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Arabidopsis thaliana Uncoupling Proteins (AtUCPs): insights into gene expression during development and stress response and epigenetic regulation

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Abstract Mitochondrial inner membrane uncoupling proteins (UCP) catalyze a proton conductance that dissipates the proton electrochemical gradient established by the respiratory chain, thus affecting the yield of ATP synthesis. UCPs are involved in mitochondrial energy flow regulation and have been implicated in oxidative stress tolerance. Based on the global gene expression profiling datasets available for *Arabidopsis thaliana*, in this review we discuss the regulation of *UCP* gene expression during development and in response to stress, and provide interesting insights on the possible existence of epigenetic regulation of *UCP* expression.

Keywords Uncoupling proteins · Gene expression · Stress · Development · Arabidopsis

Introduction

Mitochondrial inner membrane uncoupling proteins (UCP) catalyze a proton conductance that dissipates the proton electrochemical gradient established by the respiratory chain, thus affecting the yield of ATP synthesis. In plants, the proton electrochemical potential energy-dissipating pathway involving UCPs leads to a decrease in the efficiency of oxidative phosphorylation (Vercesi et al. 2006). Plants also contain a redox energy-dissipating pathway that involves an alternative oxidase (AOX). UCPs along with AOXs 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 (Vercesi et al. 2006). Such overloads are generated by 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. Additionally, UCPs and AOXs seem to protect cells against high production of reactive oxygen species (ROS) during biotic and abiotic stresses (Maxwell et al. 1999; Brandalise et al. 2003; Van Aken et al. 2009).

UCPs have been implicated in the attenuation of ROS generation in mitochondria based on their ability to decrease the coupling between substrate oxidation and ATP synthesis. The premise is that UCP alleviates ROS production by increasing the rate of respiration through regulated mitochondrial uncoupling. The importance of uncoupling proteins as components of the cellular tolerance to oxidative stress has been revealed in studies performed in vitro and in vivo using different models. In plants, activation or overexpression of UCP alleviates ROS production and enhances tolerance to oxidative stress (Kowaltowski et al. 1998; Brandalise et al. 2003). Conversely, ablation of UCP expression in a knockout *Arabidopsis* mutant promotes localized oxidative stress (Sweetlove et al. 2006). Similarly, animals lacking UCP function show enhanced levels of intracellular ROS and oxidative stress (Arsenijevic et al. 2000; Vidal-Puig et al. 2000; McLeod et al. 2005). The fact that UCP activity in mitochondria is stimulated by superoxide and/or products of lipid peroxidation (Considine et al. 2003; Smith et al. 2004) is consistent with an oxidative-stress protection function, and indicates that UCP-mediated mitochondrial uncoupling controls mitochondrial ROS formation through a negative-feedback mechanism (Pastore et al. 2007). In this regard, a proposed ancestral role for UCP was to prevent oxidative stress by reducing superoxide production in mitochondria (Brand et al. 2004). In fact, ROS are one of the major components in a wide range of biotic and abiotic stresses and mitochondria is a major intracellular

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source of ROS production (Moller 2001). Many plant adaptations to stresses involve mitochondria, and in this scenario, UCP-mediated mild uncoupling seems to be fundamental to overcome increased oxidative stress.

Contrasting the relatively well-known physiological roles of UCPs in response to stresses, little is known about their possible roles during plant development. Moreover, the modulation of *UCP* gene expression during development and/or in response to environmental and biotic stresses is poorly understood. In this review, we focus on the available information on the expression patterns of plant UCP (pUCP) coding genes during development and under different stress conditions by exploring the huge repository of gene expression data of *Arabidopsis thaliana*. We also discuss the possible role(s) of epigenetic markers on the regulation of specific members of the *Arabidopsis UCP* gene family (*AtUCPs*) based on available databases of genomic methylation and small RNA-related signatures. Additionally, we focus on comparing the *AtUCP* gene expression profiles with a highly expressed member of the *AOX* gene family, namely *AOX1a* (Saisho et al. 1997; Clifton et al. 2006). Using such information, we are able to provide interesting clues about the regulation of certain members of the *UCP* family in the model plant *Arabidopsis*.

UCP gene expression

The availability of hundreds of *Arabidopsis* whole-genome gene expression experiments allows a comprehensive overview of the expression profile of several genes in this species, including nuclear-encoded mitochondrial genes. Most of these experiments were compiled into the Genevestigator database (www.genevestigator.com) (Zimmermann et al. 2004) and the Bio-Array Resource for Plant Functional Genomics (BAR; Winter et al. 2007), which include a range of developmental stages and stress conditions. Thus, we used these databases to gain more insight into *pUCP* gene expression. Unless otherwise noted, all the expression data were analyzed based on the genetic background of *Arabidopsis thaliana* ecotype Columbia (Col-0).

