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The on/off switches of the mitochondrial uncoupling proteins

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Abstract

Mitochondrial uncoupling proteins disengage substrate oxidation from ADP phosphorylation by dissipating the proton electrochemical gradient that is required for ATP synthesis. In doing this, the archetypal uncoupling protein, UCP1, mediates adaptive thermogenesis. By contrast, its paralogues UCP2 and UCP3 are not thought to mediate whole body thermogenesis in mammals. Instead, they have been implicated in a variety of physiological and pathological processes, including protection from oxidative stress, negative regulation of glucose sensing systems and the adaptation of fatty acid oxidation capacity to starving. Although much work has been devoted to how these proteins are activated, little is known of the mechanisms that reverse this activation.

Oxidative phosphorylation and uncoupling

Mitchell's chemiosmotic hypothesis proposed a mechanism by which oxidation of nutritional substrates is coupled to ATP synthesis in mitochondria [1]. During substrate oxidation, electrons move through the respiratory chain, which concomitantly ejects protons from the mitochondrialmatrix into the intermembrane space. This process establishes a proton electrochemical gradient or protonmotive force (Δp), which is dissipated when protons move back into the matrix. Such dissipation occurs largely through the F_o/F_1 ATP synthase, whose rotary action catalyses the generation of ATP from ADP and P_i [2]. However, oxidative phosphorylation is not fully coupled. Although several explanations could account for this, a review of the experimental evidence suggests that proton leak is likely to be the main mechanism involved in uncoupling substrate oxidation and ATP synthesis (Figure 1) [3].

Proton leak flux through detergent-free liposomes made from mitochondrial inner membrane phospholipids comprises only 5% of the flux under comparable conditions in intact mitochondria [3], implying that up to 95% of proton leak in intact mitochondria is mediated by membrane proteins. The involvement of proteins in mediating proton leak has been elucidated by biochemical and genetic experiments that manipulate protein expression and by specific protein inhibitors that allow estimation of proton leak that occurs via that protein.

Uncoupling or proton leak can be mediated by uncoupling proteins (UCPs), which are the focus of this review, and by other mitochondrial inner membrane proteins, including the adenine nucleotide translocase (ANT) [4] and the glutamate carrier [5], or complexes such as the mitochondrial permeability transition pore [6].

The estimate that proton leak contributes ~25% to standard metabolic rate shows that it is metabolically expensive and likely to be an important process. In keeping with this notion, the postulated functions of UCPs include cold- and diet-induced thermogenesis, decreasing reactive oxygen species (ROS) production, metabolic and energy balance and regulation, glucose sensing and adaptation to fasting [7].

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The UCP family

Uncoupling proteins are a subfamily of the mitochondrial solute carrier family, proteins that are metabolite transporters with a characteristic tripartite structural repeat of ~100 amino acids. The canonical uncoupling protein UCP1 was first discovered in brown adipose tissue (BAT) 30 years ago and has since been characterized as the mediator of adaptive thermogenesis in mammals [8,9].

Two further paralogues of UCP1, UCP2 and UCP3, were discovered in 1997 [10]. These 'novel' uncoupling proteins show ~60% sequence identity with UCP1 and ~70% identity with each other. This sequence similarity points toward a likeness in biochemical function in which they, like UCP1, can dissipate protonmotive force. However, the novel uncoupling proteins appear to differ physiologically from UCP1. Comparison of wild type and *Ucp2* or *Ucp3* gene-ablated mice has shown that UCP2 and UCP3 are not involved in adaptive thermogenesis or regulation of body weight [11], whereas *Ucp1* knockouts are both thermosensitive [9] and obese when euthermal [12].

Unlike UCP2 and UCP3, UCP4 and UCP5 (also called BMCP1) do not branch off the UCP subfamily ancestor (Figure 2). Indeed, they show less sequence identity with UCP1 than do other members of the mitochondrial solute carrier family, such as the oxoglutarate/malate and the dicarboxylate carriers. UCP numbering reflects simply the order of their identification, and which of the UCPs most closely resembles the subfamily ancestor remains unknown [13]. Recent phylogenetic analysis showing that UCPs are not limited to mammals but also are found in many eukaryotes, such as protozoa, plants and fish, suggests that their evolutionary emergence probably occurred before the divergence of fungal, plant and animal kingdoms [13,14]. This finding lends credence to suggestions that, as a family, UCPs are not solely thermogenic, but might have additional important functions in different tissues. Indeed, although a physiological mechanism has been demonstrated for the activation and deactivation of UCP1 in BAT, its role in other tissues and the function of its orthologues remains unclear. This ambiguity is largely true for the other UCP homologues.

In addition to models suggesting that fatty acids (FAs) and alkylsulphonates are UCP substrates [15], recent bioinformatic studies postulate that the substrates of UCPs are likely to be small carboxylic or keto acids transported with protons [16]. Identification of these substrates could yield considerable insight into the physiological functions of the UCPs. However, this claim has yet to be empirically substantiated and must be regarded with caution. Furthermore, work on UCP-containing proteoliposomes [17,18] suggests that proton conductance can still occur in the absence of these small substrates. This finding implies that either the substrate transport mechanism and proton conductance pathways are distinct (as they appear to be for ANT [19]) or that UCPs have evolved sufficiently differently from other transporters that protons are now their sole substrates and the retention of positively charged residues in the substrate binding site could serve a regulatory role (rather than a transport role) by interacting with phosphate moieties of nucleotides or carboxyl groups of FAs [16].

UCP1

UCP1 has classically been associated with mediating nonshivering thermogenesis in BAT. Although a good deal has been learned about UCP1 function since its discovery four decades ago, there is still controversy surrounding its mechanism of action at the molecular level and the tissue specificity of its expression.

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UCP1 in BAT

In (neonatal) mammals, adaptive thermogenesis in BAT occurs in response to prolonged cold exposure or chronic overfeeding. This adaptation is triggered by catecholamine release from sympathetic nerves that innervate the tissue. Direct electrical stimulation of these nerves or use of β_3-adrenergic receptor (β_3-AR) agonists results in induction of the thermogenic response, whereas denervation causes selective loss of UCP1 from BAT mitochondria[20]. Importantly, Locke *et al.* showed that mitochondrial uncoupling is not caused directly by catecholamines such as norepinephrine, but instead by FAs [21], which probably work by overcoming inhibition of activity by endogenous adenine nucleotides [20].

The induction of adaptive thermogenesis is highly regulated and culminates in UCP1 activation, which dissipates mitochondrial protonmotive force as heat. *Ucp1* ablation obliterates adaptive thermogenesis [9]. Remarkably, norepinephrine stimulation of β_3-ARs results in several concerted steps (Figure 3a): (i) activation of p38 mitogen-activated protein kinase (MAPK) pathways that upregulate UCP1 synthesis [22]; (ii) activation of protein kinase A (PKA)-mediated pathways that initiate lipolysis and release of acute regulators of UCP1 such as FAs [21]; and (iii) inhibition of lysosomal pathways that degrade UCP1 [23,24]. In other words, UCP1 is controlled at the synthesis, ligand-activation and proteolysis steps.

Even 30 years after the discovery that FA ligands activate UCP1 proton conductance, the mechanism by which this occurs is a matter of intense debate. The main proposed mechanisms (which apply also to UCP2, UCP3 and perhaps ANT) include the flip-flop model, the cofactor model, and the functional competition model (Figure 4) [20].

In the flip-flop model for UCP1, which is analogous to the FA cycling mechanism for ANT [25], a protonated FA crosses the mitochondrial inner membrane into the matrix, dissociates and the fatty acid anion is translocated out of the matrix by the protein. It is suggested that UCPs are fatty acid anion transporters and that cycling of the FA anion and its protonated counterpart cause uncoupling by inducing a net flux of protons into the matrix. In the cofactor model, FA carboxyl groups can buffer protons, thereby creating a proton-conducting channel. ‘Unprotonatable’ and ‘unflippable’ lipids, such as undecanesulfonate and glucose-*O*-ω-palmitate, respectively, have been used to attempt to delineate whether activation occurs via the flip-flop model or the cofactor model; however, results are inconsistent between laboratories. The functional competition model, by contrast, proposes that FAs act as allosteric ligands that affect UCP1 conformation, without needing to be involved directly in the transport mechanism. In short, a clear consensus is still lacking regarding the mechanism of UCP action, with each camp being able to call on evidence supporting their favoured model [20]. Recent bioinformatic studies on the mitochondrial solute carrier family show that, in contrast to the carnitine/acylcarnitine transporters, UCPs lack hydrophobic residues situated asymmetrically in their cavities, indicating that the activating FAs are not the translocated substrates [15]. This report is difficult to reconcile with the flip-flop mechanism, but it is compatible with the cofactor and functional competition models.

