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Uncoupling protein 1: a short-circuit in the chemiosmotic process

Richard K. Porter

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Abstract One of the significant naturally occurring short-circuits in the chemiosmotic process is manifest through the activity of uncoupling protein 1, most notably present in the inner membrane of mitochondria in brown adipose tissue. Much has been learnt about the mechanism of action of uncoupling protein 1 since its discovery in the 1970's, yet many areas of controversy surround (i) the mechanism of its action, (ii) novel regulators of its activity, and (iii) the tissue specificity of its expression. In this mini-review, areas of controversy are highlighted from the aforementioned categories using selected key experiments to demonstrate the arguments.

Introduction

Uncoupling protein 1 (UCP 1 also known as UCP and thermogenin) is a 33 kDa mitochondrial inner membrane protein and has classically been associated with brown adipose tissue (BAT) of mammals and human infants (and in adult human patients with phaeochromocytoma). Sympathetic innervation of BAT, usually as a result of cold adaptation/acclimation, results in dissipation of the proton electrochemical gradient (Δp) across the mitochondrial inner membrane by UCP 1. The resulting futile cycle of proton pumping and proton leak increases flux through fatty acid oxidation and increases electron transport chain activity. The heat generated from the increase in metabolic flux defines the molecular basis of non-shivering thermogenesis in mammals. Extensive blood supply to the brown adipose tissue ensures that the whole animal (and human infant) is kept warm under cold conditions (for reviews see: Nicholls and Locke 1984; Nicholls 2001; Cannon and Nedergaard 2004; Nicholls 2006).

The mechanism behind the noradrenalin signal that activates mature brown adipose tissue is interesting and multifaceted in that there are three types of adrenergic receptor on the surface of mature brown adipocytes (β, α_2 and α_1), all of which are associated with activation of brown fat (reviewed in Cannon and Nedergaard 2004). β_3-adrenergic receptors are the most significant β-receptors in terms of mature brown adipocyte activation, certainly in rats and mice. Noradrenalin and agonists such as BRL-37344 (Arch et al. 1984) and CL-316243 (Himms-Hagen et al. 1994) have all been used to activate brown adipocytes through the β_3-receptor in vitro. The β_3-adrenergic receptor is coupled to a G-protein activated adenylate cyclase. The noradrenalin induced increase in intracellular cAMP levels activate protein kinase A which phosphorylates a series of proteins in the cytosol, and possibly mitochondria through A-kinase anchoring proteins (Sardinalli et al. 2006; Dyson et al. 2008). Thermogenesis in BAT is dependent on protein kinase A phosphorylation of perilipin which facilitates access of hormone sensitive lipase to intracellular fat stores, releasing free fatty acids into the cytosol (Souza et al. 2007). So, in short, activation of UCP 1 in vivo requires interaction with free fatty acids and the ability of free fatty acids to overcome the inhibition of UCP 1 by purine nucleotides (at millimolar concentrations inside the cell).

Apart from the β_3-adrenergeic response, brown adipocytes also have α_2-adrenergeic receptors. Inhibitors of the α_2-adrenergic receptor response have been shown to enhance the β_3-stimulated cAMP response (Bronnikov et al. 1999a), implying that the α_2-adrenergic response can be antagonistic to the β_3-response. α_1-adrenergic receptors are

R. K. Porter (✉)
School of Biochemistry and Immunology, Trinity College Dublin, Dublin 2, Ireland
e-mail: rkporter@tcd.ie

abundant on the surface of brown adipocytes, are predicted to result in mobilisation of intracellular $Ca^{2+}$and appear also to be antagonist to the β_3-response (Bronnikov et al. 1999b). However, Ca^{2+} is a stimulator of metabolism in mitochondria e.g. Krebs cycle enzymes and glycerol-3-phosphate dehydrogenase, and would be predicted to stimulate mitochondrial phosphatases (Lee et al. 2005; Hopper et al. 2006). Clearly, the activation of brown adipose tissue results from a balance between the β- and α-receptor responses and the activation of UCP 1 by free fatty acids, coupled with activation of metabolism in mitochondria is consistent with the thermogenic response in BAT following sympatheic innervation.

