해양과학기술논문집 제14권 제1호(2005)
Bull. Mar. Sci. & Tech., 14(1) : 33-40(2005)

BULLETIN OF
MARINE SCIENCE AND TECHHNOLOGY
© KOREA MARITIME UNIVERSITY

Uncoupling Protein 3 in the Rainbow Trout, *Oncorhynchus mykiss* Sequence, Splicing Variants, and Association with the AvaIII SINE element

Soonhag Kim*, Cheol Young Choi[1], Jooyeon Hwang, Youngyoul Kim, Chan Park, Bermseok Oh, KuChan Kimm, Scott A. Gahr[2] and Young Chang Sohn[3]

National Genome Research Institute, NIH, Eunpyung-Gu, Nokbun-dong, Seoul122-701, Korea
[1]*Laboratory of Gene Regulation and Development, National Institute of Child Health and Human Development, National Institute of Health, Bethesda, MD 20852, USA*
[2]*Division of Animal and Veterinary Sciences, Davis College of Agriculture, Forestry and Consumer Sciences, West Virginia University, Morgantown, WV 26506, USA*
[3]*Division of Marine Life Sciences, Kangnung National University, Kangnung 210-702, Korea*

A rainbow trout uncoupling protein 3 (UCP3) cDNA clone, encoding a 310 amino acid protein, was cloned and sequenced from a liver cDNA library. Two different splice variants designated UCP3-v1 and UCP3-v2, were identified through liver cDNA library screening using rainbow trout UCP3 cDNA clone as a probe. UCP3-v1 has 3 insertions in the UCP3 cDNA: the first insertion (133 bp), the second (141 bp), and the third (370 bp) were located 126 bp, 334 bp and 532 bp downstream from the start codon, respectively. UCP3-v2 contained a single insertion, identical in sequence and location to the second insertion of UCP3-v1. UCP3, a mitochondrial protein, functions to modulate the efficiency of oxidative phosphorylation. UCP3 has been detected from heart, testis, spinal cord, eye, retina, colon, muscle, brown adipose tissue and white adipose tissue in mammalian animals. Human and rodent UCP3s are highly expressed in skeletal muscle and brown adipose tissue, while they show weak expression of UCP3 in heart and white adipose tissue . In contrast to mammalian studies, RT-PCR and Southern blot analysis of the rainbow trout demonstrated that UCP3 is strongly expressed in liver and heart. UCP3, UCP3-v1, and UCP3-v2 all contain an Ava III short interspersed element (SINE), located in the 3' untraslated region (UTR). PCR using primers from the Ava III SINE and the UCP3 3' UTR region indicates that the UCP3 cDNA is structurally conserved among salmonids and that these primers may be useful for salmonid species genotyping.

Keywords: AvaIII, Rainbow trout, SINE, Splicing variants, UCP3

Introduction

Over the past 30 years, there has been extensive research into the mechanism of non-shivering thermogenesis and importance of brown adipose tissue (BAT) in thermal regulation of infants (for review see Nicholls, 2001). This work led to the identification of uncoupling protein 1 (UCP1) in the BAT, a mitochondrial carrier protein with the ability to increase heat production by altering the proton permeability of the inner mitochondrial membrane (for review see Villarroya et al., 2001). Subsequently, two other members of the UCP family have been identified, UCP2 and UCP3. UCP2, classified according to the amino acid sequence homology to UCP1, is expressed in a variety of tissues and is thought to serve a similar function to UCP1, but its true function is yet to be fully elucidated (for review see Ricquier and Bouillaud, 2000). An additional UCP (UCP3), identified by the 57% aa homology to UCP1, has been found to be primarily expressed in skeletal muscle with lower expression in BAT and cardiac muscle. (Fleury et al., 1997). In addition to the proton shunting ability, recent studies have indicated a significantly different role for UCP3 in the regulation of fatty acid metabolism. During periods of starvation, i.e. a shift in the muscle to primarily lipid catabolism, UCP3 expression is upregulated in the muscle, with the fast glycolytic muscle showing the greatest response (Hildebrandt and Neufer, 2000). In the mouse, the connection between UCP3 and fatty acid metabolism is further reflected in the mapping of markers of obesity and hyperinsulemia in close proximity to the UCP3 gene locus on chromosome 7 (Fleury et al., 1997).

