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

Mitochondrial Uncoupling: Role of Uncoupling Protein Anion Carriers and Relationship to Thermogenesis and Weight Control *"The Benefits of Losing Control"*

Anna Mae Diehl[1,3] and Jan B. Hoek[2]

Uncoupling proteins, a subgroup of the mitochondrial anion transporter superfamily, have been identified in prokaryotes, plants, and mammalian cells. Evolutionary conservation of these molecules reflects their importance as regulators of two critical mitochondrial functions, i.e., ATP synthesis and the production of reactive oxygen species (ROS). Although the amino acid sequences of the three mammalian uncoupling proteins, UCP1, UCP2 and UCP3, are very similar, each homolog is the product of a unique gene and important differences have been demonstrated in their tissue-specific expression and regulation. UCP1 and UCP3 appear to be key regulators of energy expenditure, and hence, nonshivering thermogenesis, either in brown adipose tissue (UCP1) or skeletal muscle (UCP3). UCP2 is expressed more ubiquitously, although generally at low levels, in many tissues. There is conflicting evidence about its importance as a regulator of resting metabolic rate. However, evidence suggests that this homolog might modulate the mitochondrial generation of ROS in some cell types, including macrophages and hepatocytes. While the induction of various uncoupling protein homologs provides adaptive advantages, both to the organism (e.g., thermogenesis) and to individual cells (e.g., reduced ROS), increased uncoupling protein activity also increases cellular vulnerability to necrosis by compromising the mitochondrial membrane potential. This narrow "risk–benefit" margin necessitates tight control of uncoupling protein activity in order to preserve cellular viability and much remains to be learned about the regulatory mechanisms involved.

KEY WORDS: Energy expenditure; reactive oxygen species; cellular viability; apoptosis; necrosis.

INTRODUCTION

Mitochondria play a central role in regulating cellular viability because they are the major site of energy substrate oxidation, ATP production, reactive oxygen species (ROS) generation, and the activation of certain cysteine-aspartate proteases (caspases) that are involved in apoptosis (reviewed in Lemasters, 1998; Magnotte and Vayssiere, 1998). Complex mechanisms have evolved to modulate mitochondrial functions in response to fluctuations in the supply of energy substrates or to changing energy requirements (Nicholls and Fergusan, 1992). Briefly, the proton electrochemical gradient that develops across the inner mitochondrial membrane during electron transport preserves oxidative energy in a form that is trapped efficiently by F_0-F_1-ATP synthase and used to phosphorylate ADP to produce ATP. Hence, aerobic respiration is coupled to phosphorylation. Mitochondrial ATP synthesis, in turn, is matched to cellular ATP utilization (Hardie and Carling, 1997).

Uncoupling of mitochondrial electron transport chain activity from the phosphorylation of ADP dissipates the electrochemical energy that is generated during mitochondrial respiration as heat, resulting in

[1] Departments of Medicine, The Johns Hopkins University, Baltimore, Maryland 21205
[2] Departments of Pathology, Thomas Jefferson University, Philadelphia Pennsylvania 19107.
[3] Author to whom all correspondence should be sent. Email: amdiehl@welchlink.weich.jhu.edu.

0145-479X/99/1000-0493$16.00/0

thermogenesis. It has been suggested that some degree of mitochondrial uncoupling (i.e., "partial uncoupling") is physiological because it optimizes the efficiency of oxidative phosphorylation (Stucki 1980) and prevents ROS generation by the mitochondrial respiratory chain in the resting state (Skulachev, 1996b; Skulachev, 1994.). Fatty acid anions have been implicated as carriers for H^+, facilitating the net transfer of protons from the intermembrane space into the mitochondrial matrix, thereby mediating weak uncoupling (Wojtczak *et al.*. 1993; Jezek *et al.*, 1997; Korshunov *et al.*, 1998).

Evidence has been presented that supports the importance of certain inner membrane anion carrier proteins in the recycling of the deprotonated fatty acid anions from the matrix to the cytosolic side of the inner mitochondrial membrane. The ubiquitous ADP/ATP carrier, the brown adipose tissue uncoupling protein homolog, UCP1, and the plant uncoupling protein, PUMP, may facilitate mitochondrial uncoupling by mechanisms that involve interaction of the membrane proteins with fatty acids (reviewed in (Jezek *et al.*, 1998). Two recently identified, uncoupling protein homologs, UCP2 (Fleury *et al.*, 1997) and UCP3 (Gimeno *et al.*, 1997; Boss *et al.*,1997b), have been predicted to provide white adipose tissue, skeletal muscle, and several other mammalian tissues with a mechanism for regulated fatty acid-dependent H^+ translocation across the mitochondrial inner membrane and, thus, a means to "fine-tune" physiological, mitochondrial uncoupling during normal feeding behavior. In addition, extreme alterations in the expression of UCP2 and/or UCP3 have been documented in obesity (Fleury *et al.*, 1997; Liu *et al.*, 1998), cachexia (Sauchis *et al.*, 1998), and inflammation (Faggioni *et al.*, 1998; Cortez-Pinto,1998), suggesting that dysregulation of these two proteins may contribute to the pathophysiology of these conditions. As a result, intensive efforts are underway to define the molecular mechanisms that modulate the expression and activity of these new uncoupling protein homologs. This review will summarize the evidence that there are some similarities, but several differences, among both the regulatory mechanisms and apparent biological activities of the three mammalian uncoupling proteins.

STRUCTURAL HOMOLOGIES AMONG MAMMALIAN UNCOUPLING PROTEINS

UCP1, UCP2, and UCP3, are a subgroup of the superfamily of mitochondrial anion transporters, and are believed to function as the dominant regulators of mitochondrial uncoupling activity in mammalian cells (Boss *et al.*, 1998b; Ricquier 1998). UCP1, UCP2, and UCP3 are the products of distinct genes that encode relatively homologous products. For example, the amino acid sequence of UCP2 is 59% homologous to UCP1 and 73 % homologous to UCP3 (Liu *et al.*, 1998.). Nucleotide and amino acid sequences for each UCP homolog are highly conserved across species, as evidenced by the fact that murine, rat, and human UCP2 are 86–99% identical (Liu *et al.*, 1998). Based on what is known about other mitochondrial anion transports (e.g., the ADP/ATP exchanger), as well as the structure and function of UCP1, the two most recently identified uncoupling proteins are predicted to have six transmembrane domains that span the inner mitochondrial membrane and a mitochondrial localization sequence. They may also possess nucleotide-and fatty acidbinding domains characteristic of UCP1 (Boss *et a*., 1998; Aquila *et al*., 1987; Kozok *et al*., 1988; Klingenberg *et al*., 1995). Enforced overexpression of UCP2 and UCP3 in yeast has demonstrated that, similar to UCP1, each of the new homologs can reduce the mitochondrial membrane potential and promote thermogenesis (Fleury *et al*., 1997; Gimeno *et al.*, 1997; Casteilla *et al.*, 1990; Paulek, 1998), suggesting that all mammalian uncoupling proteins permit the transport of protons from the intermembrane space into the mitochondrial matrix down the elctrochemical gradient of protons. However, differences in the sequences of the three homologs also imply that UCP1, UCP2, and UCP3 may have somewhat different functions and regulation under physiologically relevant conditions (Biengraeber *et al.*, 1998; Yameda *et al.*, 1998.). Developmental- and tissue-related differences in the expression of various UCP homologs provide additional support for this concept (Shimokawa *et al.*, 1998; Hodney *et al.*, 1998; Carmona *et al.*, 1998).

REGULATION OF THERMOGENESIS AND BODY WEIGHT BY DIFFERENT UCP HOMOLOGS

Because mitochondrial uncoupling activity decreases the mitochondrial membrane potential, uncoupling proteins have been identified by their ability to inhibit ATP synthesis while enhancing the thermogenesis that occurs as a result of mitochondrial respiration. These characteristics of uncoupling proteins imply that they regulate energy expenditure.

Thus, these molecules could conceivably play major roles in controlling both body weight and body temperature. Hence, dysregulation of uncoupling protein activity may be an important mechanism for pathological weight loss (cachexia) or weight gain (obesity), as well as fever and hypothermia. However, as detailed below, efforts to correlate altered expression of specific UCP isoforms with these conditions of pathological energy expenditure have not been entirely successful.

Thermogenesis appears to be a major function of UCP1, the uncoupling protein homolog that is restricted to brown adipose tissue (BAT). As such, UCP1 activity is also an important negative regulator of ATP production from energy substrates. In rodents, neonatal, or hibernating mammals, or other organisms that have large amounts of BAT-associated UCP1 activity, energy substrates are used to generate heat, limiting ATP production. Thus, transgenic mice with brown fat ablation have decreased weight-specific metabolic rates and are hypothermic (Klaus *et al.*, 1998.). Because UCP1 activity in BAT is relatively "wasteful" of energy substrates, these mice also become obese (Klaus *et al.*, 1998.). Taken together, this information suggests that mitochondrial uncoupling activity regulates adiposity by modulating the resting energy expenditure of fat. Additional support for this concept is provided by evidence that subcutaneous fat is reduced in both transgenic lean C57BL/6J mice and obese Avy mice in which the the fat-specific aP2 gene promoter is used to direct WAT expression of UCP1 (Kopecky, *et al.*, 1995.). However, because UCP1 is normally expressed only in BAT, and most adult mammals do not have much active BAT, it is unlikely that UCP1 activity accounts for much energy expenditure, or thermogenesis, in adulthood. Consequently, human obesity probably cannot be explained by decreased UCP1 activity, nor is it likely that increased UCP1 activity contributes importantly to cachexia or fever in people.

