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Roles of mitochondrial energy dissipation systems in plant development and acclimation to stress

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● **Background** Plants are sessile organisms that have the ability to integrate external cues into metabolic and developmental signals. The cues initiate specific signal cascades that can enhance the tolerance of plants to stress, and these mechanisms are crucial to the survival and fitness of plants. The adaption of plants to stresses is a complex process that involves decoding stress inputs as energy-deficiency signals. The process functions through vast metabolic and/or transcriptional reprogramming to re-establish the cellular energy balance. Members of the mitochondrial energy dissipation pathway (MEDP), alternative oxidases (AOXs) and uncoupling proteins (UCPs), act as energy mediators and might play crucial roles in the adaption of plants to stresses. However, their roles in plant growth and development have been relatively less explored.
● **Scope** This review summarizes current knowledge about the role of members of the MEDP in plant development as well as recent advances in identifying molecular components that regulate the expression of AOXs and UCPs. Highlighted in particular is a comparative analysis of the expression, regulation and stress responses between AOXs and UCPs when plants are exposed to stresses, and a possible signal cross-talk that orchestrates the MEDP, reactive oxygen species (ROS), calcium signalling and hormone signalling.
● **Conclusions** The MEDP might act as a cellular energy/metabolic mediator that integrates ROS signalling, energy signalling and hormone signalling with plant development and stress accumulation. However, the regulation of MEDP members is complex and occurs at transcriptional, translational, post-translational and metabolic levels. How this regulation is linked to actual fluxes through the AOX/UCP *in vivo* remains elusive.

Key words: Alternative oxidase, AOX, calcium signals, energy signalling, mitochondrial energy dissipation pathway, MEDP, mitochondrial retrograde regulation, plant stress signalling, reactive oxygen species, ROS wave, uncoupling protein, UCP.

INTRODUCTION

All chemical reactions in living cells are energy dependent. In biological processes, energy drives the cellular metabolism and transport processes that are necessary for plant growth and developmental switching (Wallimann *et al.*, 1992; Borisjuk *et al.*, 2003). Depending on the metabolic demands, production, transportation, utilization and conversion of energy within cells are typically dynamic and require the co-ordination of different organelles through diverse signalling pathways to achieve specific developmental switches (Suzuki *et al.*, 2012). For instance, changes in energy demands were found to be closely associated with the greening process in embryos of *Vicia faba* during their development (Borisjuk *et al.*, 2003), and endosperm differentiation of barley grains (Rolletschek *et al.*, 2004) was reported to be developmentally regulated in a specific temporal and spatial manner (Borisjuk *et al.*, 2003; Rolletschek *et al.*, 2004). Stresses that affect plant growth and development can result in enhanced accumulation of reactive oxygen species (ROS) (Mittler, 2002), and alterations in the cellular energy state (Suzuki *et al.*, 2012), which can cause oxidation of cellular components,

developmental defects and even arrest of plant growth. The relationship between energy balance and plant stress adaption has been well documented (Huner *et al.*, 1998; Wilson *et al.*, 2006; Baena-González and Sheen, 2008). When plants respond to stresses, there is generally a decrease in the photosynthetic performance and fluctuations in respiration, which trigger an energy-deficit signal (Baena-González and Sheen, 2008). Specifically, some abiotic stresses such as extreme temperature (Stupnikova *et al.*, 2006), hypoxia/anoxia (Huang *et al.*, 2005; Liu *et al.*, 2006; Branco-Price *et al.*, 2008), low pH (Messerli *et al.*, 2005), starvation (Vanlerberghe, 2013) and stresses that downregulate TOR (target of rapamycin) signalling (Caldana *et al.*, 2013) are more likely to result in ATP overconsumption, causing a cellular energy crisis and thereby activating energy-conserving responses. In contrast, stresses that induce the autophage pathway generally do not trigger energy deficiency (Lv *et al.*, 2014). A popular proposal has been that different stresses were partly decoded as signals of energy deficiency and that the adaption of plants to stresses is a process by which cellular metabolism and the transcriptome are reprogrammed

to re-establish the balance of cellular energy (Wilson *et al.*, 2006; Baena-González and Sheen, 2008; Rodrigues *et al.*, 2013; Lastdrager *et al.*, 2014). The metabolic re-programming has been suggested to be involved in inhibition of biosynthetic pathways, such as sugar synthesis (Stitt and Hurry, 2002), as well as induction of catabolic processes, photosynthesis, sugar remobilization (Tome *et al.*, 2014), starch and storage lipid accumulation (Caldana *et al.*, 2013), and alteration of primary and secondary metabolism (Caldana *et al.*, 2013). Similarly, transcript profiling studies have provided a snapshot of the complex energy-associated gene network that operates during stress conditions (Baena-González *et al.*, 2007; Avin-Wittenberg *et al.*, 2012; Caldana *et al.*, 2013). The changes in global gene expression in response to energy stress are followed by slowed photosynthesis, and activated energy-promoting gene networks, some of which are known to be correlated with tetrapyrrole biosynthesis, the aspartate family pathway, the tricarboxylic acid (TCA) cycle and mitochondrial electron transport chain (Avin-Wittenberg *et al.*, 2012). Thus, the ability of plants to maintain the delicate balance of energy production and utilization may be of fundamental importance for their survival and fitness, particularly when exposed to stress conditions.

Plants have developed several mechanisms to minimize any damage that might result from energy imbalances, and two of the most important mechanisms have been well characterized. One of the mechanisms takes place in the chloroplast and is known as non-photochemical quenching-mediated energy dissipation (Huner *et al.*, 1998; Szabo *et al.*, 2005; Ruban *et al.*, 2007), whereby excessively absorbed light energy is dissipated as heat (non-photochemical quenching) (Huner *et al.*, 1998; Szabo *et al.*, 2005). The other mechanism is a mitochondria–energy dissipation system, whereby ATP production and mitochondrial respiration are uncoupled by alternative oxidases (AOXs) and uncoupling proteins (UCPs) (Meeuse, 1975; Sluse and Jarmuszkiewicz, 2002). The AOXs and UCPs regulate coupling between the TCA cycle and ATP synthesis, allowing a stable carbon flow through glycolysis, which is essential for autotrophic organisms such as plants whose biosynthetic demands depend greatly on TCA intermediates (Vercesi *et al.*, 2006). AOXs and UCPs were identified originally as being functionally related to thermogenic processes in plants and animals, respectively (Meeuse, 1975; Gimeno *et al.*, 1997). It is now well documented that genes in the mitochondrial energy dissipation pathway (MEPD) are involved in a wide range of physiological and developmental processes (Chai *et al.*, 2012; Diano and Horvath, 2012; Xu *et al.*, 2012; Vanlerberghe, 2013). However, the precise mechanisms by which AOXs and UCPs are regulated are largely unknown.

In this review, we briefly summarize the advances in knowledge of the MEDP in plants with a timeline (Fig. 1) and describe the role of the MEDP in plant growth and development as well as in integrating metabolic processes in different compartments of the cell (Fig. 2). We also discuss recent advances in the understanding of components that regulate the expression of AOXs and UCPs with the focus on a comparative analysis of AOXs and UCPs in stress adaption, expression and regulation. We highlight stress-triggered ROS signals, calcium signals, redox signals and metabolic intermediates that might be important in integrating mitochondrial retrograde signalling with energy and stress signals under stress conditions.

THE MITOCHONDRIAL ENERGY DISSIPATION PATHWAY IN PLANTS

There are at least two sets of functionally related pathways in plant mitochondria. One pathway consists of members of the small nuclear gene family, which couple the oxidation of ubiquinol with the reduction of molecular oxygen to water (Vanlerberghe and McIntosh, 1997). This pathway mediates cyanide-insensitive plant respiration with a terminal AOX and is known as the alternative pathway or the cyanide-resistant respiratory pathway (Vanlerberghe and McIntosh, 1997). The alternative pathway branches at a ubiquinol pool and bypasses ATP-producing complexes III and IV, which results in uncoupling between electron transport and ATP synthesis. Thus, a direct consequence of alternative pathway engagement is a decrease of the tight coupling of oxidative phosphorylation and an increase in heat release. The other pathway is involved in energy dissipation in plant mitochondria and consists of members of the mitochondrial anion carrier protein superfamily, the UCPs, which mediate free fatty acid-activated, purine nucleotide-inhibited proton conductance (Echtay *et al.*, 2002; Jarmuszkiewicz *et al.*, 2010). The presence of UCPs creates an alternative way for the re-entry of protons into the mitochondrial matrix, and leads to uncoupled substrate oxidation from the phosphorylation of ADP to ATP to the release of energy as heat (Pecqueur *et al.*, 2009). In animals, uncoupled mitochondrial respiration mediated by UCPs was considered to be involved with the control of the NAD^+/NADH ratio and the regulation of metabolic pathways such as ketogenesis and amino acid synthesis (Ricquier and Bouillaud, 2000). Recently, Gandin *et al.* (2014) addressed the possible role of UCP1 and AOX1A in balancing the energy partitioning between nitrogen and carbon metabolism by feeding *aox1a* and *ucp1* plants with NO_3^- and $NH4^+$ (Gandin *et al.*, 2014). The rate of foliar NO_3^- assimilation was enhanced in both *aox1a* and *ucp1* mutants compared with the wild-type plants, suggesting that the decreased capacity of the mitochondrial electron transport chain and increased reductant within the cytosol probably contribute to driving foliar NO_3^- assimilation (Gandin *et al.*, 2014). These results suggested the important role of AOX and UCP in maintaining cellular metabolism.