*Corresponding author: kimsoonhag@hotmail.com

Two alternatively spliced forms of the UCP3 gene have been identified in human skeletal muscle. The long transcript ($UCP3_L$) includes a small portion of the 6th exon and a 7th exon, while the short transcript ($UCP3_S$) only the 1st 6 exons (Solanes et al., 1997). Based on the cDNA sequences of the two variants, human UCP3 is represented by proteins of 312 aa ($UCP3_L$) and 275 aa ($UCP3_S$) found on the inner mitochondrial membrane (Solanes et al., 1997). However, researches on the mouse and human indicate that the long transcript is alone translated into an active protein (for review see Harper et al., 2001).

Short interspersed repetitive elements (SINEs) are non-viral retrotransposons incorporated into the genome of a wide variety of organisms (Singer, 1982). SINEs are commonly referred to as tRNA pseudogenes, because of their homology to tRNA sequences (Okada, 1991). In salmonid fish, three classes of SINE elements have been discovered: SmaI family, HpaI family and FokI family. An additional group, the HpaI-related AvaIII family, is common to many salmonid species (Kido et al., 1994). By sequence and quantity, SINE elements are used as phylogenetic markers (Murata et al., 1996), and the identification of a common SINE element in the transcribed portion of a particular gene may indicate the evolutionary conserved gene.

In a preliminary experiment, we determined the identity of gene transcripts containing the HpaI-Related AvaIII SINE element in the rainbow trout. From this work, we isolated a partial sequence of the rainbow trout UCP3. It is the first discovery of UCP3 cDNA from aquatic animals. The availability of full length of UCP3 from rainbow trout should provide molecular resources for production of recombinant UCP3 for application in rainbow trout. As a cold water fish, rainbow trout may have unique system of thermogenesis, different from warm-blooded terrestrial mammals. With potential for metabolic regulatory role for UCP3 in the rainbow trout, we looked at the pattern of gene expression of UCP3 in tissues of the trout to know whether the function of UCP3 in a rainbow trout is different from the mammalian UCP3s. In this communication, we report the full length UCP3 sequence of the rainbow trout, its tissue distribution, identity of any splice variants and conservation of the AvaIII SINE element of the 3' UTR of the UCP3 gene in other salmonid species.

Material and Methods

Genes expressing AvaIII SINE

A liver cDNA library, prepared in λ ZapII vectors, was kindly provided by Dr. Joe Brunelli of Washington State University. During routine EST screening of this library, a clone (RTI#12) was isolated containing a putative AvaIII SINE. A single PCR primer from RTI#12 clone was designed using GeneTools 1.0 (BioTools Inc., Canada) within the SINE element. The primer sequence was 5'-TTAACCCAACCCCTCTGAATCAGAG-3', which was purchased from Gibco BRL (now Invitrogen Corp., USA). Using the AvaIII primer and either the T7 or T3 promoter primers (Promega, USA), PCR screening of the excised cDNA library was performed as follows. 24 μl of a PCR cocktail containing 2.5 μl of 10x *Taq* buffer (1.5 mM $MgCl_2$ final concentration), 0.5 μl dNTPs (final concentration 0.2 mM each), 1 μl of AvaIII primer (4 ng/ml final concentration) and 5 μl T3 or T7 primer (4 ng/ml final concentration), 0.5 μl (2.5 Units) of *Taq* DNA Polymerase (Display Systems Biotech., USA) and 14.5 μl of nuclease free water was added to 1 μl excised liver cDNA library phagemid (10 ng/μl). The PCR commenced with one cycle consisting of denaturing (94°C) for 4 min, annealing (55°C) for 1 min, and extension (72°C) for 1 min. The first cycle was followed by 30 cycles consisting of 1 min intervals of denaturing at

