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MINI REVIEW

Mitochondrial Uncoupling Proteins in Mammals and Plants

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Uncoupling proteins (UCPs) belong to a distinct cluster of the mitochondrial anion carrier family. Up to five different uncoupling protein types were found in mitochondria of mammals and plants, and recently in fishes, fungi and protozoa. They exhibit a significantly conserved structure with several motifs specific to either the whole cluster or protein type. Uncoupling proteins, as well as the whole mitochondrial anion carrier gene family, probably emerged in evolution before the separation of animal, fungi, and plant kingdoms and originate from an anion/nucleotide or anion/anion transporter ancestor. Mammalian UCP1, UCP2, UCP3, and plant uncoupling proteins pUCP1 and pUCP2 are similar and seem to form one subgroup, whereas UCP4 and BMCP1 belong to a different group. Molecular, biochemical, and phylogenic data suggest that UCP2 could be considered as an UCP-prototype. UCP1 plays its biological role mainly in the non-shivering thermogenesis while the role of the other types is unknown. However, hypotheses have suggested that they are involved in the general balance of basic energy expenditure, protection from reactive oxygen species, and, in plants, in fruit ripening and seed ontogeny.

KEY WORDS: Mitochondria; thermogenesis; uncoupling proteins

ABBREVIATIONS: BAT, brown adipose tissue; BMCP1, brain mitochondrial carrier protein; FA, fatty acid; MACF, mitochondrial anion carrier family; pUCP, plant uncoupling protein; UCP, uncoupling protein.

INTRODUCTION

The transport of metabolites across the inner mitochondrial membrane is achieved by specific integral membrane carrier proteins. Most of these carriers transport anions (fatty-acid anions, ADP, ATP, phosphate, oxoglutarate, malate, aspartate, glutamate, citrate, or pyruvate) but some of them transport zwitterionic substrates (ornithine, carnitine, or glutamine). These anion carriers belong to the gene family named Mitochondrial Anion Carrier Family (MACF)[2], consisting of about 17 clusters or subfamilies [1, 2]. In yeast, 27 putative subfamilies of 5 already known and 22 possible distinct mitochondrial carriers [3] have been described. At least 10 different

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mitochondrial carrier proteins have been purified to homogeneity and reconstituted into artificial membrane systems, which is essential for identification, detailed functional characterization, and structural studies [4]. All mitochondrial carriers exhibit molecular weights in a very narrow range of 28–34 kDa. All of them have a protein molecule consisting of three repeats of about 100 amino acids and every repeat contains two hydrophobic transmembrane domains.

Uncoupling proteins found in mammalian and plant mitochondria (UCPs) are members of this carrier gene superfamily because (1) they have a high sequence homology with other carriers and (2) transport anionic forms of fatty acids or other anions [5]. The brown adipose tissue-specific mitochondrial uncoupling protein (BAT-specific UCP1) was discovered already in 1976 [6] and its function is already known [7]. It dissipates energy of H^+ gradient across the inner mitochondrial membrane resulting in heat production, essential to awaken hibernating mammalians. Two possible hypotheses of proton transport are proposed: first, H^+ is transported directly by the UCP1, functioning as a proton channel [8]; second, H^+ transport is carried by a free fatty acid (FA) cycling. This mechanism involves protein-mediated passage of FA anions to the opposite side of the membrane where FA anions become protonated and neutral FA can readily move back across the membrane by a flip-flop mechanism, while carrying H^+ [9, 10]. Data from the authors favor the second hypothesis (31). UCP1 activity is inhibited by purine nucleotides in a pH-dependent manner.

In addition to UCP1, four mammalian members of the UCP subfamily have been described, namely the ubiquitous UCP2 [11], skeletal muscle- and BAT-specific UCP3 [12], and brain-specific UCP4 [13] and BMCP1 [14]. Expressed in bacteria and reconstituted in proteoliposomes, UCP2 and UCP3 behaved identically to UCP1, with exception of lower affinity to purine nucleotides ($K_{i,\mathrm{GDP}}$ were 20 μM for UCP1, 1.2 mM for UCP2, and 1 mM for UCP3, Ref. 15). Their role remains unclear. UCP2 may play a role in fine-tuning the efficiency of oxidative phosphorylation and in preventing of superoxide production in mitochondria [16, 17]. UCP2 and UCP3 genes are located within quantitative trait loci for obesity [11, 18] and are regulated by leptin [19, 20], implicating the role of corresponding proteins in the regulation of energy balance. UCP4 may be reasonable for the mitochondrial proton leak observed in brain tissue, and may potentially be involved in adaptational thermoregulation and heat production in both fetal and adult brains [13].

