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# Genomic Structure and Regulation of Mitochondrial Uncoupling Protein Genes in Mammals and Plants

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Uncoupling mitochondrial proteins (UCPs) belong to a discrete family within the mitochondrial anion carrier superfamily. Several uncoupling protein types have been found in mitochondria from mammals and plants, as well as in fishes, fungi, and protozoa. Mammalian UCPs and plant uncoupling proteins (PUMPs) form five distinct subfamilies. Only subfamily III contains both plant and animal uncoupling proteins, as well as UCPs from primitive eukaryotic organisms, which suggest that this group may represent an ancestral cluster from which other UCPs/PUMPs may have evolved. Genetic data indicate that *UCP*s/*PUMP*s are regulated at the transcriptional, post-transcriptional, and translational levels. Tissue/organ- and stress-specific gene expression suggests that *UCP*s/*PUMP*s are involved in the general balance of basic energy expenditure, protection against reactive oxygen species, and thermogenesis. Finally, the simultaneous occurrence of PUMP and alternative oxidase, another energy-dissipating system in plant mitochondria, raises the question of their response to biotic and abiotic stress at the transcriptional and functional levels.

**KEY WORDS:** Expression profile; mitochondria; transcription regulation; uncoupling protein.

**ABBREVIATIONS:** BAT brown adipose tissue; FA, fatty acid; MACF, mitochondrial anion carrier superfamily; PUMP, plant uncoupling mitochondrial protein; RT-PCR, reverse transcriptase-PCR.

## INTRODUCTION

In eukaryotic organisms, mitochondrial electron transport through the respiratory chain is coupled to the pumping of protons from the mitochondrial matrix, thereby generating a proton electrochemical potential ($\Delta\mu_{H+}$)across the inner membrane. Although the $\Delta\mu_{H+}$ is generally used by ATP synthase to produce ATP, the proton electrochemical potential can be dissipated by leak reactions catalyzed by endogenous proton-conductance pathways including mitochondrial uncoupling protein

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(UCP in animals or PUMP, "plant uncoupling mitochondrial protein", in plants) [1, 2]. In brown adipose tissue (BAT), proton leak triggered by UCP1 activity dissipates the energy generated by the respiratory chain as heat, resulting in a thermogenic process that is essential for the maintenance of body temperature in hibernating mammals and newborns (for a review see [3]).

In the last decade, several proteins sharing a significant degree of homology with UCP1 have been identified in mammals (for a review see [4]), suggesting novel physiological roles for uncoupling proteins. For instance, UCP3 is involved in the development of lymphocytes, thymus atrophy, and fatty acid (FA) utilization in spleen and thymus [5], whereas UCP2 may be neuroprotective by reducing mitochondrial $Ca^{2+}$ uptake and preventing the mitochondrial accumulation of reactive oxygen species (ROS) following cerebral ischemia [6].

In plants, the proton electrochemical potential energy-dissipating pathway involving PUMP and the redox energy-dissipating pathway involving an alternative oxidase (AOx, [7]) lead to the same final effect, i.e., a decrease in the efficiency of oxidative phosphorylation. PUMPs are probably involved in optimizing the $\Delta\mu_{H+}$ across the inner mitochondrial membrane, while AOx appears to function predominantly in thermogenic processes [8]. Additionally, PUMP together with AOx may have a role in controlling energy metabolism by serving as safety "valves" in the case of overloads in the redox and/or phosphate potential [9]. These overloads are consequences of an imbalance between the supply of reducing substrate and the energy and carbon demands for biosynthesis, both of which are coupled by respiratory chain activity. Another important physiological role proposed for PUMP is to protect cells against the high production of ROS during biotic and abiotic stress [10–12]. More recently, Smith *et al.* [13] suggested that the uncoupling of the mitochondrial respiration from ATP synthesis by PUMP activity may reduce the rate of mitochondrial ROS production and affect the tricarboxylic acid cycle flux.

Uncoupling proteins (UCPs/PUMPs) belong to the mitochondrial anion carrier protein superfamily (MACF), all members of which possess the energy transfer protein signature (ETPS); P-x-[DE]-x-[LIVAT]-[RK]-x-[LRH]-[LIVMFY], where x is any amino acid [8]. UCPs/PUMPs are members of the MACF because (i) they have a high sequence homology with other carriers and (ii) they transport anionic forms of FAs and other anions [8]. Alignment of the amino acid sequences of *Solanum tuberosum* and *Arabidopsis thaliana* PUMPs (StPUMP and AtPUMP1, respectively) with mammalian UCP sequences shows that these proteins are markedly similar (41% identity for both with UCP1, and 43% and 46% identity for StPUMP and AtPUMP1 with UCP2, respectively) [4, 14, 15]. Moreover, UCPs/PUMPs are proteins of ~300 amino acids with a predicted molecular mass of approximately 32 kDa [4]. Several UCP homologues have also been identified in various species and organisms, including the carnivorous marsupial *Sarcophilus harrisii*, the non-photosynthetic soil amoeba *Acanthamoeba castellannii*, the mycetozoa *Dictyostelium discoideum*, and the fungus *Candida parapsilosis* [16–19].

Although several biochemical studies of these proteins under different physiological conditions have been reported, the true role of UCP/PUMP remains to be elucidated. Attempts to determine the physiological role of these proteins in different organisms have focused on unmasking the gene structure and expression regulation

of mammalian *UCP*s [20, 21]. In contrast, there is little or no information about the gene regulation and genomic structure of plant *UCP*s.

