The Human Uncoupling Protein-3 Gene

GENOMIC STRUCTURE, CHROMOSOMAL LOCALIZATION, AND GENETIC BASIS FOR SHORT AND LONG FORM TRANSCRIPTS

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Gemma Solanes, Antonio Vidal-Puig, Danica Gruijc, Jeffrey S. Flier, and Bradford B. Lowell

From the Department of Medicine, Division of Endocrinology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02215

Uncoupling protein-3 (UCP3) is a recently identified candidate mediator of adaptive thermogenesis in humans. Unlike UCP1 and UCP2, UCP3 is expressed preferentially and at high levels in human skeletal muscle and exists as short and long form transcripts, UCP3_S and UCP3_L. UCP3_S is predicted to encode a protein which lacks the last 37 C-terminal residues of UCP3_L. In the present study, we have defined the intron-exon structure for the human UCP3 gene and determined that UCP3_S is generated when a cleavage and polyadenylation signal (AATAAA) located in the last intron prematurely terminates message elongation. In addition we have mapped UCP3 to the distal segment of human chromosome 11q13 (between framework markers D11S916 and D11S911), adjacent to UCP2. Of note, UCP2 and UCP3 in both mice and humans colocalize in P1 and BAC genomic clones indicating that these two UCPs are located within 75–150 kilobases of each other and most likely resulted from a gene duplication event. Previous studies have noted that mouse UCP2 maps to a region of chromosome 7 which is coincident with three independently mapped quantitative trait loci for obesity. Our study shows that UCP3 is also coincident with these quantitative trait loci raising the possibility that abnormalities in UCP3 are responsible for obesity in these models.

The control of body weight involves a regulated balance between energy intake and expenditure. Energy expenditure can be divided into three components (1): resting metabolic rate, physical activity, and adaptive thermogenesis, the latter being defined as the component of energy expenditure that changes in response to environmental stimuli such as cold exposure or chronic dietary excess. In rodents, an important site of adaptive thermogenesis is brown adipose tissue (reviewed in Ref. 2) where uncoupling protein-1 (UCP1), expressed exclusively in brown adipocytes (3, 4), promotes proton transport across the mitochondrial inner membrane. UCP1 decreases the proton electrochemical potential gradient, uncoupling fuel oxidation from ADP availability (reviewed in Refs. 5 and 6). Activation of UCP1, therefore, causes increased consumption of calories and generation of heat. UCP1-mediated effects on energy expenditure are regulated by changes in the level of sympathetic nervous system activity in brown fat. Cold exposure and overfeeding cause increased sympathetic stimulation of brown fat, simulating UCP1-mediated uncoupling and energy expenditure. The importance of this is demonstrated by the fact that mice lacking UCP1 are cold-intolerant (7). UCP1 is also regulated by purine di- and trinucleotides (ATP, ADP, GTP, and GDP) and free fatty acids, which inhibit and stimulate uncoupling activity, respectively (reviewed in Refs. 5 and 6).

UCP1 may be of lesser importance in humans in whom the mass of brown adipose tissue is limited. Instead, skeletal muscle is thought to be a major site of adaptive thermogenesis (8–12). UCP2 (13–15) is a recently described UCP1 homologue which, unlike UCP1, is expressed in most tissues. Because of its wide tissue distribution, UCP2 could have important effects on metabolic rate in humans. However, as UCP2 is expressed at high levels in many sites not thought to mediate adaptive thermogenesis, such as spleen, lymph node, thymus, and gastrointestinal tract (13–16), its role in mediating regulated energy expenditure is unclear.

