Developmental Changes in Uncoupling Protein 1 and F_1-ATPase Subunit Levels in the Golden Hamster Brown Adipose Tissue Mitochondria as Determined by Electron Microscopy *in situ* Immunocytochemistry

J. Bednár[1,2] and T. Soukup[1]

[1] *Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic*
[2] *Laboratoire de Spectrometrie Physique, Av. De la Physique, B.P. 87, 38 402 St. Martin d'Heres, France*

Abstract. The postnatal developmental changes in mitochondrial uncoupling protein 1 (UCP 1) and F_1-ATP synthase (ATPase) subunit levels in the interscapular brown adipose tissue (BAT) were studied in golden Syrian hamsters (*Mesocricetus auratus*) using electron microscopy *in situ* immunocytochemistry. The relatively low initial density of 5 nm gold conjugated anti-UCP 1 immunocomplexes gradually increased from 7- to 21-day-old animals and numerous immunocomplexes were found on the mitochondrial membranes of adult hamsters. At the age of 7–9 days, a positive reaction was also detected in the cytoplasm of BAT adipocytes. Immunolocalization of F_1-ATPase subunit indicated its presence in BAT mitochondria and cytoplasm of 7- to 9-day-old animals. However, contrary to UCP 1, intensity of the immunostaining of F_1-ATPase subunit rapidly decreased both in mitochondria and cytoplasm between the 10th and 21st postnatal day and it became stabilized in adult animals at a very low level restricted to mitochondria. These results confirm that profound changes in the enzymatic apparatus of BAT mitochondrial membranes, leading to formation of thermogenic mitochondria, occur not until the early postnatal period of hamster ontogenetic development.

Key words: Immunoelectron microscopy — Uncoupling protein 1 — Mitochondrial ATP synthase — Brown adipose tissue — Golden Syrian hamster

Introduction

In the mammalian body, brown adipose tissue (BAT) plays an important role in thermogenesis. The BAT's ability to generate heat is based on the function of the tissue-specific uncoupling protein 1 (UCP 1) localized in numerous mitochondria

Correspondence to: Dr. Tomáš Soukup, Institute of Physiology, Czech Academy of Sciences, Vídeňská 1083, CZ–142 20 Prague 4, Czech Republic
E-mail: tmsoukup@biomed.cas.cz

(for review see Nicholls et al. 1986). The UCP 1 is a 32 kDa protein that uncouples the proton electrochemical gradient produced by the respiratory chain from ATP synthesis, ensured by the phosphorylating action of mitochondrial ATP synthase (ATPase) (for review see Nedergaard and Cannon 1992). At present, several UCP proteins are known and genes related to different UCP proteins have recently been found to be expressed both in the BAT and in a number of other tissues (Boss et al. 1997; Fleury et al. 1997; Brauner et al. 2001, 2003; Kopecký et al. 2003; Škárka et al. 2003). Their function, however, is not yet fully understood, but it seems to be other than thermogenesis (for review see Ricquier and Bouillaud 2000; Ježek 2002).

A characteristic feature of BAT is the high amount of mitochondria with unusually low levels of the ATPase complex (Smith et al. 1966; Prusiner et al. 1970; Houštěk et al. 1991). The final ratio between oxidative and phoshorylating capacity varies not only among different species, but also during development (Cannon et al. 1977; Houštěk et al. 1988, 1990a,b,c, 1993; Kopecký et al. 1990). The critical event in the process of formation of BAT specific thermogenic mitochondria is the expression of UCP 1 gene resulting in the synthesis and incorporation of UCP 1 molecules into the inner mitochondrial membrane and the parallel decrease of the synthesis of mitochondrial ATPase.