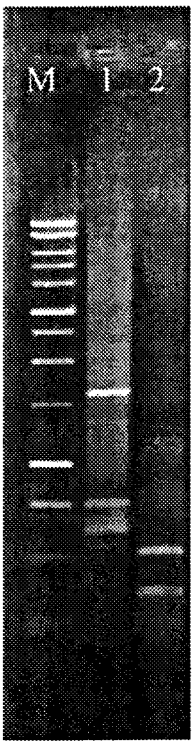

Fig. 1. Determination of amplicon size from the PCR screening of liver cDNA library with the RTI#12 Primer (see Material and Methods) and either T3 (lane 1) or T7 (lane 2) promoter primer. 1-kb molecular size marker was used and indicated as the letter M. Products were separated on 1% agarose gel and stained with ethidium bromide.

94°C followed by annealing at 55°C and extension at 72°C. These cycles were followed by a final 10 min extension step (72°C). The products were separated by gel electrophoresis (1% agarose) and stained with ethidium bromide to determine the sizes of the amplicons (see Fig. 1). The PCR products were subjected to TA cloning (pCR 4 Vector, Invitrogen Corp., USA), and plasmid insert size was determined by *Eco*RI restriction digestion and gel electrophoresis analysis. Plasmids containing unique amplicons were sequenced using the Big Dye Terminator method (PE Biosystems Co., USA) on an automated ABI 310 DNA sequencer (PE Biosystems Co., USA). The identity of each insert was determined by searching the GenBank Database with NCBI Blast program.

Liver cDNA library screening for UCP3

The UCP3 cDNA was found in the rainbow trout liver expressed sequence tag analysis. Because this gene had not been characterized, we used hybridization-based cDNA library screening to isolate the full-length expressed sequence. Briefly, plaque DNA from the cDNA library was fixed to nylon membranes to allow hybridization with ^{32}P-labeled probe. Following the pre-hybridization wash, the membranes were hybridized overnight at 42°C. Three washes of each membrane were used to remove unbound probe, these consisted of 2x SSC with 0.1% SDS at room temperature, 0.5x SSC with 0.1% SDS at 55°C, and 0.1x SSC with 0.1% SDS at 65°C. Following the washes, the membranes were exposed for at least 3 hrs to Bio-Max film (Eastman Kodak Co., USA) at 80°C. Probe DNA was generated from the plasmid DNA isolated in the preliminary experiment. Following *Eco*RI restriction digestion of the plasmid DNA, the insert fragment (~1400 bp) was gel purified using the Freeze N Squeeze column method (Bio-Rad, USA). This fragment was labeled with ^{32}P using Amersham dCTP Ready-To-Go Labeling beads according to the manufacturers protocol (Amersham Biosciences, USA). Approximately, 3.0×10^6 plaques were screened. Fifteen positive plaques, which had been subject to second and third rounds of screening, were isolated for sequence analysis.

Once the individual positive plaques were isolated, the phagemid vector (pBluescript) was excised using the ExAssist Helper Phage following Stratagenes protocol (Stratagene, USA). The resulting plasmid was sequenced from both forward and reverse directions, using the T7 and T3 promoter primers and the Big Dye Terminator Reaction (see above). A contig of the two sequences was constructed for each clone using GeneTools 1.0 (BioTools, Canada). For clones not showing overlapping sequences, primer walking was performed to determine the full-length sequence.