Some groups have investigated the chloride conductance capability of UCP1 in an attempt to validate certain transport models. For example, Echtay et al. concluded that the separate inactivation of proton and chloride transport argued against the flip-flop mechanism because chloride is likely to be transported via the same anion translocation pathway as FAs [26]. However, it can be argued that chloride is not a prerequisite for proton conductance because plant UCP transports FA anions but not chloride [27].

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Adding to the controversy, although purine nucleotides have been associated with UCP1 inhibition since the earliest studies on the protein, the nature of their interaction with FAs remains unresolved. Questions pertain to whether FAs displace nucleotides in order to activate UCP1, as well as how FAs can physiologically overcome inhibition of UCP1 by purine nucleotides (which are present at millimolar concentrations in the cell, but have binding constants for UCP in the micromolar range). This discrepancy might be explained by the fact that magnesium chelation of intracellular purine nucleotides would increase K_i to the millimolar range [28].

The reversal of uncoupling is physiologically prudent and can be achieved by altering protein activity and/or decreasing protein levels. In isolated mitochondria, FAinduced proton leak via UCP1 is reversed when FAs are washed away [21].

In addition to biochemical deactivation, UCP1-mediated uncoupling can be reversed by downregulating protein levels. Theoretically, this can be achieved by simply halting synthesis, e.g. by withdrawing adrenergic stimulation, and allowing degradation to remove the protein. However, adaptive thermogenesis also is regulated by modulation of UCP1 protein half-life. Extensive research on UCP1 degradation in BAT by Desautels and colleagues indicated that under basal conditions UCP1 has a turnover rate of 3.7 ± 0.4 days compared with 8.4 ± 0.9 days after chronic adrenergic stimulation via treatment with norepinephrine, which is the signal for initiating non-shivering thermogenesis [23]. Under these different conditions, the rates of degradation of UCP1 and other mitochondrial proteins are parallel. Along with the fact that lysosomal inhibitors also delay UCP1 turnover, this finding suggests that norepinephrine inhibits whole mitochondrial turnover by autophagy [24].

UCP1 as a therapeutic target

Although there was initially considerable pharmaceutical interest in developing β_3-AR agonists for the treatment of obesity and diabetes, until now this aim has been hindered by the inability to produce drugs that do not cross-react with β_1-AR or β_2-AR, and by the fact that BAT is found in only small quantities in humans. However, several findings might rekindle interest in this field. The first pertains to the use of a selective human β_3-AR agonist, which can increase metabolic rate in monkeys [29], and the second pertains to the quantity of BAT in adult humans. Recently, several studies demonstrated that adult humans have more functional BAT than was previously thought [30–32], with one study estimating that even at hugely submaximal activation (~10%) it could burn the energy equivalent of 4.1 kg of adipose tissue per year [32]. However, with only a small subpopulation possessing sufficient quantities of functional tissue to burn off 4.1 kg of fat per year [31], the recent discovery of a molecular switch for BAT production could provide another potential drug target for increasing BAT mass in humans [33].

UCP1 in different clades and tissues

Although UCP1 has long been thought to have co-evolved with BAT for the purpose of adaptive thermogenesis solely in eutherians (placental mammals), numerous studies are beginning to cast doubt on this theory. Recently, Jastroch and colleagues showed that the fat-tailed dunnart (*Sminthopsis crassicaudata*), a metatherian (marsupial) native to Australia, possesses BAT-like interscapular deposits whose UCP1 is upregulated in response to cold [34]. Work by the same group suggests that UCP1 is not constrained to therian lineages or to mediating thermogenesis. Conserved synteny of the region containing *Ucp1* extends to fish and amphibians, which raises the question of the role of the UCP1 orthologue in these ectotherms. Remarkably, the fish UCP1 orthologue is also activated by FAs and inhibited by purine nucleotides [35], analogous to its regulation in eutherians, but its functional

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significance remains mysterious. Although the use of synteny to establish orthology has been questioned by some, work by several groups demonstrates the presence of *Ucp1* in various taxa [13,14,36]. Interestingly, these studies show that there was an acceleration of evolution of *Ucp1* in the common ancestor of eutherians, possibly allowing the acquisition of enhanced or novel gene function.

Additionally, there is now convincing evidence that UCP1 is functionally expressed in mammalian thymus [20], with hints that it might be expressed also at the protein level in skin and pancreatic beta cells. Its role in these contexts remains elusive, but is likely not to be thermogenic.

The fact that UCP1 in different tissues retains the same biochemical properties that it displays in BAT and does not necessarily function to mediate thermogenesis suggests that it is UCP1 in the context of BAT and not just UCP1 *per se* that has evolved to mediate adaptive thermogenesis in eutherians. In this light, and with the knowledge that other UCPs can mediate mitochondrial uncoupling, calls for dismissing non-eutherian/non-BAT UCP1, UCP2 and UCP3 as *bona fide* mediators of proton leak [37] appear premature.

UCP2

In contrast to the tissue specificity of mammalian UCP1 (e.g. BAT and thymus), mammalian UCP2 is found in the kidney, pancreas, spleen, immune cells and the central nervous system [38]. The wide distribution of UCP2 has led to it being implicated in a variety of processes (e.g. regulation of ROS production [17,39], food intake [39], insulin secretion and immunity [17]) and pathologies (e.g. atherosclerosis [7], cancer [40], diabetes [17] and neuronal injury [7]).

The precise function of UCP2 in cells remains unknown [7,17]. Work in proteoliposomes, isolated mitochondria and whole cells indicates that it functions as a facilitator of proton leak, particularly when activated by superoxide and downstream lipid peroxidation products (Box 1). Yet other data suggest that UCP2 is not a *bona fide* uncoupling protein, but instead a metabolic switch that in some undefined way favours fatty acid metabolism over glucose metabolism, or that it is involved (along with UCP3) in mitochondrial calcium uniport [41]. However, these results might represent an accompanying secondary phenomenon. Furthermore, they have not been substantiated, with Brookes and colleagues demonstrating recently that UCP2 and UCP3 are not calcium uniporters or even a necessary part of such machinery [42].

BOX 1

The role of superoxide and lipid peroxidation species in activating mitochondrial UCPs

The respiratory chain generates superoxide radicals, which can be further processed into species such as the hydroxyl radical. These radicals diffuse in the inner membrane and attack membrane phospholipids and membrane-residing ω-6 polyunsaturated fatty acids (e.g. linoleic acid and arachidonic acid) to generate lipid hydroperoxides that are subsequently cleaved to form hydroxynonenal (HNE). This process can occur for unesterified FA such as arachidonic acid (Figure 1), and for esterified fatty acids in either leaflet of the phospholipid bilayer.

Based on observations that superoxide and reactive alkenals activate uncoupling in mitochondria via ANT and UCPs, Brand and colleagues suggested a physiological role for this process [17]. When HNE activates proton leak, membrane potential is decreased, thereby stimulating electron flux through the respiratory chain, which decreases the

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steady-state concentration of reduced electron carriers that are likely to donate an electron to oxygen to generate superoxide. Therefore, ROS production has a negative feedback mechanism that functions via lipid peroxidation products to protect against oxidative stress.

The mechanism(s) by which HNE activates proton conductance in UCPs and ANT is unknown. However, HNE forms adducts with proteins such as ANT [70], and it is proposed that these modifications are responsible for uncoupling. Although the link might be circumstantial, it is based on biochemical studies showing that, unlike FA-induced proton leak, HNE-induced uncoupling appears to be irreversible [19]. Theories of superoxide-mediated activation of UCPs include activation via FA products of HNE metabolism, such as nonenoic acid, as well as via hydroperoxy FAs generated during lipid peroxidation.

