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Review

Possible physiological roles of mitochondrial uncoupling proteins—UCP n

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Abstract

Five mitochondrial uncoupling proteins exist in the human genome: UCP2, expressed ubiquitously; UCP1, exclusively in brown adipose tissue (BAT); UCP3, predominantly in muscle; UCP4 and BMCP (UCP5), in brain. UCP4 is the ancestral prototype from which the other UCP n diverged. Findings on the level of organism and reconstituted recombinant proteins demonstrated that UCP n exhibit a protonophoric function, documented by overexpression in mice, L6 myotubes, INS1 cells, muscle, and yeast. In a few cases (yeast), this protonophoric function was correlated with elevated fatty acid (FA) levels. Reconstituted UCP n exhibited nucleotide-sensitive FA induced H⁺ uniport. Two mechanisms, local buffering or FA cycling were suggested as an explanation.

A basic UCP n role with mild uncoupling is to accelerate metabolism and reduce reactive oxygen species. UCP2 (UCP3) roles were inferred from transcriptional up-regulation mediated by FAs via peroxisome proliferator-activated receptors, cytokines, leptin signalling via hypothalamic pathway, and by thyroid and β 2 adrenergic stimulation. The latter indicated a role in catecholamine-induced thermogenesis in skeletal muscle. UCP2 (UCP3) may contribute to body weight regulation, although obesity was not induced in knockout (KO) mice. An obesity reduction in middle-aged humans was associated with the less common allele of –866 G/A polymorphism in the *ucp2* gene promoter enhancing the exon 8 insertion: deletion transcript ratio. Up-regulated UCP2 transcription by pyrogenic cytokines (tumour necrosis factor α (TNF α)) suggested a role in fever. UCP2 could induce type 2 diabetes as developed from obesity due to up-regulated UCP2 transcription by FAs in pancreatic β -cells. UCP n might be *pro*-apoptotic as well as *anti*-apoptotic, depending on transcriptional and biochemical regulation.

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Keywords: Mitochondrial uncoupling proteins UCP2, UCP3, UCP4, UCP5; Fatty acid-induced uncoupling; Obesity; Diabetes; Fever; Apoptosis

Abbreviations: BAT, brown adipose tissue; BMCP, i.e. UCP5; FA, fatty acid; iv, intravenous; icv, intracerebroventricular; KO-mice, knockout mice; LPS, lipopolysaccharide; NIDDM, non-insulin-dependent diabetes mellitus; PPAR, peroxisome proliferator-activated receptor; PUMP, plant uncoupling mitochondrial protein; TNF α , tumour necrosis factor α ; T₃, triiodothyronine; UCP n , uncoupling protein ($n = 1, 2, 3, 4, 5$); WAT, white adipose tissue

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1. Introduction—explosion of UCP n research

Progress in the understanding of mitochondrial function within living and dying (apoptotic) cells has been paralleled by the recent progress in research into novel mitochondrial uncoupling proteins, UCP n . This explosion of UCP n research has meant that this review cannot provide more than a snapshot of these proteins in the year 2001 (and early 2002). For

earlier accounts, the reader should refer to other less recent reviews, e.g. [1]. It should also be noted that new and fascinating developments are expected in the very near future. Our enthusiasm was recently strengthened by reports that helped eliminate some doubt concerning the physiological roles of UCP n . These include findings at the level of the organism [2–5], as well as isolated reconstituted UCP n [6–8], demonstrating that both UCP2 and UCP3 are true uncoupling proteins providing a protonophoric function. Unexpected physiological or pathological implications of the UCP n function were also indicated, such as possible UCP2 involvement in diabetes [9–11] and in apoptosis [12]. UCP2 was clearly linked to obesity [13], to direct β_2 adrenergic stimulation [14] and to the regulation of myocardial energy metabolism [15].

The high level of progress achieved is evident when we recall the classic UCP1, specific to brown adipose tissue (BAT) mitochondria. It has been known since the mid-1970s that UCP1 is a final unit in the cascade of nonshivering thermogenesis in the BAT of newborn mammals, cold-adapted rodents and hibernators [16,17]. UCP1 was recognized as a factor causing BAT mitochondria to be uncoupled when isolated without bovine serum albumin (BSA). Coupling was possible upon successive nucleotide addition. This revealed purine nucleotides as the inhibitory ligands of UCP1. UCP1 was one of the first mitochondrial membrane proteins to be isolated, sequenced and characterized in the reconstituted system. Numerous mutagenesis and photomodification studies have elucidated its structure/function relationships. Uncoupling, however, was considered as an artefact originating from isolation for the other types of mitochondria, and as a result plant uncoupling mitochondrial protein (PUMP) was discovered and reconstituted only about 20 years later [18]. It was then found that the PUMP1 sequence contains all features common to mammalian UCP n [19,20]. Recently, sequencing of the human genome and other genomic screening has led to an “inflation” of UCPs. In 1997, Tartaglia’s group first cloned and sequenced an uncoupling protein homolog UCPH [21], later called UCP2 [22]. The ubiquitous distribution of its mRNA [22] has surprised the scientific community. Predominantly muscle-specific UCP3 was described in the same year [23–25], and identification of two brain-specific proteins, called BMCP (UCP5) [26] and UCP4 [27] followed. We have annotated even

a UCP4 homo-log in the plant *Arabidopsis thaliana* [20], UCP2 homo-logs were also recognized in fish and humming-birds, and UCP4 or UCP5 homologs in *Drosophila* and *Caenorhabditis elegans* [20]. Why did the nature develop so many distinct inner membrane proteins that might uncouple mitochondria from lymphocytes to bananas? What are their major physiological roles and what are their minor ones? Answering these questions will keep bioscientists busy for a long time to come. This review aims to elucidate the current roads that may lead to such a goal.

2. Phylogenesis of uncoupling proteins

Since the *UCP3* gene in the plant *A. thaliana* is closely related to human UCP4 (Fig. 1), as well as to the analogs in *Drosophila melanogaster*, *C. elegans*, and soil amoebas (*Dictiostelium discoideum*) [20], we have suggested that UCP4 is most likely to be the ancestral UCP n prototype from which the other plant and mammalian UCPs diverged [20]. UCP2 (UCP3) are evolutionary younger, the youngest being UCP1. Speculation that UCP4 might act in apoptosis in the mammalian brain [27] is supported by the early occurrence of UCP4 homologs in the simplest multicellular organisms. Moreover, if any basic physiological UCP n function exists, it must have existed since the origin of multicellular eukaryotes and for some reason persisted in higher-level organisms only in the brain. Alternatively, such elementary functions could be relayed to UCP2.

