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Short sequence-paper

# Uncoupling protein 2 from carp and zebrafish, ectothermic vertebrates

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**Abstract**

Uncoupling protein 1 (UCP1) is of demonstrated importance in mammalian thermogenesis, and early hypotheses regarding the functions of the newly discovered UCP homologues, UCP2, UCP3 and others, have focused largely on their potential roles in thermogenesis. Here we report the amino acid sequences of two new UCPs from ectothermic vertebrates. UCPs from two fish species, the zebrafish (*Danio rerio*) and carp (*Cyprinus carpio*), were identified in expressed sequence tag databases at the European Molecular Biology Laboratory. cDNAs from a *C. carpio* 'peritoneal exudate cell' cDNA library and from a *D. rerio* 'day 0 fin regeneration' cDNA library were obtained and fully sequenced. Each cDNA encodes a 310 amino acid protein with an average 82% sequence identity to mammalian UCP2s. The fish UCP2s are about 70% identical to mammalian UCP3s, and 60% identical to mammalian UCP1s. Carp and zebrafish are ectotherms – they do not raise their body temperatures above ambient by producing excess heat. The presence of UCP2 in these fish thus suggests the protein may have function(s) not related to thermogenesis. 

Uncoupling protein (now UCP1) is a demonstrated uncoupler of oxidative phosphorylation [1]. UCP1 expression is restricted to mammalian brown adipose tissue, where it provides a pathway for protons pumped out of the mitochondrial matrix by the electron transport chain to pass back in. The resultant futile cycling of proton pumping and leaking allows an augmentation of heat production in this tissue. However, the net proton conductance catalysed by UCP1 simply augments an existing inner membrane proton conductance that is found in all animal, plant and yeast mitochondria [2]. A significant proportion of protons pumped out of the mitochondria by the electron transport chain passes back into the matrix through as yet uncharacterised proton conductance pathway(s) [2]. The recently discovered UCP1 homologues, uncoupling proteins 2 [3,4], 3 [5,6], and others [7–9], have been considered candidates for the catalysis of this basal level of uncoupling of oxidative phosphorylation that characterises all mitochondria. In part because of the high sequence similarity of the UCP homologues to UCP1, early hypotheses for their physiological role(s) have focused on thermogenesis. Here we report the identification and sequencing of two fish UCPs with very high amino acid sequence identity to the mammalian UCP2. The presence of UCP2 in these ectothermic vertebrates, which do not raise their body temperatures above ambient, suggests that UCP2 may have function(s) not related to thermogenesis.

We identified two fish UCP cDNAs in expressed sequence tag (EST) libraries at the European Molecular Biology Laboratory database (www.embl-ebi.ac.uk) using a tfasta search (www2.igh.cnrs.fr/fasta/tfasta-query.html) with a 40 amino acid sequence from the N-terminus of the human UCP2 protein.