TEMPORAL AND SPATIAL EXPRESSION PATTERNS OF MEMBERS OF THE MEDP

The availability of public databases, such as Genevestigator database (Zimmermann *et al.*, 2004; Hruz *et al.*, 2008) and BAR (Bio-Array Resource for plant biology) (Winter *et al.*, 2007), provides opportunities to explore the expression and regulation of members of the MEDP in plants. The Development tool and Anatomy tool in the condition search tools of the Genevestigator database were used to examine the expression patterns of MEDP gene members at different developmental stages of the life cycle of *Arabidopsis thaliana* (arabidopsis) as well as in different tissues and organs (Fig. 3A, B). The expression of MEDP members during seed development is retrieved
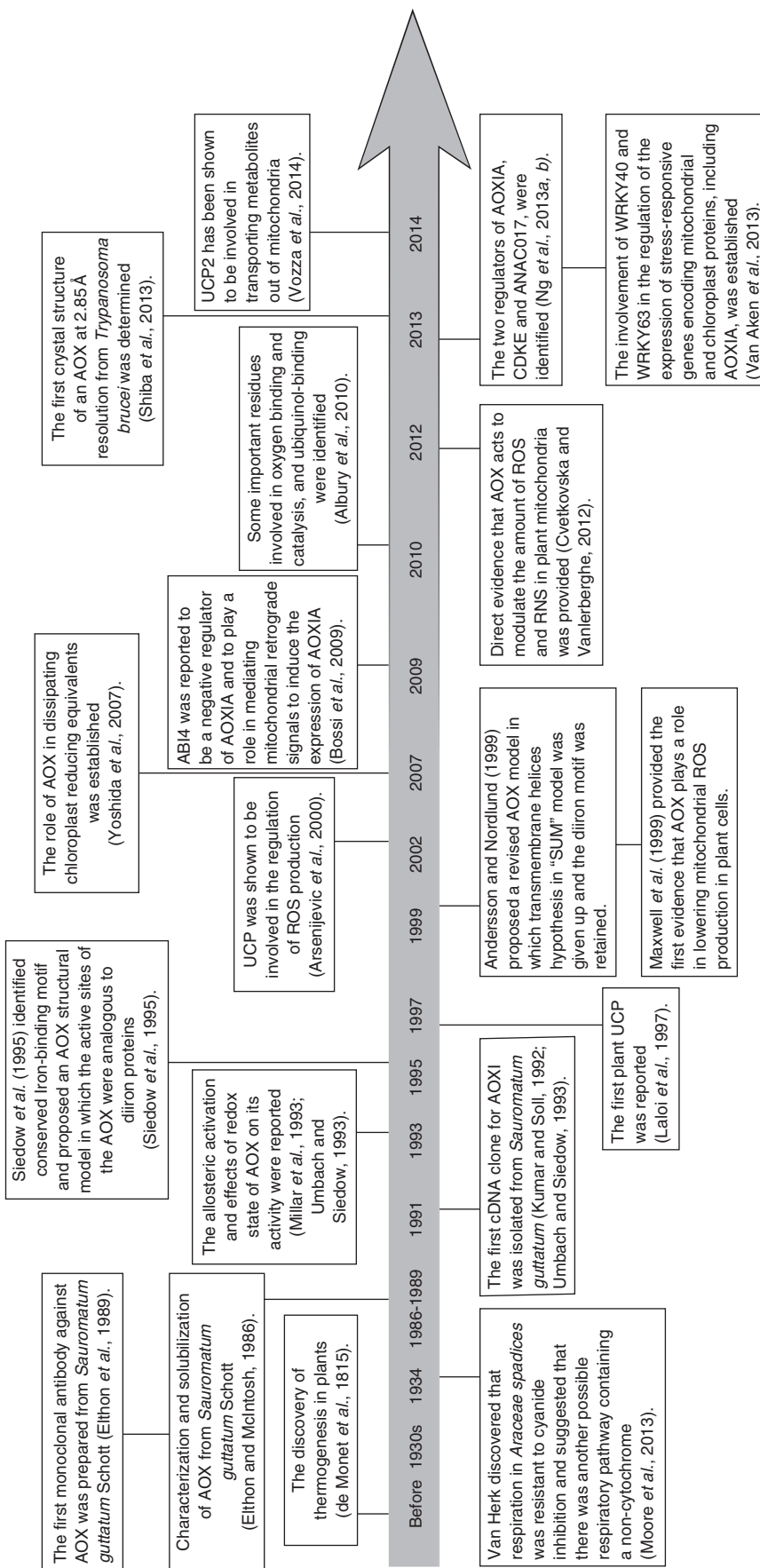
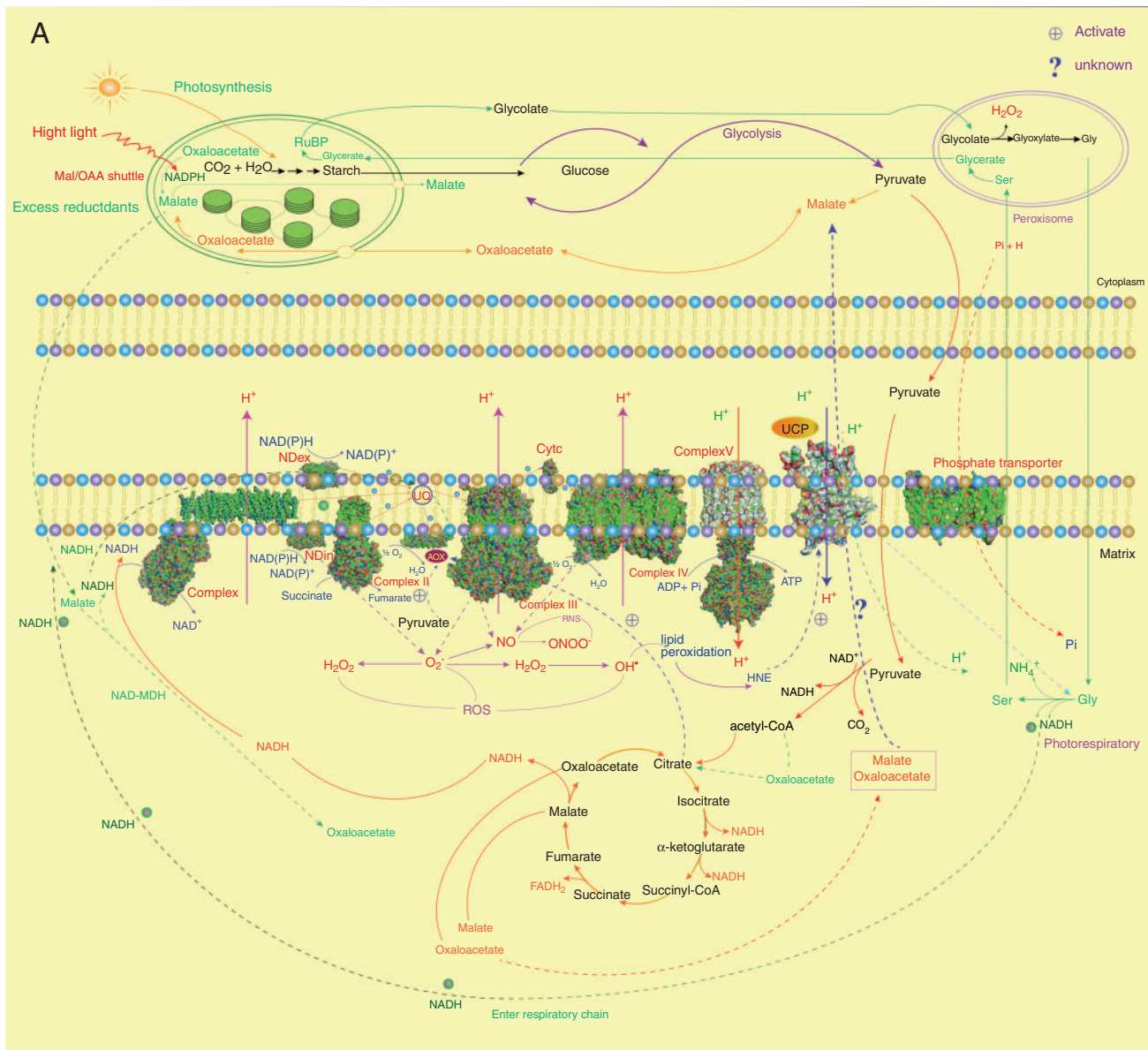Fig. 1. Timeline: a brief history of the understanding of the MEDP.

