Endogenous mutations in human uncoupling protein 3 alter its functional properties

Angela M. Brown[a], Joseph W. Dolan[b], Steven M. Willi[c], W. Timothy Garvey[a], George Argyropoulos[a,*]

[a] *Department of Medicine, Division of Endocrinology, Medical University of South Carolina and the Ralph H. Johnson Veterans Affairs Medical Center, 114 Doughty Street, Charleston, SC 29425, USA*

[b] *Department of Microbiology and Immunology, Medical University of South Carolina and the Ralph H. Johnson Veterans Affairs Medical Center, Charleston, SC 29425, USA*

[c] *Department of Pediatrics, Medical University of South Carolina and the Ralph H. Johnson Veterans Affairs Medical Center, Charleston, SC 29425, USA*

Received 10 November 1999; received in revised form 26 November 1999

Edited by Vladimir Skulachev

Abstract Human uncoupling protein 3 (UCP3) is a mitochondrial transmembrane carrier that uncouples oxidative phosphorylation and is a candidate gene for obesity. Expression of native human UCP3 mutations in yeast showed complete loss (R70W), significant reduction (R143X), or no effect (V102I and IVS6+1G > A) on the uncoupling activity of UCP3. It is concluded that certain mutations in UCP3 alter its functional impact on membrane potential ($\Delta\Psi$), possibly conferring susceptibility to develop metabolic diseases.

Key words: Mitochondrion; Obesity; Membrane potential; UCP3

1. Introduction

Uncoupling proteins are mitochondrial transporters that uncouple mitochondrial respiration from ATP production. Uncoupling protein 1 (*UCP1*) is expressed in brown adipose tissue and is involved in non-shivering and diet-induced thermogenesis [1]. Uncoupling protein 2 (*UCP2*), on the other hand, is widely expressed [2] while uncoupling protein 3 (*UCP3*) is predominantly expressed in skeletal muscle [3,4], a major tissue contributing to non-shivering thermogenesis in humans. *UCP3* shows high amino acid similarities with *UCP1* (57%) and *UCP2* (73%) [5]. Both *UCP2* and *UCP3* are located on chromosome 11 (11q13) within close proximity to each other [4]. The *UCP1*, *UCP2*, and *UCP3* genes consist of six coding exons, each of which encodes a putative transmembrane domain [4,6,7], and *UCP1* has been shown to function as an uncoupler of mitochondrial respiration when expressed in yeast [8,9]. *UCP3* encodes two forms of transcripts: $UCP3_L$ that encompasses all six transmembrane domains and $UCP3_S$ that is missing the sixth coding exon via an alternative polyadenylation site [4]. *UCP3* mRNA levels were shown to be increased by thyroid hormone, β3-adrenergic agonists, leptin, fasting, fat feeding and cold exposure [5,10–12], underscoring its potential role in energy balance.

Mutations and polymorphisms in *UCP3* have recently been identified in humans [13,14]. A nucleotide substitution that resulted in a glycine being replaced by a serine at position 84 in a healthy female was reported [13]. In addition, two polymorphisms, V102I and exon 6 splice donor (IVS6+1G > A), and a mutation, R143X, were detected in obese African-Americans [14]. Heterozygotes for the IVS6+1G > A polymorphism were found to have a 50% reduction in fat oxidation and a significant elevation in the non-protein respiratory quotient [14], both of which are metabolic traits that increase risk of future weight gain. It is noteworthy that the IVS6+1G > A polymorphism results in the insertion of a premature stop codon and the resultant protein product is identical to the short isoform of *UCP3*. Interestingly, the two polymorphisms, V102I and IVS6+1G > A, were identified in African-Americans and the Mende tribe of Sierra Leone but not in Caucasians [14]. Recently, we identified a rare mutation in *UCP3*, R70W, in a severely obese and diabetic 15 year old male of Chinese descent [15]. Alignments showed that the mutated valine and arginine residues in V102I and R70W are completely conserved in all UCPs reported to date including the plant UCP (PUMP) suggesting that these residues may play an important functional role.

In this report, the impact of endogenous mutations on mitochondrial uncoupling activity of *UCP3* when expressed in yeast was examined. We report the effect of the four mutant constructs (V102I, R70W, R143X, and IVS6+1G > A) on yeast mitochondria. We also discuss the effects of $UCP3_L$ and the four *UCP3* mutants on yeast cell growth.

