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Review

Physiological regulation of the transport activity in the uncoupling proteins UCP1 and UCP2

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Abstract

Brown fat is a thermogenic organ that allows newborns and small mammals to maintain a stable body temperature when exposed to cold. The heat generation capacity is based on the uncoupling of respiration from ATP synthesis mediated by the uncoupling protein UCP1. The first studies on the properties of these mitochondria revealed that fatty acid removal was an absolute prerequisite for respiratory control. Thus fatty acids, that are substrate for oxidation, were proposed as regulators of respiration. However, their ability to uncouple all types of mitochondria and the demonstration that several mitochondrial carriers catalyze the translocation of the fatty acid anion have made them unlikely candidates for a specific role in brown fat. Nevertheless, data strongly argue for a physiological function. First, fatty acids mimic the noradrenaline effects on adipocytes. Second, there exists a precise correlation between fatty acid sensitivity and the levels of UCP1. Finally, fatty acids increase the conductance by facilitating proton translocation, a mechanism that is distinct from the fatty acid uncoupling mediated by other mitochondrial carriers. The regulation of UCP1 and UCP2 by retinoids and the lack of effects of fatty acids on UCP2 or UCP3 are starting to set differences among the new uncoupling proteins.

Keywords: Uncoupling protein; UCP1; UCP2; Fatty acid; Retinoid; Thermogenesis; Mitochondrion

1. Introduction

The uncoupling protein from brown adipose tissue mitochondria (UCP1) was discovered nearly 25 years ago and for a long time it was considered the result of a unique adaptation of a mammalian tissue to non-shivering heat production. Over the last 3 years, proteins homologous to UCP1 have been found not only in mitochondria from other mammalian tissues but also in other organisms including plants. The function of these new uncoupling proteins is still being defined. Since the discovery of the lack of energy conservation in brown fat mitochondria and the physiology of the thermogenic response preceded the discovery of the protein, we will give a historical perspective to this review of the regulation of UCP1. Over the years, mounting evidence pointed to fatty acids as second messengers of noradrenaline to activate UCP1. However, the demonstration that members of the mitochondrial transporter family can catalyze the transport of the fatty acid anion had raised doubts on a specific regulatory role on the

Abbreviations: AAC, ADP/ATP carrier; PiC, phosphate carrier; PTP, permeability transition pore

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brown adipocyte. We will review the evidences that establish a unique physiological role for them in the regulation of the proton conductance of UCP1. Finally, the regulation of UCP1 and UCP2 by retinoids is a recent development that sets differences and analogies in the regulation of these two related proteins.

2. Thermogenesis in brown adipose tissue

Brown fat was probably first recognized as a tissue distinct from white fat by Conrad Gesner in 1551 when describing the interscapular tissue of the marmot [1]. The physiological role assigned to the tissue changed over the years and this is reflected in the many names given to it: hibernating gland, interscapular gland, lipoid gland, primitive fat organ, etc. and it was even suggested that it could produce water during hibernation (see historical reviews [2–4]). The tissue was believed to be part of the thymus until 1817 and subsequently it was regarded as an endocrine gland. From 1863 until 1902 brown fat was considered a modified fatty tissue whose function was the storage of fat but, again, in 1902 some authors believed it to be an endocrine tissue. In the early 60s, key contributions by Robert E. Smith and coworkers [5–7] and Dawkins and Hull [8,9] established the role of brown adipose tissue as a thermogenic effector. By that time, it had already been established that the sympathetic nervous system was controlling the activity of the tissue and that noradrenaline was the cause of two key phenomena: the increase in lipolysis and in oxygen consumption (reviewed in [3,10]).

