REVIEW ARTICLE

The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP

Daniel RICQUIER[1] and Frédéric BOUILLAUD

Centre de Recherche sur l'Endocrinologie Moléculaire et le Développement (CEREMOD), Centre National de la recherche Scientifique (CNRS – Unit 9078), 9 rue Jules Hetzel, 92190 Meudon, France

Animal and plant uncoupling protein (UCP) homologues form a subfamily of mitochondrial carriers that are evolutionarily related and possibly derived from a proton/anion transporter ancestor. The brown adipose tissue (BAT) UCP1 has a marked and strongly regulated uncoupling activity, essential to the maintenance of body temperature in small mammals. UCP homologues identified in plants are induced in a cold environment and may be involved in resistance to chilling. The biochemical activities and biological functions of the recently identified mammalian UCP2 and UCP3 are not well known. However, recent data support a role for these UCPs in State 4 respiration, respiration uncoupling and proton leaks in mitochondria. Moreover, genetic studies suggest that UCP2 and UCP3 play a part in energy expenditure in humans. The UCPs may also be involved in adaptation of cellular metabolism to an excessive supply of substrates in order to regulate the ATP level, the NAD^+/NADH ratio and various metabolic pathways, and to contain superoxide production. A major goal will be the analysis of mice that either lack the *UCP2* or *UCP3* gene or overexpress these genes. Other aims will be to investigate the possible roles of UCP2 and UCP3 in response to oxidative stress, lipid peroxidation, inflammatory processes, fever and regulation of temperature in certain specific parts of the body.

Key words: carrier, energy, mitochondria, proton, thermogenesis.

INTRODUCTION

This article reviews data on the brown adipose tissue (BAT) uncoupling protein 1 (UCP1), two recently discovered mammalian UCP homologues, UCP2 and UCP3, and plant UCP homologues identified in *Solanum tuberosum* (StUCP) and *Arabidopsis thaliana* (AtUCP). Other review papers on BAT mitochondria or UCPs have been published in recent years [1–15]. Two other carriers predominantly expressed in brain and showing some uncoupling activity in recombinant systems were recently identified, and referred to as BMCP1 (brain mitochondrial carrier protein 1) [16] and UCP4 [17]. These two carriers are less similar to the UCPs, and will not be considered in this review.

Uncoupling of respiration

Mitochondrial ATP synthesis from ADP, driven by electron flow from reduced substrate (mainly NADH) to oxygen, defines oxidative phosphorylation. Peter Mitchell proposed that oxidation is coupled by the electron transport chain to pumping of protons from the mitochondrial matrix, generating a proton motive force (or electrochemical potential difference) for protons across the inner membrane of the mitochondria. This force drives the protons back into the matrix through ATP synthase, which couples proton transport across the membrane to phosphorylation of ADP [18]. The observation that mitochondria still consume oxygen when ADP phosphorylation is inhibited demonstrates that the coupling of respiration to ATP synthesis is imperfect; in fact, State 4 respiration is mainly due to uncoupling [19,20]. The coupling of oxidative phosphorylation is impaired by the existence of certain leaks through the mitochondrial inner membrane [19–26].

Mechanism of respiration uncoupling: proton leaks versus slip

The existence of two types of mechanism for respiration uncoupling has been debated for several years (for a review, see [27]). On the basis of Mitchell's theory, it was proposed that an increased proton permeability of the mitochondrial inner membrane, not coupled to an energy-consuming system such as ATP synthase, would constitute a proton leak and decrease the level of coupling of respiration to ADP phosphorylation. An alternative explanation of uncoupling corresponds to the concept of 'slip'. The slippage of respiratory chains can be defined as a failure of the proton pumps of the respiratory chain, which transfers electrons without extruding protons out of the membrane. The slippage of respiratory chains implies that a higher respiratory rate is necessary for the predicted ATP synthesis.

Biological roles and 'benefits' of respiration uncoupling

Besides the biochemical mechanisms of respiration coupling/uncoupling, the biological significance of such a mechanism can be considered. Leaks and slips, which explain the partial efficiency of energy transduction through the mitochondrial membrane, favour wastage of free energy, but may also have functional roles beneficial to organisms [22]. A major consequence of the uncoupling of respiration is the activation of substrate oxidation and dissipation of oxidation energy as heat. In terms of physiology, uncoupling of respiration and dissipation of energy as heat are obviously important for energy balance and body weight control, and represent research lines for metabolic diseases such as obesity. Increased substrate oxidation and heat production due to uncoupling are precisely observed when the BAT UCP1 is working in animals exposed to the cold, in newborn mammals, or when hibernators are waking from hibernation. In

Abbreviations used: AP-1, activator protein-1; BAT, brown adipose tissue; DIT, diet-induced thermogenesis; ROS, reactive oxygen species; UCP, uncoupling protein; AtUCP, UCP homologue in *Arabidopsis thaliana*; StUCP, UCP homologue in *Solanum tuberosum*.

[1] To whom correspondence should be addressed (e-mail ricquier@infobiogen.fr).

other respects, in addition to a contribution to the basal metabolic rate and adaptive thermogenesis, it was proposed that mild uncoupling of respiration could prevent the accumulation of oxygen radicals generated by mitochondria [28]. Rolfe and Brand [20] have proposed that partial uncoupling may increase the sensitivity and rate of response of oxidative phosphorylation to effectors. Another role for uncoupling of respiration is to control the NAD^+/NADH ratio and regulate metabolic pathways such as ketogenesis, lipogenesis and amino acid synthesis, which are dependent on the levels of these coenzymes [28,29].

BAT UCP1, A MITOCHONDRIAL UNCOUPLING PROTEIN OF AN ENERGY-DISSIPATING ORGAN

The discovery of the BAT UCP (renamed UCP1 when UCP2 was discovered [30,31]) resulted from studies of the mechanism of thermogenesis in BAT. The thermogenic function of BAT was demonstrated around 1961–1964 by several groups working on rodents or lagomorphs. These groups reported that BAT produces heat, in particular under conditions requiring extra heat production, such as exposure to the cold, birth or arousal from hibernation (for reviews, see [1–3,9,32,33]).

BAT is present in almost all mammals. It is a major site of cold-induced thermogenesis in rodents and also contributes to diet-induced thermogenesis (DIT) [34]. This tissue is well developed around birth in large mammals, and throughout the lifespan in rodents. BAT is found in characteristic deposits scattered in specific areas in the body, the major deposits being interscapular, axillar, perirenal, thoracic, and between the neck muscles. The topology of BAT is such that, upon activation of brown adipocytes, heat is quickly cleared through large vessels which convey it to the thoracic spinal chord, heart, thoracic structures, brain and kidneys. BAT is composed of brown adipocytes, which differ from white adipocytes. Brown adipocytes contain several droplets of triacylglycerols, a central nucleus and many mitochondria. In addition, the mitochondria are striated and exhibit numerous cristae generated by the highly developed inner membrane. Morphological analysis of brown adipocytes suggested that these cells have a marked oxidative capacity, and this was confirmed by measurements of oxygen consumption of isolated brown adipocytes.

The role of BAT in regulatory thermogenesis implies that its activity is regulated. The main control system of BAT activity is located in the hypothalamus and acts through thermoregulatory centres and sympathetic nerves which innervate the brown adipocytes directly. Activation of BAT thermogenesis by sympathetic fibres and noradrenaline was demonstrated in studies showing that noradrenaline released at the surface of brown adipocytes activates adrenoceptors, increasing oxygen consumption and inducing heat production within 1 or 2 min after noradrenaline delivery. Since noradrenaline activates lipolysis, it was proposed that the increased oxidation of fatty acids was related to raised heat production.

Although increased respiratory activity of BAT mitochondria constitutes a thermogenic process *per se*, the existence of a particular mechanism operating in these mitochondria was suspected from 1967, when Robert Smith and Olov Lindberg and their collaborators independently observed that the respiration of mitochondria isolated from BAT was loosely coupled to ADP phosphorylation (for reviews, see [1,2]). Several researchers then demonstrated that the uncoupled respiration of BAT mitochondria was activated by fatty acids and inhibited by purine nucleotides such as GTP, GDP, ATP or ADP [1,2]. These data suggested the existence of a regulatable uncoupler in the inner membrane of BAT mitochondria.

Although it is not known if regulation of the uncoupling pathway by the binding and debinding of nucleotides is physiological, the activation of uncoupling by fatty acids has a strong physiological significance, since activation of BAT by noradrenaline obligatorily triggers lipolysis. A major breakthrough was made by David Nicholls and his collaborators when they measured a particularly high proton conductance of BAT mitochondria, and observed that this proton pathway was inhibited by GDP and activated by fatty acids. Using photoaffinity labelling experiments, they identified a 32-kDa protein as the binding site of nucleotides and the putative UCP [35]. Before the publication of these data, a 32-kDa protein abundant in the membranes of BAT mitochondria was described: this protein was absent from liver mitochondria, induced during exposure of rats to the cold for several days, and down-regulated after readaptation to room temperature [36]. Following these studies, the 32-kDa protein was purified from hamster and rat [37,38], sequenced [39], cloned as cDNAs [40–43], and its proton-translocating activity reconstituted in liposomes [44–47]. It was therefore accepted that this protein was responsible for the regulated loose coupling of BAT mitochondria, and it was generally referred to as the uncoupling protein, UCP (UCP1). Other authors referred to this protein as thermogenin [2]. Finally, the demonstration of an important role for UCP1 in adaptive thermogenesis was provided by Enerbäck et al. [48], who obtained null mutant mice unable to maintain their body temperature in a cold environment. UCP1 synthesis is strongly activated by noradrenaline, cAMP, thyroid hormones and retinoids (for reviews, see [2,8,9]). Dietary vitamin A supplementation in rats [49] or the addition of natural carotenoids to brown adipocytes induces UCP1 [50]. Chronic treatment of obese rats by nicotine was reported to induce UCP1 in white fat depots [51].

IDENTIFICATION OF UCP HOMOLOGUES: UCP2, UCP3 AND PLANT UCPS

The specific expression of UCP1 in brown adipocytes has been confirmed in many laboratories (see reviews cited in the Introduction section). Nevertheless, the molecular mechanisms of partial coupling of respiration in mitochondria of most tissues remained unclear. The debate between advocates of proton leaks and supporters of slippage of respiratory chain remains open, although it seems reasonable to assume that the two types of mechanism exist [52]. The existence of proton leaks accounting for 20–30% of total oxygen consumption of liver or skeletal-muscle mitochondria [21,22] indicates that certain components of the inner membrane of mitochondria catalyse proton leaks. Brookes et al. [53] recently concluded that the mitochondrial proton leaks are not due to phospholipids. Since the BAT UCP transports protons through the inner membrane of mitochondria, it was tempting to propose that UCP homologues were translocating protons through the inner membrane of liver or skeletal-muscle mitochondria. In fact, over the last few years we have observed that antibodies against UCP1 or UCP1 cDNAs are occasionally able to bind to protein or RNA from tissues other than BAT. Whether such interactions are due to non-specific binding to protein or RNA of other known mitochondrial carriers or UCP1 homologues had not been investigated. In addition, the complete sequencing of *Saccharomyces cerevisiae* yeast revealed the putative existence of 34 proteins related to the family of mitochondrial carriers [54], suggesting that this family was much larger than expected.