2. Materials and methods

2.1. Construction of the expression vectors

The entire coding region of *UCP3* was amplified from first strand cDNA using the expand long template PCR kit (Boehringer-Mannheim, Indianapolis, IN, USA) according to the manufacturer's protocol with the following primer pair: *UCP3F* 5′-tcgctctcgagcctctctccttggacctc-3′ and *UCP3R* 5′-agtatcatcagcggccgcgatgcaccgttttcttccat-3′. These primers contain the recognition sites for *Xho*I and *Not*I, respectively. The 1017 bp PCR product was cloned into the pYES2 (Invitrogen, Carlsbad, CA, USA) yeast expression vector between the *Xho*I and *Not*I sites. To create the exon 6 splice donor mutation, PCR was performed using UCP3F and the following reverse primer containing a *Not*I site: 5′-agtatcatcagcggccgccccttgtagaaggctgtgg-3′. To create the C208T and G304A mutations, the ExSite PCR-based site-

*Corresponding author. Fax: (1)-843-953 6444.
E-mail: argyrog@musc.edu

Abbreviations: UCP, uncoupling protein; XTT, sodium salt of (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt); $DiOC_6$, 3,3′-dihexyloxacarbocyanine iodide; EDTA, ethylenediaminetetraacetic acid; $\Delta\Psi$, membrane potential

0014-5793/99/$20.00
PII: S0014-5793(99)01708-1

directed mutagenesis kit was used according to the manufacturer's instructions (Stratagene, La Jolla, CA, USA) with the following primers: *C208TF* 5′-tggactgagggtccctgcagcccct-3′ and *C208TR* 5′-cacaatggtcaggatggtgcccagc-3′; *G304AF* 5′-atcaagcaggtgtacacccccaaa-3′ and *G304AR* 5′-ggagtcatagaggccgatgcggatg-3′. PCR was performed with the following primers to create the C427T mutation: *C427TF* 5′-tgatttcaggccagcatacacctcg-3′ and *C427TR* 5′-gaccttcaccacatctgtggctgg-3′. All clones were sequenced manually with the Amplicycle DNA sequencing kit according to the manufacturer's instructions (Perkin-Elmer, Foster City, CA, USA) to confirm the presence of the targeted mutations.

2.2. Expression in Saccharomyces cerevisiae

The haploid yeast strain W303-1A (*MAT a*: *ade2-2; trp1-1; can1-100; leu2-3, 112; his 3-11, 15; ura3-1*) was transformed with each of the cloned mutations as well as with $UCP3_L$ and pYES2 vector. Transformed yeast was selected after 2 days of growth at 30°C on SC-ura plates [16]. A representative number of clones were selected and further plated to obtain isolated colonies that were used in subsequent experiments. Expression of $UCP3_L$ and mutant *UCP3* under the control of the *GAL1* promoter was induced by growth on 2% galactose in the absence of glucose. To perform the XTT analysis, the yeast was grown overnight in SSuc-ura and for 6 h in SGal-ura before the analysis. For the flow cytometry analysis, the yeast was grown overnight at 30°C in 2 ml of SC-ura. Following the overnight growth, 0.2 ml was transferred to 2 ml of SSuc-ura and grown for approximately 15 h. After 15 h, an A_{600} reading was taken and 1×10^6 cells were added to 10 ml of SGal-ura and grown for 6 h before the analysis.

2.3. XTT analysis

After cells were grown as indicated above, 1×10^6 cells from each culture were diluted in 200 μl of SC-ura containing 2% galactose in eight wells of a 96-well plate (Falcon, Lincoln Park, NJ, USA). The sodium salt of (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt) (XTT) was added according to the manufacturer's recommendations (Sigma, St. Louis, MO, USA). Plates were incubated for 4 h at 30°C. Absorbances at 650 nm and 450 nm were read using the V_{max} kinetic microplate reader (Molecular Devices, Menlo Park, CA, USA). The reference absorbance read at 650 nm was subtracted from the 450 nm reading.