UCP3 is a third member of the uncoupling protein family (15, 16). It was identified by Boss et al. (15) using a homology-based screening method and by the present authors (16) as an expressed sequence tag (EST) deposited into the Washington University, St. Louis-Merck & Co. EST data base. UCP3 is distinguished from other UCPs by its relatively selective, high level expression in skeletal muscle (15, 16) and the existence of two RNA transcripts (15), UCP3_S and UCP3_L, which are predicted to encode long (312 amino acids) and short (275 amino acids) UCP3 proteins, differing only by the presence or absence of C-terminal 37 residues. This difference could be significant because the region in question is homologous to a domain in UCP1 thought to mediate inhibition of uncoupling activity by purine nucleotides (17, 18). The abundant and relatively selective expression of UCP3 in skeletal muscle suggests that it may be a mediator of adaptive thermogenesis in humans. Here we define the intron-exon structure of the human UCP3 gene, establish its chromosomal localization at 11q13, within 75–150 kb of UCP2, and define the genetic basis for the two UCP3_S and UCP3_L mRNA transcripts.

EXPERIMENTAL PROCEDURES

Intron-Exon Structure—Six sense and antisense PCR primer pairs corresponding to cDNA sequence were used to amplify genomic fragments from human genomic DNA (see Table I). The genomic PCR fragments were subcloned using the TA cloning system (pCR2.1 plasmid, Invitrogen, Carlsbad, CA) and were subjected to restriction fragment length analysis (RFLA) with EcoRI and HindIII. Two cDNA fragments containing the 5′ and 3′ ends of UCP3 were obtained by RT-PCR (see Table I). Of note, UCP3 differs from UCP1 and UCP2 in being a non-expressed sequence tag (EST) deposited into the Washington University, St. Louis-Merck & Co. EST data base.

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enzyme digestion plus agarose gel electrophoresis and dyeoxy sequencing using M13, T7, and internal UCP3 gene-specific primers. 3′ RACE (rapid amplification of cDNA ends) was used to clone the 3′ ends of UCP3s and UCP3l. 3′ RACE was performed using the Marathon cDNA Amplification Kit, human skeletal muscle Marathon-Ready cDNA (both from CLONTECH) and a sense UCP3 primer (TACGCCCCCTGAGACTCTA) located in exon 6 (cDNA position relative to ATG = +761 to +778).

Analysis of UCP3s and UCP3l mRNA Transcripts by RNase Protection Assay—RNase protection assays were performed as described previously (19) using two in vitro transcribed 32P-labeled RNA antisense probes, one corresponding to UCP3s, spanning exons 6 and 7 ( +631 to +925 relative to ATG), and the other corresponding to UCP3l, spanning exons 6 and the immediately adjacent UCP3l 3′ UTR ( +623 to +900 relative to ATG).

UCP3 Chromosomal Localization—The Genebridge 4 Radiation Hybrid Panel (20–22) was screened for the presence of hUCP3 (Research Genetics, Inc., Huntsville, AL) using the following PCR primer pair: sense = GCCAGAGAAATACAGCCGGACTA (exon 4, cDNA position relative to ATG = +464 to +487) and antisense = GCCAAGGCGGCTG-GTAAAAATGAACTG (intron 4, 192 to 169 bp downstream of the exon 4 splice donor). These primers amplified a 269-bp band from human genomic DNA and failed to amplify any signal from control, hamster genomic DNA.

Analysis of P1 and BAC Human and Mouse Genomic Clones for Colocalization of UCP2 and UCP3—P1 (human and mouse 129/Ola) and BAC (mouse 129/SvJ) genomic libraries were screened (Genome Systems, St. Louis, MO) using gene-specific primers shown in Table II (P1 libraries) or a 32P-labeled mUCP3 cDNA clone (BAC library). P1 and BAC DNA was isolated and analyzed for the presence of UCP2 and UCP3 using PCR (specific primer sets shown in Table II).

RESULTS

Intron-Exon Structure—As shown in Fig. 1, the human UCP3 coding sequence was found to be distributed over six exons (exons 2–7) spanning 5.25 kb of genomic DNA. To obtain 5′ upstream cDNA sequence of human UCP3, 5′ RACE on human skeletal muscle Marathon cDNA was performed (16). Different clones were obtained and sequenced, and the longest ones were found to contain 183 bp 5′ upstream of the ATG. Thus, at least one exon containing UCP3 5′-untranslated sequence was detected (exon 1). Sequence analysis indicated that the 3′-UTR of UCP3s and the intron region between exon 6 and the AATAAA box in intron 6 were identical (see Fig. 1). The protein predicted to be generated by the UCP3s transcript is truncated by an in-frame stop codon (tga) which follows a preserved glycine (G) codon (GGG) at residue position 275. This glycine codon in UCP3s (GGA) is located at the splice junction between exons 6 and 7.