WAT and skeletal muscle are the two major peripheral tissues that regulate energy homeostasis and thermogenesis in adult mammals. Thus, the identification of UCP3 generated great excitement among obesity researchers because UCP3 is constitutively expressed in those tissues. The possibility that UCP3 dysfunction contributes to obesity was further supported by the discovery that UCP3 maps to the putative "obesity loci" on human chromosome 11 and mouse chromosome 7 (Liu *et al.*, 1997) and evidence that UCP3 expression is decreased in obese, leptin-deficient *ob/ob* mice (Liu *et al.*, 1997) and leptin-resistant Wistar fatty rats (Matsada *et al.*, 1998). Furthermore, skeletal muscle UCP3 levels in *ob/ob* mice normalize as obesity regresses during leptin treatment (Liu *et al.*, 1997).

However, despite these provocative findings in animal models of obesity, efforts to correlate decreased UCP3 function or expression with obesity in humans have been frustrating. For example, variants of the coding region of the UCP3 gene were rare in a large cohort of Danish Caucasians and were not correlated with diabetes in that population (Urhammer *et al.*, 1998.). Similarly, a single UCP3 varient was found in 82 Pima Indians and was not associated with metabolic rate or obesity (Walder *et al.*, 1998.). Furthermore, as in rodents, feeding behavior tends to induce UCP3 expression, while fasting inhibits it, in both normal and obese human subjects (Millet *et al.*, 1998.). These observations suggest that obesity-related differences in UCP3 function might reflect the regulation of its activity by other modulators and have intensified efforts to characterize the mechanisms by which different energy substrates may regulate UCP3 (Table I).

Several reports have correlated diet-induced changes in skeletal muscle UCP3 expression with increased serum concentrations of free fatty acids in skeletal muscle (Wiegle *et al.*, 1998) and adipose tissue (Samec *et al.*, 1998), suggesting that lipid-regulated factors, including PPARγ, may regulate transcriptional activity of the UCP3 gene. Consistent with this concept, after 10–14 days of treatment with thiazolidinedione (TZD), a potent PPARγ agonist, UCP3 expression was increased in BAT of obese, leptin-resistant, *db/db* mice, as well as in the BAT of lean rats and mice (Kelley *et al.*). These findings support other evidence (Wiegle *et al.*, 1998) that the PPARγ-related effects on UCP3 expression in BAT do not require leptin activity in rats and suggest that certain metabolic consequences of obesity may be more important than leptin resistance per se in downregulating UCP3 in this adipose depot. However, there appear to be important tissue-related differences in the regulation of UCP3 expression *in vivo*. For example, although PPARγ agonists induce UCP3 expression in cultured rat adipocytes, TZD did not affect UCP3 mRNA levels in WAT or skeletal muscle of normal or leptin-resistant mice (Matsuda *et al.*, 1998.). Indeed, another group has reported that PPARγ agonists actually decrease UCP3 mRNA levels in the skeletal muscles of hyperglycemic and hyperinsulinemic *KK/Ta* mice (Shimokuwa *et al.*, 1998.). Thus, if there is decreased UCP3 expression in certain forms of obesity, it remains uncertain to what extent, if any, inhibited PPARγ or leptin signaling

Table I. Regulation of UCP3 mRNA Expression in Various Tissues

Change in UCP-3 mRNA	Condition	Tissue (references)
Increase	Cold	BAT[a,b]
	Fasting	Muscle[b–d]
	Refeeding	BAT[d]
	Dietary fat	BAT, muscle[a]
	Exercise	Muscle[f]
	Leptin	BAT, muscle (*ob*/*ob* mice)[d]
	Thyroid hormone	BAT, muscle[b,d]
	Glucocorticoids	Muscle[d]
	Insulin/IGF-1	WAT, muscle (STZ-diabetic rats)[a]
	NE, β-adrenergics	BAT, WAT[h]
	PPARγ	BAT, WAT[i]
	LPS	Muscle[j]
	TNFα	Muscle[k]
Decrease	Fasting	BAT[d,l]
	Refeeding	Muscle[d]
	Endurance training	Muscle[m]
	Obesity	Muscle (*fa*/*fa* rats)[l]
		BAT (*fa*/*fa* rats),[l] (*ob*/*ob* mice)[d]
	Hypothyroidism	Muscle[d]
	Glucocorticoids	BAT[d]
	NE, β_3 adrenergics	Muscle, heart[h]
	Leptin	Muscle (*ob*/*ob* mice)[n]
	PPARγ	Muscle (*KK*/*T*α mice)[e]

[a] Raskin *et al.*, 1997.
[b] Boss *et al.*, 1998d.
[c] Wiegle *et al.*, 1998.
[d] Gong *et al.*, 1997.
[e] Millet *et al.*, 1997.
[f] Tsuboyama-Kasaoka *et al.*, 1998.
[g] Kageyama *et al.*, 1998.
[h] Yoshitomi *et al.*, 1998.
[i] Kelley *et al.*, 1998.
[j] Fuggioni *et al.*, 1998.
[k] Bussquets *et al.*, 1998.
[l] Boss *et al.*, 1998a.
[m] Boss *et al.*, 1998c.
[n] Liu *et al.*, 1997.
[o] Shimokawa *et al.*, 1998.

contributes to these abnormalities in any given tissue. This confusion has peaked interest in identifying other factors that regulate the expression (summarized in Table I) and activity of this UCP homolog, as well as efforts to link UCP3 dysregulation with pathological states other than obesity.

Emerging evidence suggests that several different stressful situations induce UCP3 mRNA levels in skeletal muscle. For example, transient, 14 to 18 fold increases in UCP3 mRNA levels and energy expenditure were observed in mouse gastrocnemius muscle within a few hours after exercise. Of interest, neither exercise-related increases in UCP3 transcripts nor energy expenditure increased in denervated muscles (Tsuboyama-Kasaoka *et al.*, 1998), suggesting that neuronal factors may regulate UCP3 expression. Indeed, similar to UCP1 (Cassard-Donecia *et al.*, 1993), UCP-3 expression is likely to be regulated by the sympathethic nervous system. Norepinephrine and β_3-adrenergic agonists induce UCP3 expression in brown and white adipose tissue, but appear to inhibit UCP3 expression in skeletal muscle and the heart (Yoshitomi *et al.*, 1998). In addition, like UCP1 (Silva and Rabelo, 1997), UCP3 expression is induced by thyroid hormone (Gong *et al.*, 1997). Increased UCP3 mRNA levels have been noted in skeletal muscles of rats with streptozotocin-induced diabetes, suggesting that hyperglycemia and/or insulin may also influence UCP3 expression in this tissue (Kageyama *et al.*, 1998). In addition, lipopolysaccharide (LPS) (Fuggival *et al.*, 1998) and the LPS-inducible cytokine, tumor necrosis factor-α (TNFα) (Bussquets *et al.*, 1998), induce UCP3 mRNA levels in rat skeletal muscle. However, it is unknown if these TNFα effects are mediated via direct activation of TNF receptors on myocytes or indirectly mediated by inflammation-associated changes in other hormones and/or in the delivery of fatty acids and other energy substrates to muscle cells.

Clearly, a better understanding of the various signals that regulate UCP3 expression requires characterization of the UCP3 promoter. Additional work is also necessary to clarify the posttranscritpional mechanisms that are likely to be important regulators of UCP3 activity. Never the less, the fact that UCP3 is the predominate uncoupling protein homolog in adult skeletal muscle, as well as growing evidence that UCP3 expression in this tissue increases during stress, suggest that upregulation of UCP3 may contribute to increased energy expenditure, fever, and cachexia that often accompany chronic inflammatory states and certain endocrinopathies.

Of the three known mammalian UCP homologs, UCP2 is the most widely expressed in adult tissues. Northern blot analysis of RNA from several tissues in healthy rodents demonstrates variable UCP2 expression, with abundant UCP2 mRNA in white adipose tissue and progressively less UCP2 in skeletal muscle, heart, pancreas, lung, spleen, kidney, brain, and liver (Fluvy *et al.*, 1997). UCP-2 is also a candidate gene for obesity because it maps close to UCP3 in the "obe-

sity loci" on human chromosome 11 and mouse chromosome 7. However, similar to UCP3, there is scant evidence that UCP2 polymorphisms are directly involved in the genesis of human obesity. For example, screening obese Swedish patients with low BMR identified only one polymorphism (Ala55 → Val) in the coding regions of the UCP2 gene and there were no significant differences in the allele or genotype frequencies of this polymorphism between 55 patients with obesity and the dysmetabolic syndrome and 46 healthy controls, leading the authors of this study to conclude that mutations in the coding regions of the UCP2 gene do not affect BMR or contribute to increased susceptibility to obesity or the dysmetabolic syndrome (Klaunemark *et al.*, 1998). Similarly, only two nucleotide substitutions (Ala55 → Val and Ala232 → Thr) in the coding regions of the UCP2 gene were identified in 25 Japanese patients with obesity and NIDDM. The frequency of one of these polymorphisms (Ala55Val) was no different in normal controls, 210 other individuals with NIDDM, or 42 additional obese individuals. A single allele for the other UCP2 variant (Ala232Thr) was identified in three of the NIDDM group. However, expression of this human UCP2 variant and wild-type UCP2 in yeast revealed no difference in functional activity (Kubota *et al.*, 1998.). Two UCP2 polymorphisms (an Ala → Val substitution in exon 4 and a 45-bp insertion/deletion in the 3′-untranslated region of exon 8) were identified by screening a cohort of 82 young, unrelated, nondiabetic Pima Indians. Both UCP2 variants were associated with decreased metabolic rate. However, when an additional 790 full-blooded Pima Indians were evaluated, there was no significant association between the variants and body mass index or UCP2 mRNA levels in skeletal muscle (Walder *et al.*, 1998).