The next issue to be solved was the biochemical basis of heat production. Since noradrenaline was stimulating both lipolysis and heat production, the first hypothesis proposed a futile cycle with the breakdown of triglyceride and their subsequent resynthesis [9,11]. The role of brown fat mitochondria in thermogenesis was defined in the late 60s, although the first report suggesting that these mitochondria were uncoupled was by Lepkovsky et al. in 1959 [12]. These authors reported that while in liver P/O was 1.8, in brown fat it was close to zero even when 'oxygen uptake was similar to that of the liver particles'. Smith et al. were the first to corroborate these observations and correlate the lack of oxidative phosphorylation with the thermogenic properties of the tissue [13]. In 1967, Olov Lindberg et al. [14] reported that when liver tissue and brown fat were homogenized together, the mixed mitochondrial preparation gave a P/O ratio that was interpreted as the result of a mixture of tightly coupled and uncoupled mitochondria. This finding was an indication that the uncoupling factor was only affecting mitochondria from brown fat. One of the hypotheses put forward to explain the low P/O ratios was the lack of the oxidative phosphorylation machinery, however, in the following year several papers appeared demonstrating that brown adipose tissue mitochondria were indeed capable of ATP synthesis [15–17]. Those reports pointed to the necessity of albumin addition to remove traces of free fatty acids and authors signified the high sensitivity to fatty acid uncoupling when compared to brain or liver mitochondria. In the following year, Hittelman et al. [18] showed that albumin could be omitted if CoA, carnitine and ATP were present to oxidize endogenous fatty acids. There were, however, discrepancies in the recoupling effect when fatty acids were removed either by albumin addition or by fatty acid activation that led to the proposal that a specific fraction of endogenous fatty acids was responsible for the uncoupling [19].

An additional essential finding was reported by Rafael and coworkers when they observed that addition of millimolar ATP or GTP to the incubation together with albumin yielded tightly coupled mitochondria [20,21]. The apparent discrepancy in the fatty acid removal data was then solved since it was shown that the ATP used for fatty acid activation was also an inducer of respiratory control [22], thus in agreement with Rafael's observations. 55 μM ATP was sufficient to activate endogenous fatty acids but insufficient to induce respiratory control, whereas 0.8 mM ATP was serving the two functions [22].

As we said earlier, by the end of the 60s, it was firmly established that the hormonal control of thermogenesis in brown fat was exerted through the rich sympathetic innervation of the tissue. It was also known that thermogenesis was linked to the mobilization and oxidation of fatty acids. The demonstration that fatty acids could mimic the noradrenaline effects on intact cells and that propranolol was in-

hibiting both the lipolytic and calorigenic effects of noradrenaline but could not inhibit the effects of fatty acids on cellular respiration led to the proposal of a simple and attractive hypothesis [23]: the hormonally induced increase in the intracellular content of fatty acids was providing both the substrate and the regulator of respiration. This finding was subsequently linked to the high sensitivity of brown fat mitochondria to fatty acids [21,24]. However, the role of fatty acids as cytosolic second messengers of noradrenaline has remained controversial for many years and in the following sections we will review the nature of the debate.

3. Mitochondrial carriers, fatty acid uncoupling and permeability transition

The ability of fatty acids to uncouple oxidative phosphorylation was first described by Pressman and Lardy. In 1952 they reported that a heat-stable and acetone-soluble microsomal fraction could stimulate respiration in the absence of phosphate acceptor and could also increase the ATPase activity [25]. In 1955, the authors identified this fraction as fatty acids [26]. A lot of research was devoted over the years to investigate the mechanism of uncoupling. It become clear that at high concentrations fatty acid effects were detergent-like but at low concentrations they seem to exert a conventional protonophoric action, i.e. acting like classical uncouplers (reviewed in [27,28]). This protonophoric action requires the transbilayer movement (flip-flop) of the protonated fatty acid from the cytosolic to the matrix face of the inner membrane and the subsequent return of the anionic form to the cytosolic side. This cycle results in the net delivery of protons to the matrix side. While the flip-flop of the protonated form is extremely fast, the flip-flop rate of the anion is probably too slow ($t_{1/2}$ of the order of minutes) [29] (reviewed in [30]) and thus the mechanism of uncoupling could not be explained.

An important development took place in 1988 when Skulachev and coworkers showed that carboxyatractylate could partially inhibit the uncoupling effect of palmitate in liver mitochondria [31,32] and they interpreted these experiments as an indication of the participation of the ADP/ATP carrier (AAC) in the mechanism of uncoupling. The authors postulated that the movement of the fatty acid anion was being facilitated by the carrier [31–33]. These data were corroborated with the AAC reconstituted in proteoliposomes [34]. The AAC is one of the most prominent members of a superfamily of related proteins that includes the metabolite carriers of the mitochondrial inner membrane [35,36]. The family presents structural and functional similarities, being their tripartite structure one of the most characteristic features (they consist of three repeats of about 100 amino acids). Interestingly, the ability of the AAC to translocate the fatty acid anion seems to be a property shared also by other members of the family and thus a similar behavior has been shown with the aspartate/glutamate carrier [37], dicarboxylate carrier [38] and the phosphate carrier (PiC) [39]. The working hypothesis is that this carrier-mediated uncoupling induced by endogenous fatty acids may play a role in thermoregulatory heat production [27,33]. More recently, it has been suggested that it could also prevent the generation of reactive oxygen species [40].