It is also important to highlight that the accurate number of distinct *UCP*-like loci in the *Arabidopsis* genome is still a debatable issue mainly because some family members are closely related to malate/oxoglutarate and dicarboxylate mitochondrial carriers. Moreover, additional biochemical characterization of these family members is still needed to provide conclusive proofs of their UCP nature (Borecký et al. 2006). Therefore, in this review, we chose to focus our discussion on the expression profiles of the best characterized *Arabidopsis UCP* family members, namely *AtUCP1* (At3g54110), *AtUCP2* (At5g58970), and *AtUCP3* (At1g14140) (Maia et al. 1998; Watanabe et al. 1999; Borecký et al. 2006).

Growth and development

Plant growth and development are regulated physiological processes that are connected to specific cellular demands. In this context, particular growth stages are energy-committing steps that require specific patterns of nuclear-encoded mitochondrial gene expression. One of the key developmental stages for plant growth is seed development. By using the BAR database (Winter et al. 2007), we mined the expression profiles of *AtUCP1*-3 and *AtAOX1a* across distinct seed development stages. Based on the NASCArray (http://affymetrix.arabidopsis.info/narrays) expression data, it is clear that *UCPs* are downregulated in the later stages of seed development (Fig. 1a). Based on Harada-Goldberg LCM GeneChip dataset (Le et al. 2010), it is remarkable that in the early stages of seed development, the coat seed tissue accumulates more transcripts of *AtUCP1-3*, *AtUCP2* being the most transcriptionally active gene. Conversely, *AtAOX1a* transcripts were scarcely detected during the embryo torpedo stage but their levels slowly increased towards the last stages of embryo development. It has been proposed that UCPs and AOXs act in a complementary fashion during fruit ripening (Costa et al. 1999; Considine et al. 2001), which would reflect different catalytic efficiencies under distinct physiological conditions (Vercesi et al. 2006). Although protein activity cannot be inferred solely based on gene expression, the distinct patterns of expression of *AtUCPs* and *AtAOX1a* suggest that these respiratory components play different roles and operate at different stages during embryo and seed development. As energy metabolism changes during seed development, re-programming of the expression of specific nuclear-encoded mitochondrial genes may be one of the many adaptations embedded in the differentiation program of embryos and endosperms. Such adaptations are probably coupled to the embryo photoheterotrophic metabolism (Borisjuk et al. 2004). However, it is noteworthy that comparatively to the initial stages of seed development, all three *AtUCPs* as well as *AtAOX1a* seem to be expressed at low levels in the late stages (Fig. 1a). The reduction in the number of transcripts during these stages is probably due to mRNA turnover resulting from the general shutdown in transcriptional processes as the seed enters dormancy (Walling et al. 1986).

Seed germination is defined as the events between water uptake by the dry seed and the initiation of elongation of the embryonic root or radicle (Bewley and Black 1994). The process in the dry seed by which the capacity for dormancy in the imbibed seed is lost is called after-ripening (AR). This process can last for prolonged periods, depending on environmental cues. During AR and subsequent seed imbibition, both genetic and environmental cues are integrated to determine germination potential (for a review, see Holdsworth et al. 2008). High-throughput expression

