima et al., 1994) to chromosome 19 and 22 (Fig. 1). After a database search revealed that the 150 bp sequenced from the *hm02g01* cDNA clone were homologous to bovine and rodent *UQCRFS1*, we became interested in the nature of the chromosome 19 Rieske-homologous locus. Later, we discovered that the complete human Rieske cDNA had been sequenced, though it was not within the GenBank database (Nishikimi et al., 1990). Our initial question focused on determining whether the chromosome 19 locus represented a functional gene or a pseudogene, as many of the other nuclear-encoded respiratory chain genes have been found to have pseudogene copies dispersed on various chromosomes (Vanin, 1985; Seelan and Padmanaban, 1988; Lomax et al., 1990).

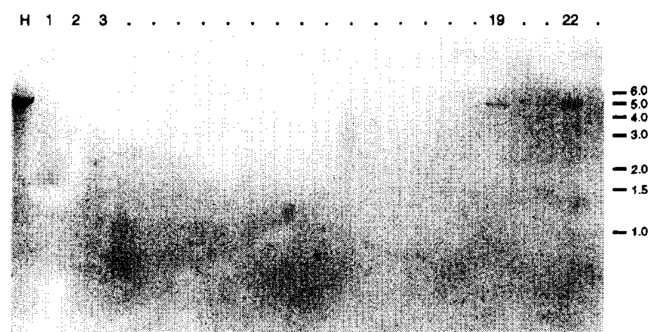

Fig. 1. Chromosomal assignment of *UQCRFS1* by Southern hybridization using an *Eco*RI-digested hybrid cell mapping panel. Lane H consists of total human genomic DNA. Numbers located on the top of each lane correspond to chromosome number and numbers to the right indicate DNA size in kb. The positive *Eco*RI fragments are approx. 4.8 kb. Human monochromosomal somatic cell Southern hybridizations were done as described previously (Fukushima et al., 1994).

(b) Chromosome 19 cosmid library screening and subcloning

To characterize the chromosome 19 Rieske locus, a probe made from PCR amplification of *hm02g01* was hybridized to our LLNL chromosome-19-specific arrayed cosmid library. Two clones were identified as hybridization positives: *f22979* and *f17252*. Cosmid clone *f17252* was verified by PCR to amplify a band of the expected size based on the *hm02g01* sequence. By Southern hybridization to an *Eco*RI digestion of this cosmid, a 4.8-kb fragment was identified that was *hm02g01* positive and, overlapping it, a 1.3-kb *Pst*I fragment. The 4.8-kb *Eco*RI cosmid fragment was not successfully subcloned, but an overlapping 1.3-kb *Eco*RI-*Pst*I fragment was subcloned.

(c) Sequence analysis

The 1.3-kb subcloned fragment yielded a sequence of approx. one-third of this gene's coding region and continued through the 3′ end of the gene. All other sequence was obtained by the direct sequencing of cosmid *f17252* (Fig. 2).

The isolation of a chromosome 19 cosmid containing the Rieske locus allowed us to determine that the chromosome 19 locus is likely to be the one from which the Rieske mRNA is transcribed, and is unlikely to be a pseudogene. Four types of data support this conclusion. First, the exonic genomic sequence is identical to that of the cDNA. Second, genomic sequence 5′ to the cDNA sequence has characteristics of a promoter (G+C-rich, with potential transcription-factor-binding sites as indicated in Fig. 2) and lacks repeated elements. It does not have an obvious TATAA or CCAAT box, and therefore resembles promoters observed from other housekeeping genes (Dynan, 1986). This gene has a GC box (GGGCGG) identical to that on other genes where the Sp1 transcription factor is known to bind (Pugh and Tjian, 1990). In many genes that lack TATA or CAAAT promoters the binding of Sp1 has been shown to be essential for the intiation of transcription (Pugh and Tjian, 1990). Third, an intron was found within the chromosome 19 genomic Rieske sequence. At the exon:intron boundaries, acceptor and donor intron consensus sequences are present. This intron may therefore indicate that the Rieske protein is composed of two domains. The sequence (Fig. 2), derived from the coding region, matched that of the published cDNA sequence, with the exception of a single intron interrupting the 72nd codon

