Topical Review

Mitochondrial uncoupling proteins: from mitochondria to the regulation of energy balance

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The coupling of oxygen consumption to ADP phosphorylation is incomplete, as is particularly evident in brown adipocyte mitochondria which use a regulated uncoupling mechanism to dissipate heat produced by substrate oxidation. In brown adipose tissue, uncoupling is effected by a specific protein in the inner mitochondrial membrane referred to as uncoupling protein-1 (UCP1). UCP1 gene disruption in mice has confirmed UCP1's role in cold-induced thermogenesis. Genetic analysis of human cohorts has suggested that UCP1 plays a minor role in the control of fat content and body weight. The recent cloning of UCP2 and UCP3, two homologues of UCP1, has boosted research on the importance of respiration control in metabolic processes, metabolic diseases and energy balance. UCP2 is widely expressed in different organs whereas UCP3 is mainly present in skeletal muscle. The chromosomal localization of UCP2 as well as UCP2 mRNA induction by a lipid-rich diet in obesity-resistant mice suggested that UCP2 is involved in diet-induced thermogenesis. A strong linkage between markers in the vicinity of human UCP2 and UCP3 (which are adjacent genes) and resting metabolic rate was calculated. UCPs are known or supposed to participate in basal and regulatory thermogenesis, but their exact biochemical and physiological functions have yet to be elucidated. UCPs may constitute novel targets in the development of drugs designed to modulate substrate oxidation. However, very recent data suggest an important role for the UCPs in the control of production of free radicals by mitochondria, and in response to oxidants.

Body weight depends on the balance between energy intake and energy expenditure. Energy is expended on basal metabolism, exercise-induced thermogenesis, and adaptative thermogenesis, which is a response to environmental changes such as cold, excess food intake, and microbial or viral infection. Little work has been devoted to the molecular and genetic bases of the biochemical processes underpinning thermogenesis. Cold-induced thermogenesis in rodents has long been known to involve brown adipose tissue and a mitochondrial uncoupling protein (UCP) (Girardier, 1983; Nicholls & Locke, 1984; Cannon & Nedergaard, 1985; Himms-Hagen & Ricquier, 1998). The discovery in 1997 of new uncoupling proteins prompted re-examination of the molecular mechanisms of thermogenesis, their potential contribution to the pathogenesis of obesity, and above all their utility in the search for drugs to combat obesity (Fleury *et al.* 1997; Gimeno *et al.* 1997; Ricquier & Bouillaud, 2000; Boss *et al.* 2000).

Importance of thermogenesis in energy balance

The regulation of body temperature is essential in homoiotherms (also called endotherms), and brings into play thermolysis or thermogenesis mechanisms, which are triggered by a change in internal or external temperature. Thermoregulation processes are not only induced by exposure to cold or heat, and by hibernation in some species, but also by a variety of pathophysiological situations that result in changes in body temperature. Such situations include fasting, food intake, physical exercise, hypo- and hyperthyroidism, alcohol consumption, infection, pheochromocytoma, malignant tumours, malignant hyperthermia and the severe hypermetabolism of Luft's syndrome (defective coupling between respiration and phosphorylation).

Thermogenesis is not only a process activated or inhibited according to the body's pathophysiological status, but also a characteristic of the cells of endothermal animals, which explains why their body temperature is spontaneously close

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to 37 °C even if exposed to low temperatures. When a mammal is exposed to the temperature of thermal neutrality (18–20 °C in humans), the heat produced corresponds to basal metabolism. This standard metabolic rate can be measured directly by means of the heat produced, or from the oxygen uptake.