Analysis of UCP3s and UCP3l mRNA Transcripts by RNase Protection Assay—An RNase protection assay probe corresponding to the UCP3s transcript, spanning exons 6 and 7, was prepared. This probe contained 193 bp of exon 6 sequence and 100 bp of exon 7 sequence. As is shown in Fig. 2, two bands were protected, one of ~290 bp representing UCP3s and another of ~190 bp representing UCP3l. Additional RNase protection assays were performed using a probe corresponding to UCP3s (data not shown). This probe contained 200 bp of exon 6 and 77 bp of adjacent 3′ sequence corresponding to the UCP3s 3′-untranslated region (3′UTR3, see Fig. 1). As would be predicted, two protected bands were obtained, one of ~280 bp representing UCP3s and another of ~200 bp representing UCP3l (data not shown). Quantitation of RNase protection assay results using in vitro transcribed sense UCP3 transcripts as a standard curve and total RNA extracted from five lean subjects (rectus abdominis muscle) revealed that there were ~15 amol (per µg of total RNA) of UCP3s transcripts and ~18 amol (per µg of total RNA) of UCP3l transcripts.

UCP3 Chromosomal Localization—A hUCP3 PCR primer set (see “Experimental Procedures”) was applied to the Genebridge 4 Radiation Hybrid Panel (20–22) generating the following data set for hybrid clones 1 through 93 (0 = no amplification, 1 = amplification and 2 = ambiguous results): 1001012001 0000010101 0000010000 0200112000 1110000000 0000100000 0000000000 0100100000 001. These data were submitted to the MIT Center for Genome Research STS mapping server.2 UCP3 was mapped to chromosome 11q13 (distal portion), 1.31 cR (lod > 3.0) below framework marker WI-6189, WI-6189 maps to 387.58 cR from the top of the Chr 11 linkage group on the Whitehead Institute Center for Genome Research radiation hybrid map, between framework markers D11S916 (384 cR) and D11S911 (391 cR). D11S916 and D11S911 have also been positioned on the Genethon human genetic linkage map, 85 and 89 cM from the top of the Chr 11 linkage group, respectively (23). It has previously been noted (13) that two ESTs representing UCP2, WI-13873 (accession number R49188) and WI-16720 (accession number T80845), have been independently mapped to this region (385.84 and 387.58 cR, respectively, Whitehead Institute Center for Genome Research). See Fig. 3 for the order of markers in this region and the position of framework markers on the genetic map.

Analysis of P1 and BAC Human and Mouse Genomic Clones for Colocalization of UCP2 and UCP3—Given the proximities of human UCP2 and UCP3 by radiation hybrid mapping, we investigated whether human UCP2 and UCP3 might be found together on P1 genomic clones. P1 genomic clones generally have genomic inserts of ~75–100 kb. In addition, we also investigated whether mouse UCP2 and UCP3 might be found together on P1 and BAC mouse genomic clones. BAC genomic

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2 http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl.