This protein family includes not only metabolite transporters but also the growing family of the uncoupling proteins. The discovery of a 32 kDa protein (now termed the uncoupling protein UCP1) as the site of energy dissipation in brown adipose tissue [41] and its subsequent purification and sequencing revealed its close relationship first to the AAC [42,43] and later on to other members of the carrier family [35,36]. The discovery in 1997 of two new uncoupling proteins in mammalians, UCP2 [44] and UCP3 [45,46], was soon followed by the cloning of another two closely related proteins that are only expressed in the brain, BMCP1 [47] and UCP4 [48]. The family has continued to grow with the identification of genes encoding UCPs in fish [49] and even in plants [50–53] (reviewed in [54]). It is not surprising that several of the UCPs have been shown to catalyze the translocation of the fatty acid anion [55,56]. At the present time, some authors consider the UCPs as fatty acid transporters [56–58] and thus the role of fatty acids as seconds messengers of noradrenaline to specifically activate UCP1 and initiate thermogenesis in brown fat is being ignored.

Data have been published recently suggesting that fatty acids may be inducers of apoptosis by opening

the permeability transition pore (PTP) [59,60]. The PTP is a complex that includes proteins from the outer and inner mitochondrial membrane and also soluble ones (reviewed in [61–63]). Two of the more significant ones are the voltage-dependent anion channel and the AAC [63]. The participation of the AAC is particularly striking since it is believed that opening of the PTP relies on the conversion of the carrier into a pore that allows the permeation of solutes of 1500 Da. This leads eventually to the collapse of the membrane potential and swelling of the matrix (reviewed in [61,62]). Several members of the carrier family have also displayed this ability to switch from the carrier mode to a channel or pore mode (reviewed in [64,65]). Thus, it has been shown that under patch clamp conditions the AAC [66], PiC [67] and UCP1 [68] display channel conductances that range from 25 pS in the PiC to 600 pS in the AAC. There are also other means to modify drastically the transport properties of the carriers, like the chemical modification of critical sulfhydryl groups [69–71]. We have also shown that deletion of nine amino acids on the third matrix loop in UCP1 leads to the formation of a pore that allows permeation of molecules of at least 1000 Da [72]. These properties may be giving important hints on the structural organization of the members of this protein family. It has been suggested that these carriers may possess two functional domains: a channel and a gating domain. Modifications in the gating domain would lead to changes in the regulation of transport and ultimately to the appearance of pore properties [64,65,70].

The above mentioned suggestion on the opening of the PTP induced by fatty acids [59,60] could be interpreted as due to the switching of the AAC to the pore mode. In this context, the so-called carrier-mediated fatty acid uncoupling would not involve the specific translocation of the fatty acid anion but rather the opening of the PTP. When other carriers are placed under similar conditions, one would also expect an analogous carrier–pore transition.

4. Fatty acid activation of UCP1

We have presented above a historical perspective of the development of the understanding of the role of brown adipose tissue as a specialized organ for thermogenesis. It is established that the molecular basis for heat production lies on the uncoupling of respiration. There is also a consensus on the idea that noradrenaline controls the activity of the adipocyte and that fatty acids released during the hormone-stimulated lipolysis are the substrate for oxidation. In the early 70s, the analysis of the bioenergetic properties of brown fat mitochondria revealed an atypical permeability to anions, particularly to chloride, and protons [73,74]. Since this permeability was inhibited by the same range of nucleotides that would recouple respiration, the pathway was quickly identified with the heat dissipatory mechanism [75]. The nucleotide sensitivity of the uncoupling pathway has been, and still is, a key tool to investigate its properties. The nucleotide binding site was located on the outer face of the inner mitochondrial membrane and binding was atractylate-insensitive [76]. This finding allowed the quantification of the protein in the membrane [76] and the demonstration that there was a direct correlation between the binding capacity and the thermogenic activity of the tissue [77]. Furthermore, nucleotide binding was the tool that allowed Nicholls and coworkers to demonstrate in 1978 that the uncoupling protein was a 32 kDa polypeptide [49]. Two years before, Ricquier and Kader had found that a 32 kDa protein band was increased in brown fat mitochondria from cold-adapted rats although at that time its function was unknown [78].

