Perspectives on the biology of uncoupling protein (UCP) homologues
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Abstract
In mammals, it is believed that a portion of tissue metabolic rate is driven by counteraction of uncoupling, in which the energetically inefficient process of proton leak acts to diminish the mitochondrial electrochemical membrane potential. It is proposed that specific proteins associated with the mitochondrion catalyse uncoupling, and the biology of such putative uncoupling proteins (UCPs) is the subject of active research efforts. UCP4 and UCP5 are interesting in light of their abundant expression in the brain, which may signal an important metabolic function in thermogenesis or regulation of reactive oxygen species in that tissue. While each is expressed to various degrees outside of the brain, their impact on whole-animal metabolism remains to be clarified further. Transgenic mice expressing murine UCPS₄, the long isoform of UCP5, using an inducible metallothionine promoter (to drive expression of the transgene in liver, testis, heart, lung, spleen, intestine, kidney and brain) did not display any overt metabolic phenotype, despite liver UCPS₄ mRNA expression equivalent to that of normal mouse brain. This highlights the need for further studies to examine the nature of UCP5 physiology. Evidence for uncoupling behaviour has recently emerged from studies of the human 2-oxoglutarate carrier (OGC), indicating that the possibility of physiological proton leak elicited by the OGC and other mitochondrial carriers warrants further experimental evaluation.

Introduction
Recent scientific and societal developments have sparked a 'renaissance' of interest in the fields of metabolic regulation and bioenergetics. This movement has been supported by rapid advancements in molecular biology and bioinformatics, enabling the identification of numerous unique metabolic players with unprecedented speed. Such progress holds promise in helping to elucidate the molecular basis of thermoregulation and energy balance, which may in turn facilitate development of therapeutic modalities to treat obesity, diabetes and other conditions associated with a breakdown of metabolic homoeostasis.

Ironically, although technological and biological advancements have occurred swiftly in the metabolic arena over the past few years, the velocity of the progression of metabolic disease has concurrently increased (see [1]). As of the mid-1990s, for instance, one in five Americans were considered obese (body-mass index > 30 kg/m²), compared with just ≈ 14% a decade earlier. It is estimated that an additional 25–40% of the U.S. population are overweight (body-mass index = 25–29.9 kg/m²). Overweight and obesity are associated with significantly higher risks of type 2 diabetes, coronary heart disease, high blood pressure and other disorders.

Therapeutic approaches which promote negative energy balance will be valuable clinically in efforts to reverse the effects of excessive weight gain. In light of this fact, the central role of mitochondria in driving fuel combustion and metabolic rate make biological processes associated with this organelle interesting subjects of research. One phenomenon studied in some detail is mitochondrial proton leak, in which a portion of the mitochondrial proton electrochemical potential (Δp) is dissipated through mechanisms independent of proton flow through the F₁Fₐ ATP synthase (a process termed uncoupling). Mitochondrial proton leak is a common feature of most mitochondria, and may account for a significant portion of the tissue metabolic rate in mammals [2,3]. Can mitochondrial proton leak be exploited therapeutically? Do specific proteins

Key words: fatty acids, metabolic rate, mitochondria, obesity, thermogenesis.
Abbreviations used: UCP, uncoupling protein; BAT, brown adipose tissue; OGC, 2-oxoglutarate carrier; CNS, central nervous system; ROS, reactive oxygen species; RT-PCR, reverse transcriptase PCR; SKM, skeletal muscle; WT, wild-type.
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catalyse the uncoupling observed in various tissues? The answers to these questions lie in the elucidation of biochemical and biophysical phenomena underlying mitochondrial proton leak.

