Journal of Bioenergetics and Biomembranes, Vol. 31, No. 5, 1999

A History of the First Uncoupling Protein, UCP1

David G. Nicholls[1] and Eduardo Rial[2,3]

The lack of energy conservation in brown adipose tissue mitochondria when prepared by conventional methods was established in the 1960s and was correlated with the thermogenic function of the tissue. In order to observe energy conservation, two requirements had to be met: the removal of the endogenous fatty acids and the addition of a purine nucleotide. These two factors have been the essential tools that led to the discovery of the energy dissipation pathway, the uncoupling protein UCP1. The activity is regulated by these two ligands. Purine nucleotides bind from the cytosolic side of the protein and inhibit transport. Fatty acids act as seconds messengers of noradrenaline and increase the proton conductance. This review presents a historical perspective of the steps that led to the discovery of UCP1, its regulation, and our current view on its mechanism of transport.

KEY WORDS: Brown adipose tissue; mitochondria; uncoupling protein; UCP1; transport; nucleotide; fatty acid.

INTRODUCTION

The bioenergetic behavior of brown adipose tissue mitochondria was elucidated in the 1970s and early 1980s and provided one of the first independent confirmations of Peter Mitchell's chemiosmotic concept of mitochondrial bioenergetics. Recently some of the fundamental concepts of the uncoupling protein function have been challenged (Garlid *et al.*, 1998) and, in view of the difficulty in accessing the literature from 25 years ago, it may be useful to review some of the findings made at that time which led to the discovery, kinetic characterization, and elucidation of the physiological regulation of the protein now referred to as UCP1.

Research in the 1960s and 1970s contributed greatly to our understanding of the anatomy and physiology of brown adipose tissue and established its role in the hibernator, the newborn, and, at least in experimental animals, in diet-induced thermogenesis (for reviews see Trayhurn and Nicholls, 1986). Brown fat is the major source of nonshivering thermogenesis in cold-adapted adult experimental animals, such as the rat (for reviews see Trayhurn and Nicholls, 1986) and from the earliest days there was interest in the molecular basis of a mechanism which could generate heat at up to 60 times the rate of an equivalent mass of liver tissue (Foster and Frydman, 1978). It was soon established that β-adrenergic stimulation of the tissue led to the rapid hydrolysis of the multilocular triglyceride droplets within the brown adipocytes and that, in some way, the abundant mitochondria were able to oxidize the resulting fatty acids at an enormous rate to account for the heat production.

Since such a dissipative process runs counter to concepts of energy conservation and respiratory control, several groups in the late 1960s began to prepare isolated brown fat mitochondria and to study their bioenergetic properties. There was a general finding that the isolated brown adipose tissue mitochondria (BATM) were capable of the rapid oxidation of substrates such as long-chain acyl carnitine and *sn*-glycerol 3-phosphate, but that classic respiratory control was absent (for review see Smith and Horwitz, 1969). This did not appear to be a consequence of artifactual

[1] Neurosciences Institute, Ninewells Medical School, Dundee University, Dundee, Scotland.
[2] Centro de Investigaciones Biológicas, CSIC, Madrid, Spain.
[3] Author to whom all correspondence should be sent. email: rial@cib.csic.es.

0145-479X/99/1000-0399$16.00/0

mitochondrial damage during preparation, since respiratory control could be induced by the addition of a purine nucleotide such as ATP or GDP (Rafael *et al.*, 1969) together with the removal of endogenous fatty acids, either by albumin (Rafael *et al.*, 1969) or by oxidation (Hittelman *et al.*, 1969). The mechanism of this "recoupling," however, remained obscure.

THE ABNORMAL ION CONDUCTANCES OF BROWN ADIPOSE TISSUE MITOCHONRIA

Starting in 1972, a series of experiments began to indicate that BATM might possess atypical ion permeability properties. First, the oxidation of NAD^+-linked substrates was abnormally sensitive to the osmolarity of the supporting medium (Nicholls *et al.*, 1972). Indeed, hamster BATM only oxidized palmitoyl-L-carnitine optimally when the osmolarity of a sucrose-based medium was decreased below about 120 mOsM (Nicholls *et al.*, 1972). Parallel studies with liver mitochondria suggested that the oxidation of NAD^+, but not flavoprotein-linked, substrates was inhibited when the matrix condensed below a critical value (Nicholls and Lindberg, 1972). In an isotonic KCl-based medium, however, the rate of respiration of BATM slowly increased to maximum as the sucrose-impermeable matrix space dilated (Nicholls *et al.*, 1972). Osmotic swelling of the matrix in a KCl-based medium requires the entry of both K^+ and Cl^- and, since mitochondria were considered to be relatively Cl^- impermeant at neutral pH, the possibility was addressed that brown fat mitochondria might posses abnormal permeability to halides (Nicholls and Lindberg, 1973). This was investigated by following the passive swelling (by decrease in light scattering) of nonrespiring mitochondria in 100 m*M* potassium salts in the presence of valinomycin to allow the free uniport entry of K^+ (Nicholls and Lindberg, 1973). Relative to liver or heart mitochondria, brown fat mitochondria from hamster, rat or guinea pig were found to be much more permeant at neutral pH to Cl^- and Br^-. Most particularly, in the absence of albumin, the rates of swelling of BATM were identical regardless of whether K^+ entry was facilitated by the uniport ionophore valinomycin or the K^+/H^+ antiport ionophore nigericin. This provided the very first indication that BATM might be proton permeable (Fig. 1)—a conclusion that may appear obvious now, but at the time provided the first indication that the chemiosmotic hypothesis might be a fruitful paradigm for these mitochondria (Nicholls and Lindberg, 1973). Importantly, swelling in the presence of nigericin was strongly inhibited by albumin and restored by FCCP, suggesting that fatty acid removal was restricting the proton permeability of the membrane.

