Mitochondrial import of the long and short isoforms of human uncoupling protein 3

Anja Renold[a], Carla M. Koehler[b], Michael P. Murphy[c,*]

[a] *Biozentrum der Universität Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland*
[b] *Department of Chemistry and Biochemistry, University of California-Los Angeles, Box 951569, Los Angeles, CA 90095-1569, USA*
[c] *Department of Biochemistry, University of Otago, Box 56, Dunedin, New Zealand*

Received 16 November 1999

Edited by Gunnar von Heijne

Abstract Human uncoupling protein 3 (UCP3) has two RNA transcripts that arise from the differential processing of the same gene product. One encodes the full length protein (UCP3L) while the other encodes a truncated version (UCP3S) lacking the sixth membrane spanning domain. The roles of the two isoforms are not known, but a mutation that decreases the proportion of UCP3L decreases fat oxidation and increases susceptibility to obesity. In the ADP/ATP carrier, a protein closely related to UCP3, the sixth membrane spanning domain is required for insertion into the inner membrane. Therefore, defective membrane insertion of UCP3S may account for the different effects of the two isoforms in vivo. We investigated mitochondrial import of the two UCP3 isoforms. When epitope-tagged versions of UCP3S and UCP3L were expressed in COS7 cells, both were inserted into the mitochondrial inner membrane. Translation in vitro followed by incubation with isolated mitochondria showed that both isoforms were inserted into the inner membrane, however, the insertion of UCP3S was significantly slower.

Key words: Uncoupling protein 3; Mitochondrial protein import

1. Introduction

The first uncoupling protein (UCP1) was discovered in the inner membrane of brown adipose tissue mitochondria, where it dissipates the mitochondrial proton electrochemical gradient as heat [1,2]. Since then, three additional human UCPs have been discovered through their sequence homologies to UCP1 [2,3]: UCP2 is expressed ubiquitously, UCP3 is expressed mainly in skeletal muscle and UCP4 is expressed in the brain [2,3]. These UCPs are all members of the mitochondrial carrier family [4], have a molecular mass of 30–35 kDa and contain six membrane spanning helices [1,2]. Homology with UCP1 suggests that the other UCPs function by increasing the proton leak through the inner membrane [1,2].

UCP3 was discovered in human skeletal muscle and is expressed there in large amounts [5,6]. As skeletal muscle is important for adaptive thermogenesis, UCP3 is an attractive candidate protein for this process [2]. Interestingly, two UCP3 mRNAs are present in vivo, the full length transcript UCP3L and a shorter form, UCP3S, which arises from a cleavage and polyadenylation signal located in the last intron that prematurely terminates about half of the transcripts [7]. UCP3L encodes a 312 amino acid (aa) protein while UCP3S encodes a 275 aa protein lacking the sixth transmembrane spanning domain [5,7]. The transcript levels for UCP3L and UCP3S were similar in a range of obese and lean subjects [5,7,8].

When UCP3 was studied as a candidate gene for pathological obesity in humans, a mutation in the splice donor site of the sixth exon was found that results in a truncated protein the same size as UCP3S [9]. Subjects heterozygous for this mutation had lower rates of fat oxidation and an increased tendency to obesity, suggesting that UCP3L favours mitochondrial uncoupling and fat oxidation while UCP3S does not [9]. How this occurs is not known, but as the two UCP3 isoforms uncouple mitochondria to similar extents [10–12], it is unlikely to arise from an intrinsic difference in proton conductance.

UCPs are imported into mitochondria and inserted into the inner membrane by an import machinery specialised for membrane proteins [13–16]. Mitochondrial targeting information for import by this pathway is present in the mature protein, rather than in an N-terminal signal sequence that is removed after import [13]. The sixth membrane spanning domain of the ADP/ATP carrier, a protein closely related to UCP3, is required for its membrane potential dependent insertion into the mitochondrial inner membrane [17]. As this domain is missing from UCP3S, there may be significant differences in the mitochondrial insertion of the two UCP3 isoforms. To investigate this hypothesis, we constructed haemagglutinin (HA)-tagged versions of UCP3L and UCP3S and expressed them in COS7 cells. Both isoforms were inserted into the mitochondrial inner membrane within cells, dissipating the membrane potential to a similar extent. We then translated the two UCP3 isoforms in vitro and showed that both were inserted into the mitochondrial inner membrane in isolated yeast and rat liver mitochondria, but that insertion was significantly slower for UCP3S.