Cellular metabolism generates heat. Outside periods of reproduction or lactation, all the energy a resting adult derives from food intake is lost in the form of heat released during cellular metabolism (Girardier, 1983). In animals, free energy is derived from the oxidation of foodstuffs: sugar, fat and protein. This oxidation is coupled to the reduction of NAD to NADH, and the oxidation of NADH by the mitochondrial electron transport chain is in turn coupled to the setting up of a proton gradient across the inner mitochondrial membrane (Mitchell's chemiosmotic theory). The synthesis of ATP by mitochondria is coupled to the return of these protons to the mitochondrial matrix by mitochondrial ATP synthase. Mitchell's theory predicts that the leaks of mitochondrial membrane protons not coupled to the phosphorylation of ADP increase the dissipation of energy in the form of heat. Moreover, processes such as protein synthesis, maintenance of sodium–potassium gradients across membranes, and muscle contraction are coupled to ATP hydrolysis and are thermogenic. It was long thought that energy metabolism was fully coupled to ATP production. In fact, it is now known that not all oxygen consumption is mitochondrial, and that a significant proportion of mitochondrial respiration is not coupled to ATP synthesis (Fig. 1).

Brown adipose tissue and the uncoupling protein-1

Shivering generates heat but prevents normal muscle movement, whereas non-shivering thermogenesis (also called metabolic thermogenesis) allows normal use of muscles. Much non-shivering thermogenesis in small mammals is achieved in the brown adipose tissue (BAT), which is located near large blood vessels. BAT is found in all small mammals and in the newborn of larger mammals, such as humans. It becomes less abundant in adult large mammals in which its role is minor (Girardier, 1983; Nicholls & Locke, 1984; Cannon & Nedergaard, 1985; Himms-Hagen & Ricquier, 1998).

BAT consists of brown adipocytes which are morphologically and functionally distinct from white adipocytes. Brown adipocytes contain droplets of triglycerides and numerous mitochondria characterized by a highly developed inner membrane. The morphology of brown adipocytes indicates that they have a high oxidative capacity. Work done in the 1960s shows that BAT produces heat which is carried by the circulation to the brain and heart. Thermogenesis in BAT is activated in newborns, in rodents exposed to cold and in animals emerging from hibernation. This activation is commanded by the central nervous system and the orthosympathetic fibres innervating each brown adipocyte. The noradrenaline released by these fibres binds to several types of adrenergic receptors on the surface of the brown adipocytes. The later steps of the activation of thermogenesis in brown adipocytes are production of cyclic AMP, activation of lipolysis and oxidation of fatty acids by the numerous mitochondria.

The release of fatty acids stimulates the respiration of brown adipocytes and heat production. The increase in respiratory rate in each cell leads to overproduction of heat. In line with Mitchell's chemiosmotic theory, proton leakage from the mitochondrial membrane controls the respiratory rate (see Fig. 1). If leakage is activated by a signal (free fatty acids in the case of the mitochondria of brown adipocytes), the respiratory rate is enhanced and more heat is released per unit time. If in addition the activated proton leakage is independent of ADP phosphorylation, respiration is uncoupled from ATP synthesis and oxidation energy is dissipated in the form of heat. This mechanism can be compared with the action of an uncoupling agent. To explain the thermogenic behaviour of brown adipocytes, it is possible to postulate the existence within their inner mitochondrial membrane of an adjustable proton pathway. The mitochondria of brown adipocytes are characterized by proton conductance, which is activated by free fatty acids and inhibited by purine nucleotides (Nicholls & Locke, 1984). The protein responsible for proton leakage within the inner mitochondrial membrane and for respiratory uncoupling was identified in 1976–1977, and purified in 1982, and its cDNA was cloned in 1984 (Nicholls & Locke, 1984; Cannon & Nedergaard, 1985; Himms-Hagen & Ricquier, 1998). It has an apparent molecular weight of 33 000, is active as a dimer, and was originally called uncoupling protein (UCP). Known as UCP1 since the discovery of UCP2 in 1997, it is abundant in the inner membrane of the mitochondria of brown adipocytes and is specific to these cells. The expression of UCP1 in yeasts or mammalian cell lines induces partial uncoupling of respiration (Ricquier & Bouillaud, 2000). UCP1 binds to purine nucleotides. The protonophoric activity of UCP1 has been reconstituted in liposomes. It can be neutralized by nucleotides and activated by free fatty acids. The mechanism of action of UCP1 is subject to debate: some scientists believe that it is a proton transporter, whilst others assert that it returns anionic fatty acids to the intermembrane space, after they have crossed the membrane in protonated form. Following the ADP/ATP translocator, UCP1 is the second mitochondrial transporter whose amino acid sequence has been determined. These two proteins are, moreover, similar in structure and derive from the same ancestral gene, like several other members of the family of mitochondrial transporters sequenced since.