In the rat and mouse, the conversion of nonthermogenic mitochondria to thermogenic ones takes place prenatally (Houštěk et al. 1988), whereas it does not occur until the first postnatal week in the hamster (Houštěk et al. 1990a). Various methods (western blotting, enzyme activity measurements, light, electron and immunoelectron microscopy) demonstrated that the content of the F_1-ATPase subunit in the hamster inner mitochondrial membrane reaches its maximum level at the end of the first postnatal week and gradually decreases thereafter in parallel with the increase in UCP 1 concentration, starting from day 7 and reaching maximum one to two weeks later (Houštěk et al. 1990a). The immunoelectron microscopy technique has revealed the transient presence of significant amounts of UCP 1 and F_1-ATPase antigens in the cytosol of hamster BAT cells between the 7th and 9th postnatal days (Houštěk et al. 1990a). In order to check the occurrence of these soluble protein antigens and the process of postnatal recruitment of thermogenic mitochondria, we have used a modified procedure combined with 5 nm gold particles instead of antibody-colloidal gold probe with 10 nm particles (GAR 10 Jansen) used previously (Houštěk et al. 1990a).

Materials and Methods

The Ethical Principles and Guidelines for scientific experiments on animals were respected throughout the studies. The maintenance and handling of experimental animals followed the recommendations of the European Union and the animals were treated in accordance with principles of the Care and Use of Animals. The Expert Committee of the Physiological Institute of the Academy of Sciences, Prague, Czech

Republic, approved the investigation. Experiments were performed on Golden Syrian hamsters (*Mesocricetus auratus*). Pregnant female hamsters were kept in a special laboratory-animal breeding unit of the Institute of Physiology in separate cages at room temperature and under natural lighting (March, April; 12/12, 13/11 h; light/dark regimen), with food (DOS 2B diet) supplied with corn, sunflower and rye seeds and water *ad libitum*.

The interscapular BAT was excised from 7, 8, 9, 10, 11, 14, 17, 21 days old and adult animals after decapitation (one to three animals were studied in each age group). In adult animals, also livers were excised for positive control of the F_1-ATPase subunit presence. Immediately thereafter, tissue samples were fixed for 24 h at 4℃ with 8% paraformaldehyde in 0.12 mol/l phosphate buffer (pH 7.3), washed for 20 min with phosphate buffered saline (PBS) and saturated with 2.1 mol/l sucrose in PBS (4×5 min) for cryoprotection, frozen in liquid nitrogen and further processed according to the Tokuyasu method (Tokuyasu 1980, 1986). Briefly, ultrathin ∼50 nm thick cryosections were cut on a Reichert Ultracut ultramicrotome with a FC4 cryo unit at −100℃ and collected onto EM grid than incubated with 5% fetal calf serum in PBS for 30 min at room temperature to eliminate nonspecific reactions, washed 2×5 min in PBS and incubated for 30 min with rabbit hyperimmune serum raised against hamster BAT UCP 1 and bovine heart F_1-ATPase subunit, prepared as described previously (Houštěk et al. 1988), diluted with 10% fetal calf serum in PBS. After washing in PBS (3×5 min), the sections were incubated for 30 min with secondary goat-anti-rabbit antibody labeled with 5 nm gold particles. After washing with PBS (6×5 min) and redistilled water (5×2 min), the samples were impregnated for 10 min with a mixture of 2% methylcelullose and 3% uranyl acetate (9 : 1) and examined under a JEM electron microscope.

Results

As expected, the immunostaining for UCP 1 and F_1-ATPase subunit of hamster BAT underwent changes during early postnatal development. Immunocomplexes, appearing as dense black grains and indicating the presence of the UCP 1 antigen associated with inner mitochondrial membranes were found already in 7-day-old hamsters (Fig. 1a). The relatively low initial intensity of immunostaining gradually increased between the 7th and the 21st day (not shown). At the age of 7–9 days, the number of mitochondria was small and the presence of the reaction against UCP 1 antigen inside mitochondria was relatively scarce (Fig. 1a). However, positive staining for UCP 1 antigen was also detected in the cytoplasm of adipocytes from 7- to 9-day-old hamsters (Fig. 1a). The cytosolic reaction disappeared after the 10th postnatal day and the immunostaining with anti-UCP 1 antibody remained confined to BAT mitochondrial membranes. The most numerous anti-UCP 1 immunocomplexes were then found on the mitochondrial membranes of adult hamsters (Fig. 1b). Immunostaining with anti-UCP 1 antibody was absent

in adult hamster liver mitochondria and in other, nonmitochondrial structures both in BAT and other tissues (not shown).