UCP n form the only group of proteins with several members, representing the annotated gene subfamily within the gene family of mitochondrial anion carriers [20]. Five subfamily members are found in the human genome and three in the *A. thaliana* genome (PUMP1, PUMP2 and PUCP4). The whole mitochondrial anion carrier family contains 35 different carriers in the yeast genome (there are no UCPs in yeast), 44 in the *Drosophila* genome, and 47 in the human genome. We have identified the so-called UCP-signatures, i.e. sequence motifs common to all UCP n but absent in other family carriers [19,20]. The dicarboxylate carrier seems to be the closest carrier to UCP n , since it contains portions of some UCP-signatures. The closest by overall homology is the oxoglutarate carrier. The UCP-signature sequences may be correlated with the

	Signatures / MACP conserved	1st transmembrane segment		1st matrix segment		2nd transmembrane helix	
		AGRA~DII## SIEG~QCRR* non	#####Q * * * * * *	+ n n G T n I n n + OH F n n * (for UCP1-3, PUMP)	* n n X + Q n n I n n + * s n N G n S n G s f H s n n P		
1 <i>hbMCP</i>	M G F P G I I L F L R K F A A A I V S G H Q K S T T S H S M S G L N W K P	F V Y G C L A S T A E F G I P D L T K T R Q G Q S D A R F K E	Y R G M F H A F R C K E G L A G C A P A L L R G A S Y C T I N	122			
2 <i>DmB MCP</i>	M A E S K D W R F V Y G G V A S I A E F G I P D L T K T R Q G G Q K D Q S F S Q	Y R G M T D A F V K S R E E G L E R A G C W P P A V L R D A T Y C T I K F	98				
3 <i>DmUCPA</i>	M A A K T D E S P V A S T S S N P A P S S R H Q L R P K F Y A D A F C T Y	F V Y S V A S I A E F G I P D L T K T R Q G G E G A A H S A G K S N X M Q Y R G M V A T A F C	R E G L L K I G G V T P A Y R H V V S G S R	124			
4 <i>DmUCP4B</i>	M M K G V N T V F R P A E W D N S E E K E R K L E Y V T N K K T P P V E I	I T A F A S A S A E I G I P D M C K T R Q G G E Q A S R V G Q K A Y R G I L A T A M C	R E G L L K I G G S A M F R H S L F S G I K	118			
5 <i>DmUCP4C</i>	M A K A E R D Y W H L R S E I E E E P R F P T N V D P L T A R N L F O I	Y V N T F G A N A A E S C V P I D V K T R Q D G E Q A K K T K A M P T A T E T N	M R V E G F K S Y A G F S A M V T R F I N S R	116			
6 <i>CeUCP4</i>	M G S A V T S T S D S T A P G N S Q T F K I A T K V F I Q Q V L P E R T S I Y F G	E F S C T A A A E T N T P D I T K T R C R N K F T K G G I W M V Q V T Y D	R E G A M A G C Y A P A T R H Y I T G I R	117			
7 <i>DdUCP4</i>			S L G L V N G T K K N E G S A N K G T P S L R E A T Y S T B	40			
8 <i>hUCP4</i>	M S V P E E E E R L L P T Q R W P R S R S S G C A T A E F G I P D L T K T R Q G C E A A L R L G D G A R E S	F Y R G M Y R A L C L E E G F L K Q G T P A Y R H V V S G S R	106				
9 <i>AiUCP4</i>	M E R S R V T R E A P T G T R I L L A S E S A M A E S I P D L T N M Q H G S G S A S G A H R I G A G V V S E A	R E G I L G K G S P A I R H E F T P R I	91				
10 <i>AiPUMP1</i>	M V V A G K S D L S L P K T F A C S A F A A C V E G I P D P D K N R Q R S A L A G	D V T L P Y R G L L C T V G T A R E G E R S L V R Q V P C L H R C L F G G R	93				
11 <i>SfPUMP1</i>	M C G G D H G G K S D I S F A C I S A S S F A A F A B A G L I P D A K V R Q Q K A V E G	D G L A L P Y R G L L C T V G T A R E G E S A L K G V P P C L H R C L F G G R	97				
12 <i>SfPUMP1</i>	M G D H G P R T E I S F A C S S R A A F A F A B A G L I P D A K V R Q Q K A V T G	D V A L P Y R G L L C T V A T A R E E G S A L K G V P P C L H R C L F G G R	94				
13 <i>AiPUMP2</i>	M A D F K P R I E I S F L E T F I C S A F A A F A F A G L I P D A K V R Q R K I P T G D G E	N L P Y R G S I G L T A T A R E E G S Q L W K V A C H R C I V G G R	95				
14 <i>UCP1 hamster</i>	M V N P T T S L V H P T T M G V I F S A G V A A C L A D I P D I T K T R Q G E G Q	S S T I Y R G V L C T I T T A K T E G L P K G P A C A G R O I S F A S T R	92				
15 <i>UCP1 human</i>	M V G L T A S D V H P T I G V L F S A P A A L A D I P D I T K T R Q G E C P	T S S V I R K G V L C T I T A V K T E G R M K Y G P A G A G R O I S S A S T R	93				
16 <i>UCP2 human</i>	M V G F K A T L V P P T A T V K I G A G T A A D I P D I T K T R Q G E S O G P V	R A T A S R O Y R G V M C T I L T M V R T E G P R S N C A G C R O M S F A S S A	97				
17 <i>UCP2 carp</i>	M V G F R A G D V P P T T V I G A G T A A A D I P D I T K T R Q G E S K P V N T G H G	P Y Y R G V F C T I S T M V R E G P R S S C V A G C R O M S F A S S A	98				
18 <i>UCP2 danio</i>	M V G F R A G D V P P T T V I G A G T A A A D I P D I T K T R Q G E S K A S T N M G R G	P Y Y R G V F C T I S T M V R E G P R S S C V A G C R O M S F A S S A	98				
19 <i>UCP3 human</i>	M H S L K P S D V P P T M A V I I G A G T A A A D I P D I T K T R R Q G E N Q A V	Q T A R L V O Y R G V E G T I L T M V R T E G P C S P N R N C A G C R O M S F A S S A	96				