**Identification of uncoupling protein (UCP) homologues**

The concept that specific proteins may drive uncoupling was proven with the discovery and subsequent characterization of a unique protein, now termed UCP1, which facilitates proton leak in thermogenic brown adipose tissue (BAT) [4,5]. In addition to biochemical evidence for uncoupling via UCP1, it is clear that the presence and induction of UCP1 in brown adipose is critical for maximal thermogenesis in response to cold in vivo [6], and its activity is necessary for catecholamine- and fatty acid-induced respiration [7]. A growing appreciation for UCP1 biology sparked the notion that additional UCP functional family members may exist, and could participate in the observed proton leak determined in tissues outside of BAT. Recently, proteins with almost 60% homology to UCP1 were discovered (UCP2 and UCP3) [8–12] and found to lower mitochondrial membrane potential (Δψm) upon overexpression, consistent with uncoupling behaviour under these conditions. Subsequently, the brain-abundant family members UCP4 [13] and UCP5 [14] were described. UCP2 and UCP3 are the subject of a number of recent reviews (i.e. [15–17]) and contributions to this colloquium, and thus will not be the focus of this report. Instead, we wish to provide some perspectives on the biology of UCP4 and UCP5, whose physiological characterization remains an interesting area of discovery. Furthermore, commentary regarding the mitochondrial carrier proteins 2-oxoglutarate carrier (OGC) and CGI-69 is presented.

**UCP4 and UCP5**

As for other tissues, proton leak is an active pathway in brain mitochondria [18], raising the possibility that UCP-like proteins reside in this tissue. Indeed, UCP2 mRNA was found to be present in numerous brain regions of the mouse, with relatively strong expression in the hypothalamus [19]. However, its brain mRNA abundance was not altered by experimental manipulations such as fasting or cold-exposure [19], and its protein levels were negligible in this tissue despite the presence of mRNA [20]. Additional UCP homologues, UCP4 and UCP5, have recently been described to display relatively high expression in the brain [13,14,21,22], and thus may play a role in regulation of metabolism in this tissue.

There is much to be learned regarding the biology of UCP4, whose almost exclusive expression in the brain as measured by Northern-blot analysis is remarkable [13]. Recently, there has been a suggestion that UCP4 represents an ‘ancestral’ UCP [23]. We have postulated a metabolic role for UCP4 in central nervous system (CNS) [13]. Interesting in this regard, whole-brain UCP4 mRNA increased significantly when mice were exposed to a cold environmental temperature [21], but the specific cell types and regions of induction were not measured. UCP4 may also be involved with regulation of reactive oxygen species (ROS) and/or thermoregulation in specific cells of the CNS. Indeed, growing evidence indicates that other UCP homologues, probably through activities that lower Δψm [24], help minimize excessive mitochondria-derived ROS production [25,26]. Finally, it is notable that quantitative real-time reverse transcriptase PCR (RT-PCR) using oligonucleotide probes recognizing UCP4 mRNA indicates expression of the gene in tissues such as BAT, skeletal muscle (SKM), liver and white adipose tissue, but the mRNA levels are relatively minor (< 0.1% of UCP1 mRNA in BAT, < 5% of UCP3 or UCP2 mRNAs in SKM and liver, respectively, and 10% of UCP2 mRNA in white adipose tissue; L. John, T. A. Stewart, X. X. Yu and S. H. Adams, unpublished work). Detectable UCP4 mRNA in a variety of tissues may signal a metabolic function for this mitochondrial carrier in certain cells body-wide. However, abundant brain expression relative to all other tissues appears to point to a primary physiological role in the CNS.

