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Review

The regulation and turnover of mitochondrial uncoupling proteins

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ABSTRACT

Uncoupling proteins (UCP1, UCP2 and UCP3) are important in regulating cellular fuel metabolism and as attenuators of reactive oxygen species production through strong or mild uncoupling. The generic function and broad tissue distribution of the uncoupling protein family means that they are increasingly implicated in a range of pathophysiological processes including obesity, insulin resistance and diabetes mellitus, neurodegeneration, cardiovascular disease, immunity and cancer. The significant recent progress describing the turnover of novel uncoupling proteins, as well as current views on the physiological roles and regulation of UCPs, is outlined.

1. Introduction

Mitochondria are the centre of metabolism in cells, coupling the oxidation of substrates to ATP synthesis by an electrochemical proton gradient. Varying this protonmotive force allows for adjustments in energy metabolism to maintain metabolic homeostasis. For this reason, the coupling of substrate oxidation is incomplete, as protons can leak across the mitochondrial inner membrane independently of ATP production. This unregulated futile proton conductance is of considerable physiological relevance, as it can account for as much as 20–70% of cellular metabolic rate depending on cell type [1,2]. A majority of proton leak can be strictly attributed to the abundance, but not activity, of mitochondrial carrier proteins such as the adenine nucleotide translocase (ANT) and, in brown adipose tissue (BAT), uncoupling protein 1 (UCP1) [3,4].

Importantly, the regulation of proton leak allows for responses to fluctuations in energy demands and controls energy transduction to maintain cellular homeostasis and body function. The first proton leak mechanism was identified in BAT, where UCP1-catalysed proton conductance generates heat to defend body temperature during cold acclimation [5]. Sequence similarity allowed the identification of its paralogous proteins UCP2 and UCP3 [6,7]. These UCPs do not contribute to basal proton conductance *in vitro* in the absence of specific activators [8]. When activated, however, all UCPs (including avian and plant UCPs) can catalyse proton leak [9]. The precise mechanisms of activation and inhibition of both UCP2 and UCP3, as well as their physiological role, remain uncertain [10,11]. There has been considerable recent progress, however, in understanding the transcriptional and translational regulation that implicates UCP2 and UCP3 in adaptation to nutritional status and oxidative stress. More recently, the unique dynamic regulation of UCP2 reveals a new mechanism for the regulation of mitochondrial energy metabolism by the novel UCPs.

2. Acute activation of uncoupling protein activity

UCP1 activity is highly regulated at the molecular level by small molecules. It is inhibited by physiological concentrations of purine nucleoside di- and tri-phosphates and stimulated when fatty acids overcome nucleotide inhibition [12].

How fatty acids activate the net protonophoric activity of UCP1 is still debated. Broadly, there are three models that can explain the dependence on fatty acids. In the first, fatty acids act as co-factors by embedding their carboxyl groups in the core of the protein to bind and release protons as they access amino acid side chains during transport [13]. Evidence that UCP1 can translocate chloride and fatty acid anions suggests a second model. In this mechanism, protonated fatty acids

Abbreviations: ANT, adenine nucleotide translocase; ATF1, Cyclic AMP-dependent transcription factor; ATP, adenosine triphosphate; BAT, brown adipose tissue; GDP, guanosine diphosphate; ORF, open reading frame; PPAR, peroxisome proliferator-activated receptor; SREBP-1c, sterol regulatory element-binding protein-1c; TRE, thyroid response element; UCP, uncoupling protein; UTR, untranslated region

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freely diffuse across the mitochondrial inner membrane. The pH gradient promotes their dissociation into fatty acid anions in the matrix, and the fatty acid anions are then exported from the matrix by UCP1 [14]. The net activity results in proton conductance across the inner membrane, though in this model UCP1 itself does not translocate protons. Thirdly, fatty acids themselves may not be directly required for UCP1 activity, but instead act as allosteric activators by promoting a conformation of the protein that is protonophoric (or that translocates hydroxide ions), since fatty acids and nucleotides appear to affect proton conductance in a manner described by simple competitive kinetics [15,16].

It remains unclear to what extent UCP2 and UCP3 are subject to the same acute molecular regulation as UCP1 (and the extent to which they share the same mechanism of uncoupling). Although they lack sequence homology in a matrix-localised region reportedly critical for fatty acid activation of UCP1 [17], proteoliposome studies show that UCP2 and UCP3 have similar fatty acid-activated proton conductance and purine nucleotide inhibition as UCP1 [18–20]. One difficulty has been the inability to directly compare UCPs in mitochondria, since UCP2 and UCP3 are expressed in different tissues and at hundred-fold lesser amounts than UCP1 [21–23]. Another difficulty relates to the fact that GDP has been shown to inhibit uncoupling via ANT [24,25] as well as by the UCPs. This complicates the calculations of UCP-mediated proton leak in tissues that express different amounts of UCP and ANT when activity is defined as GDP-sensitive uncoupling.

There is evidence that superoxide, both exogenous [26] and endogenous [27], and lipid peroxidation products such as hydroxynonenal [25,28,29] can activate uncoupling by all three UCPs, suggesting a model in which superoxide reacts with membrane phospholipids to generate the proximal activator, hydroxynonenal [28,30]. The physiological relevance of this model, which has not been reproduced in all laboratories, remains controversial [10,31–33].