In 1995, i.e., before the discovery of any UCP other than UCP1, Vercesi *et al.*, discovered a different uncoupling protein in plant mitochondria and named it PUMP (plant uncoupling mitochondrial protein, Ref. 21). The discovery of plant homologue of UCP, let us say second UCP, was the first evidence that uncoupling protein is not only the protein with some very specialized function in the specific mammalian tissue but its physiological role may be more general. To avoid a possible confusion with the term "pump," used for transporters responsible for active transport, we prefer to use the term pUCP (plant uncoupling protein). So far, five genes encoding pUCPs have been identified—StUCP from potato [22], AtUCP1 (formerly AtPUMP, [23, 24]) and AtUCP2 [25] from *Arabidopsis*, and SfUCPa and SfUCPb from spadix of skunk cabbage [26]. AtUCP1 and StUCP have been expressed in *E. coli* [27] and yeast [22], respectively. Similarly to UCP1 [9, 28], UCP2

and UCP3 [29], pUCP requires FA as a cofactor for its activity [21, 30–40] and, like other UCPs, allows for uniport of FA anions leading to FA cycling [31]. The purine nucleotide inhibition constants for StUCP [32] and AtUCP1 [27] were close to those measured for UCP2 and UCP3, suggesting that pUCPs are more similar to them than to UCP1. pUCPs are supposed to be involved together with alternative oxidase in regulating the balance between $\Delta\mu_{H^+}$ and "active phosphate" potential [41].

Several reviews describing the nature, structure, and function relationships of mitochondrial carriers or UCPs in detail have been published [8, 42–56]. Here we present the genetic data for uncoupling proteins within their gene family.

UCPs AS MEMBERS OF MITOCHONDRIAL ANION CARRIER FAMILY

About 80 protein sequences of at least 8 different mitochondrial anion carriers (UCP, ATP/ADP-carrier, phosphate carrier, citrate carrier, malate/2-oxoglutarate carrier, carnitine/acylcarnitine carrier, dicarboxylate carrier, and ornithine carrier) from various organisms are deposited in the GeneBank or SWISSPROT databases. Some carriers have isoforms encoded by different genes. For example, ATP/ADP-carrier has 3, UCP5, and pUCP at least 2 isoforms. All the protein sequences contain a highly conserved motif called Energy Transfer Proteins Signature—P-x-[DE]-x-[LIVAT]-[RK]-x-[LRH]-[LIVMFY].

Phylogenic analysis shows that mammalian and plant UCPs are concentrated in two clusters of MACF (Fig. 1). One cluster consists of 4 branches of UCP1, UCP2, UCP3, and pUCP1 and pUCP2. The smallest phylogenetic distance is between UCP2 and UCP3 and corresponds to the tandem coincidence of their genes in chromosomes. Exclusively mammalian-specific UCP1 is more distant and the next proximal branch contains all pUCPs.

UCP4 and BMCP1 form neighbor clusters together with oxoglutarate carriers. Thus, UCP1, UCP2, UCP3 together with pUCP1 and pUCP2 can be assigned to one subfamily, whereas the brain-specific UCP4 and BMCP1 carriers seem to belong to a distinct class.

PLANT- AND MAMMAL-SPECIFIC PROTEIN PATTERNS IN UNCOUPLING PROTEINS

All uncoupling proteins but UCP4 and SfUCPb have three Energy Transfer Proteins Signatures, one in each protein repeat. The signatures differ slightly (see Table 1) and retain variants which are specific either for animals or for plants.

The first signature (in the first protein repeat) has one variant, identical for UCP1–3 and pUCP1–2, and other variant identical for UCP4 and BMCP1. These findings support the hypothesis that UCP4 and BMCP1 belong to a separate subfamily. Moreover, this first signature is followed by tripeptide Gly-Φ-Gly, present in all UCPs. Its middle hydrophobic amino acid (Φ) seems to be specific because all sequences in the UCP1-3 group have leucine in this position, all pUCPs isoleucine, UCP4 methionine, and BMCP1 valine. So, there is an evidence for mammalian- or plant-specific sequences.

