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RABBIT BROWN ADIPOSE TISSUE UNCOUPLING PROTEIN MRNA: USE OF ONLY ONE OF TWO POLYADENYLATION SIGNALS IN ITS PROCESSING*

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A cDNA containing the complete coding sequence of rabbit brown adipose tissue uncoupling protein was isolated and sequenced. The coding region is 80.6% identical to rat UCP cDNA and the protein is about 86% identical to the rat and hamster proteins. Despite the presence of 2 AATAAA polyadenylation consensus sequences in rabbit UCP cDNA, only one rabbit UCP mRNA was detected indicating that only the 3'-downstream signal is used in contrast to rat and mouse where both are used.

Heat production by brown adipose tissue in, for example, rodents exposed to cold is the result of the action of uncoupling protein (UCP), a unique mitochondrial inner membrane protein which uncouples oxidative phosphorylation (1). To further understand this process, cDNAs for rat and mouse UCP (2-4) and the genes for mouse (5) and rat (6) UCP have been isolated and studied. Interestingly there are two UCP mRNAs (4,7,8) in both species reflecting the presence of two AATAAA polyadenylation consensus sequences (9) in the sequence of the gene in each species (5,6). We have studied the expression of UCP in fetal and newborn rabbits (10). Here we report the cloning of rabbit UCP cDNA and the observation of only one mRNA species despite the presence of two AATAAA polyadenylation consensus sequences.

METHODS

A cDNA library was constructed as described previously (7) from sucrose density gradient fractionated RNA obtained from

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brown adipose tissue of 31 day fetal New Zealand white rabbits. The cDNA was cloned into the Pst I site of pBR322 and a clone for rabbit UCP was selected by probing at reduced stringency with [^{32}P]-labelled rat UCP cDNA (2). The rabbit cDNA was subcloned into M13mp18, M13mp19 and pUC118 for sequence determination in both directions using standard and synthesized oligonucleotide primers prepared by the central facility of the Institute for Molecular Biology and Biotechnology, McMaster University.

Northern analysis of rat and rabbit RNA was by the method of Derman et al. (11). The probes, the complete cDNA inserts of rat and rabbit UCP and the rabbit Pst I/Hinc II cDNA fragments (Hinc II site at nucleotide 1165), were labelled with [α-^{32}P]dCTP by the random primer method (12).

RESULTS AND DISCUSSION

Sequence. The nucleotide sequence of rabbit UCP cDNA and the derived amino acid sequence are shown in Fig. 1. In addition to the deduced initiation and translation codons, two AATAAA consensus polyadenylation signals are present and are underlined. The coding regions of the rabbit and rat UCP cDNAs are 80.6% identical whereas there is only 52.4% and 50.4% identity in the 5'-untranslated regions and the overlapping portions of the 3'-untranslated regions respectively. The amino acid sequence of rabbit UCP is 86.3% and 85.3% identical to rat UCP (2,3) and hamster UCP (13) respectively but lacks proline 110 of the mature rat protein.

Most of the amino acid differences are conservative but there are four changes in amino acids 37 to 49 compared to hamster UCP which are of interest. This region was proposed to be a transmembrane amphiphilic β-strand which might be near a pore (13). However, two recent models suggest that this region of UCP or the related ADP/ATP carrier is likely to be part of a region along with residues to 50 to 70 outside of the membrane (14,15). The presence of a glutamine at residue 40 in rabbit UCP in place of leucine in hamster, mouse and rat UCP would weaken the β-structure amphilicity; the presence of proline at residue 47 in rabbit UCP in place of glutamine in hamster, mouse and rat UCP is not consistent with a β-structure since proline is found rarely in β-structures and commonly in β-turns (16). Moreover, regions 37 to 49 in rabbit UCP, as well as in the rat and mouse protein, is more hydrophilic than in hamster UCP as calculated using a consensus hydrophobicity scale (17). The differences are -0.07 for rabbit and -0.48 for rat and mouse. Overall, these three types of changes are more consistent with models placing residues 37 to 49 outside of the membrane. It is of interest

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-125                                                                    CC GGG  -121