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                 GC-Box
CCTCTCCAGGTTTGCCCCGCCCTGCAGGACTGCAGAATTTCCTTTCCGGCGGCGCGCTGT   60
CGTCACGCGTCCCGCGCCTCCTCGCGCCTGCACCGTGGTTGGAAGGTCGTCCCCTGTGAC  120

     G----------------->
CGGTTGGGCGGTTGGAGCGGCTGTCGCCATGTTGTCGGTAGCAGCCCGCTCGGGCCCGTT  180
                             M  L  S  V  A  A  R  S  G  P  F   11

                        <----------------------H
CGCGCCCGTCCTGTCGGCCACGTCCCGCGGGGTGGCGGGCGCGCTGCGGCCCTTGGTGCA  240
  A  P  V  L  S  A  T  S  R  G  V  A  G  A  L  R  P  L  V  Q   31
GGCCACGGTGCCCGCCACCCCGGAGCAGCCTGTGTTGGACCTGAAGCGGCCCTTCCTCAG  300
  A  T  V  P  A  T  P  E  Q  P  V  L  D  L  K  R  P  F  L  S   51

          A------------------->
CCGGGAGTCGCTGAGCGGCCAGGCCGTGCGCCGGCCTTTGGTCGCCTCCGTGGGCCTCAA  360
 R  E  S  L  S  G  Q  A  V  R  R  P  L  V  A  S  V  G   L N
TG[gtgagccggggcgagggccg..().gtgtttttaaccttctgtttgtcttgcag]
TCCCTGCTTCTGTTTGTTATTCCCACACAGACATCAAGGTGCCTGACTTCTCTGAATACC  422
V  P  A  S  V  C  Y  S  H  T  D  I  K  V  P  D  F  S  E  Y     71

<---------------B
GCCGCCTTGAAGTTTTAGATAGTACGAAGTCTTCAAGAGAAAGCAGCGAGGCTAGGAAAG  482
R  R  L  E  V  L  D  S  T  K  S  S  R  E  S  S  E  A  R  K     91
GTTTCTCCTATTTGGTAACTGGAGTAACTACTGTGGGTGTCGCATATGCTGCCAAGAATG  542
G  F  S  Y  L  V  T  G  V  T  T  V  G  V  A  Y  A  A  K  N    111
CCGTCACCCAGTTCGTTTCCAGCATGAGTGCTTCTGCTGATGTGTTGGCCCTGGCGAAAA  602
A  V  T  Q  F  V  S  S  M  S  A  S  A  D  V  L  A  L  A  K    131
TCGAAATCAAGTTATCCGATATTCCAGAAGGCAAGAACATGGCTTTCAAATGGAGAGGCA  662
I  E  I  K  L  S  D  I  P  E  G  K  N  M  A  F  K  W  R  G    151
AACCCCTGTTTGTGCGTCATAGAACCCAGAAGGAAATTGAGCAGGAAGCTGCAGTTGAAT  722
K  P  L  F  V  R  H  R  T  Q  K  E  I  E  Q  E  A  A  V  E    171

                                      <--------------------E
TATCACAGTTGAGGGACCCACAGCATGATCTAGATCGAGTAAAGAAACCTGAATGGGTTA  782
L  S  Q  L  R  D  P  Q  H  D  L  D  R  V  K  K  P  E  W  V    191
TCCTGATAGGTGTTTGCACTCATCTTGGCTGTGTACCCATTGCAAATGCAGGAGATTTTG  842
I  L  I  G  V  C  T  H  L  G  C  V  P  I  A  N  A  G  D  F    211
GTGGTTATTACTGCCCTTGCCATGGGTCACACTATGATGCATCTGGCAGGATCAGATTGG  902
G  G  Y  Y  C  P  C  H  G  S  H  Y  D  A  S  G  R  I  R  L    231

