UCP2 is a mitochondrial transporter with an unusual very short half-life

Sophie Rousset\textsuperscript{a,1}, Julien Mozo\textsuperscript{a,1,2}, Geneviève Dujardin\textsuperscript{b}, Yalin Emre\textsuperscript{a}, Sandrine Masscheley\textsuperscript{a}, Daniel Ricquier\textsuperscript{a}, Anne-Marie Cassard-Doulcier\textsuperscript{a,*}

\textsuperscript{a} CNRS-UPR 9078; Université Paris Descartes, Faculté de Médecine, site Necker, 75730 Paris Cedex 15, France
\textsuperscript{b} CNRS-UPR 2167, Avenue de la Terrasse, Bât. 26, 91198 Gif-Sur-Yvette Cedex, France

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Abstract This study focused on the stability of UCP2 (uncoupling protein 2), a mitochondrial carrier located in the inner membrane of mitochondrion. UCP2 is very unstable, with a half-life close to 30 min, compared to 30 h for its homologue UCP1, a difference that may highlight different physiological functions. Heat production by UCP1 in brown adipocytes is generally a long and adaptive phenomenon, whereas control of mitochondrial ROS by UCP2 needs more subtle regulation. We show that a mutation in UCP2 shown to modify its activity, actually decreases its stability.

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1. Introduction

The uncoupling proteins (UCPs) belong to the family of mitochondrial carriers located in the inner membrane. UCP1 is specifically expressed in brown adipocytes, and acts as a proton carrier in uncoupling oxidative phosphorylation \cite{1,2}, thereby ensuring the non-shivering thermogenesis of brown adipose tissue \cite{3,4}. UCP1 and its first homologue, UCP2 \cite{5}, share 59% homology, but in many respects. First, UCP1 is involved in non-shivering thermogenesis, whereas UCP2 plays no role in thermogenesis \cite{6}. Second, whereas UCP1 is specifically expressed in brown adipose tissue, UCP2 is expressed in many tissues, such as spleen, lung, intestine, pancreatic \(\beta\) cells, and immune cells \cite{7–9}. Third, UCP1 expression is transcriptionally regulated, whereas UCP2 protein level can change without any alteration in mRNA level \cite{7}.

Several physiological situations highlight the importance of regulation for UCP2 expression, since UCP2 protein level increases and decreases in a very short time. LPS injection in mice leads to rapid down regulation of UCP2 protein in macrophages, whereas its expression increases strongly and transiently in the lung 16 hours after LPS injection \cite{6,7,10,11}.

UCP2 protein level also changes during the oestrus cycle in ovary and uterus \cite{12} or during kainic acid-induced seizure in neurons \cite{13}. Taken together, these data suggest the existence of protein regulation, allowing a rapid decrease in UCP2 expression, while translational regulation accounts for the rapid increase in UCP2 expression. These results suggested that UCP2 is very less stable than its homologue UCP1 since Puigserver et al. described that the half-life of UCP1 was very long from 20 to 30 h \cite{14}. We show here that the half-life of UCP2 is short, approximately 30 min, compared with about 30 h for UCP1 \cite{14}. We also investigated the effect on UCP2 half-life of three polymorphic sites in the translated region \cite{15,16}, and of the dominant negative D212N mutation \cite{17}.

2. Materials and methods

2.1. Plasmids and mutagenesis

A \textit{pYeDP} plasmid containing human UCP2 was used \cite{18}. Mutagenesis was done using the QuikChange multisite-directed mutagenesis kit (Stratagen). All clones were sequenced to confirm the targeted mutations. Oligonucleotide sequences were as follows:

\begin{center}
\begin{tabular}{l}
CTT 164:: A55V 5\textsuperscript{GTGCGCGCTACAG} 3\textsuperscript{GTGCGCGCTACAG};
G/A 253:: G85S 5\textsuperscript{GGTTGCC} 3\textsuperscript{AAAGCCAACCTCATGACA};
G/A 694:: A232T 5\textsuperscript{ACCACTGTCATC} 3\textsuperscript{ACCACTGTCATC};
G/A 634:: D212N 5\textsuperscript{GTGCGCGCTACAG} 3\textsuperscript{GTGCGCGCTACAG};
\end{tabular}
\end{center}

2.2. Yeast culture, electroporation and half-life analysis

The W303 strain was transformed by electroporation as described previously \cite{19}, with \textit{pYeDP} plasmid containing UCP1, UCP2 or each of the mutated UCP2 clones. After 3-4 days of growth on a selected medium at 30 \(^\circ\)C, colonies were seeded in a minimum medium without uracil until OD = 0.5. Galactose (0.1\%) was added for 2 h in the absence of glucose to induce the UCP promoter. Galactose was removed, and a sample was taken for the first point of the time course. Cycloheximide (100 \(\mu\)g/ml) was added to the rest of the culture. The time course was recorded from 30 min to 24 h.

2.3. Cell culture and treatments

Stable CHO cells expressing UCP2 were obtained as previously described for UCP1 \cite{20} and were cultured in Ham/F12 medium supplemented with 10% foetal calf serum (FCS). Caco-2 (from Dr. E. Laroche) and POGRS-1 (from Dr. A. Amsterdam) cell lines were grown in DMEM/F12 supplemented with 10% FCS and 2 mM glutamine. Nonessential amino acids (1 m\textsuperscript{m}) were added to Caco-2 culture. All media contained penicillin (100 IU/ml) and streptomycin (50 \(\mu\)g/ ml). Cell lines were grown in a humidified atmosphere of 5% \(CO_2\) and 37 \(^\circ\)C. To determine UCP half-life, cells were treated with cycloheximide (10 \(\mu\)g/ml). The time course was recorded from 0 to 24 h.