-120  GTG GGA GGG CGG TTC CCG AGG TCA GAG AGA GCC CAG TGA CCA GGC AGG AAA GGG AAC TTA   -61

 -60  CAT CTT CGG AGA TTG CAG CCC TTA TCC TCT TGC ACC TGC CTC TTG CTC AGA GTG AAG ATG    -1
                                                                          Met    -1

   1  GTG GGC ACC ACG ACC ACG GAC GTG CCC CCA ACC ATG GGG GTC AAG ATC TTC TCA GCT GGA    60
   1  Val Gly Thr Thr Thr Thr Asp Val Pro Pro Thr Met Gly Val Lys Ile Phe Ser Ala Gly   20

  61  GTG GCA GCC TGC CTG GCG GAC GTG ATC ACC TTT CCG CTG GAC ACC GCC AAA GTC CGG CAA   120
  21  Val Ala Ala Cys Leu Ala Asp Val Ile Thr Phe Pro Leu Asp Thr Ala Lys Val Arg Gln   40

 121  CAG ATC CAA GGC GAG TTC CCG ATC ACC AGC GGC ATC AGG TAC AAA GGT GTC CTG GGG ACA   180
  41  Gln Ile Gln Gly Glu Phe Pro Ile Thr Ser Gly Ile Arg Tyr Lys Gly Val Leu Gly Thr   60

 181  ATC ACC ACC CTG GCA AAA ACG GAA GGG CCC CTG AAA CTC TAC AGC GGG TTG CCC GCC GGC   240
  61  Ile Thr Thr Leu Ala Lys Thr Glu Gly Pro Leu Lys Leu Tyr Ser Gly Leu Pro Ala Gly   80

 241  CTC CAG AGA CAA ATC AGC TTC GCC TCG CTC AGG ATC GGC CTC TAC GAC ACG GTG CAG GAG   300
  81  Leu Gln Arg Gln Ile Ser Phe Ala Ser Leu Arg Ile Gly Leu Tyr Asp Thr Val Gln Glu  100

 301  TTC TTC ACC TCG GGG GAA GAA ACA CCC AGT TTA GGA AGC AAG ATC TCG GCC GGC CTA ACA   360
 101  Phe Phe Thr Ser Gly Glu Glu Thr Pro Ser Leu Gly Ser Lys Ile Ser Ala Gly Leu Thr  120

 361  ACT GGA GGC GTG GCG GTG TTC ATC GGG CAG CCC ACA GAG GTC GTG AAA GTC AGG CTG CAA   420
 121  Thr Gly Gly Val Ala Val Phe Ile Gly Gln Pro Thr Glu Val Val Lys Val Arg Leu Gln  140

 421  GCG CAG AGC CAC CTG CAC GGT CTC AAG CCT CGC TAC ACG GGG ACG TAC AAT GCC TAC AGG   480
 141  Ala Gln Ser His Leu His Gly Leu Lys Pro Arg Tyr Thr Gly Thr Tyr Asn Ala Tyr Arg  160

 481  ATT ATA GCT ACA ACT GAG AGC TTG ACC AGT CTG TGG AAA GGG ACA ACT CCT AAT CTG TTA   540
 161  Ile Ile Ala Thr Thr Glu Ser Leu Thr Ser Leu Trp Lys Gly Thr Thr Pro Asn Leu Leu  180

 541  AGG AAT GTC ATT ATT AAC TGT ACA GAG CTC GTA ACC TAC GAC CTA ATG AAG GGG GCC CTT   600
 181  Arg Asn Val Ile Ile Asn Cys Thr Glu Leu Val Thr Tyr Asp Leu Met Lys Gly Ala Leu  200

 601  GTG AGA AAC GAA ATA CTA GCA GAT GAT GTT CCC TGC CAC TTA CTG TCA GCT CTT ATC GCT   660
 201  Val Arg Asn Glu Ile Leu Ala Asp Asp Val Pro Cys His Leu Leu Ser Ala Leu Ile Ala  220

 661  GGA TTT TGC ACA ACG CTT CTG TCC TCT CCA GTG GAT GTG GTG AAA ACC AGA TTT ATT AAC   720
 221  Gly Phe Cys Thr Thr Leu Leu Ser Ser Pro Val Asp Val Val Lys Thr Arg Phe Ile Asn  240

