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Review

Mitochondrial proton leak: a role for uncoupling proteins 2 and 3?

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Abstract

In mitochondria ATP synthesis is not perfectly coupled to oxygen consumption due to proton leak across the mitochondrial inner membrane. Quantitative studies have shown that proton leak contributes to ~25% of the resting oxygen consumption of mammals. Proton leak plays a role in accounting for differences in basal metabolic rate. Thyroid studies, body mass studies, phylogenic studies and obesity studies have all shown that increased mass-specific metabolic rate is linked to increased mitochondrial proton leak. The mechanism of the proton leak is unclear. Evidence suggests that proton leak occurs by a non-specific diffusion process across the mitochondrial inner membrane. However, the high degree of sequence homology of the recently cloned uncoupling proteins UCP 2 and UCP 3 to brown adipose tissue UCP 1, and their extensive tissue distribution, suggest that these novel uncoupling proteins play a role in proton leak. Early indications from reconstitution experiments and several in vitro expression studies suggest that the novel uncoupling proteins uncouple mitochondria. Furthermore, mice overexpressing UCP 3 certainly show a phenotype consistent with increased metabolism. The evidence for a role for these novel UCPs in mitochondrial proton leak is reviewed.

Keywords: Mitochondrion; Oxidative phosphorylation; Proton leak; Uncoupling protein(s); Oxygen consumption; Basal metabolic rate

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1. Introduction

In mammals, basal metabolism can conveniently and accurately be determined by measuring oxygen consumption rate under resting conditions [1]. Mitochondria are the major site of oxygen consumption in mammals. The free energy released from the combustion of substrates is converted to ATP. However, some of that energy is converted to heat due to an inefficiency in the coupling of ATP synthesis to oxygen consumption, a phenomenon known as mitochondrial proton leak. Quantitative studies have shown that proton leak contributes to ~25% of the resting oxygen consumption of the rat. Proton leak is also a determinant in accounting for differences in basal metabolic rate. Thyroid studies, body mass studies, phylogenic studies and obesity studies have all shown that increased mass-specific metabolic rate is linked to increased mitochondrial proton leak (for reviews see [2–4]).

There is also an emerging literature suggesting that uncoupling protein 2 (UCP 2) and uncoupling protein 3 (UCP 3) are important determinants of basal metabolism. The novel homologues of a known mitochondrial uncoupling protein (UCP 1), from brown adipose tissue (BAT), have chromosomal locations close to markers of basal metabolic rate. Furthermore, mutations in these proteins have been linked to reduced metabolic rates in humans. Tissue

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mRNA for UCP 2 and UCP 3 expression studies also suggest an adaptive role in energy metabolism, with UCP 3 in particular being associated with conditions that increase fatty acid mobilisation and oxidation, however few data exist correlating amounts of UCP 2 and UCP 3 protein with function (for reviews see [5–8]).

2. Oxidative phosphorylation and diffusion-based proton leak

Oxidative phosphorylation is the process by which synthesis of ATP, by the ATP synthase of mitochondria, is coupled to oxygen consumption by mitochondria via the proton electrochemical gradient (Δ*p*) (reviewed in [9]). As electrons pass along the electron transport chain, Δ*p* is generated as a result of the translocation of protons from the mitochondrial matrix to the cytosol across the mitochondrial inner membrane. The ultimate acceptor of the electrons is oxygen, which is converted to water at cytochrome oxidase. Δ*p* is used to drive ATP synthesis. However, in the absence of ATP synthesis (or in the presence of oligomycin, an F_o ATP synthase channel inhibitor), mitochondria still consume oxygen (state 4) developing a maximal Δ*p* of about 220 mV. The resulting curve is non-Ohmic and has (in the opinion of this author) been correctly interpreted as representing oxygen consumption due to leakage of protons across the mitochondrial inner membrane [2]. Proton leak shows a non-Ohmic relationship with its driving force, Δ*p* (Fig. 1), in mitochondria from all sources thus far studied. Proton leak is not an artefact of mitochondrial isolation. A similar non-Ohmic relationship between oxygen consumption due to proton leak and Δ*p* can be observed in mitochondria in situ in cells and tissues. Furthermore the contribution of proton leak to the oxygen consumption in intact systems can be quantified. Proton leak accounts for ~20% of the oxygen consumption of cells, tissues and indeed a whole animal. It is now generally accepted that mitochondrial proton leak or natural proton leak is a significant contributor to basal metabolism (for reviews [2–4]).