Mechanism of action of UCP 1

The nature of the fatty acid dependency and purine nucleotide inhibition of proton leak, through UCP 1, is crucial to the understanding of the process of non-shivering thermogenesis. Consequently, this has been investigated at the cellular and subcellular level as well as at the reductionist level of (native) UCP 1 reconstituted into liposomes membranes. It has long been known that mitochondria isolated from active BAT are essentially uncoupled, but on addition of purine nucleotides, oxygen consumption rates due to the UCP 1 catalysed proton leak are inhibited (reviewed in Nicholls and Locke 1984; Cannon and Nedergaard 2004). It has been clearly demonstrated that addition of nanomolar concentrations of long chain free fatty acids can alleviate the purine nucleotide inhibition of isolated BAT mitochondria (Nicholls and Rial 1999). This evidence for fatty acid dependent alleviation of purine nucleotide inhibition of UCP 1 activity at the subcellular level is consistent with observations at the cellular level for UCP 1 activation. Some of the most convincing studies, demonstrating fatty acid dependent UCP 1 activity emanate from work on brown adipocytes isolated from wild-type and UCP 1 knock-out mice (summarized in Cannon and Nedergaard 2004). Brown adipocytes from UCP 1 wild-type mice clearly display an increase in oxygen consumption on addition of noradrenaline, whereas there is no increase in oxygen consumption rates on addition of noradrenaline to brown adipocytes from UCP 1 knock-out mice. Moreover, the fatty acid dependency of UCP1 is further highlighted by the observation that brown adipocytes from UCP 1 wild-type mice clearly display an increase in oxygen consumption on addition of oleate, whereas brown adipocytes from UCP 1 knock-out mice do not respond to oleate to the same extent.

However the nature by which free fatty acids are involved in the UCP uncoupling process is controversial. The two main models for the role of fatty acids in the mechanism of action of UCP 1 are (a) that UCP 1 acts as a proton conduit across the mitochondrial inner membrane and importantly that fatty acids act as cofactors/activators providing an addition carboxyl group at a key intramembrane site enhancing the rate of proton movement (Fig. 1a)

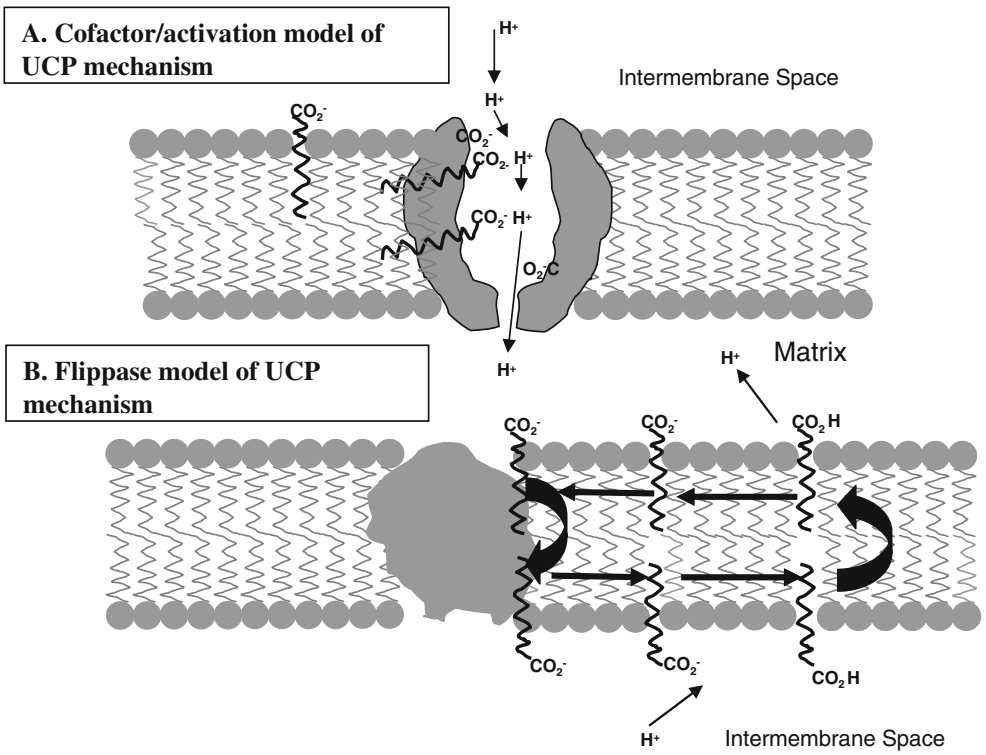

Fig. 1 The two main models for the role of fatty acids in the mechanism of action of UCP 1