2.4. Flow cytometry

Yeast cells were grown as indicated above, then 2 ml of cells were transferred to appropriate tubes for analysis in the Epics XL-MCL flow cytometer (Beckman-Coulter, Miami, FL, USA). The potential sensitive dye 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$; Molecular Probes, Eugene, OR, USA) was added to each set of tubes to a final concentration of 1 μM approximately 30 min before analysis. To an additional set of tubes, the chemical uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP; Sigma, St. Louis, MO, USA) was added to a final concentration of 10 μM 10 min before the addition of $DiOC_6$. Immediately before flow cytometry, 100 μl of 0.5 M EDTA was added to the cells, then the cells were sonicated for approximately 10 s in order to prevent clumping of the cells. The count rate was automatically set at 50 000 objects. For the expression of results, the pulse integral of fluorescence on a three-order logarithmic scale was used.

2.5. Nomenclature

Gene names and mutation/polymorphism symbolisms used in this manuscript were according to Stylianos E. Antonarakis and the Nomenclature Working Group. Details can be found on the following URL: http://journals.wiley.com/1059-7794/nomenclature.html.

3. Results

3.1. Expression of $UCP3_L$ and mutant UCP3 and effects on yeast growth

$UCP3_L$ was cloned into the pYES2 expression vector. Targeted in vitro mutagenesis was then used to create the four mutations: R70W, R143X, V102I, and IVS6+1G > A. $UCP3_L$ and mutant inserts in the pYES2 vector were under the control of the inducible GAL1 promoter; therefore, expression of *UCP3* was repressed by growth on glucose and induced by growth on galactose. Uniform growth of the yeast was observed when the transformants were plated on complete medium containing glucose as the carbon source (Fig. 1A). However, after induction of expression of the *UCP3* gene by growth on medium containing galactose as the carbon source, different growth patterns were observed among transformants expressing the various mutants (Fig. 1B). The pYES2 and R70W transformants were able to grow on minimal medium containing galactose as the carbon source (Fig. 1B). No growth of the other transformants was observed under the same conditions (Fig. 1B). To further study the effect of expression of the $UCP3_L$ and mutant *UCP3* proteins, growth rates in liquid medium containing 2% galactose were monitored by optical density readings at 600 nm (Fig. 2). The OD is an indirect measure of cell density. Yeast cells transformed with the pYES2 vector grew logarithmically after 2 h while R70W transformants began to grow logarithmically after 4 h of inoculation (Fig. 2). No noticeable increase in growth was seen in the $UCP3_L$, V102I, R143X and IVS6+1G > A transformants after 10 h of induction (Fig. 2).

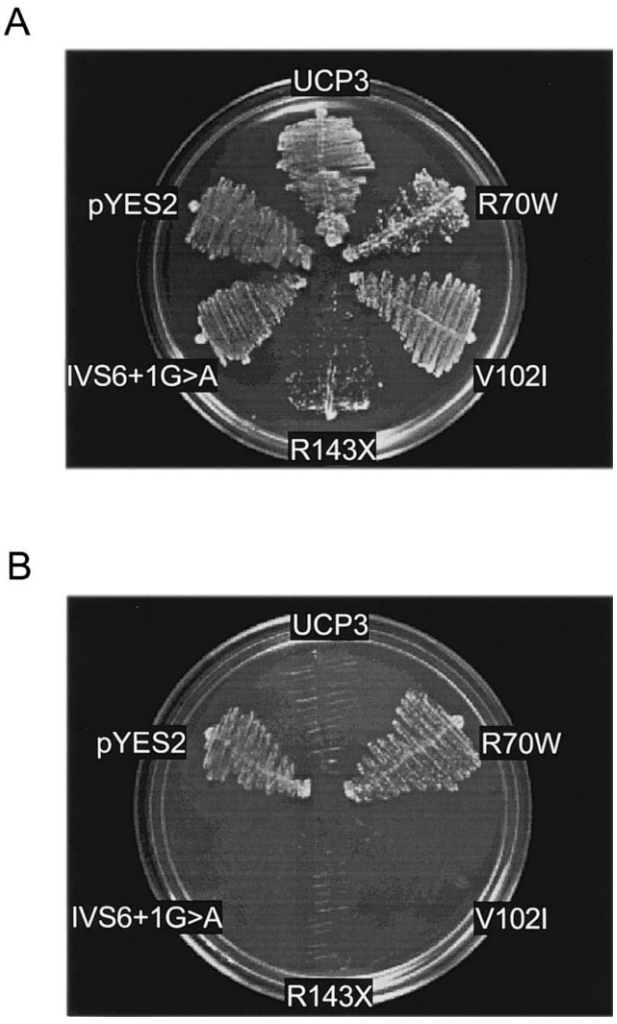

Fig. 1. Comparison of yeast growth of transformants expressing human wild-type and mutant *UCP3* constructs. A: Growth of yeast on medium containing 2% glucose. B: Growth of yeast on medium containing 2% galactose.