2. Materials and methods

2.1. Cloning and in vitro transcription and translation of UCP3L and UCP3S

Human skeletal poly(A)$^+$ RNA (Clonetech) was reverse-transcribed using oligo d(T) and the UCP3L and UCP3S cDNAs were amplified

*Corresponding author. Fax: (64)-3-479 7866.
E-mail: murphy@sanger.otago.ac.nz

Abbreviations: DMEM, Dulbecco's modified Eagle medium; FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; FITC, fluorescein isothiocyanate; HA, haemagglutinin; TPMP, methyltriphenylphosphonium; UCP, uncoupling protein; UCP3L, full length isoform; UCP3S, truncated isoform

PII: S0014-5793(99)01688-9

using the same 5′ primer (CTT CCA GGA CTA TGG TGG) but different 3′ primers (GTT CAA AAC GGT GAT TCC CG for UCP3L and GAAAGAAGCCCCTGTTCTCTG for UCP3S [5]). The PCR products were subcloned into pYES2 and sequenced. Comparison with the GenBank sequences (U82818 for UCP3S; U84763 for UCP3L) showed one silent C to T transition at nucleotide 783 in UCP3S and two silent transitions: a T to C at nucleotide 450 and a C to T at nucleotide 783. Linearised pYES2 plasmids were transcribed using T7 polymerase and translated in vitro in the presence of [^{35}S]methionine in a rabbit reticulocyte lysate [18]. Electrophoresis on a 16% Tris–Tricine gel [19] followed by fluorography gave single products of the expected molecular weights for UCP3S and UCP3L (29.7 kDa and 34.1 kDa, respectively).

2.2. Construction of HA-tagged UCP3L and UCP3S

HA-tagged UCP3S and UCP3L were constructed by adding 12 aa HA tags (CYPYDVPDYASL) to their C-termini. Plasmids containing UCP3L or UCP3S were PCR-amplified using Expand Hi Fidelity polymerase, a 5′ primer incorporating a *Hin*dIII site before the start codon and 3′ primers encoding the HA tag and incorporating an *Eco*RI site after the stop codon. The 5′ primer was GGGGAAGCTTATGGTTGGACTGAAGCCTTCA and the 3′ primers were GGGGGAATTCTCATAGGGAAGCGTAGTCAGGCACATCATATGGATAGCAAAACGGTGATTCCCGTAA and GGGGGAATTCTCATAGGGAAGCGTAGTCAGGCACATCATATGGATAGCACCCCTTGTAGAAGGCTGT for UCP3L-HA and UCP3S-HA, respectively. After digestion with *Hin*dIII and *Eco*RI, the PCR products were gel-purified, ligated into pcDNA3 (Invitrogen) and transformed into *Escherichia coli*. Clones were selected by restriction digestion of plasmids and by PCR, and then confirmed by complete DNA sequencing of the plasmid insert. Plasmids linearised with *Xho*I were transcribed using T7 polymerase and translated in a rabbit reticulocyte lysate in the presence of [^{35}S]methionine. This gave single products of the correct sizes (31.1 kDa for UCP3S-HA and 35.6 kDa for UCP3L-HA) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/fluorography.

2.3. Expression of UCP3L-HA and UCP3S-HA in COS cells

COS7 cells were grown at 37°C in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 U/ml penicillin, 100 mg/ml streptomycin sulfate and 10% foetal calf serum under 95% air/5% CO_2, then transiently transfected using lipofectin (Life Technologies, Gaithersburg, MD, USA). After 10–15 h at 37°C, the transfection medium was replaced with serum-containing DMEM and cells were harvested after further incubation. For immunohistochemistry, cells grown on glass coverslips were transfected, then Mitotracker (1 μM; Molecular Probes) was added to the incubation medium and incubated at 37°C for 30 min. After fixation with 3% paraformaldehyde in phosphate-buffered saline (PBS), cells were incubated with 50 mM NH_4Cl in PBS, permeabilised with 0.1% Triton X-100 in PBS and non-specific binding was blocked with 1% bovine serum albumin (BSA) in PBS. A mouse monoclonal antibody against the HA tag (12CA5, Babco, Richmond, CA, USA; 1:200 in BSA/PBS) was added for 1–2 h and then incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG (Organon Teknika-Cappel; 1:200 in BSA/PBS) for 30 min. Coverslips were then mounted in Mowiol (Hoechst Pharmaceuticals) and analysed using an Axiomat BSTV confocal microscope and images were processed using Datavision and Photoshop.