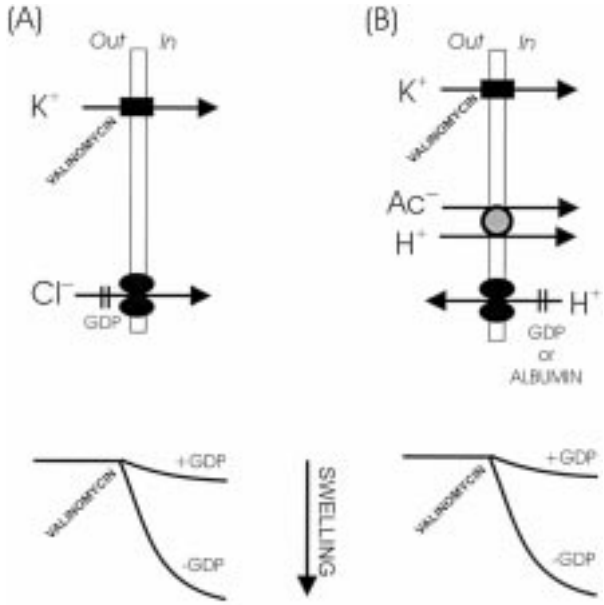

Fig. 1. Passive swelling of hamster BATM in KCl or K-acetate. (A) BATM suspended in 100 m*M* KCl in the presence of the K^+ ionophore valinomycin swell and, consequently, decrease their light scattering. Swelling is prevented by GDP, which inhibits the Cl^- conductance of the membrane. (B) BATM swell in 100 m*M* K-acetate in the presence of valinomycin. In order for this to occur, the inner membrane must be permeable to protons. Addition of GDP or albumin inhibits this proton permeability and thus decreases swelling. Both the H^+ and Cl^- permeabilities were later found to be due to UCP1. Schemes based on data in Nicholls and Lindberg (1973).

In view of the earlier findings that purine nucleotides aided recoupling of the mitochondria (Rafael *et al.*, 1969), it was expected that their addition might mimic the effects of albumin; however, GDP abolished swelling in KCl in the presence of *either* valinomycin or nigericin. Swelling was not restored by FCCP, suggesting that Cl^- permeability was being inhibited (Nicholls and Lindberg, 1973)—a conclusion confirmed by direct determination of the kinetics of $^{36}Cl^-$ exchange into the matrix (Nicholls, 1974b). An effect of GDP on proton permeability could, however, be detected if mitochondria were suspended in potassium acetate in the presence of valinomycin (Fig. 1).

The presence of a high *sn*-glycerol-3-phosphate dehydrogenase activity in brown adipose tissue mito-

chondria transferring electrons from the substrate binding site on the outer face of the inner membrane to the respiratory chain allows the respiratory chain of these mitochondria to be driven by a substrate whose oxidation would be unaffected by changes in matrix volume or in accumulation across the membrane in response to altered ion gradients. This facilitated the quantitative analysis of the proton circuit, initially in liver mitochondria (Nicholls, 1974c) and then in hamster BATM (Nicholls, 1974a). "Ohm's Law" was invoked, where the effective proton conductance of the inner membrane, C_mH^+, was equated to the proton current (respiratory rate *times* H^+/O stoichiometry) divided by the protonmotive force, Δp. The membrane potential component, $\Delta\psi_m$, was determined in low K^+ medium from the distribution of $^{86}Rb^+$ in the presence of valinomycin, while ΔpH was determined from the distribution of a weak acid and weak base (Nicholls, 1974a,c). In the absence of albumin or nucleotide, hamster BATM maintained no detectable Δp during substrate oxidation at a range of external pH values, consistent with an extremely high C_mH^+ (Nicholls, 1974a). The presence of a high concentration of essentially fatty acid free albumin (80 μM) allowed a Δp of about 80 mV to be sustained. This is well below the value of 166 mV at which respiratory control began to become apparent under these conditions, thus providing a quantitative explanation for the failure of fatty acid removal alone to induce "recoupling."