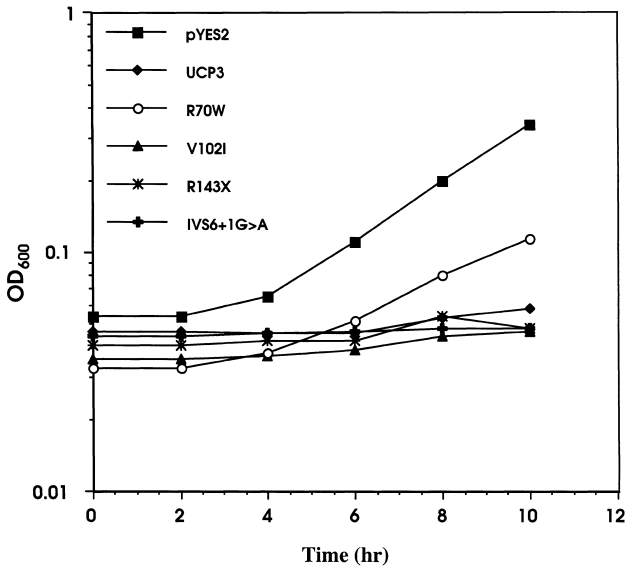

Fig. 2. Semi-log plot of yeast growth in liquid medium containing 2% galactose in a 10 h period.

3.2. Effects of $UCP3_L$ and mutant UCP3 proteins on mitochondrial respiratory activity

To examine the effect of the *UCP3* mutant proteins on mitochondrial respiration, the reduction of the sodium salt of (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt) (XTT) by mitochondrial dehydrogenase enzymes was measured since a direct relationship exists between XTT-formazen formation and mitochondrial respiratory (i.e. electron transport) activity [17]. All of the *UCP3* transformants had less formation of XTT-formazen compared to the pYES2 control transformant indicating less mitochondrial activity in these cells (Fig. 3). The R70W transformant, however, showed more XTT-formazen production than the other four *UCP3* proteins (Fig. 3).

3.3. Effects $UCP3_L$ and mutant UCP3 proteins on mitochondrial $\Delta\Psi$

Coupling between respiration and oxidative phosphorylation is dependent upon a proton gradient across the mitochondrial membrane. To assess effects on this proton gradient, the fluorescence of the mitochondrial $\Delta\Psi$ sensitive dye, $DiOC_6$, in the transformed yeast cells was measured using flow cytometry. The pYES2 and $UCP3_L$ transformants were used as coupled and uncoupled controls, respectively. Addition of the chemical uncoupler CCCP resulted in a complete shift of the fluorescence peaks of all transformants to the left (including pYES2 vector control) indicative of total loss of $\Delta\Psi$ in mitochondria (data not shown). The amount of fluorescence observed with the V102I and IVS6+1G > A transformants was similar to the level of fluorescence obtained with $UCP3_L$ suggesting similar capabilities to reduce $\Delta\Psi$ (Fig. 4A and B). The uncoupling abilities of $UCP3_L$, V102I and IVS6+1G > A could not be distinguished from the CCCP control indicating either maximal uncoupling by the UCPs or less than maximal uncoupling by CCCP (data not shown). Repetition of the experiment showed similar results. An intermediate level of fluorescence was observed with the R143X mutant corresponding to intermediate degree of uncoupling