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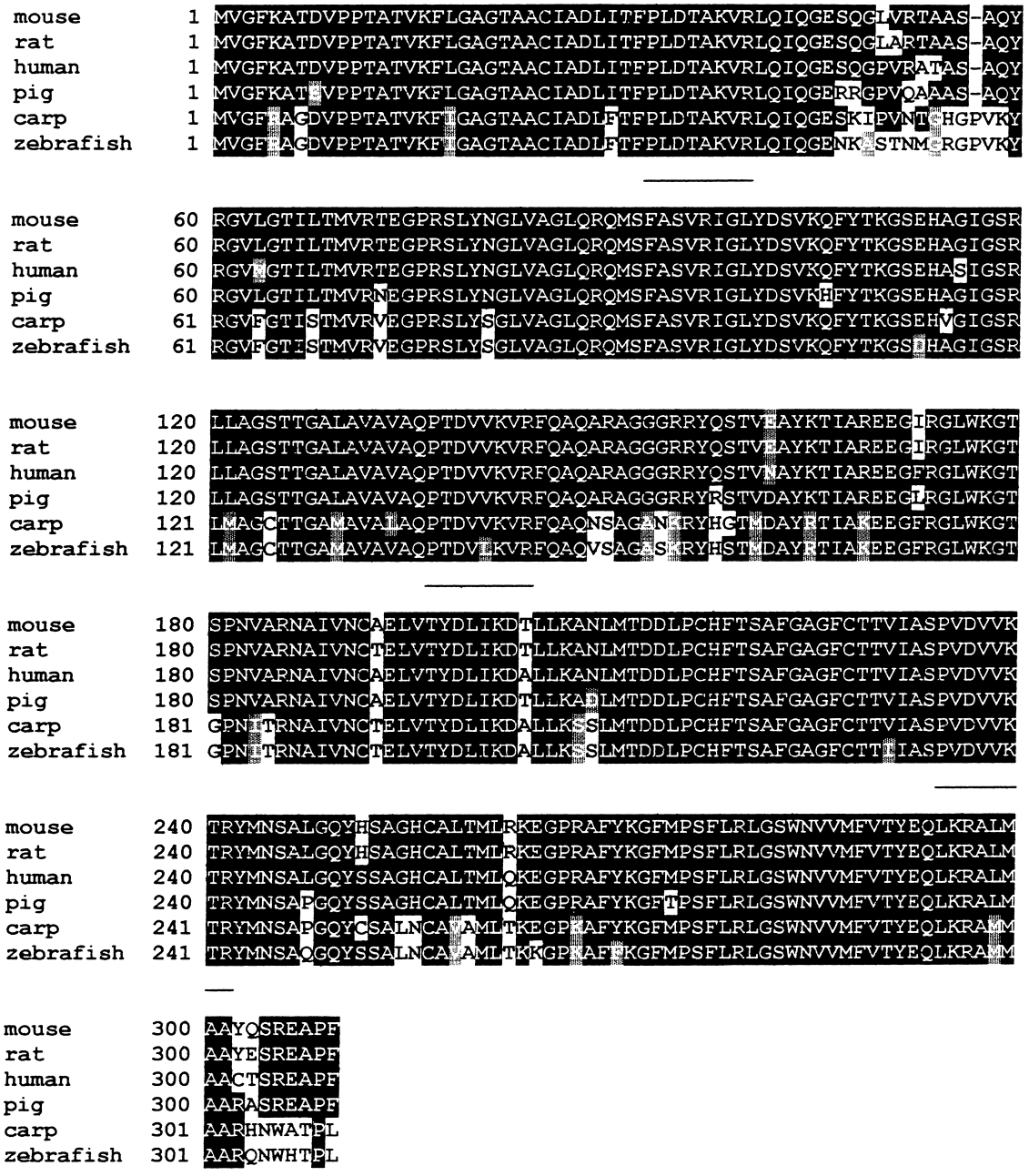


Fig. 1. Carp and zebrafish UCP2 predicted amino acid sequences aligned with mammalian UCP2s using ClustalW (K.C. Worley, Human Genome Center, Baylor College of Medicine). The three mitochondrial transporter protein signature motifs [10] are underlined. Pig UCP2 is as translated from EMBL database entry AF036757.

ESTs from the carp *Cyprinus carpio* (accession number C88392) and the zebrafish *Danio rerio* (accession number AI384260) were identified as having approximately 90% identity with this sequence.

The carp cDNA was kindly provided by the authors of the database entry (K. Fujiki, M. Nakao, D. Shin and T. Yano) in a pBK-CMV plasmid prepared from the ZAP Express (Stratagene) phage library by in vivo excision. This cDNA is from a library of carp genes differentially expressed in sodium alginate-elicited peritoneal exudate cells, identified by suppression subtractive hybridisation. Automated sequenc-