In this review, we will describe the structural and functional characteristics of plant and mammalian *UCP* genes and will discuss their possible role(s) in plant and animal physiology.

## PHYLOGENETIC ANALYSIS AND GENOMIC STRUCTURE OF THE UCP/PUMP FAMILY

Phylogenetic analysis of the available UCP amino acid sequences from mammals, plants, and other organisms revealed the presence of five well-defined subfamilies of UCPs/PUMPs, (Fig. 1). Subfamily I contains animal UCP1, UCP2, and UCP3, whereas subfamilies II and V contain only PUMP1–2 and PUMP4–6, respectively. Interestingly, the subfamily III includes mammalian UCP4, as well as PUMP3 from dicots and monocots, which clustered with the sequence of UCP from the primitive eukaryote *Caenorhabditis elegans* (CeUCP). Therefore, this subfamily may represent an ancestral cluster, from which other UCPs/PUMPs may have evolved. Family IV contains only mammalian brain mitochondrial carrier protein-1-(BMCP1/UCP5) like proteins, which suggests that BMCP1/UCP5 may have separated from other UCPs/PUMPs before the divergence of plants and animals, The sequences of the malate/2-oxoglutarate carrier (M2OM) and the dicarboxylate carrier (DIC) from various organisms, that were included as the phylogenetically closest members of the MACF in this analysis, clustered in well-defined branches (encircled clusters in Fig. 1), indicating that UCPs/PUMPs complex family consisted exclusively of uncoupling proteins of all types (Fig. 1). Whereas M2OMs are involved in importing substrates for oxidative phosphorylation, DICs have a role in gluconeogenesis [24, 25].

The genomic structure and localization of mammalian *UCP*s have been described in detail elsewhere [8], Therefore, we will focus on *PUMP*s because novel genes from the *Arabidopsis thaliana* and rice *(Oryza sativa)* genomes have recently been identified. *AtPUMP1* [25] resides on chromosome 3 whereas *AtPUMP2* (or *AtUCP2)* [26] maps to chromosome 5. *AtPUMP1 and AtPUMP2* have almost identical gene structures consisting of nine exons. Their presumed paralogs (*At-PUMP3–6*; Fig. 2) that have been recently identified (J. Borecký, F. T. S. Nogueira, I. G. Maia, F. R. da Silva, A. E. Vercesi, and P. Arruda, unpublished results) have different gene structures. *AtPUMP3* and *AtPUMP6* have only two exons (Fig. 2) and are located on chromosome 1 and 5, respectively. The other two members of the *AtPUMP* gene family, *AtPUMP4* and *AtPUMP5*, are intronless genes (Fig. 2). *AtPUMP4* resides on chromosome 4 and *AtPUMP5* resides on chromosome 2. The rice orthologs of *PUMP*s *(OsPUMP1–4)* show a similar gene structure to their *Arabidopsis* counterparts *(Fig. 2)*. *OsPUMP1* (or *OsUCP1)* [27] was mapped to chromosome 1 whereas *OsPUMP2 (or OsUCP2)* [26] resides on chromosome 11. We have identified the rice genes encoding OsPUMP3 and OsPUMP4 (J. Borecký, F. T. S. Nogueira, I. G. Maia, F. R. da Silva, A. E.Vercesi, and P. Arruda, unpublished results). As with *AtPUMP3, OsPUMP3* also has two exons and maps to chromosome *4*. *OsPUMP4* is an intronless gene like *AtPUMP4–5*, and is located on chromosome 8.