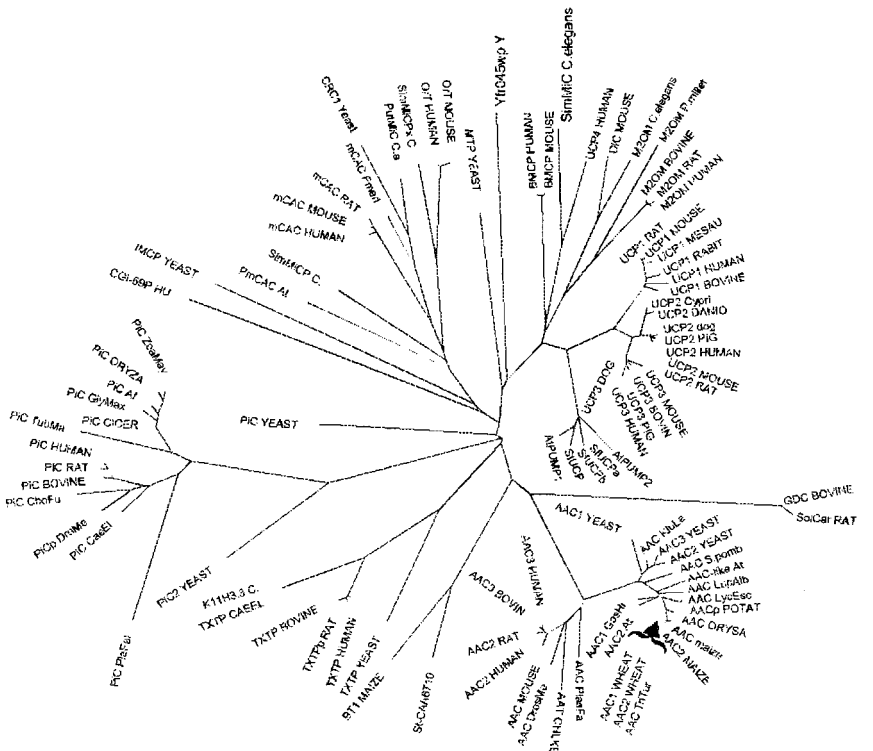

Fig. 1. Unrooted phylogenetic tree analysis of different mitochondrial carrier protein sequences using PHYLIP. The sequences were aligned by CLUSTAL W and distance scores were generated using Protdist program. The tree topology and evolutionary distance estimations were preformed by the neighbor-joining method with Dayhoff distances employing Neighbor program. Accession numbers for UCPs are: AtUCP1 (AJ223983); AtUCP2 (BAA36222); StUCP (Y11220); SfUCPa (BAA92172); SfUCPb (BAA92173); UCP1-human (P25874); UCP1-rat (P04633); UCP1-mesau (P04575); UCP1-mouse (P12242); UCP1-bovine (P10861); UCP1-rabbit (P14271); UCP2-rat (AF039033); UCP2-mouse (P70406); UCP2-human (P55851); UCP2-pig (O97562); UCP2-dog (BAA90457); UCP2-Cyprinus (CAB46248); UCP2-Danio (CAB46268); UCP3-human (P55916); UCP3-mouse (AF032902); UCP3-pig (O97649); UCP3-bovine (O77792); UCP2-dog (BAA90458); UCP4-human (O95847); BMCP1-human (O95258); BMCP1-mouse (Q9Z2B2). Protein abbreviations were taken from SWISPROT database.

The second signature has one variant for each uncoupling protein type, which is invariant for all species being considered. Thus, the signature sequences seem to be specific for a given UCP type. Note that UCP4 also possesses part of the second signature, if a deletion of two last amino acids of the whole signature is considered. On the contrary, both pUCP types exhibit only one plant-specific signature variant. The following tripeptide Gly-Φ-Gly is present in all UCPs but UCP3, while pUCPs contain Gly-Φ-Glu.

Variants of the third signature are specific for UCP1, UCP4, and BMCP1. UCP2 and UCP3 have one in common, as well as pUCP1 and pUCP2. Note that

Table 1. Changes of Energy Transfer Protein Signature with Additional Tripeptide in UCPs

Protein branch	First signature	Second signature	Third signature
UCP1		PTEVVKVR[LM]-QAQ	PVDVVKTRF-[IV]NS
UCP2	PLDTAKVRL-QLQ	PTDVVKVRF-QAQ	
UCP3		PTDVVKVRF-VVAS	PVDVVKTRY-MNS
pUCP1–2	PLDTAKVRL-QIQ	PTDLVKVRL-Q[AS]E	PVDV[VM]KSRM-MGD
UCP4*	PLDLTKTRL-QMQ	PTDLVKV••-QMQ	PADVIKSRI-MNQ
BMCP1*	PVDLTKTRL-QVQ	PTDVLKIRM-QAQ	PVDVVRTRM-MNQ

*Note that only one UCP4 and two BMCP1 sequences are stored in databanks and thus they are not representative. Possible deletions are marked as •.