                                C---------------------->
GTCCTGCTCCTCTCAACCTTGAAGTCCCCACGTATGAGTTCACCAGTGACGATATGGTGA  962
G  P  A  P  L  N  L  E  V  P  T  Y  E  F  T  S  D  D  M  V    251
TTGTTGGTTAAGAGACTTGGACTCAAGTCATAGGCTTCTTTCAGTCTTTATGTCACCTCA 1022
I  V  G  *                                                    254

<---------------------D
GGAGACTTATTTGAGAGGAAGCCTTCTGTACTTGAAGTTGATTTGAAATATGTAAGAATT 1082
GATGATGTATTTGCAAACATTAATGTGAAATAAATTGAATTTAATGTTGAATACTTTCAG 1142
GCATTCACTTAATAAAGACACTGTTAAGCACTGTTATGCTCAGTCATACACGCGAAAGGT 1202
ACAATGTCTTTTAGCTAATTCTAATTAAAAATTACAGACTGGTGTACAAGATACTTGTGA 1262
AATCTGTAACTGACTTTATTTTCTTGCCCTATATTTGGTTCCCGCTTTGCGTCAGGGACG 1322
CAGGTTCTGCACGGGCATTGGTGGGATGAAGTCAAATTAACGGG                 1366
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Fig. 2. Human chromosome 19 genomic sequence for the Rieske gene. GenBank accession Nos. *L32917* and *L32977*. The nt are numbered based on exon sequence only. Underlined regions indicate transcribed but untranslated regions. Lowercase sequence notes the intron of unknown size. Bold letters donote the invariant nt of acceptor and donor splice site sequences. A Sp1-binding site, the GC Box, is located at nt 16–21. The partial cDNA fragment from *hm02g01* corresponds to nt 912 through 925 of *L32977*. Based on the Rieske cDNA sequence (Nishikimi et al., 1990), the following sequencing primers were synthesized by the phosphoramidite method on an ABI DNA Synthesizer. A-(F4) 5′-CTGAGCGGCCAGGCCGTGCGCC, B-(R2) 5′-GGAATAACAAACAGAAGCAGGG, C-(hm02g01F1) 5′-ACGTATGAGTTCACCAGTGACG, D-(hm02g01R1) 5′-GGCTTCCACTCAAATAAGTCTCC, E-(RmidR2) 5′-CCCATTCAGGTTTCTTTACTCG, F-(R4) 5′-CTGAGCGGCCAGGCCGTGCGCC, G-(RnonCF1) 5′-CGGTTGGAGCGGCTGTCG and H-(R8) 5′-CAGCGCGCCCGCCACCCCGCGGGT. Primer orientation and location can be seen by arrows and dotted lines. PCR was performed on flow-sorted human chromosome 19 DNA with primers hm02g01F1 and hm02g01R1. The cycling conditions were as follows: 94°C 10 s, 55°C 30 s, 72°C 20 s (Perkin Elmer 9600). The product was separated on a 1.5% agarose gel, and visualized by ethidium bromide staining. The expected resulting band of 105 bp was excised and eluted by placing at 55°C in 100 μl of TE (10 mM Tris·HCl/1 mM EDTA/pH 7.4). This DNA was then labeled by asymmetric PCR using primer hm02g01R1 and [α-^{32}P]dCTP. This probe was used to screen a chromosome-19-specific cosmid library. Filters were washed at low stringency (2 × SSC/1% SDS) twice for 30 min at 65°C. Next, filters were washed twice in a high-stringency wash (0.2 × SSC/1% SDS). Filters were visualized using a Molecular Dynamics PhosphorImager. A clone positive for the 105-bp PCR probe was digested with *Eco*RI, analyzed by electrophoresis on a 0.8% agarose gel, Southern blotted and hybridized to the same assymetrical PCR probe as used above. The 4.8-kb fragment was further digested with *Pst*I, analyzed on a 1% agarose gel, Southern blotted and rehybridized with the same asymetrical PCR probe. The 1.3-kb fragment identified by the Southern blot was cloned between the *Eco*RI and *Pst*I site of pKS$^-$. Positive clones were identified by Grunstein-Hogness colony screening methods as described in Sambrook (1989). This clone was sequenced using a fluorescent dye terminator kit (SequiTherm™), 25 cyles of PCR and analyzed on an ABI Automated Sequencer. Other regions of this gene were sequenced by direct Dye Terminator sequencing of 2.5 μg of *Eco*RI-cut cosmid.