 721  TCT CCA CCG GGA CAA TAT GCG AGT GTG CCC AAC TGT GCA ATG ACA ATG TTC ACT AAG GAA   780
 241  Ser Pro Pro Gly Gln Tyr Ala Ser Val Pro Asn Cys Ala Met Thr Met Phe Thr Lys Glu  260

 781  GGA CCA ACG GCT TTT TTC AAA GGA TTT GTA CCT TCC TTC CTG CGA CTC GGA TCA TGG AAC   840
 261  Gly Pro Thr Ala Phe Phe Lys Gly Phe Val Pro Ser Phe Leu Arg Leu Gly Ser Trp Asp  280

 841  GTC ATC ATG TTC GTG TGC TTT GAA AAG CTG AAA GGA GAA CTC ATG AGG TCA AGG CAG ACT   900
 281  Val Ile Met Phe Val Cys Phe Glu Lys Leu Lys Gly Glu Leu Met Arg Ser Arg Gln Thr  300

 901  GTG GAC TGT GCC ACA TAA TCA GCT TCA AGA AAA GGA CAC CAC ATC CCA GTG GGA ACC CCT   960
 301  Val Asp Cys Ala Thr                                                            305

 961  GCA CCC GGG TCA TCA AGA AAT AAA ACC TTG TTC ACT TTA TTT TAC CCT AAA AAC TAA AGA  1020

1021  AAT CCC AGT AGG GAG TTT TGG ACT TTT TTT TTC AAA GGC AAA TGA AGA CCT ATT TTG TTT  1080

1081  AAG TTT TAT CCT CAG TGC CTT AGG AGG AGA AAG CCA AAC ATA CAT CTG GCA AAT GTA ACG  1140

1141  CCC AAA TAA GGA ACA GCA CTT GGT TGA CCA TTT TGG AGG TGC AAT GGT ATA ATT GAA TAT  1200

1201  GAA GAA CCT TTA TAT ATT TTA ATA TTT GAA ACT GAT GGT AGA GGA AAA CTG AGT GAA ATG  1260

1261  CAT TTT ATG AAT ACT TTA AAA TGA AGT TGT CAA AGA AAA TAT TAG TTT CTT TTT ATT TAT  1320

1321  TAA CCA CAC TGC CAG CTA ATA TAT TCA ATA AAG TAT TCT AAT ACC CTT TAA AAA AAA AAA  1380

1381  AAA AAA                                                                        1386
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Figure 1 Complete nucleotide sequence of rabbit UCP cDNA. Single-stranded DNA was isolated and sequenced as described in the Methods. Initiation and termination codons are underlined at positions -3 to -1 and 916-918, respectively. The consensus polyadenylation signals are located at nucleotides 979-984 and 1347-1352 and are underlined. The deduced amino acid sequence is presented below the nucleotide sequence.

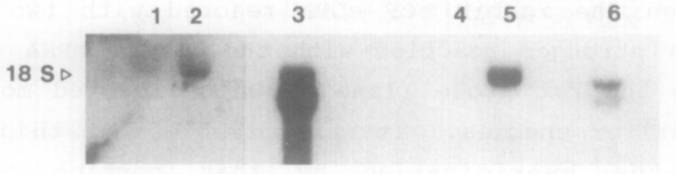

Figure 2 Autoradiogram of Northern analysis of total RNA from rabbit liver, 31 day fetal rabbit brown adipose tissue and cold acclimated rat brown adipose tissue. Total RNA was isolated, run on a denaturing agarose gel and transferred to nitrocellulose as described in the Methods. Prehybridization and hybridization to either of two radioactive probes was performed as outlined in the Methods. Lanes 1 and 4, 10 µg of rabbit liver total RNA; lanes 2 and 5, 10 µg of 31 day fetal rabbit brown adipose tissue total RNA; lanes 3 and 6, 10 µg of cold acclimated rat brown adipose tissue total RNA. Lanes 1 to 3 were probed with random primer-labelled rat UCP cDNA. Lanes 4 to 6 were probed with random primer-labelled rabbit UCP cDNA. The migration of 18 S rRNA is indicated. Lanes 1 and 2 were exposed for three times as long as lanes 3 to 6.

that the UCP gene sequence (5,6) has six exons which encode the six membrane spanning regions of the more recent model (14).

