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Minireview

Oxidative stress, thermogenesis and evolution of uncoupling proteins

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Abstract

The uncoupling protein UCP1 provides eutherian mammals with an efficient thermogenic mechanism. Recent work published in *BMC Evolutionary Biology*, following the identification of UCP1 orthologs in non-eutherians, concludes that this unique function appeared after sequence divergence and purifying selection that allowed functional co-option.

Oxidative stress, energy dissipation, and thermogenesis

Organisms living in an oxygen-rich environment have to overcome the dangers posed by highly reactive oxygen-derived free radicals, the so-called reactive oxygen species (ROS). To protect against damage by ROS, all biological systems have evolved complex antioxidant mechanisms composed of low molecular weight compounds (such as glutathione and vitamin E) and enzymes such as catalase, superoxide dismutase or glutathione peroxidase. As the mitochondrial respiratory chain is probably the major site of ROS production, and the rate of ROS formation increases when respiratory rates are low, cells also evolved means of accelerating respiration and thus reducing the damage caused by free radicals. One such mechanism involves an increase in the permeability of the inner membrane of the mitochondrion, so that protons pumped by the respiratory chain can return to the matrix. The uncoupling proteins (UCPs), a family of transporters belonging to the mitochondrial carrier protein superfamily, which is found in all eukaryotic organisms, provide the pathway for proton re-entry. Once a mechanism to increase respiration was operative, it was subsequently accommodated (co-opted in evolutionary terms) to fulfill other physiological roles such as maintenance of body temperature or even control of energy balance.

Brown fat is a thermogenic tissue only present in eutherian mammals. Heat generation in brown adipose tissue relies on the above-described modification of the mitochondrial proton circuit, which allows fast substrate oxidation without ATP synthesis. This low coupling of oxidative phosphorylation was recognized in the 1960s, and was soon related to the thermogenic activity of the tissue. The unusually high proton permeability of brown-fat mitochondria was shown to be inhibited by purine nucleotides and activated by fatty acids. In 1978, Nicholls and co-workers, using photoaffinity labeling with nucleotides, identified UCP1 (initially named UCP) as the protein responsible for the proton permeability [1]. The fatty-acid activation of UCP1 has great physiological importance: when noradrenaline signals the initiation of thermogenesis, fatty acids are released, and become both substrates for oxidation and the second messengers that activate UCP1. Non-shivering thermogenesis is particularly important in

hibernating and newborn mammals. Interestingly, in the Suidae (pigs and wild boars), the *UCP1* gene is disrupted, and therefore piglets have poor thermoregulation. This mutation event occurred some 20 million years ago, and is correlated with an intriguing behavioral adaptation in that suids are seemingly the only members of the Artiodactyla that build nests before giving birth [2].

The morphology and physiological function of brown and white adipocytes are markedly different. Brown adipocytes present a multilocular distribution of triglyceride deposits and contain numerous mitochondria packed with cristae, consistent with their high metabolic activity. White adipocytes, on the contrary, primarily have an energy storage function, and thus mitochondria are scarce. Recent work has shown that brown and white adipocytes have distinct embryonic origins. Brown adipocytes derive from the same myogenic progenitors as skeletal muscle cells; the transcriptional activator PRDM16 is the key factor determining whether muscle cells or brown adipocytes are produced [3].

The mitochondrial carrier superfamily

The sequencing of the first mitochondrial carriers (adenine nucleotide translocator (ANT), phosphate carrier (PiC) and UCP) revealed that these metabolite transporters have common structural features and thus belong to the same protein family. The most striking feature is their internally repetitive structure, in which a unit sequence of 100 amino acids is repeated three times. Each repeat contains two transmembrane segments linked by a long hydrophilic loop (Figure 1). The three loops are oriented toward the matrix side of the inner membrane, and include the conserved sequence motif that is currently used to identify potential members of the superfamily (NCBI conserved domain Pfam00153, mito_carr superfamily). The elucidation of the three-dimensional structure of the ANT has confirmed this structural arrangement [4]. Therefore, it appears that the protein superfamily evolved by triplication of a primordial protein that contained two transmembrane domains. Moreover, as mitochondrial carriers do not appear to have orthologs in prokaryotes, it has been proposed that the ancestral mitochondrial carrier may be an evolutionary innovation of the ancestral cell that became host to the bacterial endosymbiont that eventually became a mitochondrion. Subsequent diversification generated the carrier superfamily that ensures the highly dynamic traffic required for the full integration of the mitochondrion into cellular metabolism.