	2nd cytosolic segment		3rd transmembrane helix		2nd matrix segment		4th transmembrane helix		3th cytosolic segment	
	B B C OH	*	#####Q	I	+ n n + I X G T X n A I n n I p - C L not for hbMCP N P H	-	L W P N X X N n n n M C n - n n n I OH C T S C I n O H D H C I T I A F n O H F n	-	*	-
1 <i>hbMCP</i>	G T Y O S L K R L F V E R L E D E T L I N M I C G V V S G V I S S T I A N P	D V I K V R M Q R G S L F Q G S M G S I D I Y Q O E G T R L W R G V P T A C R A A I V V G V E P V Y D I T K H E I L S G M M	G D T L	236						
2 <i>DmB MCP</i>	G T Y I L K K L A N E R G L L I N E D G S E R V W S I C A A A G A I S S A I A N P	D V I K V R M Q R H C K G Q H K L G C G E G I Y K Y E G T R L W G V P T A Q R A V V I A S V E P V Y D I T K L O E M N A F G D H V G	206							
3 <i>DmUCPA</i>	C S V L M R K E F T Q N G T Q A R P W K S L C G V T A G A V Q W L A S P A D	K V Q I Q E G R R R M G E P P V H S A G H A R O R Q R G G T R L W R G S P N V Q R A A L V N L G D E T Y D T K H L I M N R L O M P D C H T	246							
4 <i>DmUCP4B</i>	L T Y D Y M E K M I V P D E D R P Q S F L G S C I S C Y L A G A T A S V E T N P E L	K T O N Q E G Q R R R G E P P I H N Y Q A L T S Y R T G G V I G V L W G T P N T W R S A L V T I G D V S C Y D F C R F A E F D V D R E	243							
5 <i>DmUCP4C</i>	V Y V D F R R P F L Y Q N E R N E E V K Y M A L G C S F T A G C I A Q A L A S P D	K V R N O T E G R R R Q G Y D V S V M Q A V D V I Y R G G P F L W G V P T Q R A A L M T T G D V S Y D I S R T F K R L L D E E C P	241							
6 <i>CeUCP4</i>	G Y E Q I R L L T F N K E V E S F P L W K S L C G A F S G L I A Q F A A S P D	K V O N Q E G L R R Q K O P L Y T E A T D C E R S Y R T Q G P F L W G V P N C Q R A A L L N M A D I A T Y D S K H G E D N F E K D N W L	240							
7 <i>DdUCP4</i>	G S V D V I N Y F I D S N G K T N L S K Y T S G A L S G A L C A C T S P D	K V R Q G S S M G V Y D S S A K E T I A K E G T R L W G V P T Q R A A L L T A S O P E Y D H I K M D H G I Q V D C I Q	156							
8 <i>hUCP4</i>	V T V E H L R E V V F G K S E D E H Y P W K S V G G M M A G V I Q Q F L A N P D	K V O N Q E G E R R K E G P L P F R O V H H A A K I L A E G G T R L W G V P N Q R A A L V N M G D E T Y D T K H Y L V L N T P E D N I	229							
9 <i>AiUCP4</i>	T E V E N E K L I V R S E T N N S E S P E A T R A L V G G F R G V I A Q V V A S P A D	K V R M Q A D C R L V S Q S I R P Y S G P E A F T K I Q S E G V R L W G V P N Q R A F L V N M G E L A C Y D H A R H F V I D K K T A E D N F	216							
10 <i>AiPUMP1</i>	G V Y E P K N L Y V G K D F V G D M P E S K K I A G L T I G A L G M V A N P D I	K V R L Q A E C L L A A G A P R R Y S G A N A S T I V R Q E G V R L W V L G L P N V A R N A I I N A A E L A S Y D O V K E T I L K I P G F T D N V	215							
11 <i>SfPUMP1</i>	G V Y E P K N L Y V G K D H V G D M P E S K K I A A A L T I G A L G T I A N P D I	K V R L Q A E C L L P A G V P R R Y S G A N A S T I V R Q E G V R L W G L G L P N G R N A I I N A A E L A S Y D O V K E A V E R I P G F T D N V	219							
12 <i>SfPUMP1</i>	G V Y E P K S F Y V G D N F V G D M P E S K K I A G L T I G A L A I I V A N P D I	K V R L Q S E C L P P G V P R R Y S G A N A S T I V R K E G G R L W G L G L P N A R N A I I N A A E L A S Y D O V K O T I K L P G F D F	216							
13 <i>AiPUMP2</i>	G V Y E P K T L L V G S D F I G D P E Y Q I A A E L I G A T A I I V A N P D I	K V R L Q S E C L P A G V P R R Y A G A V A N F I V K L E G V S A L W G L G L P N A R N A I I N A A E L A S Y D O K E T I M K I P F F R D S L	217							
14 <i>UCP1 hamster</i>	G V Y D T V Q E Y F S S G K E T P P T E G N R S A G E M I G G V A V F I G G P E Y	K V R L Q S O S H H G K P I T G T Y N A R I I A T T E S F S T L W G T T P N L N V I N C E L V Y D L M K A L I N N O L A D D V P	212							
15 <i>UCP1 human</i>	G V Y D T V Q E F L T A C K E T A P S E G S I A G L T I G G V A V F I G G P E Y	K V R L Q S O S H H G I K P I T G T Y N A R I I A T T E G F T E L W G T T P N L M R S V I I N C T E L V Y D L M K E A F N K N N L A D D V P	213							
16 <i>UCP2 human</i>	G V Y S V K Q F Y T G S E H S C S R I A G S T I G A L A V A Y A Q P D Y	K V R F Q R Q S H G C G R R Y O S T Y N A N K I A R E E G F L W G T S P N V A R N A I V N C E L V Y D L I D A L K A N L M T D D L P	215							
17 <i>UCP2 carp</i>	G V Y S V K Q F Y T G S E H G C S R I A G A C T I G A M A V A Y A Q P D Y	K V R F Q R Q S A G A N K R I H S T M A R I A R E E G F L W G T G P N I R A I V N C T E L V Y D L I D A L K S S L M T D D L P	216							
18 <i>UCP2 danio</i>	G V Y S V K Q F Y T G S D H G C S R I A G A C T I G A M A V A Y A Q P D Y	K V R F Q R Q S S A S K R Y H S T M A R I A R E E G F L W G T G P N I R A I V N C T E L V Y D L I D A L K S S L M T D D L P	216							
19 <i>UCP3 human</i>	G V Y S V K Q V Y T P K G A D N S S E T T I A G C I I G A M A V T C A G P D Y	K V R F Q A S S H G P S R S D R Y S G T M A R I A R E E G V E L W G T E P N I R A I V N C A E V Y D I E H E K I D Y H I T D N F P	218							

(A)

Fig. 1. Alignment of predicted uncoupling proteins in *D. melanogaster*, *C. elegans*, *Dictyostelium discoideum* and *A. thaliana* genomes together with known human, fish or plant UCPS. The absolutely (or predominantly) conserved residues and charges in all UCPS are white in black boxes; the semiconserved residues occurring at least in a group of UCPS are shadowed. The mitochondrial carrier family signatures are marked by number, the previously defined UCP-signatures [19] extended later [20] are written above in bold with a dotted background. Conserved charges are also indicated in the first line. Symbols represent the following items: n, neutral nonaromatic residue including M; f, aromatic residue; “+” or “-”, positive or negative charged residues, respectively; OH stands for S or T; NH stands for N or Q; and, p represents S, T, N, Q. Stars depict the residues well conserved in the mitochondrial carrier family, exclamation marks refer to the “quite conserved” residues [19]. The transmembrane regions are depicted according to Klingenberg [17].

sites of fatty acid (FA) interaction [19]. An analysis of UCP n sequence motifs revealed that all UCP n possess the residues identified in UCP1 which are important for purine nucleotide binding.

3. Transcriptional up-regulations—hints for possible physiological roles

UCP2 mRNA was found in all studied tissues but in differing amounts [21,22]. The actual protein amounts are not however quantified in a satisfactory manner. A dispaired relationship between the mRNA and protein amount might result from the revealed translational down-regulation [28]. This counter-regulatory decrease in translation most probably represents the main mechanism preventing the lethal effects of the extensive uncoupling. UCP3 mRNA was found predominantly in human skeletal muscle and mouse (rat) skeletal muscle, heart, and BAT [23,24] (traces were also found in the human heart, thyroid and bone marrow). UCP4 [27] and UCP5 [26] were identified as brain-specific proteins. In general, screening studies of UCP n mRNA have revealed many principal transcriptional up-regulations for novel UCP n . Let's consider them in details, since they provide some hints for assessment of the possible physiological roles of UCP n .