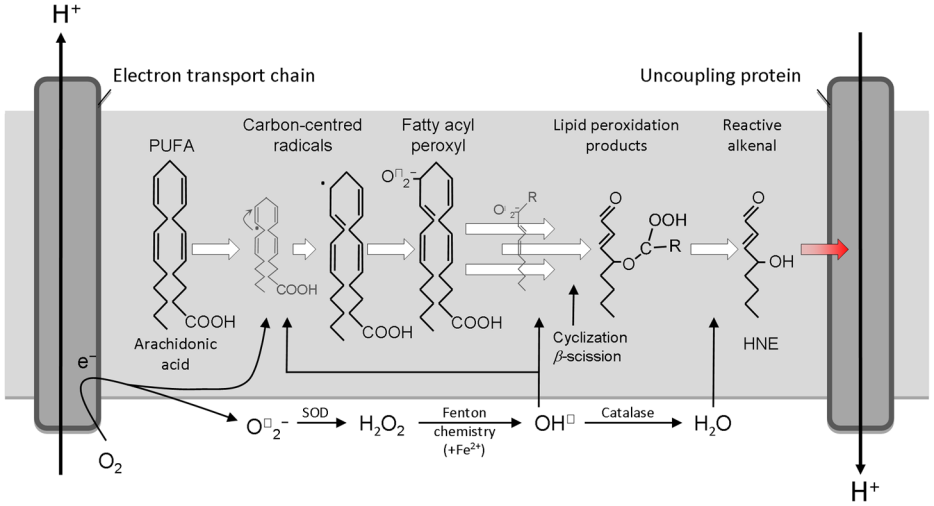

Figure I. A model for the physiological activation of uncoupling proteins by superoxide and its downstream derivatives such as HNE

A high protonmotive force set up by the respiratory chain increases the steady state concentration of reduced electron carriers that donate an electron to molecular oxygen, thereby increasing superoxide production. Formation of downstream free radicals begins a cascade of FA radical production that ultimately results in the generation of HNE, which activates uncoupling proteins, causing them to transport protons, leading to lower protonmotive force and decreased superoxide production. Abbreviations PUFA, polyunsaturated fatty acid; SOD, superoxide dismutase.

HNE, 4-hydroxynonenal; PUFA, polyunsaturated fatty acid; SOD, superoxide dismutase.

What does appear to be widely accepted is the role of UCP2 in attenuating steady-state levels of ROS [17,39]. Mitochondrial ROS production is correlated exponentially with mitochondrial membrane potential [43], the mechanism involving an alteration in the redox potential of respiratory chain carriers that leads to a greater chance of reduced components donating electrons to oxygen, thereby leading to superoxide generation. UCP2-mediated ROS attenuation is thought to occur via dissipation of mitochondrial protonmotive force, again consistent with its role as an uncoupling protein.

The effects of ROS attenuation appear to be protective or detrimental, depending on context. UCP2 promotes pancreatic alpha-cell [44] and beta-cell [45] survival and appears to protect against oxidative stress in liver [46], endothelium (thereby preventing atherosclerosis) [47], and in neurones. UCP2 is thought to have a cytoprotective role by stimulating mitochondrial

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biogeneis and preventing induction of cell death by decreasing membrane potential and calcium influx into mitochondria [48]. A large body of data suggests that UCP2 activity might be detrimental in obesity and diabetes [17] because it attenuates glucosestimulated insulin secretion (GSIS) by diminishing ROS production, an important signal in glucose sensing by the brain [39] and the pancreas [49]. Indeed, chromosomal mapping of *Ucp2* indicates that it is coincident with quantitative trait loci for obesity and diabetes [11]. A polymorphism at −866 in the promoter region of *Ucp2* has the greatest association with type 2 diabetes mellitus, with the A allele having greater UCP2 expression and lower glucose-stimulated insulin secretion than the G allele [50]. However, recent work by Collins and co-workers shows that *Ucp2* ablation in mice of different genetic backgrounds leads to opposing effects on GSIS [51]. This finding suggests that the effect of genetic background on physiology is not insignificant, and that chronic absence of UCP2 might impact on the pathophysiology of diabetes differently from acute UCP2 downregulation, e.g. by siRNAmediated knockdown [17].

As noted earlier for UCP1, the regulation of UCP2 appears to occur at numerous steps, including transcription, translation, modulation of protein activity and degradation (Figure 3b). Most studies show increased UCP2 expression in diabetes, and it is thought that glucolipotoxicity (hyperglycaemia and hyperlipidaemia) increases *Ucp2* mRNA expression via various transcription factors, such as peroxisome proliferator-activated receptors (PPARs) and forkhead box A2 (FOXA2) transcription factor [52]. Important work by Hurtaud et al. showed that changes in *Ucp2* mRNA and protein level are not synonymous; UCP2 falls under glutamine-dependent translational control by virtue of its action at an upstream open reading frame [53]. Even when UCP2 is expressed, its activity seems to be further regulated by FAs, reactive alkenals and purine nucleotides [17]. Although not widely substantiated, it was reported that the aglycone genipin and its non-cross-linking derivatives inhibit UCP2 function in pancreatic islets and, by doing so, improve GSIS [17].

Although evidence for these regulatory steps is mounting, knowledge about UCP2 degradation has materialized only recently. To date, UCP2 has been found to have an unusually short half-life in a range of tissues [54], including clonal pancreatic beta-cells [55]. We postulated that this fast turnover allows for rapid variations in UCP2 levels in response to changes in nutrient supply [55]. The short half-life and lack of UCP2 degradation in mitochondria isolated from pancreatic beta-cells [55] led us to discover that UCP2 is degraded by the cytosolic ubiquitin-proteasome system [56]. This could be a measure to regulate UCP2 in line with other components of the insulin secretion pathway, which are also regulated by the ubiquitin-proteasome system [57]. Increased levels of UCP2 seen in type 2 diabetes mellitus [50] might result from decreased degradation due to proteasomal dysfunction as well as increased UCP2 expression to protect against glucolipotoxicity. Because proteasome function is known to decrease with age and proteasomal inhibition decreases GSIS, the targeted use of proteasome activators might prove beneficial in the treatment of ageing-related diseases such as diabetes.

UCP3

UCP3 expression is tissue-restricted: it is expressed most abundantly inskeletalmuscle and, to a lesser extent, in BAT and heart [17]. As skeletal muscle is an important site of thermogenesis and UCP3 is the only uncoupling protein expressed in skeletal muscle, it was initially a candidate for mediating thermogenesis in muscle. However, several lines of evidence point to this not being the case. *Ucp3* knockout mice are not cold sensitive nor obese, are normophagic and have normal energy expenditurecompared to wild type mice [11]. Contrary to the predictions of a thermogenic model, fasting (when energy conservation is required) causes UCP3 upregulation in skeletal muscle [17]. Despite upregulation, UCP3

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does not mediate basal proton conductance [17], providing evidence that its primary role is not thermogenic. However, UCP3 does uncouple when activated [17] and, interestingly, its presence is required for whole body thermogenesis under specific pharmacological conditions: it is necessary for the thermogenic effect of themethamphetamine MDMA (colloquially known as ecstasy) [17]. Furthermore, a recent study reports the impaired cold-tolerance of hamsters with a BAT-specific loss of *Ucp3* [58].

What, then, is the function of UCP3? Although originally proposed for UCP2, Brand and colleagues suggest that the novel uncoupling proteins are attenuators of ROS production [17], particularly during FA oxidation. Others point to a role for UCP3 in FA metabolism, either in exporting excess FAs from mitochondria when FA oxidation predominates [59], or in exporting lipid hydroperoxides [60]. A recent study by Harper and colleagues showed that UCP3 is not required for FA oxidation or FA anion export, but that it is essential for adaptation to fasting, possibly via mitigated ROS [61]. Although UCP3 has been shown to mediate inducible proton leak by FAs [18] and ROS products [17] (thereby diminishing ROS production), the authors question whether the ROS mitigation occurs via proton leak as it happens when Δp is dissipated [61]. They conclude that their data are in line with the function of UCP3 (and UCP2) originally proposed by Brand, but query the underlying mechanism. Indeed, data from our laboratory suggest that ROS attenuation by UCP3 is complicated: UCP3 can diminish ROS production through both a simple uncoupling activity that can be mimicked by the chemical uncoupler carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) as well as via a membrane potential-independent effect (Toime, L.J. (2009) The role and regulation of mitochondrial uncoupling proteins. PhD thesis, University of Cambridge).

Interestingly, decreased expression of UCP3 has been associated with insulin insensitivity, and exercise or rosiglitazone treatment both restore UCP3 levels and improve insulin sensitivity [62]. If UCP3 is indeed protective against insulin resistance, and given its restricted expression in skeletal muscle, it would be an attractive therapeutic target for treating insulin resistance, a condition that precedes diabetes.

Skeletal muscle is also an appealing target for uncoupling because its large mass contributes considerably to metabolic rate. The overexpression of UCP3 in mice leads to decreased body mass despite higher food intake [63]. Although transgenic studies have been criticized for the artefactual uncoupling induced by huge expression levels, work using low-expressing lines can help to alleviate these concerns (Box 2). Irrespective of whether uncoupling is regulated, *Ucp1* [64] and *Ucp3* [65] transgenic mice with low muscle expression phenotypes show the same beneficial effects as chemical uncoupling by dinitrophenol; namely, preventing obesity and insulin resistance, decreasing blood pressure and diminishing ageing-related disease.

