The Biology of Mitochondrial Uncoupling Proteins

Sophie Rousset, Marie-Clotilde Alves-Guerra, Julien Mozo, Bruno Miroux, Anne-Marie Cassard-Doulcier, Frédéric Bouillaud, and Daniel Ricquier

Uncoupling proteins (UCPs) are mitochondrial transporters present in the inner membrane of mitochondria. They are found in all mammals and in plants. They belong to the family of anion mitochondrial carriers including adenine nucleotide transporters. The term "uncoupling protein" was originally used for UCP1, which is uniquely present in mitochondria of brown adipocytes, the thermogenic cells that maintain body temperature in small rodents. In these cells, UCP1 acts as a proton carrier activated by free fatty acids and creates a shunt between complexes of the respiratory chain and ATP synthase. Activation of UCP1 enhances respiration, and the uncoupling process results in a futile cycle and dissipation of oxidation energy as heat. UCP2 is ubiquitous and highly expressed in the lymphoid system, macrophages, and pancreatic islets. UCP3 is mainly expressed in skeletal muscles. In comparison to the established uncoupling and thermogenic activities of UCP1, UCP2 and UCP3 appear to be involved in the limitation of free radical levels in cells rather than in physiological uncoupling and thermogenesis. Moreover, UCP2 is a regulator of insulin secretion and UCP3 is involved in fatty acid metabolism.
***Diabetes* 53 (Suppl. 1):S130–S135, 2004**

Mitochondria are the cellular organelles where respiration occurs. They contain two compartments bounded by inner and outer membranes. The outer membrane is permeable to small metabolites, whereas the permeability of the inner membrane is controlled to maintain the high electrochemical gradient created by the mitochondrial respiratory chain that is necessary for energy conservation and ATP synthesis in mitochondria. The inner membrane transports anion substrates such as ADP, ATP, phosphate, oxoglutarate, citrate, glutamate, and malate. The reactions of the citric acid cycle, fatty acid oxidation, and several steps of urea synthesis and gluconeogenesis also take place in mitochondria. Energy produced by mitochondrial respiration is used for ATP synthesis by a complex mechanism referred to as "oxidative phosphorylation." In addition to oxidative phosphorylation and metabolic pathways, mitochondria are involved in thermogenesis, radical production, calcium homeostasis, protein synthesis, and apoptosis. Although respiration is coupled with ADP phosphorylation, this coupling is less than perfect and may be partially or very partially loose. The uncoupling proteins (UCPs) are particular mitochondrial transporters of the inner membrane that appear to be controlling the level of respiration coupling. Several reviews devoted to UCPs have been published in the last few years (1–14). This article is an attempt to summarize recognized as well as putative biological functions of the UCPs.

From the Centre National de la Recherche Scientifique, Unité Propre de Recherche 9078, Faculté de Médecine and Institut de Recherches Necker-Enfants Malades (IRNEM), Paris, France.

Address correspondence and reprint requests to Dr. D. Ricquier, Faculté de Médecine, Necker-Enfants Malades, CNRS UPR 9078, 7ème étage, 75730 Paris, Cedex 15, France. E-mail: ricquier@necker.fr.

Received for publication 13 March 2003 and accepted 2 April 2003.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Les Laboratoires Servier.

ROS, reactive oxygen species; UCP, uncoupling protein.

BIOLOGY OF RESPIRATION UNCOUPLING

It has long been known that respiration and mitochondrial ATP synthesis are coupled. The observation that decreased ATP utilization inhibited oxygen consumption and that respiration rate increased when mitochondria synthesized more ATP led to the concept of respiratory control by ADP phosphorylation. In fact, there is a link between mitochondrial ATP synthesis and cellular ATP demand by a feedback mechanism controlling ATP synthesis induced by mitochondrial respiration. After the seminal proposal made by Peter Mitchell (chemi-osmotic theory), it was demonstrated that the mitochondrial electrochemical proton gradient, generated as electrons are passed down the respiratory chain, is the primary source for cellular ATP synthesis. The mitochondrial respiratory chain is made of five complexes. Complexes I, III, and IV pump protons outside the inner membrane during reoxidation of coenzymes and generate a proton gradient that is consumed by complex V, which catalyzes ATP synthesis (Fig. 1). In addition to reentry of protons through ATP synthase, a proton leak represents another mechanism consuming the mitochondrial proton gradient. Mitchell's theory predicted that any proton leak not coupled with ATP synthesis would provoke uncoupling of respiration and thermogenesis. A well-known example of such an uncoupling of respiration to ADP phosphorylation is represented by the mitochondrial uncoupling protein of brown adipocytes (UCP1), which dissipates energy of substrate oxidation as heat (15–18). Besides adaptive thermogenesis, uncoupling of respiration allows continuous reoxidation of coenzymes that are essential to metabolic pathways. In fact, partial uncoupling of respiration prevents an exaggerated increase in ATP level that would inhibit respiration.