There was, however, one intriguing feature that did not fit in the picture, the lack of influence of the fatty acids on the halide permeability [74]. This fact and their ability to uncouple of oxidative phosphorylation in all types of mitochondria led to the questioning of the role of the fatty acids as regulators of the uncoupling protein [79]. This revision was put forward even when it was recognized that brown fat mitochondria were more sensitive to fatty acids than those from other sources [79,80]. Other regulatory mechanisms were evoked such as the competitive inhibition of nucleotide binding by long-chain acyl-CoA [81] or changes in the cytosolic pools of adenine nucleotides [82] (reviewed in [83]). To rationalize a transport mechanism that would allow the catalysis of the movement of anions such as chloride and that of protons, it was proposed that during energy dissipation the uncoupling pathway was transporting

OH^- rather than H^+ and thus the uncoupling protein UCP1 was an anion carrier [80].

The role of fatty acids was again reconsidered when experiments were designed to mimic the transition to the thermogenic state with cells and mitochondria [84,85]. Since brown fat mitochondria can oxidize fatty acids in the presence of ATP, CoA and carnitine [18], experiments were designed to mimic in vitro the hormone-stimulated lipolysis by the slow infusion of palmitate into a mitochondrial incubation. The simultaneous measurement of the membrane potential, rate of respiration and the level of intermediates (free palmitate, palmitoyl-CoA and palmitoyl-carnitine) demonstrated that the proton conductance correlated precisely with the concentration of free palmitate and, furthermore, that there was no detectable threshold concentration required to observe the increase in conductance [84,85]. When the infusion was stopped, palmitate disappeared from the incubation, the membrane potential was gradually restored and respiratory control was reinduced [84,85]. Since fatty acids liberated by the hormone-stimulated lipolysis are rapidly converted to acyl-CoA to be oxidized, they can only be physiological regulators if the concentration required to observe the uncoupling is within the range of concentrations that may be reached in the cytoplasm. The comparison between the concentration dependency for fatty acid activation and that required for uncoupling showed that half-maximal stimulation of palmitoyl-CoA synthase requires 12 nM free palmitate and the uncoupling displays a K_m around 100 nM [86]. Two elements seem to make the 8-fold difference in affinity compatible with a regulatory role. First, fatty acids will accumulate in the cytosol until the activation of UCP1 allows their rapid oxidation and second, there is no threshold concentration of fatty acids to activate UCP1. Additionally, the higher affinity of the fatty acid oxidation system ensures that the cytoplasmic concentration of fatty acids is lowered sufficiently to restore respiratory control when lipolysis has been halted.

An additional tool that allowed the clarification of the physiological role of fatty acids was the comparison between their uncoupling effects on mitochondria and brown adipocytes from cold-adapted and warm-adapted guinea-pigs. Thus it was demonstrated that the sensitivity of mitochondria to fatty acid uncoupling correlated with the amount of uncoupling protein [85,87]. Brown fat mitochondria from a warm-adapted animal were 9-fold less sensitive and thus resembled liver mitochondria [85]. We have already mentioned that fatty acids had been shown to mimic the noradrenaline effects on cells [23]. It was later confirmed that brown adipocytes from cold-adapted animals were more sensitive to fatty uncoupling than cells from animals reared at thermoneutral temperature [86]. This sensitivity was related with a higher presence of UCP1 in the mitochondria and with a greater ability of noradrenaline to stimulate respiration [86,88]. With the development of the recombinant expression of the UCPs in yeasts [89,90] it has been possible to demonstrate that when UCP1 is expressed, *Saccharomyces cerevisiae* mitochondria display a fatty acid sensitivity comparable to those from brown fat [91] while expression of UCP2 or UCP3 has no effects on this respect [92].