3.1. Regulation via nuclear orphan receptors *PPAR α , PPAR β , PPAR γ*

FAs, polyunsaturated FAs, prostaglandins, and eicosanoids act as transcriptional up-regulators of UCP2 [29–41] and UCP3 [42–46] by means of the orphan nuclear receptor, peroxisome proliferator-activated receptor PPAR [29], subtype PPAR α in liver [33,34], PPAR β (also called PPAR δ) in skeletal muscle [39], and PPAR γ in adipose cells [30–32,36–38]. The PPAR γ 2 isoform is adipocyte specific, whereas PPAR β is ubiquitous. FAs may interact directly with PPAR or serve as precursors for biosynthesis of higher affinity PPAR ligands. Thus, linoleic acid up-regulates UCP2 transcription in white adipose tissue (WAT) and skeletal muscle cell lines [32]. Mice fed with fish oil, which contains ω -3 polyunsaturated FAs such as eicosapentaenoic acid, had increased UCP2 mRNA in liver (activated via PPAR α) [34], or in small intestine

[35] and WAT (activated via PPAR γ) [36]. Unlike eicosapentaenoic acid, ω -6 polyunsaturated FAs, such as *cis*-8,11,14 eicosatrienoic acid, were found to be the most potent up-regulators of UCP2 transcription in skeletal muscle [39]. Most likely they serve as precursors for prostaglandin PGE $_2$ and prostacyclin PGI $_2$ biosynthesis. PGE $_2$ and PGI $_2$ may subsequently bind to PPAR β or act via protein kinase A [39]. Non-metabolized bromopalmitate also stimulated UCP2 mRNA in WAT [37], while the conjugated linoleic acid induced UCP2 mRNA in both WAT and skeletal muscle [38]. PPAR receptors act in cooperation with retinoic RXR receptors [29,30]. The PPAR ligands most likely stimulate UCP2 transcription indirectly via a regulatory cascade, when PPAR activates the expression of some, yet unknown, transcription factor(s). This (these) subsequently bind to the double E-box motif of the *ucp2* promoter [40]. A potentiation of PPAR-mediated stimulation was found for the mitochondrial biogenesis controlling thermogenic cofactor PGC-1 [41].

The PPAR agonists thiazolidinediones also up-regulate UCP n . High-fat feeding induced the UCP2 mRNA increase in the epididymal fat pads of A/J mice [30]. The PPAR γ 2 participation was confirmed by the equal responses of its agonists carbaprostacyclin (cPGI $_2$), α -bromopalmitate and BRL49653 [30]. The first two also acted in preadipocyte cell lines possessing PPAR β . Troglitazone induced UCP2 mRNA in the pancreatic islets of Zucker diabetic rats [31]. Darglitazone and troglitazone were more active than the PPAR α ligands, pirinixic acid and clofibrate, in inducing UCP2 mRNA in BAT and muscle cell lines [32]. The PPAR α -selective thiazolidinediones [33] and fenofibrate up-regulated UCP2 mRNA in liver [34]. PPAR α -activation was also observed in the pancreatic β -cells [10]. A 48-h treatment with PPAR γ agonists BRL49653 and bromopalmitate resulted in the UCP2 mRNA increase in the cultured human adipocyte explants [37]. Similarly, UCP3 mRNA was elevated in mouse skeletal muscle and C2C12 cells upon administration of oleic acid or BRL49653 (in cells), or by fasting [43]. An intralipid infusion to rats led to the increased UCP3 mRNA content in muscle [42]. UCP3 was even up-regulated in the adult rodent heart via PPAR α [44]. The chronic docosahexaenoic acid intake increased UCP3 in aged C57BL/6NJc1 mice [45]. Several FAs and PPAR

agonists also up-regulated UCP3 in 16 myotubes [46].

The stimulation of retinoid RXR receptor (a PPAR partner) was documented when 9-*cis* retinoic acid caused a dose-dependent induction of the UCP2 mRNA levels in brown adipocytes, whereas all-*trans*-retinoic acid had no effect [47]. The selective RXR agonists, methoprene and phytanic acid, elevated UCP2 mRNA, but agonists of the retinoic acid receptor (TTNPB and Am80) did not [47].

3.2. Regulation by leptin

Leptin is a peptide hormone released by adipocytes acting through the hypothalamus to decrease appetite and maintain lipostasis in rodents. Its peripheral (extra-neural) effects are not excluded. Leptin was found to up-regulate UCP n transcription. Leptin overexpression increased UCP2 mRNA six-fold in pancreatic islets and raised the mRNA of mitochondrial and peroxisomal oxidation enzymes, while decreasing mRNA for esterification enzymes [48]. Leptin stimulated UCP2 mRNA 10-fold in WAT, but not in leptin-receptor-deficient obese rats [48]. Intracerebroventricular (icv) leptin administration led to an 80% increase in PPAR γ , but to a decrease in UCP2 mRNA in young rats and as increase in old rats [49]. UCP3 mRNA (not UCP2) was enhanced in skeletal muscle of *ob/ob* mice lacking leptin [25,50]. The liver and WAT UCP2 and skeletal muscle UCP3 mRNA levels were increased upon an icv leptin infusion in rats [51]. Leptin increased UCP2 mRNA in WAT and both UCP1 and UCP3 mRNA in BAT [52]. The comparable responses to leptin under intravenous (iv) and icv infusion and their loss after denervation suggested that acute leptin effects involve central signalling pathways [53]. On the contrary, the decreased steady-state UCP2 mRNA levels were found in macrophages from leptin-deficient *ob/ob* mice [54].

3.3. Regulation by cytokines

Transcriptional up-regulations by tumour necrosis factor α (TNF α) and other “pyrogenic” cytokines support a possible UCP n role as the terminal heat-producing effector in fever. Thus lipopolysaccharide (LPS, simulating infection) stimulates UCP2 mRNA levels 28-fold in liver and 5-fold in muscle

and WAT [55]. This effect is prevented by antipyretics indomethacine. IL-1 β and TNF α increased UCP2 mRNA in liver four- and three-fold, respectively. These effects do not occur in liver macrophages and were confirmed with isolated hepatocytes [56]. Moreover, the LPS-induction of hepatocyte UCP2 expression was abolished by prior treatment of rats with neutralizing antibodies to TNF α . LPS also induced UCP2 mRNA in the brain [57], lung and stomach [28]. TNF α enhanced UCP2 mRNA in primary cultures of hepatocytes [56]. The absence of any TNF-responsive element in the *ucp2* promoter indicates that the TNF α effects are rather indirect, resulting from the unknown signaling cascades. Single iv TNF α injections to rats led to the significant increase in UCP2 (UCP3) mRNA in skeletal muscle [58]. UCP2 mRNA in BAT, WAT and skeletal muscle and UCP3 mRNA in skeletal muscle were up-regulated 16 h after TNF α administration in rats [59]. Since TNF α also activates the NF- κ B pathway, which leads to the decrease in reduced glutathione and increase in intracellular reactive oxygen species (ROS), the advantage of the elevated UCP2 levels lies in the aid to suppression of ROS production back to low levels.