FIG. 2. The intraorganelle metabolic association linked by the MEDP (A) and co-regulation gene network (B). (A) The major mitochondrial respiratory complexes (MCRs) and AOX are shown as crystal structures. For each MCR protein, structures are retrieved from the Protein Data Bank (PDB): complex I, PDB 3M9S (Efremov *et al*., 2010); complex II, PDB 1ZOY (Sun *et al*., 2005); complex III, cytochrome *bc*1, PDB 3H1J (Iwata *et al*., 1998); complex IV, cytochrome *c* oxidase, PDB 1OCO (Yoshikawa *et al*., 1998); complex V, F0, PDB 1C17 (Rastogi and Girvin, 1999), F1, PDB 1E79 (Gibbons *et al*., 2000); yeast NADH dehydrogenase, PDB 4G9K (Iwata *et al*., 2012); phosphate transporter, PDB 4J05 (Pedersen *et al*., 2013); AOX, PDB 3VV9 (Shiba *et al*., 2013); UCP, PDB 2LCK (Berardi *et al*., 2011). (B) To obtain gene sets of co-expressed MEDP members (Supplementary Data Tables S1–S5), each MEDP member is selected, respectively, to run the Perturbations tool, Co-Expression tool and Biclustering tool of the Genevestigator database (Zimmermann *et al*., 2004; Hruz *et al*., 2008). First, conditions that are relevant for each MEDP target gene are identified by using the Perturbations tool from the condition search tools to create samples based on a fold change value of 2 and *P*-value cut-off of 0·05, and then to correlate across these perturbations in the Co-Expression tool. The resulting co-expressed gene sets are used to produce the co-regulation gene network.

(continued)

from the BAR database using the Arabidopsis eFP brower (Fig. 3C). The Co-Expression tool, Perturbations tool and Biclustering tool of the Genevestigator database (Hruz *et al*., 2008) were used to analyse the expression and regulation of members of the MEDP in arabidopsis. The expression levels of *UCP* genes in arabidopsis were higher than those of *AOX* genes in different developmental stages (Fig. 3A). Similarly, high transcript levels of five *UCP* genes were detected in most cell types and tissues; whereas, of five *AOX* genes, only *AOX1A* displayed high levels of expression similar to those of the *UCP* genes (Fig. 3B). Notably, the MEDP genes were differentially expressed in most tissues, and each gene displaying a distinct expression pattern. For instance, in the ovule, *AOX1C* was the predominant transcript, while *AOX1D* and *AOX2* were predominant in pollen and seeds. A co-expression analysis based on the Co-Expression tool in Genevestigator (Hruz *et al*., 2008),
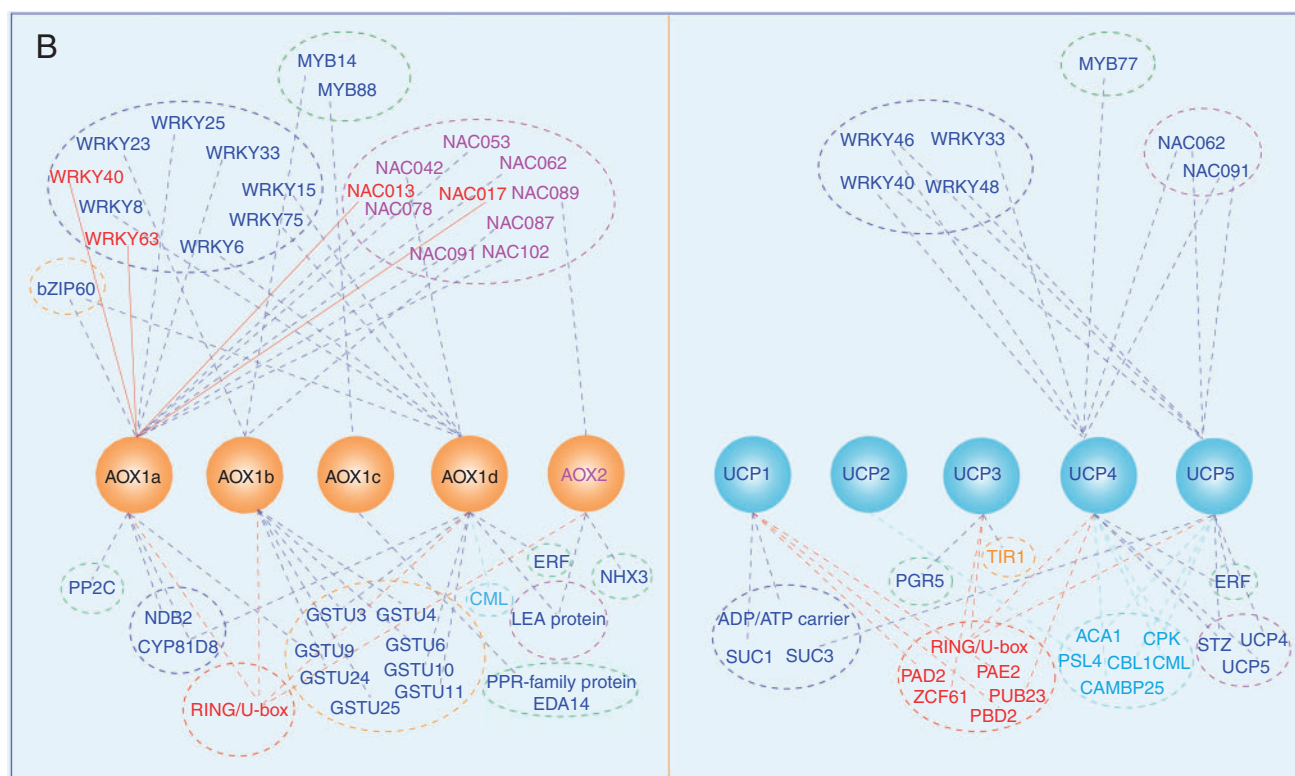
Fɪɢ. 2. Continued

further suggested that genes involved in embryo sac development arrest (AT3G60360), embryo defects (AT2G31340) and embryogenesis, such as the pentatricopeptide repeat-containing protein (AT3G49240), were co-expressed with *AOX1C*. Therefore, *AOX1C* may play a specific role in ovule development, which is consistent with a study that showed that *AOX1C* was regulated by growth and developmental signals (Ho *et al.*, 2007). Unlike the other MEDP genes, the expression of *UCP2* was ubiquitous in many tissues and organs, with higher expression levels occurring in the root protoplast, abscission zone, petal and seed coat; *UCP4* was present at much lower levels than the other *UCP* genes, particularly in root endodermis and the quiescent centre (Fig. 3B). In a recent study, a transcription factor, UPBEAT1 (UPB1), was identified as a key regulator that modulates transition from proliferation to differentiation in the root cells by regulating ROS balance and distribution (Tsukagoshi *et al.*, 2010). The *upb1* mutants exhibit an altered accumulation of ROS, with reduced accumulation of hydrogen peroxide and increased accumulation of superoxide in the elongation zone (Tsukagoshi *et al.*, 2010). The plants lacking *upb1* have longer roots due to an increase in both meristem size and root cell length (Tsukagoshi *et al.*, 2010), suggesting that alteration of the gradient and compartmentation of ROS in the root result in changes in the size of the meristem and in root length. Moreover, the size of the wild-type meristem was significantly affected by salicylhydroxamic acid (SHAM), an inhibitor of peroxidase activity as well as AOX, and KCN treatment (Tsukagoshi *et al.*, 2010), indicating that AOX might be involved in the regulation of the size of the root meristem, and that upregulation of *AOX* is not sufficient to compensate effects

