Review

UCP2, UCP3, avUCP, what do they do when proton transport is not stimulated? Possible relevance to pyruvate and glutamine metabolism

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Abstract

Uncoupling proteins (UCPs) are specialized members of the mitochondrial transporter family. They allow passive proton transport through the mitochondrial inner membrane. This activity leads to uncoupling of mitochondrial respiration and to energy waste, which is well documented with UCP1 in brown adipose tissue. The uncoupling activity of the new UCPs (discovered after 1997), such as UCP2 and UCP3 in mammals or avUCP in birds, is more difficult to characterize. However, extensive data support the idea that the new UCPs are involved in the control of reactive oxygen species (ROS) generation. This fits with the hypothesis that mild uncoupling caused by the UCPs prevents ROS production. Activators and inhibitors regulate the proton transport activity of the UCPs. In the absence of activators of proton transport, the UCP allows the permeation of other ions. We suggest that this activity has physiological significance and, for example, UCP3 expressed in glycolytic muscle fibres may be a passive pyruvate transporter ensuring equilibrium between glycolysis and oxidative phosphorylation. Induction of UCP2 expression by glutamine strengthens the proposal that new UCPs could act to determine the choice of mitochondrial substrate. This would obviously have an impact on mitochondrial bioenergetics and ROS production.

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1. Introduction

This article covers the topics presented by one of us (FB) at the Bari Meeting (17–22 December 2005). Most of the results discussed here are published [1,2] or in manuscripts that are in revision [3] or preparation [4]. They are enriched by further speculations and comments following questions raised during this meeting. Some previously unpublished results are also presented.

For reviews on uncoupling proteins (UCPs) see [5–11]. From 1976 to 1997, only one UCP, now called UCP1, was known. UCP1 is expressed in the brown adipose tissue of mammals and is necessary for the inducible thermogenic activity of this tissue. In 1997, two genes were discovered in mammals that code for proteins sharing about 60% sequence similarity with UCP1. Because of this similarity, and also because the first experiments using recombinant expression showed a modification of mitochondrial activity consistent with uncoupling, these proteins were called UCP2 and UCP3. Related proteins have been found in other phyla, including plants, and in birds (called here avUCP). We will use the term “new UCPs” to distinguish these proteins discovered after 1997 from the original UCP1. The relationships between the respiratory chain, FoF1 ATPase, and UCP are illustrated in Fig. 1. UCP simply allows passive proton return through the mitochondrial inner membrane, whereas FoF1 ATPase couples this proton return to phosphorylation of ADP into ATP. Consequently, in the presence of UCP oxidation accelerates, whereas ATP production by mitochondria diminishes. Other consequences of uncoupling are also shown on the bottom of Fig. 1. Lowering of the mitochondrial membrane potential (ΔΨ) is the first observable event when UCP activity is suddenly stimulated. The effect on production of reactive oxygen species (ROS) by the respiratory chain of mitochondria needs further explanation: in simple terms one may accept that
ROS production by mitochondria is explained by crowding of electrons within the respiratory chain complexes I and III [12]. To form ROS some of these electrons escape from the normal pathway that link movement of pairs of electrons to proton pumping. Decreasing permeability of the inner membrane to protons facilitates this normal pathway, and hence reduces the crowding of electron and ROS production, the price being increased waste of energy. Consequently, soon after the discovery of UCP2 and UCP3 it was proposed that these proteins might be involved in the control of ROS production [13]. Experimental data now support this point of view [14,15], whereas the relevance of UCP2 or UCP3 to the control of energy expenditure seems doubtful. Mitochondrial ROS formation might be of physiological relevance but is also very likely a major source of damage, at least at the mitochondrial level [16].