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**TABLE I**

| PCR primers used to amplify human UCP3 gene | Position of primers in cDNA (relative to ATG) | Position of primers in gene | Size of amplified PCR product |
|--------------------------------------------|----------------------------------------------|--------------------------------|--------------------------------|
| Sense: GAGGGGCTACCATCATTCC | -183 to -165 | Exon 1 | kb |
| Antisense: AAGGCTTCAGTCTGCAATAG | +19 to +2 | Exon 2 | 2.0 |
| Sense: AGGACTAGTGTGAGCTGAA | -6 to +14 | Exon 2 | 0.12 |
| Antisense: GCGGCCCTTGGCTGTT | +121 to +104 | Exon 2 | |
| Sense: AACCTGTTACCTTCACCTG | +83 to +102 | Exon 2 | 1.3 |
| Antisense: GTGGTCTGATGGGCTCATA | +504 to +486 | Exon 2 | 3.0 |
| Sense: AACCTGTTACCTTCACCTG | +83 to +102 | Exon 2 | |
| Antisense: GGGCCACATCTTTTATCA | +796 to +779 | Exon 2 | 1.7 |
| Sense: TCCGCAAGGGGCTGGAAGGA | +506 to +522 | Exon 4 | |
| Antisense: GTCAAGGGGCTGAAGTAC | +774 to +756 | Exon 4 | 2.5 |
| Sense: TAAGGAGAAGCCTGAGACTA | +608 to +629 | Exon 6 | |
| Antisense: CATTTAATCTGGTTCGCCACAC | +991 to +969 | Exon 7 | |
mUPC3 was possible because we had recently cloned its corresponding cDNA. 3 Mouse UCP3 is 87% identical to human UCP3 at the amino acid level, but is only 55% identical to mUCP1 and 72% identical to mUCP2. As is shown in Table II, 3 of 3 human P1 clones, 3 of 4 mouse P1 clones, and 2 of 3 mouse BAC clones contained both UCP2 and UCP3. Thus, UCP2 and UCP3 genes in mice and humans are located within 75–150 kb of each other.

3 D. Grujic, C.-Y. Zhang, G. Solanes, A. Vidal-Puig, J. S. Flier, and B. B. Lowell, unpublished data.

**TABLE II**

| Human P1 genomic clones | Mouse P1 genomic clones | Mouse BAC genomic clones |
|-------------------------|-------------------------|-------------------------|
| 324 (H6) 597 (P6) 1159 (G8) | 17 51 109 301 | 37 (L13) 280 (E17) 454 (C10) |
| **UCP2** | + + + + + + | + + + |
| **UCP3** | + + + + + | + + + |

| Position of primers in cDNA (relative to ATG) | Size of amplified PCR product (kb) |
|---------------------------------------------|----------------------------------|
| Human | UCP2 Sense: GCCCGAGCCTTCTACAAA | +795 to +813 |
| | Antisense: ATCAGTGACAGCAGGAGAG | +953 to +933 |
| Mouse | UCP2 Sense: GGGCCAGCATCATTATCCAT | +624 to +643 |
| | Antisense: CCCCCGAAGCGAAGTGAAGTGG | +796 to +778 |

**FIG. 2. UCP3 RNase protection assay.** A probe spanning exons 6 and 7 (see “Experimental Procedures” for details) was used to assess UCP3A and UCP3B. mRNA expression in human skeletal muscle total RNA isolated from quadriceps muscle, male subject, age 32, RNA purchased from CLONTECH, catalog number 64033-1. Total RNA ranging in amounts from 0–10 μg were assessed. A cyclophilin probe was used to control for quality of RNA. Expected size of each signal is shown.

**DISCUSSION**

In the present study we have analyzed the human UCP3 gene. It contains at least 7 exons spread over ~5 kb and is located on chromosome 11 (11q13), adjacent to UCP2. The UCP3 gene generates two mRNA transcripts, UCP3A and UCP3B, which are predicted to encode long and short UCP3 proteins, differing only by the presence or absence of 37 residues on the C terminus (15). These 37 residues are encoded by exon 7 which is missing from UCP3B. Intron 6 contains a cleavage and polyadenylation signal (designated AATAAA_L) located on chromosome 11 (11q13), adjacent to UCP2. The UCP3 gene maps to the distal segment of 11q13, adjacent to UCP2. Human UCP1, on the other hand, is located on chromosome 4 (24). In this context, it is noteworthy that UCP2 and UCP3 are more similar to each other than to UCP1.
quantitative trait loci for obesity (28–30), raising the possibility that abnormalities in UCP3 are responsible for obesity in these models. Thus, human linkage studies for the UCP2/UCP3 locus along with mutational analyses of mouse and human UCP2 and UCP3 genes should be the focus of future investigations.

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