Tissue distribution of UCP3

In combination with Southern blot analysis, reverse transcription-polymerase chain reaction (RT-PCR) was used to determine the tissue distribution of the UCP3 in the rainbow trout. Total RNA was isolated from adult (~2 yr old) mixed-sex rainbow trout following the TRI-Reagent (Molecular Research Center Inc., USA) modification of the guanidine isothiocyanate/phenol-chloroform method as described by Chomczynski and Sacchi (1987). The quantity of RNA was estimated by measuring the absorbance at 260 nm on a Shimadzu Spectrophotometer (Shimadzu Corp., USA). The quality of the RNA was assessed by formaldehyde agarose gel electrophoresis with the visualization of the 28S and 18S rRNA bands. RNA was isolated from brain, heart, liver, kidney, spleen, skeletal muscle, pituitary, stomach, intestines, eye, skin and adipose tissue. All RNA samples were stored at 80°C. Reverse transcription was performed with 2 μg of total RNA, 2 μg of oligo-dT primers and murine maloney leukemia virus reverse transcriptase (MMLV-RT), as previously described (Kocamis et al., 2002). *Taq* polymerase (Display Systems, USA) and gene specific primers were used for gene product amplification with the reaction setup as described above. A touchdown reaction was run with an initial annealing temperature of 68°C, reducing by 1°C for 10 cycles and then an additional 25 cycles with an annealing temperature at 58°C. The products were separated by 1% agarose gel electrophoresis containing 200 ng/ml ethidium bromide and visualized and photographed with the FlouroChem Imaging System (Alpha-Innotech Corp., USA). The identity of the products was verified by Southern blot analysis.

Conservation of the UCP3 Ava III SINE

Using Bio-Rads AquaPure Genomic DNA Isolation Kit (Bio-Rad, USA), genomic DNA samples were isolated from rainbow trout (kamloop, red band, cutthroat and steelhead strains), brook trout, brown trout, Artic char, Atlantic salmon and Pacific salmon. These samples were subjected to PCR amplification for the AvaIII SINE located in the 3' UTR of the rainbow trout UCP3 gene. The PCR reaction was set up, as described above, using 1 μl of genomic DNA (50 ng/μl) as the template. The PCR reaction consisted of 35 cycles with an annealing temperature of 60°C and primers specific to the

SINE element in 3' UTR of UCP3. The sense primer (5'-GGGGACCTCTAGCCATTC-3') and antisense primer (5'-TCAACTGAAATGTGTCCTCCGC-3') were designed to amplify a 201-bp product of UCP3 3' UTR including the AvaIII SINE. The products were separated by 1% agarose gel electrophoresis containing 200 ng/ml ethidium bromide and visualized and photographed with the FlouroChem Imaging System (Alpha-Innotech Corp., USA). The identity of the products was verified by Southern blot analysis. The probe used for the Southern analysis was a sub-clone from the 3' UTR of the rainbow trout UCP3 cDNA labeled using the Ready-to-go labeling beads (Amersham Biosciences, USA) as previously described.

Results and Discussion

As repetitive sequences constitute a substantial portion of the eukaryotic genomes, understanding the organization of genes in relation to repetitive sequences is critical for genomics research. Genes containing Ava III SINE were isolated through EST and PCR screening of the rainbow trout liver cDNA library (Table 1). The 28S ribosomal RNA (RTI#12) represents the original AvaIII SINE identified through the analysis of rainbow trout liver EST screening. To find additional cDNAs containing AvaIII SINE, we performed PCR screening of the excised liver cDNA library using the AvaIII primer from RTI#12 clone and either the T7 or T3 promoter primers (see Fig. 1). PCR screening and cloning subsequently led to the identification of two additional unique sequences containing an AvaIII SINE (see Table 1). These genes may be located in identifiable regions of the genomic DNA linked to evolutionary or phenotypic changes in salmonids (Murata et al., 1996). To investigate this possibility further, we felt the potential role of UCP3 in metabolic regulation would make it an excellent candidate for association with phenotypic changes. However, the identification of a novel gene product (unknown in Table 1) containing a phylogenic marker could represent a more useful marker specific to salmonids, a hypothesis that is currently being investigated in our laboratory. However, the role of UCP3 as a potential metabolic regulator in animal leads us to further investigate this previously unreported uncoupling protein from a cold water fish.