UNCOUPLING PROTEINS

History. Morphologists and physiologists identified the brown adipose tissue as a particular form of adipose tissue in hibernators and small mammals and observed its thermogenic activity in infants at birth, rodents exposed to the

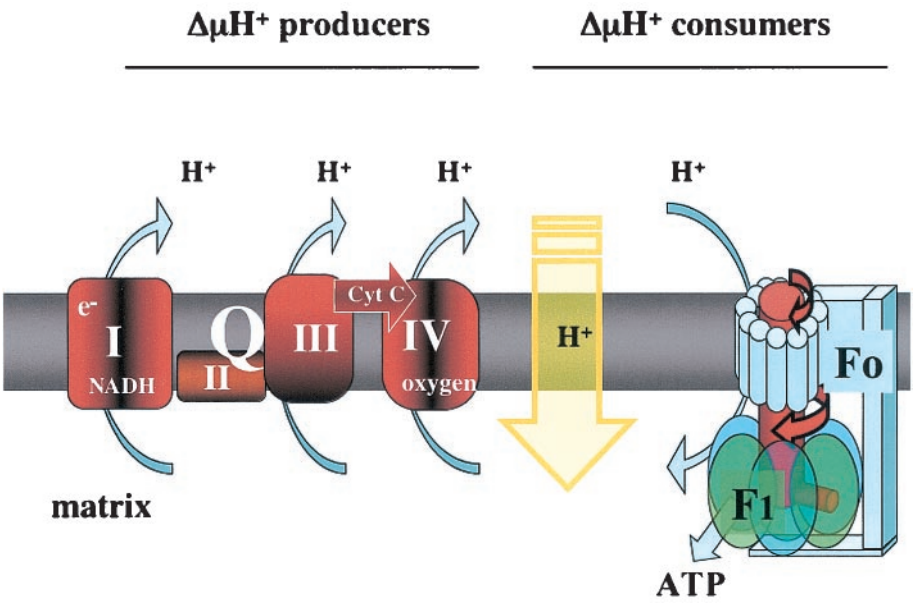

FIG. 1. The mitochondrial proton gradient generated by complexes of respiratory chain is used by F_0-F_1-ATP synthase to phosphorylate ADP. Another mechanism consuming the gradient and lowering ATP synthesis is proton leak (yellow arrow). The reentry of protons in the matrix noncoupled with ATP synthesis is an energy-dissipating mechanism. The brown fat UCP1 is an example of mitochondrial proton leak. Cyt C, cytochrome C; $\Delta\mu H^+$, proton electrochemical gradient; e^-, electron; F_0, membranous part of ATP-synthase; F_1, catalytic part of ATP-synthase.

cold, and hibernators during arousal (15–17). Brown adipocytes differ from white adipocytes by a direct sympathetic innervation, a central nucleus, many triglyceride droplets, and numerous mitochondria. Original studies of isolated brown fat mitochondria revealed an elevated respiratory rate and an uncoupled respiration not controlled by ADP. A rapid respiration not coupled with ATP synthesis represents a powerful thermogenic process. It was also established that activation of brown adipocytes by norepinephrine was immediately followed by increased respiration and heat production, a marked increase in blood flow, and evacuation of warmed blood toward the brain and cardiac regions. It appeared that fatty acids generated by stimulated lipolysis were directly activating a specific proton pathway not coupled with ADP phosphorylation in the inner mitochondrial membrane. The protein explaining this proton pathway was identified as a 33-kDa UCP (15–18). Brown fat mitochondrial UCP is unique to brown adipocytes. The UCP content reflects the thermogenic activity of brown fat deposits: the elevated thermogenic capacity of brown fat of rats adapted to cold parallels the increased UCP in mitochondria. Decrease in brown fat thermogenic capacity during postnatal development in many mammals is accompanied by a declining UCP content. The brown fat UCP belongs to the family of the anion carriers present in the inner membrane of mitochondria. Like the mitochondrial adenine nucleotide transporters, the phosphate carrier, or the citrate carrier, UCP has a triplicate structure and every third is made of two transmembrane domains attached by a more hydrophilic domain (Fig. 2).