It was possible, however, that the fatty acid-induced proton conductance was independent of the uncoupling protein UCP1 and due to another unknown factor that had appeared in parallel. This possibility was ruled out analyzing the rate of swelling of non-respiring mitochondria suspended in potassium acetate in the presence of valinomycin. The fatty acid-induced proton permeability was inhibited stoichiometrically as GDP was titrated into the incubation [93]. This experiment created, however, a new paradox. How could then fatty acids override the nucleotide inhibition under respiring conditions? The explanation lies in the different magnitude of the proton electrochemical potential gradient that is around 20 mV during swelling and some 200 mV during controlled respiration. The analysis of the current/voltage relationships in brown fat mitochondria in the presence of millimolar nucleotide shows a low proton conductance when membrane potential is below 180 mV while at higher values there is a rapid increase in the conductance with small changes in potential [93,94]. Fatty acids exert their action by lowering the 'break-point' potential at which the conductance increases rapidly [93]. The effect of the fatty acids is quite subtle, instead of merely introducing an ohmic proton leak, that would lead to an unstable membrane potential, they clamp it at a level that is insufficient for respiratory control. Besides, the level

is high enough to allow significant ATP synthesis, metabolite transport and Ca^{2+} regulation [95].

The magnitude of the driving force under which UCP1 is operating should be considered when comparing data obtained in different systems. We have already mentioned the differences in the effect of nucleotides and fatty acids in swelling experiments and under respiratory conditions [93]. Something similar should also be said about the discrepancies between experiments performed with the UCPs reconstituted into proteoliposomes. Thus, UCP1 has been shown to behave identically to UCP2 or UCP3 in proteoliposomes [56] while they are different when studied after recombinant expression in yeast mitochondria [92]. Differences may lie in the fact that in those liposome systems, membrane potentials at most reach 120 mV. A closely related observation should also be made about a recent report where a comparison of brown fat mitochondria from UCP1-ablated mice and control mice reveals the same fatty acid sensitivity hence the authors conclude that fatty acids are working independently of UCP1 [96]. It is noteworthy, however, that data presented reveal that mitochondria from wild type mice do not reach membrane potentials above 150 mV (even in the presence of GDP) while in the UCP1-ablated mice, membrane potential is around 200 mV.

5. Mechanistic aspects. Are fatty acids substrate or cofactors?

The molecular mechanism by which fatty acids increase the proton conductance in brown fat mitochondria is also controversial. There are two proposals, either fatty acids are substrates for UCP1 or they act as cofactors. As we said earlier, the fatty acid cycling hypothesis suggests that several members of the mitochondrial carrier family, including the UCPs, catalyze the permeation of the fatty acid anion [33,56,58]. Since this model requires that the protonated acid permeates across the lipid bilayer, Jezek et al. tested the model by comparing the activation of UCP1 by a number of organic acids with their ability to permeate across the bilayer and concluded that all the active compounds could also permeate [97,98]. The authors found that the bulky-planar structure of the benzene ring prevented the flip-flop of compounds like the phenylhexanoic acid. However, the demonstration that all-*trans* retinoic acid is a powerful activator of UCP1 and UCP2 would seem to violate the rule [92]. Furthermore, Klingenberg and Huang have found a fatty acid derivative, ω-glucopyranoside palmitic acid, that will not permeate but does activate UCP1 [99].

There are a number of indications that point to the alternative mechanism of activation in UCP1 and that is represented in a working model that proposes that the fatty acid acts as a prosthetic group [91,100]. The carboxylate would bind protons and deliver them to a site inside UCP1 from which translocation would take place. One of the first striking features of the fatty acid activation of UCP1 is its pH dependency. There is a sharp increase in proton conductance over the pH range 7.0–8.0 [93,101] that cannot be explained by the pH dependency of the nucleotide binding [76]. Since the pK_a for a long-chain fatty acid in a phospholipid bilayer is around 7.5 [102], the increase in the pH of the medium would result in a significant decrease in the proportion of the protonated form. This observation would be difficult to accommodate to the cycling model where the first step is the inward permeation of the protonated fatty acid. The pH dependency may be an indication of the involvement of an amino acid side chain in UCP1. Klingenberg and coworkers have extensively studied residues involved in UCP1 function (reviewed in [99]). Two reports are particularly relevant to our present discussion. In 1998 they described that His145 and His147 were required for fatty acid-dependent proton transport while anion transport was unaffected when these residues were mutated. They propose that these residues, that are located in the matrix side of the protein, could be the final acceptors in the proton translocation chain [103]. More recently, this group has also reported the involvement of Asp27 in proton but not chloride transport. The authors argue that since the mutant Asp27Asn retains a residual proton translocating activity, this amino acid may have a role in accelerating proton transfer rather than being an indispensable translocation step [104].