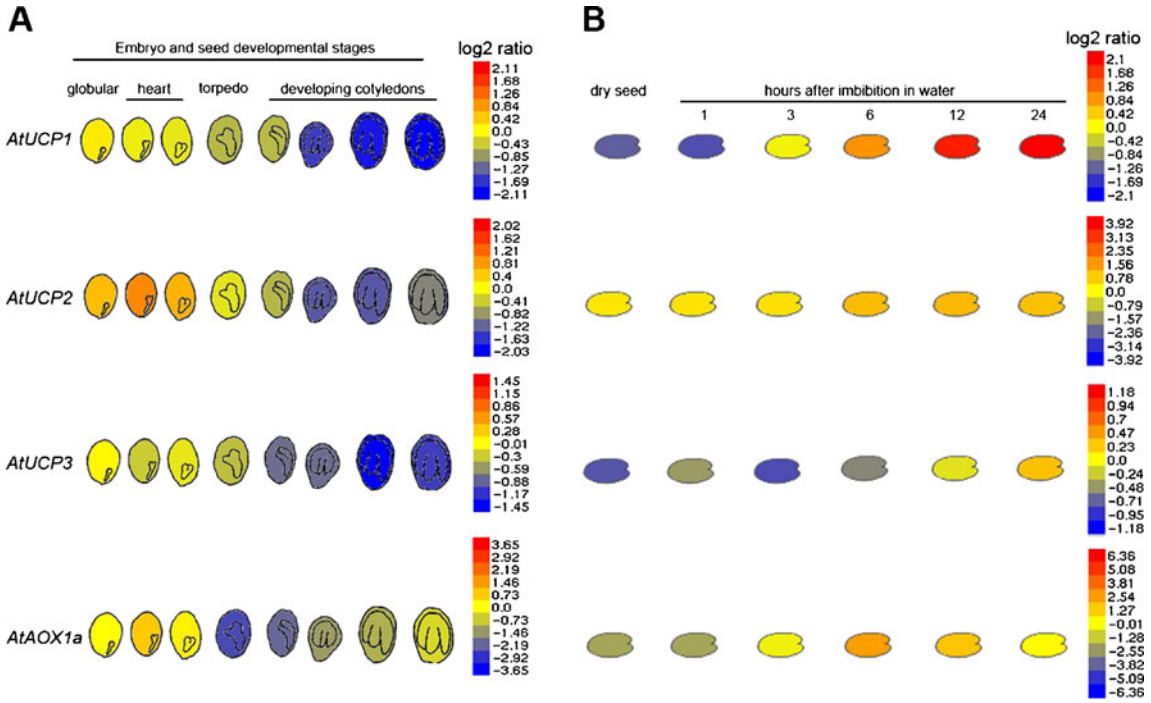

Fig. 1 *AtUCP1-3* and *AtAOX1a* display overlapping but distinct expression profiles during *Arabidopsis* seed development and germination. (**a**) BAR-based transcript accumulation of three members of the *UCP* gene family (*AtUCP1-3*) as well as the highly expressed member of *AOX* gene family, *AtAOX1a,* during seed development, as shown in the eFP browser. Raw data were normalized by the GeneChip® Operating Software (GCOS; http://www.affymetrix.com). (**b**) Expression profiles of *AtUCPs* and *AOX1a* during seed germination events. The seeds were after-ripened 2 to 4 months and imbibed under continuous light without stratification. RNA from dry to 24 h-imbibed seeds was used to hybridize GeneChip Arabidopsis ATH1 Genome Array and the raw expression data were normalized as described by Nakabayashi et al. (2005). In panels **a** and **b**, the $\log_2$ ratio graphs on the right indicate the highest level of expression in red and the lowest level of expression in blue for each gene. The data were retrieved from http://bbc.botany.utoronto.ca/efp

profiling experiments have been performed to gain insight into the transcriptional program of seed germination and its genetic regulation. Such studies provide evidence that physiological changes observed during lost of dormancy and subsequent germination are subject to both transcriptional and posttranscriptional control. The gene expression map of *Arabidopsis* during AR and subsequent seed imbibition reveals that *AtUCPs* display distinct expression profiles (Fig. 1b). While *AtUCP2* and *AtUCP3* are sparsely expressed in dry and in AR-imbibed seeds, accumulation of *AtUCP1* transcripts slowly increases, reaching maximum levels at 12 h of imbibition in water. Interestingly, mature mitochondria are detected only after 12–24 h of seed imbibition, with the formation of the inner membrane and cristae (Howell et al. 2006). Examination of the spatial expression patterns of *AtUCP1-3* at 24 h of imbibition (seeds of Arabidopsis ecotype Landersberg erecta), reveals that *AtUCP1* is more strongly expressed in the endosperm than in the embryo tissues, whereas *AtUCP2* is equally expressed in both tissues. On the other hand, expression of *AtUCP3* is higher in the embryo than in the endosperm (Penfield et al. 2006).

Among the investigated *AtUCP* genes, *AtUCP1* displays increased expression levels during seed imbibition (Fig. 1b). Contrastingly, *AtOX1a* transcripts reach a peak of accumulation at 6 h of seed imbibition but their level is then slowly reduced (Fig. 1b). It has been shown that non-enzymatic reactions are likely to occur in dry, AR seeds, including the production of ROS. In this context, ROS might be produced to release seed dormancy through the carbonylation of specific embryo proteins (Oracz et al. 2007). Nevertheless, after the releasing of dormancy, the continued production of ROS may be harmful for seed germination as it would affect protein turnover. The increase in the expression of *AtUCP1* and *AtAOX1a* may suggest that both proteins play a role in attenuating ROS effects on cell metabolism during early seed imbibition (Fig. 1b). Interestingly, the increase in the expression of *AtUCP1* observed during seed imbibition is in agreement with our data on transgenic tobacco plants constitutively overexpressing *AtUCP1*. Transgenic seeds germinate considerably faster than wild-type seeds, suggesting that ROS produced during germination may be attenuated by overexpression of UCP (Maia, IG; unpublished data). However, the role of UCP1 during *Arabidopsis* seed germination remains elusive as an *ucp1* mutant shows no alteration of seed germination and only modest increase in ROS production (Sweetlove et al. 2006). One possibility is that AtUCP2 and AtUCP3 as well as AtAOX act by buffering

the loss of AtUCP1 function in the *ucp1* mutant seeds, assuring the alleviation of ROS effects during germination. It would be interesting to evaluate whether double or triple *ucp* knockdown mutant seeds are able to complete the germination process.