Taken together, these data encouraged us to look for UCP homologues expressed in different tissues of mammals and to

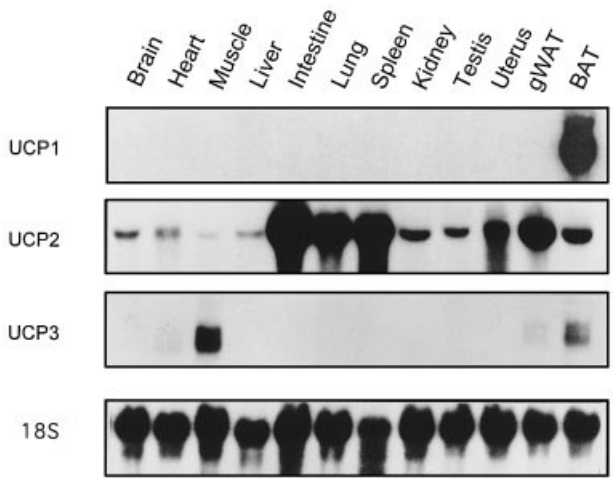

Figure 1 Northern analysis of UCP1, UCP2 and UCP3 mRNAs in mouse tissues

Portions of 20 μg of total RNA from each tissue (except BAT; 10 μg) were loaded. rRNA of 18 S was loaded as a control. Abbreviation: gWAT, gonadal white adipose tissue. This Figure was kindly provided by Dr Daniel Sanchis.

clone UCP2, a UCP1 homologue present in several tissues of mice and humans which is 59 % identical to UCP1 [30]. UCP2 was also cloned by others using sequencing of cDNA libraries or reverse transcriptase–PCR analysis [31,55]. A third UCP, referred to as UCP3 and sharing 72 % identity with UCP2, was identified by several groups a few months after the discovery of UCP2 [55–59]. Figure 1 shows the distribution of UCP1, UCP2 and UCP3 mRNAs in mouse tissues. Next, Laloi et al. [60] reported in 1997 the cloning of the first plant UCP, present in *Solanum tuberosum* and referred to as StUCP. cDNAs of a UCP homologue present in *Arabidopsis thaliana* were also cloned and referred to as AtUCP cDNA [61]. Interestingly, using antibodies against plant UCP, Jarmuszkiewicz et al. [62] detected a mitochondrial protein in the non-photosynthetic soil amoeboid protozoan *Acanthamoeba catellanii*.

Several genes encoding proteins sharing a significant degree of identity with UCP1 have therefore been identified. These UCPs may provide an explanation for the mitochondrial proton leaks. Whether such genes encode proteins with identical, related or distinct properties is discussed here, as is the contribution of these proteins to mitochondrial proton leaks. The question of mitochondrial carriers regulating the size of the proton gradient and the level of coupling of respiration is in fact complicated, since it was reported that the mitochondrial ADP/ATP carrier and aspartate/glutamate antiporter catalyse a proton transport activated by fatty acids [63–66]. In other respects, in the course of experiments on UCP1 expression in yeast carried out in collaboration with Eduardo Rial, a proton transport activity activated by ATP and inhibited by phosphate was described and named the yeast uncoupling pathway, or YUP [67]. A new homologue of mitochondrial carriers predominantly expressed in brain and referred to as BMCP1 (brain mitochondrial carrier protein 1) was also identified [16]. Curiously, although the sequence of this new protein is only moderately similar to the sequences of the UCPs (34–39 % identity), its expression in yeast induced a marked uncoupling of respiration and proton leak. Finally, another mitochondrial carrier expressed in brain and showing some uncoupling activity in mammalian cells was described by Mao et al. [17], who proposed to name it UCP4, although the amino acid identity with the UCPs is only around 30 %.

BIOCHEMISTRY OF THE UCPs

Primary and secondary structures

The alignment of the amino acid sequences of UCP1, UCP2 and UCP3 shows that these proteins are significantly similar (see Figure 2 and Table 1). These proteins exhibit a triplicated structure and a signature motif that is also present in other mitochondrial carriers. A similar organization was observed in plant UCPs [60,61]. No N-terminal mitochondrial cleavable targeting sequence is present in UCP1, and sequence comparison suggests that the other UCPs are similar. Computerized analysis of the secondary structure of the UCPs predicted the existence of six transmembrane domains linked by polar loops [39,42]. Miroux et al. [68,69] identified several antigenic sites in rat UCP1 in an approach to the topology of the protein in mitoplasts and sonicated mitoplasts. These studies validated the predicted secondary structure of UCP1. Although no other data are available, it can be proposed that the different UCPs share the same type of folding in the membrane.

Immunological detection of UCPs

There are few reports describing specific and sensitive antibodies against mitochondrial carriers, such as the ADP/ATP carrier, phosphate carrier or oxoglutarate carrier. In fact, there is almost no convincing published description of antibodies specifically reacting with UCP2 or UCP3, whereas antibodies against UCP1 usable in immunohistochemistry, Western analysis and immunoprecipitation experiments were obtained by several groups after UCP1 was purified (for reviews, see [2,6,9]). It is unclear whether the difficulty in identifying UCP2 or UCP3 in tissue mitochondria is due to very low levels of UCP2 or UCP3 in tissues, or to the poor quality of the antisera.

UCP2 was first detected immunohistochemically in rat liver by Larrouy et al. [70], who used a highly sensitive antiserum against UCP1 at 1:2000 dilution and observed a signal in Kupffer cells, which do not express UCP1 or UCP3 but express UCP2 mRNA, suggesting that the antigen was UCP2. In the same study, Western analysis was used to show that anti-UCP1 antibodies could react with UCP2 overexpressed in yeast mitochondria. Using the same anti-UCP1 antiserum, high expression of an antigen was detected by immunohistochemistry in monocytes and macrophages present in foetal liver [71]. The authors estimated that the antigen was UCP2, since foetal liver contains UCP2 mRNA, whereas UCP1 and UCP3 mRNAs are undetectable. In their immunohistochemical study of UCP2 in the liver of adult mice, Chavin et al. [72] used an antiserum directed against the N-terminal extremity of UCP2 at a 1:50 dilution. They did not detect a high level of antigen in the livers of control lean mice, whereas a significant induction of the protein was observed in hepatocytes of obese *ob*/*ob* mice, in agreement with data from Northern analysis. Two groups recently reported the detection of UCP2 in pancreatic islets of rats [73,74].

Two groups reported the detection of UCP2 in human skeletal muscle using Western analysis with antibodies directed either against the N-terminal [75] or the C-terminal [76] extremity of the protein, although the identity of the antigen was not proven. A description of an UCP2 antigen in Western analysis of rat white adipose tissue was not entirely convincing, since the authors utilized an antiserum against UCP1 and since white fat contains

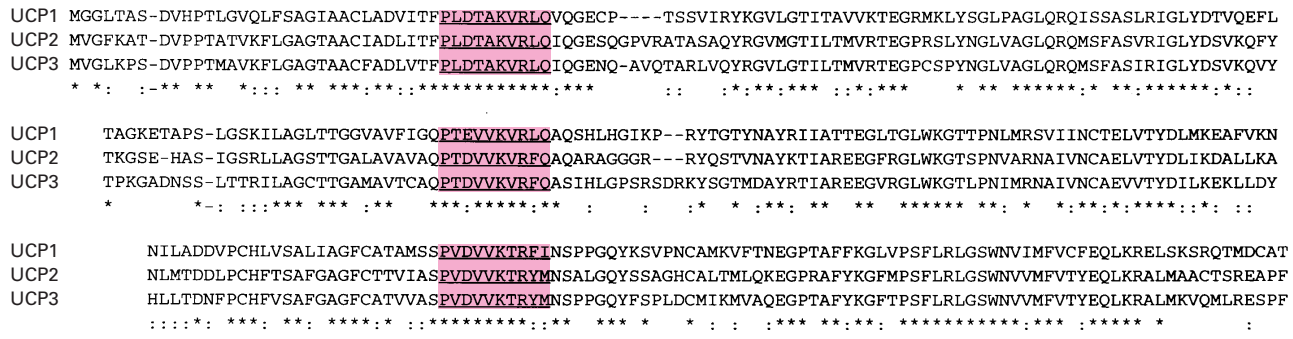

Figure 2 Alignment of UCP1, UCP2 and UCP3 amino acid sequences, showing the triplicated structure of mitochondrial carriers

Sequences of the human proteins are given. The signatures of mitochondrial carriers are underlined and shaded. Identical (*) and similar (:) amino acid residues are indicated.

Table 1 Main characteristics and relative identities of the UCPs

Molecular masses (*M*, in Da) and the numbers of amino acid (aa) residues (*n*) were calculated from proteins without the initiation methionine. mUCP, mouse UCP; rUCP, rat UCP; hUCP, human UCP; Sk.m., skeletal muscle. Two forms of human UCP3 were predicted from cDNA cloning experiments: a long form $UCP3_L$ and a short form $UCP3_S$. The level of identity corresponds to the same amino acid residues at the same positions. Most data presented were calculated using sequence data in GenBank.

				Identity (%)		
UCP	*M* (Da)	AA (*n*)	Distribution	With UCP1	With UCP2	pI
mUCP1	33117	306	BAT	100	56	9.3
rUCP1	33080	306	BAT	—	57	9.2
hUCP1	32873	306	BAT	—	57	9.3
mUCP2	33342	308	All tissues	59	100	9.8
rUCP2	33245	308	All tissues	57	—	9.7
hUCP2	33098	308	All tissues	59	—	9.7
mUCP3	33779	307	Sk.m., BAT	54	73	9.6
rUCP3	33884	307	Sk.m., BAT	54	72	9.6
$hUCP3_L$	34084	311	Sk. m.	57	72	9.3
$hUCP3_S$	29651	274	Sk.m.			
StUCP	32327	305	All organs	41	43	9.4
AtUCP	32531	305	All organs	41	46	9.6

brown adipocytes [77]. Others reported the immunodetection of UCP3 in Western analysis of BAT or gastrocnemius muscle extracts, but the immunoblot pictures and competition with antigenic peptides revealed the presence of numerous bands, such that the identification of UCP3 itself was problematic [78]. The detection of UCP2 in mitochondria from rat tissues by a group who used anti-UCP3 antibodies was not convincing [79]. In conclusion, although several immunohistochemical studies have identified an antigen probably corresponding to UCP2, there is little evidence for the specific detection of UCP2 and UCP3 using Western analysis. The specificity of the available antibodies remains to be fully demonstrated, and only microsequencing of immunodetected or immunoprecipitated tissue antigen will prove the identity of the proposed UCP2 and UCP3 antigens.