Paradoxically, although loss-of-function mutations in uncoupling proteins are predicted to underlie obesity-related decreases in energy expenditure, there is conflicting information about the expression of UCP2 in obese humans (Millet *et al.*, 1998; Millet *et al.*, 1997; Bao *et al.*, 1998, Nordkois *et al.*, 1998 Simonean *et al.*, 1998). Both abnormally increased and decreased expression of UCP2 have also been reported in obese, Wistar fatty rats and *ob/ob* mice (Fluny *et al.*, 1997; Matsuda *et al.*, 1998; Emilson *et al.*, 1998; Chavin *et al.*, 1999). However, in at least one study, increased UCP2 expression was not related to basal energy expenditure or insulin sensitivity (Simoneau *et al.*, 1998.). These findings suggest that upregulation of UCP2 in certain tissues may be a compensatory response to balance-decreased uncoupling activity (and hence, energy expenditure) in other tissues or to overcome decreases in factors that ordinarily stimulate its activity in the mitochondria. If one or both of these possibilities are true, then neither increased nor decreased UCP2 expression need be consistent feature of obesity. This, in turn, might help to explain the conflicting results mentioned earlier, as well as the variable expression of UCP2 in other animal models of obesity (Strobel *et al.*, 1998; Kushi, *et al.*, 1998). Of interest, even within WAT of the same obese individual, regional differences in UCP2 expression also occur, as illustrated by data that UCP2 expression is decreased in the intraperitoneal WAT, but not extraperitoneal WAT, of obese human subjects. This finding, coupled with the observation that UCP2 expression in intraperitoneal WAT is generally greater than in extraperitoneal WAT (Obekofler *et al.*, 1998), suggests the importance of energy substrates and/or splanchnic factors as regulators UCP2 expresion (Table II).

Consistent with this concept is evidence that, similar to UCP3, UCP2 expression is influenced by fatty acids. Rats that consume diets enriched in fatty acids have increased UCP2 mRNA levels in WAT and skeletal muscle (Wiegle *et al.*, 1998). Recently, unsaturated fatty acids, including linoleic and oleic acid, were also shown to induce UCP2 mRNA and protein levels in primary cultures of normal rat hepatocytes (Cortez-Pinto *et al.*, 1998). Confocal microscopy of hepatocytes cultured in serum-free medium without or with micromolar concentrations of these fatty acids for 24 h illustrates a substantial increase in UCP2 protein levels and colocalizes UCP2 expression with that of the B-1 subunit of ATP synthase, confirming its upregulation in hepatocyte mitochondria (Fig. 1). The latter finding may help to explain why UCP2 expression is induced in hepatocytes of obese, *ob/ob* mice, which typically exhibit increased circulating levels of triglycerides, hepatic free fatty acids, and fatty liver (Chavin *et al.*, 1999).

Evidence that the lipid-inducible transcription factor, PPARγ, upregulates UCP3 suggests that similar factors may also mediate lipid-related induction of hepatocyte UCP2. However, agonists of PPARα, and not PPARγ, induce hepatic UCP2 expression (Kelley *et al.*, 1998). Similar to UCP3, lipopolysaccharide and TNFα, are known to induce UCP2 expression in rat WAT, skeletal muscle, and liver. Pretreatment with neutralizing antibodies to TNFα inhibits LPS induction of UCP2 in hepatocytes and also prevents activation of a nucleotide fragment that encompasses the proxi-

Table II. Regulation of UCP2 mRNA Expression in Various Tissues

Change in UCP-2 mRNA	Condition	Tissue (references)
Increase	Cold	BAT, heart[a]
	Fasting	Muscle[a–c]
	Dietary fat	WAT,[b,d,e] muscle,[b] hepatocytes[f]
	Obesity	BAT (ob/ob mice),[g] WAT (*ob/ob, db/db mice*),[h] liver,[i] muscle (humans)[j]
	Thyroid hormone	Muscle, BAT, WAT, heart[k,e]
	NE, β_3 adrenergics	BAT[m]
	Leptin	WAT (young rats),[n] pancreatic islets[o]
	PPARγ	Pancreatic islets[p]
	PPARα	Liver[q]
	LPS	WAT, muscle,[r] liver,[r,s] hepatocytes,[s] heart[r]
	TNFα	WAT, muscle,[t] hepatocytes[s]
Decrease	Endurance training	Muscle[u]
	Obesity	Muscle (humans),[v] WAT (intraperitoneal, humans)[w]
	Leptin	BAT (ob/ob mice),[g] WAT (old rats)[n]
	Hypothyroidism	Heart[k]
	NE, β_3 adrenergics	Muscle, heart[m]
	LPS	Peritoneal macrophages[x]

[a] Boss *et al.*, 1997a.
[b] Wiegle *et al.*, 1998.
[c] Millet *et al.*, 1997.
[d] Fleury *et al.*, 1997.
[e] Matsuda *et al.*, 1997.
[f] Cortez-Pinto *et al.*, 1999.
[g] Gong *et al.*, 1997.
[h] Gimeno *et al.*, 1997.
[i] Chanin *et al.*, 1999.
[j] Simoneu *et al.*, 1998.
[k] Lanni *et al.*, 1997.
[l] Masaki *et al.*, 1997.
[m] Yoshitomi *et al.*, 1998.
[n] Siegrist-Kaiser *et al.*, 1997.
[o] Zhon *et al.*, 1997.
[p] Shimabukaro *et al.*, 1997.
[q] Keeley *et al.*, 1998.
[r] Faggioni *et al.*, 1998.
[s] Cortez-Pinto *et al.*, 1998.
[t] Bussquets *et al.*, 1998.
[u] Boss *et al.*, 1998.
[v] Nordfors *et al.*, 1998.
[w] Oherkofler *et al.*, 1998.
[x] Lee *et al.*, 1999.

mal 3.6 kb of the UCP2 promoter, suggesting that TNF activates hepatocyte UCP2 transcription (Cortez-Pinto *et al.*, 1998.). Although binding sites for several TNF-regulated *trans*-acting factors, including AP-1 and C/EBP, are present in this region, the TNF responsive element(s) in the UCP2 promoter have not been localized. Because TNF increases serum concentrations of free fatty acids, it is conceivable that the upregulation of UCP2 in hepatocytes of LPS-treated rats merely reflects TNF-related increases in the delivery of fatty acids to hepatocytes. On the other hand, evidence that recombinant TNFα induces the expression of UCP2 mRNA in primary hepatocytes cultured in serum-free, insulin-supplemented, glucose-based medium (Cortez-Pinto et al., 1998) suggests that TNF's effects on the UCP2 promoter may also result directly from its modulation of hormone and/or stress-related signaling cascades in hepatocytes. The latter possibility is particularly intriguing because several downstream targets of TNF, including PI-3 kinase, *Ras*, and *c-Jun*, modulate