SfUCPb does not contain this signature. However, alignment of both SfUCPs showed their very identity; the only difference is that 35 amino acids of SfUCPa (position 204–238) are missing in SfUCPb. The third signature in SfUCPa starts in position Pro235, four amino acids before end of deletion in SfUCPb. But the rest of the signature, MKSRMM, is present in SfUCPb.

Looking for other sequences, specific for mammal and plant uncoupling proteins, we employed the pattern prediction program package MEME-MAST (http://www.sdsc.edu/MEME, Refs. 57, 58). MEME motifs are represented by letter-probability matrices that specify the probability of each possible letter (of single-letter amino acid code) appearing at each possible position in an occurrence of the motif. Thirteen probable motifs were predicted in a training set of 30 uncoupling protein sequences. Table 2 summarizes eight predicted motifs that had been associated with some complete and specific groups of UCPs. Motifs 1 and 2 are long (50 and 30 amino acids, respectively) and occur in all uncoupling proteins. Four shorter motifs are specific to various groups of UCPs and two for pUCPs. Figure 2 demonstrates a model for *trans*-membrane spanning [8] with locations of proposed motifs in examples of UCP and pUCP.

The Motifs 1 and 2 form a tandem, which is repeated three times and covers about 80% of the UCP. The Motif 2 sequence includes the first transmembrane domain and the Energy Transfer Proteins Signature (highlighted in Table 2) in each protein repeat, followed by Motif 1 involving the second transmembrane domain in each of the repeats. It can be hypothesized that these tandom motifs are conserved in evolution to maintain the proper conformation of uncoupling proteins. In *trans*-membrane spanning model used in Fig. 2, Motif 2 does not begin at the same place of each repeat; so the beginning of fifth transmembrane helix should be reconsidered as starting at Phe219 (UCP2) or Ile218 (AtUCP1).

Motifs 3–7 are localized at hydrophilic loops of UCPs being exposed to intermembrane space. Motif 3 and Motif 4 are specific only for UCP1–3. Motif 3 is localized in the protein *N*-terminus, whereas Motif 4 is at the beginning of the third repeat. Motif 5 and Motif 6 are specific only for pUCPs and their locations are within the two external loops (start of second and third protein repeat, respectively). The two remaining significant motifs are even more specific—Motif 7 occurs only in UCP3 in the first external loop. Motif 8, the only motif oriented to the matrix side of the proteins, is specific exclusively for UCP2 and forms the center of the first matrix loop.

Table 2. Possible Protein Motifs in UCP Cluster of MACF

Name length	Sequence	Specificity	Localization	Topology
Motif 1 50 aa	[KR]Y[KR]GxLNAYxT[LI]x[KR]-xEGP[KR]AL[YF][KR]GLxPNLxR-xAS[YF]NA[VI]xLxTYEx[VI]KxxL	UCP1–5 pUCP	Each repeat	Second transmembrane domain
Motif 2 30 aa	[KR]LLAAxxAGxLAxx[VI]Ax-**PxDVVKVRL**QxQA*	UCP1–5 pUCP	Each repeat	First transmembrane domain
Motif 3 12 aa	MVGF[KR]ASDVPPT	UCP1–3	*N*-end	Cytosolic
Motif 4 12 aa	LKxNLLTDDLPC	UCP1–3	Start of Third repeat	Cytosolic
Motif 5 12 aa	GKDFVGD[IV]PLSK	pUCP	Start of second repeat	Cytosolic
Motif 6 12 aa	LKIPGFTDNVVT	pUCP	Start of third repeat	Cytosolic
Motif 7 9 aa	TPKG[SA]DHSS	UCP3	Start of second repeat	Cytosolic
Motif 8 12 aa	ESQGPVRAAASA	UCP2	Middle of second repeat	Matrix

*A highlighted region corresponds to energy transfer protein signature.