2.4. Preparation and analysis of mitochondria from COS cells

Cells were transiently transfected with UCP3S-HA or UCP3L-HA, harvested by treatment with trypsin/EDTA and washed in cold PBS supplemented with protease inhibitors (1.25 μg/ml leupeptin, 0.75 μg/ml antipain, 0.25 μg/ml chymostatin, 0.25 μg/ml elastinol, 5 μg/ml pepstatin). Cells were then washed in STE (250 mM sucrose, 10 mM Tris–HCl, pH 7.4, 1 mM EGTA), suspended in 1–2 ml STE supplemented with protease inhibitors on ice for 20 min and then homogenised in a 2 ml glass homogeniser [20]. Debris was pelleted ($500\times g$ for 1 min), then mitochondria were isolated from the supernatant (1 min $20\,000\times g$), suspended at 1–2 mg protein/ml in STE and the supernatant retained as the cytoplasm. Protein concentrations were determined using the bicinchoninic acid assay using BSA as a standard [21]. About 33–40% of the activity of the matrix enzyme citrate synthase [22] was recovered in the mitochondrial fraction, and only 5–7% of the total lactate dehydrogenase activity [23]. For alkali extraction, mitochondria were suspended in 50 μl 100 mM Na_2CO_3 and after 30 min incubation on ice, membranes were pelleted by centrifugation (30 min at $100\,000\times g$) and proteins in the supernatant acid precipitated. Protein samples were separated by SDS-PAGE, transferred to nitrocellulose and blocked with 5% non-fat milk powder in PBS. Blots were probed with a mouse monoclonal antibody against the HA tag (12CA5, Babco, Richmond, CA, USA; 1:1000) and against human cytochrome oxidase, subunit 2 (12C4-F12, Molecular Probes; 1:500). Blots were then incubated with rabbit antiserum raised against mouse IgG (M 7023, Sigma; 1:2000), followed by incubation with [^{125}I]protein A and bands were visualised by autoradiography.

2.5. Measurement of mitochondrial membrane potential

COS7 cells were transiently transfected with lipofectin/plasmid, or mock-transfected with lipofectin alone. Cells were harvested, washed in PBS and counted using a new improved Neubeyer haemocytometer. Cells (500 000) were pelleted by centrifugation and resuspended in 1 ml DMEM containing glucose (4.5 g/l), 10 mM HEPES–NaOH, pH 7.4, 1 μM methyltriphenylphosphonium (TPMP), 500 nM sodium tetraphenylboron and 100 nCi/ml [^{3}H]TPMP and incubated for 1 h at 37°C. Cells were pelleted by centrifugation and the supernatant and pellets separated and processed for scintillation counting [24]. TPMP binding to deenergised cells was estimated from parallel incubations in medium supplemented with 12.5 μM oligomycin, 20 μM carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), 1 μM myxothiazol, 100 nM valinomycin and 1 mM ouabain. TPMP accumulation was calculated by subtracting the amount of TPMP bound to deenergised cells and is expressed as an accumulation ratio ((TPMP/mg protein)/(TPMP/μl supernatant)).

2.6. Import of UCP3S and UCP3L into isolated mitochondria

Yeast mitochondria (200 μg protein) purified from lactate-grown yeast [25] were incubated with radiolabelled UCP3L and UCP3S proteins at 25°C in 800 μl import buffer (600 mM sorbitol, 50 mM HEPES–KOH, pH 7, 50 mM KCl, 10 mM $MgCl_2$, 2.5 mM EDTA, 2 mM KH_2PO_4, 1 mg/ml BSA and 0.75 mg/ml methionine) supplemented with 2 mM ATP and 2 mM NADH: for incubations without membrane potential, 1 μg/ml valinomycin was added [18]. Mitochondria were then pelleted and resuspended in 200 μl 0.6 M sorbitol, 20 mM HEPES–KOH, pH 7.4, supplemented with 50 μg/ml trypsin and after 30 min on ice, 200 μg/ml soy bean trypsin inhibitor was added and 5 min later, the mitochondria were pelleted. For osmotic shock, mitochondria were suspended in 200 μl 0.6 M sorbitol, 20 mM HEPES–KOH, pH 7.4, and then diluted 10-fold in 20 mM HEPES–KOH, pH 7.4, and after 30 min on ice, mitoplasts were isolated by centrifugation and the supernatant protein was acid-precipitated. Liver mitochondria were prepared from starved, female rats [26] and protein was quantitated by the biuret method using BSA as a standard [27]. Mitochondria (300 μg protein) were suspended at 30°C in 40 μl import buffer (220 mM mannitol, 70 mM sucrose, 10 mM HEPES–KOH, 1 mM $MgCl_2$, 1 mM EGTA, pH 7.6) supplemented with 1 mg/ml BSA, 1 mM dithiothreitol, 10 mM K-succinate, 2 mM ATP, 0.1 mM GTP, 1.5 mM creatine phosphate and 15 μg/ml creatine kinase [28,29]. For incubations in the absence of a membrane potential, oligomycin, antimycin and FCCP were added (5 μM of each). After incubation, the mitochondria were pelleted and resuspended in 200 μl 0.6 M sorbitol, 20 mM HEPES–KOH, pH 7.4, containing 200 μg/ml proteinase K for 30 min on ice, then 2 mM phenylmethyl sulfonyl fluoride was added and 15 min later, mitochondria were pelleted. For alkali extraction, mitochondria or mitoplasts were resuspended in 100 μl 100 mM Na_2CO_3, incubated on ice for 30 min, the membranes pelleted by centrifugation for 30 min at $100\,000\times g$ and supernatant protein acid-precipitated. All mitochondrial samples were separated by SDS-PAGE electrophoresis and the ^{35}S-labelled proteins visualised by fluorography [18].