of the aa coding region (as numbered in Fig. 2). Therefore this gene is made up of two exons and an intron. Although approx. 700 bp of single pass sequence were obtained, this intron was not fully sequenced and attempts to PCR across it were not successful. Sequence was also obtained from regions flanking the published cDNA sequence (Fig. 2). Fourth, genomic sequence found 3′ to the site of polyadenylation did not consist of poly(A) or other identifiable repeated elements, and therefore appears to represent unique sequence.

(d) Fluorescent in situ hybridization (FISH)

In order to fine-map cosmid *f17252* on chromosome 19, FISH was performed using the cosmid as a probe. Hybridizations observed from ten metaphase nuclei were scored. All 20 chromatids were localized to band 19q12 (Fig. 3), and no grains were observed over other chromosomes (including in particular chromosome 22).

(e) Conclusions

Although the nature of the chromosome 22 Rieske-homologous locus remains unknown, it may represent a pseudogene of the gene on chromosome 19. We can not rule out the possibility that the DNA prepared from the chromosome 22 somatic cell hybrid was contaminated with chromosome 19 DNA, although this seems unlikely based on the strength of the hybridization signal (Fig. 1). Recently, Duncan et al. (1994) published data complementing our initial somatic cell hybrid results, showing Rieske homologous cDNAs hybridize to chromosomes 19 and 22. Indeed, hybridization signals derived from the use of cDNA probes may be stronger to highly conserved processed pseudogene loci than to the intron-containing, and thus interrupted, transcribed loci. However, with the original probe representing an uninterrupted 200 bp from the 3′ end of the message this explanation would seem unlikely. It is also unlikely that a duplication involving a large segment of DNA has occured, since in the FISH experiment, using the entire cosmid, signal was observed only on cytogenetic band 19q12 and not on chromosome 22.

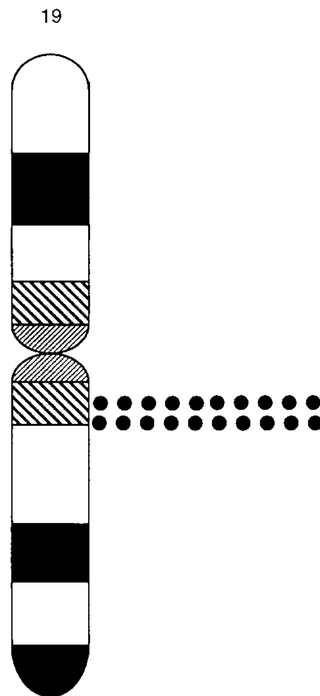

Fig. 3. The cosmid probe was hybridized to metaphase chromosomes. Idiograms of G-banded chromosome 19 showing the location of cosmid clone *f17252*. Bands are parts of a chromosome distinguishable from adjacent segments by appearing darker or lighter when treated with one or more banding techniques (Harnden et al., 1985). Fluorescent signals were seen on both chromatids for 20 metaphase chromosome spreads of peripheral blood lymphocytes of a normal donor. Fluorescent bands were produced by incubation of the slides before analysis in DAPI followed by actinomycin. No hybridization signals were seen on other chromosomes. The slide preparation and hybridization conditions used in the procedure were as described previously (Trask, 1990).

While this localization confirms that the gene is encoded within the nuclear genome, making it the third gene (after *COXVIb* and *COXVII7a*) involved in a mt respiratory chain complex to be mapped to chromosome 19, it does not appear that either of these or other similiar genes are clustered in one region of the genome. The two chromosome 19 *COX* genes map to different locations (*COXVIb* to 19q13.1 (Taanman et al., 1991); *COXVIIa* to 19p13.1 (Arnaudo et al., 1992; Hoffman et al., 1993; and personal communication)), and the two other known ubiquinol cytochrome-*c* reductases (*UQCRCI* and *UQCRCII* map to chromosomes 3 and 16, respectively (Hoffman et al., 1993; Duncan et al., 1993).

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