2.4. Animals and treatment

Studies on mice were performed in agreement with the institutional CNRS guidelines defined by the European Community guidelines.
principles, and by French decree No. 87/848. C57BL/6 J mice (Elevage Janvier) were maintained under a 12-h light, 12-h dark schedule, with food and water ad libitum. Mice were injected with cycloheximide (2 mg/kg) and sacrificed by cervical dislocation. Tissues were removed and kept at −20 °C until protein extraction.

2.5. Western blot analysis
Mitochondrial proteins were prepared from cells, tissues or yeasts as described [7,19]. In Western blots [7], membranes were hybridised with appropriate antibodies as previously published: UCP2, hUCP2-605 antibody [7], UCP1, 375-5 antibody [21]. In each experiment, the amounts of UCP1 and UCP2 were normalized by using an antibody against porin. Chemiluminescence (ECL kit, Amersham) was recorded using a CCD camera.

3. Results

3.1. Half-life of UCP1 and UCP2 in mammalian cells
The half-life of UCP2 was measured in two cell lines endogenously expressing UCP2, Caco-2, a human colon carcinoma cell line and POGRS-1, a granulosa cell line. We also used a stable CHO clone expressing UCP2 heterogeneously. Fig. 1A shows the kinetics of degradation of UCP2 in these cells after treatment with cycloheximide, a translation inhibitor. Interestingly, 25 min after the treatment, UCP2 expression was already decreased by 50%. Similar experiments in CHO cells with UCP1 showed a half-life close to 30 h (data not shown), as previously described [14]. UCP2 half-life was also measured in vivo. After injection of cycloheximide in C57BL/6 J mice, UCP2 was assayed in spleen, lung and duodenum. In agreement with our in vitro studies, UCP2 rapidly decreased in the three organs tested (Fig. 1B). These data agree with previous descriptions of rapid variations in UCP2 level in tissues [7,12].

3.2. Half-life of UCPs and UCP2 mutant proteins in yeast
We analysed whether a mutation in its sequence could modify UCP2 stability. To avoid the establishment of stable cell line expressing each mutant, we chose the yeast *Saccharomyces cerevisiae* since previous studies have shown that yeast is a relevant model to analyse UCPs function [22].

In this heterologous system, the half-life of UCP2 was calculated to be around 60 min (Fig. 2A), confirming the results obtained in mammalian cells and mammalian tissues. We tested the effect of three polymorphisms in the coding region of human UCP2, A55V, G85S and A232T. No significant differences were observed in the half-life of these isoforms compared to the wild-type UCP2 (Fig. 2B). A UCP2 mutant protein,
4. Discussion

UCP2 has a shorter half-life (around 30 min) than its UCP1 homologue (30 h) [12]. Degradation of mitochondrial proteins in the inner membrane is controlled by the AAA proteases (ATPases Associated with a variety of Cellular Activities) [23], which have been largely studied in yeast. To investigate the role of these proteases in the half-life of UCPS, we tested the stability of UCP1 and UCP2 in wild-type and mutant yeasts. We first ruled out degradation by the proteasome, since addition of inhibitor of this pathway does not modify UCP half-life. We then tested involvement of the iAAA protease (ym1) and the mAAA proteases (afg3 and rca1) by using yeast since it was described mammal proteases are able to restore degradation function of protease disuptant yeast [24,25]. However, whatever the protease mutant used, the half-life of UCP1, as well as UCP2, remained unchanged (data not shown), so these proteases are not involved in UCP degradation. Two recently reported proteases, Omal [26] or Mop112 [27], as or yet unknown proteases, may be involved in proteolysis of UCP1 and UCP2.

The difference in UCP half-lives may be related to their different physiological functions. Cold-induced thermogenesis due to UCP1 activity in brown adipocytes is generally not transient, and, therefore, it is important for UCP1 to be expressed at a high level over a long period of time. In contrast, UCP2 is present in many tissues whose role is not to produce heat. UCP2 is implicated in the immune response by regulating ROS production [6,11,28]. After infection by a pathogen, the ROS pathway plays an important role leading to activation of macrophages and other immune cells that eliminate the pathogen. However, this ROS production must not last if tissue damage is to be avoided. Moreover, during the oestrus cycle in females, UCP2 rapidly decreases to allow the production of luteinising hormone from the ovary. The regulation of ROS level must be strictly regulated in order to avoid side effects, such as a destruction of the oocyte. The regulation of ROS level must be subtle, and it is clear that UCP2, as a regulator of ROS level, is both rapidly synthesised and degraded.

The UCP2 half-life measured here is short and unusual. Although the half-lives of mitochondrial carriers have not often been investigated, it is accepted that they are over 10 h. Few data are available, but the half life of P450cc (cholesterol side chain cleavage cytochrome P450), an inner membrane mitochondrial protein, is around 20 h [29].

We analysed variations in UCP2 half-life using known mutations. For the three known polymorphisms already described, a slight, but not significant, decrease in half-life was observed. For the D212N mutation, UCP2 half-life was significantly reduced by 60%. Mills et al. demonstrated that the D212N mutation led to an increase in the mitochondrial membrane potential [17], suggesting that this mutation was impairing UCP2 activity. Our data imply that the reported decreased activity of the D212 mutation should, in fact, be explained by a decreased stability of the mutant. The absence of UCP2 in mice promotes atherosclerosis [28], chronic inflammatory diseases like experimental autoimmune encephalomyelitis [30], and cancers [31], and the search for D212N polymorphic site associations with such diseases in appropriate cohorts of patients should be considered.

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