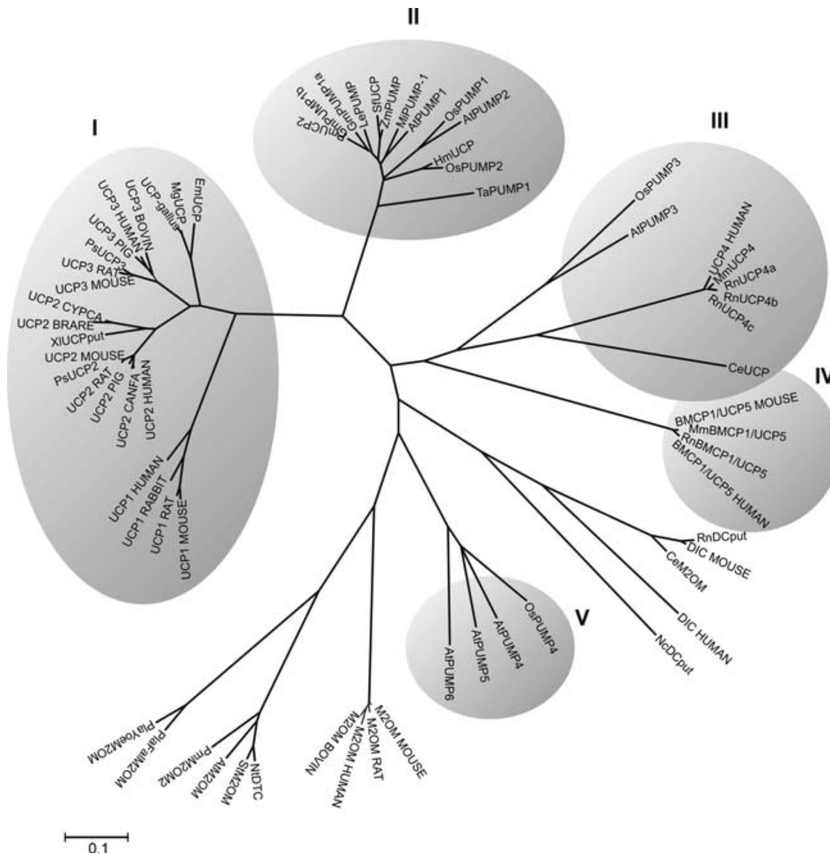


**Fig. 1.** Unrooted phylogenetic tree for UCPs/PUMPs and other mitochondrial carrier protein sequences obtained with the MEGA2 program [22]. The sequences were aligned using the CLUSTALX program [23] and the tree topology and evolutionary distances were estimated using the neighbor-joining method (1000 bootstraps). The following sequences (accession numbers in parentheses) were used for the phylogenetic analysis: UCP1_MOUSE (P12242), UCP1_RAT (P04633), UCP1_RABBIT (P14271), UCP1_HUMAN (P25874), UCP2_HUMAN (P55851), UCP2_CANFA (Q9N2J1), UCP2_RAT (P56500), PsUCP2 (AAG33984), UCP2_MOUSE (P70406), UCP2_PIG (097562), X1UCPput (AAH44682), UCP2_BRARE (Q9W720), UCP2_CYPCA (Q9W725), UCP3_MOUSE (P56501), UCP3_RAT (P56499), PsUCP3 (AAG33985), UCP3_CANFA (Q9N2I9), UCP3_PIG (097649), UCP3_HUMAN (P55916), UCP3_BO-VIN (077792), UCP-gallus (AAL35325.2), MgUCP (AAL28138), EmUCP (AAK16829), PmUCP2 (AAL92117), GmPUMPlb (AAL68563), GmPUMPla (AAL68562), MiPUMP-1 (AAK70939), LePUMP (AAL82482), AtPVMP1 (CAA11757), SfUCP (BAA92172), HmUCP (BAC06495), ZmPUMP (AAL87666), OsPUMP1 (BAB40657), OsPUMP2 (BAB40658), OsPUMP3 (CAE01569), OsPUMP4 (BAD09745), TaPUMP1 (BAB16385), AtPUMP2 (NP_568894), AtPUMP3 (F7A19_22), AtPUMP4 (F22K18_230), AtPUMP5 (F14M13_10), AtPUMP6 (T5E8_270), DIC_MOUSE (Q9QZD8), RnDCput (NP_596909), DIC_HUMAN (Q9UBX3), CeM2OM (NP_509133), NcDCput (XP_327953), M2OM_MOUSE (Q9CR62), M2OM_RAT (P97700), M2OM_HUMAN (Q02978), M2OM_BOVIN (P22292), NtDTC (CAC84545), StM2OM (CAA68164), AtM2OM (NP_197477), PmM2OM2 (S65042), P1aFa1M2OM (CAD51134), P1aYoeM2OM (EAA21506), BMCP1/UCP5 MOUSE (Q9Z2B2), MmBMCP1/UCP5 (NP_035528), RnBMCP1/UCP5 (NP_445953), BMCP1/UCP5_HUMAN (095258), UCP4_HUMAN (095847), MmUCP4 (BAC66453), RnUCP4a (CAC20898), RnUCP4b (CAC20899), RnUCP4c (CAC20900), and CeUCP (NP_505414). Protein abbreviations are from the SWISPROT database.

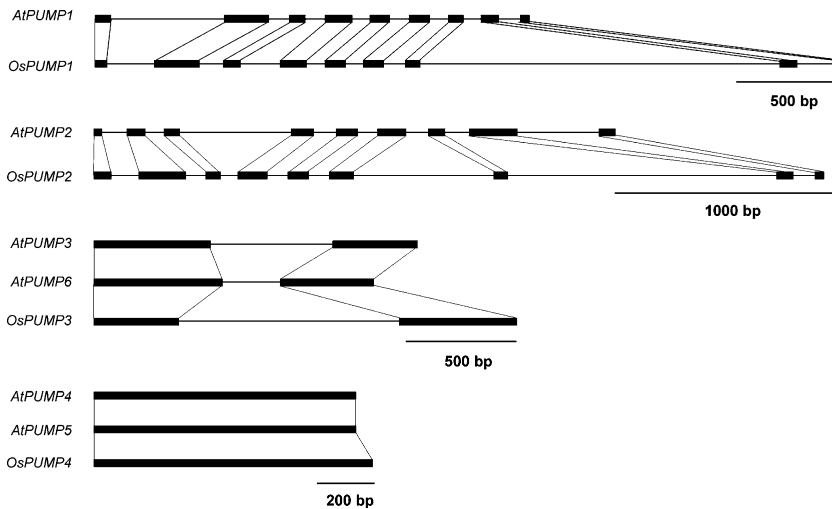


**Fig. 2.** Gene structure of *UCP*s and *PUMP*s. Filled boxes represent exons and lines represent introns according to locus data from the *Arabidopsis* information resource (TAIR) and the GenBank database. Bars indicate bp of chromosomal DNA.