BOX 2

The problem with comparing UCP2 and UCP3 to UCP1

Proteoliposome studies

Studies reconstituting UCP1, UCP2 and UCP3 into proteoliposomes suggest that all UCPs have a comparable proton conductance and similar inhibition by purine nucleotides [17,18]. The advantage of proteoliposomes is the lack of other proteins that can confound interpretation. However, many mechanistic aspects of uncoupling protein function seen in mitochondria and cells (such as activation of UCP1 by FAs in the presence of purine nucleotides) are not replicated in proteoliposomes, making extrapolation to physiology problematic.

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Using GDP as a diagnostic of UCP functionM

GDP has been used to probe the function of native folded uncoupling protein in proteoliposomes and isolated mitochondria. However, GDP also inhibits proton conductance via ANT [71], suggesting that the use of GDP sensitivity as a sole diagnostic of UCP activity in mitochondria should be treated with caution. A superior method is to compare wild type with knockout animals, but this approach can suffer from confounding effects, such as the upregulation of compensatory mechanisms.

Gene ablation studies

Ucp2- and *Ucp3*-null mice exhibit weak phenotypes when housed under normal conditions. However, these phenotypes can be exacerbated in particular genetic backgrounds or under stressful conditions [17].

Because the mitochondrial uncoupling effects of UCP2 and UCP3 are not as large as those of UCP1, some authors have suggested that these UCPs do not uncouple significantly [37]. However, the physiological contexts in which UCPs are compared are not similar. UCP1 comprises up to 10% of mitochondrial protein in cold-adapted brown adipocytes, a tissue that has low quantities of the F_o/F_1 ATP synthase and is primed for activating thermogenesis. By contrast, UCP2 and UCP3 are expressed at much lower levels in tissues whose function is not primarily thermogenic [38].

Overexpression studies

Proving causal links between UCP protein level and various physiological effects has been attempted by inducing UCP overexpression in cells [72] or transgenic animal models [63]. However, these gain-of-function studies should be approached with caution because a body of work suggests that such effects arise from artefactual (unregulated) uncoupling [73]. In yeast cells, which do not possess endogenous UCPs (and possibly also lack the machinery required for folding them) or transgenic mice that overexpress to large extents, the artefact might arise from a disruption to mitochondrial inner membrane structure due to improper insertion of the UCP into the membrane. This idea has led others to use transgenic animals that produce only low-level overexpression of UCPs [64,64], but even these studies require further validation to ensure that the observed phenotype is reflective of native function and not an artefact.

How UCP3 is deactivated or turned over has not been addressed in the literature, and it remains unknown whether UCP3 is degraded in a manner similar to UCP1 (autophagy) or UCP2 (proteasomal), or perhaps by intramitochondrial proteases (Figure 3c). Given the high level of sequence identity and the similar behaviour and regulation of UCP2 and UCP3, it seems possible that UCP3 might be turned over in a similar manner and with a half-life of the same order of magnitude as that of UCP2.

Revisiting the role of ANT

Numerous reports ranging from proteoliposome work to *in vivo* studies indicate that a subsidiary role of the ANT could be to mediate proton leak [7,19,66,67]. Indeed, basal proton leak in mitochondria is proportional to ANT content, and inducible FA-mediated leak through ANT has long been known [7].

Much like the state of affairs for UCPs, the molecular mechanism of uncoupling by ANT remains elusive. In the simplest model, a basal proton conductance pathway is formed at the protein–lipid interface or within the transport mechanism, a process that can probably be carried out by other mitochondrial solute carriers [5]. The reason why ANT contributes to proton leak so significantly is merely a numbers game: with the exception of UCP1 in cold-

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acclimated BAT, ANT is far more abundant than other carriers in all tissues, forming up to 10% of total mitochondrial protein [4].

Other models have been suggested for inducible proton leak; namely, the FA cycling hypothesis, which is reversible and inhibited by the abundant substrates of the nucleotide translocation activity of ANT [4]. By contrast, alkenal-induced proton leak via the ANT appears to be irreversible, with the uncoupling activity of ANT being distinct from its nucleotide translocation activity [19]. Interestingly, mutational studies show that UCP1 is also able to distinguish proton flux from anion (e.g. chloride) flux [26].

Given its role in nucleotide translocation and the fact that high levels of purine nucleotides would inhibit any FA-mediated activity, scepticism remains regarding whether ANT can uncouple in vivo. However, a recent study suggests that ANT mutants that retain basic kinetic properties for nucleotide exchange can cause mitochondrial uncoupling in vivo [67].

Extensive studies using chemical uncouplers, such as dinitrophenol, show that mitochondrial uncoupling is an effective way to decrease body weight, improve glucose tolerance and decrease blood pressure. The abundance of ANT in nearly all tissues, its ability to mediate uncouplerinduced proton leak [66], and the recent finding that a system exists to recover energy balance in response to the toxicity (loss of ATP-synthesizing capacity) of mild uncoupling [68] raises the question of whether simple uncouplers should be reconsidered for the treatment of obesity.

Concluding remarks

A significant proportion of metabolic rate is diverted towards proton leak pathways *in vivo*. Uncoupling proteins, which mediate some of this proton leak, are upregulated in response to nutritional status: overfeeding in the case of UCP1 and starvation in the cases of UCP2 and UCP3, suggesting a function for the latter two in the metabolic adaptation to fasting. Although mammalian UCP1 in BAT has been convincingly shown to be required for adaptive thermogenesis, UCP3 and UCP2 (and UCP1 in non-eutherians or other tissues) are not thought to be significantly thermogenic, with the possible exception of UCP3 in avian muscle [69]. Despite their hitherto undetermined function, strong evidence suggests that they mediate a regulated proton leak.

On a functional level, it is important to note that the regulation of uncoupling protein levels and activity occurs in a concerted fashion at multiple steps from transcription to degradation. This is best demonstrated by UCP1 in BAT; noradrenergic stimulation of this tissue upregulates UCP1 levels, liberates activating ligands of the protein and inhibits pathways that degrade the protein. Recently, ever greater knowledge is being gained of the regulatory steps for controlling UCP2 levels and activity, but knowledge of such pathways for UCP3 remains hazy.

The fact that uncoupling proteins have been implicated in a wide range of pathophysiological processes makes them potentially important drug targets. Indeed, the elucidation of the molecular pathways governing their levels and activities should provide increasing possibilities for modulating uncoupling protein function.

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References

1. Mitchell P. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature. 1961; 191:144–148. [PubMed: 13771349]
2. Hirono-Hara Y, et al. Pause and rotation of F1-ATPase during catalysis. Proc Natl Acad Sci USA. 2001; 98:13649–13654. [PubMed: 11707579]
3. Brand MD, et al. Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. Free Radic Biol Med. 2004; 37:755–767. [PubMed: 15304252]
4. Brand MD, et al. The basal proton conductance of mitochondria depends on adenine nucleotide translocase content. Biochem J. 2005; 392:353–362. [PubMed: 16076285]
5. Samartsev VN, et al. Involvement of aspartate/glutamate antiporter in fatty acid-induced uncoupling of liver mitochondria. Biochim Biophys Acta. 1997; 1319:251–257. [PubMed: 9131047]
6. Hunter DR, et al. Relationship between configuration, function, and permeability in calcium-treated mitochondria. J Biol Chem. 1976; 251:5069–5077. [PubMed: 134035]
7. Brand MD, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. Cell Metab. 2005; 2:85–93. [PubMed: 16098826]
8. Nicholls DG, Locke RM. Thermogenic mechanisms in brown fat. Physiol Rev. 1984; 64:1–64. [PubMed: 6320232]
9. Enerback S, et al. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature. 1997; 387:90–94. [PubMed: 9139827]
10. Ricquier D, Bouillaud F. The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. Biochem J. 2000; 345:161–179. [PubMed: 10620491]
11. Harper ME, Himms-Hagen J. Mitochondrial efficiency: lessons learned from transgenic mice. Biochim Biophys Acta. 2001; 1504:159–172. [PubMed: 11239492]
12. Feldmann HM, et al. UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. Cell Metab. 2009; 9:203–209. [PubMed: 19187776]
13. Saito S, et al. Adaptive evolution of the uncoupling protein 1 gene contributed to the acquisition of novel nonshivering thermogenesis in ancestral eutherian mammals. Gene. 2008; 408:37–44. [PubMed: 18023297]
14. Hughes J, Criscuolo F. Evolutionary history of the UCP gene family: gene duplication and selection. BMC Evol Biol. 2008; 8:306. [PubMed: 18980678]
15. Jaburek M, et al. Transport function and regulation of mitochondrial uncoupling proteins 2 and 3. J Biol Chem. 1999; 274:26003–26007. [PubMed: 10473545]
16. Robinson AJ, et al. The mechanism of transport by mitochondrial carriers based on analysis of symmetry. Proc Natl Acad Sci USA. 2008; 105:17766–17771. [PubMed: 19001266]
17. Affourtit C, et al. Novel uncoupling proteins. Novartis Found Symp. 2007; 287:70–91. [PubMed: 18074632]
18. Zackova M, Jezek P. Reconstitution of novel mitochondrial uncoupling proteins UCP2 and UCP3. Biosci Rep. 2002; 22:33–46. [PubMed: 12418549]
19. Azzu V, et al. High membrane potential promotes alkenal induced mitochondrial uncoupling and influences adenine nucleotide translocase conformation. Biochem J. 2008; 413:323–332. [PubMed: 18426390]
20. Porter RK. Uncoupling protein 1: a short-circuit in the chemiosmotic process. J Bioenerg Biomembr. 2008; 40:457–461. [PubMed: 18958609]
21. Locke RM, et al. Fatty acids as acute regulators of the proton conductance of hamster brown-fat mitochondria. Eur J Biochem. 1982; 129:373–380. [PubMed: 6295765]
22. Collins S, et al. Learning new tricks from old dogs: beta-adrenergic receptors teach new lessons on firing up adipose tissue metabolism. Mol Endocrinol. 2004; 18:2123–2131. [PubMed: 15243132]
23. Moazed B, Desautels M. Differentiation-dependent expression of cathepsin D and importance of lysosomal proteolysis in the degradation of UCP1 in brown adipocytes. Can J Physiol Pharmacol. 2002; 80:515–525. [PubMed: 12117300]