Data obtained from Genevestigator also revealed that the investigated *AtUCP* genes are co-expressed in various organs/tissues over nine different developmental stages but with distinct expression patterns (Fig. 2). In this context, the expression of *AtUCP1* was always higher (3–9 fold) than that of *AtUCP2* and *AtUCP3*, regardless of the developmental stage (Fig. 2). *AtUCP1* is expressed throughout the plant with the highest expression levels detected in germinated seeds (1–6 days), and the lowest in mature silique stage (45–50 days after germination - DAG). After the germination stage, *AtUCP1* transcript levels oscillated down (3–4 fold) from seedling (6–14 DAG) to developed flower stages (29–36 DAG). Another decrease in *AtUCP1* expression was also observed in the transition from flower and silique (36–45 DAG) to mature silique stages (45–50 DAG). It is worth noting that besides germinated seed, two organs also showed increased levels of *AtUCP1* expression, namely mature polen and stamen in stage flower 12 (database from Schimd et al. 2005). *AtUCP2* and *AtUCP3* were expressed at low levels during all stages of development, and both were less expressed in the young flower stage (25–29 DAG). The highest expression levels of *AtUCP2* were observed in germinated seeds and in flower and silique stages (36–45 DAG), while the lowest level was observed in mature silique stage (45–50 DAG). Besides germinated seed, *AtUCP2* transcripts were highly concentrated in the second internode, and in silique stages 3–5 (database from Schimd et al. 2005). The highest expression level of *AtUCP3* was detected in the bolting stage (21–25 DAG). Interestingly, expression of *AtAox1a* was relatively low in the germination seed stage relative to that of *AtUCP1* (Fig. 2). By contrast, expression levels of *AtAox1a* were very high in young rosette (14–18 DAG) and young flower stages (25–29 DAG). A remarkable difference in transcript accumulation between *AtUCP1* and *AtAOX1*a is also observed in the early stages of plant postgermination (1–6 DAG; Fig. 2). This finding may reflect a transcriptional activation of *AtUCP1* by changes in the fatty acid composition during this early developmental stage.

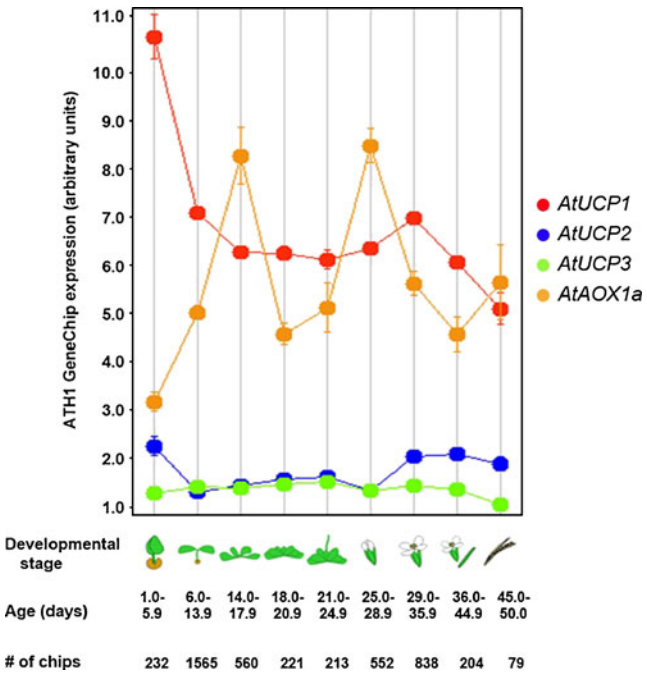

Fig. 2 Genevestigator expression map describing the expression patterns of *AtUCP1-3* and *AtAOX1a* over *Arabidopsis thaliana* development. Growth stages from seed germination to senescence are arbitrarily grouped into subcategories based on Boyes et al. (2001). Each category contains averaged raw signal data and standard error derived from all ATH1 chips hybridized with RNA from the corresponding growth stages in the Genevestigator database, including all organs available at that stage. Each of the *Arabidopsis* growth stages is shown and plant age in each category is indicated underneath the graph, as is the number of chips contributing to each category. *Error bars* represent standard error. Data were retrieved from https://www.genevestigator.ethz.ch

Although attaining different expression levels, the overlapping expression of *AtUCP1-3* throughout various development stages may be indicative of a combinatorial action of these proteins in mitochondria. Whether these proteins act redundantly remains to be determined. The aforementioned data also point out the existence of stage-specific modulation of *AtUCP* gene expression, especially for *AtUCP1*. In the later case, spatiotemporal expression of *AtUCP1* suggests relevant physiological functions in certain developmental stages, such as seed germination and postgermination.

Stress

Although not exhaustively investigated, the members of the UCP gene family were found to be differentially expressed under specific stress conditions, implying that distinct patterns of expression regulation must exist for each member. One example is the differential expression profiles of *AtUCP1* (responsive) and *AtUCP2* (not responsive) in response to low temperature (Watanabe et al. 1999; Borecký et al. 2006).

A survey of different Arabidopsis microarray datasets using the Genevestigator software revealed that the expression of *AtUCP1* and *AtUCP2* is differentially affected by a modest number of external stimuli or conditions. Conditions that down-regulated *AtUCP2* expression included hypoxia, induced programmed cell death and treatment with paclobutrazole. Decreased *AtUCP1* expression was observed

under drought and low nitrogen supply. Both genes were also negatively modulated by six-benzyladenine, a synthetic cytokinin. Up-regulation of *AtUCP1* expression was observed under potassium starvation, sucrose availability, cold treatment, and in Col-0 seedlings treated with flg22, a synthetic peptide derived from the N-terminal portion of *Pseudomonas aeruginosa* flagellin. Curiously, *AtUCP2* showed increased expression levels in a gibberellic acid (GA) insensitive mutant treated with flg22 or methyl jasmonate. In contrast, this gene was down-regulated by the flg22 elicitor in the Col-0 background. Despite the changes observed, the most significant alterations in the expression levels of both genes (up and down) occurred in response to several biotic stresses including fungus, bacteria, nematodes and insects. Most notable were the up-regulation of *AtUCP1* expression in response to avirulent and virulent *P. syringae*, and the down-regulation of *AtUCP2* in the same conditions. It should be highlighted, however, that in all cases only a moderate intensity of fold changes in the expression levels could be observed. *AtUCP3*, on the other hand, is likely to be constitutively expressed since its expression remained almost invariant under the subset of stress and environmental conditions available in the Genevestigator database.