of KCN on other antioxidant systems. Despite the fact that *UCP4* (At4g24570) is not a direct target of UPB1, the *UCP4* expression was significantly upregulated in *UPB1*-overexpressing plants (Tsukagoshi *et al.* (2010). Whether *AOX* genes and *UCP4* are directly involved in the regulation of the root meristem needs further investigation. The highest expression levels of *UCP3* were observed in the shoot apical meristem, auxiliary bud, leaf primordia and guard cell (Fig. 3B), and in stomatal closure under perturbations, suggesting that *UCP3* may play a specific role in shoot meristem development. Interestingly, regulators responsible for stomatal development, such as the epidermal patterning factors EPF1 (Hara *et al.*, 2007) and STOMAGEN (Sugano *et al.*, 2009), as well as proteins involved in the regulation of metabolic fluxes during abscisic acid (ABA)-controlled seed development and germination (Pudelski *et al.*, 2012), were co-expressed with UCP3. However, it remains unknown whether UCP3 is involved in the regulation of the shoot meristem. Nitric oxide (NO) and ROS are known to regulate stomatal closure in response to ABA in various species (Desikan *et al.*, 2004). The inter-relationship between NO, ROS and AOX was confirmed in a study that found that the knock-down of *AOX* in tobacco plants led to increased content of NO in guard cells and decreased hydrogen peroxide levels compared with wild-type plants (Cvetkovska *et al.*, 2014). While no noticeable impact on stomatal function was found in *AOX* knock-down plants, the repression of AOX reduced the response of guard cells to NO and compromised the ability of stomata to change the irradiance response (Cvetkovska *et al.*, 2014), highlighting the role of AOX in stomata and NO homeostasis regulation. Therefore, the differential
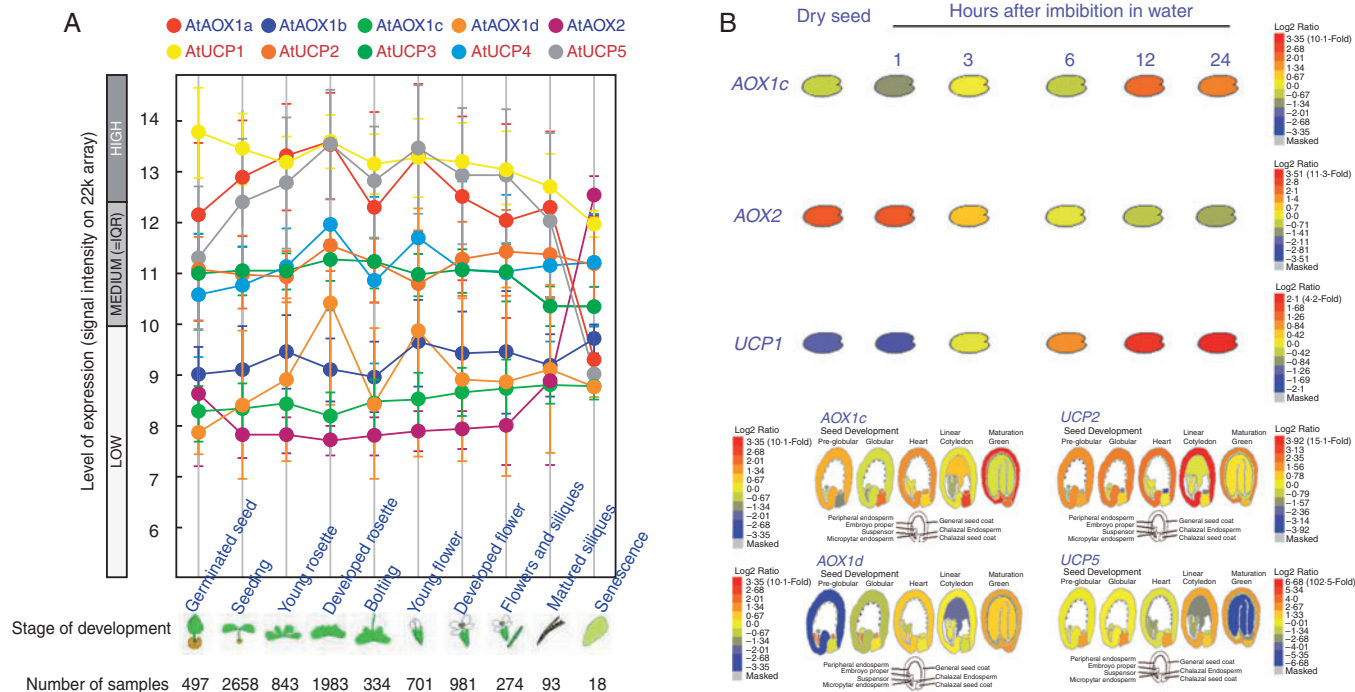
Fig. 3. Analysis of *MEDP* gene expression during development and organ distribution. (A) Genevestigator expression map describing the expression patterns of MEDP members during arabidopsis development. Growth stages from seed germination to senescence are arbitrarily grouped into sub-categories based on Boyes standard (Boyes *et al*., 2001). Each category contains averaged raw signal data and the 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 the standard error. Data were retrieved from the Genevestigator database (https://genevestigator.com/gv/plant.jsp) using the Development tool. (B) Transcript expression of the MEDP members in wild-type arabidopsis. Relative expression level of MEDP members in plant tissues and organs and in particular cell types. Data were obtained from the Genevestigator database using the condition search tool set, the Anatomy tool. (C) The expression level of MEDP members during seed development and germination. The log2 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 the BAR database (http://bar.utoronto.ca/).

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INVOLVEMENT OF THE MEDP IN POLLEN GERMINATION AND POLLEN TUBE GROWTH

The growth and development stages of plants have distinct cellular demands (Clifton *et al*., 2006). Co-ordination between the cellular energy status and gene expression is required to achieve specific development programmes. Thus, the increased expression of a specific sub-set of genes involved in cellular energy processes at particular growth stages or in specific tissues/organs is probably indicative of specific cellular energy demands. Pollen germination (PG) and pollen tube growth (PTG) are complex processes that requires high rates of energy flow (Rounds *et al*., 2011), the co-ordination of ion fluxes (Holdaway-Clarke and Hepler, 2003) and the regulation of ROS production (Martin *et al*., 2013; Lassig *et al*., 2014). As an energy mediator as well as a regulator of ROS production, it is tempting to speculate that the MEDP might play a role in these developmental processes. It has been suggested that the expression of *AOX1B* from arabidopsis was specific to floral tissues (Saisho *et al*., 1997; Clifton *et al*., 2006), while transcripts of *AOX1D* were highly expressed in stamens and pollen (Fig. 3B).

Similarly, high expression levels of *UCP5* were found in stamens and pollen, suggesting that *UCP5* may play a role similar to *AOX1D* in floral development (Fig. 3B). Generation of an *AOX1b*/*UCP5* double mutant would resolve this question and provide more insight into the role of MEDP genes in floral development. The links between the MEDP genes and PG and PTG were supported by a study in arabidopsis that found that the MEDP genes were upregulated during the transition from mature pollen grains to germinating pollen and to growing pollen tubes (Wang *et al*., 2008). In their study, Wang *et al.* (2008) showed that the expression of *AOX1A*, *AOX1B*, *UCP1*, *UCP4* and *UCP5* was detected during PG and PTG; the expression of *UCP4* and *UCP5* increased during PG, while increases in *AOX1A* and *UCP4* expression were detected only during PTG, indicating that each stage of PG and PTG had different energy demands and growth rate oscillations. In a separate study of gene expression profiles associated with PG and PTG, 137 and 186 genes (both included *AOX1A*), respectively, were identified. The expression levels of all these genes increased during pollen hydration and PTG *in vitro*. The expression of *AOX1A* in 4 h PTG was upregulated >2-fold compared with its expression in dry pollen (Qin *et al*., 2009). It has been demonstrated that ion fluxes and transporters play critical roles in controlling PTG by controlling pollen tube polarity and also by providing
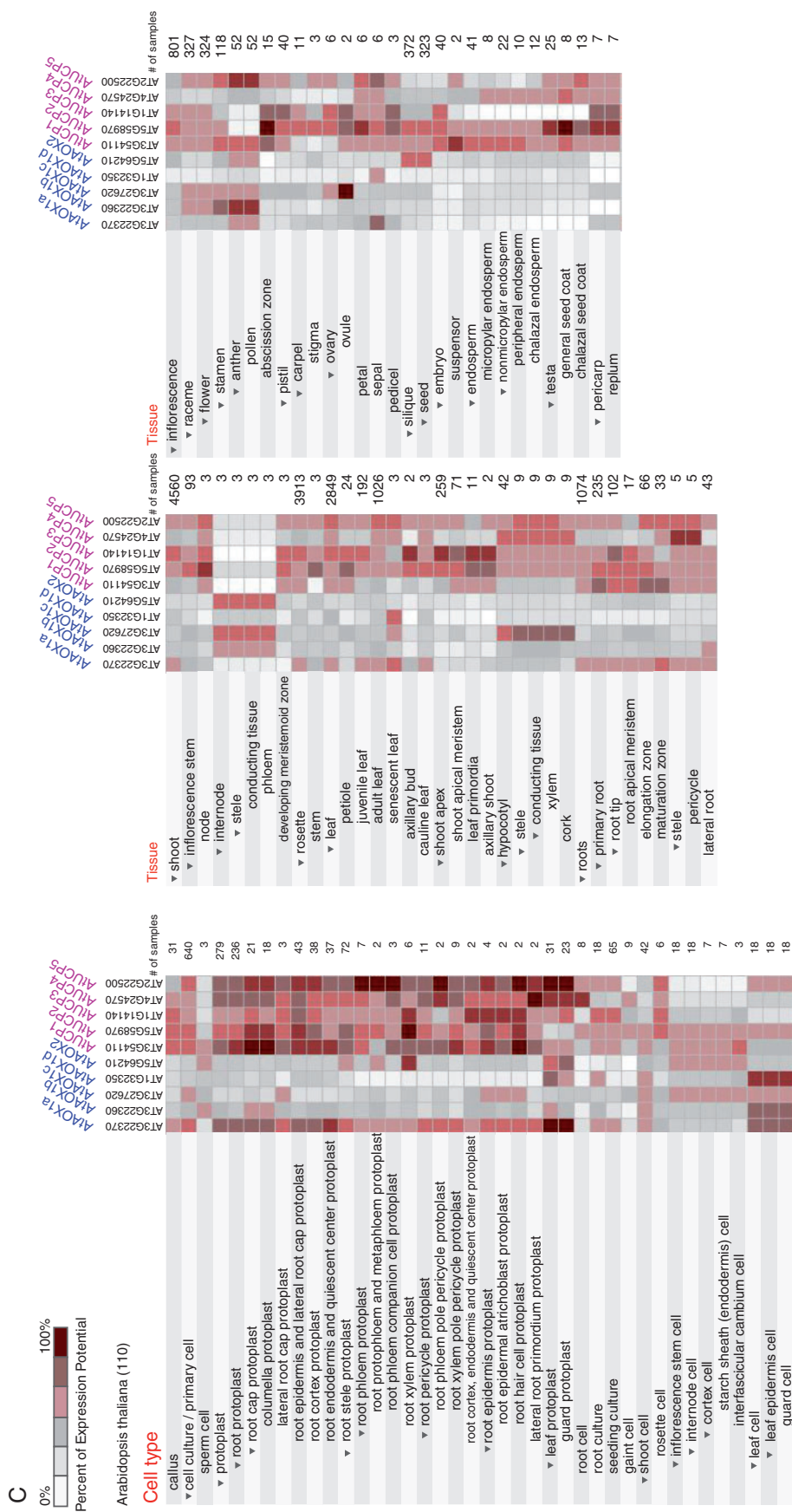
Fig. 3. Continued.