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24. Moazed B, Desautels M. Control of proteolysis by norepinephrine and insulin in brown adipocytes: role of ATP, phosphatidylinositol 3-kinase, and p70 S6K. Can J Physiol Pharmacol. 2002; 80:541–552. [PubMed: 12117303]
25. Skulachev VP. Fatty acid circuit as a physiological mechanism of uncoupling of oxidative phosphorylation. FEBS Lett. 1991; 294:158–162. [PubMed: 1756853]
26. Echtay KS, et al. Site-directed mutagenesis identifies residues in uncoupling protein (UCP1) involved in three different functions. Biochemistry. 2000; 39:3311–3317. [PubMed: 10727223]
27. Jezek P, et al. Evidence for anion-translocating plant uncoupling mitochondrial protein in potato mitochondria. J Biol Chem. 1996; 271:32743–32748. [PubMed: 8955108]
28. LaNoue KF, et al. Mechanism of action of norepinephrine in hamster brown adipocytes. J Biol Chem. 1982; 257:13740–13748. [PubMed: 7142176]
29. Fisher MH, et al. A selective human beta3 adrenergic receptor agonist increases metabolic rate in rhesus monkeys. J Clin Invest. 1998; 101:2387–2393. [PubMed: 9616210]
30. Nedergaard J, et al. Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab. 2007; 293:E444–E452. [PubMed: 17473055]
31. Cypess AM, et al. Identification and importance of brown adipose tissue in adult humans. N Engl J Med. 2009; 360:1509–1517. [PubMed: 19357406]
32. Virtanen KA, et al. Functional brown adipose tissue in healthy adults. N Engl J Med. 2009; 360:1518–1525. [PubMed: 19357407]
33. Seale P, et al. PRDM16 controls a brown fat/skeletal muscle switch. Nature. 2008; 454:961–967. [PubMed: 18719582]
34. Jastroch M, et al. Marsupial uncoupling protein 1 sheds light on the evolution of mammalian nonshivering thermogenesis. Physiol Genom. 2008; 32:161–169.
35. Jastroch M, et al. Functional characterisation of UCP1 in the common carp: uncoupling activity in liver mitochondria and coldinduced expression in the brain. J Comp Physiol B. 2007; 177:743–752. [PubMed: 17576568]
36. Hughes DA, et al. Molecular evolution of UCP1 and the evolutionary history of mammalian non-shivering thermogenesis. BMC Evol Biol. 2009; 9:4. [PubMed: 19128480]
37. Nedergaard J, Cannon B. The ‘novel’ ‘uncoupling’ proteins UCP2 and UCP3: what do they really do? Pros and cons for suggested functions. Exp Physiol. 2003; 88:65–84. [PubMed: 12525856]
38. Pecqueur C, et al. Uncoupling protein 2, in vivo distribution, induction upon oxidative stress, and evidence for translational regulation. J Biol Chem. 2001; 276:8705–8712. [PubMed: 11098051]
39. Andrews Z, et al. UCP2 mediates ghrelin’s action on NPY/AgRP neurons by lowering free radicals. Nature. 2008; 454:846–851. [PubMed: 18668043]
40. Derdak Z, et al. The mitochondrial uncoupling protein-2 promotes chemoresistance in cancer cells. Cancer Res. 2008; 68:2813–2819. [PubMed: 18413749]
41. Trenker M, et al. Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca^{2+} uniport. Nat Cell Biol. 2007; 9:445–452. [PubMed: 17351641]
42. Brookes PS, et al. UCPs – unlikely calcium porters. Nat Cell Biol. 2008; 10:1235–1240. [PubMed: 18978830]
43. Korshunov SS, et al. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. FEBS Lett. 1997; 416:15–18. [PubMed: 9369223]
44. Diao J, et al. UCP2 is highly expressed in pancreatic alpha-cells and influences secretion and survival. Proc Natl Acad Sci USA. 2008; 105:12057–12062. [PubMed: 18701716]
45. Emre Y, et al. Role of uncoupling protein UCP2 in cell-mediated immunity: how macrophage-mediated insulitis is accelerated in a model of autoimmune diabetes. Proc Natl Acad Sci USA. 2007; 104:19085–19090. [PubMed: 18006654]
46. Lee FY, et al. Tumor necrosis factor increases mitochondrial oxidant production and induces expression of uncoupling protein-2 in the regenerating mice [correction of rat] liver. Hepatology. 1999; 29:677–687. [PubMed: 10051468]
47. Mattiasson G, Sullivan PG. The emerging functions of UCP2 in health, disease, and therapeutics. Antioxid Redox Signal. 2006; 8:1–38. [PubMed: 16487034]

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48. Mehta SL, Li PA. Neuroprotective role of mitochondrial uncoupling protein 2 in cerebral stroke. J Cereb Blood Flow Metab. 2009; 29:1069–1078. [PubMed: 19240738]
49. Leloup C, et al. Mitochondrial reactive oxygen species are obligatory signals for glucose-induced insulin secretion. Diabetes. 2009; 58:673–681. [PubMed: 19073765]
50. Sasahara M, et al. Uncoupling protein 2 promoter polymorphism -866G/A affects its expression in beta-cells and modulates clinical profiles of Japanese type 2 diabetic patients. Diabetes. 2004; 53:482–485. [PubMed: 14747301]
51. Pi J, et al. Persistent oxidative stress due to absence of uncoupling protein 2 associated with impaired pancreatic beta-cell function. Endocrinology. 2009; 150:3040–3048. [PubMed: 19246534]
52. Chan CB, Harper ME. Uncoupling proteins: role in insulin resistance and insulin insufficiency. Curr Diabetes Rev. 2006; 2:271–283. [PubMed: 18220632]
53. Hurtaud C, et al. Glutamine stimulates translation of uncoupling protein 2 mRNA. Cell Mol Life Sci. 2007; 64:1853–1860. [PubMed: 17514359]
54. Rousset S, et al. UCP2 is a mitochondrial transporter with an unusual very short half-life. FEBS Lett. 2007; 581:479–482. [PubMed: 17240372]
55. Azzu V, et al. Dynamic regulation of uncoupling protein 2 content in INS-1E insulinoma cells. Biochim Biophys Acta. 2008; 1777:1378–1383. [PubMed: 18692019]
56. Azzu V, Brand MD. Degradation of an intramitochondrial protein by the cytosolic proteasome. J Cell Sci. 2009
57. Kawaguchi M, et al. Essential role of ubiquitin-proteasome system in normal regulation of insulin secretion. J Biol Chem. 2006; 281:13015–13020. [PubMed: 16543239]
58. Nau K, et al. Brown adipose tissue specific lack of uncoupling protein 3 is associated with impaired cold tolerance and reduced transcript levels of metabolic genes. J Comp Physiol B. 2008; 178:269–277. [PubMed: 17987299]
59. Himms-Hagen J, Harper ME. Physiological role of UCP3 may be export of fatty acids from mitochondria when fatty acid oxidation predominates: an hypothesis. Exp Biol Med. 2001; 226:78–84.
60. Goglia F, Skulachev VP. A function for novel uncoupling proteins: antioxidant defense of mitochondrial matrix by translocating fatty acid peroxides from the inner to the outer membrane leaflet. FASEB J. 2003; 17:1585–1591. [PubMed: 12958165]
61. Seifert EL, et al. Essential role for uncoupling protein-3 in mitochondrial adaptation to fasting but not in fatty acid oxidation or fatty acid anion export. J Biol Chem. 2008; 283:25124–25131. [PubMed: 18628202]
62. Mensink M, et al. Skeletal muscle uncoupling protein-3 restores upon intervention in the prediabetic and diabetic state: implications for diabetes pathogenesis? Diabetes Obes Metab. 2007; 9:594–596. [PubMed: 17587402]
63. Clapham JC, et al. Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. Nature. 2000; 406:415–418. [PubMed: 10935638]
64. Gates AC, et al. Respiratory uncoupling in skeletal muscle delays death and diminishes age-related disease. Cell Metab. 2007; 6:497–505. [PubMed: 18054318]
65. Costford SR, et al. Long-term high-fat feeding induces greater fat storage in mice lacking UCP3. Am J Physiol Endocrinol Metab. 2008; 295:E1018–E1024. [PubMed: 18713955]
66. Lou PH, et al. Mitochondrial uncouplers with an extraordinary dynamic range. Biochem J. 2007; 407:129–140. [PubMed: 17608618]
67. Wang X, et al. Dominant membrane uncoupling by mutant adenine nucleotide translocase in mitochondrial diseases. Hum Mol Genet. 2008; 17:4036–4044. [PubMed: 18809618]
68. Rohas LM, et al. A fundamental system of cellular energy homeostasis regulated by PGC-1alpha. Proc Natl Acad Sci USA. 2007; 104:7933–7938. [PubMed: 17470778]
69. Talbot DA, et al. Uncoupling protein and ATP/ADP carrier increase mitochondrial proton conductance after cold adaptation of king penguins. J Physiol. 2004; 558:123–135. [PubMed: 15146050]