In another dataset containing the expression profiles of 670 genes encoding mitochondrial proteins in response to stress, the abundance of *AtUCP1* transcripts was modulated (up or down) in 10 out of 219 stress conditions investigated. It should be highlighted that *AtUCP2* expression remained invariable in all conditions tested (Clifton et al. 2006). Interestingly, abundance of *AtUCP1* transcripts was reduced (2.15-fold) in an Arabidopsis *aox1a* knockout mutant subjected to moderate light and drought treatments (Giraud et al. 2008). Similarly, a decreased level of AOx protein was observed in an Arabidopsis *ucp1* knockout mutant (Sweetlove et al. 2006). These findings indicate that the endogenous levels of both energy-dissipating proteins are coordinated, with important consequences on mitochondrial function.

According to Van Aken et al. (2009), the UCP coding genes were not amongst the most strongly stress-responsive mitochondrial genes of Arabidopsis. In this regard, the transcript profiles of *AtUCP1* and *AtUCP2* were reported to be stable under a range of abiotic stress treatments affecting plant metabolism and stress signaling, except for glucose and cysteine, which slightly increased the expression of *AtUCP2*, and mannitol that decreased *AtUCP1* expression (Clifton et al. 2005). These authors also documented that compounds that lead to mitochondrial dysfunction, such as rotenone and antimycin A, have little or no effect on the expression of *AtUCP1* and *AtUCP2*. The exceptions were malonate that promoted down-regulation of *AtUCP1* and up-regulation of *AtUCP2*, and FCCP, which down-regulated the expression of *AtUCP1*. However, care should be taken in interpreting such data since the analyses described were performed using Arabidopsis cell suspensions. Discrepancies in transcript abundances between cell suspensions and whole plant tissues have already been observed (Lehmann et al. 2009). Such discrepancies could be illustrated by comparing, for example, the expression data of *AtUCP1* in response to exogenous hydrogen peroxide as published by Ho et al. (2008), *i.e.* while in Arabidopsis suspension cells, *AtUCP1* was listed as an up-regulated gene, it was not listed as up-regulated in leaf tissues submitted to the same treatment. It should be mentioned that the validity of such large-scale data needs to be confirmed by other approaches.

Collectively, these data indicate that at least in *Arabidopsis*, transcriptional regulation of *UCP* gene expression, when present, occurs only in response to very specific stimuli. Since one important physiological role of UCP is to protect cells against oxidative stress, its almost invariable expression levels under stressful conditions indicate that the constitutive protein pool is sufficient to sustain protection, and suggest the existence of regulatory mechanisms acting at the post-transcriptional level. In mammals, UCP expression is proposed to be regulated at multiple levels, which include several post-transcriptional events such as translation efficiency, modulation of catalytic activity and proteolysis (reviewed in Azzu et al. 2010). In this context, the modulation of UCP activity by specific regulators seems to be a plausible strategy to guarantee protection in *Arabidopsis*.

Promoter analysis

To gain further insights into the regulation of *AtUCP1-2* expression the upstream promoter sequences of both genes (~1,200 bp) were used for the prediction of putative regulatory *cis*-elements. In a first step, the Athena database (http://www.bioinformatics2.wsu.edu/cgi-bin/Athena/cgi/home.pl) was employed to detect common transcription factor (TF) binding sites within the investigated promoters regions. The detection of shared regulatory elements within these promoter sequences could be indicative of common regulatory mechanisms controlling gene expression. However, apart from the canonical eukaryotic promoter elements such as the TATA box, this search revealed no enrichment of specific TF-binding sites within the promoters investigated.

Further analyses of the *AtUCP1* promoter using PlantCare and PLACE identified two regulatory elements (Table 1) that are normally enriched in the promoter sequences of cold-responsive genes, namely a core sequence related to an abscisic acid (ABA)-responsive element (ABRE) and four G-box elements. The G-box represents a larger group of regulatory elements present in plant gene promoters shown to be regulated by various signals such as cold, UV irradiation,

Table 1 Putative *cis*-acting regulatory elements found in the 5′ upstream region (1,200 nucleotides) of *AtUCP1* and *AtUCP2*