The possible functions associated with these shorter motifs (Motif 3–8) remain unknown. However, it is interesting to note that almost the whole molecule of uncoupling proteins consists of conserved structures that are common for either the whole MACF or for a specific uncoupling protein subfamily. These findings suggest that members of MACF emerged early in the evolution of eukaryots and are essential for their survival.

UCPs AND pUCPs IN GENOMES

The UCP1, 2 and 3 genes were located in the human, mouse, and rat genomes. UCP1 was mapped to human chromosome 4 (position ~157 cM; Ref. 59), mouse chromosome 8 [60] or rat chromosome 19 [61–63]. UCP2 and UCP3 are adjacent genes at a distance of 8 or 7 kb from each other for human or mouse, respectively, suggesting possible gene duplication [64–66]. They reside on human chromosome 11 (position 81–82 cM; Refs. 18, 19, 67), mouse chromosome 7, and rat chromosome 1 [68], in a region that has been linked to hyperinsulinaemia and obesity [11, 12, 69]. All three genes have six exons, each one encoding a transmembrane domain of the protein [70]. In addition to the coding exons, one or two untranslated exons were found in UCP3 and UCP2, respectively [64, 65, 71].

UCP4 maps to human chromosome 6p11.2-q12 [13]. BMCP1 was assigned to chromosome X in mouse and to Xq24 in human [14].

AtUCP1 and AtUCP2 were located in the *Arabidopsis thaliana* genome. AtUCP1 resides on chromosome 3 (position 76 cM; in clone F24B22, Ref. 72) and AtUCP2 on chromosome 5 (position 116 cM; in clone K19M22, Ref. 25). AtUCP1 is the more homologous to UCP2 (49–50% identity, 65–66% similarity) then to

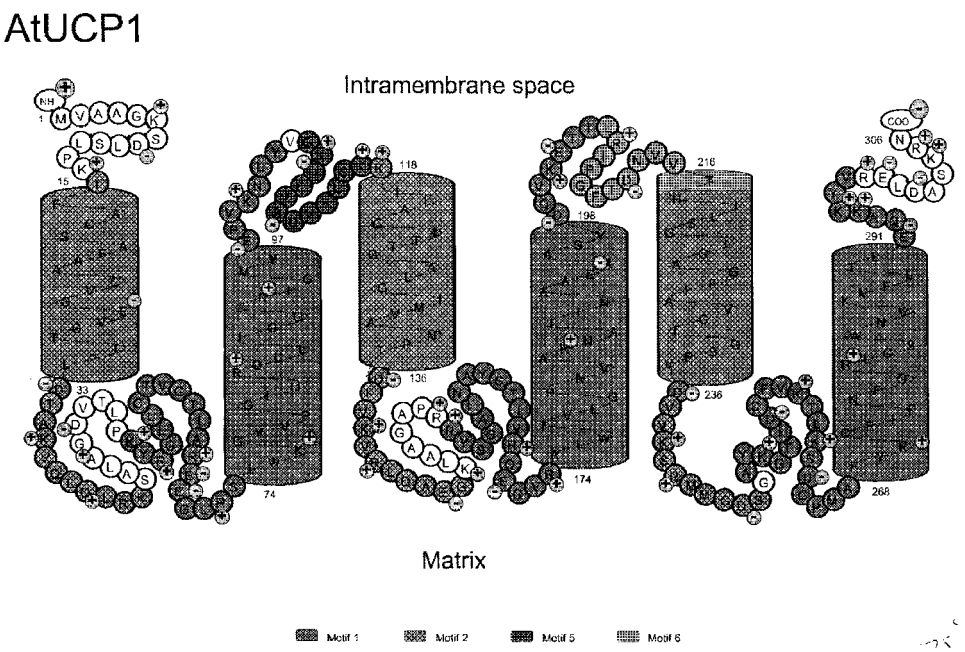

Fig. 2. Examples of predicted protein motifs in uncoupling protein gene subfamily. (A) UCP2. (B) AtUCP1. Protein Motifs 1 and 2 are common for all members of the subfamily and involve first transmembrane protein domain (Motif 2) or the second domain (Motif 1) of each protein repeat. Motifs 3 and 4 are specific for UCP1–3, and Motifs 5 and 6 for pUCPs, being all exposed to outside of inner mitochondrial membrane. Motif 8 is specific only for UCP2 and located on the matrix side of the membrane.