3. Results

3.1. Expression of UCP3S-HA and UCP3L-HA in mammalian cells

To determine the locations of the two UCP3 isoforms within mammalian cells, we incorporated HA tags and transiently expressed the tagged proteins in COS7 cells (Fig. 1). Immuno-

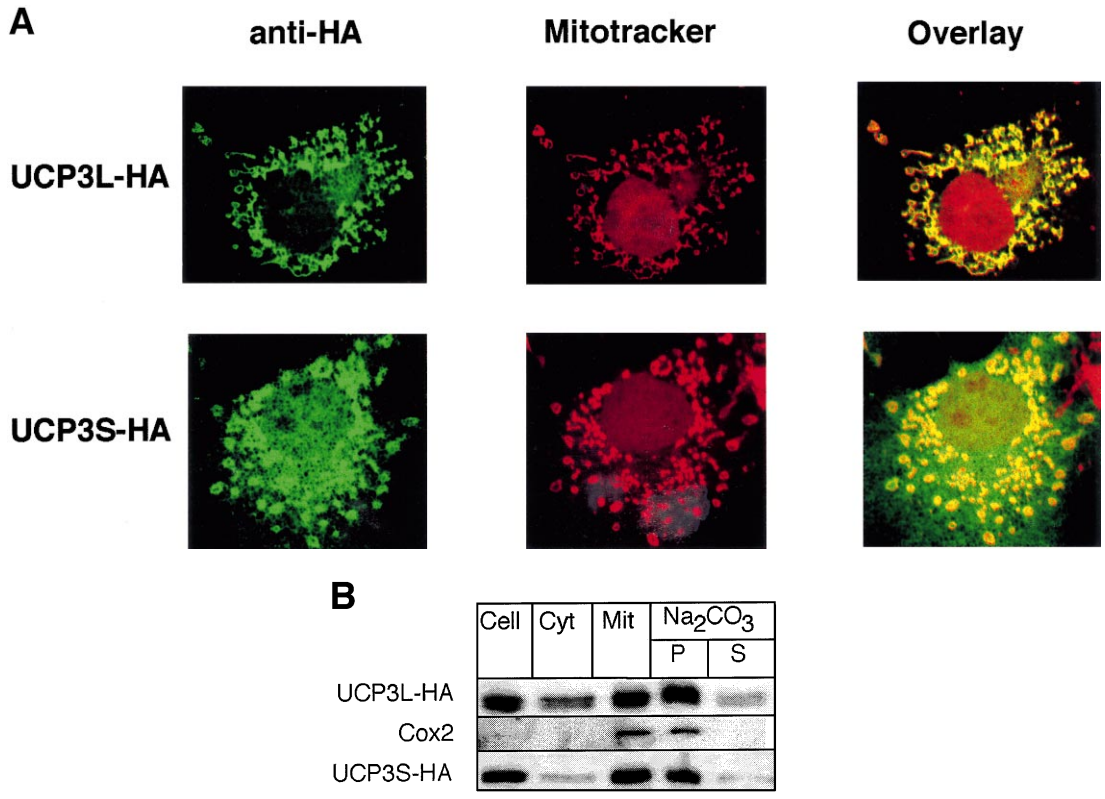

Fig. 1. Mitochondrial localisation of UCP3L-HA and UCP3S-HA within cells. COS7 cells were transiently transfected with UCP3L-HA and UCP3S-HA. In A, cells were incubated with Mitotracker before being fixed and immunostained with an antibody against HA tag which was then linked to a FITC-conjugated antibody. Under fluorescent microscopy, the HA-tagged UCP3s are green, mitochondria are red and colocalised HA-tagged UCPs and mitochondria appear yellow in digitally overlaid images. In B, cells were harvested 24 h after transfection and separated into mitochondrial and cytoplasmic fractions. The mitochondria were further separated into integral membrane proteins (P) and other proteins (S) by alkali extraction with Na_2CO_3. Protein (50 μg) was separated by SDS-PAGE and immunoblotted with antibodies against the HA tag or against cytochrome oxidase subunit 2 (Cox2) and visualised by decoration with [^{125}I]protein A followed by autoradiography.