(for reviews see Klingenberg and Huang 1999; Nicholls and Rial 1999). The second model (b) proposes that protonated fatty acids freely flip across the mitochondrial inner membrane, independently of UCP 1, and uncouple the mitochondria. In this instance, uncoupling proteins act as 'flippases' translocating the resulting anionic fatty acids back across the bilayer leaflets of the mitochondrial inner membrane (Fig. 1b)(for review see Garlid et al. 2001). This latter mechanism was originally proposed by Skulachev (1991) and has been championed by Garlid and Ježek (Ježek and Garlid 1990; Garlid et al. 2000). Thus in this latter model, UCP 1 doesn't transport protons *per se* but facilitates a cycle of uncoupling by free fatty acids.

Remarkably, there is evidence for both models. A key piece of evidence, for the cofactor/activator model, stems from tabulations in two reviews by Klingenberg reporting that the chemically modified, unflippable yet α-carboxyl unmodified fatty acid, glucose pyranoside-O-ω-palmitate were able to "activate" native reconstituted UCP 1 present in liposome membranes (Klingenberg and Huang 1999; Klingenberg et al. 1999). In fact, the rate of proton translocation through UCP 1 in the presence of glucose pyranoside-O-ω-palmitate was shown to be twice that of using palmitate alone. Clearly if UCP 1 was a flippase then glucose pyranoside-O-ω-palmitate should not work because, of the hydrophilic glucose on the ω-end of the fatty acid. Unfortunately, no information was published by Klingenberg for the synthesis of glucose pyranoside-O-ω-palmitate nor indeed was the original data published demonstrating that glucose pyranoside-O-ω-palmitate could "activate" UCP 1.

In contrast, the key evidence for the flippase model stems from the many observations that native UCP 1 reconstituted into liposome membranes can transfer charge across the liposome membrane using fatty acids and most notably a non-protonatable fatty acid analogue, namely undecanesulphonate ($C_{11}SO_3^-$, pK~ 2)(reviewed in Garlid et al. 2000). A requirement for the cofactor/activator model is a protonatable and deprotonatable carboxyl group provided by the α-carboxyl of long chain fatty acids. The sulphonate group on undecanesulphonate is unprotonatable at neutral pH and therefore should not facilitate proton translocation/charge transfer according to the cofactor/activation model. Yet the only logical explanation for the observed (GDP-sensitive) charge transfer in the presence of undecanesulphonate is that UCP 1 flipped the anionic fatty acid analogue across the lipid bilayer.

In an endeavour to reconcile the two models, our laboratory revisited the aforementioned key pieces of evidence for both models. Using liposomes containing native UCP 1, it was possible to confirm that undecanesulphonate could catalyse charge transfer across liposome membranes, in accordance with the key evidence for the "flippase" model. In the case of the key evidence for the cofactor/activation model we had to synthesize glucose pyranoside-O-ω-palmitate (or glucose-O-ω-palmitate as denoted in our publications) de novo. Importantly, it was possible to demonstrate that the de novo synthesized glucose-O-ω-palmitate had >93% purity and was stable at neutral pH (Gouin et al. 2005). Dramatically, and in contrast to the data reported by Klingenberg in his reviews, we demonstrated that glucose-O-ω-palmitate could not "activate" proton translocation in liposomes containing native reconstituted UCP 1 nor could it "activate" oxygen consumption by mitochondria from brown adipose tissue in the presence of purine nucleotides (Breen et al. 2006). Although these latter observations do not distinguish between the two models, they do question key evidence for the cofactor/activation model for UCP 1.

Controversy also surrounds the nature of the interaction between the fatty acids and the purine nucleotides on UCP 1. Using isolated mitochondria from BAT Shabalina et al. (2004) demonstrated that oleate competes with GDP for the GDP-binding site. This conclusion however, is at odds with data for isolated BAT mitochondria demonstrating that nanomolar concentrations of fatty acids can alleviate inhibition of UCP1 at micromolar concentrations of purine nucleotide, an observation which intuitively suggests an allosteric interactions between two separate binding sites, one for purine nucleotides and one for fatty acids. Two independent binding sites are also inferred from structural studies. Like all mitochondrial inner membrane transporters so far characterized, UCP 1 is predicted to have a tripartite structure (Klingenberg et al. 1999). Photoaffinity studies suggest that residues in the third domain and the C terminus are required for purine nucleotide binding (Klingenberg et al. 1999). Chimeric studies have predicted that the central domain is required for fatty acid binding (Jimenez-Jimenez et al. 2006).