Further evidence for the importance of proton leak as a determinant of basal metabolism comes from studies on factors that effect basal metabolism.

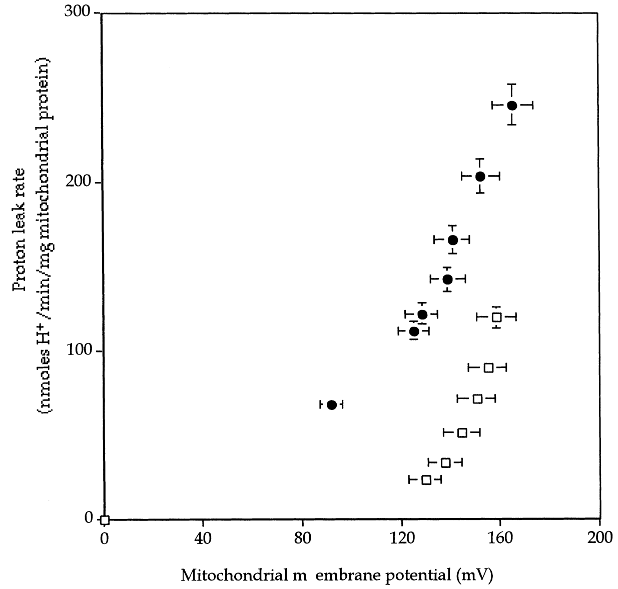

Fig. 1. Kinetics of the proton leak for liver mitochondria from lean, obese mice. Mouse liver mitochondria (3.3 mg) were incubated in a Clark-type oxygen electrode at 37°C in 3.3 ml of medium containing 120 mM KCl, 1 mM EGTA, 1 μg/mg oligomycin, nigericin (100 pmol/mg) and 5 mM HEPES adjusted to pH 7.2 with KOH. Five sequential addition of methytriphenylphosphonium iodide ($TPMP^+I^-$) were made to the chamber of the Clark-type oxygen electrode to facilitate calibration of the TPMP-electrode giving a final concentration of 5 μM. 7.5 mM succinate (K^+ salt, pH 7.2) was added to initiate oxygen consumption; titration of the membrane potential was with 0–10 mM malonate (K^+ salt, pH 7.2), shows proton leak kinetics for mitochondria from lean (□) and obese (●) mice.

Thyroid status is a major determinant of basal metabolism. Animals made hyperthyroid have a higher metabolic rate than sham treated euthyroid animals, and part of this increase is due to an increased mitochondrial proton leak. Animals made hypothyroid have a lower metabolic rate than euthyroids and mitochondrial proton leak is decreased [10–13]. In addition, an interspecies comparison of proton leak was made from mammals of differing body mass [14,15]. It has long been known that the mass-specific metabolic rate of larger animals is greater than that of smaller animals. In accordance with these differences in mass-specific metabolic rate, it was observed that proton leak was greater in mitochondria, both isolated and in situ, from smaller mammals compared to larger mammals. In a further consolidation of the evidence for the importance of proton leak to basal metabolism, reptiles of similar mass to mammals have lower metabolic rates and lower mitochondrial proton leak [16]. And in obese (ob/ob) mice, where lean mass-specific metabolic rate is greater than that of lean animals [17], proton leak has been shown to be increased (Fig. 1) [18,19].