3. Role and regulation of uncoupling proteins

The archetypal uncoupling protein, UCP1, is best known for its role in adaptive non-shivering thermogenesis and control of body weight, whereby a cold stimulus or over-feeding results in sympathomimetic stimulation of β_3-adrenergic receptors in BAT. This leads to upregulation of *Ucp1* mRNA expression via a BAT-specific enhancer box [34], activation of UCP1 by fatty acids [35] produced from lipolysis [36], and the transduction of the mitochondrial protonmotive force into heat [37]. Indeed *Ucp1* knockout results in the absence of non-shivering thermogenesis [38], loss of cold tolerance [39] and appearance of obesity at thermoneutrality [40]. Beyond thermogenesis, the role of UCP1 in thymus [41,42] and in ectotherms [43] remains speculative.

The UCP1 paralogues, UCP2 and UCP3, probably evolved from a duplication event in vertebrates. This is supported by their juxtaposition in the genome and their high sequence identity with each other (72–74% from fish to mammals). Sequence analysis shows that unlike UCP1, UCP2 and UCP3 are under strong purifying selection, suggesting that they have not changed function during evolution [44].

The literature varies on whether or not UCP2 and UCP3 are upregulated in response to cold in various organisms and tissues [45–47], but they are not thought to be significantly thermogenic [48], primarily because of their low abundance. However, rodent UCP3 can participate in thermogenesis under particular conditions [49,50]. UCP2 and UCP3 are also upregulated in response to starvation, and have been linked with a number of processes including insulin secretion from pancreatic β-cells [51] and insulin resistance [52] in peripheral tissues, as well as modulation of reactive oxygen species production and immune responses [10,53–55].

3.1. UCP2 function

An ever-increasing number of studies highlight the significance of UCP2 in a broad range of physiological and pathological processes, including cytoprotection [55–58], immune cell modulation [53,59] as well as the regulation of glucose sensing in the brain [60] and pancreas [51].

In thymocytes [61] and the intact INS-1E pancreatic β-cell model [62], UCP2 decreases the coupling between substrate oxidation and ATP production. Since mitochondrial ROS production is highly sensitive to decreases in protonmotive force [63–65], UCP2-mediated dissipation of the mitochondrial membrane potential and pH gradient results in decreased reactive oxygen species production [66,67], particularly during reverse electron transport [65].

In glucose-sensing cells in the pancreas and brain, UCP2 attenuates insulin secretion, likely acting in two ways. By lowering the coupling efficiency of oxidative phosphorylation, UCP2 decreases the ATP/ADP ratio, resulting in the decreased stimulation of K_{ATP} channels and lowered insulin secretion [51,68]. It may also function by decreasing ROS production [67], which is important signal in glucose-sensing systems [69,70].

As well as improving the diabetic phenotype via increased insulin secretion [51], UCP2 downregulation also improves insulin resistance in peripheral tissues such as white adipose [71]. Although much work indicates that UCP2 exacerbates the diabetic phenotype, recent work from the Collins group suggests that this effect is dependent on genetic background, and that the chronic absence of UCP2 causes persistent oxidative stress in general and impairs β-cell function [72]. However, it is unlikely that these findings simply invalidate all previous work demonstrating attenuation of glucose-stimulated insulin secretion by UCP2. For example, acute *in vivo* knockdown of UCP2 using antisense oligonucleotides in two animal models of diabetes and insulin resistance causes a significant improvement in insulin secretion and enhanced whole-body sensitivity to insulin [73]. In the light of the cytoprotective effects conferred by UCP2, however, Pi *et al.* [72] question the validity of the approach of inhibiting UCP2 function in order to improve glucose-stimulated insulin secretion in diabetes [74]. Numerous studies have shown that by attenuating oxidative stress, UCP2 promotes cell survival in pancreatic α- [58] and β- [55] cells and in neurones [57], and can regulate colon tumour formation [75] and atherosclerosis [56,76].

Newell and colleagues propose an interesting hypothesis in which a cell's ability to efficiently metabolise fat confers immune privilege. Specifically, they suggest that UCP2 is a part of the mechanism controlling the change from one metabolic strategy (glucose metabolism) to another (primarily lipid metabolism), and by doing this, UCP2 plays a role in preventing immune-mediated pathology [54,77]. This, of course, is in line with the cytoprotective effect of UCP2 described earlier. Bouillaud has recently proposed that this function could be explained by a uniport for anionic pyruvate that lowers the preference for pyruvate oxidation as membrane potential increases [78]. However, this hypothesis has yet to be experimentally verified and remains speculative.

3.2. Regulation of UCP2 concentration

UCP2 regulation occurs in a concerted manner by modulation of protein activity and protein content. Ligands (such as fatty acids and ROS derivatives) that stimulate UCP2 catalytic activity in isolated mitochondria [11] may also play a role in upregulating UCP2 content [79].

In hyperglycaemia and hyperlipidaemia, as occurs in diabetes mellitus, *Ucp2* gene transcription is activated by key regulatory proteins such as peroxisome proliferator-activated receptors (PPARs), forkhead transcription factors, and sterol regulatory element-binding protein-1c (SREBP-1c) [80]. Sirt1, a protein that has been implicated in metabolic stress resistance, suppresses the function of these proteins, thus decreasing *Ucp2* expression and promoting insulin secretion [81]. Additionally, reactive oxygen species and their products have been implicated in the upregulation of UCP2