Table 1. Genes containing the AvaIII SINE element identified by expressed sequence tag and PCR screening of liver cDNA library, and length of the SINE element found in each transcript

Clone ID	Gene identity	SINE location	SINE length
RTI#12	28s Ribosomal RNA		25 bp
Ava#6	Uncoupling protein 3	3' UTR	111 bp
Ava#34	Unknown	3' UTR	231 bp

Using a cloned partial sequence isolated by PCR screening of the liver cDNA library, we identified 15 clones that hybridized specifically to the UCP3 probe. Thirteen of the 15 clones were found to contain the identical 1685 bp sequence (see Fig. 2) in which the longest open reading frame encodes for a 310-aa polypeptide with 69% aa homology to porcine (GenBank Accession No. AAD33396) and canine (GenBank Accession No. Q9N2I9) UCP3s. The rainbow trout UCP3 cDNA (Fig. 2) is transcribed as a 1685 bp mRNA consisting of a 101-bp 5' UTR, a 930-bp open reading frame, and a 654-bp 3' UTR. We have identified the AvaIII SINE as a 111-bp sequence located in the 3' UTR. The two additional clones (UCP3v1 and UCP3v2) were identified as potential splice variants of the UCP3 gene. UCP3v1 contained three insertions, while UCP3v2 only one, which was identical to the second insertion in UCP3v1 in location of the sequence (see Fig. 2). The first insertion was found 126-bp downstream from the translation start codon, and was identified as 133-bp in length. The second insertion was 134-bp in length and was located 334-bp downstream from the start codon, not including the first insert. The final inserted sequence was 370-bp long and was found 533-bp from the start codon. Following the GT-AG sequence located at the ends of each insertion, we can conclude that these are the result of splice variations in the UCP3 gene. Considering the open reading frame of the UCP3 cDNA, each variant would result in the translation of a truncated form of the protein. This may support a similar situation as in human, in which a truncated form of UCP3 ($UCP3_S$), results from the splice variation; it is suggested to be a non-functional form of the protein (Solanes et al., 1997).

The similarity in the amino acid sequence with the mammalian homologues (swine and dog) suggests a similar structure for the UCP3 identified in the present study. Based on the homology, we suggest, that the protein in the rainbow trout would have a conserved function. The next point was to determine the tissue distribution of UCP3 in the rainbow trout tissues. We performed reverse transcription-polymerase chain reaction to confirm gene expression pattern of UCP3 in tissues of rainbow trout. The magnitude of gene expression of UCP3 was normalized by measuring the level of b-actin messenger RNA. We found that UCP3 was expressed in a wide variety of tissues scanned (Fig. 3). This was somewhat