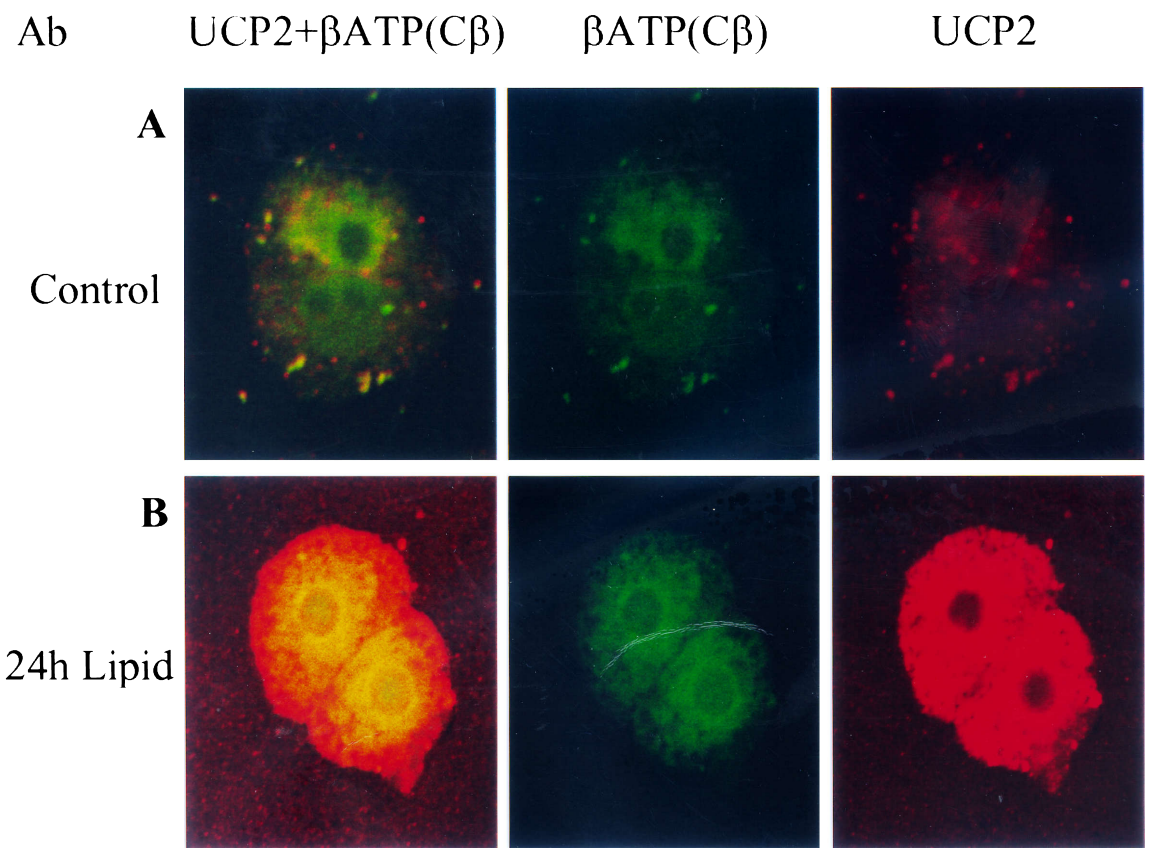

Fig. 1. Expression of UCP2 protein in rat hepatocyte mitochondria. Primary cultures of rat hepatocytes were incubated in serum-free medium without (A) or with (B) an emulsion of linoleic acid (150 m*M*) and oleic acid (75 m*M*) for 24 h. UCP2 expression was evaluated by confocal laser microscopy (Zeiss Laser Scanning Microscope) using Cy-3 conjugated primary goat antisera to a recombinant UCP2 peptide (from Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and FITC-conjugated primary rabbit antisera to b-1 subunit of rat ATP synthase (generously provided by Peter Pedersen, Dept. Biol. Chem., Johns Hopkins University). All images were obtained at the same laser power (default setting 5 0); the brightness value stayed at 9999. Cy-3-conjugated antisera (550 nm excitation; 565 nm emission) was excited at 543 nm with a helium-neon laser and appears red; FITC-conjugated antisera (494 nm excitation; 535 nm emission) was excited at 488 nm with crypton-argon laser and appears green. Colocalization of UCP2 and b-1 ATP synthase is demonstrated by yellow images. Final magnification 5 13123.

insulin-related induction of UCP1 in brown adipocytes (Ternel *et al.*, 1998; Yuhero *et al.*, 1998). Finally, similar to UCP1 (Scarpace and Matheny 1998) and UCP3 (Liu *et al.*, 1998; Gong *et al.*, 1997), leptin is likely to regulate UCP2 mRNA expression in cells with long forms of the OB receptor (OB-Rb), including adipocytes (Siegrist-Kaiser *et al.*, 1997; Kutah *et al.*, 1998; Kielar *et al.*, 1998). Leptin induction of UCP2 expression has already been demonstrated for pancreatic islet cells, which express OB-Rb (Zhon *et al.*, 1997.).

Taken together, published information about UCP2 (Table II) and UCP3 (Table I), the two most recently identified uncoupling protein homologs, suggests that their structures and regulation differ somewhat from UCP1 (Table III). Strong evidence supports the importance of UCP1 in the control of resting energy expenditure and, hence, body temperature and body weight, in animals with a large amount of BAT. However, to date, evidence that either endogenous UCP2 or UCP3 can ™substituteſ for these actions of UCP1 in animals that lack large BAT depots is not very compelling. Thus, even if UCP2 and/or UCP3 do influence the resting energy expenditure, this action may not be sufficient to modulate adiposity or thermogenesis appreciably in humans. The latter possibility is consistent with very recent evidence that subjects who become obese during overfeeding do not have decreased resting metabolic rates (Levine *et al.*, 1999).

Table III. Regulation of UCP1 mRNA Expression in BAT

Change in UCP-1 mRNA	Condition (references)
Increase	Cold[a,b]
	Refeeding[c]
	High dietary fat[d]
	Leptin (*ob/ob* mice)[e]
	Thyroid hormone[f,g]
	Insulin[h,i]
	PPAR-γ agonists[j]
	Retinoic acid[k–n]
	Norepinephrine, α-1, β(1–3) agonists[a,o–q]
Decrease	Fasting[c,r,s]
	Obesity (ob/ob mice),[e] (fa/fa rats)[a,p]
	Hypothyroidism[f,t]
	Glucocorticoids[h,u,v]

[a] Ricquier *et al.*, 1986.
[b] Wiesinger *et al.*, 1990.
[c] Champigny and Ricquier, 1990.
[d] Giaraudo *et al.*, 1994.
[e] Gong *et al.*, 1977.
[f] Bianco and Silva, 1987.
[g] Bianco and Silva, 1988.
[h] Strack *et al.*, 1995b.
[i] Klaus *et al.*, 1995.
[j] Foellmi-Adams *et al.*, 1996.
[k] Cassard-Doulcier *et al.*, 1993.
[l] Cassard-Doulcier *et al.*, 1994.
[m] Alvarez *et al.*, 1995.
[n] Puigserner *et al.*, 1996.
[o] Mory *et al.*, 1984.
[p] Mozzin *et al.*, 1989.
[q] Rehnmark *et al.*, 1990.
[r] Knott *et al.*, 1992.
[s] Matamala *et al.*, 1996.
[t] Obregon *et al.*, 1987.
[u] Moriocot *et al.*, 1993.
[v] Strack *et al.*, 1995a.

Furthermore, important, tissue-related and diet-dependent differences in the expressions of UCP2 and UCP3 have also been described, suggesting that the latter two UCP homologs may not serve the same primary function(s) during health. Consequently, relatively selective dysregulation of the expression of specific uncoupling protein(s) might occur during disease states. Additional work is required to clarify the respective roles of UCP2 and UCP3 during pathological states that disturb energy homeostasis. It is conceivable that disease-related changes in UCP2 and/or UCP3 activity are not motivated primarily to change energy expenditure, but rather, to accomplish some other goal(s). If so, then any alterations in body weight or temperature that result are merely secondary phenomena.

UNCOUPLING PROTEINS AS REGULATORS OF MITOCHONDRIAL ROS PRODUCTION

Cells that rely largely on aerobic respiration to produce ATP are confronted with the challenge of constraining the reduction of molecular oxygen to superoxide and other reactive oxygen species (ROS) that can occur during mitochondrial electron transport. Because superoxide anion (O_2^-) is a highly reactive molecule that is capable of damaging cellular components, cells have developed numerous strategies to dissipate O_2^- and remove its oxidation products. A variety of systems have also evolved to facilitate the repair of vital macromolecules that inadvertently become damaged by these ROS. Failure to control ROS damage can cause the collapse of multiple vital functions, including mitochondrial energy conservation, culminating in loss of membrane integrity and cell death by necrosis. Mitochondrially generated ROS also appear to influence programmed cell death, or apoptosis. The possibility that death might actually be programmed by ROS-related signals that are generated during mitochondrial respiration is provocative and could help to explain the variable influences of different energy substrates on cellular senescence. It is also conceivable that mitochondrially generated ROS might prompt the induction of protective responses that check the propagation of these apoptotic signals, permitting conditional cellular survival.

Compared to the hundreds of references that document regulation of energy expenditure by uncoupling proteins, relatively little has been written concerning their role as potential regulators of mitochondrial ROS production. However, there is good evidence that certain pharmacological uncoupling agents (e.g., FCCP) and plant uncoupling proteins (e.g., PUMP) can perform such a function. It is possibile that regulation of mitochondrial ROS production and, hence, cell survival, may be a primary function of some of the mammalian uncoupling proteins. However, it is equally important to acknowledge that very little information exists that correlates variations in UCP expression or activity with altered cellular ROS production or viability.

Nevertheless, emerging evidence raises the possibility that regulation of ROS production might be a

particularly important function of UCP2, the most ubiquitously expressed uncoupling protein homolog. Although far from definitive, such a role for UCP2 has been most clearly demonstrated in macrophages, which express UCP2 mRNA constitutively (Fleury *et al.*, 1997; Larrony *et al.*, 1997). Negre-Salvayre and colleagues (1997) have shown that macrophage ROS production is influenced by cellular GDP content. Addition of GDP, which, presumably, inhibits UCP2 activity, increases H_2O_2 production by macrophages, suggesting that the constitutive expression of UCP2 represses basal production of H_2O_2 by these cells.

In cells that express long forms of the leptin receptor (OB-Rb), such as pancreatic islet cells, treatment with leptin increases UCP2 mRNA expression (Zheru *et al.*, 1997). Recombinant leptin is known to influence the phenotype of cultured macrophages, suggesting that macrophages also express functional leptin receptors (Faggioni *et al.*, 1998; Gainsford *et al.*, 1996; Loffreda *et al.*, 1998). Taken together, these observations suggest that leptin may regulate UCP2 expression in macrophages, raising the possibility that basal UCP2 expression might be decreased in macrophages from *ob/ob* mice, which lack leptin (Campfield *et al.*, 1996.). Consistent with this possibility, we found that levels of UCP2 mRNA were decreased in peritoneal macrophages that were freshly harvested from *ob/ob* mice compared to similar cells that were obtained from lean control mice. In addition, as predicted by Negre-Salvayre's *et al.* work' (1997), *ob/ob* macrophages (which expressed less UCP2) produced significantly higher basal levels of O_2^- and H_2O_2 than macrophages from lean controls. Interestingly, when macrophages from *ob/ob* or normal, lean mice were exposed to lipopolysaccharide for 90 min, the expression of UCP2 mRNA was significantly inhibited and there was a severalfold increase in the generation of O_2^- and H_2O_2. The latter could be inhibited by rotenone (which blocks the entry of electrons into the mitochondrial electron transport chain at complex I), myxothiazol (an inhibitor of superoxide anion generation at complex III), or pharmacologic agents (FCCP) that uncouple mitochondrial respiration, suggesting that mitochondria are an important source of ROS in macrophages and that inhibition of UCP2 may be one mechanism that contributes to LPS-related increases in macrophage ROS production (Lee *et al.*, 1999).