Comparison of the distribution of exons of various *PUMP*s (Fig. 2) suggests that these genes may have originated through gene duplication events. Indeed, the location of *AtPUMP1, AtPUMP2, AtPUMP4*, and *AtPUMP5* within or close to duplicated regions of the *Arabidopsis* genome [28] corroborates this hypothesis (J. Borecký, F. T. S. Nogueira, I .G. Maia, F. R. da Silva, A. E. Vercesi, and P. Arruda, unpublished results). These findings are in keeping with the phylogenetic analysis shown in Fig. 1, and corroborate the existence of distinct subfamilies of *PUMP*s.

## TRANSCRIPTIONAL REGULATION OF UNCOUPLING PROTEIN GENES

The regulation of UCP gene expression was first investigated in mammals. BAT-specific *UCP1* was isolated in 1985 [29] and detailed studies provided considerable information of the molecular mechanisms regulating its transcription (for review see [30]). The transcription of *UCP1* occurs in brown fat cells and is regulated by hormonal signals during cell differentiation [31]. For instance, norepinephrine upregulates *UCP1* expression by acting through ($\beta_1$- and $\beta_3$-adrenoreceptors to increase production of cyclic AMP (cAMP). Thyroid hormone ($T_3$) and insulin also activate *UCP1* transcription [32]. However, it is still unclear whether the $T_3$ transcriptional regulation of *UCP1* is a direct effect on regulatory elements in the gene itself, or whether thyroid hormone is essential for processes in the adrenergic receptor-stimulated intracellular cascade.

Initially, the same genomic region was shown to control the tissue-specific expression of *UCP1* and its response to hormonal factors [33]. These authors proposed that a *cis*-acting regulatory element located in the 5′-flanking region, possibly at a DNase I-hypersensitive site, was required to control *UCP1* expression *in vivo*

and that this regulation may occur via a cAMP responsive element (CRE) that binds the transcription factor ATF-2 [33]. Another study of the rat *UCP1* promoter region, based on the transfection of cultured brown adipocytes with *UCP1* promoter region–chloramphenicol acetyltransferase gene constructs, showed the importance of a strong −211 bp enhancer element (regulatory region 1, R1) located between bp −2494 and −2283 [34]. In this same study, a second region (regulatory region 2, R2), located between bp −400 and −157, was supposed to contain negative *cis*-acting elements. Analogous regions were identified in the murine *UCP1* [35], suggesting that the genomic regions involved in *UCP1* transcriptional regulation are conserved between close-related species. In the R1 regulatory region, the FP1 (at bp −2444 to −2423) and FP2 (at bp −2352 to −2319) domains were shown to interact with different factors *in vitro*. The former domain binds factors related to nuclear factor 1 (NF-1) and transcriptional activator Ets 1, whereas the latter domain binds the retinoid X and triiodothyronine receptors [32]. Additional sites, designed as boxes A and B, were detected on both sides of the 5′-extremity of the R2 regulatory region. Box A contains repeated core sequence CACCC whereas box B has been suggested to bind an Etsl-related protein. The *Ets* oncogene family encodes a class of sequence-specific DNA-binding proteins involved in cell growth and differentiation [36]. Other elements present in the proximal region of the *UCP1* promoter intervene negatively in the regulation of its transcription [37].

Recently, del Mar Gonzalez-Barroso *et al.* [38] demonstrated that although the regulatory genomic region of human *UCP1* was partly homologous to the rodent enhancers, the molecular mechanisms involved in the stimulation by drugs were different, with a former having a complex region, in which the binding of transcriptional factors occurred according to a hierarchy. To fully understand the signaling pathways that mediate drug stimulation, especially the permissive effect of retinoids on the human *UCP1* gene, it is necessary to identify cofactors and transcription factors that directly bind to the human *UCP1* promoter region. Transcription factors that form obligate heterodimers with the retinoid X receptor and bind to defined elements in the promoter region of several genes belong to the nuclear receptor superfamily known as peroxisome proliferator-activated receptors (PPARs) [39]. A well-studied PPAR subgroup consists of three closely related members, PPAR$\alpha$, $\gamma$, and $\delta$. These receptors are activated by a variety of FAs, FA derivatives, and synthetic compounds [39]. Each member has a tissue-selective expression profile, with PPAR$\alpha$ and PPAR$\gamma$ occurring predominantly in the liver and adipose tissue, respectively, and PPAR$\delta$ in many tissues [40]. PPAR$\alpha$ is also present in BAT and regulates the expression of the *UCP1* gene in primary brown adipocytes and in BAT *in vivo*. A PPAR$\alpha$-responsive element (PPRE) was identified in the enhancer region upstream to the rat *UCP1* [34]. In this same study, highly comparable elements were found in the human and mouse *UCP1* promoters, suggesting that these sequences could play an important regulatory role in the response to PPAR$\alpha$. The authors proposed that PPAR$\alpha$ could interact directly with CREB binding protein (CBP) and with PPAR$\gamma$-coactivator-1 (PGC-1). These proteins may interact physically with each other, to provide multiple contact points to stabilize the intricate assembly [34].