Mitochondrial localization of the UCPs

The subcellular localization of animal and plant UCPs is strongly suggested by their primary structure and the presence of the signature of mitochondrial carriers (see above), although it was reported that certain proteins of this family may be present in peroxisomes [80]. In the case of UCP1, the localization in mitochondria was demonstrated by functional assays, immunodetection and purification. Moreover, the marked uncoupling activity of UCP1 overexpressed in yeast [7,81,82], adipose tissue mitochondria [83] and skeletal-muscle mitochondria (F. Bouillaud, A. M. Cassard-Doulcier, C. Fleury, D. Ricquier and P. Diolez, unpublished work), as well as the analysis of $UCP1^{-/-}$ mice [48], convincingly established the mitochondrial localization of UCP1. In the case of UCP2, UCP3 and the plant UCPs, fewer data are available. However, the significant effect of these UCPs on the mitochondrial membrane potential of recombinant yeast [30,31,59,60,84,85] supported their mitochondrial localization. In addition, UCP2 and UCP3 were immunologically detected in the mitochondrial fraction of recombinant yeast [70,85]. Expression of UCP2 in COS cells and cell-free synthesis of UCP2 followed by *in vitro* import experiments demonstrated that this UCP was targeted to mitochondria (C. Lévi-Meyrueis, C. Gelly, D. Sanchis, I. Cohen, C. Prips-Buus and D. Ricquier, unpublished work). Finally, expression of UCP3 fused to an epitope in human breast carcinoma MCF7 cells demonstrated its subcellular localization in mitochondria [17].

Uncoupling mechanism of UCP1

The uncoupled state of BAT mitochondria is explained by an exceptional permeability of the inner membrane to protons and anions [86], related to the presence of UCP1 [1]. Studies with respiring mitochondria demonstrated that both removal of non-esterified fatty acids and the presence of nucleotides were necessary to ensure proper recoupling and UCP1 inhibition [2] [86]. Concerning the regulation of UCP1 activity, most researchers agree on its inhibition by nucleotides and its activation by non-esterified fatty acids, a requirement for the carboxy groups of fatty acids, the ability of UCP1 also to transport various anions, the existence of distinct fatty acid- and nucleotide-binding sites, and the identification of certain amino acid residues at the nucleotide-binding site. However, the mechanism of proton transport by UCP1 is still debated (for reviews, see [7,13,18,87]).

The amino acid sequence of UCP1 indicates that it is a member of the family of the anion carriers present in the mitochondrial inner membrane. The distinction between a carrier and a channel relies on the maximal rate of transport, which is much higher in a channel, and on the type of interaction between transported molecules and the protein. In carriers, a more intimate interaction results in a change of conformation of the

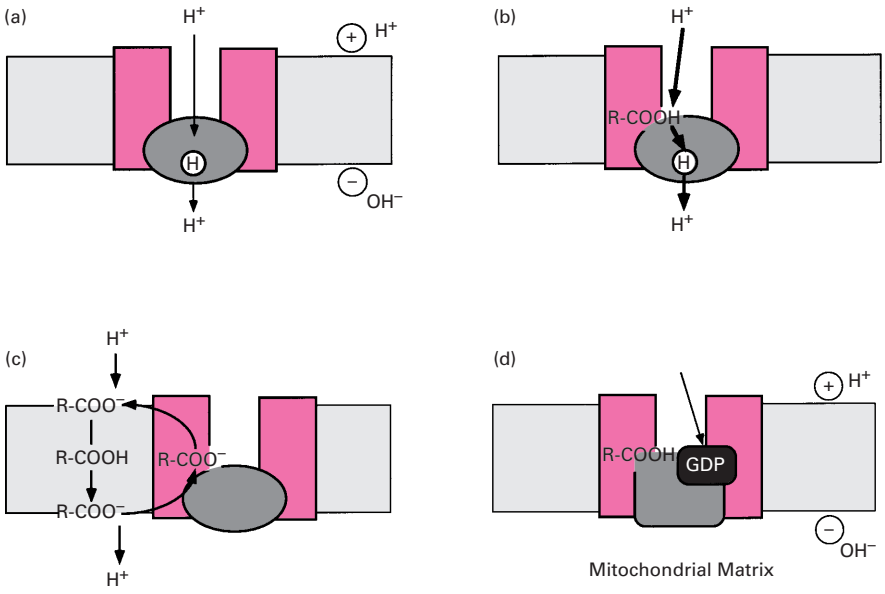

Figure 3 Models of the mechanism of action of UCP1, showing H^+ transport and the participation of fatty acids

Shown are a schematic representation in the membrane and a model of proton transport in UCP1 in the mitochondrial inner membrane. Pink rectangles represent regions of the protein participating in the constitution of the channel domain, and which are probably transmembranous α-helices. The grey oval represents the gating domain. The orientations of the electrical potential and of the ΔpH component are indicated. (**a**), (**b**) Hypothesis of a proton pathway present in UCP1. This pathway may operate in the absence (**a**) or presence (**b**) of fatty acids. The carboxy group of a fatty acid participates in the constitution of the proton pathway, and allows or enhances proton transport (**b**); this mechanism lowers the membrane potential and therefore increases respiration. (**c**) Fatty acid cycling hypothesis: the anion carrier (UCP1) allows the completion of a protonophoric cycle of fatty acids in the membrane; the fatty acid anion transport is due to the protein, whereas the protonated form diffuses freely in the membrane. We propose that this mechanism operates when the fatty acid concentration is relatively high. (**d**) Whatever the mechanism (**a**, **b** or **c**), binding of nucleotide induces a conformational change that makes transport impossible. Data suggest that there is no competition between nucleotides and fatty acids for the same site.

protein that is necessary for transport [88], whereas, in a channel, ions go through a pore [89]. Kinetic analysis of proton transport through UCP1 indicated that, although a carrier mechanism was suspected, it was instead functioning as a channel [90]. The study of mitochondrial anion carriers has shown that limited modifications can convert carriers into channels. These modifications include manipulation of the ionic composition of the medium in the case of the ADP/ATP carrier [91], chemical modification in the case of the phosphate carrier [92], or site-directed mutagenesis in the case of UCP1 [93]. Following such modifications, a loss of specificity of the transport was noted, and sometimes the channel exhibited certain pore-like properties and allowed large molecules to go through. This suggested that the anion carriers of the mitochondrial inner membrane contain two distinct domains: a channel-like structure and a gating domain. The involvement of such a shift between carrier and channel properties was suggested to occur *in vivo* for the ADP/ATP translocator, and would explain the phenomenon referred to as the mitochondrial transition pore. As opening of the mitochondrial transition pore is thought to be a primary event in the execution programme of apoptosis [94], the identification of mechanisms underlying carrier/channel conversion is of great interest.

The high ionic permeability of BAT mitochondria was assigned to UCP1 activity, since purine nucleotides, and more specifically GDP, could inhibit this leak. It was concluded from these pioneer studies that UCP1 was able to transport protons and several anions, such as chloride, bromide and nitrate [86]. In later studies, additional anions were also found to be transported by UCP1 [96]. In theory, proton transport by UCP1 contradicts its relationship with anion carriers. However, co-transport of a proton by the mitochondrial phosphate carrier ensures electroneutrality [97], or makes the exchange sensitive to the electrochemical gradient across the inner membrane in case of the aspartate/glutamate carrier [98]. Therefore proton pathways existed in mitochondrial carriers before the appearance of UCP1 in mammals.

The study of BAT mitochondria from mice deficient in UCP1 showed the absence of the GDP-sensitive proton leak and confirmed the proton transport function of UCP1 [99]. Overexpression of UCP1 in skeletal muscle of mice induced a marked mitochondrial proton leak that was activated by non-esterified fatty acids and inhibited by GDP (A. M. Cassard-Doulcier, C. Fleury, D. Ricquier, M. Goubern and F. Bouillaud, unpublished work). Matthias et al. [95] recently analysed the bioenergetics of BAT mitochondria from UCP1-ablated mice and observed that, surprisingly, these mitochondria were as sensitive to the uncoupling effect of non-esterified fatty acids as were UCP1-containing mitochondria. In contradiction of a well accepted mechanism, they concluded that fatty acids do not seem to be the intracellular physiological activator of UCP1. There are currently two proposed mechanisms to explain the uncoupling activity of UCP1 and the role of fatty acids (see Figure 3). In the first model, proposed by Klingenberg [13], UCP1 actually transports protons, and fatty acids provide an essential free carboxy group making proton transport possible or more efficient (Figures 3a and 3b). The second model, proposed by Garlid [87], involves cycling of fatty acids in the membrane, with UCP1 ensuring the transport of the anionic form of fatty acids (Figure 3c).

The fatty acid cycling model is supported by experiments carried out with both reconstituted systems and mitochondria. For example, the inhibition of proton transport when azido fatty acids were immobilized on UCP1 indicated that movement of the

fatty acid is necessary to transport protons under the experimental conditions used [100]. However, it is not certain if this model explains UCP1 activity *in vivo*. Firstly, this mechanism was originally proposed to explain the uncoupling effect of fatty acids in mitochondria devoid of UCP1, the proteins involved being other anion carriers of the same family, and the ADP/ATP carrier in particular [101,102]. This proposal suggests that all the transporters of the mitochondrial inner membrane are potential uncoupling proteins in the presence of fatty acids [64,87,103]. Secondly, in the case of UCP1, experiments supporting this model were carried out using high concentrations of fatty acids able, according to the authors, to induce a significant partial lysis of mitochondria [104]. Therefore, although this does not invalidate this mechanistic model, it is likely that under similar experimental conditions many other carriers would behave as uncoupling proteins [63]. In terms of this model, the purpose of UCP1 gene expression in BAT could be understood, in its more extreme interpretation, as a mere overloading of the mitochondrial inner membrane with a carrier protein, which by virtue of a characteristic common to all these proteins would uncouple respiration when fatty acids are present.

It still makes sense to consider the possibility that, besides the fatty acid cycling mechanism, UCP1 possesses a specific pathway for proton transport, thus making UCP1 different from the other carriers. Several arguments can be put forward to support the contention that a proton pathway exists in UCP1 and that fatty acid cycling is not absolutely necessary for uncoupling to occur. Mitochondria endowed with a variety of other carriers are more coupled than are BAT mitochondria. The introduction of UCP1 either in CHO cell mitochondria, or in yeast mitochondria using recombinant DNA technology, induced a partially uncoupled state [81,105]. When yeast mitochondria containing UCP1 were challenged with fatty acids, they showed a much higher sensitivity to the fatty acids than the control mitochondria [106]. The inhibition of both the basal and the fatty acid-induced uncoupling by nucleotides indicated that the proton transport was due to UCP1 [106]. As expected, addition of a higher concentration of fatty acids partially uncoupled control mitochondria without UCP1, but, in contrast, this uncoupling could not be inhibited by nucleotides [106]. Certain modified fatty acids with hydrophilic substitutions make the 'flip flop' across the hydrophobic phase very unlikely. Such modified fatty acids were still able to activate proton transport through UCP1 [13]. These data suggest that cycling is not absolutely required for proton transport to occur.

In the fatty acid cycling model, fatty acids are obligatory for proton flux and uncoupling. However, the observation of UCP1-mediated uncoupling in the absence of fatty acids would indicate that the proton pathway still operates when fatty acid cycling cannot take place. As indicated above, data obtained with respiring mitochondria showed that, even when fatty acids were trapped by albumin, mitochondria were still uncoupled, and lowering of the respiratory rate required the further addition of nucleotides [106]. The efficiency of fatty acid removal by albumin has been questioned, and therefore remaining non-exchangeable fatty acids have been proposed to explain the persistence of the uncoupling activity of UCP1 in BAT mitochondria [87]. This possibility is, however, poorly compatible with the fact that UCP1 activation in reconstitution experiments requires relatively high concentrations of fatty acids. Moreover, the fatty acid cycling model implicates a pool of fatty acids in equilibrium between UCP1 and the lipid phase of the membrane, and it is therefore difficult to understand why this fatty acid pool would not equilibrate with albumin. Finally, in the yeast recombinant expression system, the basal rate of proton leak catalysed by UCP1 in respiring mitochondria was not influenced by a mutation increasing UCP1 sensitivity towards fatty acids [106]. These observations suggest that in respiring mitochondria, where a high and stable membrane potential value was reached, UCP1 was able to catalyse a proton backflow, inducing a high uncoupled respiratory rate not dependent on fatty acid activation.