Heat production by brown adipocytes therefore results from the activation and regulated uncoupling of respiration, this uncoupling being due to activation by free fatty acids produced by lipolysis of UCP1, a specific membrane protein.

The uncoupling proteins

Given the specific role of brown adipose tissue in thermogenesis, it has always seemed logical that brown adipocytes be equipped with an original mechanism, partial uncoupling of respiration, brought into play by a specific protein, UCP1, which induces proton leakage. The corollary of these considerations was that the UCP1 gene could not be expressed in cells other than brown adipocytes, and this has been verified. A second corollary was that the thermogenesis mechanisms in the other cells were not associated with respiratory uncoupling systems, and we now know that this is untrue.

In fact, an explanation had to be sought for the heat-generating mechanisms in tissues other than brown adipose tissue. All metabolic reactions produce heat. However, it was known (see above) that mitochondrial respiration is accompanied by heat production as it is imperfectly coupled to ADP phosphorylation, and almost completely uncoupled in activated brown adipocytes. To explain this incomplete coupling of respiration and the energy loss mechanism, some authors have invoked slippage of the respiratory chains, while others refer to proton leaks. It has been calculated that proton leaks from the inner membrane of mitochondria of hepatocytes and myocytes could explain 26% of the liver's oxygen consumption and 5% of the skeletal muscle's, i.e. about 20% of the body's basal metabolism (Brand *et al.* 1994). The same authors proposed that these leaks of mitochondrial protons may be explained by the membrane's intrinsic properties, mainly in terms of phospholipids.

UCP2: a gene transcribed in various tissues

Knowing that the UCP of brown adipocytes acts as a proton transporter in their mitochondria, we hypothesized that the leaks of mitochondrial membrane protons were due to homologous proteins. By comparing the cDNA of the UCP of brown fat with a library of cDNAs of mouse skeletal muscle, we, and others, isolated a clone corresponding to a protein with 59% sequence identity with the UCP of brown fat. This new UCP, called UCP2, was also identified in humans. A major difference between the two UCPs is that the mRNA of UCP2 is present in many tissues and cell types, such as adipose tissue, muscle, heart, kidney, digestive tract, brain, spleen, thymus, adipocytes, myocytes, lymphocytes and macrophages (Fleury *et al.* 1997; Gimeno *et al.* 1997). Expressed in yeasts, murine UCP2 lowers the potential of the mitochondrial membrane, raises the respiration rate and reduces sensitivity to uncouplers: UCP2 therefore uncouples respiration and is a second mitochondrial uncoupling protein (Fleury *et al.* 1997; Gimeno *et al.* 1997; Rial *et al.* 1999; Ricquier & Bouillaud, 2000; Boss *et al.* 2000).