GENE	MOTIF	SEQUENCE	POSITION (STRAND)[a]	FUNCTION
AtUCP1	5′UTR Py-rich stretch	TTTCTTCTCT	−57 (+) −151 (−)	Transcriptional enhancer
	ABRE core	CACGTG	−213 (+)	Abscisic acid-responsive element
	BOX-W1	TTGACC	−918 (+) −1186 (+)	Fungal elicitor responsive element
	EIRE	TTCGACC	−427 (+)	Elicitor-responsive element
	G-BOX	CACGTG(T)	−212 (+) −605 (−) −719 (+)	Stress responses
	CGTCA-motif	CGTCA	−221 (+) −492 (−) −519 (−) −881 (+)	MeJA-responsiveness
	GARE-motif	TAACAAA	−233 (+)	Gibberellin-responsive element
	TC-rich repeats	ATTTTCTTCA	−276 (+)	Defense and stress-response
	TGACG-motif	TGACG	−221 (−) −492 (+) −529 (+) −881 (−)	MeJA-responsiveness
	W-Box		−912 (+) −1186 (+)	Defense and stress responses
AtUCP2	ABRE core	CACGTG	−417 (+)	Abscisic acid-responsive element
	GARE-like motif	AAACAGA	−281 (+) −1049 (−)	Gibberellin-responsive element
	G-BOX	CACGTG(T)	−322 (+) −424 (+)	Stress responses
	AuxRR-core	GGTCCAT	−962 (−)	Auxin-responsiveness

[a] Numbers represent the locations of the regulatory elements relative to the ATG start codon (A +1)

anaerobiosis, ABA and light. In addition, the *AtUCP1* promoter also contained a low-temperature responsive element (named LTRE; CCGAAA) previously described in barley (Dunn et al. 1998). The presence of such regulatory elements is consistent with the experimentally determined cold-inducible expression of *AtUCP1* at both transcript and protein levels (Maia et al. 1998; Armstrong et al. 2008). The *AtUCP1* promoter is also enriched with regulatory sequences known to be associated with different types of biotic stress responses, such as two W-box motifs, two box-W1 elements (fungal elicitor responsive element) and a TC-rich repeat element, and those involved in mediating hormonal regulation (Table 1).

Parallel inspection of the *AtUCP2* upstream promoter region also revealed the presence of hormone responsive regulatory elements, such an ABRE element, a GARE-like motif associated with GA response and an AuxRR-core. Other *cis*-acting elements such as a G-box binding site and a TC-rich repeat were also noted (Table 1).

Curiously, a functional regulatory element (CGTGAT; named B element) shown to be required for *AtAOX1a* expression in response to rotenone and hydrogen peroxide (Ho et al. 2008) was detected in the promoter sequences of both *AtUCP1* and *AtUCP2*. As already mentioned *AtAOX1a* is known to be a highly stress-responsive gene (Saisho et al. 1997; Clifton et al. 2006). Notably, in the *AtUCP2* promoter, B element overlaps a second regulatory element (named I; ACGTG) that acts as positive regulator of *AOX1a* response to rotenone and hydrogen peroxide (Ho et al. 2008). The presence of these regulatory motifs is intriguing since neither gene is reported to be induced by oxidative stress in the meta-profile data of the Genevestigator database. However, the functional relevance of such motifs is supported by the fact that *AtUCP1* is listed as an up-regulated gene in *Arabidopsis* cell culture treated with hydrogen peroxide (Ho et al. 2008).

In summary, the absence of shared regulatory elements does not support the existence of common regulatory mechanism governing the expression of *AtUCP1* and *AtUCP2*. Moreover, the differences observed in putative promoter elements suggest that these genes are probably not regulated in the same way by abiotic and biotic stresses.

Obviously, the biological significance of such *cis*-acting elements should be validated and their effective involvement in different stress responses and across plant development investigated.

Possible epigenetic regulation of *UCP* expression

Cytosine methylation (CG), a common form of DNA modification that antagonizes transcriptional initiation, is generally found at transposons and repeats in vertebrates, plants, and fungi genomes (for review see Chan et al. 2005; Goll and Bestor 2005; Klose and Bird 2006). In addition to transposons and repeats, cytosine methylation can also be found within genes (Zilberman et al. 2007). Methylation within genes is strongly influenced by their transcription status, *i.e.* moderately transcribed genes are most likely to be methylated, while genes at either extremes are less methylated. Additionally, small RNAs are generally enriched at methylated genes, suggesting that aberrant transcription can trigger gene methylation (Zilberman et al. 2007).

The *Arabidopsis* Next Generation Sequence Database from the groups of Meyers and Green integrates genome-wide methylation data and small RNA signatures (http://mpss.udel.edu/at_sbs/; German et al. 2008). By mining this database, we found that all three *UCP* loci are methylated at CG sites within the gene body, though at variable levels (Fig. 3a). No significant methylation at CG or other sites (CHH and CHG) is observed in upstream sequences, suggesting that the putative promoter regions of *AtUCP1-3* are not under this type of epigenetic control (Fig. 3a). The observed CG methylation is biased away from gene ends, suggesting a dependence on RNA polymerase transit. This could affect transcript elongation and, consequently, transcript decay and turnover (Zilberman et al. 2007). Recently, Zemach et al. (2010) found that gene body methylation is conserved between plants and animals, and is thus an ancient property of eukaryotic genomes.