Immunostaining indicating the presence of F_1-ATPase subunit was strongly positive in BAT mitochondria from 7- to 9-day-old animals (Fig. 2a), but contrary to the anti-UCP 1 reaction, it rapidly decreased during the subsequent period and exhibited very low staining level in adult animals (Fig. 2b). The cytosolic reaction was present only in 7- to 9-day-old hamsters and disappeared after the 10th postnatal day, similarly as in the case of UCP 1. The anti-F_1-ATPase subunit reaction, in contrast with the reaction of anti-UCP 1 antiserum, intensively stained the mitochondria in all examined tissues of adult hamsters except BAT, as is shown by the positive reaction in the liver mitochondria from adult hamsters (Fig. 3).

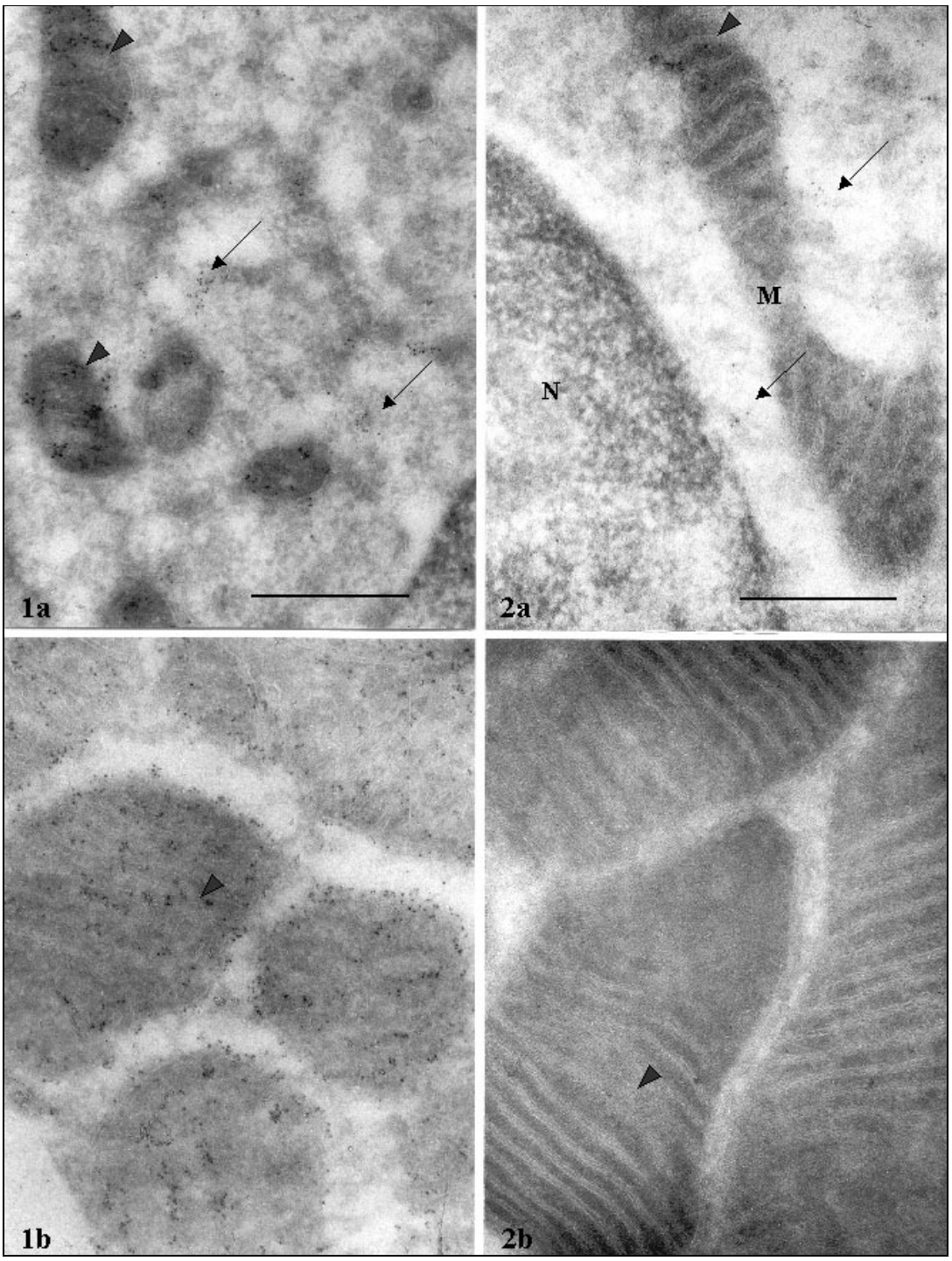
Discussion

The present results are fully consistent with the previous immunocytochemical, immunoblotting and biochemical data (Sundin et al. 1981; Houštěk et al. 1990a,b). All these experiments have confirmed that profound changes in the enzymatic apparatus of mitochondrial membranes occur not until the early postnatal period of hamster ontogenetic development. In mammals, there are three main types of perinatal development of BAT, namely newborns with well developed BAT at birth like pigs and lambs, newborns with little BAT at birth, but recruiting more BAT during the first week after birth, for example rats and mice and finally immature newborns unable to generate heat and attaining BAT with thermogenic mitochondria only after some weeks of their early postnatal development, as e.g. hamsters (Nedergaard et al. 1986).

Our data were obtained on material treated with fixation intended to preserve the maximum of antigenicity, partly sacrificing optimal preservation of the ultrastructure (Johnson 1985). The use of smaller gold conjugates increased the penetration capacity of the labeled secondary antibodies complexes into tissue sections and thus minimized the possibility of false-negative results in the case of low antigen concentrations. This method has also proved to be highly sensitive, as it detected both proteins already incorporated into the mitochondrial membrane as well as small amounts of non-assembled protein molecules ("preproteins") located in the cytoplasm (Figs. 1a and 2a).

It has been shown that mitochondria in hamster BAT increase in number and complexity during the first few postnatal weeks (Houštěk et al. 1990a). They contain considerable amounts of cytochrome c oxidase and F_0F_1-ATPase, but they

Figures 1 and 2. Immunoelectron microscopy of the uncoupling protein 1 (Fig. 1a,b) and the F_1-ATPase subunit (Fig. 2a,b) in 7-day-old (a) and adult (b) hamster brown adipose tissue. Immunocomplexes visualized with 5 nm gold particles are either present in the cytosol as extramitochondrial labeling (arrows) or bound to the mitochondrial membranes (arrowheads). N, nucleus; M, mitochondrion. Scale bar = 0.5 μm (×60,000).