fluorescence microscopy showed that both UCP3L-HA and UCP3S-HA colocalised with the mitochondrial marker Mitotracker (Fig. 1A). Cells transfected with the HA-tagged proteins were subfractionated and immunoblotting with an anti-HA antibody confirmed that most UCP3L-HA and UCP3S-HA expressed in these cells was mitochondrial (Fig. 1B). Alkali extraction of these mitochondria with Na_2CO_3, which leaves only integral membrane proteins [30], showed that both UCP3L-HA and UCP3S-HA were found predominantly in the membrane pellet, along with the mitochondrial inner membrane protein cytochrome oxidase (Fig. 1B). Hence, both UCP3S-HA and UCP3L-HA are integral, mitochondrial inner membrane proteins.

The time courses of expression of UCP3L-HA and UCP3S-HA in COS7 cells were determined (Fig. 2). From 12 to 36 h after transfection, the specific amounts of both UCP3-HA isoforms in whole cells were similar (Fig. 2A, upper row). When mitochondrial fractions were prepared from these cells, the specific amounts of the two isoforms in mitochondria were also similar at each time point (Fig. 2A, lower row). Quantification of these expression levels in mitochondria by scanning densitometry of autoradiographs (Fig. 2B) confirmed that the specific amounts of UCP3S and UCP3L in mitochondria were similar at each time point. Therefore, both UCP3 isoforms have similar time courses of expression and mitochondrial localisation within cells.

3.2. *Effect of UCP3S-HA and UCP3L-HA on mitochondrial membrane potential*

As the specific amounts of the two isoforms within mitochondria were similar, we could compare their uncoupling efficacies by measuring the membrane potential at various times after transfection. The membrane potential of cells transfected with either UCP3L-HA or UCP3S-HA was measured and compared with that of mock-transfected cells (Fig. 3). At 18 h after transfection, neither UCP3 isoform affected membrane potential, but after 24–36 h, both isoforms decreased the membrane potential to similar extents (Fig. 3), suggesting that both isoforms have similar effects on the membrane potential under these conditions.

3.3. *Import of UCP3L and UCP3S into isolated mitochondria*

To determine whether there were kinetic differences in the import of both isoforms of UCP3 into isolated mitochondria, we translated UCP3L and UCP3S in vitro in a reticulocyte lysate and incubated the radiolabelled proteins with isolated mitochondria (Fig. 4). Both UCP3L and UCP3S were imported into yeast mitochondria and became inaccessible to added protease, indicating that they had been transported across the mitochondrial outer membrane (Fig. 4A). To determine their location within mitochondria, we disrupted the outer membrane by osmotic shock, which released about 75% of cytochrome b_2 from the intermembrane space into the

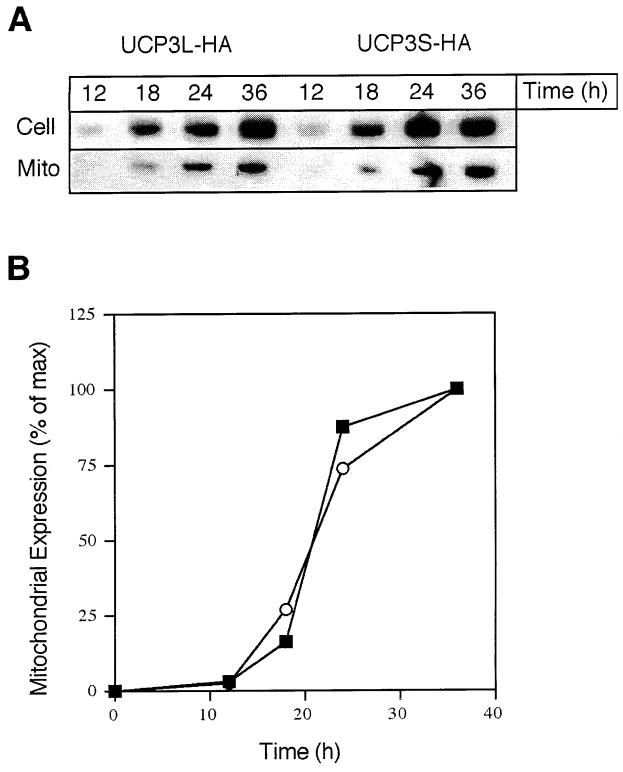

Fig. 2. Time course of expression of UCP3s in cells. COS7 cells expressing UCPL-HA or UCPS-HA were isolated at various times after transfection and a mitochondrial fraction was prepared. In A, cell and mitochondrial protein (50 μg) for each time point were separated by SDS-PAGE, immunoblotted with antibodies against the HA tag and visualised by decoration with [^{125}I]protein A followed by autoradiography. This is a typical experiment that was repeated three times. In B, the UCP3 contents of the mitochondrial fractions in A were quantitated by scanning densitometry and plotted against time, as a percentage of the expression at 36 h (UCP3L-HA, circles; UCP3S-HA, squares).