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70. Choksi KB, et al. Oxidatively damaged proteins of heart mitochondrial electron transport complexes. Biochim Biophys Acta. 2004; 1688:95–101. [PubMed: 14990339]

71. Khailova LS, et al. Participation of ATP/ADP antiporter in oleate- and oleate hydroperoxide-induced uncoupling suppressed by GDP and carboxyatractylate. Biochim Biophys Acta. 2006; 1757:1324–1329. [PubMed: 16765906]

72. Hinz W, et al. Recombinant human uncoupling protein-3 increases thermogenesis in yeast cells. FEBS Lett. 1999; 448:57–61. [PubMed: 10217410]

73. Cadenas S, et al. The basal proton conductance of skeletal muscle mitochondria from transgenic mice overexpressing or lacking uncoupling protein-3. J Biol Chem. 2002; 277:2773–2778. [PubMed: 11707458]

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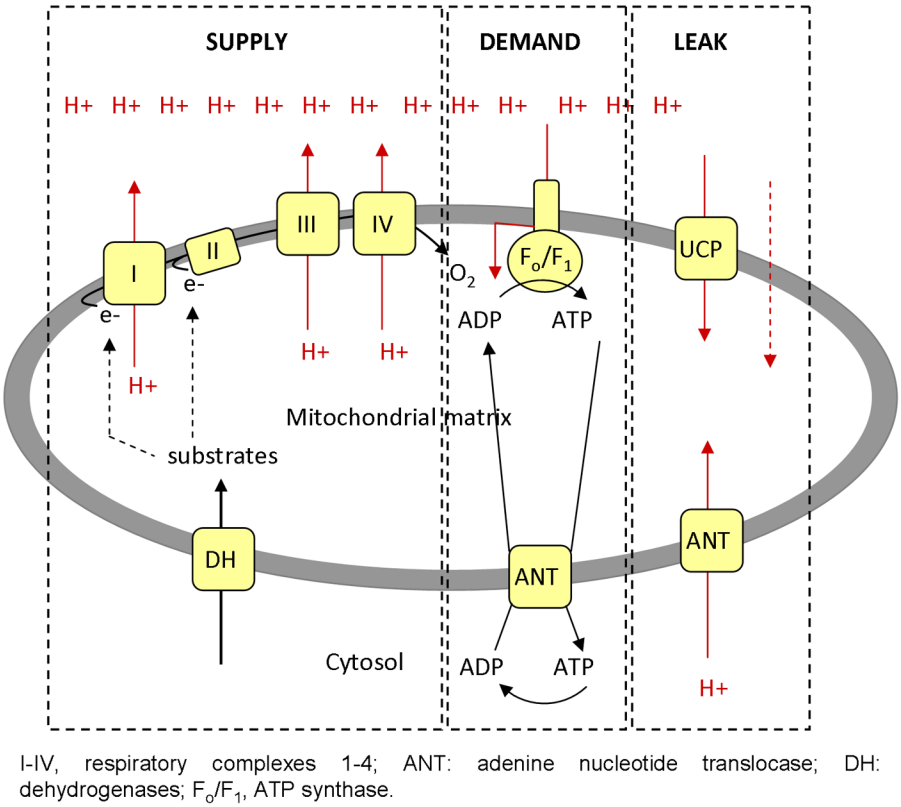

I-IV, respiratory complexes 1-4; ANT: adenine nucleotide translocase; DH: dehydrogenases; F_o/F_1, ATP synthase.

Figure 1. Oxidative phosphorylation and proton leak pathways in mitochondria

Respiratory substrates are oxidized at mitochondrial respiratory complexes I–IV, leading to the ejection of protons (H^+) into the intermembrane space (for diagramatic simplicity, the intermembrane space is depicted as being continuous with the cytosol). This proton electrochemical gradient is consumed by demand pathways via the F_o/F_1 ATP synthase to produce ATP or by proton leak pathways, which release energy in the form of heat. Proton leak pathways can be mediated by UCP or by ANT.

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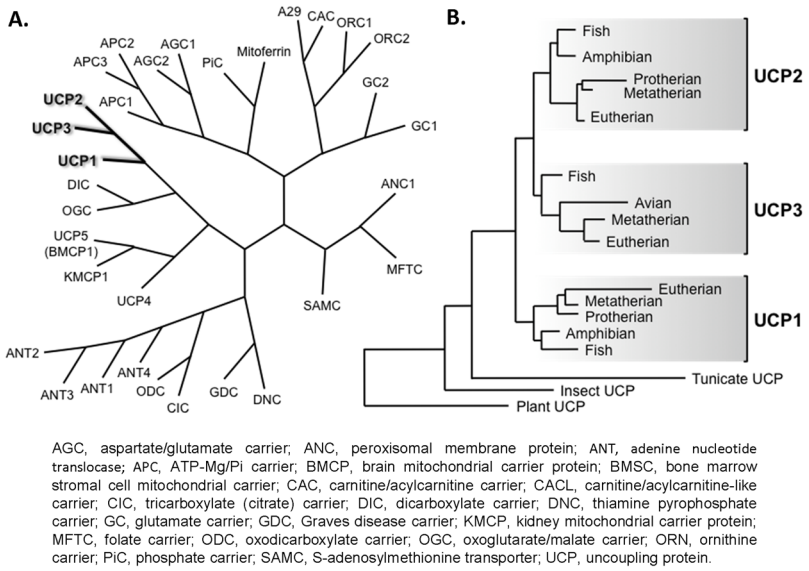

AGC, aspartate/glutamate carrier; ANC, peroxisomal membrane protein; ANT, adenine nucleotide translocase; APC, ATP-Mg/Pi carrier; BMCP, brain mitochondrial carrier protein; BMSC, bone marrow stromal cell mitochondrial carrier; CAC, carnitine/acylcarnitine carrier; CACL, carnitine/acylcarnitine-like carrier; CIC, tricarboxylate (citrate) carrier; DIC, dicarboxylate carrier; DNC, thiamine pyrophosphate carrier; GC, glutamate carrier; GDC, Graves disease carrier; KMCP, kidney mitochondrial carrier protein; MFTC, folate carrier; ODC, oxodicarboxylate carrier; OGC, oxoglutarate/malate carrier; ORN, ornithine carrier; PiC, phosphate carrier; SAMC, S-adenosylmethionine transporter; UCP, uncoupling protein.