From the early studies with UCP1 it became apparent that the activity of UCP itself is regulated [17]. This occurs via binding of activating or inhibiting ligands to the UCP. Nucleotides (ATP, ADP, GTP, GDP) inhibit and fatty acids activate UCP1 [18]. After discovery of new UCPs, more or less similar mechanisms of the regulation of their proton transport activity have been proposed [19–21]. The UCP may therefore exist in three states (Fig. 2): (1) inactive with a bound inhibitor (black), (2) proton transport mode with an activator of proton transport linked to the UCP (grey), (3) with no ligand (white). The latter state is supposed to be of little significance for UCP1 since intracellular nucleotide concentrations are expected to ensure that UCP1 is largely inhibited. However, data obtained with isolated mitochondria and with UCP1 reconstituted in proteoliposomes support the fact that, in the absence of any ligands, UCPs exhibit transport activity. This transport activity concerns anions such as chloride but also organic anions including pyruvate [22]. Does proton transport occur too, when no activating compound is bound to the UCP? We believe that this is the case with UCP1 [23]. This is much more doubtful with the other UCPs (UCP2, UCP3, avUCP). In fact, a consensus has more or less been reached considering that the new UCPs need specific activation for the proton transport to occur [2,19–21,24]. In contrast, the anion transport can occur without activation [25].

The purpose of this paper is to promote the idea that transport by the UCP might be of physiological relevance even in the absence of the ligand(s) promoting proton transport. This hypothesis is based on several observations that led us to consider that whereas proton conductance through UCP3 did not take place within cells, the cell metabolism was still influenced by the presence of UCP3. A second set of arguments came from the observation that UCP2 expression was stimulated by glutamine, a known substrate for mitochondrial oxidation, pointing to the possibility that UCP2 and UCP3 function is more linked to the choice of mitochondrial substrate than to the determination of the passive proton conductance of the inner membrane. This effect is expected to have complex consequences for mitochondrial metabolism, including, but not limited to, ROS production.

At this point it should be underlined that the proposal that UCPs influence substrate use by mitochondria is not entirely new. It has been proposed that the new UCPs control fatty acid metabolism in mitochondria [11,26].
is the export of fatty acid out of mitochondria [27,28]. However, we will explain later why the distinction between this activity and uncoupling is problematic.

2. Failure to find uncoupling

Recombinant expression in cell lines has been used to study UCP activity. Using this system, we aimed to study the influence of UCP expression at the cellular level. A study was undertaken with a series of CHO cell lines expressing UCP3 and a large set of control CHO cell lines expressing no recombinant protein or other proteins supposed to be of no relevance to mitochondrial activity. A UCP1-expressing clone [29] was also included. It was observed that under standard conditions the presence of UCP3 somehow seemed to influence mitochondrial activity [1]. Many (not all) of these initial observations agreed with the consequences of uncoupling presented above (Fig. 1). However, we failed each time we attempted to manipulate experimental conditions to enhance the influence of UCP3 within the theoretical framework of a limited uncoupling [1]. This observation was not restricted to UCP3 (or UCP1) in CHO cells: our early attempts with cells expressing UCP2 led to a similar conclusion (unpublished data). Fig. 3 shows experiments with the avian UCP expressed in COS cells. In the basal respiratory state, ROS production was lower in the avUCP-expressing cells than in controls (Fig. 3 circles). Addition of the ATPase inhibitor oligomycin reduced the respiratory rate and caused hyperpolarization of mitochondria (not shown here but see [1]), and therefore increased ROS production. The uncoupling hypothesis would predict that oligomycin increases the difference between the avUCP clone and its control. In fact, in the presence of oligomycin this difference disappeared (Fig. 3 squares). As with UCP3, attempts to promote the relative influence of avUCP within the framework of the uncoupling hypothesis failed. Finally, the UCP1 clone showed that within the CHO cells the uncoupling activity of the UCP1 is inhibited [1], which was observed before with transgenic mice [30]. Consequently, it was concluded that within our cells uncoupling did not take place through UCP1, avUCP, UCP2 or UCP3.