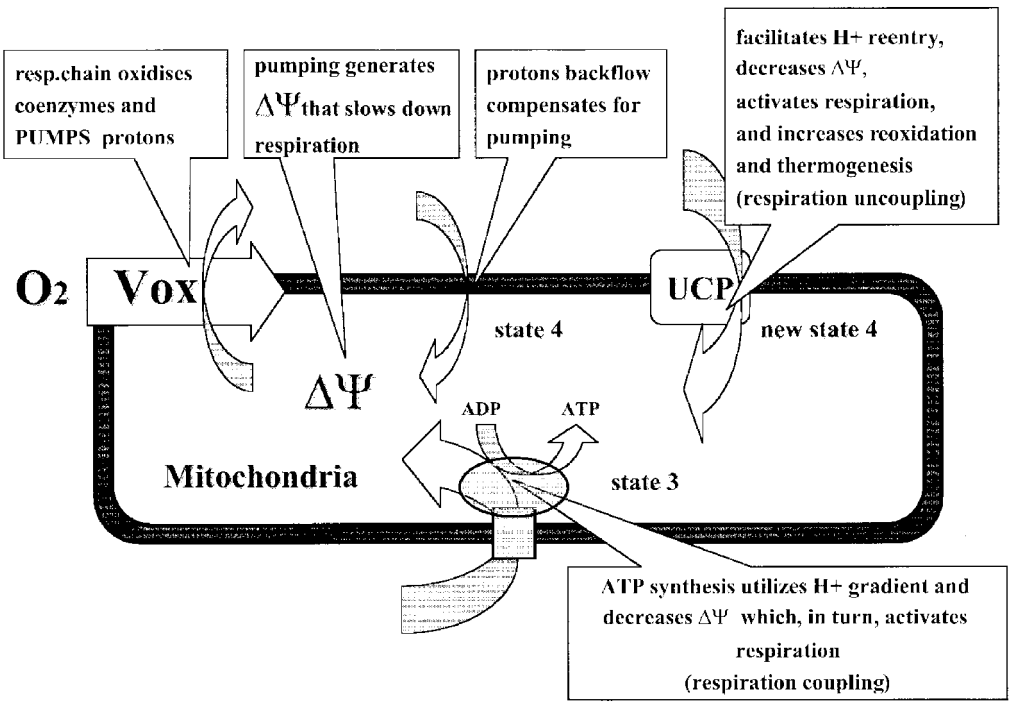

Figure 1. Schematic representation of mechanisms linking respiration to ATP synthesis and thermogenesis in mitochondria

Only the inner membrane of mitochondria is schematised. Vox identifies the respiratory chain. The respiratory chain works as a proton pump which generates a proton gradient and a membrane potential ($\Delta\Psi$). The proton gradient is used by ATP-synthase to phosphorylate ADP. During this process, the proton gradient is decreased and this activates the respiration. The UCPs function as a proton translocator; they decrease the proton gradient and activate coenzyme reoxidation.

The UCP2 gene is located on chromosome 7 of the mouse and chromosome 11 of humans, near a region linked to diabetes and obesity (Fleury *et al.* 1997; Solanes *et al.* 1997). To seek a putative physiological role of UCP2 in metabolism and in the control of body weight, we measured the expression of UCP2 mRNA in obesity-prone and obesity-resistant mice given a lipid-rich diet: the obesity-resistant mice overexpressed UCP2 mRNA in their adipose tissue. These findings, together with the function of the protein and the chromosomal location of its gene, led us to propose a role for UCP2 in food-induced thermogenesis (Fleury *et al.* 1997).

UCP3: a gene transcribed predominantly in skeletal muscle

Soon after the discovery of UCP2, cDNAs corresponding to UCP3, a protein homologous to UCP1 and UCP2, were cloned (Boss *et al.* 1997; Vidal-Puig *et al.* 1997; Gong *et al.* 1997). The amino acid sequence of UCP3 is 73 % identical to that of UCP2 and 57 % identical to that of UCP1. UCP3 also seems able to modulate the coupling of respiration (Gong *et al.* 1997; Boss *et al.* 2000). UCP3 mRNA is principally expressed in the skeletal muscles of rodents and humans. In mice, apart from the muscles, UCP3 mRNA is abundant in the brown adipose tissue and in the white adipose tissue and the heart. Some traces of UCP3 mRNA are found in human heart. Several expressed sequence tags were localized on human chromosomes, indicating that the UCP2 and UCP3 genes are very close (Solanes *et al.* 1997), and recent work has shown that human and murine UCP2 and UCP3 genes are separated by a few kilobases (Pecqueur *et al.* 1999; Ricquier & Bouillaud, 2000). These two genes probably arise from duplication and have an organisation similar to that of the UCP1 gene.

It is therefore likely that most mammalian tissues possess one or more mitochondrial uncoupling proteins.