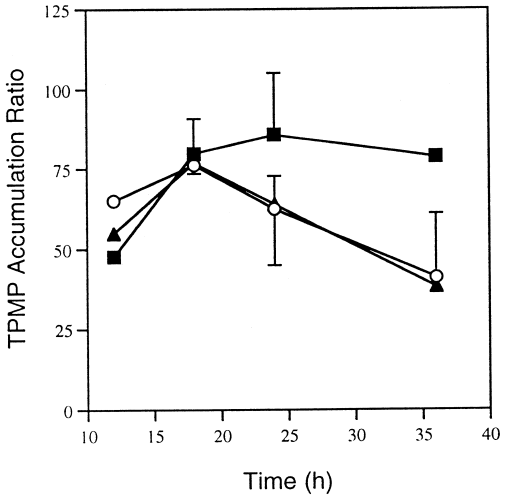

Fig. 3. Mitochondrial membrane potential in cells expressing UCP3L-HA and UCP3S-HA. Mock-transfected COS cells (squares) and cells transfected with UCPL-HA (circles) or UCPS-HA (triangles) were isolated at various times after transfection. The mitochondrial membrane potential was measured from the TPMP accumulation ratio. Data are mean ± S.E.M. of experiments on three different cell preparations.

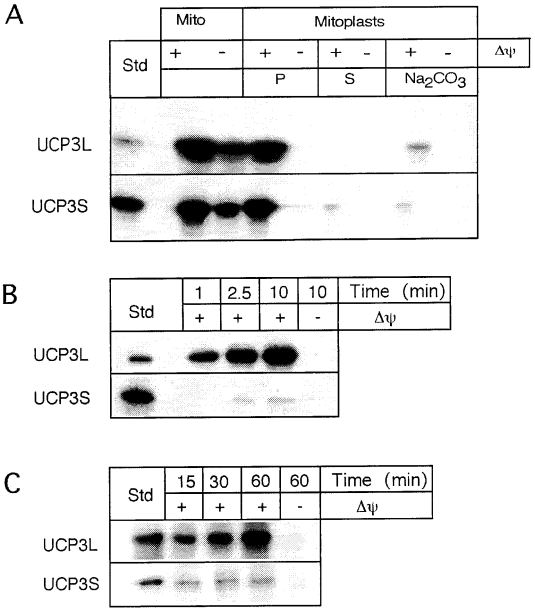

Fig. 4. UCP3L and UCP3S import into isolated mitochondria. Radiolabelled UCP3L and UCP3S were incubated with mitochondria in the presence or absence of a membrane potential ($\Delta\Psi$). After import, mitochondria were treated with protease to remove non-imported precursor. All protein samples were separated by SDS-PAGE and visualised by fluorography. Data shown are typical experiments which were all repeated four or five times. 'STD' refers to a percentage of the radiolabelled precursor used in the import. In A, UCP3L and UCP3S were imported into yeast mitochondria for 20 min (Mito). Mitoplasts were then prepared by osmotic shock (P) and proteins remaining in the supernatant were acid-precipitated (S). Mitoplasts were alkali-extracted with Na_2CO_3 and the membrane pellets analysed. STD = 10%. In B, UCP3L and UCP3S were imported into yeast mitochondria for various times, then alkali-extracted and the membrane pellets analysed. STD = 2.5%. In C, UCP3L and UCP3S were imported into rat liver mitochondria for various times and the membrane pellets after alkali extraction were analysed. STD = 20%.