UCP2, UCP3, avian UCP, plant UCPs, and other proteins related to UCP1. More recently, cDNAs encoding homologues of the brown fat UCP were isolated and named UCP2 and UCP3, whereas the brown adipocyte UCP was renamed UCP1. UCP2 and UCP3 share, respectively, 72 and 57% amino acid identity toward UCP1 (19–23). UCP2 and UCP3 are adjacent genes on human chromosome 11 and mouse chromosome 7. Whereas UCP1 is only detected in brown adipocytes, UCP2 is present in many organs and cell types, and UCP3 is predominantly expressed in skeletal muscle. The tissue distribution of these novel UCPs suggested a role other than adaptive thermogenesis. Immediately after the discovery of UCP2 and UCP3, a plant UCP cDNA was isolated from potato (24). This finding followed functional data suggesting the presence of UCPs in plants, fungi, and protozoa. The identification of a plant UCP indicated that the UCPs form an ancient and conserved family. A UCP was also identified in chicken skeletal muscle (25). Another putative mitochondrial carrier more distant from the UCPs was identified and, maybe clumsily, named UCP4. A brain mitochondrial carrier referred to as BMCP1 was also renamed UCP5 by others. Ledesma et al. (14) listed 45 genes coding for members of the UCP family and proposed a classification in six families. The following description of the roles of the UCPs will only refer to mammalian UCP1, UCP2, and UCP3 (Table 1).

ESTABLISHED BIOLOGICAL ROLES OF THE UCPs

UCP1, a mitochondrial membrane transporter essential to nonshivering thermogenesis and control of body temperature. Until the discovery of UCP2, UCP3 and a plant UCP in 1997, the brown fat UCP (UCP1),

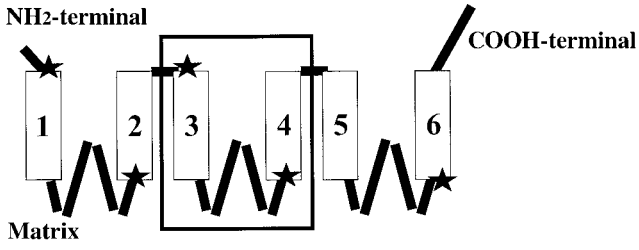

FIG. 2. Model for UCPs and anion carriers of the mitochondrial inner membrane. The protein is made of six transmembrane domains (numbers 1–6) linked by hydrophilic segments. The UCPs and mitochondrial anion transporters have a triplicated structure, with every third (the central third is framed) being made of two α-helices and a polar domain. Stars indicate position of extremities of transmembrane domains of UCP1 identified using specific antibodies (work performed in the laboratory of the authors [3,5]). The three UCPs and all anion transporters of the inner mitochondrial membrane share the same organization.

TABLE 1
Roles attributed to the different UCPs according to experimental methods

Method	Demonstrated or proposed roles		
	UCP1	UCP2	UCP3
Liposome	Proton leak	Proton leak	Proton leak
Yeast	Proton leak	Proton leak	Proton leak
Transgenic mice	Respiration uncoupling	Not determined	Proton leak or not Increased oxidation
–/– mice	Respiration uncoupling Thermogenesis	ATP increase or not ROS scavenging Insulin secretion	Respiration uncoupling or not ROS scavenging
Normal animals	Cold-induced thermogenesis	ROS scavenging Insulin secretion Diet-induced thermogenesis Fatty acid oxidation	ROS scavenging T3 thermogenesis Diet-induced thermogenesis Fatty acid oxidation
Human genetics	Body fat content	Resting metabolic rate Anorexia nervosa	Resting metabolics rate Body fat content Anorexia nervosa

Transgenic mice refer to animals overexpressing UCP. Most biochemical studies supported a proton transport activity and a potential uncoupling activity of the UCPs. However, contrary to UCP1, analysis of normal mice or mice made null for *Ucp2* or *Ucp3* did not confirm the respiration uncoupling activity of UCP2 or UCP3.

represented a very specific type of protein. UCP1 is uniquely present in brown adipocytes, and its function is to create a fatty acid–activated uncoupling of respiration. UCP1 is expressed at a very high level in brown adipocytes, where it may account for up to 4% of total protein and 8% of mitochondrial protein. The reason why UCP1 is present at a high level is unknown, but it suggests that a rapid and full uncoupling of respiration leading to a marked thermogenesis requires a large number of UCP1 molecules, with the activity of each molecule probably being weak. Physiological, pharmacological, biochemical, and genetic studies established the role of UCP1 in uncoupling of respiration and adaptive thermogenesis. Cold exposure of rodents is the most illustrative way of UCP1 induction. This depends on many hormones such as the thyroid hormones, but many studies based on the use of drugs activating β_3-adrenoceptors and other adrenoceptors confirmed that the sympathetic nervous system was the main trigger of UCP1 activation and induction (15–17). Either reconstitution studies of UCP1 activity into liposomes or ectopic expression of UCP1 in yeast, mammalian cells, or transgenic animals confirmed the respiration uncoupling activity of UCP1 (1,18,26,27). Finally, interruption of the murine *Ucp1* gene using homologous recombination proved that UCP1 was the true effector of adaptive thermogenesis in mice exposed to cold (28).