Mitochondrial uncoupling proteins also exist in the plant kingdom

Following suggestions by some authors that plants contain mitochondrial uncoupling proteins, cDNA from such a protein was isolated from a potato bank (Laloi *et al.* 1997). The sequence of the corresponding protein, called stUCP, was 44 % identical to that of UCP1 and 47 % identical to that of UCP2, stUCP possessing motifs characteristic of the members of the family of mitochondrial transporters. The mRNA of stUCP is present in most plant organs. The most surprising result was that stUCP2 mRNA is strongly induced in the leaves of plants exposed to a temperature of 4 °C (Laloi *et al.* 1997). Cold therefore induces stUCP as it induces UCP1 in the brown adipose tissue of animals. The signalling system controlling the induction of stUCP by cold has not been identified. Plant and animal UCPs may have different functions.

A new understanding of mitochondrial biochemistry and the molecular basis of thermogenesis

The discovery of new UCPs points to proton leakage in mitochondria, in line with the existence of a slippage mechanism of the respiratory chains. UCPs control mitochondrial activity in most tissues, and their presence in plants suggests that they are universal. They appear to modulate respiration rate (by altering the potential difference across the mitochondrial membrane) and perhaps the quantity of ATP produced by respiration. In certain cases, such as UCP1 in brown adipose tissue, the respiratory uncoupling has an obvious thermogenic function. UCP2 and UCP3 are perhaps involved in food-induced thermogenesis or fever, but this requires further analysis. It is possible that UCPs, or some of them, have other functions such as modulation of superoxide ion levels (Nègre-Salvayre *et al.* 1997; Skulachev, 1998; Lee *et al.* 1999). This hypothesis was confirmed by studies of mitochondria isolated from skeletal muscle of UCP3-deficient mice (Vidal-Puig *et al.* 2000). However, numerous questions remain unanswered, notably concerning the exact catalytic activity of each UCP, the nature of the endogenous ligands of UCP2 and UCP3, and the mechanisms of transcriptional control of the UCP2 and UCP3 genes. Figure 2 shows two postulated mechanisms of respiration uncoupling by the UCPs. These proteins may simply translocate protons. The proton translocating activity of UCP1 is markedly activated by free fatty acids. Others proposed that the UCPs were active as fatty acid cyclers through the membrane (Garlid & Jaburek, 1998; Jaburek *et al.* 1999). According to this second hypothesis, protonated fatty acids cross the membrane and release a proton on the matricial side; then, the UCP facilitates the translocation of the anionic form of fatty acids.

Physiological and pathological situations modifying UCP expression in animals and humans

Although new UCPs have only recently been discovered, data have already been gathered on how UCP gene expression varies in different physiological, pharmacological and pathological situations. In accordance with the known role of brown adipose tissue in cold-induced thermogenesis and the maintenance of body temperature in rodents, several studies have shown that the UCP1 gene is activated in response to exposure to cold and plays a part in the maintenance of body temperature, as demonstrated in cold-exposed mice in which the UCP1 gene has been disabled (Enerback *et al.* 1997). In these mice, overexpression of the UCP2 gene suggests a compensation process and involvement of this gene in cold-induced thermogenesis. However, this apparent compensation is not efficient since these animals are still cold sensitive. It suggests that UCP2 cannot compensate the role of UCP1 in cold-induced thermogenesis. Recently, Matthias *et al.* have studied the biochemical properties of cells and mitochondria isolated

from the brown fat of UCP1-null mutant mice. They observed that the high expression of UCP2 mRNA (and UCP3 mRNA) in the tissue was not associated with a highly uncoupled state of respiration, nor with an ability of noradrenaline or fatty acids to induce uncoupled respiration in the cells (Matthias *et al.* 2000). The explanation of this apparent paradox came from trials to quantify the UCP proteins in brown adipose tissue of these mice. In fact, although UCP2 mRNA is increased in brown fat of the mice deficient for the UCP1 gene, we did not detect any induction of the UCP2 protein (C. Pecqueur, M. Alves-Guerra, D. Ricquier & B. Miroux, unpublished data).