supernatant but none of the matrix enzyme α-ketoglutarate dehydrogenase (data not shown). In the presence of a membrane potential, both UCP3 isoforms remained associated with the mitoplast pellet (Fig. 4A). Alkali extraction of the mitoplasts with Na_2CO_3 showed that both UCP3 isoforms were inserted into the inner membrane in the presence of a membrane potential (Fig. 4A). The amount of UCP3S inserted was less than UCP3L (Fig. 4A), so to analyse this difference, we measured the time courses of insertion of the two proteins into the inner membrane (Fig. 4B). Aliquots from the import reaction were removed at various times, protease-treated and then alkali-extracted (Fig. 4B). UCP3L was rapidly inserted into the inner membrane over 10 min, but the amount of UCP3S inserted into the membrane was significantly less (Fig. 4B). Quantitation of the autoradiographs by scanning densitometry showed that the rate of insertion of UCP3S was 50–80-fold slower than for UCP3L. This quantitative difference may be due to importing mammalian proteins into yeast mitochondria, therefore, we imported UCP3L and UCP3S into rat liver mitochondria (Fig. 4C). After import, the mitochondria were protease-treated and extracted with alkali to leave only proteins that had inserted into the membrane. Both UCP3L and UCP3S inserted into the inner membrane of rat liver mitochondria in a potential dependent

fashion, however, the rate of insertion of UCP3S was still 10–20-fold slower than for UCP3L (Fig. 4C). We conclude that both UCP3 isoforms are imported into mitochondria and inserted into the inner membrane in vitro, but that UCP3S inserts more slowly than UCP3L. Import into mitochondria occurs without proteolytic processing as there was no size difference between the standards and imported proteins in Fig. 4.

4. Discussion

We investigated possible mechanisms by which UCP3L could favour fat oxidation over UCP3S in vivo. When COS7 cells were transiently transfected with HA-tagged UCP3L and UCP3S, both were found in mitochondria and subfractionation of these mitochondria showed that they were present in the inner membrane. Therefore, the absence of the sixth membrane spanning domain in UCP3S does not prevent its insertion into the mitochondrial inner membrane. The rates of expression and insertion into mitochondria of the two isoforms within COS7 cells were similar and both isoforms decreased the membrane potential to the same extent. This suggests that both UCP3 isoforms have similar mitochondrial uncoupling activities, consistent with results obtained by expressing both UCP3 isoforms in yeast [10–12]. As no functional or localisation differences between the two isoforms were found in cells, we investigated their kinetics of import into isolated mitochondria in more detail. Both isoforms were taken up by mitochondria in vitro and inserted into the inner membrane in a membrane potential dependent fashion. This occurred without proteolytic processing, indicating that targeting information for both isoforms is present within the mature protein, confirming that the sixth transmembrane domain is not essential for directing insertion of UCP3 into the mitochondrial inner membrane. However, insertion of UCP3S was significantly slower than that of UCP3L in both yeast and rat liver mitochondria, suggesting that the sixth transmembrane domain does facilitate the incorporation of UCP3L into the membrane. The slower membrane insertion of UCP3S may contribute to the decreased fat oxidation and increased obesity in subjects heterozygous for the mutation that truncates UCP3L, leaving a protein the same size as UCP3S [9].

Several questions remain. The sixth transmembrane domain of UCP3L could act in other ways than through facilitating protein insertion. In UCP1, binding of purine nucleotides to this domain inhibits uncoupling and its absence from UCP3S may enable both regulated and unregulated forms of UCP3 to coexist [5]. However, the regulation of UCP3 may be quite different from UCP1, and if UCP3L is inhibited by nucleotides, then UCP3S should have the greater uncoupling activity [31]. In COS7 cells, the UCP3 content may be greater than in skeletal muscle, decreasing the membrane potential non-specifically by disrupting the inner membrane. Possibly the two UCP3 isoforms have quite different effects on membrane potential at the expression levels and regulatory environment that occur in vivo, and the HA tag may also affect UCP3 function. It is unclear why the dramatic difference in the rate of insertion into mitochondria found in vitro did not affect the relative steady state levels of the two UCP3 isoforms in mitochondria within cells. We conclude that both the long and short isoforms of UCP3 are imported into mitochondria and inserted into the inner membrane where they have similar effects on the membrane potential. The only major difference between the two isoforms was that the rate of insertion into isolated mitochondria of the short isoform was slower than that of the long isoform.

Acknowledgements: We thank Dr Martin D. Brand, MRC-Dunn Human Nutrition Unit, Cambridge, UK, for initially suggesting this investigation and for helpful comments throughout, Professors Gottfried Schatz and Martin Spiess, Biozentrum, der Universität Basel, Switzerland, for support and Dr Annemarie Simpson for supplying the UCP3S and UCP3L plasmids. This work was supported by Swiss National Science Foundation Grant 31-43483.95 to Professor Martin Spiess.