3. Effect of UCP3 in the absence of uncoupling

The uncoupling activity of any UCP (UCP1, UCP2, UCP3 or avUCP) could therefore not be observed in these CHO cell lines because the appropriate activator was missing. However, it was observed that the hyperpolarization due to oligomycin was remarkably constant in the five different UCP3-expressing clones (22 mV ±1), whereas it varied much more with the controls (25 mV ±10) [1]. This suggested that, even in the absence of an activator of the uncoupling activity, UCP3 could influence mitochondrial activity. How could UCP3 guarantee that hyperpolarization would be precisely controlled? Since UCP3 is supposed to be a passive transporter in the mitochondrial inner membrane of CHO cells, we proposed that this hyperpolarization control was due to UCP3, which ensures equilibration according to the Nernst law governing the concentration of an ion between the mitochondrial matrix and the cytosol. Accordingly, a variation of 22 mV would change by a factor 2.4 the ratio between the external and internal concentration of a monovalent ion. A hypothesis was formulated [1], which should be divided into two separate tenets: (1) UCP3 acts as a uniporter controlling the distribution of an ion relevant to mitochondrial metabolism, and (2) this ion is pyruvate.

4. The uniport hypothesis

The first tenet is supported by the experimental evidence that the hyperpolarization of mitochondria after oligomycin addition in the UCP3 clones is constant (22 mV). It should be added that this value was independent of UCP3 expression level in the cellular clones. In relative terms the expression of UCP3 in these clones varied from 1 to 10 (1 being a value close to the physiological expression of UCP3 in muscle mitochondria preparations). This tallies with the equilibration of an ion through a specific transporter: the transporter needs to be present, but its amount would simply make the equilibration faster and would not influence the final state. This observation is a supplementary argument against a “cryptic” uncoupling activity that would have escaped other investigations, because, in the case of uncoupling, UCP abundance would matter.

UCPs belong to a large family of transporters of the mitochondrial inner membrane [31]. Most of these transporters are symporters such as the proton–phosphate symporter or exchangers such as the ATP/ADP translocase. In contrast, the UCPs have been characterized as uniporters. It should be recalled that cations are attracted inside negatively polarized mitochondria whereas anions are driven out of the matrix. The mitochondrial substrates are organic anions, and it therefore...
makes sense that their transporters mediate symport or antiport. On the other hand, the existence of uniporters would lead to accumulation of the cation or loss of the anion transported. The history of UCPs supports the hypothesis of an anionic uniport.

In view of these considerations, we can re-examine some of our data obtained with avUCP. This protein was studied using the yeast expression system [2], where it gave results close to those obtained before with mouse UCP2 [19]. For example, retinoic acid addition to yeast mitochondria expressing avUCP resulted in significant uncoupling [2]. In the absence of retinoic acid, although the respiratory activity of avUCP mitochondria remained higher than that of controls, no evidence was found for an increased proton conductance in these avUCP (Fig. 2 in [2]). In subsequent studies (Fig. 4), it was observed that neither the state 4 rate of respiration nor the proton conductance was affected after addition of the potassium–proton exchanger nigericin to the avUCP mitochondria (Fig. 4, middle). In contrast, the same addition of nigericin increased the state 4 rate of control mitochondria to a value not different from that of avUCP (Fig. 4, bottom). In other words “control+nigericin” = “avUCP”. It appeared difficult at that time to propose a similar activity for UCP3 and nigericin. Nigericin addition was expected to convert any remaining delta pH into delta psi (ΔΨ) and therefore we anticipated an increase in membrane potential with little change in respiratory rate. This did not happen, since only an increase in respiratory rate was observed (Fig. 4). A slight upward shift of the conductance curve (Fig. 4, top) suggests a limited increase in proton conductance in the presence of nigericin with control mitochondria. Therefore, the energy stored in delta pH appeared to be lost when nigericin was added. No such loss occurred in the presence of avUCP (Fig. 4, middle), probably because delta pH was already dissipated. Possible interpretations are given in the insets of Fig. 4. In the case of control mitochondria in the presence of nigericin, a potassium conductance is proposed to explain the loss in delta pH. With avUCP we propose the participation of a proton–anion symport (via a normal yeast mitochondrial carrier?) coupled to the anionic conductance of the avUCP. The anion is unknown and must have been introduced by the mitochondrial preparation. Both mechanisms (insets of Fig. 4) would dissipate delta pH without creating a delta psi counterpart. In the absence of nigericin, the avUCP mitochondria would differ from their control by a null delta pH. This may explain the relative protection of these mitochondria against oxidative stress [2] since it has been demonstrated that delta pH promotes ROS production by mitochondria [32]. However, this delta pH-dependent ROS formation was shown with mammalian complex I, something very different from the situation found in yeast mitochondria.