The behavior of Δp when GDP was added to BATM without removing endogenous fatty acids with albumin was particularly interesting. At pH 6.7, a Δp of 200 mV was attained, accompanied by slight respiratory control. As the pH was raised, a sharp decrease in the maintained Δp was observed, consistent with a C_mH^+, which increased from 1.5 to 6 nmol H^+ min/mg/mV over the pH range 6.7–8.0 (Nicholls, 1974a). Recent suggestions have been made, that UCP1 may catalyze the transport of the fatty acid anion, thus completing a classic protonophoric uncoupling loop together with the undissociated fatty acid (Garlid *et al.*, 1996). The pK_a for a long-chain fatty acid in a phospholipid bilayer is about 7.5 (Hamilton and Cistola, 1986). Thus, within the above pH range, the proportion of protonated fatty acid falls from 83 to 24% as C_mH^+ increases. It is clearly difficult to reconcile this with a simplistic concept of facilitated fatty acid uncoupling where the "inward" leg is catalyzed by permeation of the protonated fatty acid through the bilayer. In the presence of GDP *plus* 80 μM albumin, BATM displayed respiratory control comparable to mitochondria from other tissues. C_mH^+ was still pH dependent, although a Δp of $>$220 mV could be attained at neutral pH. The relationship between respiration and Δp, when the concentration of GDP was decreased, was exactly superimposable upon that when FCCP was titrated, consistent with purine nucleotide control of a proton-conducting pathway in the inner membrane (Nicholls, 1974a).

THE DISCOVERY OF UCP1

How do purine nucleotides control C_mH^+? No covalent modification or equilibration of the guanine nucleotides with the matrix pools could be detected (Nicholls, 1976). However, a high-affinity binding site for [^{3}H]GDP (or for [^{3}H]ADP in the presence of atractylate to inhibit the adenine, nucleotide translocator) could be detected in hamster brown fat mitochondria, with a B_{max} of 0.7 nmol/mg protein. The apparent dissociation constant was highly pH dependent, increasing from 4 μM at pH 6.7 to 34 μM at pH 7.9 (Nicholls, 1976), thus providing an explanation for the high pH dependency of the nucleotide inhibition of C_mH^+. It was proposed that "a specific protein component in the inner membrane is responsible for the abnormal ion conductances of these mitochondria" and that "purine nucleotides can bind to a site on the outer face of the inner membrane, which is either identical with, or in close contact with, the component of the inner membrane responsible for conducting protons across the membrane" (Nicholls, 1976). Ten purine nucleotide analogs were examined and, in each case, their ability to compete with [^{3}H]GDP for binding to hamster BATM correlated with their ability to raise Δp (Heaton and Nicholls, 1977).

Identification of this putative protein was possible following the synthesis of a photoaffinity purine nucleotide analog, 8-azido-ATP. This analog was found to compete with [^{3}H]GDP in the dark in the same way as the other analogs and to induce respiratory control in the presence of albumin. The synthesis of [γ-^{32}P]-8-azido-ATP and its covalent attachment to the mitochondria by UV irradiation allowed two proteins bands to be identified: one (M_r = 30 kDa) was competed by atractylate and was, therefore, assigned to the adenine nucleotide translocator, while the second (M_r = 32 kDa) was abolished by competition with 1 mM GDP (Fig. 2)(Heaton *et al.*, 1978). This 32-kDa protein, therefore, contained the nucleotide-binding site responsible for controlling C_mH^+ in these mitochon-

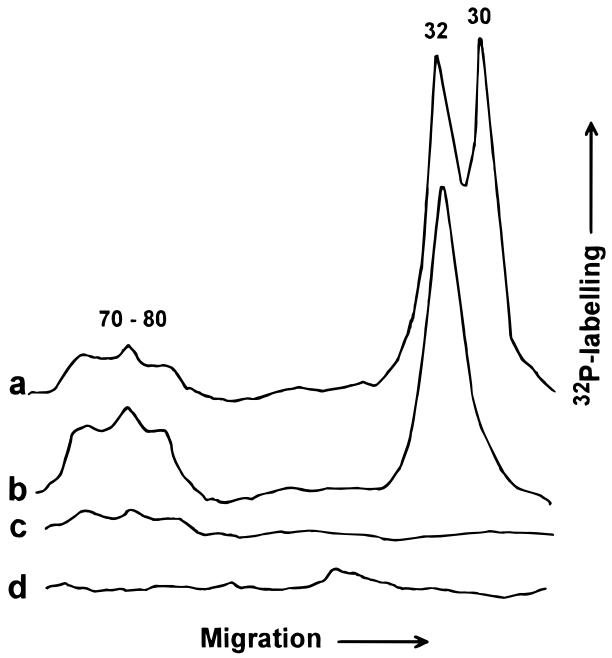

Fig. 2. Identification of the site of energy dissipation. BATM were photoaffinity labeled with [γ-^{32}P]-8-azido-ATP under the following conditions: (a) no further additions; (b) 30 μ*M* carboxyatractylate present; (c) 30 μ*M* carboxyatractylate and 1 m*M* GDP; (d) 0.1 m*M* [γ-^{32}P]ATP. Mitochondria were sonicated and proteins in the submitochondrial particles separated by SDS-PAGE. Traces represent the densitometric analysis of the autoradiographs. Adapted from Heaton *et al.*, 1978.

dria. The subsequent purification (Lin and Klingenberg, 1982) and reconstitution of this 32-kDa "uncoupling" protein showed that it was necessary and sufficient to induce GDP-sensitive proton permeability in model membranes (Klingenberg and Winkler, 1985). Of GDP bound/g of purified protein (Lin and Klingenberg, 1982), 17 μmol corresponding to one nucleotide per 60,000 M_r suggested that the protein acts as a dimer. On this basis, the protein is present in surprisingly high amounts, accounting for up to 14% of the inner membrane protein.