PPAR$\gamma$ also positively regulates the transcript accumulation of *UCP1* [41] in a manner similar to PPAR$\alpha$ [34]. Indeed, the predominance of each receptor type

could depend on several factors, such as the relative intracellular amount of each protein, cross-talk with other signaling pathways (including regulation of PPAR transcriptional activity by MAP kinase-dependent phosphorylation, which improves PPAR$\alpha$ but reduces PPAR$\gamma$ activity), ligand availability, interaction with coregulators, and tissue differentiation [42–45]. Thus, PPAR$\alpha$ and PPAR$\gamma$ factors have distinct and tissue-restricted functions in the transcription of the *UCP1* gene. Furthermore, Wang *et al.* [46] demonstrated that PPAR$\delta$ acts as a key metabolic regulator of fat burning and promotes the transcription of *UCP1*. The regulation of *UCP1* has been shown to be extremely complex, including the recently discovered activation of ATF-2 and PGC-1 by phosphorylation via p38 mitogen-activated protein kinase (MAPS) in brown adipocytes [47]. p38 MAPK controls the expression of the *UCP1* gene by modulating the respective interactions of ATF-2 and PGC-1 with a cAMP response element and a PPAR response element, both located within a critical enhancer motif of the *UCP1* gene [47].

As discussed above, most studies have focused on the transcriptional regulation of mammalian *UCP1*. However, the regulation of mammalian *UCP2* and *UCP3* genes has also received attention. Several putative regulatory elements within *UCP2* and *UCP3* promoter regions, such as CREB, MyoD, glucocorticoid receptor, myocyte enhancer factor-2, and PPARs have been identified (for a review see [4]). These findings reinforce the idea that the transcriptional regulation of each member of the mammalian UCP subfamily (Fig. 1) varies among different cells, tissues/organs, and physiological condition.

Some studies have shown that mammalian *UCP*s are also regulated at the translational level. *UCP2* mRNA contains an open reading frame (ORF) located in exon 2 upstream to ATG of *UCP2* in exon 3 (Fig. 2; [48]) that strongly inhibits the expression of *UCP2*. As a result, the *UCP2* gene can be down-regulated in *cis* at the translational level by an upstream ORF [48]. The stimulation of the *UCP2* expression under stress conditions can occur without any change in the *UCP2* mRNA levels. Since a discrepancy between mRNA levels and protein expression has also been suggested for *UCP3* [49], caution is required in interpreting variations in the mRNA levels of *UCP2* and *UCP3*.

Unlike mammalian *UCP*s, no experimental studies have focused on *cis*-elements (or binding sites) of *PUMP* promoter regions. However, computational inspection of the regulatory region (within a 1.0-kb region upstream to the transcription initiation site) of *AtPUMP*s using PLACE [50] and PlantCARE [51] identified common *cis*-element binding sites (Table 1). Although the functional activity of these binding sites has not yet been demonstrated, their presence in promoter regions of all *At*-

**Table 1.** Conserved Binding Motifs Found in the Promoter Region of *AtPUMP*s

| *cis* Element | *cis* Element sequence |
|---|---|
| ACGTATERD1 | ACGT |
| CAATBOX1 | CAAT |
| DOFCOREZM | AAAG |
| GATABOX | GATA |
| WBOXATNPR1 | TTGAC |

*PUMP* genes suggests that they may have an essential role in the transcriptional regulation of *PUMP*. Additionally, Watanabe and Hirai [27] have shown that the processing of the pre-mRNAs of two rice nuclear genes, *OsPUMP1/OsUCP1* and *OsPUMP2/OsUCP2*, was defective. The defects in the processing of pre-mRNA result in multiple abnormal transcripts, the translation products of which do not have normal UCP activities.

## *UCP/PUMP* TISSUE-SPECIFIC GENE EXPRESSION

Whereas *UCP1* is exclusively expressed in BAT specialized for adaptive thermogenesis [3], *UCP2* is expressed in several tissues including BAT, and high levels of *UCP3* mRNA have been reported in skeletal muscle and BAT of rodents [4]. In contrast, *BMCP1/UCP5* is expressed in brain and, to a lesser extent, in other tissues in both humans and rodents [52]. Finally, *UCP4*, the most recently identified mammalian uncoupling protein gene, is exclusively expressed in fetal and adult brain tissues [53]. These findings suggest that each mammalian *UCP* is subject to tissue-specific transcriptional regulation that may reflect its physiological role in different cell types. However, significant amounts of all five *UCP* mRNAs were recently detected in the brain cortex, with distinct proportions in the central nervous system [54]. In this case, each *UCP* gene is apparently subject to specific transcriptional regulation in the same cell type [54].

Similarly to mammalian *UCP*s*, PUMP*s are widely expressed in several tissues/organs. For instance, *AtPUMP1* is expressed ubiquitously in several plant organs [55]. *AtPUMP2/AtUCP2* transcripts were found in seedlings [26] and in green siliques (J. Borecký, F. T. S. Nogueira, I. G. Maia, F. R. da Silva, A. E.Vercesi, and P. Arruda, unpublished results). *AtPUMP3* transcripts have been detected only in roots, whereas *AtPUMP4* and *AtPUMP5* were ubiquitously expressed and at greater levels than the other *AtPUMP*s (J. Borecký, F. T. S. Nogueira, I. G. Maia, F. R. da Silva, A. E.Vercesi, and P. Arruda, unpublished results). Interestingly, we have not detected any *AtPUMP6* transcripts in tissues/organs, using *in silico* and experimental (RNA gel-blot, RT-PCR, etc.) approaches, which suggests that *AtPUMP6* could be a pseudogene.