For two decades the function of UCP1 from brown adipose tissue was considered to be a unique mechanism evolved in eutherian mammals to allow regulated dissipation of the proton gradient when non-shivering thermogenesis was

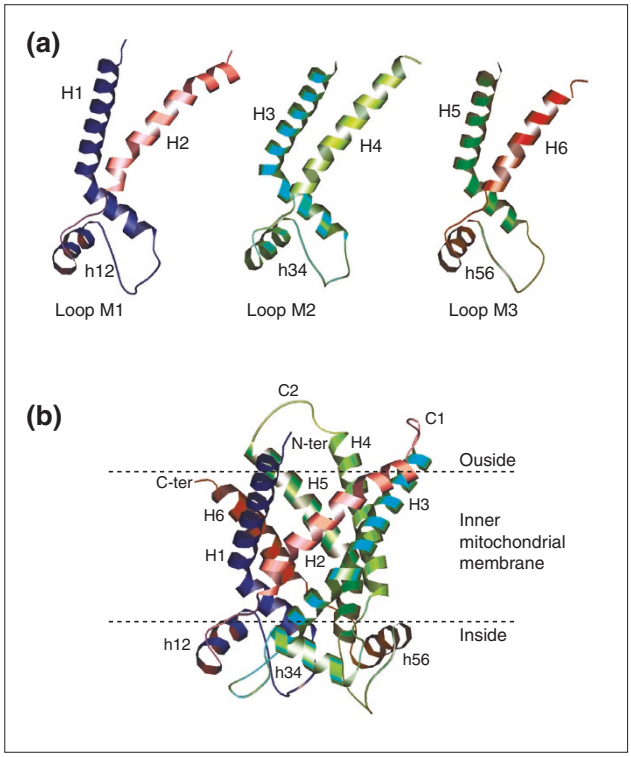

Figure 1
Three-dimensional structure of the adenine nucleotide translocator. **(a)** Ribbon representation of the structure of the three sequence repeats that constitute the transporter. **(b)** Lateral view of the complete three-dimensional structure of the carrier. Modified from [4] with permission from Gérard Brandolin.

required. Furthermore, the presence of a nucleotide-binding site in UCP1 was considered reminiscent of that found in the ANT. Since 1997, however, proteins with relatively high sequence similarity to UCP1 have been found in plants and other animals, including invertebrates, making up a distinct UCP protein family within the larger mitochondrial carrier protein superfamily. The functions of these other members of the UCP protein family are not fully established, but available data point to a general role in protection against oxidative stress. As mentioned earlier, the acceleration of respiration due to UCP-mediated uncoupling would lead to a reduction in ROS production by the respiratory chain. There are now many known examples of UCPs being upregulated in physiological situations of oxidative stress, and thus they are widely considered to be part of the antioxidant defense system of eukaryotes [5].

In a phylogenetic analysis of the mitochondrial carrier protein superfamily made by our group in 2006 [6] (inset in Figure 2), each member was recovered as a distinct paralog (except UCP3). According to our reconstructed phylogeny,