The mechanism behind the 'basal proton leak' is not that clear. Differences in proton leak rate (at any given membrane potential) correlate with differences in mitochondrial inner membrane surface area as evidenced from thyroid status, mammal/reptile comparisons and body mass studies [2,16,20]. The non-Ohmic nature of the proton leak kinetics is characteristic of diffusion processes [21,22] and certainly if proton leak occurs by delocalised diffusion, than inner membrane surface area differences could account for the majority of differences in proton leak rate observed. Differences in proton leak rate have also been correlated with differences in mitochondrial inner membrane phospholipid fatty acid composition. In general, phospholipids from leakier membranes have more polyunsaturated fatty acids [16,20,23,24]. However there is no significant difference in permeability of deproteinised phospholipid unilamellar vesicles made from mitochondria in which proton leak differences do occur [24], suggesting a role for proteins in basal proton leak.

However, one does not have to invoke specific uncoupling proteins to account for this basal proton leak. Mitochondria from yeast have proton leak but do not have inner membrane proteins that have high homology to the UCPs [4,25]. Mitochondria in and from parenchyma cells of the liver are leaky to protons [10,15] but do not have inner membrane proteins that have high homology to the UCPs [26]. Differences in basal proton leak may be due in part to interaction of different phospholipid fatty acids with membrane proteins, in general, as would be suggested by the work of Clandinin et al. [27]. Measured differences in basal proton leak rates have also been attributed to differences in the amount of free non-esterified fatty acids associated with mitochondrial preparations. Incorporation of defatted bovine serum albumin to mop up fatty acids in the preparation has been shown efficacious in minimising the uncoupling effect of free fatty acids [12,13,28].

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RATUCP1 MVSSTTSEVQPTMGVKIFSAGVSACLADIITFPLDTAKVRLQIQGEGQ----ASSTIRYKGV
RATUCP2 MVGFKATDVPPTATVKFLGAGTAACIADLITFPLDTAKVRLQIQGESQGLARTAASAQYRGV
RATUCP3 MVGLQPSEVPPTTVVKFLGAGTAACFADLLTFPLDTAKVRLQIQGENP----GVQSVQYRGV
        **.  .::* **  **::.**.:**:**::****************.        : :*:**

RATUCP1 LGTITTLAKTEGLPKLYSGLPAGIQRQISFASLRIGLYDTVQEYFSSGRETPASLGSKISAG
RATUCP2 LGTILTMVRTEGPRSLYNGLVAGLQRQMSFASVRIGLYDSVKQFYTKGSE-HAGIGSRLLAG
RATUCP3 LGTILTMVRTEGPRSPYSGLVAGLHRQMSFASIRIGLYDSVKQFYTPKGTDHSSVAIRILAG
        **** *:.:***  . *.** **::**:****:******:*:::::      :.:. :: **

RATUCP1 LMTGGVAVFIGQPTEVVKVRMQAQSHLHGIKP-RYTGTYNAYRVIATTESLSTLWKGTTPNL
RATUCP1 STTGALAVAVAQPTDVVKVRFQAQARAGGGR--RYQSTVEAYKTIAREEGIRGLWKGTSPNV
RATUCP3 CTTGAMAVTCAQPTDVVKVRFQAMIRLGTGGERKYRGTMDAYRTIAREEGVRGLWKGTWPNI
          **.:**  .***:*****:**  :       :* .* :**:.**  *.:  ***** **:

RATUCP1 MRNVIINCTELVTYDLMKGALVNHHILADDVPCHLLSALVAGFCTTLLASPVDVVKTRFINS
RATUCP2 ARNAIVNCTELVTYDLIKDTLLKANLMTDDLPCHFTSAFGAGFCTTVIASPVDVVKTRYMNS
RATUCP3 TRNAIVNCAEMVTYDIIKEKLLDSHLFTDNFPCHFVSAFGAGFCATVVASPVDVVKTRYMNA
         **.*:**:*:****::*  *:. ::::*:.***: **: ****:*::**********::*:

RATUCP1 LPGQYPSVPSCAMTMYTKEGPAAFFKGFAPSFLRLGSWNVIMFVCFEQLKKE
RATUCP2 ALGQYHSAGHCALTMLRKEGPRAFYKGFMPSFLRLGSWNVVMFVTYEQLKRA
RATUCP3 PPGRYRSPLHCMLRMVAQEGPTAFYKGFMPSFLRLGSWNVMMFVTYEQLKRA
          *:* *   * : *  :*** **:*** ***********:*** :****:

RATUCP1 LMKSRQTVDCTT
RATUCP2 LMAAYESREAPF
RATUCP3 LMKVQVLRESPF
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Fig. 2. Multiple sequence alignment of rat UCP 1, UCP 2 and UCP 3. The diagram shows alignment of UCP 1 (accession number M11814), UCP 2 (accession number AF039033) and UCP 3 (accession number AB 006614) from rat. The tripartite signature motif common to mitochondrial anion transporters, is highlighted in bold. The figure also highlights the consensus sequence EGPAAFFKG in UCP 1 and its equivalent (EGPAAFYKG) in UCP 2 and UCP 3 (EGPTAFYKGF) at the C-terminal end of the proteins. Also in bold is Arg-277 of rat UCP 1, equivalently positioned Arg-279 of rat UCP 2 and Arg-278 of rat UCP 3. The contiguous sequence EGPAAFFKG and the Arg-276 (Arg-277 in rat) of mouse UCP 1 have been implicated in purine nucleotide binding.

3. Mitochondrial function in brown adipose tissue

The non-linear relationship between oxygen consumption due to proton leak and Δp has been observed for mitochondria from a variety of tissues. In mitochondria from brown adipose tissue (BAT) there is a proton leak catalysed by uncoupling protein 1 (UCP 1) (originally called thermogenin or uncoupling protein) that is superimposed upon this basal proton leak. BAT is innervated by the sympathetic nervous system and noradrenaline causes the expression of UCP 1 concomitant with an increase in brown adipose tissue mitochondrial volume density. Expression of UCP 1, and an increase in BAT mitochondrial volume density, occur in all new-born infants and animals and is an adaptive feature for heat generation. BAT persists in adult rodents and is triggered in a cold environment and preparation/reawakening from torpor. BAT is the source of non-shivering thermogenesis (for reviews see [8,29–31]).

When isolated, mitochondria from active BAT are uncoupled. Thus they have a high oxygen consumption rate and a low or negligible Δp. If measurable, an Ohmic relationship is observed between leak rate and Δp. Addition of purine nucleotides recouple isolated BAT mitochondria and state 4 oxygen consumption occurs to a similar extent as mitochondria from tissue not expressing UCP 1. In the presence of purine nucleotides high Δp values are attainable and oxygen consumption rates due to proton leak display a non-Ohmic relationship with Δp. Purine nucleotides bind to UCP 1 and prevent proton (and chloride) translocation. Some of the amino acids known to be involved in purine nucleotide binding in UCP 1 have been identified from chemical modification, photoaffinity labelling and site-directed mutagenesis studies [30,32–34]. The purine nucleotide binding site of UCP 1 is located on the cytosolic side towards the C-terminus of the protein (Fig. 2). The contiguous sequence EGPAAFFKG, associated with the sixth putative helix, has been reported to be involved in purine nucleotide binding in mouse (and rat) UCP 1. Similar contiguous sequences EGPRAFYKG and EGPTAFYKG are found at a similar location in UCP 2 and UCP 3 from rat. In addition, site-directed mutagenesis studies have shown that three arginines equivalent to Arg-84 (helix 2), Arg-183 (helix 4) and Arg-277 (helix 6) in the rat UCP 1 sequence are essential for purine nucleotide binding. Arginines at similar locations are found in UCP 2 and UCP 3. It is therefore possible that UCP 2 and UCP 3 could bind purine nucleotides and that proton (and chloride) translocation by them may be purine nucleotide sensitive.