In contrast to the LPS-related inhibition of UCP2 that has been observed in macrophages, LPS treatment appears to increase UCP2 expression several tissues, including skeletal muscle, heart, and liver (Faggioni *et al.*, 1998; Cortez-Pinto *et al.*, 1998). These targets are not known to express full-length leptin receptors (Tortaglia *et al.*, 1995), suggesting that factors other than leptin may regulate LPS-related induction of UCP2 in these cells. At least in hepatocytes, this response is mediated by TNFα, because pretreatment with anti-TNF antibodies blocks the ability of LPS to induce UCP2 in hepatocytes (Cortez-Pinto *et al.* 1998). Recombinant TNFα also increases UCP2 mRNA levels in cultures of primary hepatocytes and the kinetics of this response are similar to the induction of hepatocyte UCP2 in LPS-treated rats. In both cases, there is a lag period of more than 6 h before increases in UCP2 expression are detected (Cortez-Pinto *et al.*, 1998).

We have suggested that such TNF-related increases in UCP2 reflect an attempt by hepatocytes to control increases in mitochondrial ROS production that are induced rapidly by TNF (Stadler *et al.*, 1992). Consistent with this possibility, increased hepatocyte expression of UCP2 mRNA and protein follow the TNF-dependent induction of mitochondrial H_2O_2 production in regenerating mouse livers (Lee *et al.*, 1999) and temporally correlate with the regenerative induction of mRNAs that encode other mitochondrial proteins that may regulate mitochondrial ROS release (Kren *et al.* 1996.). In addition, we have shown that pharmacological agents that increase hepatocyte mitochondrial ROS, such as ethanol (Rashid *et al.*, 1999) or *tert*-butylhydroperoxide (Cortez-Pinto*et al.*, 1999) increase hepatocyte UCP2 expression in mice. Other, all be it more indirect, support for a possible relationship between UCP2 induction and hepatocyte mitochondrial ROS regulation is provided by recent reports that linoleic acid, which induces UCP2 expression in hepatocytes (Cortez-Pinto *et al.*, 1999), also inhibits mitochondrial ROS production in plants by inducing PUMP, a plant uncoupling protein homolog (Kowaltowski *et al.* 1998). However, whether or not increased UCP2 expression leads to decreased mitochondrial ROS release has not been tested directly in hepatocytes. Furthermore, if increased uncoupling protein expression does, indeed, decrease mitochondrial ROS production, the molecular mechanisms involved remain obscure.

It has been suggested that decreases in O_2^- formation occur because uncoupling increases the rate of electron transport, lowering the steady state level or accessibility of the semiquinone form of ubiquinone, thereby diminishing the probability that electrons will "escape" from the respiratory chain and interact with molecular oxygen (Skulacher, 1995, 1999). However,

the extent to which this might be effective would depend on the activity state of UCP2. Little is known about the mechanism by which the protein exerts its uncoupling effect or how its activity is regulated. UCP2 lacks the histidine residues that are required for UCP1 to conduct protons (Biengracher *et al.,* 1998). Furthermore, UCP1 proton conductance is regulated independently by the fatty acid binding and nucleotide-binding domains of that molecule, such that the uncoupling activity is activated by free fatty acids and inhibited by di-and triphopho purine nucleotides (Bonillaud *et al.*, 1994; Modriansky *et al.*, 1997; Winkler *et al.*, 1997; Jezek *et al.,* 1994.). By contrast, UCP-2 mediated uncoupling activity has not been consistently found to be regulated by either free fatty acids or purine nucleotides (Chavin *et al.*, 1999; Simonyan and Skedacher, 1998). It is likely that additional, as yet uncharacterized factors modulate UCP2 activity. In addition, it can not be excluded that UCP2 affects mitochondrial ROS formation by mechanisms that do not require mitochondrial uncoupling.

UCP2 AND APOPTOSIS

We have recently observed a temporal correlation between the induction of UCP2 and anti-apoptotic bcl-2 family members in hepatocytes from obesity-related and ethanol-induced fatty livers (Rashid *et al.*, 1999), and also in prereplicative hepatocytes during the early phases of liver regeneration after partial hepatectomy (Lee *et al.*, 1999). Although this may be merely coincidental, the concurrent induction of these mitochondrial membrane proteins in viable hepatocytes suggests that UCP2 itself may serve an antiapoptotic role in the cell. Several mechanisms might be involved. First, the suppression of ROS formation in mitochondria by UCP2 may help prevent the opening of the mitochondrial permeability transition pore (MPTP), an event that has been associated with the onset of apoptosis and the release of apoptosis-promoting factors, such as cytochrome *c* (Kroemer *et al.*, 1998). In addition, the mitochondrial depolarization induced by UCP2-dependent uncoupling would inhibit mitochondrial Ca^{2+} accumulation and prevent excessive alkalinization of the mitochondrial matrix, both of which are conditions that strongly promote the opening of the MPT pore (Bernadi *et al.*, 1998). However, a more direct interaction of UCP2 with bcl-2 family member proteins is also conceivable. Both pro- and antiapoptotic bcl-2 family members have a close association with the mitochondrial outer membrane, where they can form homo or hetero dimers. The balance between pro- and antiapoptotic proteins may contribute to the net effect on mitochondrial integrity, with an excess of proapoptotic proteins signaling mitochondrial demise. For instance, overexpression of the proapoptotic family member Bax induces apoptosis associated with the MPTP in Jurkat T cells (Pastorino *et al.*, 1998), and recombinant Bax induces the MPT in isolated mitochondria (Narita *et al.*, 1998). Recent evidence (Marzo *et al.*, 1998b) suggests that Bax homo dimers trigger this response by interacting with inner membrane proteins, specifically with the adenine nucleotide translocator (ANT). There is strong evidence that the ANT is an integral component of the MPT pore complex (Halestrap and Davidson, 1990; Marzo *et al.*, 1998a). UCP2 (as well as other uncoupling proteins) have considerable structural homology to the ANT (Klingenberg et al., 1995) and, thus, it is conceivable that UCP2 physically interacts with either the ANT or Bax. Either interaction might decrease the formation of Bax–ANT complexes and thereby suppress the assembly of the MPT pore complex. Other studies (Shimizu *et al.*, 1998) reported that liver mitochondria from rats that have selective hepatic overexpression of the antiapoptotic protein Bcl-2 exhibit decreased proton translocation across the mitochondrial membrane, although the mechanism of this effect was not elucidated. Thus, there is reason to believe pro- and antiapoptotic Bcl-2 family proteins have a direct interaction with proteins that mediate the proton translocation across the mitochondrial inner membrane.

Finally, UCP2 might modulate the targeting of Bcl-2 related proteins to the mitochondrial membrane. UCP2 activity results in localized thermogenesis (Paulik *et al.*, 1998) and the resulting heat may cause activation of heat shock proteins, important molecular chaperones that are required for the insertion of several proteins, including ATP synthase, UCP1, porin, p53, and Bcl-2, into mitochondrial membranes (Merrick *et al.*, 1996; Ricart *et al.*, 1997; Komiya *et al.*, 1997; Schleiff and Turnull, 1998; Polla *et al.*, 1996). Thus, increased UCP2 activity might provide a signal, i.e., heat, that promotes the targeting of Bcl-2 or other antiapoptotic Bcl-2-related proteins, such as BAG-1 (Takayama *et al.*, 1998), to mitochondria. By increasing the local concentration of antiapoptotic Bcl-2 family members, increased UCP2 activity might favor the formation of Bax–Bcl-2 hetero dimers or Bcl-2 homo dimers, decreasing the number of Bax homo dimers that are available for MPT pore formation.

Although MPT pore opening decreases the mitochondrial membrane potential, it also depletes mitochondrial NAD(P) levels and inhibits mitochondrial respiration. Under some conditions where the supply of electrons to ubiquinone still occurs, this may promote the formation of ROS (Skulacher, 1996a), as well as the release of mitochondrial factors, such as cytochrome *c*, that promote apoptosis. Thus, if increased UCP2 inhibits opening of the MPTP, this may help to limit both caspase activation and mitochondrial ROS production, permitting cells to survive exposure to TNFα, or other agents that affect cell death by mechanisms that involve opening of the MPT pore (Mignotte and Voyssiere, 1998). In this regard, it is interesting that, similar to TNFα, which promotes hepatocyte proliferation after partial hepatectomy (Akerman *et al.*, 1992), several other hepatocyte mitogens, including transforming growth factor α (TGFα) (Factor *et al.*, 1998) and estrogen (Chen *et al.*, 1998), can promote mitochondrial ROS production. Hepatocytes must have developed mechanisms to control mitogen-related generation of ROS, because clonal expansion of neoplastic hepatocytes is thought to involve autocrine production of TGFα (Factor *et al.*, 1998). Recently, Carretero and colleagues (1998) reported that H4IIE hepatoma cells express much more UCP2 mRNA than normal rat hepatocytes. Furthermore, these authors found that UCP2 was hypomethylated in several transformed liver cell lines, suggesting that upregulation of this gene may be a fairly general response to malignant transformation. Taken together, these observations suggest that UCP2 may convey a survival advantage to hepatocytes, at least in some circumstances when there is pressure to increase ROS production.