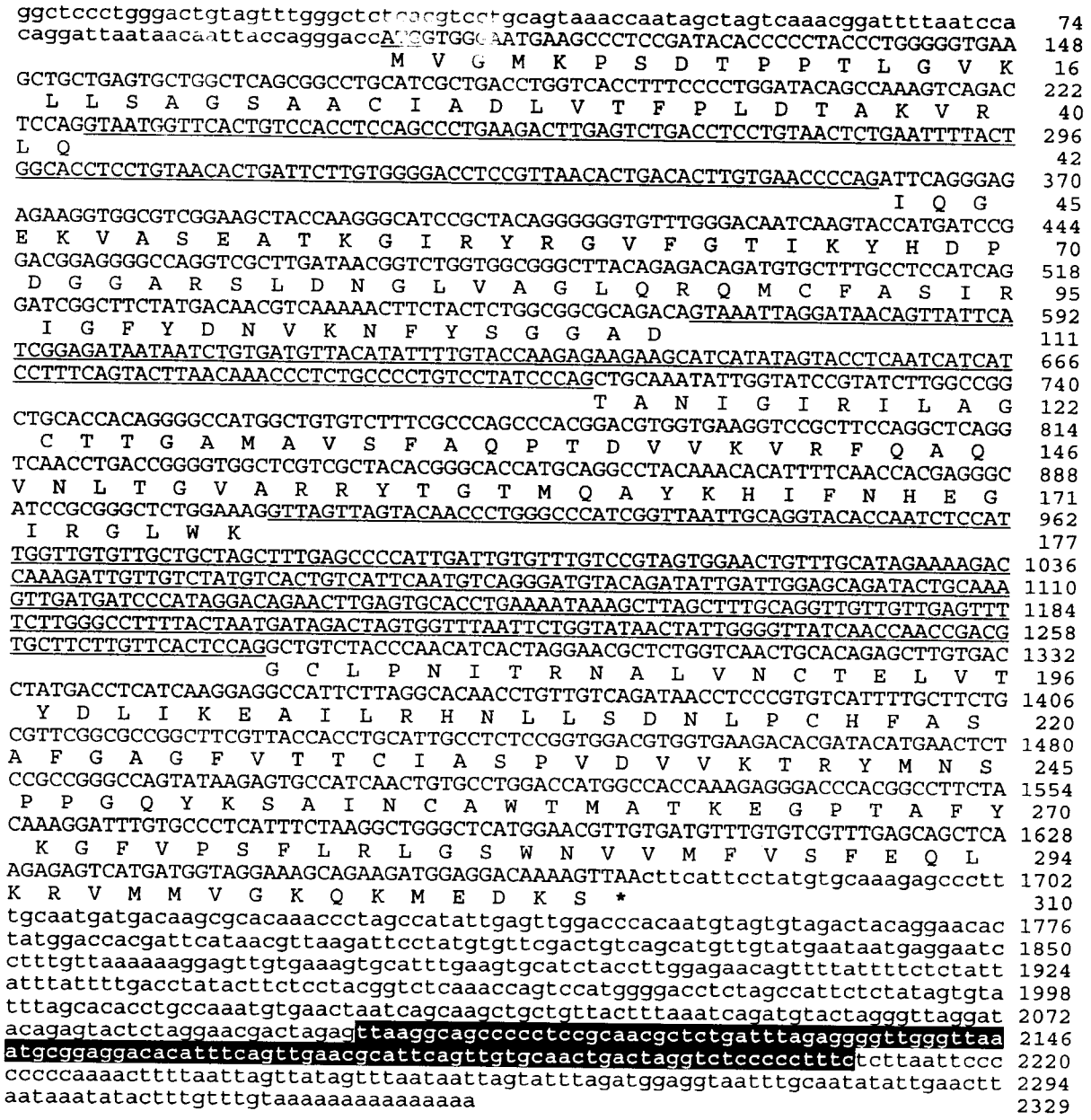

Fig. 2. cDNA sequence of rainbow trout UCP3 with the translated 309 aa peptide sequence. Nucleotide sequences are numbered on the top, far right, and the amino acid sequence on the bottom, far right. The coding region is in uppercase letters and the noncoding region in lowercase letters. The deduced amino acid sequences are indicated below the nucleotide sequence in the one-letter amino acid code. The first amino acid in the mature peptide is underlined. The stop codon is indicated by a bold asterisk. Three insertions included in UCP3v1 and UCP3v2 are in uppercase letters and underlined. The AvaIII SINE is highlighted.

unexpected, as previous reports showed that UCP3 expression was limited to skeletal muscle and BAT, with significantly weak expression in the cardiac muscle (for review see Fleury et al., 1997). In the present study, a strong expression was recorded in the liver followed by the gastro-intestinal organs (stomach and intestines), which suggest a significantly different role for the UCP3 in metabolic regulation of the rainbow trout. In tuna and lamnid sharks, a heat exchange system surrounding the viscera has been suggested to play a key role in maintenance of elevated organ temperature by increasing the digestive capabilities of the fish (for review see Carey et al., 1971). Similarly, we can suggest a similar role for the expression of UCP3 in the visceral organs of the rainbow trout, a hypothesis which could be tested by comparing the expression of the gene in trout reared at different temperatures.