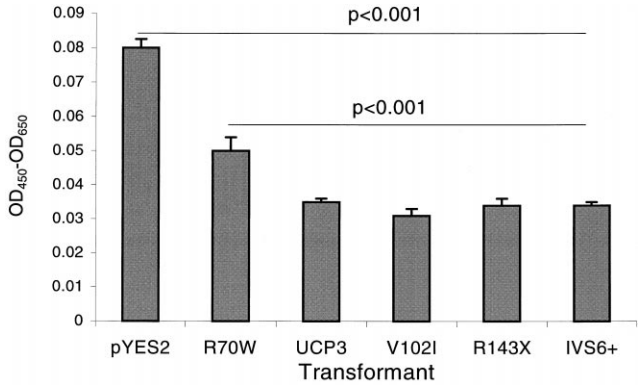

Fig. 3. Measurement of XTT-formazen formation in yeast transformants. Statistical comparisons (ANOVA and Bonferroni multiple comparison) were performed. The horizontal bars indicate significant differences between pYES2 and the other five transformants and between R70W and the remaining four transformants, with *P*-values as shown. The data represent means ± S.E.M. of 16 measurements from two independent experiments.

(Fig. 4C). The fluorescence measured in yeast cells transformed with the R70W mutant construct was identical to that obtained with the pYES2 vector control indicating that the $\Delta\Psi$ was not decreased and uncoupling did not occur (Fig. 4D).

4. Discussion

UCP3 appears to participate in thermogenesis and energy balance [3–5], and significant linkage and associations have been reported between markers at the *UCP2/UCP3* gene locus with resting metabolic rate [18] and *UCP2* polymorphisms with metabolic rate during sleep and over 24 h [19]. In the present study, a novel, non-conservative mutation, R70W, was found to abolish activity of *UCP3* when expressed in yeast. Another mutation, R143X [14], was found to significantly reduce activity of *UCP3* in yeast, whereas two other mutant constructs (V102I and IVS6+1G > A) [13] showed equal activity to wild-type *UCP3*.

Induction of $UCP3_L$, V102I, R143X, and IVS6+1G > A transformants resulted in cessation of growth compared to normal growth of yeast expressing the R70W mutant. Similar observations in growth rate of yeast with $UCP3_L$ compared to pYES2 vector control after induction with galactose have been reported previously [20]. A common characteristic of non-dividing cells is a decrease in mitochondrial activity [20]. Measurement of XTT-formazen production revealed that each of these cell lines expressing *UCP3* genes had a decrease in mitochondrial respiratory activity (i.e. a reduction in electron transport) when compared to the pYES2 vector control. XTT does not measure changes in $\Delta\Psi$ but rather acts as an artificial electron acceptor. Thus, the reduction of XTT by dehydrogenase enzymes of the electron transport chain can be used to measure mitochondrial activity [17]. The lack of growth and the decreased mitochondrial activity observed in yeast containing all *UCP3* constructs except for the R70W mutant suggests an interference with normal yeast mitochondrial activity due to the presence of the exogenous, yet active, *UCP3* proteins.

Surprisingly, the R143X mutant protein that contains only three transmembrane domains still had significant ability to