UCP2 mRNA increases in rats exposed to the cold for 48 h, but not in mice exposed to 4 °C for 20 h (Boss *et al.* 2000). Fasting in animals (Boss *et al.* 1998; Samec *et al.* 1998) and caloric restriction for 5 days in humans (Millet *et al.* 1997) raise levels of UCP2 and UCP3 mRNAs in skeletal muscles. Caloric restriction resulting in a 10% decrease in body weight in humans is associated with an increase in UCP2 in skeletal muscles (Simoneau *et al.* 1998). This seems paradoxical since a decrease in the expression of energy expenditure genes would be expected in fasting. Overexpression of UCP2 and UCP3 genes in the muscles following fasting may reflect increased thermogenesis to prevent a drop in body temperature. There may also be a regulation in response to fasting-induced changes in certain parameters (flow of free fatty acids, level of superoxide ions). A lipid-rich diet stimulates the expression of the UCP2 gene in the adipose tissue of mice, particularly in obesity-resistant strains (Fleury *et al.* 1997; Boss *et al.* 2000). This suggests that the UCP2 gene plays a part in food-induced thermogenesis. It has been shown that the levels of UCP2 and UCP3 mRNAs increase in the skeletal muscles of rodents with artificially increased levels of plasma free fatty acids (Weigle *et al.* 1998). This indicates that free fatty acids have an effect since this treatment greatly increases their use by the muscles. After physical training, the expression of UCP2 and UCP3 mRNAs decreases in the muscles (Boss *et al.* 2000). These results can be interpreted as indicating a regulation induced by increases in body temperature and in metabolism (and hence temperature) of the muscle cells. Preliminary results indicate that injection of pyretic substances into rats is accompanied by the induction of UCP3 mRNA (B. Lowell, personal communication), or the UCP2 protein (C. Pecqueur, D. Ricquier & B. Miroux, unpublished data), suggesting that UCP3 and UCP2 mediate fever. All physiological situations involving notable changes in energy balance (fasting, overeating, physical exercise, exposure to cold, infection) alter the expression of the UCP1, UCP2 and UCP3 genes, thus pointing to a role of UCPs in energy expenditure. Interestingly, other authors have studied the level of expression of UCP2 and UCP3 mRNAs in skeletal muscles of obese patients during prolonged hypocaloric diet and during the period of stabilization at reduced body weight. Vidal-Puig *et al.* (1999) measured a decrease in UCP3 mRNA in muscles of weight-reduced patients with obesity at stable body weight and proposed that reduced UCP3 expression could contribute to decreased energy expenditure in these individuals. Similarly, Esterbauer *et al.* (1999) also observed a decreased level of UCP3 mRNA in muscles of obese individuals submitted to a prolonged caloric restriction.

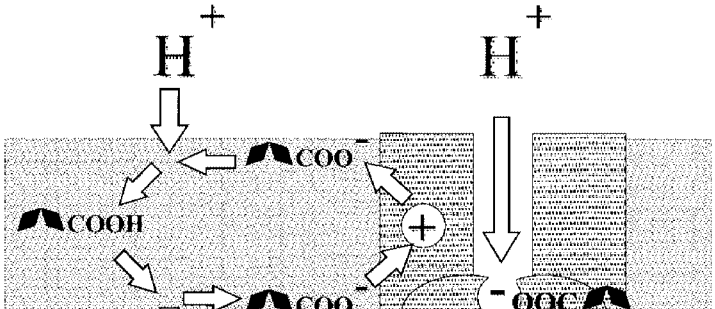

Figure 2. Schematic mechanism of proton transport by the UCPs: direct proton translocation, or proton release associated with cycling of fatty acid

The inner mitochondrial membrane is shown as the two outer stippled rectangles. The matricial side is below the membrane. The UCP is represented as the two inner striped grey rectangles and ovals corresponding to membranous domain and gating domain, respectively. The right-hand side part of the figure shows a direct transport of proton by UCP; the proton is provided by the carboxyl group of either amino acid residues or fatty acid. The left-hand side of the figure illustrates the free diffusion of protonated fatty acid through the membrane followed by release of proton on the matricial side. Then, UCP returns the anionic form of fatty acid through the membrane.