Another area of controversy, under the heading of "UCP 1 mechanism", relates to the requirement of ubiquinone for UCP 1 activity. As already mentioned, native UCP 1 from rat or hamster BAT can be reconstituted into liposomes (Klingenberg and Huang 1999; Nicholls and Rial 1999; Garlid et al. 2001) and using a fluorimeter and pH-sensitive fluorescent dyes, GDP sensitive, fatty acid dependent proton (and chloride) transport through UCP1 can be demonstrated. In addition to natively purified UCP1, hamster, rat and human UCP 1 (Breen et al. 2006) has also been expressed in *Escherichia coli* and reconstituted in liposomes where proton (and chloride) transport studies were undertaken. Data using the UCP 1 reconstitution and assay systems in the Klingenberg laboratory show that proton flux is fatty acid (micromolar) dependent, ubiquinone dependent and inhibited by nanomolar purine nucleotide concentrations (Echtay et al. 2000). Chloride flux could also be measured.

On the other hand data from the Garlid laboratory using a different reconstitution system and assay systems, gave different results. In this instance, native UCP 1 or *E. coli* or yeast expressed UCP 1 were reconstituted into liposomes and a fluorimeter using indirect measurements of proton flux and charge movement [to assay flippase activity/ uncoupling activity] was undertaken. Chloride flux was not measured but proton flux was shown to be dependent on micromolar fatty acids and nucleotide sensitive (micromolar) (Jabůrek et al. 1999; Jabůrek and Garlid 2003). The bottom line was that flippase activity showed no ubiquinone dependence. The discrepancy over ubiquinone dependency may reflect methodological difference in UCP 1 reconstitution and assay systems between the Garlid and Klingenberg laboratories.

Novel regulators of UCP 1 activity

A relatively recent protagonist in the area of UCP 1 function has been the discovery that fatty acid breakdown products (e.g. 4-hydroxy-2-nonenals) can activate UCP 1 and other mitochondrial transporters, presumably through direct covalent modification (Echtay et al. 2003; Esteves and Brand 2005). 4-hydroxy-2-nonenals can occur as a result of superoxide damage to polyunsaturated fatty acids associated with phospholipids in membranes. Consequently, a role for UCP's in increasing proton leak to reduce free radical production by mitochondria has been proposed, particularly for UCP's other than UCP 1. However data also exist demonstrating that 4-hydroxl-2-nonenals increase proton leak across the mitochondrial inner membrane of BAT mitochondria from UCP1 wild-type and UCP1 knock-out mice suggesting that the proton leak catalyzed by these aldehydes is a non-UCP specific leak (Shabalina et al. 2006).

More recently, further evidence for covalent modification of UCP1 has come from our laboratory (Carroll et al. 2008). Mass spectrometry has identified phosphorylation on serine 51 in UCP1 purified from cold-acclimated rats. Furthermore, immunoblot analysis of UCP 1 purified from BAT mitochondria, using antibodies to serine phosphate, demonstrates that there is a greater proportion of serine phosphorylation associated with UCP 1 purified from cold-acclimated rats when compared to rats kept at room temperature. Our observation is consistent with the possibility of mitochondrial phosphorylation events via protein kinase A downstream of noradrenalin activation of brown adipose tissue (Sardinalli et al. 2006; Dyson et al. 2008), but inconsistent with the predicted dephosphorylation of mitochondrial proteins through the same noradrenalin event (Lee et al. 2005). Furthermore it is hard to postulate what role phosphorylation of serine 51 might have in UCP 1 function. As already mentioned the third domain and the C terminus of UCP1 are required for purine nucleotide binding (Klingenberg et al. 1999). The central domain is required for fatty acid binding (Jimenez-Jimenez et al. 2006). Serine 51 is in the first domain, so the role of this domain or the significance associated with its phosphorylation has yet to be determined.