3.4. Regulation by thyroid hormones

Triiodothyronine (T $_3$) produces an organ-specific enhancement of UCP2 mRNA in rats, namely in the heart. Moderate T $_3$ effects were found in skeletal muscle, but not in kidney or liver [60]. Chronic T $_3$ treatment increased UCP2 mRNA in BAT, WAT and soleus muscle, whereas it decreased *ob* (leptin) mRNA in WAT [61]. Muscle UCP3 levels decreased three-fold in hypothyroid rats and increased six-fold in hyperthyroid rats [25]. Administration of T $_3$ led to up-regulation of UCP3 mRNA in BAT and skeletal muscle, while cold treatment up-regulated UCP3 only in BAT [62]. Mitochondrial state 3 (4) respiration rates were low in the skeletal muscle of hypothyroid rats, higher in euthyroid rats, and five-fold stimulated (versus euthyroid) in hyperthyroid rats [63]. A similar pattern was also found for potential dependencies (plus minus BSA) reflecting H $^+$ leak kinetics. This suggests that UCP3 mediates the part of T $_3$'s effects on energy metabolism [63]. Resting metabolism increased in parallel with UCP2 (UCP3) mRNA but not

with nonphosphorylating mitochondrial respiration in mouse liver and skeletal muscle [64].

3.5. Regulation via β 2-adrenergic receptor

Originally, the decreased, unchanged, or increased UCP3 expression upon cold exposure in skeletal muscle was reported, as well as no or opposite effects of β 3 agonists [1]. However, L6 myotubes or WAT and BAT cells exhibited a direct β 2 adrenergic stimulation of UCP2 (UCP3) transcription, observed as the effects of epinephrine or β 2 agonist salbutamol, sensitive to β 2 antagonist ICI-118,551 [14]. These findings indicate the UCP n participation in catecholamine-induced skeletal muscle thermogenesis, which is a significant component of whole-body energy expenditure.

3.6. Other possible transcriptional regulations

Transcriptional regulations act via the corresponding DNA elements which are analyzed experimentally or in silico in relation to human (mouse) *ucp2* or *ucp3* genes [1,65]. The regulatory elements were found for the specificity protein Sp1, activator proteins AP-1, AP-2, cAMP-response-element binding protein (CREBP), muscle-regulatory protein (MyoD), and glucocorticoid receptors. The in silico analysis of the *ucp2* promoter predicted binding motifs for the BAT regulatory element, CCAAT box, and Y box. The latter two may determine the ubiquitous UCP2 expression [65]. The $-86/-44$ bp promoter region of *ucp2*, responsible for PPAR γ stimulation, contains Sp1, the sterol response element (SRE) and double E-box [40]. The analysis of from -879 to -839 bp of the *ucp2* promoter indicated [13] the presence of binding sites for (i) dimers of the aryl-hydrocarbon receptor nuclear translocator (ARNT) with the aryl-hydrocarbon receptors (AHR) and (ii) ARNT dimers with hypoxia inducible factor 1a (HIF1a, their presence indicates a link of hypoxic and toxic signals with UCP2 expression). The analysis also indicated that the insulin promoter factor 1 (IPF1) and the paired box-containing 6 protein (PAX6), which are acting in the nutrient regulation of glucose homeostasis. The human *ucp3* gene also contains motifs for the E-box, MyoD, myocyte enhancer factor-2, and responsive elements for PPAR and thyroid hormones [1]. Recently, the insulin-like

growth factor type 1 was also found to up-regulate UCP3 [66].

4. Transgenic animals—definitive answers?

Today's paradigm assumes that the function(s) of a protein can be revealed by deleting it genetically. Indeed studies of transgenic mice contributed to the understanding of physiological roles of novel UCP n , but some were not so straightforward. Interpreting overexpression results, one cannot consider only a direct relationship—"protein is present hence is functional"—since multiple, ligand-gated or cofactor regulations exist, as well as post-translational regulatory covalent modifications. Interpreting knockouts is possible only when gene deletion is not lethal, but the fact itself that it is not lethal, or a lack of the expected effects may result from compensatory mechanisms.

UCP2 deficiency in UCP2 knockout (KO) mice had no effect on body weight [67]. But these mice were resistant to *Toxoplasma gondii* infection due to the enhanced toxoplasmaicidal activity of the infected UCP2-deficient macrophages. This indicates that a lack of UCP2 leads to increased ROS. Another UCP-KO-mice study revealed that UCP2 negatively regulates insulin secretion by pancreatic β -cells and that UCP2 could be responsible for the development of type 2 diabetes due to obesity [9].

UCP-KO-mice were found not to be obese and had normal serum insulin, triglyceride and leptin levels, normal circadian rhythm in body temperature and motor activity, and normal body temperature responses to fasting, T₃ and cold exposure [68]. The H⁺ leak was reduced in their skeletal muscle mitochondria (contrary to no leak change in liver) [68,69]. The basal metabolic rate and respiratory exchange ratio was the same as in the control mice [68], while the ROS production substantially increased [69]. The fact that UCP3 was not required for body weight regulation, exercise tolerance, FA-oxidation or cold-induced thermogenesis, indicates that some compensatory mechanism could not be excluded. Another study of UCP3-KO-mice provided much better evidence of UCP3 uncoupling activity in vivo [70]. At the whole body level, there were no changes in energy expenditure. However, ATP-synthesis rates increased four-fold in the skeletal muscle of UCP-KO-mice

with no changes in the Krebs cycle rate [70]. This resulted in an increased ATP: ADP ratio (5.9. versus 4.5 in controls).

The 66-fold UCP3-overexpression in mice skeletal muscle led to hyperphagia, but mice weighed less and had a striking reduction in WAT mass [3]. They exhibited lower fasting plasma glucose and insulin levels and increased glucose clearance rates. Resting O₂ consumption was increased by 25% with the normal diet (by 40% with the palatable diet), accounting for an increase of 77%, (91%, when corrected for body weight) [3]. The core temperature was not affected but muscle temperature increased by 1.2 °C. Uncoupling of muscle mitochondria was indicated by the reduced respiratory control ratio from 3.4 to 2.4, caused by the ~26% increase of state 4 and ~12% decrease of state 3 respiration. Mitochondrial membrane potential dropped by 12 mV. Consequently, these results provide evidence that UCP3 affects metabolic rate and glucose homeostasis.