As for UCP4, UCP5 represents a UCP homologue whose brain-abundant mRNA [14,21,22] is consistent with an important physiological relevance for this protein in the CNS. Indeed, UCP5 expression is widespread in the brain [14,21,22], suggestive of a broad role in CNS metabolism. UCP5 isoforms have been discovered [UCP5 short-form (UCP5s), UCP5 long-form (UCP5l) and UCP5l with insert (UCP5lI)], with apparent differences in potency for lowering Δψm when overexpressed in vitro [21]. Notably, in addition to lowering Δψm, UCP5-transformed yeast mitochondria display proton leak kinetics consistent with increased uncoupling behaviour [14]. A function for UCP5 activity in regulation of CNS ROS production and regional thermogenesis...
has been proposed [14,21]. Relevant in this regard, whole-brain UCP5 mRNA increased significantly in cold-challenged mice [21]. Importantly, UCP5 mRNA is present body-wide, with expression in testis, heart, kidney, uterus and elsewhere [14,21,22]. This expression pattern is consistent with a metabolic function of UCP5 outside the CNS. Modulation of UCP5 expression in the SKM and liver after lipopolysaccharide in mice was significant, indicating that the gene is regulated via signals generated following this immune challenge [27]. UCP5 mRNA levels generally tracked changes in body temperature following lipopolysaccharide [27], and liver mRNA was increased upon cold-exposure [21]. However, considering the relatively low liver or SKM mRNA levels compared with the brain, and the lack of altered uncoupling in isolated mitochondria from tissues displaying large changes in UCP5 mRNA [27], the impact of physiological changes in UCP5 activity on global thermogenesis or ROS production remains to be clarified further.

Analyses of UCP4 and UCP5 physiological function will benefit from experiments which titrate their abundance in vivo through genetic modification of animals. To this end, transgenic constructs were prepared for generation of mice overexpressing murine UCP5L driven by the inducible metallothionein (MT1) promoter (G. Pan, unpublished work), thus enabling expression in tissues sensitive to this promoter (liver, testis, heart, lung, spleen, intestine, kidney and brain) upon introduction of ZnSO4 (25 mM) into the drinking water at weaning. Expression levels of UCP5L in MT1-mUCP5L-transgenic (mUCP5L-Tg) mice were checked by quantitative real-time RT-PCR analysis of liver biopsies taken after \( \approx 3-4 \) weeks on zinc/water. For mUCP5L-Tg mice used for physiological evaluations, liver expression levels were of the same magnitude as that observed in normal mouse whole brain (results not shown).

No overt metabolic phenotype was detected in mUCP5L-Tg mice in our preliminary studies. For instance, metabolic rate (via indirect calorimetry) in adult male mUCP5L-Tg founders (\( n = 4 \)) was not different from wild-type (WT, \( n = 9 \)) mice during the light cycle (3.72±0.37 versus 3.87±0.35 ml of \( O_2/g \) per h in WT and mUCP5L-Tg, respectively; means±S.D.) or dark cycle (3.66±0.34 versus 3.77±0.22 ml of \( O_2/g \) per h in WT and mUCP5L-Tg, respectively). Metabolic rate was also unchanged in founder mUCP5L-Tg females compared with WT mice (results not shown). Consistent with these findings, body temperature in male or female \( F_1 \) mUCP5L-Tg progeny (as measured by use of a rectal probe at 2 and 3 months of age, or via continuous telemetry using an implanted thermocouple) did not differ relative to WT mice (results not shown). Body weights of \( F_1 \) mUCP5L-Tg progeny are depicted in Figure 1. Although mUCP5L-Tg males displayed a lower mean body weight (\( \approx 1 \) g) at 2 or 3 months of age, and female Tg mice were slightly smaller (\( \approx 1 \) g) at 3 months of age, these differences were not statistically significant (\( P > 0.1 \)).

The data generated from mUCP5L-Tg mice emphasize the need for further studies to more completely characterize the physiological function of UCP5. The lack of significant alterations in whole-animal metabolic rate in mUCP5L-Tg
mice, for instance, may have resulted from a need for even higher expression and translation of the gene (e.g. in SKM) to elicit a robust response impacting metabolic rate. Alternatively, the data may signal that alternative metabolic functions for UCP5 exist beyond thermogenesis.

Do unsuspected mitochondrial carriers participate in physiological uncoupling?