The "Ohm's Law" approach to the mitochondrial proton circuit allowed current–voltage relationships to be obtained for UCP1. Cold-adapted guinea pig BATM displayed a voltage-independent C_mH^+ of 16 nmol H^+ min/mg/mV (Nicholls, 1977). On the basis that these mitochondria have 0.7 nmol of UCP1 dimer/mg, this implied a conductance of only 20 H^+ min/mV per dimer, which is many orders of magnitude less than that catalyzed by pore-forming antibiotics, such as gramicidin.

Early studies by Rafael and colleagues (Rafael *et al.*, 1969) showed that the purine nucleotide requirement for respiratory control in guinea pig BATM was decreased if the animals had been reared under warm conditions. Following the detection of nucleotide binding to hamster mitochondria, Rafael and Heldt (1976) found that the B_{max} for GDP binding to guinea pig BATM correlated with the thermogenic capacity of the tissue: being increased by cold adaptation. In the same year, Ricquier and Kader (1976) showed that a 32-kDa M_r mitochondrial protein of unknown function was enhanced in BATM from cold-adapted rats and speculated that this might represent a cytochrome or flavoprotein, however, following the discovery of the uncoupling protein as M_r = 32 kDa band (Heaton *et al.*, 1978), the stage was set to investigate the adaptive induction of UCP1 in response to environmental conditions.

THE ACUTE PHYSIOLOGICAL REGULATION OF UCP1

Although GDP has been generally used to inhibit UCP1 *in vitro*, the protein shows a low specificity for purine nucleoside di -and triphosphates (Heaton *et al.*, 1978). The physiologically relevant nucleotide is ATP, whose cytoplasmic concentration should be sufficient always to saturate the nucleotide binding site. The question, therefore, arose as to the mechanism by which the cell regulates the activity of UCP1. Four putative regulators had been pointed out over the years: purine nucleotides, cytosolic pH, and two intermediates of the hormone-stimulated lipolysis: fatty acids and long-chain acyl-CoA (for review see Nicholls and Locke, 1984).

The earliest accounts on the bioenergetic properties of brown fat mitochondria pointed out an unusual high sensitivity to fatty acid uncoupling (Fig. 3) (Rafael *et al.*, 1969; Hittelman *et al.*, 1969) and thus soon they were proposed as regulators of the conductance (for review see Flatmark and Pedersen, 1975). However, two lines of evidence seemed to indicate that they were not the specific regulators of UCP1 activity (Heaton and Nicholls, 1976; Nicholls, 1979). First, the lack of specificity, because fatty acids were uncouplers of all types of mitochondria, and, second, they appeared not to act through the nucleotide-sensitive pathway because they did not influence chloride permeability (Nicholls and Lindberg, 1973; Nicholls, 1979). The turning point in the understanding of the regulatory of role of fatty acids came in the early 1980s.

BATM in the presence of ATP, carnitine, and CoA oxidize endogenous fatty acids and subsequently demon-

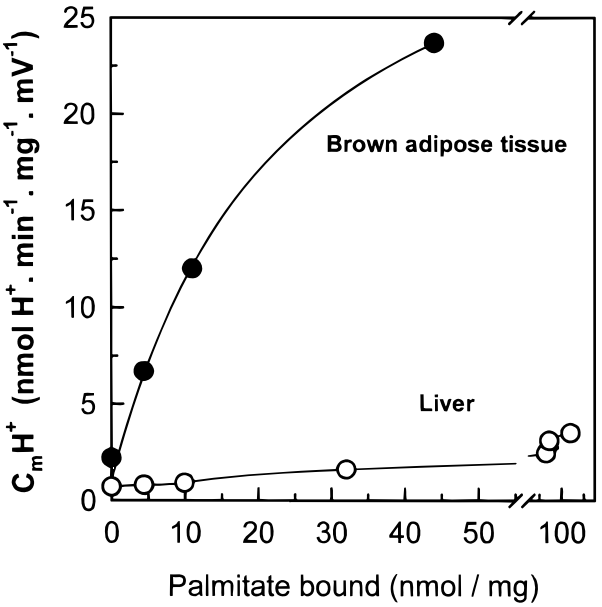

Fig. 3. Mitochondria from brown adipose tissue are more sensitive to fatty acid uncoupling than those from liver. Mitochondria were equilibrated with different palmitate to albumin ratios and then 60 μ*M* palmitoyl-L-carnitine added to initiate respiration. The respiratory rate and membrane potential were determined and from them the proton conductance. Palmitate binding was determined in parallel experiments. Adapted from Heaton and Nicholls (1976).