Ito *et al.* [56] isolated a gene from *Helicodiceros muscivorus*, a highly thermogenic arum lily, that encodes a putative uncoupling protein (HmUCPa). Expression profile analysis revealed that *HmUCPa was* ubiquitously expressed in thermogenic male florets and appendix and in non-thermogenic female florets, spathe, and club-shaped organs of the spadix. These results suggested that this gene is not primarily involved in organ-specific heat production. These findings, together with the tissue-specific expression profiles observed for some *AtPUMP*s and their homologues (for a review see [57]), indicate that PUMPs are involved in more subtle functions in cell metabolism during the plant life cycle. Furthermore, since several *PUMP*s are expressed in non-thermogenic plant species [57] and form distinct subfamilies (Fig. 1), it is possible that most members of the PUMP subfamilies have completely different functions from animal UCPs.

## STRESS RESPONSE OF UCPs AND PUMPs

Since stress affects mitochondrial energy metabolism, it is important to investigate the physiological aspects of mammalian UCPs in response to harmful situations. The expression profiles of the genes encoding the different UCPs have been evaluated in various physiological, pathological, and pharmacological conditions. In particular, since UCPs have been implicated in the control of cellular and tissue energetics, most physiological studies in animals and humans have been done under conditions that affect the energy equilibrium, such as exposure to cold, disease, or altered nutritional status. Table 2 summarizes the effects of various stressful situations on the expression of *UCP1, UCP2, UCP3, UCP4*, and *BMCP1/UCP5*. In agreement with its physiological role in thermogenesis, *UCP1* mRNA levels rise when adult mice are exposed to cold stress [58]. *UCP4* and *BMCP1/UCP5* are also upregulated by cold stress, suggesting their involvement in thermoregulatory processes [63]. In contrast, the response of *UCP2* and *UCP3* to cold is not well established [58], indicating that these members of mammalian UCP family may be involved in other metabolic processes. Indeed, the stimulation of *UCP2* and *UCP3* expression by starvation [59] is inconsistent with a role in promoting thermoregulation since starvation activates FA metabolism and decreases energy expenditure [57]. Although the amount of data is limited, the responses of *UCP2* and *UCP3* to several physiological conditions [60–62, Table 2] suggest that they may be involved in adaptations of cellular metabolism to an excess of substrates in order to regulate ATP levels, the $NAD^+$/NADH ratio and various metabolic pathways, and to reduce ROS production.

Reactive oxygen species, such as hydrogen peroxide ($H_2O_2$), superoxide ($O_2^{\cdot-}$), and hydroxyl radicals ($OH^{\cdot}$), can react with DNA, lipids, and proteins, to cause severe cellular damage. ROS-detoxification systems consist of multiple enzymes that are thought to function according to stress situations in different cell compartments [64]. For instance, the mitochondrial electron transport chain can produce consid-

**Table 2.** Summary of the Effects of Different Conditions on the Expression of Mammalian *UCP* Genes

| Gene family member[a] | Experiment type | Reference |
|---|---|---|
| *UCP1* | Cold exposure | Carmona *et al.* [58] |
| | High-lipid diet | Ricquier and Bouillaud [4] |
| *UCP2* | Cold exposure | Carmona *et al.* [58] |
| | High-lipid diet | Ricquier and Bouillaud [4] |
| | Starvation | Cadenas *et al.* [59] |
| | Obesity | Oberkofler *et al.* [60] |
| | Diabetes | Ricquier and Bouillaud [4] |
| *UCP3* | Starvation | Cadenas *et al.* [59] |
| | High-lipid diet | Ricquier and Bouillaud [4] |
| | Diabetes | Ricquier and Bouillaud [4] |
| | Exercise | Hildebrandt *et al.* [61] |
| | Chronic hypobaric hypoxia | Essop *et al.* [62] |
| *UCP4* | Cold exposure | Yu *et al.* [63] |
| *BMCP1/UCP5* | Cold exposure | Yu *et al.* [63] |
| | High-lipid diet | Yu *et al.* [63] |

[a]Mammalian *UCP* family member found to be induced or repressed in each condition.

erable quantities of ROS, primarily because of the presence of the ubisemiquinone radical that can transfer a single electron to oxygen and produce $O_2^{\cdot-}$ [65]. Since mitochondria represent one of the major sources of ROS during stress in plant cells, PUMP, together with AOx, may serve to prevent ROS production in this organelle [66, 67]. Indeed, PUMPs play a potential role in protecting cells against oxidative stress [11, 12, 68] by regulating energy metabolism in mitochondria [4, 10], i.e., by preventing situations with extremely high $\Delta\mu_{H+}$ that can lead to excessive ROS production [10, 68]. Accordingly, the inhibition of PUMP strongly correlates with increased $H_2O_2$ production in potato mitochondria, while the activation of PUMP by linoleic acid decreases mitochondrial ROS formation [66]. Pastore *et al.* [69] demonstrated that ROS activated PUMP, thereby protecting the cell against the overproduction of mitochondrial ROS. Taken together, these data strongly support the idea that mild uncoupling of respiration by UCP/PUMP has a fundamental role in regulating ROS generation in plants, mainly under biotic and abiotic stress. Several types of stresses are accompanied with elevated ROS production [70], hence plant cells exposed to harmful conditions may increase their PUMP and AOx activities.