UCP2 AND NECROSIS

However, such induction of UCP2 must be viewed as a "double-edged sword" because mitochondrial uncoupling is predicted to increase vulnerability to secondary stresses that further depolarize the mitochondrial membrane and/or deplete cellular ATP stores. Thus, cells that adapt successfully to minor apoptotic stress by inducing UCP2 remain viable, but become particularly vulnerable to necrosis. Such vulnerability to necrosis develops because the additive actions of UCP2 and conditions that inhibit mitochondrial electron transport (e.g., hypoxia) could abolish the proton electrochemical gradient across the inner mitochondrial membrane, preventing oxidative phosphorylation, and causing energy failure with the consequent violation of mitochondrial membrane integrity and massive release of ROS from this compartment. In addition, because even slight increases in UCP2 activity decrease the efficiency of mitochondrial ATP synthesis, UCP2 induction increases cellular requirements for energy substrates in order to maintain ATP stores in a range that is compatible with life. Thus, slight, but sudden, decreases in substrate availability or increases in ATP requirements may be lethal for cells that have upregulated UCP2. We have suggested that UCP2 induction contributes to the lowered threshold for hypoxic, ischemic, or endotoxin-induced necrosis that occurs in obesity- or ethanol-related fatty livers (Chavin *et al.*, 1999; Lashid *et al.*, 1999). It is tempting to speculate that increases in UCP2 might also play a role in the enhanced vulnerability that neoplastic hepatocytes exhibit to energy substrate depletion Grasl-Kraupp *et al.*, 1994.).

SUMMARY

UCP2 and UCP3, the predominant UCP homologs in most adult mammals, are capable of increasing thermogenesis in certain experimental circumstances, such as enforced overexpression in yeast cultures. However, efforts to link variations in the expressions of these UCP homologs with physiological variations in body temperature or energy expenditure have produced confusing results. Thus, at present, it is not clear if, or how, increases (or decreases) in either of these homologs cause increased (or decreased) energy expenditure or thermogenesis in humans. Striking, tissue-specific and developmental variations in the expressions of UCP2 and UCP3 suggest that these homologs are tightly regulated and, hence, likely to provide some important function(s) that enables cells to adapt to changing environmental signals, including local variations in energy substrates and growth factors. Consistent with this possibility, there is growing evidence that unsaturated fatty acids and cytokines induce UCP2 and UCP3 expression in several cell types. Because these factors are known to influence mitochondrial production of reactive oxygen species and uncoupling protein homologs are capable of modulating ROS generation, it is conceivable that the latter is a key biological function of the two most recently identified UCP homologs. Such UCP regulation of mitochondrial ROS production might have critical

implications for cellular viability, given the importance of mitochondrial ROS as signaling molecules, as well as their potential cellular toxicity. This would be consistent with observations that correlate the upregulation of UCP2 with the induction of other mitochondrial proteins, including Bcl-2 and Bcl-x, that protect cells from apoptosis. However, because increased uncoupling protein activity decreases the efficiency of ATP synthesis, cells which upregulate uncoupling protein homologs, escape apoptosis only by increasing their vulnerability to necrosis.

REFERENCES

Akerman, P., Cote, P., Yang, S. Q., McClain, C., Nelson, S., Bagby, G. J., and Diehl, A. M. (1992). *Amer. J. Physiol.* **263**, G579–G585.

Alvarez, R., DeAndres, J., Yubero, P., Vinas, O., Mampel, T., Iglesias, R., Giralt, J., and Villarroya, F. (1995). *J. Biol. Chem.* **270**, 5666–5673.

Aquila, H., Link, T. A., and Klingenberg, M. (1987). *FEBS Lett.* **212**, 1–9.

Bao, S., Kennedy, A., Wojciechowski, B., Wallace, P., Ganaway, E., and Garvey, W. T. (1998). *Diabetes.* **47**, 1935–1940.

Bernadi, P., Basso, E., Colonna, R., Costantini, P., Di Lisa, F., Eriksson, O., Fontaine, E., Forte, M., Ichas, F., Massari, S., Nicolli, A., Petronilli, V., and Scorrano, L. (1998). *Biochim Biophys Acta* **1365**, 200–206.

Bianco, A. C., and Silva, J. E. (1987). *J. Clin. Invest.* **79**, 295–230.

Bianco, A. C., and Silva, J. E. (1988). *Amer. J. Physiol.* **328**, 207–215.

Biengraeber, M., Echtay, K. S., and Klingenberg, M. (1998). *Biochemistry* **37**, 3–8.

Boss, O., Samec, S., Dulloo, A., Seydoux, J., Muzzin, P., and Giacobino, J.-P. (1997a). *FEBS Lett.* **412**, 111–114.

Boss, O., Samec, S., Paoloni-Giacobino, A., Rossier, C., Dulloo, A., J., S., Muzzin, P., and Giacobino, J.-P. (1997b). *FEBS Lett.* **408**, 39–42.

Boss, O., Bobbioni-Harsch, E., Assimacopoulos-Jeannet, F., Muzzin, P., Munger, R., Giacobino, J.-P., and Golay, A. (1998a). *Lancet.* **351**, 1933.

Boss, O., Muzzin, P., and Giacobino, J. P. (1998b). *Eur. J. Endocrinol.* **139**, 1–9.

Boss, O., Samec, S., Desplanches, D., Mayet, M. H., Seydoux, J., Muzzin, P., and Giacobino, J. P. (1998c). *FASEB J.* **12**, 335–339.

Boss, O., Samec, S., Kuhne, F., Bijlenga, P., Assimacopoulos-Jeannet, F., Seydoux, J., Giacobino, J. P., and Muzzin, P. (1998d). *J. Biol. Chem.* **273**, 5–8.

Bouillaud, F., Arechaga, I., Petit, P. X., Raimbault, S., Levi-Meyrueis, C., Casteilla, L., Laurent, M., Rial, E., and Ricquier, D. (1994). *EMBO J.* **13**, 1990–1997.

Bussquets, S., Sanchis, D., Alvarez, B., Ricquier, D., Lopez-Soriano, F. J., and Argiles, J. M. (1998). *FEBS Lett.* **440**, 348–350.

Campfield, L. A., Smith, F. J., and Burn, P. (1996). *Hormone Metab. Res.* **28**, 619–632.

Carmona, M. C., Valmaseda, A., Brun, S., Vinas, O., T., M., Iglesias, R., Giralt, M., and Villarroya, F. (1998). *Biochem. Biophys. Res. Commun.* **243**, 224–228.

Carretero, M. V., Torres, L., Latasa, U., Garci-Treviajano, E. R., Prieto, J., Mato, J. M., and Avila, M. A. (1998). *FEBS Lett.* **439**, 55–58.

Cassard-Doulcier, A. M., Gelly, C., Fox, N., Schrementi, J., Raimbault, S., Klaus, S., Forest, C., Bouillaud, F., and Ricquier, D. (1993). *Mol. Endocrinol.* **7**, 497–506.

Cassard-Doulcier, A. M., Larose, M., Matamala, J. C., Champigny, O., Bouillaud, F., and Ricquier, D. (1994). *J. Biol. Chem.* **269**, 24335–24342.

Casteilla, L., Blondel, O., Klaus, S., Raimbault, S., Diolez, P., Moreau, F., Bouillaud, F., and Ricquier, D. (1990). *Proc. Natl. Acad. Sci. USA* **87**, 5124–5128.

Champigny, O., and Ricquier, D. (1990). *J. Nutr.* **120**, 1730–1736.

Chavin, K., Yang, S. Q., Lin, H. Z., Chatham, J., Chacko, V. P., Hoek, J. B., Walajtyzs, Rode, E., Rashid, A., Chen, C.-H., Huang, C.-C., Lane, M. D., and Diehl, A. M. (1999). *J. Biol. Chem.*, **274**, 5692–5700.

Chen, J., Gokhale, M., Li, Y., Trush, M. A., and Yager, J. D. (1998). *Carcinogenesis* **19**, 2187–2193.

Cortez-Pinto, H., Yang, S. Q., Lin, H. Z., Rashid, A., and Diehl, A. M. (1999). *Gastroenterology* **116**, 1184–93.

Cortez-Pinto, H., Yang, S. Q., Lin, H. Z.,Costa, S., Hwang, C.-S., Lane, M. D., Bagby, G., and Diehl, A. M. (1998). *Biochem. Biophys. Res. Commun.* **251**, 313–319.

Emilsson, V., Summers, R. J., Hamilton, S., Liu, Y. L., and Cawthorne, M. A. (1998). *Biochem. Biophys. Res. Commun.* **252**, 450–454.

Factor, V. M., Kiss, A., Woitach, J. T., Wirth, P. J., and Thorgeirsson, S. S. (1998). *J. Biol. Chem.* **273**, 15846–15853.

Faggioni, R., Shigenaga, J., Moser, A., Feingold, K. R., and Grunfeld, C. (1998). *Biochem. Biophys. Res. Commun.* **244**, 75–78.

Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, R., Seldin, M. F., Surwit, R. S., Ricquier, D., and Warden, C. H. (1997). *Nature Genet.* **15**, 269–272.

Foellmi-Adams, L. A., Wyse, B. M., Herron, D., Nedergaard, J., and Kletzien, R. F. (1996). *Biochem. Pharmacol.* **52**, 693–701.

Gainsford, T., Willson, T. A., Metcalf, D., Handman, E., McFarlane, C., Ng, A., Nicola, N. A., Alexander, W. S., and Hilton, D. J. (1996). *Proc. Natl. Acad. Sci. USA* **93**, 14564–14568.