sufficient materials for PTG (Hepler *et al.*, 2001; Holdaway-Clarke and Hepler, 2003). In the pre-germination phase, the respiration rate is high with the conversion of sugar to starch; as PG continues, respiration slows (Rounds *et al.*, 2011). The upregulation of transporters such as *UCP4* and *UCP5* during PG probably reflects an increased demand for nutrients for PG and PTG. Conversely, increased expression of *AOX1A* and *UCP4* during PTG might be associated with the control of ROS accumulation. It was reported that ROS preferentially accumulated in central cells of the embryo sac before fertilization, and that stigma pollination triggered a localized oxidative burst that was restricted to the synergid cells of mature embryo sacs and was maintained until pollen tube arrival (Martin *et al.*, 2013). Although an oxidative environment was postulated to be involved in pollen tube reception (Martin *et al.*, 2013, 2014), the incorrect localization of ROS could result in developmental defects. In a mitochondrial manganese superoxide dismutase (MSD) mutant plant, the disruption of temporal and spatial ROS homeostasis along the embryo sac during development was found to lead to aberrant female gametophytes, because impaired MSD expression resulted in high levels of ROS in central cells, which further disturbed the determination of central cell fates (Martin *et al.*, 2013). The production of ROS by pollen tube NAD(P)H oxidases has also been shown to be associated with stabilization of PTG rate oscillations (Lassig *et al.*, 2014). These results suggested that the compartmentation and gradient of ROS is critical to initiate specific developmental processes, such as PG and PTG. The differential expression of MEDP genes at the PG and PTG stages may reflect specific respiration demands and a possible mechanism by which the balance of both ROS and NAD^+ could be regulated precisely.

INVOLVEMENT OF THE MEDP IN SEED DEVELOPMENT AND FRUIT RIPENESS

Seed germination is a complex process that integrates environmental cues with internal signals to initiate a developmental programme that orchestrates the temporal and spatial expression of a set of genes. This process involves mitochondrial energy metabolism, hormone metabolism, RNA processing and degradation, protein synthesis, degradation and transportation, which re-establish mitochondrial respiratory metabolism and the subsequent emergence of radicals (Nakabayashi *et al.*, 2005; Finch-Savage and Leubner-Metzger, 2006; Holdsworth *et al.*, 2008; Nonogaki *et al.*, 2010; Narsai *et al.*, 2011). A prominent feature of seed germination is that genes related to energy metabolism and mitochondrial electron transport were activated specifically in the micropylar and chalazal endosperm (Dekkers *et al.*, 2013), and it is accompanied by a rapid acceleration in oxygen consumption and mitochondrial respiration activity (Ehrenshaft and Brambl, 1990). The production of ROS has been observed during seed germination in various species (Oracz *et al.*, 2009; Leymarie *et al.*, 2012; Ishibashi *et al.*, 2013) and was considered to act as a positive signal in seed dormancy release (El-Maarouf-Bouteau and Bailly, 2008). Based on these findings, it is reasonable to suppose that MEDP proteins such as AOXs and UCPs play roles in seed development. Indeed, from seed germination to maturation, the expression of MEDP genes occurs in a temporal- and spatial-specific manner.

During germination stages, the *AOX1A* and *AOX2* genes were found to be differentially expressed in dry seed and in stratified and germinating seedlings, whereas all the *UCP* genes were found to be differentially expressed during stratification, germination and post-germination (Narsai *et al.*, 2011). The genome-wide network model that captured seed germination also showed that *AOX1A* was upregulated by dormancy in wheat, whereas *UCP1* was upregulated by germination (Bassel *et al.*, 2011). Furthermore, the transcripts of *AOX1A* and *UCP5* decreased >3-fold from embryo emergence at around 24 h after imbibition in the light to 48 h after imbibition in the light. Conversely, the expression of *UCP1* was upregulated >3-fold by stratification, reaching a maximum at 48 h stratification (Narsai *et al.*, 2011). It has been suggested that the expression of *AOX2* may be mature seed specific and that the expression decreased upon imbibition (Clifton *et al.*, 2006), implying that *AOX2* probably plays a role in mature seed development rather than seed germination. Direct evidence for the involvement of MEDP genes in seed development was demonstrated in a study that showed that the suppression of *AOX2A* expression in soybean resulted in reduced pod production, and thus seed yields (Chai *et al.*, 2012). Interestingly, some MEDP genes were found in previously reported differentially expressed gene data sets, revealing transcriptomic changes associated with seed development, including arabidopsis seed germination, rice seed germination (Howell *et al.*, 2009) and legume seed maturation (Verdier *et al.*, 2013*a*, *b*). This result suggested that the upregulation or downregulation of mitochondrial energy components may be an evolutionarily conserved mechanism that is associated with the developmental processes that initiate seed germination or development.

Genes of the MEDP have also been demonstrated to be involved in the process of fruit ripening as regulators of cellular energy and ROS (Almeida *et al.*, 1999; Considine *et al.*, 2001; Xu *et al.*, 2012). It was recently reported that explosive seed discharge in dwarf mistletoe is triggered by thermogenesis, and that the timing of explosive seed discharge in dwarf mistletoe corresponds to the presence of AOX in its mitochondria (deBruyn *et al.*, 2015). It is therefore reasonable to suppose that AOX may play a critical role in triggering seed discharge in dwarf mistletoe. To the best of our knowledge, the MEDP may regulate ROS and the energy status of cells, and thus participate in these developmental processes.

LINKS BETWEEN MEDP MEMBERS, STRESS AND ENERGY SIGNALLING

Controlling energy and metabolic homeostasis is a great challenge for plants, and adaptations to stress conditions are typically linked to adjustment of cellular energy and/or metabolism (Baena-González and Sheen, 2008). In plants, two important protein kinases, plant Snf-1-related kinase 1 (SnRK1) homologue of yeast sucrose non-fermenting-1 (SNF1) kinase and the plant TOR kinase, are central regulators integrating plant growth, stress and energy signalling (Lastdrager *et al.*, 2014; Tome *et al.*, 2014). The arabidopsis SnRK superfamily consists of three distinct sub-families, i.e. the SnRK1, SnRK2 and SnRK3 sub-families. The SnRK2 and SnRK3 sub-family members have been suggested to be involved in ABA-mediated

signalling and other signalling pathways that regulate plant response to abiotic stresses such as cold, drought and salt (Hey *et al.*, 2010), whereas SnRK1 is a metabolic sensor that mediates an extensive metabolic reprogramming when plants are subjected to an energy crisis (Baena-González *et al.*, 2007). In arabidopsis, the activation of KIN10, a catalytic subunit of SnRK1, initiates a global gene expression, promoting catabolism and suppressing anabolism (Baena-González *et al.*, 2007). In addition to SnRK genes, MEDP members also play roles in stress and energy signalling, possibly by integrating ROS signalling with controlling the metabolic/redox state.

The expression of AOXs is known to be induced by a wide range of biotic and abiotic stresses (for reviews, see Vanlerberghe *et al.*, 2009; Vanlerberghe, 2013), whereas the induction of UCPs by stress is less well characterized despite evidence from the Genevestigator microarray database suggesting that it might be involved in a range of biotic and abiotic stress responses (Fig. 4). The induced expression of MEDP genes under stress conditions is partly associated with the demand for decreasing ROS production and remodelling of the cellular metabolic status. Low temperature is known to reduce membrane fluidity by affecting the positional redistribution of saturated and unsaturated fatty acids within lipid molecules (Moon *et al.*, 1995; Somerville, 1995), thereby reducing proton leakage, which can cause an increase in the potential for adenylate restriction of the electron transport chain (Atkin and Tjoelker, 2003). In this scenario, electron transport from ubiquinone to oxygen through AOX can occur without being restricted by adenylate control or the cellular ATP/ADP ratio, resulting in an increase in the respiration rate and heat release, which might, in turn, rescue membrane fluidity and growth. There is evidence that a chilling-sensitive maize cultivar showed higher electron partitioning through the alternative pathway compared with a chilling-tolerant cultivar when exposed to low temperatures (Ribas-Carbo *et al.*, 2000), suggesting that upregulation of genes in the alternative pathway helped alleviate cold damage so as to sustain growth after recovery from chilling. Exposure of plants to low temperature results in accumulation of ROS (Suzuki and Mittler, 2006) and soluble sugars (Guy *et al.*, 1992). Accumulation of ROS can cause oxidative damage, whereas accumulation of soluble sugars can protect plants against oxidative stress via their impact on ascorbate synthesis (Couée *et al.*, 2006), as they can serve as a substrate for the synthesis of the antioxidant ascorbate (Smirnoff *et al.*, 2001). Temperature acclimation is a complex process that involves extensive reprogramming of gene expression and metabolism (Stitt and Hurry, 2002; Zhu *et al.*, 2007); for example, low temperature acclimation is closely associated with a selective stimulation of sucrose synthesis and re-establishment of a high rate of photosynthesis (Stitt and Hurry, 2002). Several studies have shown that MEDP genes play an important role under low temperature conditions. Suppressing *AOX1A* in arabidopsis plants leads to reduction of growth, with impaired leaf area development and smaller rosette size, whereas overexpressing it has the opposite effect at low temperatures (Fiorani *et al.*, 2005). Interestingly, transgenic tobacco plants with enhanced AOX levels showed enhanced sugar accumulation upon exposure to low temperature (Wang *et al.*, 2011). Furthermore, the compensatory upregulation of *UCP1* expression was observed in an *AOX1A* arabidopsis mutant when it was exposed to low temperatures (Watanabe *et al.*, 2008), suggesting that both AOX and UCP are involved in the response to low temperatures. It has been shown that MEDP members function at different time points following exposure of arabidopsis to cold, with increased alternative pathway activity being important only in the early stages of cold treatment (Armstrong *et al.*, 2008), whereas an increase in *UCP* transcripts (Watanabe *et al.*, 2008) and protein levels (Armstrong *et al.*, 2008) was observed during long-term exposure to cold. It has also been shown that the transcripts of both *AOX* and *UCP* greatly increased in a chilling-tolerant tomato (Zhefen No. 208) but not in chilling-sensitive plants during chilling (Shi *et al.*, 2013), which has been associated with a decrease in the mitochondrial production of ROS. It should be noted that the expression of some glycolysis-metabolic genes, such as PPi-dependent phosphofructokinase and sucrose synthase, was also upregulated in that study. The simultaneous engagement of the two energy dissipation systems would theoretically lead to greatly decreased ATP yields. Therefore, cytosol glycolysis-metabolic genes may be switched on to compensate for the loss of energy, reflecting the flexible energetic and metabolic cross-talk between the two organelles and the cytosol. Taken together, it is probable that AOX plays a significant role in short-term cold acclimation by providing plants with a means, independent of the energy state, to oxidize excess cellular reductant during early stages of cold stress and a way to maintain the respiration rate. Given that cold acclimation involves re-establishment of a high rate of photosynthesis (Stitt and Hurry, 2002), it is possible that induction of *UCP* transcripts and protein levels facilitate photosynthesis as the absence of *UCP1* results in restriction of photorespiration, and thereby a decreased photosynthetic carbon assimilation rate (Sweetlove *et al.*, 2006). It should be noted that the response of MEDP members to low temperature might be species/stress dependent; for example, in tomato, the alternative splicing event in AOX pre-mRNAs occurred preferentially at low temperature (Fung *et al.*, 2006), whereas UCP but not AOX experienced such defective pre-mRNA processing when rice is exposed to cold environments (Watanabe and Hirai, 2002).