The mechanisms proposed in the insets of Fig. 4 result in a net proton transport, which would cause complete uncoupling of mitochondria unless one of them, presumably the uniport, is gated according to membrane potential and therefore occurs with a significant amplitude only at high potential values. In the absence of avUCP or nigericin (top of Fig. 4 empty circles), the proton conductance is also somehow gated since it increases abruptly above a certain potential. This is known as the non-ohmic proton leak of the mitochondrial inner membrane. It is noteworthy that the mechanisms proposed in the inset of Fig. 4
must show a gating potential close to the threshold observed for the non-ohmic proton leak. Therefore, it is tempting to speculate that this threshold is in fact a limit at which a suddenly increasing number of mechanisms similar to those shown in the insets can be recruited. This would reveal functional characteristics shared by a number of components of the inner membrane. The consequence is that there would be no unique explanation for the non-ohmic proton leak, a phenomenon thought to influence resting metabolic rate and energy expenditure [33].

5. The pyruvate hypothesis

The second tenet is that the ion is pyruvate. This is a speculation based on the assumption that a mitochondrial substrate is a good candidate as a controlling ion and on the available literature, since it has been shown that UCP1 is able to transport pyruvate [22,34]. Therefore, this activity is supposed to be the main function of UCP3 in muscle. It is important to recognize here that till now there has been no experimental evidence for this. Further arguments could be put forward for the support of our hypothesis: (1) the greatest UCP3 expression has been shown in glycolytic fibres [35]; (2) in our study, some of the modifications produced by UCP3 in CHO clones disappeared when glucose was removed from the medium [1]. Both arguments strongly suggest a link between the presence of UCP3 and glycolysis. Accordingly, the pyruvate hypothesis would ensure coexistence between intense glycolytic activity and mitochondria. According to our model, polarization of mitochondria would lead to a decrease in the internal concentration of pyruvate since this substrate is taken out by the anionic uniporter UCP3 in a membrane potential-dependent manner. Therefore, mitochondria in glycolytic cells would be equipped with a self-inhibiting mechanism that removes mitochondrial substrate as polarization increases and would moderate the Pasteur effect by which mitochondria inhibit glycolysis. Moreover, reduction of components of the respiratory chain, hence ROS formation (see above), is controlled by substrate availability and membrane potential. By linking these two parameters UCP3 would control ROS formation without any energy waste. While our data are consistent with this proposal when cells are observed in their basal state, this was no longer the case in the presence of oligomycin (Fig. 3) and [1]. Both arguments strongly suggest a link between the presence of UCP3 and glycolysis. Accordingly, the pyruvate hypothesis would ensure coexistence between intense glycolytic activity and mitochondria.

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It was remarked during the meeting that pyruvate is a leaky substrate during reconstitution experiments and therefore attribution of the pyruvate transport activity to the UCP may be questionable. The term leaky substrate means that the uncharged protonated form of this organic acid is able to cross the phospholipid bilayer. This deserves two comments: (1) the surface ratio between phospholipids and proteins is very different in liposomes and mitochondria; (2) this remark should also be addressed to the pyruvate carrier, since its activity (Fig. 5) matches precisely the permeation process mentioned above. In this respect, it should be pointed out that on the one hand the pyruvate carrier is supposed to be present in all mitochondria, and on the other hand the molecular identification of the protein remains elusive.

6. Glutamine and UCP2

The role of the new UCPs in mitochondrial metabolism remains unclear. The study of factors controlling the expression
of these new UCPs may help to clarify their physiological role, and a relevant hypothesis about the transport(s) involved might then be formulated and validated. In this respect, it should be recalled that the relevance of UCP1 expression to cold adaptation was established before its transport activity was known [39]. Our studies in several models have shown that UCP2 expression can be controlled exclusively at the level of translation: while mRNA levels did not change, protein levels increased in animals after various stimuli [40]. These stimuli shared the common property of increasing oxidative stress. Therefore, UCP2 appeared as a protein whose expression can be quickly induced by the mobilization of an existing mRNA pool whose translation is stimulated.