Tissue specificity of constitutively expressed UCP 1

There have been two reports of constitutive UCP 1 protein expression in tissues other than BAT. Detection of UCP 1 in uterine longitudinal smooth muscle cells was reported by Nibbelink et al. (2001) using (a) a commercially available peptide antibodies (11A) to the C terminus of UCP 1 and (b) antibodies raised to full-length rat UCP 1 (Ricquier et al. 1983). However, investigations by Rousset et al. (2003) challenged these data based on the non-specificity of the 11A peptide antibodies to the C terminus of UCP 1 used by Nibbelink et al. (2001).

Our laboratory has recently discovered UCP 1 in mitochondria from rat and mouse whole thymus and thymocytes (Carroll et al. 2004; Carroll et al. 2005; Porter, 2006). The thymus is the site of T-helper and cytotoxic T-cell maturation and selection (Ritter and Crispe 1992). There is a sizeable (>90%) attrition rate of thymocytes, by apoptosis, in the thymus as a result of positive and negative selection. Evidence for the existence of UCP 1 in thymus includes (a) reverse transcriptase-polymerase chain reaction detection of RNA transcripts for UCP 1 in whole thymus and in isolated thymocytes of rats and mice (thymocytes were not contaminated with brown adipocytes, but contained >99% Thy-1 (CD90) positive lymphocytes as identified by FACS analysis and cell size (Cinti et al. 1997; Adams et al. 2008), (b) antibodies specific to UCP 1 peptide, detected protein of appropriate molecular mass in mitochondria isolated from whole thymus and thymocytes of rats and mice, but not in thymus mitochondria from UCP 1 knock-out mice (c) confocal images of UCP 1 associated with mitochondria in situ in thymocytes from wild-type but not UCP 1 knock-out mice (Adams et al. 2007, 2008).

The existence of constitutively expressed UCP 1 in thymus has been challenged by Frontini et al. (2007) who showed histological/confocal images of UCP 1 detection associated with BAT in the vicinity of thymus tissue from mouse/rat, using antibodies to full-length UCP 1. These authors suggest that any detection of UCP 1 in thymus is solely due to associated BAT. However, it has been clearly demonstrated that the full-length antibody used by Frontini et al. (2007) is not specific for UCP 1 as it detects many unidentified proteins (Adams et al. 2008). However, although the role of UCP 1 in brown adipose tissue in

mammals is non-shivering thermogenesis, it does not have that role in thymus (Brennan et al. 2006). Ongoing phenotypic studies aim to decipher its role in thymus function.

In summary, UCP 1 was identified over 30 years ago, yet key elements of its mechanism, regulation and tissue localization are still not fully known but are being fervently investigated.