Hormonal factors modulating the expression of UCPs

Many hormones and neuromediators such as catecholamines, thyroid hormones and leptin act on metabolism and energy expenditure, and so their effects on various UCPs have been analysed. References are quoted in recent review papers (Ricquier & Bouillaud, 2000; Boss *et al.* 2000).

Transcription of the UCP1 gene is greatly activated by catecholamines, retinoids and thyroid hormones, through direct effects on brown adipocytes. The free fatty acids and the ligands of the peroxisome proliferator-activated receptors (PPARs) stimulate the transcription of the UCP1 and UCP2 genes in cultured adipocytes. The administration of thyroid hormones to rats strongly induces the expression of UCP3 mRNA in the skeletal muscles, and T3 induces UCP2 mRNA in rat heart. Corticosterone lowers UCP1 levels in brown adipose tissue. Chronic administration of leptin to rats raises expression of UCP2 mRNA 6-fold in adipose tissue and 10-fold in the islets of Langerhans, in which expression of fatty acid oxidation enzymes is strongly stimulated. Leptin increases the expression of UCP1 mRNA in brown adipose tissue, UCP2 mRNA in the liver and white adipose tissue, and UCP3 mRNA in the muscles of rats and *ob/ob* mice. Leptin's induction of various UCPs is probably secondary to its central action and depends on its effects on food intake, and could explain its thermogenic effects. Lastly, the administration of lipopolysaccharides to rats or mice greatly raises levels of UCP3 mRNA in the muscles, and UCP2 mRNA in the liver, muscles and adipose tissue.

Most hormones known to control energy metabolism therefore modulate the expression of UCPs, in accord with a role of UCP genes in energy balance.

The UCP2/UCP3 locus is linked to basal metabolism

Analysis of anonymous markers located near the human UCP2–UCP3 genes shows that this locus is very strongly linked ($P < 0{\cdot}00002$) to resting metabolic rate (Bouchard *et al.* 1997). These findings suggest that this locus plays a part in energy expenditure, but do not allow identification of the gene(s) concerned. Given that it is mainly expressed in muscles, whose bulk is considerable, the UCP3 gene may be the most important in terms of basal metabolism. However, the recent isolation of mice null for the UCP3 gene does not support this hypothesis. The UCP3 −/− mice have normal basal oxygen consumption and body temperature although their mitochondria exhibit a decreased proton leak (Vidal-Puig *et al.* 2000; Gong *et al.* 2000).

UCPs and obesity

Energy expenditure is an important factor in the regulation of body weight. Various studies have shown that low thermogenesis of the brown adipose tissue of rodents is associated with weight gain and fattening. Overexpression of UCP1 mRNA in brown adipose tissue and UCP2 mRNA in white adipose tissue, in A/J mice, which are resistant to fattening induced by a lipid-rich diet, suggests that these two UCPs play a role in nutritional thermogenesis and resistance to obesity (Ricquier & Bouillaud, 2000; Boss *et al.* 2000). Studies of humans by the groups of E. Jécquier, E. Ravussin, R. Leibel and J. Roberts have shown that a decrease in postprandial thermogenesis is associated with certain types of obesity, and that a decrease in thermogenesis is one predictive feature of weight gain (Jequier, 1983; Ravussin *et al.* 1988; Roberts *et al.* 1988). The identification of the potentially thermogenic UCP1, UCP2 and UCP3 has stimulated research into how their expression is altered in obese animals and humans, and studies of association and linkage between candidate genes and obesity-related phenotypes are also under way.