UCP3 (47–48% identity, 63–64% similarity), UCP1 (43–44% identity, 58–60% similarity), UCP4 (38% identity, 60% similarity) or BMCP1 (37% identity, 56% similarity). In the case of AtUCP2, the most homologous vertebrate uncoupling protein are both UCP2 and UCP3 (both 41–44% identity, 57% similarity) the next are UCP1 (36–38% identity, 53–54% similarity) and UCP4 (37% identity, 56% similarity), and the last is BMCP1 (35% identity, 51% similarity). Both of the known AtUCP genes have 9 exons that differ from the mammalian counterpartners and do not correspond to either protein repeats of transmembrane region [25, 72].

A comparison of the distribution of coding regions of exons for various UCPs and pUCPs is illustrated in Fig. 3. UCP1–3 genes are similarly organized; however, UCP1 has much larger introns than UCP2 or UCP3. BMCP1 gene is organized in a different pattern, indicating its weaker relationship to the other three UCPs. Structures of AtUCP1 and AtUCP2 are almost identical, with only exon 1 and introns 1 and 6 differing in their size.

EVOLUTION OF UNCOUPLING PROTEINS

The updated unrooted phylogenic tree (Fig. 1) shows that sequences corresponding to every distinct mitochondrial anion carrier are localized in a separate

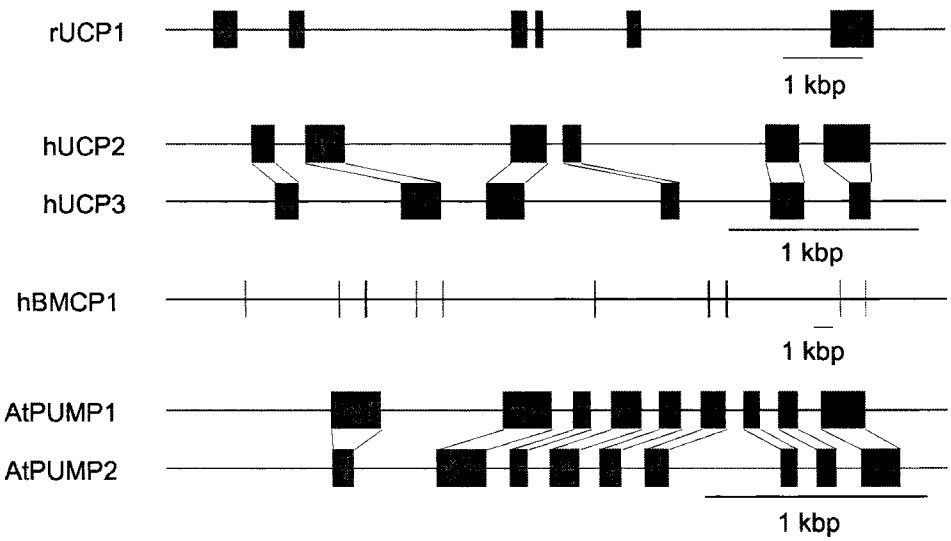

Fig. 3. Distribution of coding region of genes for UCPs and pUCPs. UCP2 and UCP3 exhibit very similar gene organization, as well as AtUCP1 and AtUCP2. UCP1 exons are separated by longer introns than other UCPs and BMCP1 distribution in genome is significantly different from the others. Bars correspond to 1 kbp of chromosomal DNA.

cluster, that consists of usually two branches of animal and plant homologues, as is apparent in the case of UCPs, AACs, or PiCs. Such a distribution suggests that the genes of MACF emerged phylogenetically very early and the sequences are conserved over a long evolution time. Only carriers found in yeast usually exhibit higher phylogenetic distance from the apparent carrier clusters.

The existence of uncoupling proteins is not limited to mammals and plants. Figure 1 already includes the two novel UCP2 sequences, found in ectothermic vertebrates, carp (*Ciprinus carpio*) and zebrafish (*Danio rerio*) [73]. Recently, UCP has also been identified in mitochondria from *Acanthamoeba castellanii*, a nonphotosynthetic soil amoeboid protozoon, using antibodies raised against recombinant AtUCP [74]. There is also the first evidence of an uncoupling protein in the fungi kingdom, CpUCP from *Candida parapsilosis*, parasitic non-fermenting yeast [75]. Finally, UCP was also found in trophozoites of malaria parasite *Plasmodium Berghei* [76]. The existence of UCPs in protozoa, fungi, and fishes suggests that uncoupling proteins emerged early during evolution as a distinct member of MACF, probably before the divergence of plant, animal, and fungi kingdoms. Thus, uncoupling proteins are supposed to be found in all eurkaryotic organisms. The only exception known is *Saccharomyces cerevisiae* that does not possess any form of UCP [3].