In addition to low temperature, poor oxygen availability is also a factor that leads to a local energy crisis within the tissue (van Dongen and Licausi, 2015). Unlike animals, plants lack efficient systems to distribute oxygen to heterotrophic tissues, such as roots, tubers, meristems, germinating pollen and developing seeds, making them vulnerable to oxygen deprivation (Geigenberger, 2003; Mustroph *et al.*, 2010). When plants suffer from oxygen deficiency, cytochrome oxidase activity becomes oxygen limited as the oxygen concentration drops, and ATP production through oxidative phosphorylation is inhibited (Geigenberger, 2003). Thus, hypoxia/anoxia restricts respiration and leads to a decrease in the ATP/ADP ratio and adenylate energy status (Geigenberger, 2003; Geigenberger *et al.*, 2010). Low oxygen and/or re-oxygenation have also been shown to be associated with ROS signalling (van Dongen and Licausi, 2015), cellular metabolic remodelling (Branco-Price *et al.*, 2008; Gupta *et al.*, 2012; Shingaki-Wells *et al.*, 2014) and mitochondrial responses, including the TCA cycle and respiratory chain (Shingaki-Wells *et al.*, 2014). It is probable that MEDP members play roles in hypoxia/anoxia similar to that in low temperature. On the one hand, AOX was downregulated by oxygen deprivation at early stages due to its extreme affinity

A   Response of MEDP members to chemical treatments

C   Response of MEDP members to biotic stresses

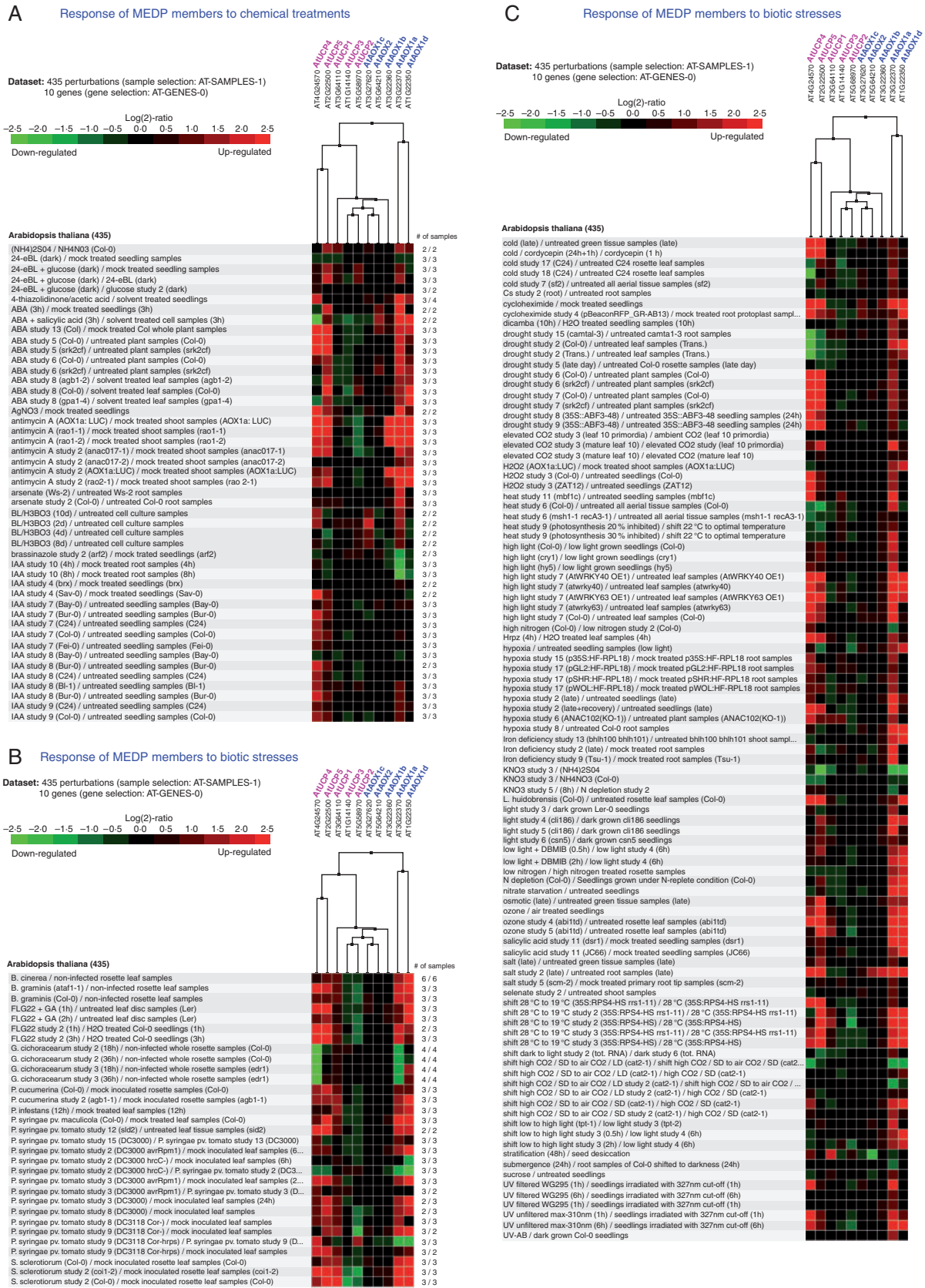**B**   Response of MEDP members to biotic stresses

Fig. 4. Clustering of responses of *MEDP* genes to a wide range of perturbations. Data are taken from Genevestigator using the Perturbations tool and Biclustering tool (Zimmermann *et al.*, 2004, 2008). Red indicates that gene expression was induced by the treatment, and green indicates the opposite. A dendrogram is shown using the Euclidean distance.

for oxygen (Igamberdiev *et al.*, 2005), which may facilitate transient ROS accumulation. Accumulated ROS might act as a signal to trigger a signal cascade to activate genes involved in ROS scavenging (van Dongen and Licausi, 2015). On the other hand, hypoxia-induced production of NO inhibits aconitase and increases the level of citrate, which, in turn, induces the expression of *AOX*. The increased level of *AOX* expression might contribute to minimizing oxidative damage by regulating ROS production when ROS are extensively accumulated in the post-anoxic stages. Although there is little evidence that UCP is involved in the plant hypoxia/anoxia response, the induced expression of UCP4 and UCP5 is observed based on Genevestigator microarray analysis (Fig. 4C).

In animal cells, an involvement of UCPs in hypoxia/anoxia has been verified by several studies (Lu and Sack, 2008; Deng *et al.*, 2012; Dromparis *et al.*, 2013) and this is partly achieved by controlling ROS production (Lu and Sack, 2008; Deng *et al.*, 2012), calcium transfer from the endoplasmic reticulum into the mitochondria (Dromparis *et al.*, 2013) and possibly a metabolic switch toward glycolysis (Greer *et al.*, 2012). In rice, hypoxia-induced elevation of cytosolic calcium is involved in anaerobic repression of *AOX1A* expression (Tsuji *et al.*, 2000). Comparative analysis based on 21 organisms from four kingdoms has further revealed that low oxygen stress is involved in metabolic reconfigurations which enhance ATP production and NAD^+ regeneration, and that some genes encoding the ROS network, such as UCP, appear to be conserved in both plants and animals in response to low oxygen conditions (Mustroph *et al.*, 2010).

A co-expression analysis suggested that some WRKY transcription factors such as WRKY33 and WRKY40 are co-expressed with some AOX and UCP members (Fig. 2B), suggesting that AOX and UCP might share similar regulation mechanisms.