What is a probable mechanism underlying epigenetic regulation of *AtUCP* expression? The CG methylation pattern at *UCP* loci seems to depend on the DNA methyltransferase MET1. In a *met1-3* mutant (Col-0 background), methylation at CG sites is completely lost within *UCP* genes (Fig. 3a, right panel). In addition to maintaining methylation in symmetrical CG dinucleotides during DNA replication, MET1 contributes to CG de novo methylation in the presence of small RNA signals (Aufsatz et al. 2004). Small RNA signatures were found to overlap with methylation regions at *UCP* loci, mainly at the *AtUCP1* locus (Fig. 3b). It is possible that *AtUCP1* expression is regulated at least to some extent, via RNA-direct DNA methylation (RdDM) of exons and introns within the gene body. It was initially proposed that RdDM is implicated in transcriptional regulation (TGS) via silencing of plant promoters (Aufsatz et al. 2002). More recently, RdDM has also been associated with the control of methylation within the gene body (Zilberman et al. 2007). By evaluating high-density oligonucleotide microarray data from wild-type and *met1* mutant plants, these authors showed that RdDM affects expression levels of a large fraction of *Arabidopsis* protein-encoding genes that may include *UCPs*. Although we detected no probe representing *UCP* genes in their microarray chips, *AtUCP1* probes were found in *Arabidopsis* cDNA microarray experiments in which wild-type plantlets were grown either in the presence of 5-aza-2′ deoxycytosine (aza-dC) or trichostatin A (TSA). Aza-dC blocks cytosine methylation whereas TSA blocks histone deacetylation, both derepressing silenced genes in multicellular eukaryotes (Chang and Pikaard 2005). *AtUCP1* transcript accumulation seems to be affected by Aza-dC (1.5 fold-change) but not by TSA (Fig. 3c, Chang and Pikaard 2005), suggesting that DNA methylation is the primary epigenetic modification of this gene. It remains to be investigated whether or not the methylation status of *AtUCP* genes influences their expression during certain developmental stages and/or in response to biotic or abiotic stresses.

Future epigenetic studies on *AtUCP* genes should include a comparison with the *AtAOX1a* locus, as CG and other sites are not methylated within its gene body. Instead, upstream sequences are heavily methylated (Fig. 3a). These upstream sequences are likely part of the *AtAOX1a* promoter region, which may contribute to its transcriptional regulation in a particular developmental stage and/or stress response. Interestingly, CG methylation at the *AtAOX1a* promoter region depends on MET1 (Fig. 3a, right panel) and is concentrated on a repeat-associated region (shown as a shaded yellowish box in Fig. 3a). Moreover, this region contains several small RNA signatures (Fig. 3b) as does the gene body. Similarly to the *AtUCP* genes, *AtAOX1a* expression may be epigenetically regulated via RdDM, although it likely acts through distinct mechanisms (Fig. 3a and b). We propose that such mechanisms contribute to the differential expression patterns and levels observed between the *AtUCP1-3* and *AtAOX1a* genes during development and responses to stresses. Substantiation or not of this hypothesis awaits future investigations.

Conclusions and perspectives

The available literature points out an involvement of uncoupling proteins in plant response to stresses, especially those associated with ROS generation. However, there is still uncertainty as to whether modulation of UCP activity in response to stress is paralleled by an increase in transcript levels and protein content. There are only very few examples