In conclusion, we propose the following hypothesis, whereby the mechanism of the uncoupling activity of UCP1 changes according to the fatty acid concentration (Figure 3): a proton pathway exists in UCP1; this pathway is sufficient to generate a partial uncoupling of mitochondria in State 4 of respiration when a high membrane potential is present (Figure 3a); addition of small amounts of fatty acids greatly increases the proton conductance through UCP1 (Figure 3b); at the same time, a fatty acid-induced uncoupling occurs in UCP1, as it does in other anion carriers, and could be the dominant mode of transport when high concentrations of fatty acids are present (Figure 3c); binding of nucleotides to UCP1 induces a change in conformation that inhibits all of these transport modes (Figure 3d).

Mutagenesis studies and functional organization of UCP1

Using recombinant expression of UCP1 in various systems, site-directed mutagenesis was used to test several hypotheses on the activity and the regulation of UCP1 in terms of nucleotide inhibition, pH-sensitivity of nucleotide binding, and proton transport. Mutagenesis of single arginine residues abolished the inhibition by nucleotides of transport through UCP1 [107]. Arginine residues at positions 83, 182 and 276 were predicted to be located in the transmembranous α-helices. These data supported the existence of a pocket in the plane of the membrane where the nucleotide binds. When the amino acid sequence of UCP1 became available, a short region of greater similarity to the ADP/ATP carrier was noted [42]. Subsequently it was observed that this small domain, corresponding to amino acid residues 261–269 in rat UCP1, was very similar to an α-helix interacting with DNA in nuclear receptors such as the oestrogen receptor [82]. This led to the prediction that this region is part of the nucleotide-binding site and of its α-helical arrangement. In agreement with this prediction, the deletion of amino acids 267–269 resulted in a UCP which could still be activated by fatty acids, as expected, but was no longer inhibited by nucleotides [82].

The binding of nucleotides to UCP1 is highly sensitive to pH. When the pH rises, the affinity of UCP1 for nucleotides declines, notably above pH 7.2 [108]. pH changes have therefore been considered as a possible mechanism of regulation of UCP1 in the brown adipocyte. The glutamic acid residue at position 190 has been demonstrated to be essential for the pH-sensitivity of nucleotide binding [108,109].

Mutagenesis of histidine residues in UCP1 indicated that a pair of histidine residues (His-145 and His-147) is important for proton transport [110]. The two histidine residues were predicted to be located in the second matrix loop of UCP1. Whereas proton transport was suppressed, mutation of the two histidines had no effect on chloride transport, suggesting that the transport mechanism was still intact, but that its specificity was changed [110]. These data suggested the existence of a proton pathway inside the hydrophilic domain of UCP1 involving proton-exchanging amino acids, and were poorly compatible with the fatty acid cycling model. It is notable that these two histidines are not conserved in UCP2 and UCP3. This suggested to Bienengraeber et al. [110] that a common mechanism of proton transport in UCP1, UCP2 and UCP3 was unlikely, or that UCP2 was not a proton transporter. However, these two hypotheses were ruled out: (i) Zhang et al. [111] reported that mutagenesis of the two

Table 2 Findings for and against an uncoupling activity or a role in energy expenditure for UCP2, UCP3 and StUCP

Findings are from references quoted in the text; * abstract ([254]); †F. Bouillaud and M. Goubern, unpublished work.

Role	For	Against
Uncoupling activity	41–59% amino acid identity with UCP1	Amounts of mRNAs do not match mitochondrial H^+ conductance
	Decrease in mitochondrial membrane potential in recombinant yeast expressing UCP2, UCP3 or plant UCPs	No UCP detected in hepatocytes of normal rodents
	Increase in respiratory rate and decreased respiratory control ratio in recombinant UCP2 or UCP3 yeast	Up-regulation of mRNAs in starvation is not associated with increased H^+ conductance*
	Increase in proton leak in UCP2 spheroplast†	
	Proton transport activity of UCP2 and UCP3 in liposomes	
Role in energy expenditure	Thermogenesis in yeast expressing UCP2 or UCP3	
	Up-regulation of UCP2 and UCP mRNAs in mice and plants exposed to the cold	No increase in UCP3 mRNA expression in cold-exposed animals
	Up-regulation of mRNAs by tri-iodothyronine (T_3)	Up-regulation in skeletal muscles after starvation
	Up-regulation of mRNAs by pyretic compounds	Up-regulation after one bout of exercise
	Correlation of mRNA level with resting energy expenditure	
	Strong genetic linkage to resting metabolic rate in humans	
	Association of gene polymorphisms with resting energy expenditure	No association of gene polymorphisms with resting energy expenditure

histidine residues in UCP1 did not impair its ability to uncouple respiration in yeast mitochondria, and (ii) Jaburek et al. [112] demonstrated that UCP2 and UCP3 catalysed the electrophoretic flux of protons in liposomes.

The complete deletion of the domain formed by residues 261–269 in UCP1 generated a mutant protein with pore-like properties, since ionic specificity was lost and solutes up to 1000 Da in molecular mass could pass through [93]. This short region was predicted to be localized at the extremity of the third hydrophilic loop, and therefore to constitute the hinge between this domain and the sixth transmembranous α-helix [69]. The activity of this mutant supported the existence of a gating domain, the destruction of which resulted in the formation of a pore-like protein with no specificity and with a cut-off close to that of the mitochondrial transition pore. A 20-amino-acid peptide containing residues 261–269 of a matrix loop was analysed by NMR and circular dichroism [113]. These studies confirmed that matrix loops contribute to the gating domain in UCP1.

Proton transport activity of UCP2, UCP3 and plant UCPs, and regulation by ligands

A limited number of analyses of the biochemical activity of the novel animal and plant UCPs are available. The present data indicate that UCP2, UCP3, StUCP and AtUCP alter the mitochondrial membrane potential recorded *in vivo* in recombinant yeast and mammalian cells, and probably uncouple respiration [17,30,31,57,59–61,84,85]. The respiration of isolated yeast mitochondria into which UCP2 had been introduced was less coupled than that of control mitochondria [30]. The portion of cellular respiration coupled to oxidative phosphorylation was decreased in yeast overexpressing UCP3 [85,111]. Increased thermogenesis was recorded in yeast overexpressing either UCP2 [114] or UCP3 [85]. Interestingly, hepatocytes of mutant obese *ob/ob* mice express UCP2, whereas hepatocytes of *ob/+* mice do not [72]. Comparison of the proton leak in liver mitochondria from these two strains of mice revealed an increased proton permeability in the liver mitochondria of *ob/ob* mice in which UCP2 is present [72]. This finding suggested that UCP2 levels are correlated with mitochondrial proton leak.

A major advance was made by Garlid and his co-workers. This team reconstituted the transport activity of human UCP2 and UCP3 purified from recombinant bacteria into liposomes, and observed that the two proteins catalysed an electrophoretic flux of protons, similarly to UCP1 [112]. In addition, the same group reported that fatty acids were obligatory for the proton transport activity of UCP2 and UCP3, again similar to UCP1 [112]. These data led the authors to conclude that UCP2 and UCP3 are true UCPs, having transport properties that are qualitatively identical with those of UCP1 with respect to transport of protons and alkylsulphonates [112]. However, using yeast mitochondria containing UCP2 or UCP3, we were unable to measure any activation of the proteins by fatty acids, under conditions where a net activation of UCP1 was recorded [115]. We and others were also unable to inhibit the uncoupling activity of UCP2 and UCP3 by adding nucleotides to isolated mitochondria [111,115]. Interestingly, using liposomes, Jaburek et al. [112] observed that the proton transport activity of UCP2 and UCP3 was not inhibited by low concentrations of nucleotides, with such an inhibition being observed only with high levels of nucleotides. In contrast, Echtay et al. [116] reported the reconstitution of the chloride transport activity of UCP3 and observed an inhibition by nucleotides such that UCP3 was more sensitive to ADP than to ADP, unlike UCP1.

Retinoids were identified as novel regulators of the uncoupling activity of UCP1 and UCP2 when tested in yeast mitochondria [115]. Using the same experimental conditions, these regulators did not affect UCP3 activity expressed in yeast [115]. Taken together, all these data suggest that the regulation of UCP1, UCP2 and UCP3 activity by ligands may somehow differ.

These *in vitro* experiments supported an uncoupling activity of the novel UCPs, but they did not demonstrate that this is the main physiological role of these proteins. UCP3 is predominantly

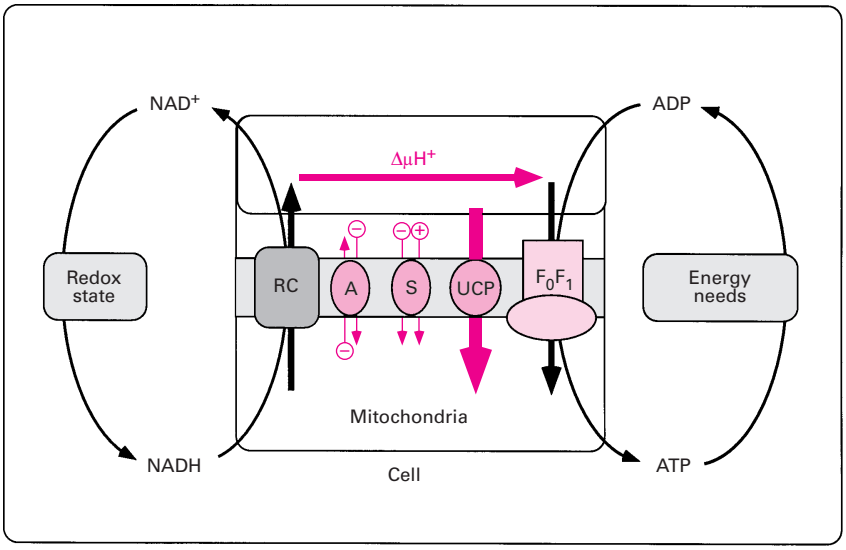

Figure 4 Proposed links between the redox state of coenzymes and ATP production by mitochondria

The mitochondrion is depicted as a box. Four components are represented: the respiratory chain (RC), the regenerating oxidized coenzymes, the F_0F_1-ATPase, and the transporters of the inner membrane (A, S, UCP). These transporters usually exchange anions through antiport (A), or co-translocate anions and protons though symport (S), resulting in electroneutral transport; UCPs are proton transporters. The intermediate used to convert redox energy into ATP is the proton electrochemical gradient, $\Delta\mu H^+$. When a higher turnover of redox equivalents is needed (shown here as NADH turnover), the increased proton pumping rate increases the electrochemical potential of the membrane which, in turn, makes proton pumping more difficult, unless ATP turnover is increased. To increase the flexibility of the system, and allow re-oxidation without changing ATP turnover, two mechanisms can operate. One possibility is to change the stoichiometry of proton pumping through the respiratory chain or the stoichiometry of proton utilization by ATPase. Such a phenomenon is known as 'slipping' of the respiratory chain. The second type of mechanism, referred to as respiration uncoupling, is the dissipation of the proton gradient across the inner membrane through UCPs, which catalyse proton leaks not coupled to ATP production. When the membrane is leaky to protons, proton pumping by the respiratory chain occurs at a higher rate of respiration, without changing the ATP production level or $\Delta\mu H^+$. Respiration uncoupling facilitates NADH re-oxidation without increasing ATP production. When respiration is uncoupled, the increased oxidation rate generates heat as a by-product. We propose that this pathway was originally present for metabolic purpose and was transformed into a thermogenic pathway in the case of the UCP1 in the BAT of mammals. In this respect, proteins such as UCP2 or UCP3 may represent the ancestors of UCP1.

expressed in skeletal muscle, and although the expression of UCP2 is broad, there are probably cells that do not express it, such as hepatocytes in normal rats [70]. Therefore UCP2 and UCP3 are not absolutely required for normal mitochondrial function. On the other hand, it remains possible that UCP2 and/or UCP3 are UCPs that are subject to specific regulation operating in several cell types.