Assays of UCP2 and UCP3 mRNAs in human adipose tissue and skeletal muscle shows that there is a positive correlation between the UCP2 mRNA levels in adipose tissue and the body mass index (ratio between weight and height squared) used to assess obesity (Millet *et al.* 1997). Obesity is therefore accompanied by overexpression of UCP2 mRNA in adipose tissue as has been described in rodents. Assay of UCP2 in human skeletal muscle confirms these findings. However, in other studies, no difference in levels of UCP2 mRNA and UCP3 mRNA was observed between lean and obese subjects (Vidal-Puig *et al.* 1999; Esterbauer *et al.* 1999). In humans on a low-calorie diet for 5 days, UCP2 mRNA levels increase 2-fold in adipose tissue and muscle, as do UCP3 mRNA levels in skeletal muscle, in lean and obese subjects alike (Millet *et al.* 1997). Taken together, these results do not point to a role of the UCP2 and UCP3 genes in determining human obesity. This conclusion is strongly supported by the absence of obesity of UCP3 −/− mice (Vidal-Puig *et al.* 2000; Gong *et al.* 2000), and UCP2 −/− mice (laboratories of D. Ricquier & S. Collins, unpublished data).

Human UCP1 gene polymorphism has been associated with fattening and weight gain, and with resistance to weight loss despite a low-calorie diet (Oppert *et al.* 1994; Fumeron *et al.* 1996; Clément *et al.* 1996). Data on UCP2 gene polymorphism are less clear. Calculation of the frequency of several gene variants revealed no very significant association in patients presenting morbid obesity often associated with type 2 diabetes, except for a weak association with nocturnal energy expenditure in obese Pima Indians (review in Ricquier & Bouillaud, 2000). In contrast, another ongoing study suggests that one of the variants of the UCP2 gene is associated with obesity, this association not being observed in type 2 diabetics. Analyses of associations of certain variants of the UCP3 gene are under way. It is unclear whether the UCP2 and UCP3 genes will be shown to play a part in determining human obesity, and it is necessary to wait for data from analyses of the variants of promoters of these genes. It is also important to study the relation to metabolic parameters such as food-induced energy expenditure and gain in body fat. The most striking result from genetic studies is that there is a strong link between the UCP2/UCP3 locus and human basal metabolism.

Conclusions and prospects: UCPs, anti-obesity drugs, control of reactive oxygen species

Most cells seem to have one or more UCPs in their mitochondria. Further investigation is needed to define the biological roles and biochemical functions of UCP2 and UCP3, which must be better characterized and also quantified in different tissues. Expression of UCP2 and UCP3 seems to be related to lipid metabolism, and the relation between free fatty acids and UCPs needs to be defined, as does any role of UCPs in the metabolism of superoxide ions. UCPs probably explain a large fraction of leakage of mitochondrial protons. By lowering the potential across the mitochondrial membrane, they activate respiration, and modulate coupling of the respiratory chains, i.e. the metabolic yield. Understanding of UCPs, notably UCP2 and UCP3, shows that they and their genes should be considered as putative targets of new drugs designed to facilitate oxidation of fat and energy expenditure. The hope is that this research will identify new drugs that activate futile cycles (energetically inefficient reduction cycles which dissipate energy), thereby lowering metabolic efficiency.

Recent data also indicate that manipulation of the mitochondrial UCPs will be of interest for controlling the level of reactive oxygen species in pathological situations. This statement is based upon the fact that any change in the level of coupling of mitochondrial respiration to ADP phosphorylation has an effect on the production of free radicals and the cellular level of reactive oxygen species (Skulachev, 1998; Nicholls & Budd, 2000). Nègre-Salvayre *et al.* have demonstrated that the inhibition of UCP1 activated the formation of reactive oxygen species in brown fat mitochondria (Nègre-Salvayre *et al.* 1997). These authors and others suggested a role for UCP2 in the prevention of free radical formation (Cortez-Pinto *et al.* 1999; Lee *et al.* 1999). Interestingly, skeletal muscle of mice null for the UCP3 gene exhibit a higher level of reactive oxygen species (Vidal-Puig *et al.* 2000). A higher level of reactive oxygen species was also measured in certain cells of UCP2-null mutant mice (D. Richard, S. Collins & D. Ricquier, unpublished data). Since changes in oxidative metabolism in cells and tissues provoke marked changes in free radical synthesis, the novel UCPs could represent important mechanisms of control and limitation of these ions which have deleterious effects on cellular machinery.

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