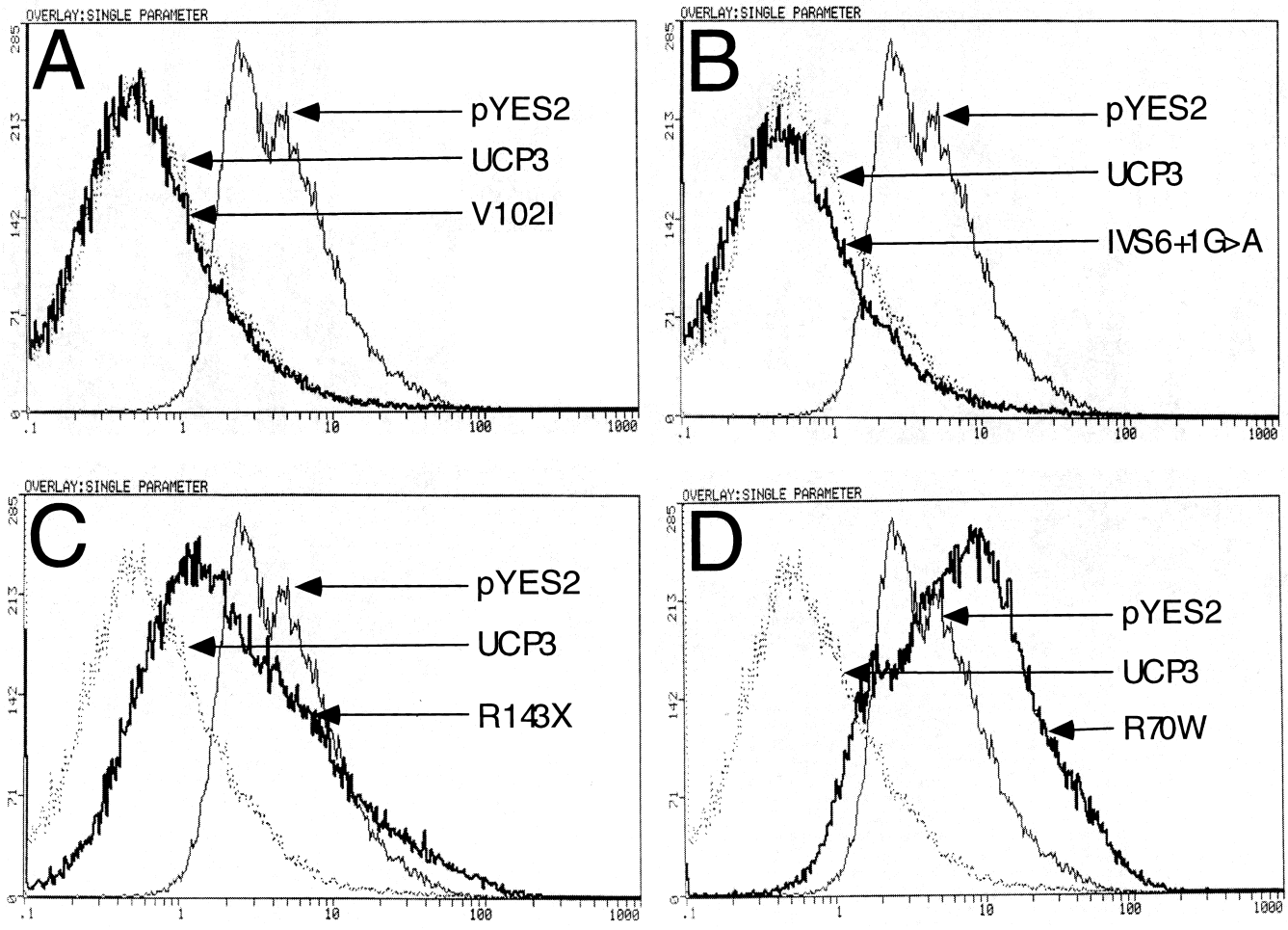

Fig. 4. Flow cytometry analysis of the mitochondrial membrane potential gradient in yeast expressing wild-type and mutant *UCP3*. The number of cells (*y*-axis) relative to logarithmic intensity of fluorescence of the potential sensitive dye $DiOC_6$ (*x*-axis) is shown. Each panel compares the fluorescence of a mutant *UCP3* protein with wild-type *UCP3* and pYES2 vector control. A: V102I; B: IVS6+1G > A; C: R143X; D: R70W. Consistent results were obtained from three independent flow cytometry experiments.

alter $\Delta\Psi$ in yeast, albeit half that of $UCP3_L$ and the IVS6+1G > A mutant. This finding suggests that the first three transmembrane domains of *UCP3* retain a native ability to alter $\Delta\Psi$ in yeast mitochondria. It would be of interest to examine whether the distal transmembrane domains of *UCP3* have similar effects on $\Delta\Psi$ in yeast.