When the mitochondrial membrane potential is close to 180 mV then, assuming that the mitochondrial inner membrane is 10 nm thick, the resulting electrical field is greater than 10^5 V/cm. With such an electrical field, the occurrence of leaks across a membrane able to perform many ionic exchanges is not surprising. On the other hand, the observation that the leak could vary according to the physiological situation suggested that the leak was something more than an unavoidable waste of energy. By maintaining respiration active, the leak helps re-oxidation of coenzymes which is associated with heat production and an increase in energy expenditure. Several explanations for this leak and the associated waste of energy have been put forward: the need for thermogenesis, the control of metabolic fluxes, and the maintenance of a correct redox balance, including defence against reduced forms of coenzyme Q, which constitutes a source of hazardous free radicals (reviewed in [20,28]).

The discovery of new genes closely related to that of the BAT UCP, which catalyses the regulated proton leak, led to the proposal that UCP2 and UCP3 participate in regulation of the proton leak. Different experimental approaches, and in particular reconstitution studies in liposomes [112], support a proton-translocating activity of UCP2 and UCP3. Analysis of tomato mitochondria also revealed a proton-translocating activity of plant mitochondria [117]. Table 2 summarizes data for and against an uncoupling activity of UCP2, UCP3 and StUCP.

Uncoupling of respiration may regulate the level of coenzymes and metabolic pathways

Lipogenesis from acetyl-CoA consumes ATP and requires NADPH, but the first steps of lipogenesis from glucose generate NADH which, when oxidized by mitochondria, results in ATP formation. Flatt [118] calculated that, surprisingly, the synthesis of fatty acids from glucose may result in a net synthesis of ATP. Since a significant part of this ATP comes from mitochondria re-oxidizing NADH, the coupling state of mitochondria could limit the speed of fatty acid synthesis from glucose. Therefore the rate at which an animal could store energy would be limited by the capacity of adipocytes to waste it [29]. In fact, this is exactly what happens in brown adipocytes, which have to maintain concomitant lipogenesis and fatty acid oxidation. In these cells, uncoupling of respiration through UCP1 limits ATP synthesis, activates the respiratory chain and maintains oxidation of NADH [29]. Although no data are available, it may be hypothesized that the roles of UCP2 and UCP3 could be to maintain the mitochondrial membrane potential below a certain value, limit ATP production by the mitochondria (otherwise, in the presence of high levels of substrates, an increase of ATP would inhibit the respiratory chain), maintain the oxidation of reduced coenzymes, control the ratio between oxidized and reduced coenzymes, and modulate lipogenesis, ketogenesis and amino acid metabolism. This proposed role is depicted in Figure 4.

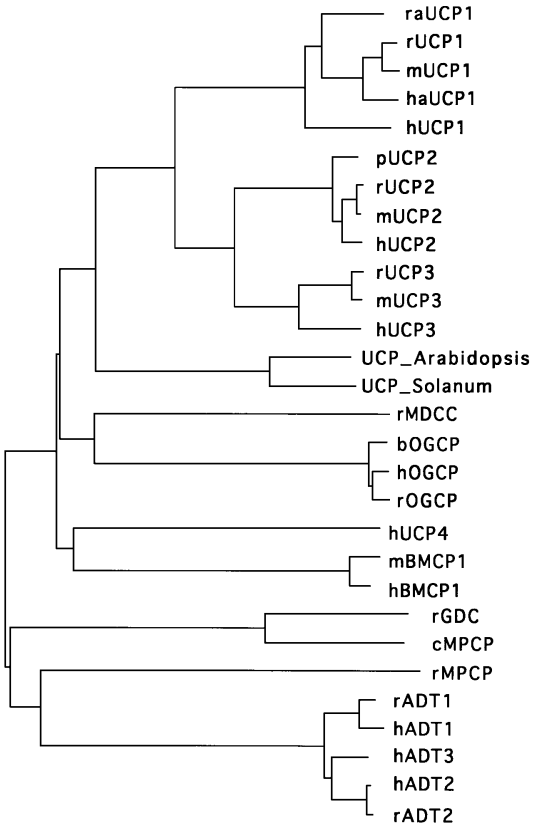

Figure 5 Evolutionary tree of UCPs and other mitochondrial carriers

The length of horizontal branches is proportional to distances between sequences. Abbreviations: MDCC, mitochondrial dicarboxylate carrier; OGCP, oxoglutarate malate carrier; BMCP, brain mitochondrial carrier protein; MPCP, mitochondrial phosphate carrier protein; GDC, Graves' disease carrier protein; ADT, adenine nucleotide translocator or ADP/ATP carrier; ra, rabbit; m, mouse; r, rat; ha, hamster; h, human; p, pig; b, bovine. The plant UCPs (*Arabidopsis thaliana* UCP and *Solanum tuberosum* UCP) are more closely related to mammalian UCPs than to other mammalian mitochondrial carriers. UCP4 refers to a gene expressed in brain and encoding a putative protein moderately similar to UCP1, UCP2 and UCP3. All sequences are available from GenBank.

Regulation of reactive oxygen species (ROS)

During respiration, a small proportion of the oxygen molecules are converted into superoxide radicals ($O_2^{-\cdot}$) by the one-electron reduction of O_2, mainly by mitochondrial complexes I and III [119,120]. H_2O_2 is produced as a secondary product via $O_2^{-\cdot}$ dismutation. Moreover, H_2O_2 itself is the source of more reactive intermediates, such as the hydroxyl radical OH^-. Under hyperoxic conditions, mitochondrial $O_2^{-\cdot}$ generation is increased linearly with oxygen concentration [120]. ROS are also produced in the destruction of cells during infection, in the degradation of fatty acids and other molecules by peroxisomes, and as by-products of processes that defend against toxic chemicals [121]. Excess superoxide ions may cause peroxidation of phospholipids, damage mitochondrial DNA, alter proteins or induce transcriptional factors [122]. Oxygen radicals can damage DNA, phospholipids and proteins, and also affect the transcription of genes.

The State 4 conditions of high redox pressure combined with a limited rate of utilization of the H^+ gradient in an oxygen-rich environment promotes $O_2^{-\cdot}$ formation, and oxygen radical production decreases when ADP phosphorylation increases (State 3) [123]. Skulachev [28] proposed that aerobic cells develop a special protective system against toxicity, and suggested the existence of the mild uncoupling of respiration and phosphorylation by means of increased H^+ leak through the mitochondrial inner membrane. In fact, uncouplers increase the rate of electron transfer and inhibit $O_2^{-\cdot}$ formation by mitochondria [119]. The explanation of this effect is that partial uncoupling stimulates O_2 consumption, shortens the lifetime of $CoQ^{-\cdot}$ (which is an excellent one-electron O_2 reductant and an initiator of ROS formation), and inhibits ROS production [123]. Therefore respiration uncoupling represents a powerful system for the limitation of ROS production. This suggested that one of the functions of proteins that uncouple respiration could be the limitation of ROS synthesis. Interestingly, Nègre-Salvayre et al. [124] have shown that this system operates in BAT mitochondria. These authors observed that H_2O_2 production was stimulated by the inhibition of BAT mitochondrial respiration through the addition of 0.4 mM GDP, which directly inhibits UCP1. The same authors also observed a similar effect of GDP on mitochondria isolated from the spleen or thymus. Since these tissues contain no UCP1, but express the UCP2 gene, the authors concluded that UCP2 is a regulator of mitochondrial H_2O_2 production [124]. Although such a role for UCP2 may exist, the conclusion of the authors remains questionable, since the inhibition of UCP2 activity by GDP was not demonstrated and since no inhibition or a partial inhibition of UCP2 by nucleotides has been observed by others [112,115].

Diehl and her collaborators have recently obtained data supporting a role for UCP2 in the response to mitochondrial oxidant production in macrophages and hepatocytes of *ob/ob* mice. They reported changes in UCP2 mRNA and protein levels associated with H_2O_2 production [72,125]. These authors tested the hypothesis that tumour necrosis factor-α increases mitochondrial oxidant production after partial hepatectomy. They observed that the tumour necrosis factor-α-dependent increase in oxidant production by liver mitochondria promoted UCP2 induction, which may represent an antioxidant defence mechanism [122]. The same authors observed an increased level of UCP2 in hepatocytes cultured in the presence of lipid emulsion [126]. In that study, lipids increased ROS formation prior to UCP2 induction in hepatocytes. It was proposed that the liver may adapt to an excessive supply of substrates by inducing UCP2 to facilitate substrate disposal (see Figure 4), while containing ROS production [126]. A role for UCP2 in fatty liver to protect hepatocytes against apoptosis was also proposed [127].

Evolutionary aspects

UCPs belong to the superfamily of anion carriers of the mitochondrial inner membrane. The characterization of an uncoupling mechanism in a protozoan strongly suggests that the UCPs emerged, as specialized proteins for proton transport, early during phylogenesis and occur in the whole eukaryotic world [62]. Figure 5 shows that the three animal UCPs and the plant UCPs have the same root. The closest related mitochondrial carriers are the mitochondrial dicarboxylate carrier and the oxoglutarate carrier, whereas the genes for the mitochondrial phosphate carrier and ADP/ATP carrier are slightly more distant in evolutionary terms. It is therefore tempting to speculate that the proton transport of UCP1 originates from a previous activity related to an energy-conserving process. It has been suggested that UCP1 was derived from an anion/proton symporter [39]. The membrane potential of respiring mitochondria opposes the net accumulation of anions in this organelle, and therefore

proton co-transport would ensure electroneutrality, as with the phosphate carrier [97]. The binding of a nucleotide to UCP1 induces a conformational change that results in the marked stability of the interaction of the nucleotide with UCP1, and is consistent with the hypothesis of an aborted transport of nucleotide. This suggests that nucleotides could have been transported by an ancestor of UCP1. Therefore a possibility is that UCP1 originated from a proton/nucleotide symporter in which conformational changes induced by nucleotides regulate the proton pathway. A further proposal is that the binding of fatty acids could open a by-pass, allowing protons to go through independently of the process of normal transport.