THE MEDP AS AN INTEGRATOR OF INTRAORGANELLE CROSS-TALK

The AOXs and UCPs provide plants with considerable metabolic flexibility by regulating cellular energy expenditure, and thus exert control over the cellular energy balance. In good agreement with this, under micronutrient- (phosphorus or nitrogen) limited conditions, the amounts of the AOX proteins were greatly increased in tobacco cells, whereas the lack of AOXs resulted in enhanced carbohydrate accumulation, suggesting that the upregulation of AOXs may help the cell to consume excess carbohydrates, thus maintaining the cellular carbon balance (Sieger *et al.*, 2005). Similarly, the carbon to nitrogen ratio in an arabidopsis *AOX1A* mutant increased under low temperatures compared with the ratio in the wild-type plants (Watanabe *et al.*, 2008), which indicated the role of AOX in maintaining cellular metabolic balance. Additional studies have suggested that the accumulation of NO in wild-type plants under hypoxia resulted in the inhibition of aconitase and accumulation of citrate, which in turn activated AOX and caused a shift of metabolism toward amino acid synthesis (Gupta *et al.*, 2012). AOX is known to be activated by pyruvate or succinate, depending on whether a cysteine or serine is present in the conserved position (Millar *et al.*, 1993; Holtzapffel *et al.*, 2003; Umbach *et al.*,

2006; Grant *et al.*, 2009). This flexible biochemical control of AOX activity by specific α-keto acids might confer an additional layer of regulation for reprogramming cellular metabolism through integrated cellular compartment or organelle signals. Indeed, it was found that a decrease in cytosolic pyruvate in transgenic potato plants that exhibited silencing of pyruvate kinase genes resulted in the repression of the AOXs and changes in carbon partitioning, with glycolysis being diverted towards starch synthesis (Oliver *et al.*, 2008). This result suggested that signals originating from alterations of glycolytic metabolism in the cytosol could be transmitted through AOX to the mitochondria, resulting in the readjustment of cellular metabolism. In animals, UCP2 has been identified as a key regulator of cellular fuel utilization and whole-body glucose and lipid metabolism (Diano and Horvath, 2012). It was shown recently that UCP2 may be involved in transportation of C4 metabolites out of the mitochondria (Vozza *et al.*, 2014), making it possible for UCP2 to regulate influxes of the TCA cycle. The overexpression of arabidopsis *UCP1* in tobacco was found to alter mitochondrial–nuclear communication, which resulted in decreased cellular ATP levels, and this in turn triggered a signal that initiated the expression of mitochondrial and stress-related genes (Barreto *et al.*, 2014).

In addition to being a mediator that regulates cellular energy expenditure and metabolic balance, the involvement of AOXs in maintaining redox balance in chloroplasts has also been established. Under high light conditions, excess reducing equivalents were accumulated in the chloroplasts, which is concomitant with the upregulation of AOX, resulting in excess reductants that were dissipated in the mitochondria through an AOX and malate/oxalacetate shuttle (Yoshida *et al.*, 2007; Noguchi and Yoshida, 2008; Taniguchi and Miyake, 2012). Interestingly, after etiolated seedlings were exposed to high light, delayed accumulation of chlorophyll and the increased accumulation of reducing reductant were observed in *AOX1A* mutant plants compared with the wild-type plants (Zhang *et al.*, 2010), suggesting that AOXs play a role in optimizing photosynthesis. When cyanide-resistant respiration is inhibited, excess NADH accumulates in mitochondria. In this situation, excess NADH can be exported from mitochondria to chloroplasts, probably via the malate/oxaloacetate shuttle, resulting in an increase in the plastid NADPH/NADP ratio, which subsequently blocks the importation of plastidial protein (Zhang *et al.*, 2014). This result suggests that the cross-talk between mitochondria and chloroplasts at the metabolic level is partly mediated by MEDP members. Furthermore, it was found that increased AOX activity correlated well with the elevation of intracellular pyruvate and that increased cellular pyruvate and malate levels paralleled increases in AOX under high light (Dinakar *et al.*, 2010). These results suggested that the post-translational regulation of AOX by metabolites under high light may be critical to optimal photosynthesis.

As sensors for environmental perturbations and metabolic status, mitochondria not only can dissipate reducing equivalents, but also can generate reductants; in particular, in the photorespiratory process, a large number of NADHs were produced through the oxidation of glycine. The increased availability of NADH and electron flow into the mitochondrial electron transport chain through the photorespiratory pathway might result in the accumulation of NADH when the malate/

oxalacetate shuttle cannot consume all of the NADH produced. This could result in the build-up a high proton gradient across the inner mitochondrial membrane when cellular ATP requirements are low, which will ultimately impose great constraints on electron flux and photorespiratory flux (Sweetlove *et al.*, 2006). Under such conditions, up-regulation of MEDP genes could help to consume excess NADH, thus maintaining the cellular redox balance. The loss of *UCP1* in arabidopsis restricted the photorespiratory flux of glycine to serine, suggesting that UCP allows NADH to be oxidized in the mitochondrial matrix by regulating the dissipation of the proton gradient across the inner mitochondrial membrane, thus ensuring continued photorespiration (Sweetlove *et al.*, 2006). Interestingly, calcium-mediated signalling cross-talk in different organelles, including the endoplasmic reticulum (ER), mitochondria and nucleus, was also found to be involved in the induction of *AOX1A* in arabidopsis during salt stress conditions (Vanderauwera *et al.*, 2012; Ng *et al.*, 2014), suggesting that the ER might also be involved in plant mitochondrial retrograde regulation. Indeed, an analysis of transcription profiling using the Diff-Expression tools in Genevestigator revealed the differential gene expression of *AOX1A* in wild-type arabidopsis after treatment with tunicamycin compared with mock-treated (control) plants (Mishiba *et al.*, 2013; Supplementary Data Table S5D), suggesting that an unfolded protein response or the ER stress response could induce the expression of *AOX* genes. Remarkably, the transcription factor bZIP60 involved in the unfolded protein response was found to be co-expressed with *AOX1A* and *AOX1D* under perturbations (Fig. 2B). Thus it is possible that bZIP60 could regulate the expression of *AOX1A* and *AOX1D* during ER stress. Together, these findings support the idea that interactions among different organelles are mediated partly through the MEDP by regulating NADH, NADPH or metabolite signals.

CROSS-TALK OF MEDP, PHYTOHORMONE AND CALCIUM SIGNALLING

Plant hormones regulate many physiological and developmental processes. They are also involved in integrating diverse environmental cues with a plant's genetic programme (Santner and Estelle, 2009), and in cross-talk between stress signalling pathways (Fujita *et al.*, 2006). ROS signalling has been shown to interact with plant hormone signalling (Fujita *et al.*, 2006; Kwak *et al.*, 2006). Hormone stability may be affected by unbalanced ROS accumulation (Kokkinakis and Brooks, 1979; Sewelam *et al.*, 2014). The involvement of members of the MEDP in limiting the generation of ROS and in the adaptation of plants to stresses might be the link that integrates mitochondrial energy systems to hormone signalling. Indeed, it has been shown that some plant hormones, including salicylic acid (Lennon *et al.*, 1997), ABA (Giraud *et al.*, 2009; Liu *et al.*, 2010), methyl salicylate and methyl jasmonate (Fung *et al.*, 2004, 2006) and ethylene (Ederli *et al.*, 2006; Wang *et al.*, 2010), may be involved in the induction of AOXs, whereas auxin was shown to repress the induction of *AOX1A* by antimycin A (Ivanova *et al.*, 2014). An examination of the Genevestigator database suggested that of the five *UCP* genes, *UCP4* and *UCP5* were stress-responsive genes and that the

expression of *UCP4* and *UCP5* could be induced by ABA and auxin in arabidopsis seedlings (Fig. 4A). As an integrator of light and hormone, the HY5 transcription factor and its close homologue HYH have been suggested to play roles in suppressing auxin signalling (Sibout *et al.*, 2006). Interestingly, the differential expression of *UCP5* in HY5 and HYH mutants was observed, and its expression was further induced in a HY5/HYH double mutant (Supplementary Data Table S5A, B), suggesting that the derepression of HY5 and/or HYH on auxin signalling contributed to the expression of *UCP* genes. Therefore, auxin signalling and MEDP signalling were reciprocally regulated, depending on the growth and stress conditions.

Reactive oxygen species are signalling molecules that mediate a diverse range of cellular responses in plant cells, including development (Swanson and Gilroy, 2010), hormone signalling (Gapper and Dolan, 2006; Kwak *et al.*, 2006), programmed cell death (Lam *et al.*, 2001) and plant immunity (Kadota *et al.*, 2014). Furthermore, ROS have been shown to interact directly or indirectly with other signalling pathways, such as NO and calcium signalling (Overmyer *et al.*, 2003; Mittler *et al.*, 2004; Mazars *et al.*, 2010). Therefore, cross-talk between members of the MEDP and plant hormones may be mediated partly by ROS and/or calcium signals. Consistent with this finding, it has been observed that chilling-induced expression of *AOX* genes in arabidopsis callus was blocked completely by a calcium chelator and NADPH oxidase inhibitor (Wang *et al.*, 2010, 2012).