We observed that increasing the glutamine concentration in the culture medium stimulated UCP2 mRNA translation in several cell lines representing relevant sites of UCP2 expression: macrophages, pancreatic beta cells, colonocytes [4]. It should be recalled that macrophage activity and insulin secretion were altered in UCP2-knockout mice. Glutamine is known to be a substrate for the immune system and the gut epithelium, two sites of UCP2 expression. Induction occurs at physiological concentrations of glutamine (around 1 mM). In contrast, our attempts to promote UCP2 expression by increasing oxidative stress in cell cultures have failed.

The mechanism underlying regulation of UCP2 translation by glutamine has been deciphered [4]: the translation of UCP2 from the UCP2 mRNA is normally inhibited because of the existence of an upstream open reading frame (uORF) in the long 5' untranslated region of the UCP2 mRNA [3,40]. When stimulation of UCP2 translation occurs, the inhibitory effect of this uORF is lost (Fig. 6).

This raises the question of a link between UCP2 and the mitochondrial utilization of glutamine as a substrate. It is unlikely that UCP2 is the mitochondrial glutamine transporter since the latter was purified from kidney mitochondria and has a different molecular weight from UCP2 [41]. Moreover, UCP2 was undetectable in kidney mitochondria [40,42]. Ongoing studies are comparing intracellular glutamine metabolism in wild-type and UCP2-knockout animals.

7. Concluding remarks

Studies using recombinant expression of UCPs have well documented the proton transport activity of UCP2, UCP3, and avUCP. However, there are still unresolved issues concerning...
UCPs. How big is the UCP family? Is the proton transport by all UCPs regulated in the same manner? What are the regulators? Which ones are physiologically relevant? Finally, is the transport of other ions of more physiological relevance than proton transport in the case of the new UCPs?

There is ample evidence that UCP2, and to a lesser extent UCP3, plays a role in the control of oxygen radicals in vivo, but this evidence is rarely associated with a clear demonstration of respiration uncoupling. It is likely that while uncoupling may occur in some models, its detection would be difficult. But does uncoupling explain all the effects of the UCPs reported so far? For example, data recorded in vivo show that the health of mice worsened or improves according to the level of UCP2 expression [43,44]. Whatever the mechanism involved, UCP2 is a negative modulator of macrophage activity [14]. When lesions are examined in mouse models, it is difficult to distinguish the direct and indirect damage due to the “cleaning” activity of macrophages. In other words, what has been attributed to the protective effect of UCP2, in preventing ROS formation within one cell, might also have been attributed to a moderation of macrophage activity around this cell.

In studies with yeast mitochondria, we have demonstrated a yeast uncoupling pathway (YUP) [45], which appears to work as a safety valve. Uncoupling by YUP is activated by ATP, and inhibited by ADP and phosphate [46]. Therefore, when mitochondria approach state 4 because all the cellular ADP had been phosphorylated into ATP, YUP uncoupling activity is triggered. In contrast, UCPs are all thought to be inhibited by the binding of the nucleotides (ATP, ADP, GTP, GDP) initially shown to be inhibitors of UCP1 activity. In this respect, UCPs seem poorly adapted to work as a safety valve similar to YUP. It appears easier to reconcile nucleotide inhibition with the anionic conductance proposed here (Fig. 7). If mitochondria contribute efficiently to ATP production, then the UCP is inhibited. Inhibition in fact means raising of the threshold at which conductance occurs, and so substrates remain within the mitochondria (Fig. 7, top). Conversely, if mitochondria cease to provide ATP then the drop in ATP lowers the threshold at which the loss of substrate occurs. The worst case scenario is shown with AMP, which does not inhibit UCP (Fig. 7, bottom).

As a final remark we would like to stress that the expression of UCP3 in glycolytic fibres and the induction of UCP2 by glutamine point to situations where the fate of pyruvate is not to enter the tricarboxylic cycle (Fig. 8). This is well in line with the proposed hypothesis.

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