1a
2a
M
N
1b
2b

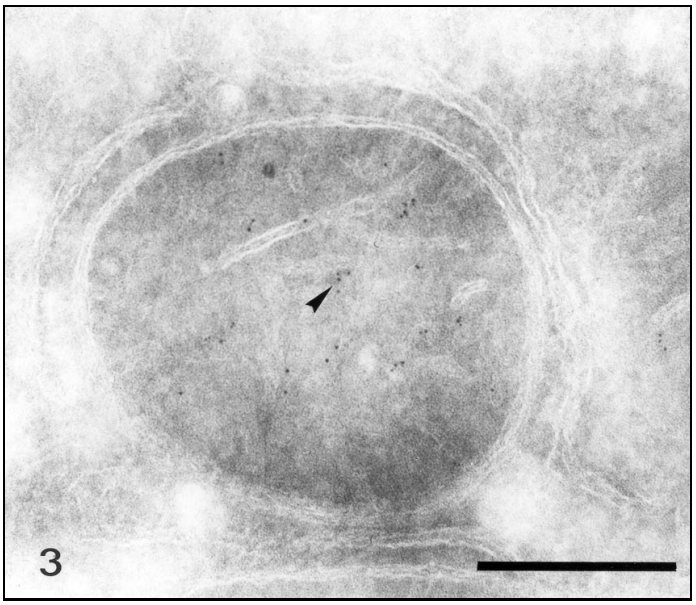


Figure 3. Control anti-F_1-ATPase subunit labeling of adult hamster liver tissue. Note the evidently higher amount of antigen in liver mitochondria (arrowhead) in comparison with that present in BAT mitochondria as shown in Fig. 2b. Scale bar = 0.5 μm (×60,000).

lack the critical thermogenic component – UCP 1 – and have to be considered as nonthermogenic, phosphorylating mitochondria (Houštěk et al. 1990a,b). After the 7th day, the amount of UCP 1 increases, thus enhancing the thermogenic capacity, while the content and activity of F_0F_1-ATPase decrease. These events eventually lead to the formation of thermogenic mitochondria. It has been suggested that the recruitment of thermogenic mitochondria may be associated with different types of mitochondria existing in parallel and replacing one another during the perinatal differentiation of BAT (Houštěk et al. 1988, 1989, 1990a,b; Drahota et al. 1989; Soukup et al. 1989, 1992).

Our results confirmed that, contrary to the UCP 1, the F_0F_1-ATPase content in BAT mitochondria of adult hamsters is low. It is generally accepted that the ATPase content in BAT mitochondria of adult mammals is significantly lower than that of other components of oxidative phosphorylation (Houštěk et al. 1988, 1990a,b). The F_0F_1-ATPase is composed of two molecular units: the F_1 oligomer composed of five subunits which form the "headpiece" and central stalk, and the membrane-bound F_0 oligomer. The central stalk provides the structural link between the catalytic sites in F_1 (residing primarily on β-subunits) and the c subunit ring in F_0. In recent years, a bulk of knowledge concerning the biogenesis and assembly of ATP synthase in mitochondria has accumulated (Houštěk et al. 1995; Andersson et al. 1997, 2000; Andersson 1998). Generally, the biosynthesis

of mitochondrial ATPase involves coordinated expression of many nuclearly and mitochondrially encoded genes and is regulated in several steps at different levels, namely the transcriptional as well as post-transcriptional and translational (Houštěk et al. 1991, 1995; Andersson 1998; Andersson et al. 2000; Tvrdík et al. 1992; Antonická 2000; Kramarova 2003). The expression of subunit c (P1 isogene) is significantly lower in BAT compared to other tissues and it is believed that the primary assembly of a c-subunit ring in the membrane is a crucial restricting step for the formation of the F_0 structure and thus for the assembly of a whole ATP synthase molecule. The other ATPase subunits in the BAT are initially expressed in excess, but subsequently their protein levels diminish and follow the low expression of subunit c (Houštěk et al. 1995; Andersson et al. 1997, 2000; Andersson 1998). Our present results seem to be consistent with these findings. However, additional experiments would be necessary for understanding whether the cytosolic reaction observed in 7- to 9-day-old hamsters reflects events in the ATPase recruitment. Interestingly, although the BAT mitochondria contain low amounts of ATPase protein, β-F_1-ATPase mRNA is present in high amounts, but it has a short half-time, which indicates the involvement of post-transcriptional regulation of its translational efficiency (Houštěk and Drahota 1977; Houštěk et al. 1991; Tvrdík et al. 1992).