The uncoupling activity of UCP1 is explained by its ability to transport protons in particular when fatty acids bind to the protein. The question of the catalytic activity of UCP1 is still debated between those who believe that UCP1 is a pure proton transporter activated by fatty acids, and others who consider that UCP1 mediates fatty acid-induced uncoupling by trans-bilayer movement (flip-flop) of the protonated fatty acid from the cytosolic to the matrix face of the inner membrane, with subsequent return of the anionic form to the cytosolic side (1,14,18).

UCP2 and UCP3 differ from UCP1: they do not contribute to adaptive thermogenesis but may be involved in the resting metabolic rate. The cloning of cDNAs potentially encoding proteins homologous to the brown fat UCP prompted their discoverers to name them UCP2 and UCP3. This terminology may have been clumsy because it was implied that UCP2 and UCP3 were functionally similar to the brown fat UCP1, both in terms of uncoupling of respiration and regulatory thermogenesis. In agreement with such assumptions, the proton transport activity of UCP2 or UCP3 was observed during reconstitution experiments (29). Moreover, UCP2 and UCP3 expressed in yeast or mammalian cells can uncouple respiration from ATP synthesis (5,14,30). However, such an uncoupling was generally obtained when UCP2 or UCP3 was expressed at a much higher level than that measured in tissues. Also, uncoupling was not inhibited by purine nucleotides as it is for UCP1. Although these data were in favor of an uncoupling activity of these novel UCPs, mice made null either for *Ucp2* or *Ucp3* maintain their body temperature in a cold environment. Therefore, unlike UCP1, UCP2 and UCP3 are not involved in cold-induced thermogenesis. However, the possibility that these novel UCPs participate in basal thermogenesis was supported by genetic studies of humans showing that the polymorphism of anonymous markers encompassing the UCP2-UCP3 locus was strongly genetically linked to the resting metabolic rate (31). Similarly, polymorphisms in the coding region of the *Ucp2* gene were calculated to be associated with the level of energy expenditure during sleep (32). These data may be connected to the fact that a basal proton leak in the mitochondrial inner membrane is associated with the resting metabolic rate in most tissues (33).

UCP2 and UCP3 limit the level of reactive oxygen species. Respiration is associated with production of reactive oxygen species (ROS) because the oxygen molecule is capable of accepting an additional electron to create the superoxide ion, a more reactive form of oxygen (34). Mitochondria can produce a large part of the total ROS made in cells. This is associated with the activity of respiratory complexes I and III and with ubisemiquinone generated in the course of electron transport reactions in the respiratory chain. It is known that mild uncoupling of respiration diminishes mitochondrial ROS formation by complexes I and III. The explanation for the control of

ROS production by respiration uncoupling is that ROS formation depends on the mitochondrial proton gradient and the mitochondrial potential. In particular, Skulachev (35) postulated that fatty acids may prevent an increase in the mitochondrial electrochemical gradient and thus decrease ROS generation. In other words, a mild uncoupling of respiration may participate in antioxidant defense and the UCPs may be the effectors of such a defense mechanism.

In agreement with such a proposal, the first reports of *Ucp3* or *Ucp2* knockout mice referred to stimulated production of ROS. Vidal-Puig et al. (36) observed elevated production of ROS in skeletal muscle of $Ucp3^{-/-}$ mice. When Arsenijevic et al. (37) isolated $Ucp2^{-/-}$ mice, they were unable to observe the expected increase in body fat content (19). Knowing that UCP2 was expressed at a high level in macrophages, they tested the ability of macrophages from the null mice to destroy parasites and observed that the deletion of the *Ucp2* gene markedly enhanced the microbicidal activity of the macrophages, with the enhanced activity of the macrophages being associated with an elevated level of ROS. These data, confirmed by other studies, supported the existence of uncoupling activity in UCP2, defining it as a protein devoted to the limitation of ROS (2). Whether the hyperactivity of macrophages of $Ucp2^{-/-}$ mice indicates that UCP2 plays a role in natural innate immunity remains to be investigated.

A role for UCP2 in the control of insulin secretion. In the original description of UCP2, it was mentioned that UCP2 mRNA was present in the pancreas and that the *Ucp2* gene was linked to hyperinsulinemia (19). The expression of the *Ucp2* gene was verified in pancreatic islets and pancreatic β-cells. Because it is known that the ATP/ADP ratio controls insulin secretion, it may be postulated that inactivation of a mitochondrial uncoupler would increase this ratio and activate glucose-induced insulin secretion. In agreement with this proposal, it was observed that overexpression of UCP2 in pancreatic cells blunted glucose-induced insulin secretion (38). In addition, Zhang et al. (39) measured an increased ATP level and a net increase in glucose-induced insulin secretion in islets of $Ucp2^{-/-}$ mice. These data confirmed the uncoupling activity of UCP2 and established its role as a negative regulator of insulin secretion.