With regard to the IVS6+1G > A polymorphism, it had an equal ability to alter $\Delta\Psi$ as $UCP3_L$. It is also noteworthy that the IVS6+1G > A polymorphism predicts a protein identical to the short isoform of *UCP3* ($UCP3_S$). This suggests that $UCP3_S$ may have similar effects on $\Delta\Psi$ as the long isoform of *UCP3* ($UCP3_L$) as has been reported previously [21–23]. However, both $UCP3_L$ and the IVS6+1G > A mutant had uncoupling abilities equal to that of CCCP, a chemical uncoupler. Therefore, it would be difficult to detect small differences in uncoupling between the two UCP3 proteins. An association has been reported for the IVS6+1G > A polymorphism with elevated respiratory quotient and reduced ability of heterozygotes to oxidize fat [14]. However, in a recent report by Chung et al. [22], resting metabolic rate, respiratory quotient and metabolic response to graded exercise were all normal in individuals with the exon 6 splice variant of *UCP3* [22]. The inconsistencies between the two studies may be explained by the differences in Caucasian admixture proportions in the two African-American populations. The estimate of admixture in the African-American population from Maywood, IL, USA examined by Chung et al. [22] is 18.8% [24] while the Charleston, S.C. Gullah-speaking African-American population studied by Argyropoulos et al. [14] is estimated to have Caucasian admixture levels of 6.7% (Dr. Shriver, personal communication). The elevated respiratory quotient and the reduced ability to oxidize fat could, therefore, be attributed to the lower Caucasian contribution to the Gullah-speaking African-American population. Furthermore, the ability of the IVS6+1G > A variant to alter $\Delta\Psi$ in yeast does not reflect all possible functions of *UCP3*. Indeed, polarographic measurement of the effect of *UCP3* on state 4 respiration in isolated mitochondria has shown that *UCP3* has true uncoupling activity [21] and micro-calorimetric measurement of direct heat production of whole yeast cells showed that *UCP3* has intrinsic thermogenic properties [25]. It is, therefore, conceivable that these uncoupling and thermogenic properties of *UCP3* could be compromised by the IVS6+1G > A polymorphism in humans and could account for the apparent lack of correlation between the reported phenotype in humans [14] and the yeast studies presented here.

The R70W mutation, on the other hand, had the most

significant impact on *UCP3* by completely abolishing its ability to alter $\Delta\Psi$. Moreover, this mutant protein had no deleterious effect on yeast growth, unlike the wild-type *UCP3* isoform, suggesting that this mutation may alter a critical functional domain in the protein. It is tempting to speculate that the mutant R70W *UCP3* protein loses its capacity to enter the mitochondrial membrane or that it is inserted into the mitochondrial membrane but loses its ability to alter $\Delta\Psi$. Furthermore, it has been demonstrated that certain arginines (R83, R182 and R276) are essential for nucleotide interaction with *UCP1* [26]. Based on the putative topology of *UCP3* [13,24], the R70W missense mutation is located in the second, matrix-orientated, extramembranous loop. Using simulation software [27], substitution of the arginine residue by tryptophan at position 70 changed the predicted secondary structure of *UCP3* by elongating a β-sheet at the point of the mutation. Therefore, the R70W mutation may also result in conformational changes that could affect functional properties of the *UCP3* protein.

In conclusion, native mutations in *UCP3* abolished (R70W) or reduced (R143X) the ability of the protein to alter $\Delta\Psi$. Expression of *UCP3* constructs in yeast is useful for studying relative differences in function. However, the yeast system may not reflect *UCP3* function as it may exist in mammalian mitochondria and a primary role for *UCP3* in regulating fuel availability in mammals may not be evident in yeast transformants. Nevertheless, endogenous *UCP3* mutations can clearly alter its ability to change $\Delta\Psi$ and may potentially confer susceptibility to develop metabolic disease. Further experiments in mammalian cells and/or animal models may be required to assess the physiological properties of *UCP3* gene variants in mammalian mitochondria.

Acknowledgements: The authors wish to thank Dr. Sally Self and Carey Wiggins of the Flow Cytometry Shared Facility at the Medical University of South Carolina for performing flow cytometry, Andrea Semler for help with the XTT analysis and David C. McLean, Jr. for help with the statistical analyses. This work was supported by Grants to W.T.G. from the NIH (DK-47461), Department of Veterans Affairs (merit review), and facilitated by support of the Sea Islands Families Project from the W.M. Keck Foundation. A.M.B. is the recipient of post-doctoral fellowship support from training Grant NIH-HL 07260.