One of the fundamental aspects of protein targeting onto mitochondria is the transfer of nuclearly encoded, cytoplasmatically synthesized proteins into the mitochondrial membranes. This process is coordinated with the targeting of a few, but important, proteins encoded by mitochondrial DNA, synthesized on mitochondrial ribosomes and inserted from the matrix side into the inner membrane. Several pathways directing "preproteins" into and within the mitochondria have been described, including those of translocase complexes of the outer (Tom) and inner (Tim) membranes, as well as existence of the multiple cytosolic factors with targeting function or chaperone-like molecules exerting multiple functions in translocation, sorting and assembly of newly imported proteins (for review see Neupert 1997). The mechanism of targeting and insertion of the UCP 1 into the inner mitochondrial membrane is still unknown. It was, however, suggested that the central matrix loop containing the site for binding hTom20 is carrying a complete targeting signal for directing UCP 1 to the inner membrane (Schleiff and McBride 2000). The positive reaction observed between the 7th and 9th postnatal day in the cytoplasm of BAT adipocytes can thus reflect that, at this stage, the UCP 1 and some ATPase components are synthesized on free polyribosomes in the cytosol and gradually transported into the mitochondria. The high cytosolic reaction for F_1-ATPase may reflect accumulation of newly synthesized subunits of the F_1 component that are not immediately imported into mitochondria and assembled into mature enzyme subunits or it may indicate an over-expression of some newly synthesized molecules.

It can be concluded that polyclonal antibodies used in our study reacted well with the membrane-assembled and soluble forms of mitochondrial UCP 1 and F_1-ATPase subunit. However, the polyclonal character of our antibodies does not

allow us to decide more conclusively about the character of their cytosolic reaction. In order to do this, it will be necessary to use more specific, preferably monoclonal antibodies against well characterized UCP 1 and individual ATPase subunits.

Acknowledgements. The authors wish to thank Dr. J. Houštěk for the anti-UCP 1 and anti F_1-ATPase antibodies and Dr. P. Hník, Dr. P. Brauner and Dr. T. Mráček for valuable comments and critical reading of the manuscript. The work was supported by grant No. 304/00/1653 from the Grant Agency of the Czech Republic and NATO grant 979876 (Research project No. AVOZ 5011922).

References

Andersson U. (1998): On the biosynthesis of ATP synthase. (Ph.D. Thesis). The Wenner-Gren Institute, Stockholm University, Stockholm

Andersson U., Houštěk J., Cannon B. (1997): ATP synthase subunit c expression: physiological regulation of the P1 and P2 genes. Biochem. J. **323**, 329—385

Andersson U., Antonická H., Houštěk J., Cannon B. (2000): A novel principle for conferring selectivity to poly(A)-binding proteins: interdependence of two ATP synthase β-subunit mRNA-binding proteins. Biochem. J. **346**, 33—39

Antonická H. (2000): Post-transcriptional events in the biosynthesis of mitochondrial ATP synthase. (Ph.D. Thesis). pp. 1—97, 1st Faculty of Medicine, Pediatric Clinic, Charles University, Prague, Institute of Physiology AS CR, Prague

Boss O., Samec S., Paoloni G. A., Rossier C., Dulloo A., Seydoux J., Muzzin P., Giancobino J. P. (1997): Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. FEBS Lett. **408**, 39—42

Brauner P., Nibbelink M., Flachs P., Vítková I., Kopecký P., Mertelíková I., Janderová L., Pénicaud L., Casteilla L., Plavka R., Kopecký J. (2001): Fast decline of haematopoiesis and uncoupling protein 2 content in human liver after birth: Location of the protein in Kupffer cells. Pediatr. Res. **49**, 440—447

Brauner P., Kopecký P., Flachs P., Ruffer J., Sebroň V., Plavka R., Vítková I., Vorlíček J., Kopecký J. (2003): Induction of uncoupling protein 3 gene expression in skeletal muscle of preterm newborns. Pediatr. Res. **53**, 691—697

Cannon B., Romert L., Sundin U., Barnard T. (1977): Morphology and biochemical properties of perirenal adipose tissue from lamb *(Ovis aries)*. A comparison with brown adipose tissue. Comp. Biochem. Physiol., B: Biochem. Mol. Biol. **56**, 87—99