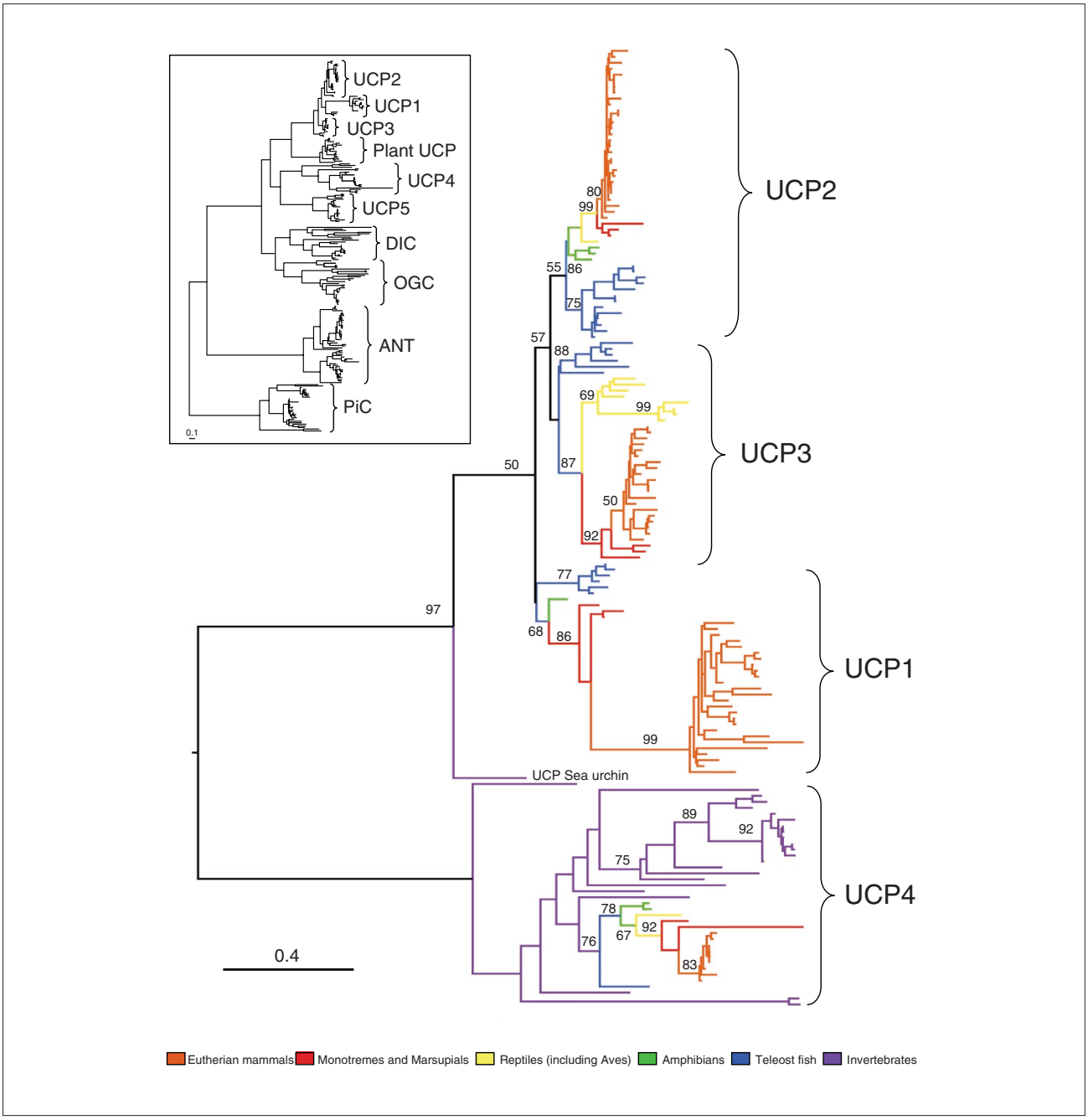

Figure 2
Evolutionary relationships of UCP1-3 family members. We have reconstructed a phylogeny using a total of 161 protein sequences of UCP1-4 retrieved from GenBank, and aligned using Mafft v. 6.626 with the L-INS-i strategy. A final alignment of 281 positions was obtained after removing ambiguous positions using Gblocks v.0.91b. The JTT+I+G was selected as the best-fit evolutionary model using Prottest v. 2.0. The maximum likelihood tree (-lnL = 15417.6) was inferred using PhyML v. 2.4.4 with midpoint rooting. An approximately unbiased test performed using RaxMLv. 7.0.4 and Consel v. 0.1 determined that the constrained tree (-lnL = 15433.2) shown in the figure was not significantly different ($P > 0.05$) and, thus, within the confidence set. Bootstrap analysis was performed using RaxML at the Cipres Portal, and bootstrap values for relevant nodes are shown in the tree. Taxonomic groups are represented by different colors. Inset: phylogeny of mitochondrial carrier proteins adapted from [6]. Our reconstructed phylogeny shows animal UCP4 and UCP5 (also termed BMCP1) as a sister group of plant UCPs and animal UCP1-3. The other members of the superfamily analyzed - PiC, ANT, OGC (oxoglutarate carrier) and DIC (dicarboxylate carrier) - were found to be more distantly related paralogs.

new functions within the superfamily have generally been achieved through gene duplication and subsequent functional diversification leading to high substrate specificity. Nomenclature of protein families should be based on homology, which is determined through phylogenetic analyses. In this regard, the reconstructed phylogeny of the mitochondrial carrier protein superfamily may prompt revision of its current nomenclature. UCP1-3 and plant UCPs share a common ancestor to the exclusion of animal UCP4 and 5, which therefore may need to be renamed. The definition of a mitochondrial transporter as an 'uncoupling protein' implies the recognition that its activity results in a controlled dissipation of the proton gradient. However, the consensus on the transport activities of the different UCPs gets poorer as we move away from UCP1. The scenario is even more complex because there is evidence that some mitochondrial carriers may also act as uncoupling proteins. Thus, the ANT or the PiC can increase the proton conductance in the presence of high concentrations of fatty acids. Future research will probably reveal differences in the molecular mechanism used by the different members of the UCP family to achieve the increase in respiration, in the regulation of their activity or even in their physiological roles.