5. Fatty acid-induced uncoupling in mitochondria

5.1. Observed uncoupling *in vivo* or with overexpressed UCP2 and UCP3

Besides the UCP2 (UCP3) overexpression in mice, the uncoupling function of novel UCPs was clearly documented by overexpression in L6 myotubes [71] and INS1 cells [2,10,11], by the UCP2 gene transfer into muscle [72], and by expression in yeast which otherwise lack UCPs [21,22,25]. Also, the increased UCP2 expression in leptin-deficient *ob/ob* mice led to increased rates of mitochondrial H⁺ leak [73]. However, it was almost impossible to correlate increased uncoupling with elevated FA levels (with the exception of *ob/ob* mice [73]). Some correlation could be found between the increased rate of FA oxidation in INS1 cells expressing UCP2 (UCP3), the resulting decrease of the ADP:O ratio and the increased state 4 respiration [2]. But L6-myotubes uncoupled by UCP3 overexpression responded by increasing their glucose uptake (increased GLUT4 translocation to cell surface, wortmannin-sensitive) and metabolism by activating a phosphoinositide 3-kinase [71].

5.2. Fatty-acid-induced uncoupling in mitochondria

Evidently, no direct proof has been reported for FA-induced uncoupling in mitochondria related to natural UCP2 (UCP3) *in vivo*. Some evidence comes from the UCP2 (UCP3) expression in yeast, subsequent studies of yeast mitochondrial energization [74–78], and from reconstitution of recombinant proteins [6–8]. Claims were made that UCP2 is specifically activated (state 4 respiration in yeast mitochondria is stimulated) by all-*trans*-retinoic acid and that it is insensitive to palmitic acid, 9-*cis*-retinoic acid and other FAs tested [74]. It is difficult to imagine a physiochemical basis for such a strange FA specificity, and one that is incompatible with the previous survey of various FA interactions with UCP1 [79,80]. More results were reported for UCP3. Uncoupling was higher for the short splicing isoform UCP3S [75], while UCP3L had only a small effect on the state 4 respiration [76]. Also no stimulatory effects of FAs were found [77,78]. FA activation was reported to be restored in chimeric UCP3 by having its 2nd repeat replaced by the corresponding UCP1 sequence [78]. In summary, even for UCP2 (UCP3) expressed in yeast, a systematic study of FA activation of uncoupling in mitochondria is lacking. The FA interaction has only been indicated for yeast-expressed UCP2 and UCP3 after their extraction and reconstitution into liposomes [8], and for reconstituted UCP2 and UCP3 expressed in *E. coli* [6,7].

5.3. Possible mechanisms of fatty-acid-induced uncoupling

Two hypotheses have been developed to explain the mechanism of UCP_n protonophoric function. The first hypothesis (the Klingenberg model) considers the existence of an H⁺ translocation pathway within the structure of UCP_n [17] and explains the observed FA-activation of UCP_n-mediated H⁺ transport by *local buffering*. According to this hypothesis, ionized (anionic) FAs participate in jumps of H⁺ over an array of sites which form the H⁺ translocation pathway. But no such array was identified in any UCP_n structure [16,20,21]. Only H⁺ jumps over a few sites would be possible, and a water filled space penetrated deeply into the membrane would be required to achieve this

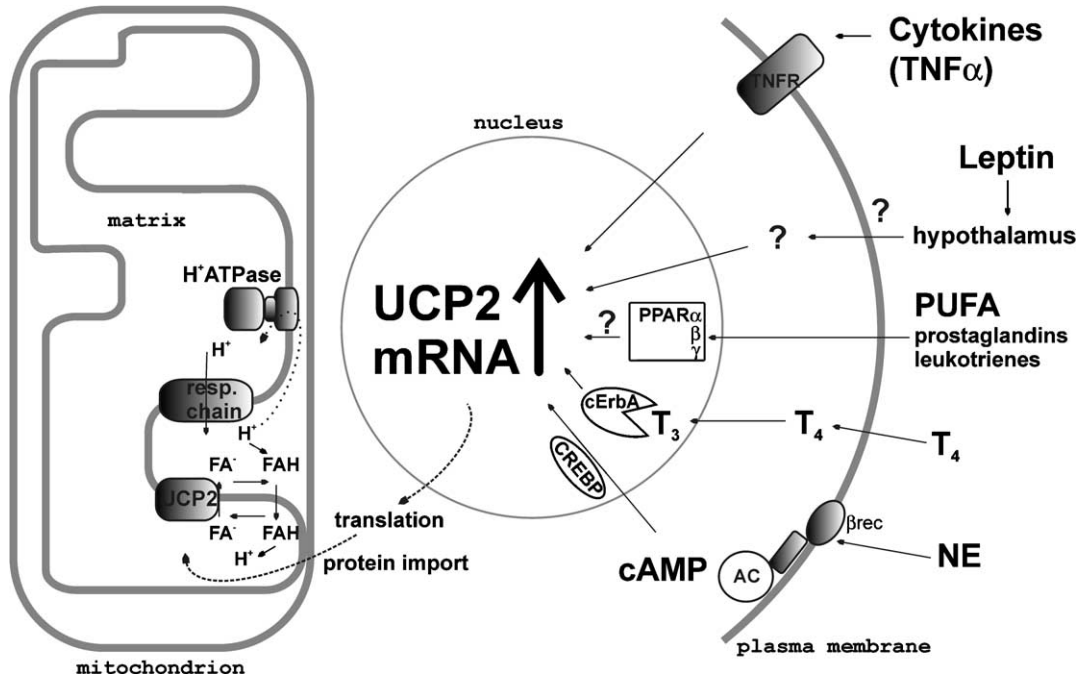


Fig. 2. Major regulations of UCP2 transcription and function. Scheme summarizes known transcriptional up-regulations of UCP2 (mostly valid also for UCP3) and depicts the fatty acid cycling mechanism of UCP-mediated uncoupling. UCP2 (UCP3) transcription is enhanced by cytokines such as $\text{TNF}\alpha$ via its receptor (subsequent complex cascade is not drawn), by leptin via a central pathway involving hypothalamic signalling (unknown steps marked by ?); by fatty acids and other lipid compounds via PPAR receptors which most probably up-regulate transcription of other transfactor (marked by ?); by thyroid hormones (involving degradation of T_4 into T_3 by deiodases and binding to cErbA nuclear receptors, isoforms $\alpha 1$ or $\beta 1$); or by β_2 adrenergic stimulation involving β_2 -receptor G-protein coupled adenylyl cyclase (“AC”) synthesis of cAMP acting via a cAMP-response element binding protein (CREBP). UCP2 protein import is most probably ensured by TOM and TIM complexes. In the presence of fatty acids and upon release of inhibitory regulations (e.g. inhibition by nucleotides), protonmotive force created by the respiratory chain across the inner membrane is dissipated by FA cycling, instead of using it for ATP synthesis.

efficient H^+ transport. The second hypothesis, originally suggested by Skulachev [81], predicts the *mechanism of fatty acid cycling* (Fig. 2), as supported by the experiments of Garlid and Ježek et al. [82,83]. UCPs and several other carriers are considered to conduct FA anions. The uniport of FA anions then leads to FA cycling, as FA anions can be protonated on a membrane *trans*-side and neutral FA can readily flip-flop back. FA dissociation releases H^+ and the resulting FA anion can enter the cycle again until all nonesterified FAs are depleted. Support for FA cycling has been inferred from the properties of undecanesulfonate uniport via UCP1. It competes with FAs for an internal (or hydrophobic binding) site on UCPs but it does not induce H^+ uniport, since it cannot be protonated (only below $\text{pH}\sim 2$). It can however be protonated, when the

H^+ carrying propranolol ion-pair is formed [83]. Further support of FA cycling is provided by the behavior of the so-called inactive FAs [79,80]. Inactive FAs are unable to flip-flop across the lipid bilayer, but they are also unable to induce H^+ transport with UCP1 [80]. Thus, when the flip-flop is inhibited, one observes no H^+ uniport. We can now extend the existence of FA cycling to all UCP n and predict that UCP n are able to mediate uniport of anionic FA, as experimentally supported for UCP2, UCP3 [6], and PUMP1 [16].