Drahota Z., Svoboda P., Soukup T., Rauchová H., Dosoudilová J., Vítek V., Kotyk A. (1989): Structural and functional alterations of brown adipose tissue in animals with genetically induced obesity. In: Molecular Aspects of Human Diseases (Eds. J. W. Gorrod, O. Albano and S. Papa) Vol. 1, pp. 147—150, Ellis Horwood, Chichester

Fleury C., Neverova M., Collins S., Raimbault S., Champigny O., Levi M. C., Bouillaud F., Seldin M. F., Surwit R. S., Ricquier D., Warden C. H. (1997): Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nat. Genet. **15**, 269—272

Houštěk J., Drahota Z. (1977): Purification and properties of mitochondrial adenosine triphosphatase of hamster brown adipose tissue. Biochim. Biophys. Acta **484**, 127—139

Houštěk J., Kopecký J., Rychter Z., Soukup T. (1988): Uncoupling protein in embryonic brown adipose tissue – existence of nonthermogenic and thermogenic mitochondria. Biochim. Biophys. Acta **935**, 19—25

Houštěk J., Kopecký J., Janíková D., Bednár J., Holub M., Soukup T., Miková M., Vízek K. (1989): Acute and long-term regulation of brown adipose tissue thermogenesis: physiological and pathological implications. In: Molecular Basis of Membrane-Associated Diseases (Eds. A. Azzi, Z. Drahota and S. Papa) pp. 265—284, Springer-Verlag, Berlin Heildelberg

Houštěk J., Janíková D., Bednár J., Kopecký J., Šebestián J., Soukup T. (1990a): Postnatal appearance of uncoupling protein and formation of thermogenic mitochondria in hamster brown adipose tissue. Biochim. Biophys. Acta **1015**, 441—449

Houštěk J., Kopecký J., Baudyšová M., Janíková D., Pavelka S., Klement P. (1990b): Differentiation of brown adipose tissue and biogenesis of thermogenic mitochondria in situ and in culture. Biochim. Biophys. Acta **1018**, 243—247

Houštěk J., Janíková D., Rychter Z., Baudyšová M., Bednár J., Šebestián J., Soukup T., Kopecký J. (1990c): Perinatal and *in vitro* formation of thermogenic brown fat mitochondria. In: Structure, Function and Biogenesis of Energy Transport system (Eds. J. Quagliariello, S. Papa, F. Palmieri and C. Saccone) pp. 87—90, Elsevier, Amsterdam

Houštěk J., Tvrdík P., Pavelka S., Baudyšová M. (1991): Low content of mitochondrial ATPase in brown adipose tissue is the result of post-transcriptional regulation. FEBS Lett. **294**, 191—194

Houštěk J., Vízek K., Pavelka S., Kopecký J., Krejčová E., Heřmanská J., Čermáková S. (1993): Type II iodothyronine 5-deiodinase and uncoupling protein in brown adipose tissue of human newborns. J. Clin. Endocrinol. Metab. **77**, 382—387

Houštěk J., Andersson U., Tvrdík P., Nedergaard J., Cannon B. (1995): The expression of subunit c correlates with and thus may limit the biosynthesis of the mitochondrial F_0F_1-ATPase in brown adipose tissue. J. Biol. Chem. **270**, 7689—7694

Ježek P. (2002): Possible physiological roles of mitochondrial uncoupling proteins-UCP*n*. Int. J. Biochem. Cell Biol. **34**, 1190—1206

Johnson T. J. A. (1985): Glutaraldehyde fixation chemistry: a scheme for rapid crosslinking and evidence for rapid oxygen consummation. Science of Biological Specimen Preparation, pp. 51—62, SEM Inc., AMF O'Hare, Chicago

Kramarova T. (2003): Post-transcriptional regulation: protein interactions with the beta-subunit F_1-ATPase mRNA, pp. 1—33, Department of Physiology, The Wenner-Gren Institute, Stockholm University, Stockholm