A parallel has been drawn between UCP1, where fatty acids could act as prosthetic groups, and bacteriorhodopsin, where the prosthetic group is the retinal bound to the protein [128]. Moreover, it was noticed that, in certain conditions, the bacteriorhodopsin molecule can transport either proton or chloride [129]. This characteristic was also observed for UCP1. To summarize, we propose two working hypotheses: (i) UCP1 activity changes according to the absence or presence of fatty acids and also according to the amount of fatty acid, and (ii) the activities of UCP2 and UCP3 are more related to the activity of UCP1 ancestors than to that of UCP1, and the physiological relevance of their transport activity and/or uncoupling activity is unknown.

GENETICS OF UCPs

The genomic organization of the mouse, rat and human *UCP1* genes has been analysed in several laboratories [7,8,130–145]. The structure of the *UCP1* gene is highly conserved in these three species. The *UCP1* gene is composed of six exons encompassing the coding sequence, and every exon encodes a membranous domain of the protein (Figure 6). The structure of the *UCP2* and *UCP3* genes is similar to that of the *UCP1* gene, the coding sequence being distributed over six exons in each of the three genes. However, the *UCP2* and *UCP3* genes differ from the *UCP1* gene by the presence of two or one untranslated exons located on the 5′ side of the *UCP2* and *UCP3* gene respectively [146–151]. The structure of the genes of plant UCPs is somewhat different, since nine exons covering the coding sequence were detected in the gene encoding AtUCP (M. Laloi, unpublished work).

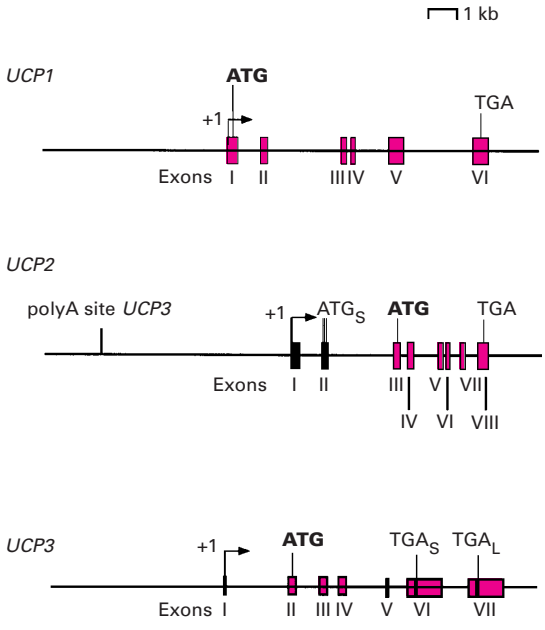

Figure 6 Alignments of the *UCP1*, *UCP2* and *UCP3* genes

Exons are indicated using roman numerals. Translation initiation (ATG; emboldened) and termination (TGA) codons are shown. The +1 position of the *UCP2* gene is located 7.0 or 8.2 kb respectively downstream of the polyA site of the human or mouse *UCP3* gene [149]. Human UCP3 mRNAs exist in short (TGA_S) and long (TGA_L) forms. Untranslated exons of *UCP2* and *UCP3* genes are shown as black boxes. Translated codons are shown as pink boxes.

The mouse, rat and human *UCP1* genes have been assigned to chromosomes 8 [130], 19 [152–154] and 4 [133] respectively. Following the initial cloning of UCP2 and UCP3 cDNAs, it was observed that these two genes are within less than 100 kb of each other [57,146,155]. In fact, it was demonstrated that the two genes are adjacent, the *UCP2* gene being only 7 or 8 kb downstream of the *UCP3* gene in mouse and human respectively [149,151,156]. The immediate vicinity of the two genes strongly suggested a duplication event. The *UCP3*/*UCP2* locus is localized on mouse chromosome 7, human chromosome 11 [30,57,146], and rat chromosome 1 [155].

Functional dissection of the *UCP1* gene

Detailed studies yielded rather good knowledge of the molecular mechanisms regulating *UCP1* gene transcription (for reviews, see [7,8]). The two areas to be studied were (i) the mechanisms explaining the unique expression of UCP1 in brown adipocytes, and (ii) the identification of genomic regions and transcriptional factors participating in the regulation of transcription in response to physiological situations, such as exposure of rodents to the cold. In fact, it was shown that the same genomic region controls both the tissue-specific expression of the gene and its response to hormonal factors. Studies in transgenic mice demonstrated the essential role of the 5′ end of the mouse [157] or rat *UCP1* gene in its specific expression in BAT [134,141,158]. Cassard-Doulcier et al. [134] identified a potent 200-bp enhancer located at position −2.4 kb in the rat gene. This enhancer was also identified in the mouse *UCP1* gene [135], as well as in the human *UCP1* gene (M. M. Gonzalez-Barroso, D. Ricquier and A. M. Cassard-Doulcier, unpublished work).

The *UCP1* gene enhancer has a complex organization and mediates the transcriptional activation of the *UCP1* gene by retinoids, thyroid hormone, cAMP, activator protein-1 (AP-1)-related factors and thiazolidinediones (which are synthetic counterparts of fatty acids and certain prostaglandins). The response elements for these factors were functionally identified in the enhancer [132,136–141,159]. Response elements for retinoids seem to be particularly important for the control of *UCP1* gene transcription. This is in agreement with previous studies demonstrating that thiazolidinediones were able to induce *UCP1* gene expression, not only in brown adipocytes [160] but also in non-differentiated 10T1/2 fibroblasts [161], or in rabbit cervical pre-adipocytes, which do not express UCP1 under basal conditions [162]. It was recently demonstrated that the 200-bp enhancer alone not only mediates hormonal responses but is sufficient to drive the specific expression of a reporter gene to BAT [158]. A new co-activator, referred to as PGC-1 (peroxisome gamma co-activator 1), was identified in BAT and skeletal muscle; this protein co-activates the transcriptional effect of peroxisome-proliferator-activated receptor γ and thyroid-hormone receptors in the *UCP1* gene [142]. Other elements present in the proximal region of the *UCP1* gene promoter also mediate regulation of *UCP1* gene transcription to cAMP, C/EBP (CCAAT-enhancer-binding protein) α, C/EBP β and c-Jun [135,143–145].

Table 3 Linkage or association studies of the human *UCP1*, *UCP2* and *UCP2* genes

RMR, resting metabolic rate; BMI, body mass index [ratio between height (m) and square of body weight (kg^2)]. The genetic markers used were either D11S911 and D11S916, which are anonymous markers close to the *UCP2*/*UCP3* locus, or the indicated variants. The 45 bp exon 8 variant corresponds to a 45-bp sequence which was present or absent in exon 8 of the human *UCP2* gene. The exon 6 variant corresponds to a mutation of the human *UCP3* gene at the level of the splice donor site of exon 6, resulting in shortening of the protein. Tyr-99-Tyr is a silent mutation in the *UCP3* gene. N.S., non-significant statistical difference. References are given in the text.

UCP1		*UCP2*		*UCP3*	
Trait; *P* value	Marker	Trait; *P* value	Marker	Trait; *P* value	Marker
		RMR			
		$P = 0.000002$	D11S911		
		$P = 0.006$	D11S916		
		Anorexia nervosa			
		$P = 0.0002$	D11S911		
		$P = 0.09$	D11S916		
				Obesity; *P*: NS	D11S916
Fat gain; $P < 0.005$	C-3826G	Sleeping energy expenditure		Decreased fat oxidation;	Exon 6 splice
		$P = 0.016$	Ala-55 → Val	$P = 0.019$	
		$P = 0.016$	45 bp exon 8		
Weight gain; $P < 0.002$	C-3826G	BMI; $P = 0.018$	45 bp exon 8	Obesity; $P = 0.02$	Tyr-99-Tyr
		Leptinaemia; $P = 0.006$	45 bp exon 8	Juvenile obesity; *P*: N.S.	Tyr-99-Tyr
		Weight loss		Obesity	
Weight loss after diet; $P < 0.005$	C-3826G	Weight loss after diet; *P*: N.S.	45 bp exon 8		
		BMI; $P = 0.7$	Ala-55 → Val		

Functional organization of the *UCP2* and *UCP3* genes

The gross and detailed structure of the human and mouse *UCP2* [147–150,163,164] and *UCP3* [146,151,165–168] genes have been determined (Figure 6). A particular feature of the mouse and human *UCP2* genes is the presence of several ATGs in-frame with an open reading frame for an unknown peptide of 36 amino acids in exon 2, whereas the UCP2 coding sequence begins in exon 3. Exon 2 did not prevent UCP2 synthesis *in vitro*, and it was demonstrated that the UCP2 initiation codon for translation is in exon 3 [149]. cDNA cloning of UCP3 revealed that the human gene is expressed as two splice variants generated by alternative splicing of the last intron; the predicted amino acid sequences correspond to a putative 312-amino-acid protein ($UCP3_L$) and a 275-amino-acid protein ($UCP3_S$) lacking the last potential transmembrane domain [55,56]. The two UCP3 mRNAs are expressed in similar amounts in human skeletal muscle [146,169]. The existence of two forms of UCP3 mRNA has not been reported in rodents.

Unlike the *UCP1* gene, the mouse and human *UCP2* promoter regions lack TATA and CAAT boxes, but are GC-rich. [148,149]. Primer extension experiments have identified transcription start sites in the mouse and human *UCP2* genes [148,149]. Possible regulatory elements for Sp1, AP-2, AP-1, CREB (cAMP-response element-binding protein), MyoD, glucocorticoid receptor and peroxisome-proliferator-activated receptors were visualized [148,149,170]. Sequencing of DNA upstream of the transcriptional start site of the mouse and human *UCP2* genes also revealed the presence of a consensus site for nuclear factor κB (C. Pecqueur, S. Raimbault and D. Ricquier, unpublished work). Although the functional activity of these binding sites for various factors has not yet been demonstrated, a possible role for these sites was suggested by the observed effects of thiazolidinediones, phorbol esters and endotoxin on UCP2 mRNA levels in animals or in cultures [122,171–175]. Yamada et al. [148] reported that 1250 bp of the promoter region of the mouse *UCP2* gene showed significant promoter activity in L6, 3T3-L1, HeLa, NIH 3T3 and CV-1 cells, in agreement with the ubiquitous expression of the gene. Interestingly, these authors measured a very high promoter activity in GHAC1 cells derived from rat pituitary. The same authors observed that the region between bp -160 and -678 exhibited a strong positive regulatory activity. A significant activity of 1400 bp of the promoter region of the human *UCP2* gene was measured in 1B8 adipocytes [149]. It was also shown that this region contains an activatory element [149]. Another study revealed that the mouse *UCP2* promoter responded to the cAMP-dependent protein kinase and delineated an enhancer element between bp -233 and -34 [170]. The same authors identified silencer elements upstream of position -602 and in intron 1 [170]. Others reported that the co-activator PGC-1 controls mitochondriogenesis and was able to induce UCP2 mRNA in myocytes [176].