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References

Adams AE, Hanrahan O, Nolan DN, Voorheis HP, Fallon PG, Porter RK (2007) Biochim Biophys Acta 1777:115–117
Adams AE, Carroll AM, Fallon PG, Porter RK (2008) Biochim Biophys Acta 1777:772–776
Arch JAT, Ainsworth AT, Cawthorne MA, Piercy V, Sennitt MV, Thody VE, Wilson C, Wilson S (1984) Nature 309:163–165
Breen EP, Gouin S, Brennan CM, Murphy AF, Haines LR, Pearson AM, Jackson TW, Murphy PV, Porter RK (2006) J Biol Chem 281:2114–2119
Brennan CM, Breen EP, Porter RK (2006) Biochim Biophys Acta 1757:1463–1468
Bronnikov GE, Bengtsson T, Kramarova L, Golozoubova V, Cannon B, Nedergaard J (1999a) Endocrinol 110:47–50
Bronnikov GE, Zhang S-J, Cannon B, Nedergaard J (1999b) J Biol Chem 274:37770–37780
Cannon B, Nedergaard J (2004) Physiol Rev 84:277–359
Carroll AM, Haines LR, Pearson TW, Brennan C, Breen EP, Porter RK (2004) Biochem Soc Trans 32:1066–1067
Carroll AM, Haines LR, Pearson TW, Fallon PG, Walsh C, Brennan C, Breen EP, Porter RK (2005) J Biol Chem 280:15534–15543
Carroll AM, Porter RK, Morrice NA (2008) Biochim Biophys Acta 1777:1060–1065
Cinti S, Frederich RC, Zingaretti MC, De Matteis R, Flier JS, Lowell RR (1997) Endocrinol 138:797–804
Dyson MT, Jones JK, Kowalewski MP, Manna PR, Alonso M, Gottesman ME, Stocco DM (2008) Biol Reprod 78:267–277
Echtay KS, Winkler E, Klingenberg M (2000) Nature 408:609–613
Echtay KS, Esteves TC, Pakay JL, Jekabsons MB, Lambert AJ, Portero-Otin M, Pamplona R, Vidal-Puig AJ, Wang S, Roebuck SJ, Brand MD (2003) EMBO J 22:4103–4110
Esteves TC, Brand MD (2005) Biochim Biphys Acta 1709:35–44
Frontini A, Rousset S, Cassard-Doulcier AM, Zingaretti C, Ricquier D, Cinti S (2007) J Hist Cytochem 55:183–189
Garlid KD, Jabůrek M, Ježek P, Vařecha M (2000) Biochim Biophys Acta 1459:383–389
Garlid KD, Jabůrek M, Ježek P (2001) Biochem Soc Trans 29:803–806
Gouin S, Pilgrim W, Porter RK, Murphy PV (2005) Carb Res 340:1547–1552
Himms-Hagen J, Cui J, Danforth E, Taatjes DJ, Lang SS, Waters BL, Claus TH (1994) Am J Physiol Regul Integr Comp Physiol 226: R1371–R1382
Hopper RK, Carroll S, Aponte AM, Johnson DT, French S, Shen RF, Witzmann FA, Harris RA, Balaban RS (2006) Biochemistry 45:2524–2536
Jabůrek M, Garlid KD (2003) J Biol Chem 278:25825–25831
Jabůrek M, Vařecha M, Gimeno RE, Dembski M, Ježek P, Zhang M, Burn P, Tartaglia LA, Garlid KD (1999) J Biol Chem 274:26003–26007
Ježek P, Garlid KD (1990) J Biol Chem 265:19303–19311
Jimenez-Jimenez J, Ledesma A, Zaragoza P, Gonzalez-Barroso MM, Rial E (2006) Biochim Biophys Acta 1757:1292–1296
Klingenberg M, Huang S-G (1999) Biochim Biophys Acta 1415:271–296
Klingenberg M, Echtay KS, Bienengraeber M, Winkler E, Huang SG (1999) Int J Obesity Rel Metab Disord 23:S24–S29
Lee I, Salomon AR, Ficarro S, Mathes I, Lottspeich F, Grossmann LI, Hütteman M (2005) J Biol Chem 280:6094–6100
Nibbelink M, Moulin K, Arnaud E, Duval C, Penicaud L, Casteilla L (2001) J Biol Chem 276:47291–47295
Nicholls DG (2001) Biochem Soc Trans 29:751–755
Nicholls DG (2006) Biochim Biophys Acta 1757:459–466
Nicholls DG, Locke RM (1984) Physiol Rev 64:1–64
Nicholls DG, Rial E (1999) J Bioenerg Biomembr 5:399–406
Porter RK (2006) Biochim Biophys Acta 1757:446–448
Ricquier D, Barlet JP, Garel JM, Combes-George M, Dubois MP (1983) Biochem J 210:859–866
Ritter MA, Crispe IN (1992) The Thymus. In: Rickwood D, Male D (eds.) IRL Press at Oxford University Press, Oxford
Rousset S, Alves-Guerra M-C, Ouadghiri-Bencherif S, Kozak LP, Miroux B, Richard D, Bouillaud F, Ricquier D, Cassard-Doulcier A-M (2003) J Biol Chem 278:45843–45847
Sardinalli AM, Signorile A, Nuzzi R, De Rasmo D, Technikova-Dobrova Z, Drahota Z, Occhiello A, Pica A, Papa S (2006) FEBS Letts 580:5690–5696
Shabalina IG, Jacobsson A, Cannon B, Nedergaard J (2004) J Biol Chem 279:38236–38248
Shabalina IG, Petrovic N, Kramarova TV, Hoeks J, Cannon B, Nedergaard J (2006) 281:13882–13889
Skulachev VP (1991) FEBS Letts 294:158–162
Souza SC, Christoffolete MA, Ribeiro MO, Miyoshi H, Strissel KJ, Stancheva ZS, Rogers NH, D'Eon TM, Perfield JW, Imachi H, Obin MS, Bianco AC, Greenberg AS (2007) J Lipid Res 48:1273–1279