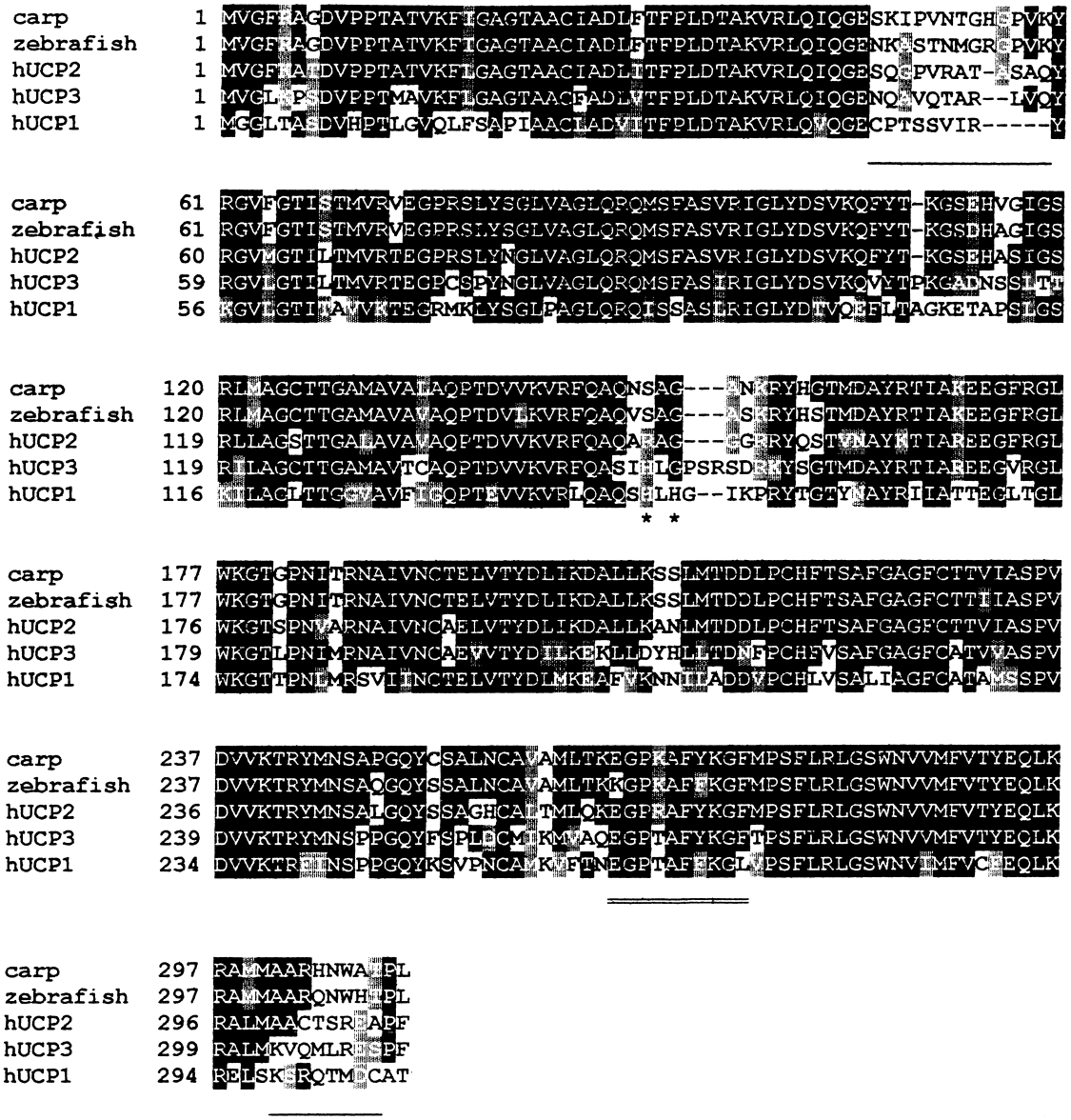


Fig. 2. Multiple sequence alignment (as in Fig. 1) of carp and zebrafish UCP2 translations with human UCP1, UCP2 and UCP3. Two sites of low homology between all UCPs are underlined. Two histidines believed to be essential for hUCP1 function [11] are indicated by asterisks. The putative UCP1 purine nucleotide binding site [19] is double underlined.