strate respiratory control—the so-called "carnitine cycle" (Hittelman *et al.*, 1969). A model for the acute regulation of UCP1 was developed where the transition to the thermogenic state was mimicked *in vitro* (Locke and Nicholls, 1981; Locke *et al.*, 1982a, b). Isolated mitochondria from brown fat were placed in an incubation chamber where the membrane potential and respiration rate could be monitored simultaneously. Since noradrenaline stimulation of the brown adipocyte initiates a lipolytic cascade, the release of fatty acids was simulated by the slow infusion of palmitate into the incubation chamber. As expected, there was an immediate depolarization and concomitant increase in the respiratory rate. When the infusion was stopped, the membrane potential was gradually restored and respiratory control was reinduced (Fig. 4) (Locke and Nicholls, 1981; Locke *et al.*, 1982a,b). The identification of the regulator of the conductance was approached by analyzing the level of acyl derivates when [^{14}C]palmitate was infused. The study showed conclusively that free fatty acid, rather than acyl-CoA was the activating species (Locke *et al.*, 1982a). Furthermore, when brown fat mitochondria from cold-adapted guinea pigs were compared with those from warm-adapted animals, it was found that warm-adapted mitochondria were ninefold less sensitive and thus resembled liver mitochondria (Locke *et al.*, 1982b). This suggested a mechanism in which free fatty acids were acting both as a self-regulating second messenger and substrate for UCP1-mediated thermogenesis.

However, the possibility remained that the fatty acid-induced conductance change could be due to a pathway not related to the nucleotide-sensitive one. This possibility was ruled out when it was demonstrated, with swelling experiments in potassium acetate plus valinomycin, that the purine nucleotide could inhibit the palmitate-induced permeability (Rial *et al.*, 1983). However, this ability of the nucleotide was in contrast to the observed failure of even high nucleotide concentrations to prevent fatty acid action under respiring conditions. Subsequent experiments demonstrated that this was due to the difference in the magnitude of the driving force for proton movement, low under passive swelling conditions and high under respiratory conditions (Rial *et al.*, 1983). The study was completed with an investigation of the requirements and specificity of the fatty acid activation of the UCP1. It was found that long-chain fatty acids were the most effective ones and that a free carboxylic group was necessary (Rial *et al.*, 1983).

Evidence for the role of fatty acids as second messengers of noradrenaline was completed with the determination of the concentration of fatty acids required to induce uncoupling both in intact cells and in isolated mitochondria. Intact brown adipocytes show a greatly enhanced respiration in response to noradrenergic activation of their β_3 adrenoreceptors (Prusiner *et al.*, 1968; Locke *et. al.*, 1982b). This models the thermogenic signal *in vivo* and, in order to confirm that UCP1 expression is required for this response, brown adipocytes were prepared from both warm- and cold-adapted guinea pigs (W cells and C cells). C cells responded to noradrenaline with a large, oligomycin-insensitive response and increase in respiration, while W cells showed little response and retained uncoupler-releasable respiratory control (Locket *et al.*, 1982b; Cunningham *et al.*, 1986; Rafael *et al.*, 1986). Importantly, activation of fatty acids to acyl-CoA by the mitochondria displayed a higher affinity than fatty acid activation of UCP1. Thus, at the termination of lipolysis, the mitochondria will decrease the fatty acid concentration by activation and oxidation sufficiently to remove the fatty acid second messenger from UCP1 (Cunningham *et al.*, 1986).

Once the role of fatty acids as second messengers has been clarified, attention focused on the mechanism

by which they increase the proton conductance. Two main hypotheses are currently being considered. A first model proposes that since UCP1 can transport anions, it catalyzes the transport of the fatty acid anion and the protonophoric action is accomplished with the flip-flop in the membrane of protonated form (Garlid *et al.*, 1996). This model is an extension of the one proposed for the ADP/ATP carrier (and also applied to other carriers) and could provide a mechanistic explanation to the so-called "nonspecific fatty acid uncoupling"(Skulachev, 1991). The second model proposes that fatty acids act as a prosthetic group in UCP1, delivering protons to a site from which they are translocated to the other side of the membrane (Winkler and Klingenberg, 1994).

Although, there are no conclusive data in support for either model, we have recently readdressed the question of the high affinity of fatty acids for UCP1 (González-Barroso *et al.*, 1998). The new approach used the recombinant expression of UCP1 in yeasts and compared yeast mitochondria without UCP with those from yeasts expressing either the wild-type protein or a UCP mutant (Cys304Gly) that presents an increased sensitivity toward fatty acids (González-Barroso *et al.*, 1996). It is observed that concentrations of fatty acids that are within the range required to activate UCP1 produces no significant effect on the control yeast strain (González-Barroso *et al.*, 1998). The K_m values obtained for the wild-type protein were in perfect agreement with those we previously reported for mitochondria from brown fat (Cunningham *et al.*, 1986). Therefore, the long-standing observation that BATM are more sensitive to fatty acids is due to the presence of the uncoupling protein, since our yeast expressing the protein become undistinguishable from them. Our current view (González-Barroso *et al.*, 1998) is that there exist two modes of fatty acid uncoupling: low concentrations, which can actually be generated in cells by hormone-sensitive lipolysis activate proton transport through UCP1, while much higher concentrations *in vitro* may be transported in the anionic form by other mitochondrial carriers and probably also by the newly discovered uncoupling proteins (UCP2, UCP3, and plant UCPs).