A final consideration was the conservation of the AvaIII SINE element found in the 3' UTR of the rainbow trout UCP3

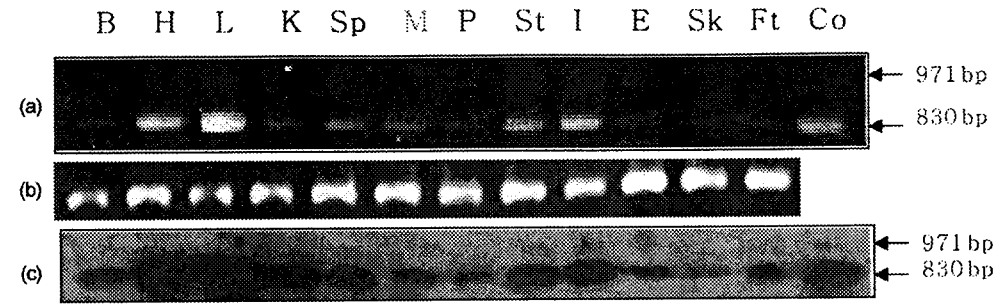

Fig. 3. UCP3 gene expression pattern in tissues from rainbow trout. This shows both the electrophoresis of PCR products (A), b-actin control (B) and the Southern blot analysis (C). UCP3 (830 bp) and UCP3v2 (971 bp) were the only transcripts detected in the tissues, with highest expression of UCP3 in the liver and the heart. UCP3v1 (1473 bp) transcript was not detected in the tissues screened. Tissues screened: B-brain, H-heart, L-liver, K-kidney, M-skeletal muscle, P-pituitary, St-stomach, I-intestines, E-eye, Sk-skin, Ft-fat tissue, Co-control plasmid.

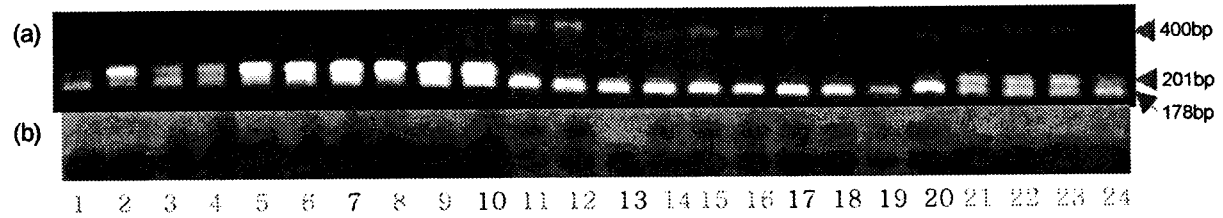

Fig. 4. Conservation of the AvaIII SINE element in the 3 UTR of the UCP3 transcripts among rainbow trout strains and other Salmonid species as determined by gel electrophoresis of PCR products (A) and Southern blot analysis (B). In addition to the expected 201 bp band, 178 bp product common to all of the samples screened, and 400 bp band were identified in a few samples. Samples: Lanes 1-6 rainbow trout kamloop, Lane 7 rainbow trout red band, Lanes 8-9 rainbow trout cutthroat, Lane 10 rainbow trout steelhead, Lanes 11-12 brook trout, Lanes 13 brown trout, Lanes 14-16 artic char, Lanes 17-20 atlantic salmon, Lanes 21-24 pacific salmon.

gene. Using PCR of salmonids genomic DNA and Southern blot analysis of the PCR products with probe containing both AvaIII SINE sequences and 3' UTR of the rainbow trout UCP3 gene, we demonstrated the presence of the AvaIII SINE in a number of salmonid species (Fig. 4). However, this investigation revealed two interesting results. Firstly, a 178-bp fragment was common to all the salmonid species, besides the expected 201-bp fragment detected in all rainbow trout strains and Pacific salmon (see Fig. 4). The Southern blot data indicated that this fragment shares close homology to the 201-bp fragment, and thus may indicate the presence of a closely related gene in all salmonid species. Based on the high homology and close localization on chromosome 11, Solanes et al. (1997) suggested that UCP3 resulted from a duplication event of UCP2. Although we have not specifically determined either the location of the gene or the identity of the sequence around the 178-bp band, this may indicate a similar event occurred in the salmonids. Moreover, we found a 400-bp amplicon associated with the lack of the 201-bp band including the 178-bp band discussed above in the brook trout, brown trout, Artic char and Atlantic salmon. This raises the interesting possibility that there is an alteration in the 3' UTR of the UCP3 in these salmonid species. Though it is beyond the scope of the present study, this change may result in a significant alteration in metabolic efficiency.