Giaraudo, S. Q., Kotz, C. M., Grace, M. K., Levine, A. S., and Billington, C. J. (1994). *Amer. J. Physiol.* **266**, R1578–R1583.

Gimeno, R. E., Dembski, M., Weng, X., Deng, N., Shyjan, A. W., Gimeno, C. J., Iris, F., Ellis, S. J., Woolf, E. A., and Tartagli, L. A. (1997). *Diabetes* **46**, 900–906.

Gong, D.-W., He, Y., Karas, M., and Reitman, M. (1997). *J. Biol. Chem.* **272**, 24129–24132.

Grasl-Kraupp, B., Bursch, W., Ruttkay-Nedecky, B., Wagner, A., Lauer, B., and Schulte-Hermann, R. (1994). *Proc. Natl. Acad. Sci. USA* **91**, 9995–9999.

Halestrap, A. P., and Davidson, A. M. (1990). *Biochem. J.* **268**, 153–160.

Hardie, D. G., and Carling, D. (1997).*Eur. J. Biochem.* **246**, 259–273.

Hodny, S., Kolarova, P., M., R., Horakova, M., Nibbelink, M., Penicaud, L., Casteilla, L., and Kopecky, J. (1998). *FEBS Lett.* **425**, 185–190.

Jezek, P., Orosz, D. E., Modriansky, M., and Garlid, K. D. (1994). *J. Biol. Chem.* **269**, 26184–26190.

Jezek, P., Modriansky, M., and Garlid, K. D. (1997). *FEBS Lett.* **408**, 161–165.

Jezek, P., Engstova, H., Zackova, M., Vercesi, A. E., Costa, A. D., Arruda, P., and Garlid, K. D. (1998). *Biochim. Biophys. Acta* **1365**, 319–327.

Kageyama, H., Suga, A., Kashiba, M., Oka, J., Osaka, T., Kashiwa, T., Hiraon, T., Nemoto, K., Namba, Y., Ricquier, D., Giacobino, J. P., and Inoue, S. (1998). *FEBS Lett.* **4**, 450–453.

Kelley, L. J., Vacario, P. P., Thompson, G. C., Candelore, M. R., Doebber, T. W., Ventre, J., Wu, M. S., Meurer, R., Forrest, M. J., Conner, M. W., Cascieri, M. A., and Moller, D. E. (1998). *Endocrinology* **139**, 4920–4927.

Kielar, D., Clark, J. S., Ciechanowicz, A., Kurzawski, G., Sulikowski, T., and Naruszewicz, M. (1998). *Metabolism.* **47**, 844–847.
Klannemark, M., Orho, M., and Groop, L. (1998). *Eur. J. Endocrinol.* **139**, 217–223.
Klaus, S., Ely, M., Encke, D., and Heldmaier, G. (1995). *J. Cell. Sci.* **108**, 3171–3180.
Klaus, S., Munzberg, H., Truloff, C., and Heldmaier, G. (1998). *Amer. J. Physiol.* **274**, R287–R293.
Klingenberg, M., Winkler, E., and Huang, S. (1995). *Methods Enzymol.* **260**, 369–389.
Knott, R. M., Trayburn, P., and Hesketh, J. E. (1992). *Brit. J. Nutr.* **68**, 583–592.
Komiya, T., *et al.* (1997). *EMBO. J.* **16**, 4267–4275.
Kopecky, J., Clarke, G., Enerback, S., Spiegelman, B., and Kozak, L. P. (1995). *J. Clin. Invest.* **96**, 2914–2923.
Korshunov, S. S., Korkina, O. V., Ruuge, E. K., Skulachev, V. P., and Starkov, A. A. (1998). *FEBS Lett.* **435**, 215–218.
Kowaltowski, A. J., Costa, A. D., and Vercesi, A. E. (1998). *FEBS Lett.* **425**, 213–216.
Kozak, L. P., Britton, J. H., Kozak, U. C., and Wells, J. M. (1988). *J. Biol. Chem.* **263**, 12274–12277.
Kren, B. T., Trembley, J. H., Krarewski, S., Behrens, T. W., Reed, J. C., and Steer, C. J. (1996). *Cell Growth Diff.* **7**, 1633–1642.
Kroemer, G., Dallaporta, B., and Resche-Rignon, M. (1998). *Annu. Rev. Physiol.* **60**, 619–642.
Kubota, T., Mori, H., Tamori, U., Okazawa, H., Kukuda, T., Midi, M., Ito, C., Fleury, C., Bouillaud, F., and Kasuga, M. (1998). *J. Clin. Endocrinol Metab.* **83**, 2800–2804.
Kushi, A., Sasai, H., Koizumi, H., Takeda, N., Yodoyama, M., and Nakamura, M. (1998). *Proc. Natl. Acad. Sci. USA* **95**, 15659–15664.
Kutoh, E., Boss, O., Levasseur, F., and Giacobino, J. P. (1998). *Life Sci.* **62**, 445–451.
Lanni, A., DeFelice, M., Lombardi, A., Moreno, M., Fleury, C., Ricquier, D., and Goglia, P. (1997). *FEBS Lett.* **418**, 171–174.
Larkin, S., Mull, E., Miao, W., Pittner, R., Albrandt, K., Moore, C., Young, A., Denaro, M., and Beaumont, K. (1997). *Biochem. Biophys. Res. Commun.* **240**, 222–227.
Larrouy, D., Laharrague, P., Carrera, G., Viguerie-Bascands, N., Levi-Meyrueis, C., Pecqeur, C., Nibbelink, M., Andre, M., Casteilla, L., and Ricquier, D. (1997). *Biochem. Biophys. Res. Commun.* **235**, 760–764.
Lee, F.-Y., Li, Y., Yang, E. K., Yang, S. Q., Lin, H. Z., Trush, M. A., Dannenberg, A. J., and Diehl, A. M. (1999). *Amer. J. Physiol: Cell Physiol.* **276**, C386–C394.
Lee, F. Y. J., Li, Y., Yang, S. Q., Trush, M., and Diehl, A. M. (1999). *Hepatology,* **29**, 677–687.
Lemasters, J. J. (1998). *Gastroenterology* **115**, 783–786.
Levine, J. A., Eberhardt, N. L., and Jensen, M. D. (1999). *Science* **283**, 212–214.
Liu, Q., Solanes, G., Vidal-Puig, A., Grujic, D., Flier, J. S., and Lowell, B. B. (1997). *J. Biol. Chem.* **2720**, 25433–25436.
Liu, Q., Bai, C., Chen, F., Wang, R., MacDonald, T., Gu, M., Zhang, Q., Morsy, M. A., and Caskey, C. T. (1998). *Gene* **207**, 1–7.
Loffreda, S., Yang, S. Q., Lin, H. Z., Bulkley, G., Bregman, M., Nobel, P., and Diehl, A. M. (1998). *FASEB J.* **12**, 57–65.
Marzo, I., Brenner, C., Zamzami, N., Susin, S. A., Beutner, G., Brdiczka, D., Remy, R., Xie, Z. H., Reed, J. C., and Kroemer, G. (1998a). *J. Exp. Med.* **187**, 1261–1271.
Marzo, I., Brenner, C., Zamzami, N., Jurgensmeier, J. M., Susin, S. A., Vierira, H. L. A., Prevost, M.-C., Xie, Z., Matsuyama, S., Reed, J. C., and Kroemer, G. (1998b). *Science* **281**, 2027–2031.
Masaki, T., Yoshimatsa, H., Kakuma, T., Hidaka, S., Kurokawa, M., and Sakata, T. (1997). *FEBS Lett.* **418**, 323–326.
Matamala, J. C., Gianotti, M., Pericas, J., Quevedo, S., Roca, P., Palou, A., and Garcia-Palmer, F. J. (1996). *Biochem. J.* **319**, 529–534.
Matsuda, J., Hosoda, K., Itoh, H., Son, C., Doi, K., Tanaka, T., Fukunaga, Y., Inoe, G., Nishimura, H., Yoshimasa, Y., Yamori, Y., and Nakao, K. (1997). *FEBS Lett.* **418**, 200–204.
Matsuda, J., Hosoda, K., Itoh, H., Son, C., Doi, K., Hanaoka, I., Inoe, G., Nishimura, H., Yoshimasa, Y., Yamori, Y., Odaka, H., and Nakao, K. (1998). *Diabetes* **47**, 1809–1914.
Merrick, B. A., He, C., Witcher, L. L., Patterson, R. M., Reid, J. J., Pence-Pawlowski, P. M., and Selkirk, J. K. (1996). *Biochim. Biophys. Acta* **1297**, 57–68.
Mignotte, B., and Vayssiere, J. L. (1998). *Eur. J. Biochem.* **252**, 1–15.
Millet, L., Vidal, H., Andreelli, F., Larrouy, D., Riou, J. P., Ricquier, D., Laville, M., and Langin, D. (1997). *J. Clin. Invest.* **100**, 2665–2670.
Millet, L., Vidal, H., Larrouy, D., Andreelli, F., Lavill, M., and Langin, D. (1998). *Diabetologia* **41**, 829–832.
Modriansky, M., Murdza-Inglis, D. L., Patel, H. V., Freeman, K., and Garlid, K. D. (1997). *J. Biol. Chem.* **272**, 24759–24762.
Moriscot, A., Rabcio, R., and Bianco, A. C. (1993). *Amer. J. Physiol.* **265**, E81–E87.
Mory, G., Bouillaud, F., Combes-George, M., and Ricquier, D. (1984). *FEBS Lett.* **166**, 393–396.
Muzzin, P., Revelli, J. P., Ricquier, D., Meier, M. K., Assimacoupoulos-Jeannet, F., and Giacobino, J. P. (1989). *Biochem. J.* **261**, 721–721.
Narita, M., Shimizu, S., Ito, T., Chittenden, T., Lutz, R. J., Matusuda, H., and Tsujimoto, Y. (1998). *Proc. Natl. Acad. Sci. USA* **95**, 14681–14686.
Negre-Salvayre, A., Hirtz, C., Carrera, G., Cazenave, R., Troly, M., Salvayre, R., Penicaud, L., and Casteilla, L. (1997). *FASEB J.* **11**, 809–815.
Nicholls, D. G., and Ferguson, S. J. (1992). *Bioenergetics: An Introduction to the Chemiosmotic Theory* (2nd ed.), Academic Press, New York.
Nordfors, L., Hoffstedt, J., Nyberg, C., Thorne, A., Arner, P., Schalling, M., and Lonnqvist, F. (1998). *Diabetologia* **41**, 935–939.
Oberkofler, H., Liu, Y. M., Esterbauer, H., Hell, E., Drempler, F., and Patsch, W. (1998). *Diabetologia* **41**, 940–946.
Obregon, M. J., Pitamber, R., Jacobsson, A., Nedergaard, J., and Cannon, B. (1987). *Biochem. Biophys. Res. Commun.* **148**, 9–14.
Pastorino, J. G., Chen, S.-T., Tafani, M., Snyder, J. W., and Farber, J. L. (1998). *J. Biol. Chem.* **273**, 7770–7775.
Paulik, M. A., Buckholz, R. G., Lancaster, M. E., Dallas, W. S., Hull-Ryde, E. A., Weiel, J. E., and Lenhand, J. M. (1998). *Pharmacol. Res.* **15**, 944–999.
Polla, B. S., Kantengwa, S., Francois, D., Salvioli, S., Franceschi, C., Marsac, C., and Cossarizza, A. (1996). *Proc. Natl. Acad. Sci. USA* **93**, 6458–6463.
Puigserver, P., Vazquez, P., Bonet, M. I., Pico, C., and Palou, A. (1996). *Biochem. J.* **317**, 827–833.
Rashid, A., Wu, T.-C., Huang, C.-H., Chen, H.Z., Lin, H.Z., Yang, S.Q., Lee, F.Y.J., and Diehl, A. M. (1999). *Hepatology* **29**, 1131–1138..
Rehnmark, S., Nechad, M., Herron, D., B., C., and Nedergaard, J. (1990). *J. Biol. Chem.* **265**, 16464–16471.
Ricart, J., Egea, G., Izquierdo, J. M., San Martin, C., and Cuezva, J. M. (1997). *Biochem. J.* **324**, 635–643.
Ricquier, D. (1998). *Biochem. Soc. Trans.* **26**, 120–123.
Ricquier, D., Bouillaud, f., Toumelin, P., Mory, G., Bazin, R., J., A., and Penicaud, L.(1986). *J. Biol. Chem.* **261**, 13905–13910.
Samec, S., Seydoux, J., and Dulloo, A. G. (1998). *FASEB J.* **12**, 715–724.
Sanchis, D., Busquets, S., lvarez, B., Ricquier, D., Lopez-Soriano, F. J.,and Argiles, J. M. (1998). *FEBS Lett.* **436**, 415–418.
Scarpace, P. J., and Matheny, M. (1998). *Amer. J. Physiol.* **275**,E259–E264.