UCP2 and UCP3 regulate fatty acid metabolism. UCP2 and UCP3 are expressed in several cell types participating in intermediary metabolism and in particular in fatty acid metabolism: adipose cells, skeletal muscle, and macrophages. Numerous reports have described significant changes in the expression of UCP2 and/or UCP3 in situations known to modify the level of free fatty acid in blood or the intensity of fatty acid oxidation, such as starvation, high-lipid diet, lipid infusion, and short- or long-term exercise. Certain data, such as the induction of UCP3 mRNA in skeletal muscle of animals or humans during starvation, are not consonant with an uncoupling activity and energy-dissipating activity of this novel UCP, because it is difficult to understand why organisms should recruit an energy-dissipating system during starvation. The paradigm of the induction of an uncoupler during starvation was strongly debated. It was suggested that the function of UCP2 and UCP3 is to export fatty acid anions outside of the mitochondrial matrix when a large excess of fatty acids is inside mitochondria (40). Actually, this is in agreement with the hypothesis of Garlid and Jaburek (1) that all UCPs function as fatty acid anion transporters after the spontaneous entry of protonated fatty acid in the matrix.

Spectacular data were obtained from analysis of transgenic mice overexpressing UCP3 in their skeletal muscle (41). These mice are lean and resist diet-induced obesity and diabetes. Whether these effects directly resulted from activation of the respiration uncoupling was not completely demonstrated. However, the conclusion from this study was that UCP3, expressed at high level in skeletal muscle, constitutes an energy-dissipating mechanism and a target for anti-obesity compounds. In agreement with such a conclusion, Harper et al. (40) measured a decreased mitochondrial proton leak associated with reduced UCP3 level in the skeletal muscle of obese diet-resistant women. These data, as well as genetic studies (42), strongly suggested that UCP3 may be involved in fat oxidation and the regulation of fat content.

PUTATIVE ROLES FOR UCPs

Thyroid hormone–induced thermogenesis and catabolism. Given that the novel UCPs contribute to the basal proton conductance of mitochondria, and knowing that such a basal conductance is positively regulated by thyroid hormones, several investigators have analyzed the effect of thyroid status on the expression level of UCP2 and UCP3. It appeared that UCP3 expression was significantly activated in hyperthyroid animals, and De Lange et al. (43) proposed that this UCP was an effector of mitochondrial thermogenesis in skeletal muscle. Conflicting data were obtained regarding the control of expression of UCP2 or UCP3 by cytokines or endotoxins and their implication in fever. In particular, an elevation of muscle UCP3 mRNA level was observed in patients with gastrointestinal adenocarcinoma; such an elevation may enhance energy expenditure and contribute to tissue catabolism (44).

Protection against free radicals and degenerative processes: a protective role of UCP2 in atherosclerosis. Several independent studies supported a role for the UCPs in defense against oxidants (2,36,37,45). The *Ucp2* gene is induced in apoptosis-sensitive lymphocytes after irradiation (46), in HeLa cells during oncosis (47), in spinal cord of mice during experimental autoimmune encephalitis (48), and in the salivary glands of a mouse model of Sjögren's syndrome (49). Whether such effects are mediated by net uncoupling activity of UCP2 or by its ability to downregulate ROS and radicals is unknown. Because UCP2 is present in cells such as macrophages, which are important for the development of atherosclerosis, and because ROS are involved in plaque formation and arterial inflammation, the influence of UCP2 on the development of atherosclerosis was tested in mice. Irradiated, LDL receptor–deficient mice were transplanted with bone marrow from either $Ucp2^{-/-}$ mice or $Ucp2^{+/+}$ mice before being fed an atherogenic diet. Using this procedure, Blanc et al. (50) observed that $Ucp2^{-/-}$ transplanted mice compared with control $Ucp2^{+/+}$ transplanted mice exhibited a marked increase in atherosclerotic lesion size in thoracic aorta as well as in aortic sinus. These data suggest that

UCP2 protects against atherosclerosis, with such an effect being associated with ROS limitation in cells.

CONCLUSION AND PERSPECTIVES

The UCPs form a small family of proteins belonging to the large family of the anion mitochondrial transporters. UCP1 is well characterized: it works as a regulated uncoupler in brown adipocytes and controls heat production. UCP2 and UCP3 are probably ancestors of UCP1, and their functions are not yet entirely understood. It may be proposed that the ancestral functions of the UCPs were not to uncouple respiration strongly, as does UCP1, but rather to facilitate adaptation of cells to oxygen molecules through a mild uncoupling of respiration, thus restricting production of ROS. UCP1 may be the only UCP inducing a marked uncoupling of respiration dedicated to regulatory thermogenesis. UCP2 appears to be involved in limitation of ROS in macrophages and other cells and may be important for resistance to degenerative processes. A possible role for UCP2 in neuroprotection warrants investigation because a protective role for UCP2 in brain injury was described in transgenic mice overexpressing UCP2 (51,52). The analysis of microsatellite markers at the UCP2/UCP3 locus on human chromosome g11 revealing a link to anorexia nervosa is intriguing (53). UCP2 and UCP3 participate in metabolic regulation. UCP2 downregulates insulin secretion and may control fat deposition. UCP3 is probably involved in fatty acid metabolism in skeletal muscle, but this remains to be clarified (54). A particular point is the question of natural ligands and regulators of the different UCPs. Nucleotides, fatty acids, quinones, and superoxide may inhibit or activate certain UCPs, but this is debated (18,55,56). It is certainly necessary to evaluate the physiological importance of these regulators. Finally, the exact functions of the novel UCPs will be clarified when their precise transport activities are elucidated.