TOWARD THE MECHANISM OF TRANSPORT

The elucidation of the molecular mechanism of transport in UCP1 and its regulation is currently one of our main areas of interest. The physiological role of the anion transport activity of UCP1 and its relation to the energy dissipation pathway in BATM has been controversial. However, the fact that they were sharing a common pathway has rarely been disputed and this could be due to the unequivocal demonstration of the competition between chloride and protons for the path and their identical nucleotide sensitivity (Nicholls and Lindberg, 1973; Nicholls, 1974b; Nicholls and Heaton, 1978). The proposal put forward, to explain how protons and anions could share the same pathway, was that during energy dissipation the ions that were actually moving were OH^- rather than H^+ (Nicholls and Heaton, 1978). However, the puzzling observation of the lack of influence of albumin, i.e., low fatty acid concentrations, on the chloride permeability (Nicholls and Lindberg, 1973; Rial *et al.*, 1983) and the demonstration that fatty acids were indeed regulators of proton transport through UCP1 (see above) had to be fitted onto the model. We then hypothesized the existence of two pathways inside the protein—one for protons and the other for anions, such as chloride (Nicholls *et al.*, 1984). This view was substantiated with data from chemical modifications where it was observed that modification of cysteine residues was differentially affecting the nucleotide regulation of the two permeabilities (Rial and Nicholls, 1986). This view is obviously disputed by groups that defend that UCP1 is purely an anion carrier and that fatty acids are not regulators, but substrates of the protein (Garlid *et al.*, 1996).

The modification of sulfhydryl groups in UCP1 with maleimides displayed unusual kinetics and a detailed analysis revealed that the modification was taking place in two stages (Rial *et al.*, 1989). In the first, both transport and nucleotide binding were inhibited, as a result of the reaction with a residue located in a hydrophobic environment. The second step led to the recovery of both binding and transport. Nucleotide was bound with an slightly lower affinity but could inhibit transport. The rate of transport was, however, strikingly increased (Rial *et al.*, 1989). This second finding was particularly interesting, since soon afterward it was reported that modification of sulfhydryl groups in other mitochondrial carriers resulted in the induction of channellike properties (for review see Dierks *et al.*, 1994; Krämer, 1998). Interestingly, various members of the carrier family, including UCP1, have been shown to behave as channels under patch-clamp conditions (for review see Krämer, 1998; Rial *et al.*, 1998). The possibility of the induction of these

two transport modes, carrier/channel, could be providing clues as to their structural organization. Thus the existence of two protein domains was proposed, one responsible for the channel properties while the other would constitute the gate and would, therefore, confer on the carrier its specific transport properties (for review see Dierks *et al.*, 1994). This model has also been suggested for UCP1 (González-Barroso *et al.*, 1997; Rial *et al.*, 1998).

The location of this putative gating domain in UCP1 has been suggested by site-directed mutagenesis studies. The origin of these lies in the description by Bouillaud *et al.* (1992) of the striking homology between the DNA binding domain of the estrogen receptor and the residues 261–269 of UCP1. It was hypothesized that this UCP1 region could be a "nucleotide recognition element" and, therefore, a deletion mutant was constructed where residues 267–269 were eliminated. The resulting mutant lost the nucleotide inhibition of transport (Bouillaud *et al.*, 1994) The same effect is observed when the deletion is restricted to Gly269 (Rial *et al.*, 1998). However, the deletion of the entire domain (261–269) produced a highly altered protein with a porelike behavior, allowing the permeation of solutes of at least 1000 Da without charge discrimination (González-Barroso *et al.*, 1997). These residues are located at the N-terminal end of the sixth transmembrane helix, thus facing the matrix side of the membrane (Fig. 5). The carrier family presents a tripartite structure with three repeated sequences of 100 amino acids (for review see Walker and Runswick, 1993). Gly269 is conserved in the other two repeats of the protein (Fig. 4) and thus when the corresponding residues of the first and second loop were deleted, there was also a loss of the nucleotide inhibition (Rial *et al.*, 1998).

UCP1 has two tryptophan residues, Trp173 and Trp280. Trp280 is located in sixth transmembrane helix, while Trp173 is in the homologous region of the second matrix loop. Fluorescence quenching-resolved spectroscopy demonstrated that acrylamide had access to both tryptophans, while iodide only quenched the fluorescence of Trp173. However, the most important feature is that nucleotide binding partially shielded Trp173 from the interaction with iodide (Viguera *et al.*, 1992). These data further support the idea of the interreaction of the nucleotide with the matrix loops of the protein. A working model