Schleiff, E., and Turnbull, J. L. (1998). *Biochemistry* **37**,13043–13051.

Shimabukaro, M., Zhou, Y. t., Lee, Y., and Unger, R. H. (1997). *Biochem. Biophys. Res. Commun.* **237**, 359–361.

Shimizu, S., Eguchi, Y., Kamiike, W., Funahashi, Y., Mignon, A., Lacronique, V., Matsuda, H., and Tsujimoto, Y. (1998). *Proc. Natl. Acad. Sci. USA* **95**,1455–1459.

Shimokawa, T., Kato, M., Watanabe, Y., Hirayama, R., Kurosaki, E., Shikama, H., and Hashimoto, S. (1998). *Bhichem. Biophys. Res. Commun.* **25**, 374–378.

Siegrist-Kaiser, C. A., Pauli, V., Juge-Aubry, C. E., Boss, O., Pernin, A., Chin, W. W.,Cusin, I., Rohner-Jeanrenaud, F., Burger, A. G., Zapf, J., and Meier, C. A. (1997). *J. Clin. Invest.* **100**, 2858–2864.

Silva, J. E., and Rabelo, R. (1997). *Eur. J. Endocrinol.* **136**, 251–164.

Simoneau, J. A., Kelley, D. E., Neverova, M., and Warden, C. H. (1998).*FASEB J.* **12**, 1739–1745.

Simonyan, R. A., and Skulachev, V. P. (1998). *FEBS Lett.* **436**, 81–84.

Skulachev, V. P. (1994). *Biochemistry* **59**, 1433–1434.

Skulachev, V. P. (1995). *Mol. Biol.* **29**, 709–715.

Skulachev, V. P. (1996a). *FEBS Lett.* **397**, 7–10.

Skulachev, V. P. (1996b). *Quart. Rev. Biophys.* **29**, 169–202.

Skulachev, V. P. (1997). *Biosci. Rept.* **17**, 347–366.

Stadler, J., Bentz, B. C., Harbrecht, B. G., Di silvio, M., Curran, R. D., Billiar, T. R.,Hoffman, R. A., and Simmons, R. L. (1992). *Ann. Surg.* **216**, 539–546.

Strack, A. M., Bradbury, M. J., and Dallman, M. F. (1995a). *Amer. J. Physiol.* **268**, R183–R191.

Strack, A. M., Horsley, C. J., Sebastian, R. J., Akana, S. F., and Dallman, M. F. (1995b). *Amer. J. Physiol.* **268**, R1209–R1216.

Strobel, A., Combettes-Souverain, M., Doare, L., Strosberg, A. D., and Issad, R. (1998).*Interm. J. Obesity Related Metab Disorders* **22**, 1121–1126.

Stucki, J. W. (1980). *Eur. J. Biochem.* **109**, 269–283.

Takayama, S., Krajewski, S., Krajewska, M., Kiada, S., Zapata, J. M., Kochel, K., Knee,D., Scudiero, D., Tudor, G., Miller, G. J., Miyashita, T.,Yamada, M., and Reed, J. C. (1998). *Cancer Res.* **58**, 116–131.

Tartaglia, L. A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Rchards, G. J., Campfield, L. A., Clark, F. T., Deeds, J., *et al.* (1995).*Cell* **83**(7), 1263–1271.

Teruel, T., Valverde, A. M., Navarro, P., Benito, M., and Lorenzo, M. (1998). *J. Cell Physiol.* **176**, 99–109.

Tsuboyama-Kasaoka, N., Tsunoda, N., Maruyama, K., Takahashi, M., Kim, H., Ikemoto,S., and Ezaki, O. (1998). *Biochem. Biophys. Res. Commun.* **274**, 498–503.

Urhammer, S. A., Dalgaard, L. T., Sorensen, T. I., Tybjaerg-Hanse, A., Echwald, S. M., Andersen, T., Clausen, J. O., and Pedersen, O. (1998). *Diabetologia* **41**, 241–244.

Walder, K., Norman, R. A., Hanson, R. L., Schrauwen, P., Neverova, M., Jenkinson, C. P., Easlick, J., Warden, C. H., Pecqueur, C., Raimbault, S., Ricquier, D., Silver, M. H. K., Shuldiner, A. R., Solanes, G., Lowell, B. B., Chung, W. K., Leibel, R. L., Pratley, R., andRavussin, E. (1998). *Human Mol. Genet.* **7**, 1431–1435.

Wiegle, D. S., Selfridge, L. I., Schwartz, M. W., Seeley, R. J., Cummings, D. E., Havel,P. J., Kuijper, J. L., and BeltrandelRio, H. (1998). *Diabetes* **47**, 298–302.

Wiesinger, H., Klaus, S., Heldmaier, G., Champigny, O., and Ricquier, D.(1990). *Can. J. Physiol. Pharmacol.* **68**, 195–200.

Winkler, E., Wachler, E., and Klingenberg, M. (1997). *Biochemistry* **36**,148–155.

Wojtczak, L., and Schonfeld, P., (1993). *Biochim. Biophys. Acta* **1183**, 41–57.

Yamada, M., Hashida, T., Shibusawa, N., Iwasaki, T., Murakami, M., Monden, T., Satoh,T., and Mori, M. (1998). *FEBS Lett.* **432**, 65–69.

Yoshitomi, H., Yamazaki, K., Abe, S., and Tanaka, I. (1998). *Biochem.Biophys. Res. Commun.* **253**, 85–91.

Yubero, P., Barbera, M. J., Alvarez, R., Vinal, O., Mampel, T., Iglesias, R., Villarroya, F.,and Giralt, M. (1998). *Mol. Endocrinol.* **12**, 1023–1037.

Zhou, Y. T., Shimabukuro, M., Koyama, K., Lee, Y., Wang, M. Y., Trieu, F., Newgard, C. B., and Unger, R. H. (1997). *Proc. Natl. Acad. Sci. USA* **94**,6386–6390.