The closest phylogenic neighbor of the UCP subfamily is the branch of malate/2-oxoglutarate carriers, whose function is to import substrates for oxidative phosphorylation and dicarboxylate carriers, taking a role in gluconeogenesis (Fig. 1). The subfamilies of phosphate carriers and ADP/ATP-carriers involved in oxidative phosphorylation are more distant.

When only UCP1 was known, it was hypothesized that UCP1 might be derived from anion/proton symporter, transporting anions by electroneutral way into the negatively charged mitochondrial matrix [77]. The reported relative wide spectrum of anions that are transported by UCP1 (chloride, pyruvate, phenylpyruvate, alkylsulfonates, and fatty acids; Ref. 5), functioning as a possible proton channel, is coherent with this hypothesis. However, the fatty acid cycling mechanism of H^+ transport mediated by UCPs and the small phylogenic distance between UCPs and malate/2-oxoglutarate carriers oppose that, corroborating more with some anion/anion antiporter.

Another hypothesis is that the ancester of UCP1 could be a nucleotide/proton symporter with aborted nucleotide transport [70]. The fact that binding a nucleotide to UCP1 causes protein conformational changes stabilizing the resulting complex, supports this hypothesis. Similarly to the previous hypothesis, the possibility of anion/nucleotide antiporter has to be considered.

Functional similarities between UCP and bacteriorhodopsin have also been reported [78]. The fatty acid could act as a prosthetic group of UCP, as well as retinal in case of bacteriorhodopsin. In addition, bacteriorhodopsin is able to transport proton or chloride under specific conditions [79]. On the other hand, retinoids were identified as novel regulators of uncoupling activity of UCP1 and UCP2 [80].

CONCLUSIONS

Uncoupling proteins in eukaryotes form a subfamily of mitochondrial anion carrier family. They originated probably from anion/anion or anion/nucleotide transporter in the evolution period before divergence of eukaryotes to animals, plants, and fungi.

Considering the phylogenic tree, mammalian BAT-specific UCP1 seems to be the most specialized and the newest member of the UCP subfamily, taking a key role in non-shivering thermogenesis in newborns, man inclusive, hibernating and cold-acclimated mammals. The genes for UCP2 and UCP3 are adjacent, with a distance of 8 or 7 kb from each other for human or mouse, respectively, suggesting a possible gene duplication. UCP2 is expressed in all tissues, whereas UCP3 expression is limited to skeletal muscle and rodent BAT. Their function is unknown but they are supposed to regulate resting energy expenditure of the body. Processes of inflammation, fever, or oxidative stress could be affected by UCP2 and UCP3, too. The finding of the fish UCP2 gene, its ubiquitous expression, and the highest homology with pUCPs open the hypothesis that UCP2 represents the subfamily member that is the closest to subfamily ancestor.

Plant uncoupling proteins exhibit properties more similar to UCP2 and UCP3 and are phylogenetically closer to them than to UCP1. pUCPs were found either in climacteric or non-climacteric, as well as in thermogenic or non-thermogenic plants. Their role remains largely unclear. It is proposed that they are involved in fruit ripening [42], seed formation and in low-temperature adaptation processes [32]. They also may function in balance of oxidative phosphorylation, controlling the proton potential across the inner mitochondrial membrane, together with alternative oxidase that controls potential of ''active phosphatases'' [41]. The free fatty acid can

have a key role in regulation of these potentials because it influences the activity of both pUCP and alternative oxidase [38, 40]. Increasing concentration of free FA activates the activity of pUCP whereas inhibits activity of alternative oxidase.

UCP4 and BMCP1 are located on the phylogenic tree at a greater distance from other uncoupling proteins and are close to oxoglutarate carriers. These proteins are preferentially expressed in the brain. Their role might be in adaptational thermoregulation and heat production in both fetal and adult brains.

The studies of properties of uncoupling proteins in other different plant types could reveal their physiological role and, together with studies of structure/function relationships of novel mammal uncoupling proteins, could establish their general role in all living organisms. Searching for UCP presence in evolutionary older organisms will be necessary to understand the nature of mechanisms involved in temperature and energy expenditure regulation at all.

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