Northern Analysis. The two AATAAA consensus polyadenylation sequences in the rabbit UCP cDNA show that it was derived from an mRNA in which the second polyadenylation signal was used for processing. It is possible that UCP mRNA derived by use of the first signal is also formed. However, as seen in Fig. 2, only one mRNA was detected by Northern analysis of rabbit brown adipose tissue RNA probing with either rat UCP cDNA (lane 2) or rabbit UCP cDNA (lane 5). In contrast, both probes detected two rat UCP mRNA species (lanes 3 and 6) and no UCP mRNA in rabbit liver (lanes 1 and 4).

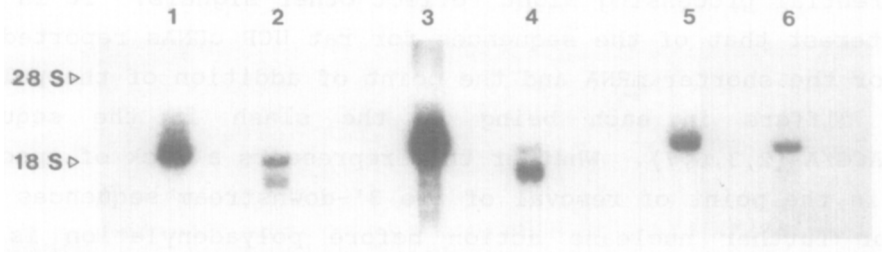

Figure 3 Autoradiogram of Northern analysis of total RNA from 31 day fetal rabbit brown adipose tissue and cold acclimated rat brown adipose tissue. Lanes 1, 3 and 5, 10 µg of 31 day fetal rabbit brown adipose tissue total RNA; lanes 2, 4 and 6, 10 µg of cold acclimated rat brown adipose tissue total RNA. Lanes 1 and 2 were probed with the full length rabbit UCP cDNA insert; lanes 3 and 4 were probed with the large 5' Pst I/Hinc II fragment of rabbit UCP cDNA; lanes 5 and 6 were probed with the small 3' Hinc II/Pst I fragment of rabbit UCP cDNA.

Although the rabbit UCP cDNA reacted with two rat mRNAs, there was a stronger reaction with the larger mRNA (lane 6) in contrast to the rat probe (lane 3) which reacted more strongly with the smaller species. It was possible that this difference reflected the hybridization by that portion of the 3'-untranslated region of the rabbit cDNA not present in the rat cDNA but present in a rat UCP mRNA with this region at its 3'-end. As shown in Fig. 3, this region hybridized far more strongly to the larger species (lane 6) than the rest of the rabbit cDNA which hybridized most strongly to the smaller species (lane 4 cf. lane 2). Although it is probable that the portion of the 3'-untranslated portion of the rabbit cDNA used as a probe is hybridizing to rat UCP mRNA, this region is only 56% identical to the analogous sequence in the rat UCP gene. However, the latter half is 72% identical.

The results of the Northern analysis are consistent with the idea that there is only one rabbit UCP mRNA produced by processing using the second polyadenylation signal only, whereas for rat and mouse UCP, both signals are used (5,6). In contrast, there is only the larger UCP mRNA in the bovine, ovine (6,18) and human (19) cases reflecting, at least for bovine UCP, the presence of only the second polyadenylation signal (6). The sequence for the mouse (5) and rat (6) UCP genes have two AATAAA consensus polyadenylation signals in similar positions to the two in the rabbit cDNA. A number of other sequences, GU- and U-rich, have been reported to be often present about 30 residues downstream from the AATAAA signal (see 20-22 for a summary). None of these are clearly present after the first signal in the rabbit cDNA or the rat or mouse UCP genes (5,6) so that the differential processing might reflect other signals. It is also of interest that of the sequences for rat UCP cDNAs reported all are for the shorter mRNA and the point of addition of the poly(A) tract differs in each being at the slash in the sequence C/AG/ACG/A (2,3,6,7). Whether this represents a lack of specificity in the point of removal of the 3'-downstream sequences (21, 22) or further nuclease action before polyadenylation is not known.

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