The 5′ flanking region of the human *UCP3* gene has been deposited in GenBank (accession no. AF032871) and was also described in two papers reporting the presence of a putative TATA box in both the mouse and human *UCP3* genes [166,168]. Acin et al. [168] identified the transcriptional start site of the human *UCP3* gene and described several potential binding sites for regulatory factors and motifs for the E-box, MyoD, myocyte enhancer factor-2, peroxisome-proliferator-activated receptors and thyroid hormones. The presence of such recognition motifs is in agreement with the expression of the *UCP3* gene in muscles and its regulation by retinoids [177] and 3,3′,5-tri-iodothyronine [178,179].

Linkage and association studies of human UCP genes

Except for Luft's hypermetabolism syndrome, which was described many years before the identification of muscle UCPs (for review, see [121]), there is no report of any disease related to the uncoupling of respiration of muscle mitochondria. A *Bcl*I polymorphic site located at bp -3826 upstream of the TATA

box was identified in the human *UCP1* gene and was shown to be associated with fat gain over time [180,181], body mass index [182], change in body mass index in response to a hypocaloric diet [183], and the level of UCP1 mRNA expression in intraperitoneal fat [184]. Several groups reported an additive effect of this *UCP1* gene variant and the Trp-64 → Tyr mutation of the β_3-adrenoceptor gene in obese individuals [185–187]. In contrast with what was found in other populations, no association of the UCP1 A3826G sequence variation with obesity was found in Swedish and German cohorts [188,189]. In other respects, several amino acid variants have been identified in human UCP1, but these genetic variations of the coding region are not common factors contributing to obesity in Caucasians [190,191].

A genetic study of markers encompassing the human *UCP2/UCP3* locus revealed a highly significant linkage ($P < 0.000002$) with resting metabolic rate [192] (Table 3). A linkage to this locus was also observed in patients with anorexia nervosa [193]. Kaisaki et al. [155] localized the *UCP2* and *UCP3* genes to a region of rat chromosome 1 linked to glucose intolerance and adiposity in the Goto–Kakizaki type II diabetic rat. Two frequent polymorphic sites were identified in the human *UCP2* gene, one in exon 4 (Ala-55 → Val substitution) and another corresponding to a 45-bp insertion/deletion in the untranslated part of exon 8 [163,194–197]. A rare Ala-232 → Thr mutation was also detected in Japanese individuals [198]. Walder et al. [197] calculated that *UCP2* gene polymorphisms were genetically associated with sleeping energy expenditure in Pima Indians, whereas no such association was found in a Scandinavian cohort [195]. An association between the UCP2 exon 8 variant and body mass index was measured in two groups of South Indians [199]. This association was not observed in British subjects, but the UCP2 genotype of obese women was correlated with the serum leptin concentration, suggesting that the UCP2 variant may affect susceptibility to weight gain by influencing regulation of leptin [199]. No significant association between UCP2 polymorphisms and obesity or type II diabetes was noted by others [147,163,164,194,196,198–200].

Several genetic variants have been detected in the human *UCP3* gene, but no strong association with juvenile-onset or adult obesity was calculated [167,201,202]. A polymorphism in the splice donor junction of exon 6 was identified by Argyropoulos et al. [201] in Gullah-speaking African Americans and in the Mende tribe of Sierra Leone, whereas the mutation was not detected in Caucasians. Interestingly, this polymorphism resulted in loss of the splice junction and premature termination of the protein product, which lacked the sixth transmembranous fragment. The authors determined that, in individuals bearing this mutation, basal fat oxidation was reduced by 50 %, and suggested a role for UCP3 in metabolic fuel partitioning [201]. Table 3 summarizes the data from the genetic studies.

PHYSIOLOGY OF UCPs

In addition to biochemical and genetic studies, physiological aspects of UCPs have been investigated. The level of expression of the genes encoding the different UCPs has been measured in various physiological, pathological and pharmacological situations. In particular, since UCPs are supposed to be implicated in the control of cellular and tissue energetics, most physiological studies have been carried out in animals or humans under conditions affecting energy equilibrium, such as exposure to cold or nutritional changes. Tables 4 and 5 summarize the effects of various situations, hormones or compounds on the expression of the *UCP1*, *UCP2* or *UCP3* genes. In the absence of published data referring to null mutants for UCP2 or UCP3, and due to the difficulty of quantifying the proteins with specific antibodies, most studies of expression of the *UCP2* or *UCP3* genes were carried out through measurement of the mRNA level. The putative functions of the UCPs are related to resting or adaptive thermogenesis (including fever), metabolic adaptation to fluxes of substrates, energy partitioning and body weight regulation. In other respects, the high level of expression of the *UCP2* gene in macrophages of adult mammals [30,70] and in monocytes and macrophages in foetal rodent liver [71] suggest other functions of UCP2 related to inflammation or haematopoietic system development.

Role of UCP2 and UCP3 in resting energy expenditure

Two independent genetic studies (linkage or association studies; see Table 3) in Canadians and in Pima Indians [192,197] suggested a role for the *UCP2/UCP3* locus in determining resting metabolic rate or resting energy expenditure. Contrasting data were obtained during the analysis of a Swedish cohort [195]. In order to approach the biological roles of these UCPs, attempts were made to correlate levels of mRNAs encoding the novel UCPs with certain biological parameters. Two groups measured a positive association between the UCP3 mRNA level in human muscle [203], or the UCP2 mRNA level in human white fat [204], and resting metabolic rate. These studies suggested that the *UCP2* and *UCP3* genes are determinants of basal energy expenditure in humans. Conversely, others found no association between the level of UCP2 mRNA or protein in skeletal muscle and resting metabolic rate or resting energy expenditure in obese and diabetic humans [76,205].

A major role for UCP1, StUCP and AtUCP in response to cold exposure in animals and plants, and a possible role for UCP2 and UCP3 in fever or control of temperature of specific parts of the body

The role of the regulated uncoupling of respiration of brown adipocytes and of UCP1 in adaptive thermogenesis in response to cold exposure was accepted for many years, but not fully demonstrated until 1997. The demonstration by Kozak and his collaborators that UCP1$^{-/-}$ mice were sensitive to cold and unable to maintain their body temperature proved that the function of the *UCP1* gene is to induce respiratory uncoupling and thermogenesis in order to maintain body temperature in a cold environment [48]. Interestingly, the authors noted an up-regulation of UCP2 mRNA in the BAT of transgenic mice whose *UCP1* gene was interrupted. Matthias et al. [95] found that the level of UCP2 mRNA in BAT of UCP1-ablated mice was 14-fold higher than in BAT of wild-type mice. Although it is difficult to interpret the significance of such a regulation of UCP2, it could be viewed as a compensatory mechanism. However, this compensation was not efficient or sufficient, since the body temperature of the cold-exposed mice decreased. From this it may be inferred that UCP2 cannot significantly contribute to thermogenesis in a cold environment. In agreement with this, we did not find elevated UCP2 expression in tissues of mice exposed to 4 °C for 16 h [30]. However, other groups reported an increased UCP2 mRNA level in BAT of mice exposed to the cold for 5 h [206], or in BAT, heart and skeletal muscle of rats exposed to 5 °C for 2 days [207]. An up-regulation of UCP2 and UCP3 mRNAs was reported in interscapular BAT of rats exposed to 4 °C for 7 days [208]. Whether these data reflect a true thermogenic potential of UCP2 remains to be demonstrated. An increased level of UCP3 mRNA was also measured in BAT of

Table 4 Effects of different situations on expression of UCP genes in rodents and humans

In the case of UCP1, the same results were obtained for the protein and the mRNA; in the case of UCP2 or UCP3, almost all data correspond to assay of mRNA levels. VMH, Ventro-Median-Hypothalamus-lesioned; WAT, white adipose tissue; Sk.m., skeletal muscle. +, Increased level; −, decreased level; U, unchanged. Data are from references quoted in the text, and [255–257].

	UCP1	UCP2 mRNA				UCP3 mRNA		
Situation	BAT	BAT	WAT	Sk.m.	Liver	BAT	WAT	Sk.m.
Cold exposure (rodents)	+	+/U	U	+/U	U	+/U		U
High-lipid diet (rodents)	+	+	+					+
Starvation								
Rats	−	−		+		−		+
Humans			+	+				+
Obesity								
ob/ob mouse	−		+		+			
db/db mouse	−		+					
fa/fa rats	−	+	+			−	−	−
VMH rats	−		U		U			
Humans			+	+/−				
Diabetes								
Diabetic rats	−	−	+	U		−		+
Humans with type II diabetes			+				+/−	
Exercise (rats)								
One bout								+
Chronic								−/+

Table 5 Effects of hormones and other factors on expression of UCP genes in rodents

In the case of UCP1, the same results were obtained for the protein and the mRNA; in the case of UCP2 or UCP3, all data correspond to assay of mRNAs. WAT, white adipose tissue; Sk.m., skeletal muscle; T_3, tri-iodothyronine; LPS, lipopolysaccharide; TNFα, tumour necrosis factor α. Leptin results are those obtained upon prolonged delivery of leptin; decreased levels of UCP2 and UCP3 mRNAs were measured upon acute treatment with leptin [258]. +, Increased level; −, decreased level; U, unchanged. References are given in the text and in [259]. Data are from references quoted in the text and from [260–273].

		UCP1	UCP2 mRNA				UCP3 mRNA		
Compound	*In vivo/in vitro*	BAT	BAT	WAT	Sk.m.	Liver	BAT	WAT	Sk.m
Fatty acids	*In vivo*								+
	In vitro	+	+	+	+				
Thiazolidinediones	*In vivo*	+	+	+	+		+		−
	In vitro	+	+	+	+		+	+	
Retinoids	*In vitro*	+	+						
T_3	*In vivo*	+	+	+	+		+		+
Leptin	*In vivo*	+	+	+	+		+		+
Insulin	*In vivo*	+							
β_3-agonist	*In vivo*	+	+/U	+/U	+/−		+/−	+/−	+/−
Dexamethasone	*In vivo*	−					−		+
LPS	*In vivo*					+			+
TNFα	*In vivo*	+	+	+	+	+			+
Interferon-γ	*In vivo*					+			

cold-exposed rats by Larkin et al. [178], whereas other authors noted no effect of cold on UCP3 gene expression in mice [206]. There is agreement that cold exposure does not activate UCP3 mRNA expression in skeletal muscle, indicating that this protein is not primarily implicated in body temperature regulation [84,178,207]. The significance of high levels of UCP2 mRNA in white fat and of UCP3 mRNA in skeletal muscle of hibernating squirrels is unknown [209].

Thermogenic hormones and factors such as 3,3′,5-tri-iodothyronine [57,178,179,210–213], leptin [57,214,215], lipopolysaccharide and tumour necrosis factor-α [173,174,216,217] increase expression of the *UCP2* and *UCP3* genes in animals, suggesting a contribution to thermogenesis regulated by hormones or cytokines. Induction of UCP2 and UCP3 in tissues of rodents by lipopolysaccharide, interleukin-1β and other cytokines, and inhibition of the effect of lipopolysaccharide on UCP2 expression by a cyclo-oxygenase inhibitor [173], point to a possible role for the novel UCPs in the still unknown thermogenic mechanisms of fever.

Although the *UCP2* gene is expressed throughout the body, it is not expressed uniformly in the organs. In the case of the brain, *UCP2* gene expression is restricted to certain specific areas, such as a few nuclei of the hypothalamus (arcuate, surprachiamastic and paraventricular nuclei) [218]. It is tempting to speculate that UCP2 may regulate the temperature, and therefore the activity, of certain specific nuclei in the brain.