ACKNOWLEDGMENTS

Our research was supported by the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, the Institut de Recherche Servier, the Ministère de la Recherche, and the Association de Recherche sur le Cancer. M.-C.A.-G. was supported by a grant from GlaxoSmithKline, and J.M. was supported by a grant from Servier and the Ministry of Industry.

REFERENCES

1. Garlid KD, Jaburek M: The mechanism of proton transport mediated by mitochondrial uncoupling proteins. *FEBS Lett* 438:10–14, 1998
2. Diehl AM, Hoek JB: Mitochondrial uncoupling: role of uncoupling protein anion carriers and relationship to thermogenesis and weight control "The benefits of losing control." *J Bioenerg Biomembr* 31:493–505, 1999
3. Ricquier D, Miroux B, Cassard-Doulcier AM, Lévi-Meyrueis C, Gelly C, Raimbault S, Bouillaud F: Contribution to the identification and analysis of the mitochondrial uncoupling proteins. *J Bioenerg Biomembr* 31:407–418, 1999
4. Kozak LP, Harper ME: Mitochondrial uncoupling proteins in energy expenditure. *Annu Rev Nutr* 20:339–363, 2000
5. Ricquier D, Bouillaud F: The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *Biochem J* 345:161–179, 2000
6. Boss O, Hagen T, Lowell BB: Uncoupling proteins 2 and 3: potential regulators of mitochondrial energy metabolism. *Diabetes* 49:143–156, 2000
7. Dulloo AG, Samec S: Uncoupling proteins: do they have a role in body weight regulation? *News Physiol Sci* 15:313–318, 2000
8. Argiles JM, Busquets S, Lopez-Soriano FJ: The role of uncoupling proteins in pathophysiological states. *Biochem Biophys Res Commun* 293:1145–1152, 2002
9. Schrauwen P, Hesselink M: UCP2 and UCP3 in muscle controlling body metabolism. *J Exp Biol* 205:2275–2285, 2002
10. Jezek P: Possible physiological roles of mitochondrial uncoupling proteins-UCPn. *Int J Biochem Cell Biol* 34:1190–1206, 2002
11. Erlanson-Albertsson C: Uncoupling proteins: a new family of proteins with unknown function. *Nutr Neurosci* 5:1–11, 2002
12. Argyropoulos G, Harper ME: Uncoupling proteins and thermoregulation. *J Appl Physiol* 92:2187–2198, 2002
13. Nedergaard J, Cannon B: Pros and cons for suggested functions. *Exp Physiol* 88:65–84, 2003
14. Ledesma A, Garcia de Lacoba M, Rial E: The mitochondrial uncoupling proteins [article online]. *Genome Biology*. Available from http://genomebiology.com/home/. Accessed 29 November 2002
15. Nicholls DG, Locke RM: Thermogenic mechanisms in brown fat. *Physiol Rev* 64:1–64, 1984
16. Cannon B, Nedergaard J: The biochemistry of an inefficient tissue: brown adipose tissue. *Essays Biochem* 20:110–164, 1985
17. Himms-Hagen J, Ricquier D: Brown adipose tissue. In *Handbook of Obesity*. Bray G, Bouchard C, James W, Eds. New York, Marcel Dekker, 1998, p. 415–441
18. Klingenberg M, Echtay KS: Uncoupling proteins: the issues from a biochemist's point of view. *Biochim Biophys Acta* 1504:128–143, 2001
19. Fleury C, Neverova S, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouillaud F, Seldin M, Surwitt R, Ricquier D, Warden C: Uncoupling-protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat Genet* 15:269–272, 1997
20. Gimeno RE, Dembski M, Weng X, Deng NH, Shyjan AW, Gimeno CJ, Iris F, Ellis SJ, Woolf F, Tartaglia LA: Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. *Diabetes* 46:900–906, 1997
21. Vidal-Puig A, Solanes G, Grujic D, Flier JS, Lowell BB: UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. *Biochem Biophys Res Commun* 235:79–82, 1997
22. Boss O, Samec S, Paoloni-Giacobino A, Rossier C, Dulloo A, Seydoux J, Muzzin P, Giacobino JP: Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett* 408:39–42, 1997
23. Gong DW, He Y, Karas M, Reitman M: Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin. *J Biol Chem* 272:24129–24132, 1997
24. Laloi M, Klein M, Riesmeier JW, MullerRober B, Fleury C, Bouillaud F, Ricquier D: A plant cold-induced uncoupling protein. *Nature* 389:135–136, 1997
25. Raimbault S, Dridi S, Denjean F, Lachuer J, Couplan E, Bouillaud F, Bordas F, Duchamp C, Taouis M, Ricquier D: An uncoupling protein homologue putatively involved in facultative muscle thermogenesis in birds. *Biochem J* 353:441–444, 2001
26. Li B, Nolte LA, Ju JS, Han DH, Coleman T, Holloszy JO, Semenkovich CF: Skeletal muscle respiratory uncoupling prevents diet-induced obesity and insulin resistance in mice. *Nat Med* 6:1115–1120, 2000
27. Couplan E, Gelly C, Goubern M, Fleury C, Quesson B, Silberberg M, Thiaudiere E, Mateo P, Lonchampt M, Levens N, de Montrion C, Ortmann S, Klaus S, Gonzalez-Barroso MD, Cassard-Doulcier A-M, Ricquier D, Bigard X, Diolez P, Bouillaud F: High level of UCP1 expression in muscle of transgenic mice selectively affects muscles at rest and decreases their IIb fiber content. *J Biol Chem* 277:43079–43088, 2002
28. Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper E, Kozak LP: Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 387:90–94, 1997
29. Jaburek M, Varecha M, Gimeno RE, Dembski M, Jezek P, Tartaglia LA, Garlid KD: Transport function and regulation of mitochondrial uncoupling proteins 2 and 3. *J Biol Chem* 274:26003–26007, 1999
30. Rial E, Gonzalez-Barroso M, Fleury C, Iturrizaga J, Jimenez-Jimenez J, Ricquier D, Goubern M, Bouillaud F: Retinoids activate proton transport by the uncoupling proteins UCP1 and UCP2. *EMBO J* 18:5827–5833, 1999
31. Bouchard C, Pérusse L, Chagnon YC, Warden C, Ricquier D: Linkage between markers in the vicinity of the uncoupling protein 2 gene and resting metabolic rate in humans. *Hum Mol Genet* 6:1887–1889, 1997
32. Walder K, Norman RA, Hanson RL, Schrauwen P, Neverova M, Jenkinson CP, Easlick J, Warden CH, Pecqueur C, Raimbault S, Ricquier D, Silver MHK, Shuldiner AR, Solanes G, Lowell BB, Chung WK, Leibel RL, Pratley R, Ravussin E: Association between uncoupling protein polymorphisms