UCP 1 also has a requirement for free fatty acids that are thought to directly interact with the protein. However, there is evidence that UCP 1 activity exists but is submaximal in the absence of free fatty acids [6,31]. Mitochondria containing UCP 1 or reconstituted native UCP 1 can also catalyse transport of chloride ions, a function that is purine nucleotide sensitive and fatty acid insensitive. The mechanism by which free fatty acids are involved in the UCP 1 uncoupling process is still a matter of investigation with two basic models being proposed: (i) the fatty acid flip-flop model and (ii) the model where fatty acid acts as a prosthetic group to UCP 1 (for reviews see [31–35]). The possibility therefore exists that the novel uncoupling proteins, UCP 2 and UCP 3, may also require fatty acids for (maximal) activity.

4. The novel mitochondrial UCPs

The recent cloning of membrane proteins UCP 2 [36,37] and UCP 3 [38,39], which have ~56% homology to UCP 1 and ~73% homology to each other, has generated a fervent if not as yet very fruitful avenue of research. UCP 1 is a member of a family of mitochondrial anion transporters [40] and is found exclusively in BAT. UCP 2 is most widely distributed being present at high mRNA levels in spleen, lung intestine, white adipose tissue (WAT) and uterus, with prominent expression in kidney, testes, uterus, BAT, brain, heart, and detectable expression in skeletal muscle and liver [36,37]. The UCP 2 mRNA in the liver is thought to be attributable to the Kupffer cells and not the parenchyma cells [26].

UCP 3 transcript on the other hand is detectable in BAT and in rat skeletal muscle with particular prominence in tensor fascia latae (fast-twitch, glycolytic), tibialis anterior (fast-twitch, oxidative/glycolytic) and gastrocnemius (mixed) muscles and to a lower level in the soleus muscle (slow-twitch, oxidative) [38,39]. UCP 3 also exists in two forms: long (L) which is the full-length protein, and short (S) which lacks ~30 amino acids from its C-terminus. A mitochondrial subcellular localisation is strongly suggested by the primary sequences of UCP 2 and UCP 3. In particular, the tripartite recurrent motif that is characteristic of mitochondrial anion transport protein is present in UCP 2 and UCP 3 (Fig. 2). In addition their size and molecular weight are similar to those of mitochondrial anion transporters [40]. Immunodetection, functional assays and purification, combined with UCP 1 knockout studies have firmly established UCP 1 as a mitochondrial inner membrane protein [6,32,41]. The data also suggest that the novel UCPs have mitochondrial localisation. In the case of UCP 2, immunodetection has been performed using UCP 2 peptide antibodies, antibodies to whole UCP 2 expressed in *Escherichia coli* or antiserum to whole UCP 1. UCP 2 was first detected immunohistochemically by Larrouy et al. [26] using a highly sensitive antiserum against UCP 1. A ~33 kDa signal was observed in the mitochondrial fraction of Kupffer cells which do not express UCP 1 or UCP 3 but express UCP 2 mRNA, suggesting that the antigen was UCP 2. Cadenas et al. [28] found that peptide antibodies to the 20 N-terminus of human or rat UCP 2 could not detect an appropriate molecular weight band in rat muscle or mitochondria isolated from skeletal muscle, however antigen could be detected in WAT with reference to a HEK293/UCP 2 expression system. Chavin et al. [18] detect mitochondrial associated UCP 2 protein using immunohistochemistry in liver slices of ob/ob mice with no detection in liver of non-obese mice using primary antisera to a UCP 2 peptide. Horvath et al. [42] used rabbit anti-UCP 2 antibody and found that UCP 2 was associated with mitochondria (immunohistochemistry) of neurons, predominantly in axons and axon terminals in divergent hypothalamic and limbic sites.

With regard to immunodetection of UCP 3, antisera to UCP 1 and peptide antibodies to UCP 3 have been successful. Cadenas et al. [28] have shown that peptide antibodies to a 14 amino acid human UCP 3 peptide detect a 32 kDa protein in rat hind limb skeletal muscle rats. In addition there is a 2-fold increase in UCP 3 protein in the whole muscle and mitochondria isolated from that muscle upon starvation. Their UCP 3 peptide antibody was also specific for UCP 3, it not being able to detected UCP 2. Interestingly, antisera to whole UCP 1 failed to detect UCP 2/3 in BAT mitochondria in UCP 1 ablated mice, despite the increased UCP 2/3 mRNA levels [43].