In the present study, we have identified the SINE elements present in the transcribed regions of the gene. However, this has also resulted in the identification of the UCP3 cDNA in the rainbow trout, and two splice variants. Based on the tissue distribution, we have projected that this protein may have a role to play in the base maintenance of core body temperature. Variations in the sequence and copy number of the AvaIII SINE in the salmonid species have allowed their use as a marker of genetic variation. Therefore, the relationship between this potential metabolic regulator and the phylogenetic marker may prove useful as a genetic marker of body partitioning or other marketable traits.

References

Carey, F. G., J. M. Teal, J. W. Kanwisher, K. D. Lawson and J. S. Beckett, 1971. Warm-bodied fish. Am. Zoologist, **11**: 137–145.

Chomczynski, P. and N. Sacchi, 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem., **162**: 156–159.

Fleury, C., M. Neverova, S. Collins, S. Raimbault, O. Champigny,

C. Levi-Meyrueis, F. Bouillaud, M. F. Seldin, R. S. Surwit, D. Ricquier, and C. H. Warden, 1997. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nat. Genet., **15**: 269–272.

Harper, M. E., R. M. Dent, V. Bezaire, A. Antoniou, A. Gauthier, S. Monemdjou and R. McPherson, 2001. UCP3 and its putative function: consistencies and controversies. Biochem. Soc. Trans., **29**: 768–773.

Hildebrandt, A. L. and P. D. Neufer, 2000. Exercise attenuates the fasting-induced transcriptional activation of metabolic genes in skeletal muscle. Am. J. Physiol. Endocrinol. Metab., **278**: E1078–E1086.

Kido, Y., M. Himberg, N. Takasaki and N. Okada, 1994. Amplification of distinct subfamilies of short interspersed elements during evolution of the Salmonidae. J. Mol. Biol., **241**: 633–644.

Kocamis, H., S. A. Gahr, L. Batelli, A. F. Hubbs. and J. Killefer, 2002. IGF-I, IGF-II and IGF-receptor-1 transcript and IGF-II protein expression in myostatin knockout mice tissues. Muscle Nerve, **26**: 55–63.

Murata, S., N. Takasaki, M. Saitoh, H. Tachida and N. Okada, 1996. Details of retropositional genome dynamics that provide a rationale for a generic division: the distinct branching of all the Pacific salmon and trout (*Oncorhynchus*) from the Atlantic salmon and trout (*Salmo*). Genetics, **142**: 915–926.

Nicholls, D. G., 2001. A history of UCP1. Biochem. Soc. Trans., **29**: 751–755.

Okada, N., 1991. SINEs. Curr. Opin. Genet. Dev., **1**: 498–504.

Ricquier, D. and F. Bouillaud, 2000. The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. Biochem. J., **345**: 161–179.

Singer, M. F., 1982. SINEs and LINEs: highly repeated short and long interspersed sequences in mammalian genomes. Cell, **28**: 433–434.

Solanes, G., A. Vidal-Puig, D. Grujic, J. S. Flier and B. B. Lowell, 1997. The human uncoupling protein-3 gene. Genomic structure, chromosomal localization, and genetic basis for short and long form transcripts. J. Biol. Chem., **272**: 25433–25436.

Villarroya, F., S. Brun, M. Giralt, Y. Cámara, G. Solanes and R. Iglesias, 2001. Gene expression of leptin and uncoupling proteins: molecular end-points of fetal development. Biochem. Soc. Trans., **29**: 76–80.

Manuscript Received: 2003
Revision Accepted: 2003
Responsible Editorial Member:

Source : Journal of Aquaculture, (2004), 17(1): 1-7