(UCP2-UCP3) and energy metabolism/obesity in Pima Indians. *Hum Mol Genet* 7:1431–1435, 1998
33. Stuart JA, Cadenas S, Jekabsons MB, Roussel D, Brand MD: Mitochondrial proton leak and the uncoupling protein 1 homologues. *Biochim Biophys Acta* 504:144–158, 2001
34. Raha S, Robinson BH: Mitochondria, oxygen free radicals, disease and ageing. *TIBS* 25:502–508, 2000
35. Skulachev VP: Uncoupling: new approaches to an old problem of bioenergetics. *Biochim Biophys Acta* 1363:100–124, 1998
36. Vidal-Puig AJ, Grujic D, Zhang CY, Hagen T, Boss O, Ido Y, Szczepanik A, Wade J, Mootha V, Cortright R, Muoio DM, Lowell BB: Energy metabolism in uncoupling protein 3 gene knockout mice. *J Biol Chem* 275:16258–16266, 2000
37. Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning B, Miroux B, Goubern M, Alves-Guerra MC, Couplan E, Surwit R, Bouillaud F, Richard D, Collins S, Ricquier D: Mice lacking uncoupling protein-2 survive to Toxoplasma gondii infection: a link with reactive oxygen species production and immunity. *Nat Genet* 26:435–439, 2000
38. Saleh MC, Wheeler MB, Chan CB: Uncoupling protein-2: evidence for its function as a metabolic regulator. *Diabetologia* 45:174–187, 2002
39. Zhang C, Baffy G, Perret P, Krauss S, Peroni O, Grujic D, Hagen T, Vidal-Puig AJ, Boss O, Kim Y, Zheng XX, Wheeler MB, Shulman GI, Chan CB, Lowell BB: Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. *Cell* 105:745–755, 2001
40. Harper ME, Dent R, Monemdjou S, Bezaire V, Van Wyck L, Wells G, Kavaslar GN, Gauthier A, Tesson F, McPherson R: Decreased mitochondrial proton leak and reduced expression of uncoupling protein 3 in skeletal muscle of obese diet-resistant women. *Diabetes* 51:2459–2466, 2002
41. Clapham C, Arch JR, Chapman H, Haynes A, Lister C, Moore GB, Piercy V, Carter SA, Lehner I, Smith SA, Beeley LJ, Godden RJ, Herrity N, Skehel M, Changani KK, Hockings PD, Reid DG, Squires SM, Hatcher J, Trail B, Latcham J, Rastan S, Harper AJ, Cadenas S, Buckingham JA, Brand MD: Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. *Nature* 406:415–418, 2000
42. Lanouette CM, Giacobino JP, Perusse L, Lacaille M, Yvon C, Chagnon M, Kuhne F, Bouchard C, Muzzin P, Chagnon YC: Association between uncoupling protein 3 gene and obesity-related phenotypes in the Quebec Family Study. *Mol Med* 7:433–441, 2001
43. De Lange P, Lanni A, Beneduce L, Moreno M, Lombardi A, Silvestri E, Goglia F: Uncoupling protein-3 is a molecular determinant for the regulation of resting metabolic rate by thyroid hormone. *Endocrinology* 142: 3414–3420, 2001
44. Collins P, Bing C, McGulloch P, Williams G: Muscle UCP-3 mRNA levels are elevated in weight loss associated with gastrointestinal adenocarcinoma in humans. *Br J Cancer* 86:372–375, 2002