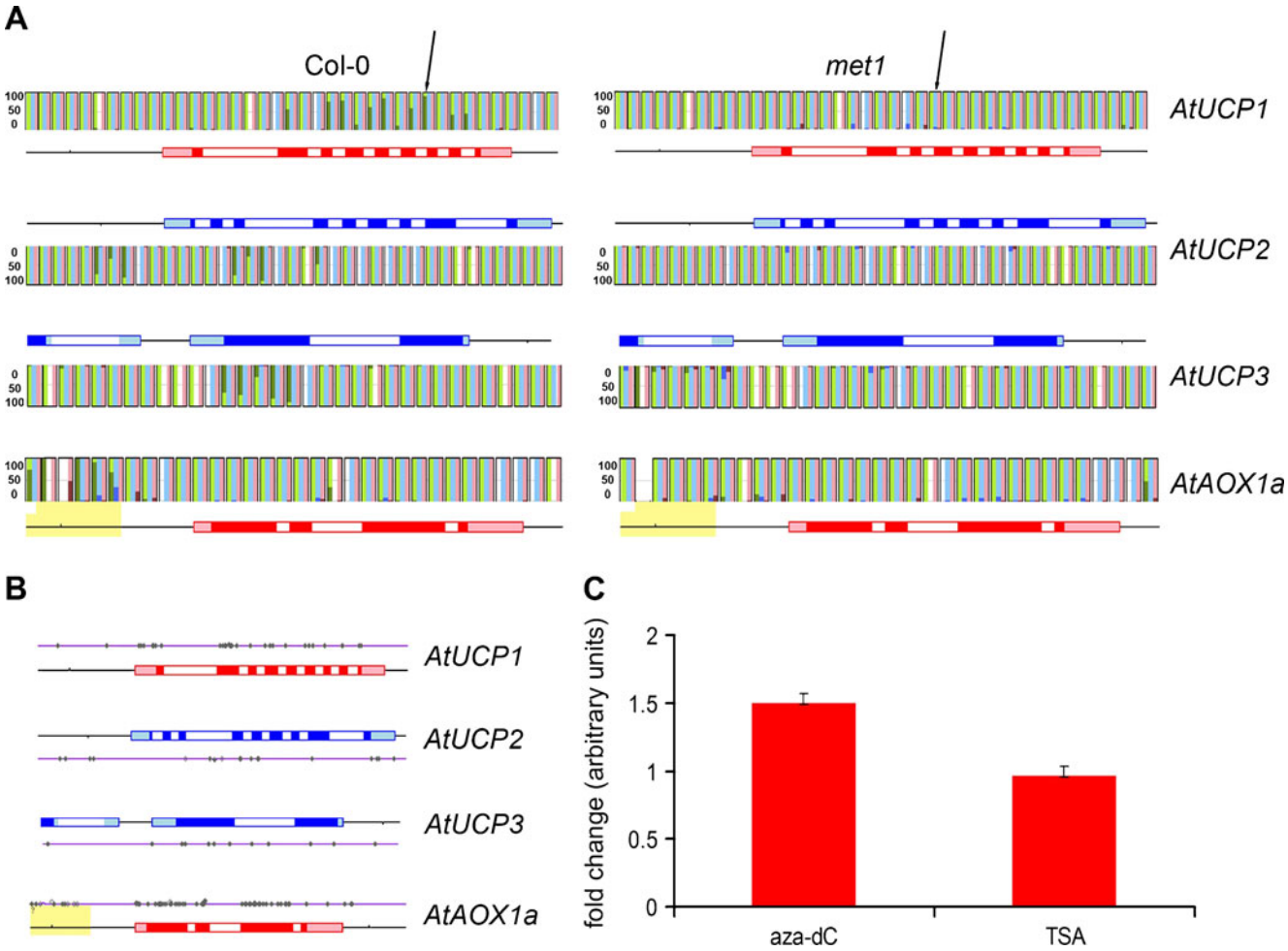

Fig. 3 Epigenetic regulation of *AtUCP* gene expression. (**a**) Methylation landscapes of *AtUCP1-3* and *AtAOX1a* loci in wild-type and *met1-3 Arabidopsis* unopened, immature flower buds (http://mpss.udel.edu/at_sbs/). Methylation status is shown across the entire locus, including transcribed as well as partial upstream and downstream untranscribed regions. Exons are separated in distinct boxes, but connected with lines to indicate a single gene. Genes on top DNA strand and their untranslated regions (UTRs) are shown as dark and light versions of red boxes, respectively. Genes on bottom DNA strand and their respective UTRs are shown as dark and light versions of blue boxes, respectively. Each rectangle on top or underneath each locus (depending of which DNA strand the gene is located) summarizes the data (in percentage) of three types of DNA methylation on every 100 nucleotides. Each type is indicated by a distinct color: CG, green; CHG, blue; CHH, magenta. Light versions indicate the unmethylated bases while dark colors indicate methylated bases (*arrows*). The shadowed yellowish box near *AtAOX1a* gene indicates a repeat-associated region. (**b**) Small RNA signatures are represented on top or underneath each locus as gray dots connected with lines. (**c**) Averaged fold-change of *AtUCP1* expression in the presence of 5-aza-2′ deoxycytosine (aza-dC) or trichostatin A (TSA). Total RNA from whole 16-day-old plantlets grown in presence or absence of aza-dC and/or TSA was used to hybridize cDNA microarrays as described (Chang and Pikaard 2005). *Error bars* represent standard error

in the literature trying to correlate UCP transcript and protein levels in plants (Daley et al. 2003; Armstrong et al. 2008). In one such an example, Taylor et al. (2010), using a quantitative peptide level analysis, did not observe a good correlation between protein and transcript abundance of certain mitochondrial carriers in rice seeds submitted to various conditions. Thus, knowledge of the interplay between UCP synthesis and degradation, as already established for mammalian UCP1 (reviewed in Azzu et al. 2010), is essential to further strengthen our understanding of the fine tuning regulation of UCP expression in plants.

Our attempt to predict the functional interplay of different *AtUCP* family members based on their expression behavior was not indicative of functional specialization. The most important clues derived from our expression analysis were a presumed physiological role for *AtUCP1* during seed germination and the high modulation of the *AtUCP1-2* genes under biotic stress. Thus, the functional contexts in which these genes are embedded could not be easily distinguished using global transcriptional regulation patterns. In this regard, whole-plant phenotypes associated with *UCP* silencing or overexpression would be highly informative to

evaluate the impact of UCP levels on development and stress tolerance. However, due to the multigene nature of the UCP family in Arabidopsis, the use of single knockout mutants to reveal UCP function may be obscured by functional redundancy. Therefore, to reduce the impact of redundancy and clearly assess the physiological relevance of UCP activity, especially in stress response, more elaborate knockout (KO)/silencing strategies should be employed. In this scenario, the use of multiple KO mutants represents an interesting approach that merits further investigation.

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