A final question should also be considered. The fatty acid cycling hypothesis proposes that the UCPs are carriers for the fatty acid anion hence in UCP1 there would be no transport activity in UCP1

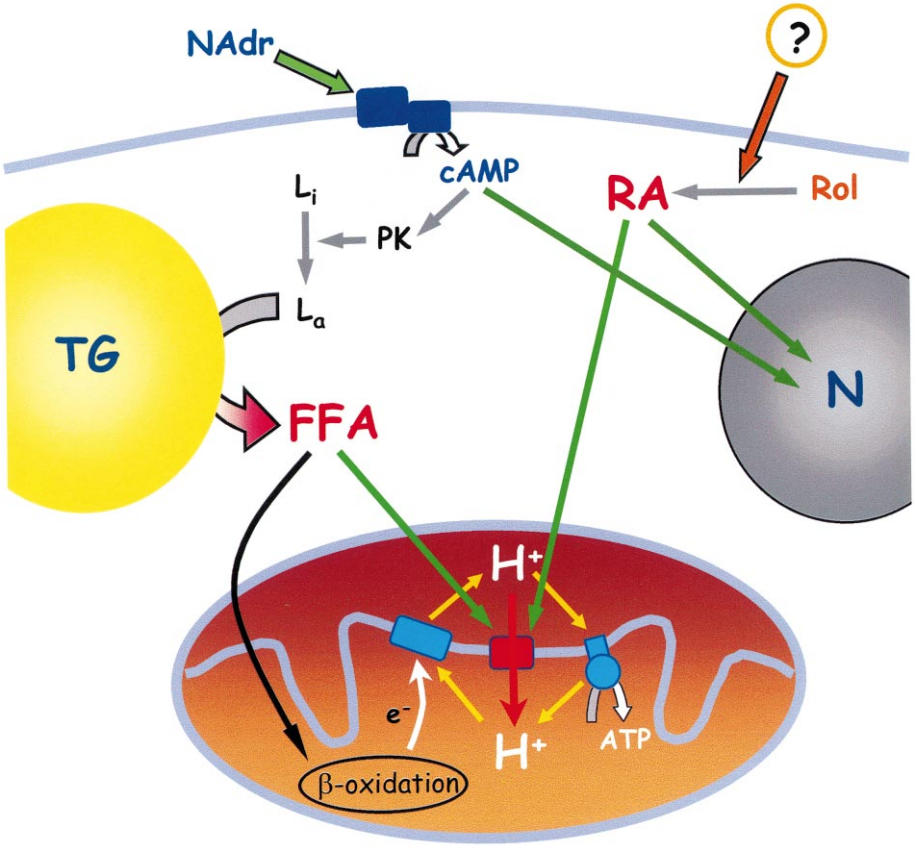

Fig. 1. Regulation of thermogenesis in the brown adipocyte. Noradrenaline (NAdr) liberated by the sympathetic nervous system binds to a β-receptor and activates a lipolytic cascade. The elevation of cAMP levels activates a protein kinase (PK) that in turn activates a hormone-sensitive lipase (L_i). The free fatty acids (FFA) liberated from the triglyceride stores (TG) will be oxidized in the mitochondria but they will also act as second messengers of noradrenaline and activate the uncoupling protein UCP1 (in red). All-*trans* retinoic acid (RA) is also a potent activator of proton transport in UCP1. The hormonal signals that may control the generation of retinoic acid from retinol (Rol) are unknown. Noradrenaline (through cAMP) and retinoic acid are also activators of the transcription of the *ucp1* gene.

in their absence [58]. This argument collides with the earliest observations of the bioenergetic properties of brown fat mitochondria that signified that even upon removal of all traces of endogenous fatty acids, mitochondria remained uncoupled. Thus for example, authors failed to obtain coupled mitochondria after the addition of up to 25 mg/ml of albumin to the homogenization and isolation media (reviewed in [3]). Similar conclusions were reached after their removal by oxidation in the presence of ATP, CoA and carnitine [22]. We have also readdressed this question by analyzing the properties of isolated mitochondria from yeasts expressing UCP1 and we concluded that there is statistically significant nucleotide-sensitive proton transport in the absence of fatty acids [91]. Thus, fatty acids are activators of proton transport but they are not an absolute requirement for UCP1 function.