5.4. Ligand gating of novel uncoupling proteins

The control over FA cycling should be provided by proper regulatory mechanisms. It is unlikely that the only such mechanism would be a translational

down-regulation [28]. Notably the inhibitory mechanisms had to be developed during phylogenesis, otherwise the presence of active UCP n would be fatal (e.g. UCP2 in the beating heart). The nature of such regulations is presently not understood in any detail. The availability of activating FAs would represent only one such regulatory line. The basic ligand regulation might be achieved by nucleotides, which were found weakly inhibiting reconstituted recombinant UCP2 (UCP3) in sulfate media [6,8] and inhibiting with $\sim 100 \mu\text{M}$ K_i in sucrose media [7]. Nevertheless, nucleotide inhibition was hardly found in mitochondria. In the first measurements the putative nucleotide effect was only assumed [84]. Recently, only superoxide-induced uncoupling states (dependent on FA) were inhibited (transferred to more coupled states) by purine nucleotide di- and tri-phosphates in skeletal muscle, kidney, spleen and pancreatic β -cell mitochondria [85]. A similar specificity for nucleotides was found in skeletal muscle and kidney as found for UCP1 in BAT [85].

Nucleotide inhibition may be negatively and positively modulated by cofactors or by putative covalent modifications. For UCP1, Mg^{2+} and alkaline pH prevent nucleotide inhibition [16,17]. Coenzyme Q_{10} [7] or superoxide [85] might be good candidates for positive modulation. Covalent post-translational modifications might be required for nucleotides to bind with stronger affinity. Cofactors like Coenzyme Q_{10} are claimed to keep the conformation or stability of recombinant UCP n [7], as found for *E. coli*-expressed UCP1 [86]. Coenzyme Q_{10} was even suggested to be a part of the translocation mechanism. However, in our reconstitution experiments with either yeast- or *E. coli*-expressed UCP2 (UCP3), an activation by coenzyme Q_{10} reached a maximum of $\sim 130\%$. Alternatively, CoQ_{10} might stimulate uncoupling through the production of superoxide [85]. Indeed, superoxide increased mitochondrial uncoupling which was dependent on FAs and related to UCP1, UCP2 and UCP3 [85]. The effect was abolished in mitochondria from UCP-KO-mice [85].

6. Possible physiological roles of novel uncoupling proteins

One might predict three basic physiological roles for novel UCPs, when their activity is particularly low,

and hence, *mild* (partial) uncoupling of mitochondria occurs. Mild uncoupling can be defined as states when ATP synthesis is still largely unaffected. Such uncoupling slightly enhances respiration rate and concomitant metabolic rate (*the basic function 1*) This is beneficial to numerous physiological processes. It also prevents excessive formation of reactive oxygen species [85,87] (*the basic function 2*) and is inevitably paralleled by concomitant mild thermogenesis (*the basic function 3*). Mild uncoupling is likely to exist in mitochondria of all tissues of all species where UCP n were found. It also maintains the mitochondrial membrane potential below a certain limit which decreases ATP production. Without such limitation, high ATP would inhibit the respiratory chain [1].

6.1. Mild uncoupling and prevention of reactive oxygen species formation

Skulachev first hypothesized that mild uncoupling can be beneficial to cells and organisms, as it causes a decrease in ROS formation [87]. The elevated respiration rates decrease the lifetime of the semi-quinone coenzyme Q forms and the probability of superoxide anion formation through the electron transfer from coenzyme Q-O_2 . Indeed, a decrease in membrane potential, most likely induced by UCP2 in the mitochondria of nonparenchymal liver cells, thymus and spleen, inhibited ROS generation [85]. In leptin-deficient *ob/ob* mice, which have low UCP2 levels in macrophages, an increased macrophage mitochondrial ROS production was found [54]. UCP2 participation in β -cell defence against oxidants was reflected by an up-regulation of UCP2 mRNA in INS1 cells after H_2O_2 exposure [88]. UCP2 overexpression led to higher survival rates after H_2O_2 exposure [88], demonstrating the beneficial role of UCP2. Vitamin E and selenite down-regulated UCP2 mRNA [88]. Also, UCP2-KO [67] or UCP3-KO-mice [69] exhibited higher levels of ROS in macrophages and muscle, respectively. Hence, the basic role of UCP n is to avoid injury to organelles and tissues. Balanced regulations of UCP n may contribute to a prolonged lifetime in organisms.

6.2. UCP n and regulation of body weight

UCP2 may be involved in body weight regulation, although obesity was not induced in the UCP2-

KO-mice [67]. Nevertheless, an increase of 1% in ATP production would result in a gain of ~1 kg fat per year. Only a small decrease (increase) in energy expenditure could result from a reduced (enhanced) UCP2 expression. These subtle changes accompanied by significant phenotypic changes would be noticeable without measurable changes in body temperature. In humans, resting skeletal muscle metabolism is a significant determinant of whole body energy expenditure and hence could play a major role.

A significant link to a decreased risk of obesity relating to UCP2 was found in the common, -866 G/A polymorphism in the *ucp2* gene promoter [13]. The decreased risk was found to be associated with enhanced adipose tissue UCP2 mRNA expression in vivo and resulted in an increased transcription of a reporter gene in the human BAT cell line PAZ-6. A significant reduction in obesity in middle-aged humans was associated with the less common allele, which enhanced the exon 8 insertion:deletion transcript ratio [13]. In turn, this strongly indicates that this locus contributes to obesity. Previous positive findings on the UCP n relation to obesity involved down-regulated UCP2 mRNA (but not UCP3) in skeletal muscle [89] or WAT [90] in human obese subjects. An association was found between val/ala-55 polymorphism of human *ucp2* and exercise efficiency (higher for val/val) [91]; between val/ala-55 and a 45 bp insertion/deletion in the 3' untranslated region of exon 8 of *ucp2*, and energy metabolism or obesity in Pima Indians [92]. Heterozygotes for variants were associated with higher metabolic rates during sleeping. Heterozygote individuals over 45 years had the lowest body mass index. Hence, the contribution from *ucp2*-determined variation in the metabolic rates of young Pima Indians may increase the overall body fat later in life [92]. A *ucp2* polymorphism was associated with a reduced basal fat oxidation rate and an increased respiratory quotient in an African American population [93]. Also UCP3 could be involved in the regulation of body weight [94,95]. Consistent with this, a contribution of -55 C-T polymorphisms in the *ucp3* promoter in Palauans (population prone to diabetes and obesity) correlated with plasma hemoglobin A1c, fasting blood glucose in males, and body fat (%) in females [94]. Other relationships between UCP2 (UCP3) function and body weight can be seen in their transcriptional up-regulations by lipids and FAs (mostly via PPAR) and by leptin.