45. Nègre-Salvayre A, Hirtz C, Carrera G, Cazenave R, Troly M, Salvayre R, Penicaud L, Casteilla L: A role for uncoupling protein-2 as a regulator of mitochondrial hydrogen peroxide generation. *FASEB J* 11:809–815, 1997
46. Voehrinher DW, Hirschberg DL, Xiao J, Lu Q, Roederer M, Lock CB, Herzenberg LA, Steinman L, Herzenberg LA: Gene microarray identification of redox and mitochondrial elements that control resistance or sensitivity to apoptosis. *Proc Natl Acad Sci U S A* 97:2680–2685, 2000
47. Mills EM, Xu D, Fergusson MM, Combs CA, Xu Y, Finkel T: Regulation of cellular oncosis by uncoupling protein 2. *J Biol Chem* 277:27385–27392, 2002
48. Ibrahim SM, Mix E, Böttcher T, Koczan D, Gold R, Rolfs A, Thiesen HJ: Gene expression profiling of the nervous system in murine experimental autoimmune encephalomyelitis. *Brain* 124:1927–1938, 2000
49. Azuma T, Takei M, Yoshikawa T, Nagasugi Y, Kato M, Otsuka M, Shiraiwa H, Sungano S, Mitamura K, Sawada S, Masuho Y, Naohiko S: Identification of candidate genes for Sjögren's syndrome using MRL/lpr mouse model of Sjögren's syndrome and cDNA microarray analysis. *Immunol Lett* 81:171–176, 2002
50. Blanc J, Alves-Guerra MC, Esposito B, Rousset S, Gourdy P, Ricquier D, Tedgui A, Miroux B, Mallat Z: Protective role of uncoupling protein 2 in atherosclerosis. *Circulation* 107:388–390, 2003
51. Bechmann I, Diano S, Warden CH, Bartfai T, Nitsch R, Horvath TL: Brain mitochondrial uncoupling protein 2 (UCP2): a protective stress signal in neuronal injury. *Biochem Pharmacol* 64:363–367, 2002
52. Horvath TL, Warden CH, Hajos M, Lombardi A, Goglia F, Diano S: Brain uncoupling protein 2: uncoupled mitochondria predict thermal synapses in homeostatic centers. *J Neurosci* 19:10417–10427, 1999
53. Hu X, Murphy F, Karwautz A, Li T, Giotakis O, Treasure J, Collier DA: Analysis of microsatellite markers at the UCP2/UCP3 locus on chromosome 11q13 in anorexia nervosa. *Mol Psychiatry* 7:814–814, 2002
54. Hesselink MKC, Greenhaff PL, Constantin-Teodosiu D, Hultma E, Saris WHM, Nieuwlaat R, Schaart G, Kornips E, Schrauwein P: Increased uncoupling protein 3 content does not affect mitochondrial function in human skeletal muscle in vivo. *J Clin Invest* 111:479–486, 2003
55. Echtay KS, Roussel D, St-Pierre J, Jekabsons MB, Cadenas S, Stuart JA, Harper JA, Roebuck SJ, Morrison A, Pickering S, Clapham JC, Brand MD: Superoxide activates mitochondrial uncoupling proteins. *Nature* 415:96–99, 2002
56. Couplan E, Gonzalez-Barroso MM, Alves-Guerra MC, Ricquier D, Goubern M, Bouillaud F: No evidence for a basal, retinoic or superoxide-induced uncoupling activity of the UCP2 present in spleen or lung mitochondria. *J Biol Chem* 277:26268–26275, 2002