ing of the carp cDNA was performed with an Applied Biosystems Model 373 DNA sequencing system. Fluorescent dye labels were incorporated into DNA using 3′-dye labelled dideoxynucleotide terminators with AmpliTaq polymerase in a cyclic sequencing reaction. Approximately 500 ng of DNA was used per sequencing reaction mixed with 10–20 pmol of primer. Following the cyclic sequencing the samples were ethanol precipitated and 3 μl gel loading buffer added. The sense strand was sequenced using a T3 primer and antisense using an M13 primer. The middle of the approximately 1.4 kb insert was sequenced using two forward primers (GCCGCGCAGTCTCTACAGCG and CACGATCAGCACCATGGTGCGT) and two backward primers (GCTGGGCTCCTGGAATGTGG and TTCATAGGTGACAAACATAACCA).

The zebrafish cDNA is from a zebrafish ‘day 0 fin regeneration’ library. It was obtained from Research Genetics (Alabama, USA). The zebrafish cDNA was

fully sequenced by Research Genetics, using double end sequencing and two primers for middle sequencing.

The nucleotide sequences of both cDNAs have been deposited at the European Molecular Biology Laboratory's database (www.embl-ebi.ac.uk), under accession numbers AJ243486 (carp) and AJ243250 (zebrafish).

The carp and zebrafish cDNAs each encode a 310 amino acid protein with approximately 82% sequence similarity to the mammalian UCP2s (88% allowing for conservative amino acid substitutions) (Fig. 1). They have somewhat lower sequence identity with other mammalian UCPs: about 70% identity with mammalian UCP3s, 59% identity with UCP1s (Fig. 2) and 47% identity with the plant uncoupling protein, stUCP (not shown). We have therefore designated them carp and zebrafish UCP2s. By comparison, the amino acid sequence of the fish UCP2s are approximately as similar to human UCP1 (59%) as are human UCP2 (59%) and UCP3 (58%) (Fig. 2), which is perhaps remarkable given the great evolutionary distance of fish from mammals.

The three mitochondrial transporter protein signature motifs found in all members of the mitochondrial transporter protein family [10] are clearly present in both fish UCP2s (Fig. 1). As observed for mammalian UCP2, the putative purine nucleotide binding domain of UCP1 is not fully conserved in the fish UCP2s (Fig. 2). The amino acid sequence of carp UCP2 contains two regions which show high divergence from mammalian UCP2s (Fig. 2). These regions correspond to: (1) the first mitochondrial matrix-exposed segment at the N-terminus of the protein (based on the Klingenberg model of UCP1 [11]), and (2) the last eight carboxy-terminal amino acids. It is evident, however, that these regions also vary significantly amongst human UCP1, 2 and 3. Two histidines which occur in UCP1, and are thought to be essential for the uncoupling function of UCP1, are absent in the fish UCP2s (Fig. 2), as they are in mammalian UCP2 and UCP3 [12].

The demonstration of a fish UCP2 provides a new perspective on the debate regarding the physiological role(s) of UCP2 and UCP3. The interpretation of experimental results in some studies appears to have been constrained by the presupposition that the UCP1 homologues must function in thermogenesis [7,9,13,14]. However, empirical evidence for the involvement of UCP2 and UCP3 in thermogenesis has remained equivocal. Three studies in particular have provided data which argue against a thermogenic role for UCP2 and UCP3 in mammals [15–17]. The expression of UCP2 in cells of an ectotherm strongly supports the idea that this protein has a function not related to thermogenesis. Heat is conducted, and thus dissipated, in water at a rate some 25 times that in air. In fish, such as carp, where specific anatomical adaptations to retain metabolic heat (e.g. retia, epidermal fat layers) are absent, a strategy of thermogenesis is untenable [18]. Thus, the identification of UCP1 homologues in any tissue should not be considered diagnostic of a thermogenic function.

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