The evolution of the UCP family

To throw more light on the diversification of the UCP family and the evolution of the apparently unique function of UCP1 in thermogenesis, several recent phylogenetic analyses have focused on vertebrate UCP1-3 relationships [7-11]. The work of Hughes and Criscuolo published recently in *BMC Evolutionary Biology* [7] has confirmed previous studies indicating that the UCP family evolved through a series of gene duplications [8]. We have made a reconstructed phylogeny of vertebrate UCP1-3 using animal UCP4 as outgroup (Figure 2) that is in good agreement with those previously published [7,10,11]. As shown in the figure, UCP4 has been widely reported both in invertebrates and vertebrates, but apparently no duplications occurred during the evolution of this paralog. In contrast, vertebrate UCP1-3 acquired much of their diversity through two rounds of gene duplication [7,8,10]. The ancestor of vertebrate UCP1-3 first duplicated into UCP1 and the common ancestor of UCP2-3, which subsequently duplicated into UCP2 and UCP3. Each of the three paralogs is found in fish, amphibians, and mammals. Strikingly, UCP1 and UCP2 have not been reported in birds, nor UCP1 in sauropsids. These proteins have a single ortholog in invertebrates (it has been reported in, for example, a deuterostome (the sea urchin), but not in the fully sequenced protostome genomes of *Drosophila* and *Caenorhabditis*).

The phylogenetic analyses based on vertebrate UCP protein sequences, together with the reported conservation of syntenic regions, demonstrates that there are orthologs of UCP1 in mammals, amphibians, and fish [7,8,10,11]. Hence, UCP1 is found in vertebrates with and without non-shivering thermogenesis. The long branch leading to eutherian UCP1 is indicative of strong structural divergence, and the studies of Hughes and Criscuolo [7] and Hughes *et al*. also in *BMC Evolutionary Biology* [10] indicate that observed amino acid changes are due to purifying rather than positive selection. UCP1 from eutherian mammals presents two distinct biochemical properties: a high nucleotide-sensitive basal proton conductance in the absence of fatty acids; and a high affinity for fatty acids (physiological activators). Hence, it seems clear that structural divergence was accompanied by a functional shift. It can be envisaged that ancestral UCP1 probably had a role in protection against oxidative stress in the tissues where it was expressed, and that the coexistence of paralogs (UCP2 and 3) that could fulfill this function, together with the restriction of UCP1 expression to brown adipose tissue, allowed it to assume the thermogenic role in eutherians [10]. The recovered phylogeny should prompt further characterization of the biochemical activity and regulation of fish and marsupial UCP1 orthologs, which are likely to be different from that of eutherian UCP1. Interestingly, the expression of the carp UCP1 in the liver decreases when fish are exposed to cold, thus ruling out a thermogenic function [12].

Although the uniqueness of the properties of UCP1 in eutherians has provoked lengthy discussions in the literature, the biochemical characterization of mutants designed to test the molecular basis of differences between UCP paralogs is now providing clear answers. Thus, the substitution of Glu134 by Asp in UCP1 results in a marked decrease in the basal proton conductance [6]. Glu134 is a shared derived residue of eutherian UCP1, this position being occupied by Asp in all other UCPs. Even in the carp UCP1, position 134 is Asp, and the biochemical characterization of carp UCP1 revealed no nucleotide-sensitive basal proton conductance [12]. In addition, several groups have searched for the domain that confers the high affinity for fatty acids on UCP1 by generating protein chimeras of domains from UCP1, UCP2 and UCP3. These studies showed that the hydrophilic loop that connects transmembrane domains 3 and 4 is responsible for the high fatty-acid affinity of UCP1. These specific transport properties fit with the regulation of thermogenesis, and provide evidence that eutherian UCP1 has evolved to achieve its heat-generating capacity in the physiological context provided by the brown adipocyte.

Acknowledgements

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