The limited amount of Western blot data on UCP 2 and UCP 3 in non-transgenic tissues (and in isolated mitochondria from those tissues) could be due to poor antibodies or poor technique. However, in cases where UCP 2 or UCP 3 is detected, the amounts fall way short of those found for UCP 1 in BAT. It just might be that amounts of novel UCP proteins expressed constitutively in skeletal muscle and BAT, are just very low, as alluded to by Cadenas et al. [28] for UCP 2 and Matthias et al. [43] for UCP 2 and UCP 3 in BAT of UCP 1 ablated mice, respectively.

5. Functional analyses of UCP 2 and 3 in cells and mitochondria

Due to the possible uncoupling nature of these novel uncoupling proteins, functional studies have tended to focus on whether resting cellular/state 4 mitochondrial oxygen consumption rates increased and/or resting cellular membrane potentials decreased as a result of varying the expression of UCP 2 and UCP 3. Decreases in resting in situ mitochondrial membrane potentials alone, measured using fluorescent probe detection systems and UCP 2 or UCP 3 expression systems, have been taken as evidence of the uncoupling effect and by default the mitochondrial location of UCP 2 and UCP 3. However, concomitant measurement of an increase in resting oxygen consumption is required to add credibility to such an interpretation as inhibition of substrate supply to in situ mitochondria could also decrease resting membrane potential.

Diminished resting membrane potentials in yeast [36,37,44–46] are reported when these cells are transfected with novel UCPs. Resting mitochondrial membrane potential has also been shown to be diminished in the breast cancer cell line MCF7 transfected with UCP 3 [47] and C_2C_{12} myotubules (modestly diminished) [5] transfected with UCP 3. Increased oxygen consumption has been seen in mitochondria from the yeast transfected with UCP3L and UCP3s [45]. Expression of the oxoglutarate carrier in the same yeast had no effect on oxygen consumption rate or resting membrane potential [48]. Hinz et al. [44] also see increases in whole yeast oxygen consumption and heat production in UCP3L transfected yeast cells. Rial et al. [49] also demonstrate that state 4 rates are increased in mitochondria isolated from yeast cells expressing UCP 1, UCP 2 and UCP 3. There are also unpublished data, reported in Ricquier and Bouillaud [6], which show increased oxygen consumption rate, compared with controls, at any selected membrane potential, in spheroplasts of yeast expressing UCP 2 (F. Bouillaud and M. Goubern, unpublished data). Direct regulation of novel UCP function has also been investigated. Data in the literature show that mitochondrial oxygen consumption from UCP 2 transfected yeast is insensitive to addition of fatty acids and purine nucleotides but increased on addition of retinoic acid [49]. Oxygen consumption by UCP 3 transfected yeast is insensitive to addition of free fatty acids, purine nucleotides or retinoic acid [45,49,50]. Retinoic acid would therefore appear to be a putative 'activator' of UCP 2 function.

An interesting conflict of function comes from mutagenesis studies on UCP 1. Bienebraeger et al. [51] have shown that the histidine pair (H145 and H147) is essential for proton translocation. They conclude that because UCP 2 and UCP 3 lack the histidine pair, they cannot function as proton translocators. Similar mutagenesis studies in the yeast expression system would seem to conflict with this observation [48].