6. Regulation of UCP1 and UCP2 by retinoids

UCP1 presents a low specificity towards the activating species, thus compounds with a free carboxylate group and with sufficient lipid solubility will increase its proton conductance [93,98,99]. Retinoic acid is a powerful activator of the transcription of the gene [105,106] and thus we recently investigated whether it could also act on the protein itself. The results showed that UCP1 displays a higher affinity for all-*trans* retinoic acid than for palmitate although the maximum rate of transport is lower [92]. The

concentration of retinoic acid required for activation of the protein is within the range generally employed to observe increased transcription of the *ucp1* gene and therefore the pathway may be of physiological relevance and even more when considering that adipocytes play an active role in the metabolism of retinoids (reviewed in [107]).

UCP2 is a protein homologous to UCP1 but that in contrast to the later one is expressed in many tissues [44]. There have been two reports on the regulation of its transport activity. The first one was in line with the above mentioned carrier-mediated fatty acid uncoupling and thus claims that UCP2 is also a fatty acid transporter with properties similar to UCP1 [56]. A second report has failed to observe a fatty acid activation of UCP2 using concentrations that would bring UCP1 close to its maximal activity [92]. UCP2 is also activated by all-*trans* retinoic acid although the conditions required to observe uncoupling differ from those in UCP1. Thus, UCP2 displays a strong pH dependency so that retinoic acid is inactive at pH values below 7.0 and it has a very high activity at 7.4. UCP2 also differs from UCP1 in its high specificity for the activating ligand [92]. Many compounds that share structural similarities to retinoic acid are ineffective and we could only find one activator, the retinoid TTNPB that is a specific ligand for nuclear retinoic acid receptors of the type RAR. The related retinoid AM580 is also inactive [92]. Therefore, the specificity of UCP2 for its activator resembles that of a nuclear receptor. The physiological significance of the pH dependency and retinoid specificity in UCP2 remains to be established.

7. Conclusions: the acute control of thermogenesis in the brown adipocyte

In the present review an effort has been made to integrate from the earlier findings on the bioenergetics of brown adipose tissue and its mitochondria up to the current thoughts on the transport properties of the uncoupling protein UCP1. It is striking to note that already in the 60s, evidences pointed to fatty acids as regulators of heat dissipation in the adipocyte. However, their ubiquitous uncoupling activity has for decades hampered their consideration as physiological regulators. Data summarized here strongly argue in favor of such a role.

In summary, the sequence of metabolic events that would take place on the initiation of thermogenesis would be as follows (Fig. 1). (1) Noradrenaline released from the sympathetic nervous systems binds to the plasma membrane. (2) Hormone-sensitive lipases are activated via cAMP-dependent pathways. (3) Fatty acids are released from the triglyceride stores and serve two functions: (3a) they are activated to acyl-CoA, then converted to acyl-carnitine and transported into the mitochondrial matrix where they will substrate for oxidation and (3b) free fatty acids bind to UCP1 to increase its proton conductance. (4) The membrane potential drops and respiratory control is released, fast respiration and heat production take place. With the cessation of the hormonal stimulus, lipolysis ceases and when fatty acids are oxidized the proton conductance decreases, membrane potential is restored and thus the phosphorylation efficiency is recovered.

There is a second regulatory route that involves all-*trans* retinoic acid. The signalling pathways that may control the generation of the cytosolic active compound (retinoic acid) from its precursor (retinol) are still unknown. It should be noted that both pathways (adrenergic and retinoid) of regulation of UCP1 are also activating the transcription of the *ucp1* gene [105,106,108]. Noradrenaline activates gene transcription through the increased levels of cAMP, which allows the binding of the nuclear receptor to specific response elements (CREs) in the gene promoter [109,110]. Similarly, all-*trans* retinoic acid binds to specific nuclear receptors (RAR) that then interact with specific retinoic acid response elements (RARES) that are also present in the *ucp1* promoter [111,112].

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