Although a relationship between abiotic stress and PUMP activity has been demonstrated [71], there is little information on the effects of stress on transcriptional regulation of *PUMP*. The first investigation of *PUMP* gene expression was provided by Laloi *et al.* [72], who demonstrated an accumulation of *StUCP* transcript under cold stress in potato plants. Maia *et al.* [55] reported that *AtPUMP1* was a cold-inducible gene, whereas Murayama and Handa [73] found that the wheat *PUMP* isoforms *WhUCP1a* and *WhUCP1b* were insensitive to low temperature. Similar results were observed for the rice *OsUCP1* and *OsUCP22* genes [27]. However, neither of the latter two studies examined gene expression under other types of stress that could increase mitochondrial ROS production, such as dehydration, biotic stresses, or $H_2O_2^-$ and menadione-induced oxidative stress [68, 74, 75]. Therefore, we cannot exclude the possibility that *WhUCP*s and *OsUCP*s are regulated by such conditions. In addition, some reports [27, 73] have suggested that higher plant UCPs may exist in several isoforms, as do animal UCPs (Fig. 1). Hence, still unidentified *WhUCP* and *OsUCP* family members may well respond to low temperatures.

We recently analyzed the expression profiles of *PUMP* genes in response to chilling and oxidative stress in several plant species. *PUMP* gene family members from *Arabidopsis* and sugarcane showed different patterns of temporal- and plant species-dependent regulation under chilling stress (J. Borecký, F. T. S. Nogueira, I. G. Maia, F. R. da Silva, A. E. Vercesi, and P. Arruda, unpublished results). Brandalise *et al.* [76] identified a *PUMP* gene in maize that was not responsive to chilling stress but its expression increased after $H_2O_2^-$ or menadione-induced oxidative stress, suggesting a protective role against ROS formation. Moreover, Brandalise *et al.* [68] demonstrated that the overexpression of a cDNA clone encoding AtPUMP1 in transgenic tobacco led to a significant increase in tolerance to oxidative stress promoted by exogenous $H_2O_2$ as compared to wild-type plants. Together, these results suggest that, regardless of the plant species, PUMP is involved in protecting cells against damage by oxidative stress.

**Table 3.** Summary of the Public *Arabidopsis* Microarray Experiments

| Gene family member[a] | CHR Iocus[b] | Experiment type | Reference |
|---|---|---|---|
| *AtPUMP1* | At3g54110 | *Alternaria brassicicola* infection | van Wees *et al.* [77] |
| | | RNA viruses | Whitham *et al.* [78] |
| | | Salt, osmotic, and cold stress | Kreps *et al.* [79] |
| | | Induced programmed cell death | ´Swidzinski *et al.* [80] |
| *AtPUMP2/AtUCP2* | At5g58970 | Induced programmed cell death | Swidzinski *et al.* [80] |
| *AtPUMP4* | At4g24570 | Oxidative stress | Desikan *et al.* [75] |
| | | Cold, drought and high-salinity stress | Seki *et al.* [81] |
| | | ABA | Seki *et al.* [82] |
| *AtPUMP5* | At2g22500 | Cold, drought and high-salinity stress | Seki *et al.* [81] |
| | | ABA | Seki *et al.* [82] |
| | | *Alternaria brassicicola* infection | van Wees *et al.* [77] |
| | | RNA viruses | Whitham *et al.* [78] |
| | | Salt, osmotic, and cold stress | Kreps *et al.* [79] |
| | | Wounding | Cheong *et al.* [83] |

[a]*AtPUMP* family member found to be significantly induced or repressed in the public microarray data.
[b]Chromosome locus based on MIPS (http://mips.gsf.de/proj/thal/proj/thal_overview.html).

Additional evidence of a stress response of *PUMP* family members can be obtained by analyzing the expression profile data available in public databases of microarray experiments. Selected experiments based on specific treatments of wild-type plants, together with *AtPUMP* genes that were reported as being up- or downregulated by each stress, are summarized in Table 3. Several of the experiments from the microarray data (Table 3) were selected in order to investigate in detail the expression profiles of significantly induced or repressed *AtPUMP* gene family members under different time courses (Fig. 3).

As shown in Fig. 3 and Table 3*, AtPUMP1, AtPUMP4*, and *AtPUMP5* were identified as being cold- and drought-inducible [79, 81]. *AtPUMP4* and -5 were also reported to be long-term downregulated by high-salinity stress (Fig. 3a). Both of these genes were upregulated by the phytoregulator abscisic acid (ABA), which suggested that they could be regulated by abiotic stress via an ABA-dependent pathway, as reported for other cold- and drought-induced genes [82, 85]. *AtPUMP1* and *At-PUMP5* have also been reported to be upregulated by biotic stress, such as infection with *Alternaria brassicicola* and RNA viruses (Fig. 3b, c, and Table 3; [77, 78]). In addition, *AtPUMP5* was also upregulated by wounding (Table 3), caused by abiotic stress factors such as wind, rain, and hail, and by biotic factors, especially insect feeding [83]. Finally, the steady state levels of the transcripts encoding *Arabidopsis* uncoupling proteins 1 and 2 were increased during heat-induced programmed cell death (PCD) but not during senescence [80]. Based on these findings and in contrast to the physiological role of animal UCP2 during apoptosis [86], PUMP 1 and PUMP2 may play a role in limiting ROS formation following exposure to heat.

An alternative oxidase has been implicated in defence mechanisms against ROS production [67]. In agreement with this, *AtAOx1a* was upregulated by biotic and abiotic stresses but displaying distinct expression profiles from that of *PUMP*s (Fig. 3a, b). Therefore, it is possible that these two energy-dissipating systems have a complementary role in regulating the metabolic and energy balance during stress conditions.