Figure 2. Phylogenetic analysis of mitochondrial anion carriers and UCPs

(a) Analysis of the mitochondrial anion carriers suggests that UCP1–3 naturally fall into a subfamily that does not include UCP4 or UCP5. The figure shows the human SLC25 mitochondrial anion carrier members A1–A33 displayed using an unrooted topological algorithm. (b) Topological view of uncoupling proteins in different organisms shows that the archetypal eutherian UCP1 has undergone rapid evolution and is more distantly related to the uncoupling protein subancestor than its eutherian paralogues UCP2 and UCP3. Sequences are based on the accession IDs in Ref. 34 (the sequences were courtesy of Dr Martin Jastroch). Full-length protein sequences were aligned using default settings on ClustalW and the tree was generated using TreeTop (http://www.genebee.msu.su/services/phtree_reduced.html). Abbreviations: AGC, aspartate/glutamate carrier; ANC, peroxisomal membrane protein; APC, ATP-Mg/P_i carrier; BMSC, bone marrow stromal cell mitochondrial carrier; CAC, carnitine/acylcarnitine carrier; CACL, carnitine/acylcarnitine-like carrier; CIC, tricarboxylate (citrate) carrier; DIC, dicarboxylate carrier; DNC, thiamine pyrophosphate carrier; GC, glutamate carrier; GDC, Graves disease carrier; KMCP, kidney mitochondrial carrier protein; MFTC, folate carrier; ODC, oxodicarboxylate carrier; OGC, oxoglutarate/malate carrier; ORN, ornithine carrier; PiC, phosphate carrier; SAMC, S-adenosylmethionine transporter.

AGC, aspartate/glutamate carrier; ANC, peroxisomal membrane protein; ANT, adenine nucleotide translocase; APC, ATP-Mg/Pi carrier; BMCP, brain mitochondrial carrier protein; BMSC, bone marrow stromal cell mitochondrial carrier; CAC, carnitine/acylcarnitine carrier; CACL, carnitine/acylcarnitine-like carrier; CIC, tricarboxylate (citrate) carrier; DIC, dicarboxylate carrier; DNC, thiamine pyrophosphate carrier; GC, glutamate carrier; GDC, Graves disease carrier; KMCP, kidney mitochondrial carrier protein; MFTC, folate carrier; ODC, oxodicarboxylate carrier; OGC, oxoglutarate/malate carrier; ORN, ornithine carrier; PiC, phosphate carrier; SAMC, S-adenosylmethionine transporter; UCP, uncoupling protein.

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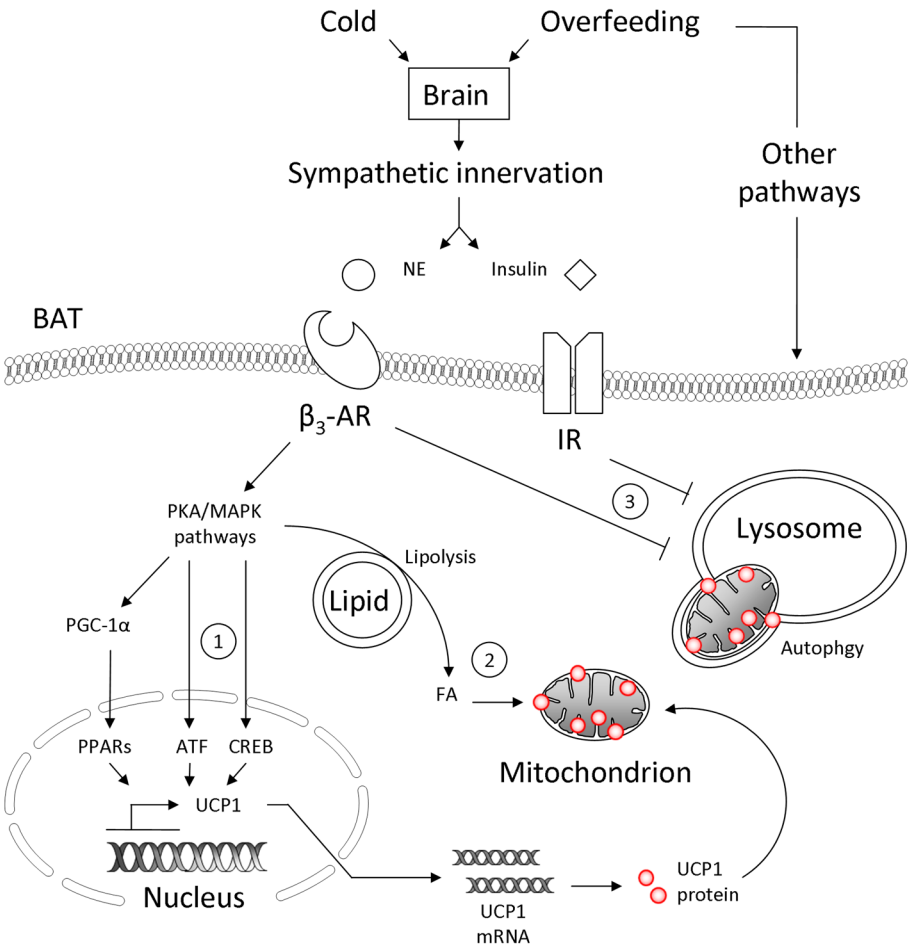

β_3-AR, β_3-adrenergic receptor; AFT, activating transcription factor; BAT, brown adipose tissue; CREB, cAMP response element binding; FA, fatty acid; IR, insulin receptor; NE, norepinephrine; PPAR, peroxisome proliferator-activated receptor; UCP1, uncoupling protein 1.

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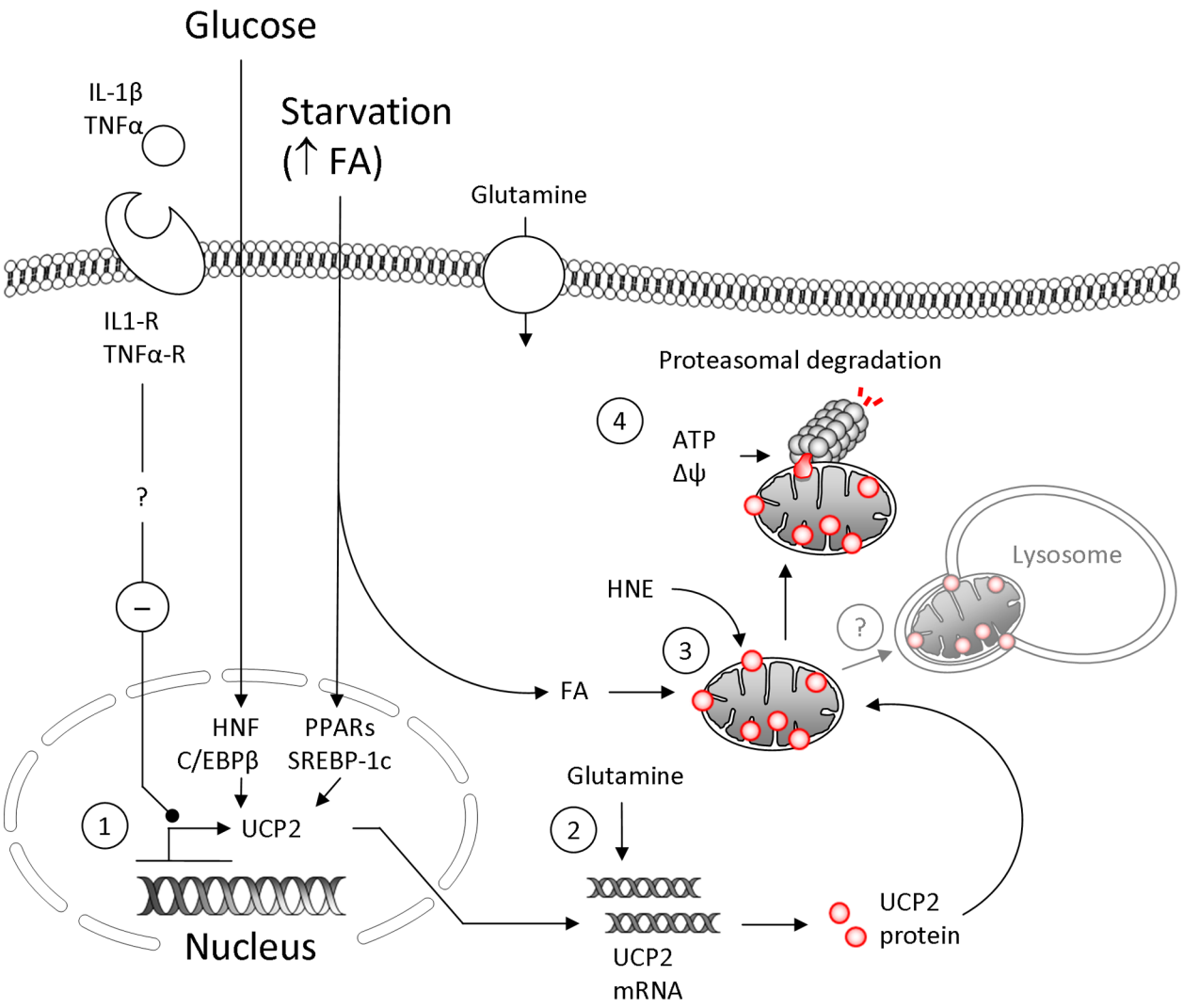

Δψ, mitochondrial membrane potential; C/EBPβ, CCAAT-enhancer-binding protein-β; FA, fatty acid; HNE, 4-hydroxynonenal; HNF, hepatic nuclear factor; IL-1β, interleukin-1β; PPAR, peroxisome proliferator-activated receptor; SREBP-1c, sterol regulatory element binding protein-1c; UCP2, uncoupling protein 2.