6.3. Adaptive thermogenesis, fever

Adaptive thermogenesis could represent the major UCP n function under conditions when the existing inhibitory regulations are substantially released and FAs are available. It is not presently known in which cell types and by which second messengers such conditions are initiated. The most plausible example is the participation of UCP2 (UCP3) in catecholamine-induced thermogenesis in skeletal muscle, due to β 2-adrenergic stimulation of their transcription [14]. The second possibility is thyroid hormone-induced thermogenesis. UCP4 [27] or UCP5 [26] could have a thermogenic function in the brain. Another specific thermogenic response is fever [96]. Even if it is not possible to consider febrile response as a simple elevation of body temperature, there are reasonable speculations claiming that UCP n could be the terminal heat-producing effectors during fever. The stimulation of UCP2 transcription by pyrogenic cytokines TNF α and IL-1 β [96] suggested such a role for UCP2 [55], although there is no direct evidence as yet.

6.4. UCP2 and diabetes

Glucose homeostasis is maintained namely by pancreatic β -cells secreting insulin in proportion to increasing glucose concentration. The β -cells sense glucose through its metabolism thus increasing the ATP/ADP ratio. It leads to closing the plasma membrane K_{ATP} channel and depolarization activating the Ca^{2+} channel. The induced Ca^{2+} influx activates insulin secretion. Insulin decreases glucose production in the liver and stimulates glucose uptake into skeletal muscle and WAT by recruiting glucose carriers to plasma membrane. Diabetes mellitus is hyperglycemia resulting either from a deficiency of the β -cell mass (type 1) or from a resistance to insulin's function (type 2, or non-insulin-dependent diabetes mellitus (NIDDM)), i.e. resistance to lowering blood glucose levels, due to β -cell dysfunction. UCP2 participation in diabetes was indicated by the revealed stimulation of UCP2 transcription in the pancreatic islets [31,48]. Stimulation was induced either by Troglitazone [31] (a PPAR agonist) in Zucker diabetic lean (+/+) and fatty (*fa/fa*) rats (homozygous for the mutation in the leptin receptor) or by leptin overexpression (by means

of adenovirus) in Zucker lean rats [48]. Subsequently, UCP2 overexpression was recognized to inhibit glucose-stimulated insulin secretion [97]. Clear indications were inferred from in vivo studies and ex vivo examinations of UCP2-KO-mice and their isolated pancreatic islets [9,67]. It has been confirmed that β -cells of UCP2-KO-mice secreted more insulin in response to glucose and that their ATP levels were higher [9]. This reflects the absence of uncoupling. Hence, it was suggested that NIDDM could develop as the dispairment between glucose metabolism and insulin secretion due to the elevated UCP2-related uncoupling [9–11]. UCP2 could be regarded as a negative regulator of insulin secretion. Indeed, the glucose-stimulated coupling of mitochondria was reduced in β -cells overexpressing UCP2 [11]. Since UCP2 transcription is up-regulated by FAs in β -cells [9,10], it has been hypothesized, that obesity, which is considered a major risk factor in acquiring NIDDM, induces this diabetes by activating UCP2. In turn, the development of artificial UCP2 inhibitors could lead to drug cures for NIDDM. This view is also supported by the finding that a diabetic *ob/ob* mouse, when crossed with a UCP2 KO mouse, restored first-phase insulin secretion and exhibited greatly decreased levels of glycemia [9]. This hypothesis is also strongly supported by the finding [10] that exposure of INS1 cells to oleate leads not only to decreased insulin secretion upon glucose stimuli and to UCP2 mRNA increase, but also to uncoupling monitored as a decreased mitochondrial membrane potential in situ. Clofibrate (a PPAR α ligand) stimulated UCP2 transcription, but Ly171-883 (a PPAR β + PPAR α agonist) or ciglitazone (a PPAR γ agonist) did not [10]. In conclusion, the reported findings provide solid evidence that pathologically increased UCP2 transcription in pancreatic islets can lead to a dispaired insulin-secretion response to glucose.

6.5. UCPn and apoptosis

Gene microarray technology monitoring gene transcription in response to radiation revealed that FA binding protein, VDAC and UCP2 (but not UCP1 and UCP3) genes belong to a cluster of genes that are rapidly induced in apoptosis-sensitive cells [12]. This was inferred from comparison of radiation effects on B cell lymphoma apoptosis-sensitive and

apoptosis-resistant cell lines. UCP2 mRNA levels increased significantly in response to radiation. Such UCP2 expression was the early event preceding any mitochondrial dysfunction. It demonstrated UCP2 involvement in this cellular response to stress. The meaning of such a response could lie either in an attempt to survive or in promoting apoptosis. The detailed UCPn role is not known, although independent findings were reported [98]. UCP4 was also suggested as a participant in apoptosis [27]. Note that the role of UCPn might be *pro*-apoptotic as well as *anti*-apoptotic, depending on transcriptional and biochemical regulations. Transcriptional enhancement and release of inhibitory regulations, which both stimulate uncoupling, might be *anti*-apoptotic, since they prevent the elevated ROS formation. The decreased mitochondrial membrane potential would inhibit Ca²⁺ accumulation into the matrix and prevent its excessive alkalization [99]. Both these events inhibit the opening of the mitochondrial permeability transition pore which is supposed to trigger apoptosis dependent on mitochondria. It is most likely triggered by cytochrome c release. Moreover, temporal correlation between an induction of UCP2 and *anti*-apoptotic Bcl-2-family members has been observed in fatty liver [99]. *Pro*-apoptotic Bax is known to interact with the ADP/ATP carrier, but since UCP2 is homologous to this carrier, one might predict the possibility of Bax interaction with UCP2 [99]. This speculation seems to be plausible, since Bcl-2 overexpression in liver decreased mitochondrial energization [99]. However, all these considerations await experimental evidence.

6.6. Other possible physiological roles of UCPn

The regulation of FA oxidation was suggested as another plausible physiological role of UCPn [1]. UCP2 could play an important role in inflammation and hematopoietic system development [1]. Adaptations inducing UCP2 transcription and constraining ROS production have been suggested for liver [99] and heart development [100].

7. Conclusions

There is no doubt that UCPn are uncoupling proteins, participating in various important physiological

and pathological situations. Revealed transcriptional up-regulations involve leptin signalling, stimulation by FAs via PPAR receptors; cytokines or thyroid hormones, and β_2 adrenergic stimulation. UCP2 (UCP3) may contribute to body weight regulation, reduced ROS production, to various types of adaptive thermogenesis including fever, and to balancing apoptotic processes. Additionally, pathological implications in obesity, type 2 diabetes, and heart failure were revealed.

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