Kopecký J., Baudyšová M., Zanotti F., Janíková D., Pavelka S., Houštěk J. (1990): Synthesis of mitochondrial uncoupling protein in brown adipocytes differentiated in cell culture. J. Biol. Chem. **265**, 22204—22209

Kopecký J., Rossmeisl M., Flachs P., Kmoch S., Bardová K., Brauner P., Matějková O., Šponarová J., Pražák T., Thomason-Hughes M., Hardie G. (2003): UCP1 expression in white adipose tissue and lipid metabolism. In: Progress in Obesity Research: 9. (Eds. G. Medeiros-Neto, A. Halpern and C. Bouchard) pp. 214—219, John Libbey Eurotext Ltd., Montrouge, France

Nedergaard J., Connolly E., Cannon B. (1986): Brown adipose tissue in the mammalian neonate. In: Brown Adipose Tissue (Eds. P. Trayhurn and D. G. Nicholls) pp. 152—213, Edward Arnold Ltd., London

Nedergaard J., Cannon B. (1992): The uncoupling protein thermogenin and mitochondrial thermogenesis. In: Molecular Mechanisms in Bioenergetics (Ed. L. Ernster) pp. 385—420, Elsevier, Amsterdam

Neupert W. (1997): Protein import into mitochondria. Annu. Rev. Biochem. **66**, 863—917

Nicholls D. G., Cunningham S. A., Rial E. (1986): The bioenergetic mechanisms of brown adipose tissue thermogenesis. In: Brown Adipose Tissue (Eds. P. Trayhurn and D. G. Nicholls) pp. 52—85, Edward Arnold Ltd., London

Prusiner S., Cannon B., Lindberg O. (1970): Mechanisms controlling oxidative metabolism in brown adipose tissue. In: Brown Adipose Tissue (Ed. O. Lindberg) pp. 283—318, American Elsevier, New York

Ricquier D., Bouillaud F. (2000): The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. Biochem. J. **345**, 161—179

Schleiff E., McBride H. (2000): The central matrix loop drives import of uncoupling protein 1 into mitochondria. J. Cell Sci. **113**, 2267—2272

Škárka L., Bardová K., Brauner P., Flachs P., Jarkovská D., Kopecký J., Ošťádal B. (2003): Expression of mitochondrial uncoupling protein 3 and adenine nucleotide translocase 1 genes in developing rat heart: Putative involvement in control of mitochondrial membrane potential. J. Mol. Cell. Cardiol. **35**, 321—330

Smith R. E., Roberts J. C., Hittelman K. J. (1966): Nonphosphorylating respiration of mitochondria from brown adipose tissue of rats. Science (Washington D. C.) **154**, 653—654

Soukup T., Rauchová H., Dosoudilová J., Drahota Z. (1989): Brown adipose tissue, diet-induced thermogenesis and genetic obesity. In: Molecular Basis of Membrane-Associated Diseases (Eds. A. Azzi, Z. Drahota and S. Papa) pp. 257—264, Springer-Verlag, Berlin Heildelberg

Soukup T., Bednár J., Houštěk J. (1992): Immunoelectron microscopy of brown adipose tissue mitochondria in the Golden hamster. Electron Microscopy. Vol. 3, pp. 371—372, EUREM, Granada, Spain

Sundin U., Herron D., Cannon B. (1981): Brown fat thermoregulation in developing hamsters (Mesocricetus auratus): a GDP-binding study. Biol. Neonate **39**, 141—149

Tokuyasu K. T. (1980): Immunochemistry of ultrathin frozen sections. Histochem. J. **12**, 381—403

Tokuyasu K. T. (1986): Application of cryoultramicrotomy to immunocytochemistry. J. Microsc. **143**, 139—149

Tvrdík P., Kužela S., Houštěk J. (1992): Low translational efficiency of the F_1-ATPase β-subunit mRNA largely accounts for the decreased ATPase content in brown adipose tissue mitochondria. FEBS Lett. **313**, 23—26

Final version accepted: June 23, 2003