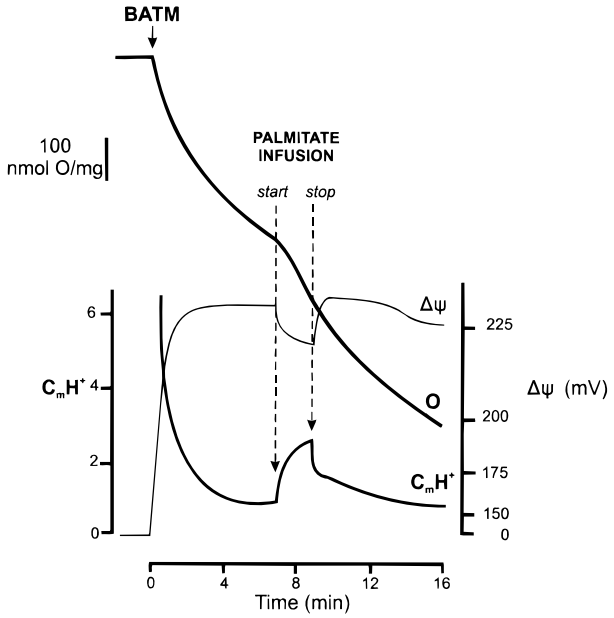

Fig. 4. Fatty acids are self-regulating second messengers and substrates for the control of UCP1. BATM were incubated in the presence of CoA, L-carnitine, and ATP to allow the activation and oxidation of fatty acids. Respiration (O) and $\Delta\Psi_m$ ($\Delta\Psi$) were monitored by oxygen and tetraphenylphosphonium electrodes, and C_mH^+ was calculated from the relationship $JH^+/\Delta p$ as in Fig. 2. Between the times given by the two arrows, palmitate was steadily infused at a rate of 13 nmol/min/mg protein, mimicking the noradrenaline-induced lipolysis of triglyceride in the brown adipocyte. Palmitate is initially activated to acyl CoA, but the oxidation of the resulting acyl-carnitine is inhibited as respiration is controlled by the low C_mH^+ of the membrane. As palmitate accumulates, it binds to UCP1, increasing C_mH^+, lowering $\Delta\Psi_m$, and accelerating respiration. When the infusion stops, the palmitate continues to be activated and oxidized until UCP1 closes. Thus fatty acids are both substrate and self-regulating second messenger for BAT thermogenesis. Data from Locke *et al.* (1982a).

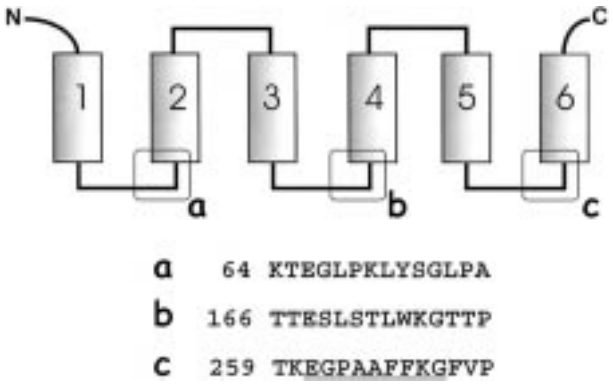

Fig. 5. Folding model for the uncoupling protein UCP1. There are six transmembrane regions with the C- and N-terminal ends of the protein facing the cytosolic side of the inner membrane. Amino acids corresponding to boxes a, b, and c are given below; numbers indicate the sequence position of the first residue listed. Underlined amino acids correspond to the region homologous to the DNA binding domain of the estrogen receptor (Bouillaud *et al.*, 1992).

is envisaged where the transmembrane regions form a barrel that could constitute the channel domain while the three matrix loops contribute to the formation of the gate. The necleotide binding site would be deep inside the protein interacting with the three matrix loops (Rial *et al.*, 1998).

CONCLUDING REMARKS

The uncoupling protein UCP1 is the key to the thermogenic capacity of brown adipose tissue. Its transport properties and regulatory mechanism are well fitted to fulfill its role and respond to the signals received by the adipocyte to initiate thermogenesis. New uncoupling proteins have been described in the last 2 years and now it is necessary to establish their respective physiological functions. There is no doubt that the determination of the regulatory signals that make them operative will help to define their role.

ACKNOWLEDGMENTS

This work is supported by a grant of the Spanish Ministry of Education and Culture (PB95-0118) to ER.