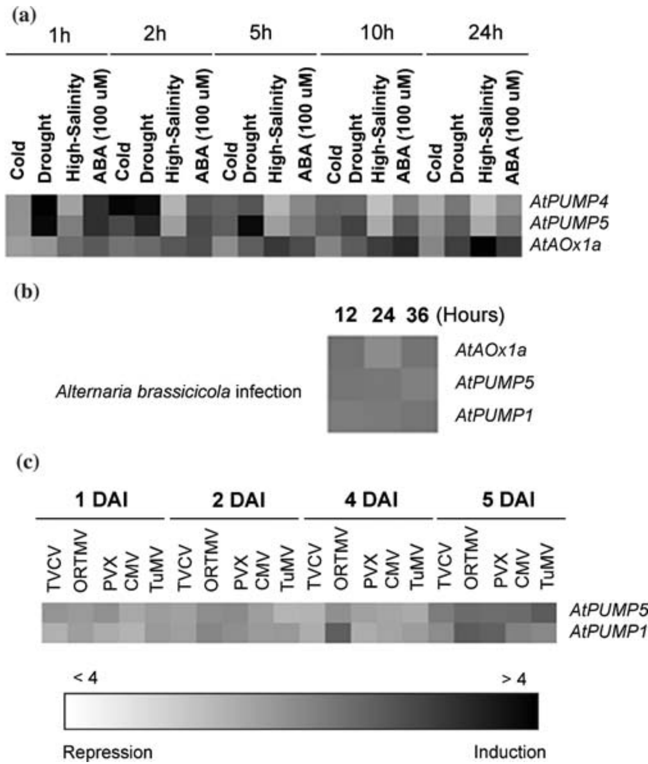


**Fig. 3.** Relative abundance of *AtPUMP*s significantly induced or repressed by different conditions. Each *AtPUMP* is represented by a single row and each hormonal or stress time point is represented by a single column. Since *AOx1a* responds to harmful situations, it was included as a comparative mitochondrial gene. The normalized intensity values were obtained from public array data (references quoted in Table 3). The averaged differences were $\log_{10}$ transformed and the data then processed with a self-organizing map algorithm followed by complete linkage hierarchical clustering of the genes and experiments (as applicable) using the Pearson correlation (non-centered) and the CLUSTER program [84]. The final image was generated by TreeView software (http://rana.lbl.gov). The *AtPUMP* and *AOxla* names are shown on the right and the color intensity scale representing the relative abundance is indicated at the bottom. TVCV, turnip vein clearing tobamovirus; ORTMV, oilseed rape tobamovirus; PVX, potato virus X potexvirus; CMV, cucumber mosaic cucumovirus, and TuMV, turnip mosaicpotyvirus. DAI, days after inoculation.

## CONCLUSIONS

Uncoupling proteins in eukaryotes form a distinct family within the superfamily mitochondrial anion carriers. UCPs probably originated from anion/anion or anion/nucleotide transporters before the divergence of eukaryotes into animals, plants, and fungi [8]. Phylogenetic analysis suggests that the family of uncoupling proteins consists of five subfamilies, including mammalian-specific UCP subfamilies (I and

IV), plant-specific UCP/PUMP subfamilies (II and V), and a probably ancestral subfamily of uncoupling proteins (III). All UCP/PUMP subfamilies are distinct from the phylogenetically close-related subfamilies of M2OM and DIC members. The *PUMP4, PUMP5*, and *BMCP/UCP5* genes are upregulated by low temperature, but their role in adaptation to cold stress appears to be different since animal BMCP1/UCP5 is involved in thermoregulation and heat production whereas PUMP4 and PUMP5 are probably involved in reducing ROS production during harmful conditions. Finally, phylogenetic and genomic structure analyses of the PUMP subfamilies have shown that almost all *Arabidopsis PUMP* orthologs are present in the monocot rice genome. This finding means that these genes predate the monocot/dicot divergence and retain a conserved gene structure in each subfamily.

In animals, the amount of UCP1 is regulated primarily at the transcriptional level (probably mainly through the ($\beta_1$- and $\beta_3$-adrenoceptor/CREB pathway). Mammalian *UCP2* and *UCP3* are also regulated at the transcriptional and translational levels, indicating that the control of *UCP* gene regulation is extremely complex and tissue/age-dependent, and involves different transcription factors, hormones, and the influence of additional ORFs in the 5′-untranslated region of the mRNA [48].

Plant uncoupling proteins show properties similar to mammalian UCPs and occur in thermogenic or non-thermogenic plants. Although their true physiological role remains unclear, the expression profiles in several tissues/organs and their response to different biotic or abiotic stress indicate that PUMPs may serve to tune oxidative phosphorylation up by controlling the proton potential across the inner mitochondrial membrane. Together with alternative oxidases that control the potential of "active phosphates", these proteins can buffer the effects of ROS production in mitochondria [57].

Further studies of the properties of uncoupling proteins in various plant and animal species are necessary to establish the physiological roles of these proteins. While UCP1 has a well-established physiological role in the thermogenesis, the roles of other mammalian UCPs and of PUMPs are far from being completely elucidated. Major goals for future research will be to functionally test novel UCPs and PUMPs, and to analyze null mutants and transgenic plants for mammalian *UCP* and *PUMP* genes, respectively.

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