In an effort to elucidate the primary players driving mitochondrial proton leak in mammals, much attention has focused on the discovery and characterization of genes encoding proteins with various degrees of homology to UCP1, the archetypal UCP. The initial discoveries of UCP2 and UCP3 were particularly exciting in light of their potential relevance to whole-body uncoupling and hence thermogenesis. However, results from UCP2- and UCP3-knockout mice would indicate that the loss of these proteins has a negligible effect on global metabolic rate or body weight [25,26,28,29]. At least four possibilities emerge from such findings.

First, it is possible that certain UCP homologues such as UCP2 or UCP3 do in fact facilitate important mitochondrial activities, but in a manner limited to very specific cell types and/or mitochondrial subpopulations, thus yielding a negligible impact on whole-animal thermogenesis.

Indeed, the loss of UCP2 or UCP3 in knockouts yielded increased ROS production concurrent with elevated $\Delta \psi_m$ specifically in those tissues normally expressing the missing protein [25,26], indicative of a function for these proteins in maintaining a normal $\Delta \psi_m$ below the threshold of excessive ROS generation, yet with an undetectable effect on body weight or whole-animal metabolic rate [25,26,28,29].

A second, albeit rarely mentioned, possibility is that widely accepted estimates regarding the contribution of physiological proton leak towards the metabolic rate (i.e. $\approx 20-40\%$ of tissue metabolic rate) [2,3] are excessive. If true, the loss of a degree of uncoupling through genetic ablation of certain *bona fide* UCPs *in vivo* would not be expected to alter whole-animal metabolic rate appreciably.

Third, it has been proposed that the uncoupling observed after experimental overexpression of various UCP homologues *in vitro* is artifactual and probably the result of mitochondrial damage [30], and therefore not reflective of an uncoupling role *in vivo*. Such assertions, supported by careful studies of UCP homologue protein titration in transformed yeast, warrant serious consideration. However, it is not clear if these experiments reflect the situation in mammalian cells, where cell-specific factors would be expected to regulate UCPs *in situ*. As noted above, UCP2- and UCP3-knockout mice do in fact display mitochondrial changes consistent with a diminution of uncoupling in certain cell types [25,26,28,29]. Furthermore, we have observed that overexpression of the unique mitochondrial carrier protein CGI-69 does not impact $\Delta \psi_m$ in transfected HEK-293 cells, despite a $>30$-fold increase in its mRNA abundance compared with non-transfected cells [31]. Although assessments of the impact of CGI-69 on uncoupling must ultimately track changes in its protein levels in affected cells, the results to date for CGI-69 suggest that artifactual proton leak due to mitochondrial damage is not the inevitable result of overexpression of mitochondrial carriers.

Fourth, the proton leak observed in most tissues may reflect the aggregate activities of multiple mitochondrial proteins, each contributing some fraction of uncoupling activity towards the whole. Relevant in this regard, overexpression of the human OGC in HEK-293 cells was found to significantly lower $\Delta \psi_m$, suggestive of previously unappreciated uncoupling behaviour [31]. Should the OGC in fact display UCP activity *in vivo*, its
broad expression pattern (Figure 2) suggests its impact on uncoupling would be widespread. Therefore, additional research is warranted to assess the degree to which the OGC and other carriers may participate in physiological uncoupling.

Closing remarks

The biology of thermoregulation and bioenergetics has progressed rapidly over the last few years, with the characterization of a number of mitochondrial carrier proteins which appear to have important roles in metabolic processes. Despite such strides, it has become clear that the molecular basis of energy expenditure and mitochondrial proton leak is exceedingly complex, sparking a number of lively discussions regarding UCP homologue physiological function. From a therapeutic viewpoint, however, it is clear that acceleration of mitochondrial respiration, via administration of chemical uncoupling agents [32], through strategies designed to induce UCPS in appropriate tissues [33] or by other means, is an attractive strategy to counteract inordinate weight gain and associated metabolic disorders.

References


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