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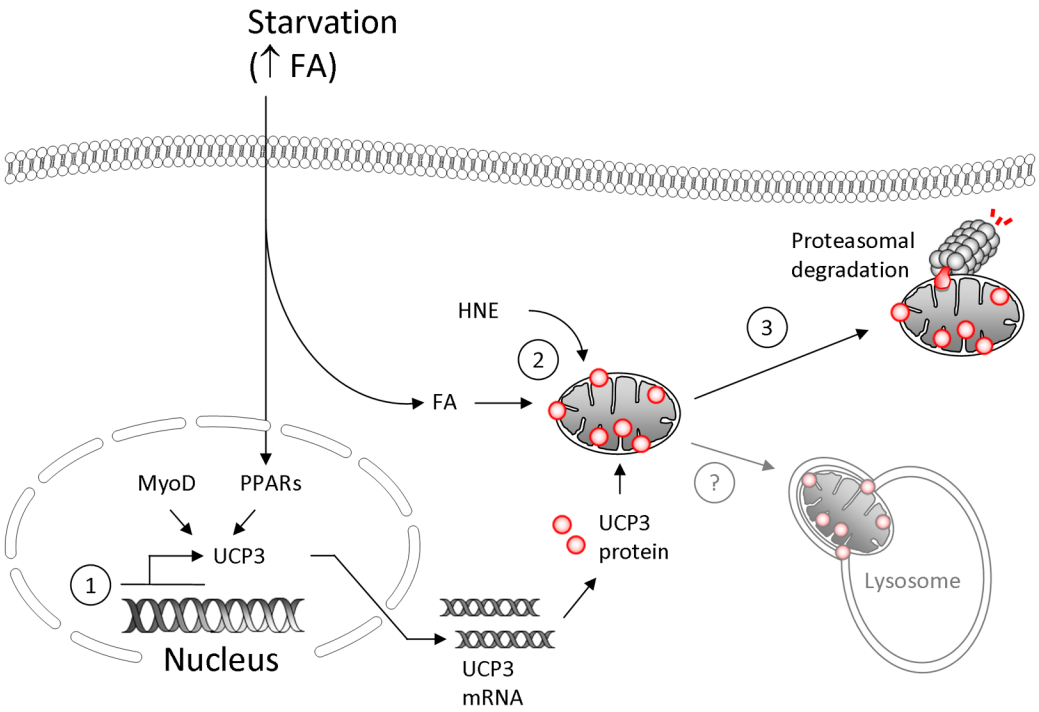

FA, fatty acid; HNE, 4-hydroxynonenal; MyoD, myogenic regulatory factor family protein; PPAR, peroxisome proliferator-activated receptor; UCP3, uncoupling protein 3.

Figure 3. The concerted regulation of the mitochondrial uncoupling proteins

Uncoupling proteins are likely to be controlled at multiple levels, such as transcription, translocation, ligand activation or inhibition and protein turnover. (a) (i) During adaptive thermogenesis in BAT, UCP1 synthesis is stimulated by transcription factors of the PKA and MAPK pathways. (ii) These pathways also induce lipolysis, which generates fatty acid (FA) ligands that activate UCP-mediated proton leak. (iii) Stimulation of β3-AR and insulin receptors also inhibit UCP1 and whole mitochondrial degradation by autophagic pathways. (b) UCP2 synthesis is regulated at the (i) transcriptional and (ii) translational levels. (iii) Protein activity can be further regulated by acute activators such as FA and HNE. (iv) Protein deactivation can occur by ligand inhibition and by rapid turnover of protein UCP2, probably by the ubiquitin proteasome system. (c) (i) UCP3 synthesis is regulated at the transcriptional level by starvation and muscle transcription factors. (ii) Protein activity can be further regulated by functional ligands, such as FA and HNE. The mechanism of protein deactivation can occur by ligand inhibition. In (b) and (c), UCP2 and UCP3 turnover can also theoretically occur as the result of mitochondrial turnover via the lysosomal pathway, although this pathway is slow compared with the likely degradation via the proteasomal pathway. Abbreviations: ATF, activating transcription factor; CREB, cAMP response element binding; IR, insulin receptor; NE, norepinephrine; Δ ψ, mitochondrial membrane potential; C/EBPβ, CCAAT-enhancer-binding protein-β; FA, fatty acid; HNF, hepatic nuclear factor; IL-1β, interleukin-1β; PPAR, peroxisome proliferator-activated receptor; SREBP-1c, sterol regulatory element binding protein-1c; MyoD, myogenic regulatory factor family protein.

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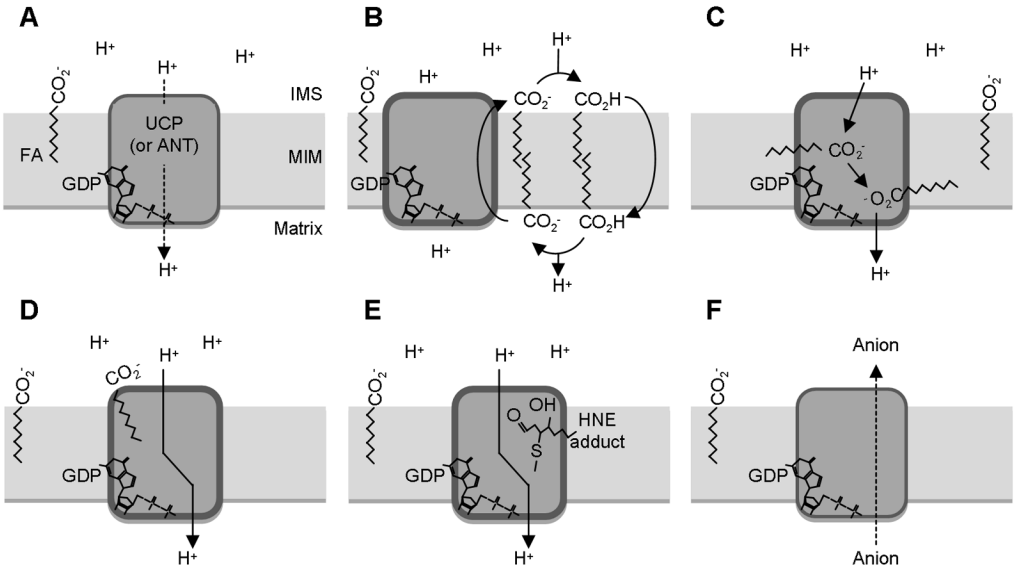

ANT, adenine nucleotide tranlocase; FA, fatty acid; GDP, guanosine dinucleotide; HNE, 4-hydroxynonenal; IMS, intermembrane space; MIM, mitochondrial inner membrane; UCP, uncoupling protein.

Figure 4. Models of UCP (or ANT) activation and inhibition

The proton conductance activity of uncoupling proteins under different conditions. (a) In the basal state in the presence of nucleotides such as GDP, there is minimal proton conductance through UCP (or ANT). (b–d) Different models in which FA anions induce a large proton conductance through UCP by overcoming GDP inhibition. (b) In the flip-flop model, UCP exports FA anions, which become protonated and the neutral species flips back across the mitochondrial inner membrane (MIM) and deprotonates, resulting in a net flux of protons into the matrix. (c) In the co-factor model, FA anions associate with UCP and act as negative charges, which assists proton flux into the matrix. (d) In the functional competition model, FAs allosterically overcome the inhibitory effect of GDP. (e) HNE interacts with UCP, e.g. via an -SH group adduct, which activates UCP proton conductance. As appears to be the case for ANT, this pathway differs from the FA activation pathway and might be irreversible [19]. (f) Although uncoupling proteins have an anion conductance pathway in which they can translocate molecules such as halides, this pathway is functionally distinct from the proton conductance pathway [26]. Therefore, it remains unclear as to whether FAs, which are activators of the proton conductance pathway, can be exported via this anion conductance pathway. Abbreviations: IMS, intermembrane space; MIM, mitochondrial inner membrane.