The identification of UCP homologues in plants [60,61] followed reports of the existence of regulated respiration un-

Table 6 Known and possible biochemical and physiological functions of the UCPs

	Biochemical activity	Physiological role
UCP1	Uncoupling of respiration Proton translocation Fatty acid cycling	Thermogenesis and control of body temperature
UCP2 and UCP3	Uncoupling of respiration Proton translocation Fatty acid cycling	Hypothesis: control of ATP level, $NADH/NAD^+$ ratio, ROS level – metabolic adaptation to fatty acid and glucose fluxes
StUCP and AtUCP	Uncoupling of respiration Unknown mechanism	Hypothesis: resistance to chilling

coupling mechanisms in plants [219]. Although a true uncoupling activity of plant UCPs has not yet been demonstrated, the marked effect of StUCP on mitochondrial membrane potential strongly suggests that this protein is an uncoupler [60]. However, the exact physiological role of such an uncoupling in plants was unclear. Since the *UCP1* gene is activated upon exposure of animals to the cold, the response of the genes of the UCP homologues was tested in plants kept in the cold. Surprisingly, there was a pronounced increase in StUCP mRNA and AtUCP mRNA levels in *Solanum tuberosum* and *Arabidopsis thaliana* respectively when kept at 5 °C for 2 days [60,61]. These data argued for a role for the UCPs in thermogenesis and resistance to chilling in plants. They also suggested that uncoupling of respiration can be used to generate heat and that the alternative oxidase pathway is not the only process involved in heat production by plants. Whether such a proposal is valid for the different organs of plants remains to be analysed.

In conclusion, the essential role of UCP1 in maintaining the body temperature of mice is firmly established. Although null mutant mice are needed for definitive conclusions to be drawn, the *UCP2* and *UCP3* genes do not seem to be related to adaptation to the cold, although a role in regulation of body temperature or the temperature of particular parts of the body, or a role in fever, cannot be excluded. In other respects, increased expression of the *UCP2* and *UCP3* genes in skeletal muscle during starvation or immediately after the cessation of exercise (see below) is inconsistent with a role for these UCPs in thermogenesis and wasting of energy. Conversely, the marked up-regulation of plant UCP mRNAs in organs of plants kept at 4 °C suggests a thermogenic role for the plant UCPs, or at least a role in defence against the cold.

Contribution of UCPs to DIT, energy partitioning and lipid metabolism

Utilization of food induces extra heat production. This corresponds to obligatory and adaptive energy dissipation due to digestion and metabolism, and is referred to as DIT. Previous studies have shown that BAT can buffer excess calorie intake under certain nutritional conditions in rats [34]. Theoretically, any increase in respiratory uncoupling in response to food intake induces thermogenesis and decreases food efficiency. Therefore the UCPs may be considered as candidates for the regulation of DIT and energy partitioning. The role of UCP1 in the wastage of energy generated by diet was demonstrated in starved animals [9,220], and in transgenic mice overexpressing UCP1 in their white adipose tissue [221] or underexpressing UCP1 in their BAT [222,223].

UCP2 and UCP3 also appear to be able to control energy partitioning and food efficiency. A high-lipid diet activated UCP2 expression in white fat of mice, and in particular in two strains of mice which are resistant to diet-induced obesity [30,58,156]. In addition, a two-fold induction of the UCP3 mRNA level in skeletal muscle of rats or mice receiving a high-fat diet was also reported [58,151]. Compared with carbohydrate feeding, fish-oil feeding up-regulated UCP2 mRNA in liver and UCP3 mRNA in skeletal muscle of mice [224]. Samec et al. [225] measured a marked increased in UCP2 and UCP3 mRNA levels in the gastrocnemius muscle of rats put on a restricted diet and then fed on a high-fat diet. During the analysis of UCP2 and UCP3 mRNA levels in obese individuals after stable weight reduction or during prolonged weight loss, expression of the UCP3 mRNA level decreased, suggesting that reduced UCP3 expression could contribute to decreased energy expenditure in weight-stable, weight-reduced individuals [226,227].

A role for fatty acids in up-regulating UCP3 expression in skeletal muscle was confirmed by infusing rats with a lipid emulsion and heparin [228]. *In vitro* and *in vivo* experiments demonstrated that fatty acids or certain retinoids promote UCP2 and/or UCP3 expression in white and brown adipocytes, myocytes and pancreatic islets [171,172,177,206,229–238]. Expression of UCP2 and/or UCP3 in skeletal muscles, heart or adipose tissue can be induced by various situations characterized by elevated levels of circulating non-esterified fatty acids, such as starvation in humans or rodents [84,169,239,240], high-fat feeding in rodents [30,177] and genetic or experimental diabetes in rodents [31,241,242]. It is not known whether the very high level of ketone bodies observed in diabetic rats is related to UCP induction [241]. The increased expression of the *UCP2* and *UCP3* genes in skeletal muscle of starved rats [207] or humans [239] questions their exact roles, and argues against a thermogenic role for UCPs, suggesting instead regulation of lipids or lipid metabolites as substrates [84,240,243]. In terms of this hypothesis, although the precise roles of UCPs in lipid metabolism remain to be elucidated, it should be recalled that a possible role for UCPs is to transport fatty acids into (or out of) mitochondria (see section on biochemistry of UCPs). Such a hypothesis is in agreement with studies of UCP2 and UCP3 reconstitution in liposomes [112].

UCPs and glucose utilization: a role in insulin resistance?

The observed increase in expression of muscle and heart UCP3 or UCP2 mRNA in rodents with type I diabetes [241,242] and in humans with type II diabetes [205] is not easy to interpret, since several metabolic parameters are altered in diabetes. However,

recent studies have implicated UCPs in glucose utilization, insulin resistance and even insulin secretion. Matsuda et al. [58] proposed that increased expression of UCPs may provide a defence against high-fat-induced obesity and impairment of glucose metabolism. Interestingly, there is a correlation between UCP3 mRNA levels in the human vastus lateralis and whole-body insulin-stimulated glucose uptake [75]. Similarly, Western analysis of the role of UCP2 and UCP3 in the partitioning of metabolic fuels in humans suggested that muscle UCP2 and UCP3 levels were correlated with carbohydrate oxidation and higher insulin-mediated glucose uptake [244].

Other data supporting a link between muscle UCPs and glucose utilization have come from studies of exercised or immobilized animals. Increased expression of UCP3 and UCP2 mRNA levels was observed in muscles of exercised mice 0–3 h after the end of the last bout of exercise, under conditions where GLUT4 levels and insulin-induced glucose uptake are increased [245,246]. Conversely, 20–30 h after cessation of the last bout of exercise, when the insulin-induced glucose uptake has returned to its basal value, no increase in UCP3 or UCP2 mRNA was observed [245,247]. In addition, an up-regulation of skeletal muscle UCP3 mRNA was measured in immobilized rats under conditions where glucose uptake was increased (M. Marzolo, D. Ricquier, X. Bigard and B. Serrurier, unpublished work). Studies based on the use of anti-diabetic compounds such as thiazolidinediones also support a role for muscle UCPs in glucose utilization [172,230,248]. Interestingly, Hjeltnes et al. [249] measured an increased level of UCP2 and UCP3 mRNAs in skeletal muscle from tetraplegic subjects, and a normalization after electrically stimulated leg cycling under conditions where insulin-stimulated glucose uptake was increased. Shimokawa et al. [248] demonstrated that the UCP3 mRNA level in skeletal muscle was correlated strongly with levels of circulating glucose in rats treated with a thiazolidinedione, suggesting that the thiazolidinediones increased glucose catabolism by up-regulating UCP2 expression.

Samec et al. [250] reported a positive association between muscle UCP2 and UCP3 mRNA expression and increased plasma glucose levels over 2 h after a glucose load in rats. They concluded that the up-regulation of muscle UCP2 or UCP3 by a high-fat diet may be more closely linked to insulin resistance than to changes in circulating non-esterified fatty acids. These authors proposed that such a positive association between muscle UCP2 or UCP3 expression and insulin resistance would be consistent with a role for muscle UCPs in the regulation of lipid as a substrate, because, in the early stages of the development of insulin resistance, increased fatty acid oxidation limits glucose utilization by muscle cells, in agreement with the Randle hypothesis [251]. Tsuboyama-Kasaoka et al. [252] measured an up-regulation of UCP3 mRNA in skeletal muscles of transgenic mice with increased GLUT4 content; they suggested that increasing the rate of glucose uptake was inducing UCP3 expression and that UCP3 was involved in glucose utilization by skeletal muscle.

The *UCP2* gene is expressed at high levels in pancreatic islets [30,229,253]. Up-regulation of UCP2 associated with increased fatty acid oxidation in pancreatic islets of rats receiving leptin has been described [253]. Since it is known that the ATP/ADP ratio in β-cells controls insulin secretion and that ATP participates in granule priming and exocytosis, it is tempting to speculate that UCP2 could modulate the secretion of insulin through changes in ATP. Wang et al. [73] reported that overexpression of UCP2 in pancreatic islets of Zucker diabetic fatty rats normalized the underexpression of glucose-stimulated insulin secretion, whereas Chan et al. [74] decribed an inhibition of glucose-stimulated insulin secretion from rat islets overexpressing UCP2. This latter report agrees with an uncoupling activity of this protein, a decreased ATP content and the expected reduced insulin secretion. It supports a potential role for UCP2 as an important modulator of β-cell function [74].

CONCLUSIONS AND PERSPECTIVES

Animal and plant UCP homologues form a subfamily of mitochondrial carriers that are evolutionarily related and possibly derived from a proton/anion transporter. The BAT UCP1 induces uncoupling of respiration and thermogenesis to maintain body temperature at 37 °C. The plant UCPs may be concerned with response to the cold and resistance to chilling. The biochemical activities and biological roles of the recently identified UCP2 and UCP3 are poorly understood (see Table 6). Analysis of recent data points to a role for these UCPs in fatty acid or glucose utilization, regulation of ATP production by mitochondria, control of the NADH/NAD^+ ratio, and limitation of the level of ROS. Whether these UCPs contribute to proton leaks measured in mitochondria from most tissues remains to be demonstrated *in vivo*. Data from genetic studies and measurements made in humans support a contribution of UCP2 and UCP3 to resting energy expenditure. Major goals for future research will be the identification of the structure of these novel UCPs, as well as the analysis of mice null for the *UCP2* or *UCP3* gene. Other aims will be to investigate the roles of UCP2 and UCP3 in insulin secretion, control of temperature in brain nuclei, inflammatory processes, fever, response to oxidative stress, cell proliferation and apoptosis.

We express our gratitude to Serge Raimbault and Dr. Daniel Sanchis for help in preparing the Figures, and to Dr. David Marsh for critical reading of the manuscript. D. R. and F. B. are established CNRS and INSERM investigators respectively. Our research is supported by Centre National de la Recherche Scientifique (CNRS), Human Frontier Science Program organization (HFSP), Association de Recherches sur le Cancer (ARC), Association Française contre les Myopathies (AFM), Human Training Mobility Programme of the European Union, and Institut de Recherches Internationales Servier.

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