References

[1] Silva, J.E. and Rabelo, R. (1997) Eur. J. Endocrinol. 136, 251–264.
[2] Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M.F., Surwit, R.S., Ricquier, D. and Warden, C.H. (1997) Nat. Genet. 15, 269–272.
[3] Boss, O., Samec, S., Paolini-Giacobino, C., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P. and Giacobino, J.-P. (1997) FEBS Lett. 408, 39–42.
[4] Solanes, G., Vidal-Puig, A., Grujic, D., Flier, J.S. and Lowell, B.B. (1997) J. Biol. Chem. 272, 25433–25436.
[5] Vidal-Puig, A., Solanes, G., Grujic, D., Flier, J.S. and Lowell, B.B. (1997) Biochem. Biophys. Res. Commun. 235, 79–82.
[6] Cassard, A.-M., Bouillaud, F., Mattei, M.-G., Henz, E., Raimbault, S., Thomas, M. and Ricquier, D. (1990) J. Cell. Biochem. 43, 255–264.
[7] Argyropoulos, G., Brown, A.M., Peterson, R., Watson, D.K. and Garvey, W.T. (1998) Diabetes 47, 685–687.
[8] Gong, D.-W., He, Y., Karas, M. and Reitman, M. (1997) J. Biol. Chem. 272, 24129–24131.
[9] 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.
[10] 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.
[11] Matsuda, J., Hosoda, K., Itoh, H., Son, C., Doi, K., Tanaka, T., Fukunaga, Y., Inoue, G., Nishimura, H., Yoshimasa, Y., Yamori, Y. and Nakao, K. (1997) FEBS Lett. 418, 200–204.
[12] Lin, B., Coughlin, S. and Pilch, P.F. (1998) Am. J. Physiol. 275, E386–E391.
[13] Urhammer, S.A., Dalgaard, L.T., Sørensen, T.I.A., Tybjærg, A., Echwald, S.M., Andersen, T., Clausen, J.O. and Pedersen, O. (1998) Diabetologia 41, 241–244.
[14] Argyropoulos, G., Brown, A.M., Willi, S.M., Zhu, J., He, Y., Reitman, M., Gevao, S.M., Spruill, I. and Garvey, W.T. (1998) J. Clin. Invest. 102, 1345–1351.
[15] Brown, A.M., Willi, S.M., Argyropoulos, G. and Garvey, W.T. (1999) Hum. Mutat. 13, 506.
[16] Sherman, F. (1991) Methods Enzymol. 194, 3–21.
[17] Jahn, B., Martin, E., Stueben, A. and Bhakdi, S. (1995) J. Clin. Microbiol. 33, 661–667.
[18] Bouchard, C., Pérusse, L., Chagnon, Y.C., Warden, C. and Ricquier, D. (1997) Hum. Mol. Genet. 6, 1887–1889.
[19] 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. and Ravussin, E. (1998) Hum. Mol. Genet. 7, 1431–1435.
[20] 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.
[21] Zhang, C.-Y., Hagen, T., Mootha, V.K., Slieker, L.J. and Lowell, B.B. (1999) FEBS Lett. 449, 129–134.
[22] Chung, W.K., Luke, A., Cooper, R.S., Rotini, C., Vidal-Puig, A., Rosenbaum, M., Chua, M., Solanes, G., Zheng, M., Zhao, L., LeDuc, C., Eisberg, A., Chu, F., Murphy, E., Schreier, M., Aronne, L., Caprio, S., Kahle, B., Gordon, D., Leal, S.M., Goldsmith, R., Andreu, A.L., Bruno, C., DiMauro, S., Moonseong, H., Lowe Jr., W.L., Lowell, B.B., Allison, D.B. and Leibel, R.L. (1999) Diabetes 48, 1890–1895.
[23] Hagen, T., Zhang, C.-H., Slieker, L.J., Chung, W.K., Leibel, R.L. and Lowell, B.B. (1999) FEBS Lett. 454, 201–206.
[24] Parra, E.J., Marcini, A., Akey, J., Martinson, J., Batzer, M.A., Cooper, R., Forrester, T., Allison, D.B., Deka, R., Ferrell, R.E. and Shriver, M.D. (1998) Am. J. Hum. Genet. 63, 1939–1951.
[25] Hinz, W., Faller, B., Grüninger, S., Gazzotti, P. and Chiesi, M. (1999) FEBS Lett. 448, 57–61.
[26] Modiansky, M., Murdza-Inglis, D.L., Patel, H.V., Freeman, K.B. and Garlid, K.D. (1997) J. Biol. Chem. 272, 24759–24762.
[27] Solovyev, V.V. and Salamov, A.A. (1994) CABIOS 10, 661–669.