Indications and context of function also come from mRNA expression studies. Northern blot analyses have shown that there are a wide variety of hormones, hormone agonists and physiological conditions that influence UCP 2 and UCP 3 mRNA expression both using in vivo (whole animal) and in vitro (cell) systems (see reviews [6,7,52]). UCP 2 expression is increased by factors that affect energy metabolism such as thyroid status, leptin status, starvation, and pyrogenic elements. UCP 3 expression is also affected by factors that affect energy metabolism in particular factors that increase fatty mobilisation and oxidation. As examples, UCP 3 mRNA expression in rat skeletal muscle is increased by starvation (10-fold in soleus) [38], chronic exercise (15-fold; non-discriminate muscle) [53] and T_3 administration [12] (5-fold). Parallel increases in UCP 3 protein were not reported in the aforementioned. UCP 2 message is increased 14-fold in BAT of UCP 1 ablated mice [43] compared to wild-type, 5-fold in skeletal muscle (non-discriminate muscle) [28] of starved animals compared to controls and 28-fold in liver of lipopolysaccharide treated rats [54]. Matthias et al. [43] and Cadenas et al. [28] could not detect any UCP 2 protein increases to correspond to these mRNA changes. No reported attempts to measure UCP 2 protein have been recorded in the case of lipopolysaccharide administration.

Detection and quantification of protein changes are central to predicting function in non-transfected expression systems. Large changes in mRNA levels, such as the aforementioned, are the most promising experimental regimes when looking for changes in protein expression. To that end, Cadenas et al. [28] have demonstrated that UCP 3 mRNA level in skeletal muscle increased 4-fold (non-discriminate muscle) which manifest itself as a 2-fold increase in UCP 3 protein. When proton leak rate was measured, in the presence of defatted BSA (to mop up free fatty acids), no difference was observed between the leak rates in skeletal muscle mitochondria from starved rats when compared to mitochondria from unstarved animals. The authors conclude that UCP 3 does not catalyse the basal conductance in skeletal muscle mitochondria. Interestingly, skeletal muscle mitochondria isolated from UCP 3 ablated mice have decreased state 4/proton leak rates [55,56], whilst mitochondria from mice overexpressing UCP have increased state 4 oxygen consumption [57].

6. Reconstituted UCP 2 and UCP 3

Expressed UCP 2 and UCP 3 have been reconstituted in proteoliposomes and proton and chloride transport studies undertaken. Echtay et al. [58] expressed UCP 1 and UCP 3 in *E. coli* and reconstituted into liposomes. Chloride flux was measured and was shown to be inhibited by exogenous submicromolar GDP/GTP concentrations. Unlike in native UCP 1 reconstituted systems, proton flux in the

E. coli expressed UCP 1-reconstituted system could not be detected even upon edition of 125 μM laurate. Jaburek et al. [59] expressed UCP 1 in yeast and UCP 2 and UCP 3 in *E. coli*. The solubilised proteins were reconstituted into liposomes. Chloride flux was not measured but proton flux was detected, and dependent on fatty acid (40 μM palmitate) and was shown to be moderately GDP sensitive. Taken together, the results suggest an uncoupling function for UCP 2 and UCP 3 similar to that of UCP 1.

7. Transgenic mice

Recent studies on transgenic animals have been interesting. Mice in which UCP 3 has been ablated lacked obvious phenotypes (e.g. no effects on body mass and metabolic rate) [55,56]. However mice overexpressing human UCP 3 are hyperphagic and are leaner than their wild-type counterparts [57], a phenotype consistent with an uncoupling effect on mitochondria.

8. Conclusion

Quantitatively proton leak contributes to ~25% of the resting oxygen consumption of mammals. Proton leak is also a determinant in accounting for differences in basal metabolic rate. The mechanism of the proton leak is unclear, but the data in the literature suggest that a diffusion-mediated basal proton leak occurs across mitochondria. The high degree of sequence homology of the recently cloned uncoupling proteins UCP 2 and UCP 3 to brown adipose tissue UCP 1, and the extensive tissue distribution, suggest that they play a role in proton leak. The expression patterns and the phenotype of the mice overexpressing UCP 3 suggest that the novel uncoupling proteins UCP 2 and UCP 3 are important determinants of basal metabolism. In vitro and reconstitution experiments strongly suggest that the novel uncoupling proteins uncouple mitochondria, an effect that is probably superimposed upon the basal proton leak that occurs in all mitochondria investigated.

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