REFERENCES

Bouillaud, F., Casteilla, L., and Ricquier, D. (1992). *Mol. Biol. Evol.* **9**, 970–975.
Bouillaud, F., Arechaga, I., Petit, P. X., Raimbault, S., Levi-Meyrueis, C., Casteilla, L., Laurent, M., Rial, E., and Ricquier, D. (1994). *EMBO J.* **13**, 1990–1997.
Cunningham, S. A., Wiesinger, H., and Nicholls, D. G. (1986). *Eur. J. Biochem.* **157**, 415–420.
Dierks, T., Stappen, R., and Krámer, R. (1994). In *Molecular Biology of Mitochondrial Transport Systems* (Forte, M., and Colombini, M., eds.), Springer-Verlag, Berlin, pp. 117–129.
Flatmark, T., and Pedersen, J. I. (1975). *Biochim. Biophys. Acta* **416**, 53–103.
Foster, D. O., and Frydman, L. (1978). *Can. J. Physiol. Pharmacol.* **56**, 110–122.
Garlid, K. D., Orosz, D. E., Modriansky, M., Vassanelli, M., and Jezek, P. (1996) *J. Biol. Chem.* **271**, 2615–2620.
Garlid, K. D., Jaburek, M., and Jezek, P. (1998). *FEBS Lett.* **438**, 10–14.
González-Barroso, M. M., Fleury, C., Arechaga, I., Zaragoza, P., Levi-Meyrueis, C., Raimbault, S., Bouillaud, F., and Rial, E. (1996). *Eur. J. Biochem.* **239**, 445–450.
González-Barroso, M. M., Fleury, C., Levi-Meyrueis, C., Zaragoza, P., Bouillaud, F., and Rial, E. (1997). *Biochemistry* **36**, 10930–10935.
González-Barroso, M. M., Fleury, C., Bouillaud, F., Nicholls, D. G., and Rial, E. (1998). *J. Biol. Chem.* **273**, 15528–15532.
Hamilton, J. A., and Cistola, D. P. (1986). *Proc. Natl. Acad. Sci. USA* **83**, 82–86.
Hittelman, K. J., Lindberg, O., and Cannon, B. (1969). *Eur. J. Biochem.* **11**, 183–192.
Heaton, G. M., and Nicholls, D. G. (1976). *Eur. J. Biochem.* **67**, 511–517.
Heaton, G. M., and Nicholls, D. G. (1977). *Biochem. Soc. Trans.* **5**, 210–212.
Heaton, G. M., Wagenvoord, R. J., Kemp, A., and Nicholls, D. G. (1978). *Eur. J. Biochem.* **82**, 515–521.
Krämer, R. (1998). *Exp. Physiol.* **83**, 259–265.
Klingenberg, M., and Winkler, E. (1985). *EMBO J.* **4**, 3087–3092.
Lin, C. S., and Klingenberg, M. (1982). *Biochemistry* **21**, 2950–2956.
Locke, R. M., and Nicholls, D. G. (1981). *FEBS Lett.* **135**, 249–252.
Locke, R. M., Rial, E., Scott, I. D., and Nicholls, D. G. (1982a). *Eur. J. Biochem.* **129**, 373–380.
Locke, R. M., Rial, E., and Nicholls, D. G. (1982b). *Eur. J. Biochem.* **129**, 381–387.
Nicholls, D. G. (1974a). *Eur. J. Biochem.* **49**, 573–583.
Nicholls, D. G. (1974b). *Eur. J. Biochem.* **49**, 585–593.
Nicholls, D. G. (1974c). *Eur. J. Biochem.* **50**, 305–315.
Nicholls, D. G. (1976). *Eur. J. Biochem.* **62**, 223–228.
Nicholls, D. G. (1977). *Eur. J. Biochem.* **77**, 349–356.
Nicholls, D. G. (1979). *Biochim. Biophys. Acta* **549**, 1–29.
Nicholls, D. G., and Heaton, G. M.. (1978). In *The Proton and Calcium Pumps* (Azzone, A., Awron, M., Metcalfe, J. C., Quagliariello, E., and Siliprandi, N, eds.), Elsevier, Amsterdam, pp.309–318.
Nicholls, D. G., and Lindberg, O. (1972). *FEBS Lett.* **25**, 61–64.
Nicholls, D. G., and Lindberg, O. (1973). *Eur. J. Biochem.* **37**, 523–530.
Nicholls, D. G., and Locke, R. M. (1984). *Physiol. Rev.* **64**, 1–64.
Nicholls, D. G., Grav, H. J., and Lindberg, O. (1972). *Eur. J. Biochem.* **31**, 526–533.
Nicholls, D. G., Snelling, R., and Rial, E. (1984). *Biochem. Soc. Trans.* **12**, 388–390.
Prusiner, S. B., Cannon, B., Ching, T. M., and Lindberg, O. (1968). *Eur. J. Biochem.* **7**, 51–57.
Rafael, J., and Heldt, H. W. (1976). *FEBS Lett.* **63**, 304–308.
Rafael, J., Ludolph, H.-J., and Hohorst, H.-J. (1969). *Hoppe-Seyler's Z. Physiol. Chem.* **350**, 1121–1131.
Rafael, J., Fesser, W., and Nicholls, D. G. (1986). *Amer. J. Physiol.* **250**, C228–C235.
Rial, E., and Nicholls, D. G. (1986). *Eur. J. Biochem.* **161**, 689–694.
Rial, E., Poustie, A., and Nicholls, D. G. (1983). *Eur. J. Biochem.* **137**, 197–203.
Rial, E., Arechaga, I., Sainz-de-la-Maza, E., and Nicholls, D. G. (1989). *Eur. J. Biochem.* **182**, 187–193.
Rial, E., González-Barroso, M. M., Fleury, C., and Bouillaud, F. (1998). *Biofactors* **8**, 209–219.
Ricquier, D., and Kader, J. C. (1976). *Biochem. Biophys. Res. Commun.* **73**, 577–583.
Skulachev, V. P. (1991). *FEBS Lett.* **294**, 158–162.
Smith, R. E., and Horwitz, B. A. (1969). *Physiol. Rev.* **49**, 330–425.
Trayhurn, P., and Nicholls, D. G., eds. (1986). In *Brown Adipose Tissue*, Edward Arnold, London.
Viguera, A. R., Goñi, F. M., and Rial, E. (1992). *Eur. J. Biochem.* **210**, 893–899.
Walker, J. E., and Runswick, M. J. (1993). *J. Bioenerg. Biomembr.* **25**, 435–446.
Winkler, E., and Klingenberg, M. (1994). *J. Biol. Chem.* **269**, 2508–2515.