References

[1] Klingenberg, M. and Huang, S.G. (1999) Biochim. Biophys. Acta 1415, 271–296.
[2] Boss, O., Muzzin, P. and Giacobino, J.-P. (1998) Eur. J. Endocrinol. 139, 1–9.
[3] Mao, W., Yu, X.X., Zhong, A.W.L., Bruch, J., Sherwood, S.W., Adams, S.H. and Pan, G. (1999) FEBS Lett. 443, 326–330.
[4] Palmieri, F., Bisaccia, F., Capobianco, L., Dolce, V., Fiermonte, G., Iacobazzi, V., Indiveri, C. and Palmieri, L. (1996) Biochim. Biophys. Acta 1275, 127–132.
[5] Boss, O., Samec, S., Paolini-Giacobino, A., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P. and Giacobino, J.-P. (1997) FEBS Lett. 408, 39–42.
[6] Vidal-Puig, A., Solanes, G., Grujic, D., Flier, J.S. and Lowell, B.B. (1997) Biochem. Biophys. Res. Commun. 235, 79–82.
[7] Solanes, G., Vidal-Puig, A., Grujic, D., Flier, J.S. and Lowell, B.B. (1997) J. Biol. Chem. 272, 25433–25436.
[8] Esterbauer, H., Oberkofler, H., Dallinger, G., Breban, D., Hell, E., Krempler, F. and Patsch, W. (1999) Diabetologia 42, 302–309.
[9] 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.
[10] Zhang, C.Y., Hagen, T., Mootha, V.K., Slieker, L.J. and Lowell, B.B. (1999) FEBS Lett. 449, 129–134.
[11] Hinz, W., Faller, B., Gruninger, S., Gazzotti, P. and Chiesi, M. (1999) FEBS Lett. 448, 57–61.
[12] Hagen, T., Zhang, C.Y., Slieker, L.J., Chung, W.K., Leibel, R.L. and Lowell, B.B. (1999) FEBS Lett. 454, 201–206.
[13] Koehler, C.M., Merchant, S. and Schatz, G. (1999) Trends Biochem. Sci. 24, 428–432.
[14] Koehler, C.M., Jarosch, E., Tokatlidis, K., Schmid, K., Schweyen, R.J. and Schatz, G. (1998) Science 279, 369–373.
[15] Kerscher, O., Holder, J., Srinivasan, M., Leung, R.S. and Jensen, R.E. (1997) J. Cell. Biol. 139, 1663–1675.
[16] Sirrenberg, C., Bauer, M.F., Guiard, B., Neupert, W. and Brunner, M. (1996) Nature 384, 582–585.
[17] Endres, M., Neupert, W. and Brunner, M. (1999) EMBO J. 18, 3214–3221.
[18] Rospert, S. and Schatz, G. (1998) in: Cell Biology: a Laboratory Handbook (Cellis, J.E., Ed.), Vol. 2, pp. 227–285, Academic Press, San Diego, CA.
[19] Schagger, H. and Von jagow, G. (1987) Anal. Biochem. 166, 368–379.
[20] Appleby, R.D., Porteous, W.K., Hughes, G., James, A.M., Shannon, D., Wei, Y.-H. and Murphy, M.P. (1999) Eur. J. Biochem.
[21] Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C. (1985) Anal. Biochem. 150, 76–85.
[22] Robinson, J.B., Jr., Brent, L.G., Sumegi, B. and Srere, P.A. (1987) in: Mitochondria - a Practical Approach (Darley-Usmar, V.M., Rickwood, D. and Wilson, M.T., Eds.), pp. 153–170, IRL Press, Oxford.
[23] Bergmeyer, H.U. and Bernt, E. (1974) in: Methods of Enzymatic Analysis (Bergmeyer, H.U., Ed.), Vol. 2, pp. 574–577, Academic Press, New York.

[24] James, A.M., Wei, Y.-H., Pang, C.-Y. and Murphy, M.P. (1996) Biochem. J. 318, 401–407.
[25] Glick, B.S. and Pon, L. (1995) Methods Enzymol. 260, 213–233.
[26] Chappell, J.B. and Hansford, R.G. (1972) in: Subcellular Components: Preparation and Fractionation (Birnie, G.D., Ed.), pp. 77–91, Butterworths, London.
[27] Gornall, A.G., Bardawill, C.J. and David, M.M. (1949) J. Biol. Chem. 177, 751–766.
[28] Mihara, K. and Omura, T. (1995) Methods Enzymol. 260, 302–310.
[29] Wada, J. and Kanwur, Y.S. (1998) Proc. Natl. Acad. Sci. USA 95, 144–149.
[30] Hunziker, A., Orme, F.W. and Macey, R.I. (1985) J. Membr. Biol. 84, 147–156.
[31] Echtay, K.S., Liu, Q., Caskey, T., Winkler, E., Frischmuth, K., Bienengraber, M. and Klingenberg, M. (1999) FEBS Lett. 450, 8–12.