Mitochondria, chloroplasts and peroxisomes are considered as sources of ROS in plants (Overmyer *et al.*, 2003). The production of ROS was enhanced when plants were exposed to stress conditions (Mittler *et al.*, 2004). Two sources of ROS production have been well characterized: metabolism-triggered ROS and enzyme-generating ROS. Metabolism-triggered ROS arise from metabolic imbalances after the changes in environmental conditions and/or electron leakage from the electron transportation chain of mitochondria and chloroplasts. Enzyme-generating ROS are produced by enzymes, most notably plant NADPH oxidases, also known as respiratory burst oxidase homologues (RBOHs) (Mittler *et al.*, 2004; Suzuki *et al.*, 2011; Vaahtera *et al.*, 2014). The production of ROS by RBOHs was found to be associated closely with a variety of growth and development processes and stress responses (Suzuki *et al.*, 2011), and RBOHD and RBOHF have been shown to be involved in the response of plants to biotic stresses (Drerup *et al.*, 2013). Although the role of MEDP genes in dampening ROS production in mitochondria has been well characterized (Maxwell *et al.*, 1999; Smith *et al.*, 2004; Cvetkovska and Vanlerberghe, 2012), members of the MEDP were also found to be involved in protecting plants from pathogen infection and in decreasing the generation of ROS triggered by pathogen infection (Fu *et al.*, 2010). These results suggested that the MEDP might play an additional role in limiting other non-mitochondrial sources of ROS. The exogenous application of hydrogen peroxide has been shown to increase the amount of AOX in many species and tissues (Vanlerberghe and McIntosh, 1996; Ho *et al.*, 2008), suggesting that the induction of AOX by hydrogen peroxide is not source specific. It has been proposed that cells activate their own ROS-producing mechanism, resulting in the generation of ROS, which in turn triggers an autopropagating wave in the adjacent cell (Mittler *et al.*, 2011). ROS waves of this type have been suggested to depend largely on RBOHD
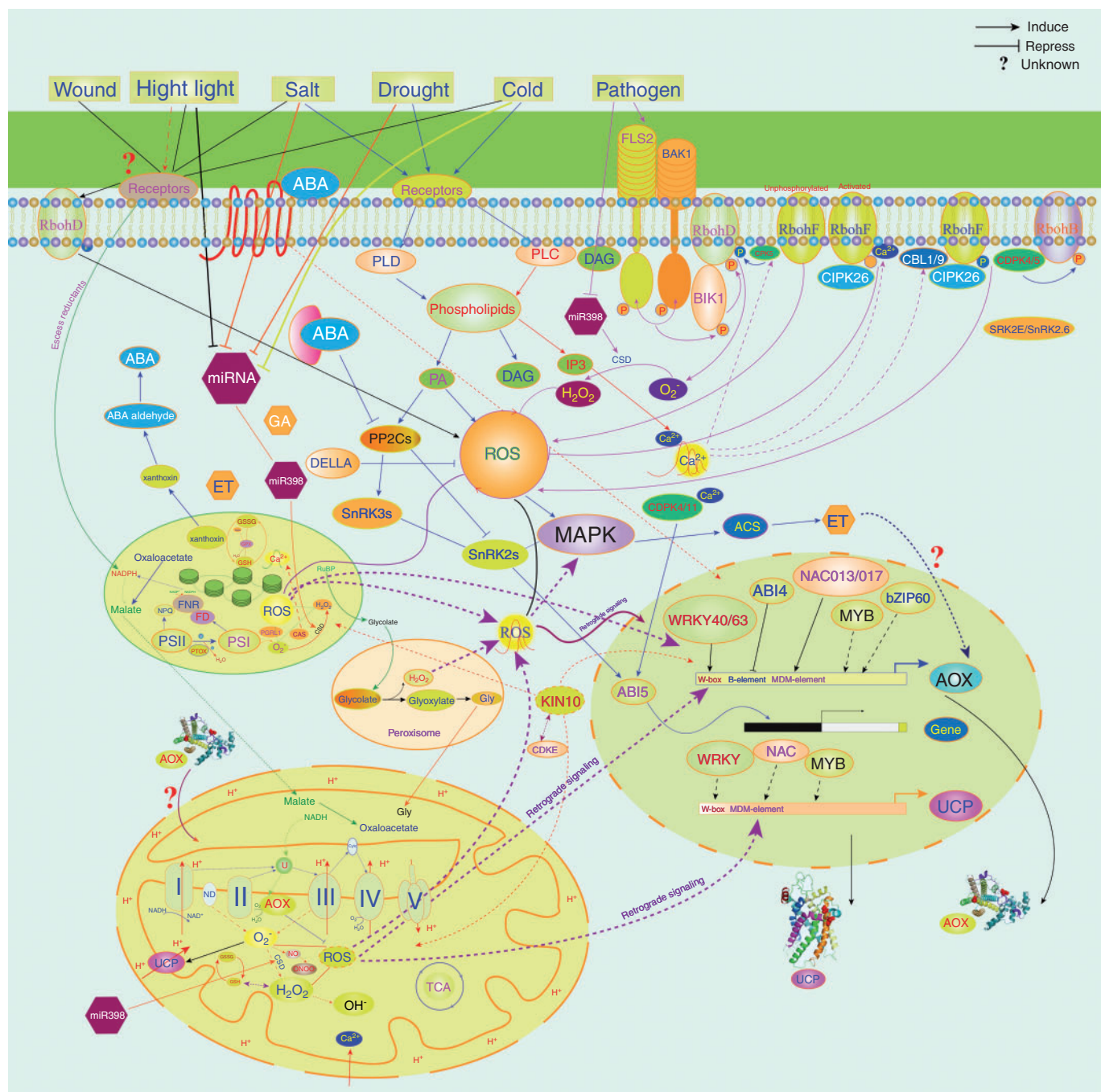
FIG. 5. Signal cross-talk between the MEDP, ROS, calcium signalling and hormone signalling. Different stress signals coming from outside the cell are transmitted by a variety of messenger molecules, leading to alteration of the calcium signature and/or the production of ROS, which could trigger cell to cell communication events and ROS wave propagation between organelles (Mittler *et al.*, 2011). This is concomitant with (de)activation of transcription factors, which, in turn, regulate the cellular antioxidant response. As mediators of ROS and energy, MEDP genes may play a role in controlling organellar ROS signalling, cellular redox and energy homeostasis.

and are considered to be an engine of systematic signalling (Gilroy *et al.*, 2014). It has been suggested that ROS could be used as rapid, long-distance autopropagating signals that spread from the initial site to the entire plant via a cell to cell communication mechanism and that the ROS waves integrate with other signalling pathways, such as calcium, electrical and hormone signals (Miller *et al.*, 2009; Mittler *et al.*, 2011; Dubiella

et al., 2013; Suzuki *et al.*, 2013). In accordance with this idea, it has been shown that RBOHB, RBOHD and RBOHF can be phosphorylated by calcium-dependent protein kinases (CDPK4/5, CPK5) (Kobayashi *et al.*, 2007; Dubiella *et al.*, 2013) and calcineurin B-like protein-interacting protein kinase (CIPK26), respectively (Drerup *et al.*, 2013; Kimura *et al.*, 2013). Therefore, the regulation of ROS production by calcium

signalling partly accounts for the reported inhibition of the expression of MEDP genes by the calcium chelator. Interestingly, we found that components involved in calcium signalling, such as the calcium-dependent protein kinases, calmodulin-like protein and calmodulin-binding protein, were co-expressed with MEDP genes under perturbations (Fig. 2C). This observation further suggested that the induced expression of members of the MEDP under stress conditions might be involved in both ROS production and calcium signals.

In addition to RBOH-derived ROS that induce the expression of MEDP genes, metabolic-derived ROS can also affect the transcription of MEDP genes. A recent study showed that different organelle-derived ROS differentially affected the expression of MEDP genes (Sewelam *et al.*, 2014). It was found that the expression of *AOX1D* (At1g32350) was specifically upregulated after 8 h of induction of hydrogen peroxide in chloroplasts, whereas *AOX1A* was induced specifically by chloroplast-produced and peroxisomal-produced hydrogen peroxide (Sewelam *et al.*, 2014), indicating that ROS might not induce the expression of MEDP genes directly. Several regulators involved in the regulation of *AOX* expression have been identified, including cyclin-dependent kinase E1 (CDKE1) (Ng *et al.*, 2013*a*), WRKY40 and WRKY60 (Van Aken *et al.*, 2013), and ANAC017 (Ng *et al.*, 2013*b*). CDKE1 was shown to play a role in mitochondrial retrograde signalling and was proposed to integrate environmental signals with plant development (Ng *et al.*, 2013*a*). CDKE1 was also predicted to interact with arabidopsis kinase 10 (KIN10), a central regulator involved in integrating stress, darkness and energy signalling with growth (Baena-González and Sheen, 2007, 2008). Therefore, it is possible that ROS produced in specific cellular compartments or organelles use a general signal to initiate and integrate different cellular signalling pathways of the cells and that ROS from different sources converge with mitochondrial retrograde regulation (MRR), thus deciding plant growth, development and the stress response. Moreover, as a player in MRR, members of the MEDP may, to some extent, act as sensors that transmit the signals from different organelles (e.g. chloroplass, mitochondria and peroxisomes) to the nucleus by sensing the balance between energy and NADPH or/and NADH levels. Furthermore, the small RNAs, including microRNAs and small interfering RNAs, have been shown to be involved in stress responses of plants, controlling ROS production and SnRK1-dependent energy signalling in arabidopsis (Khraiwesh *et al.*, 2012; Confraria *et al.*, 2013). Therefore, the regulation of the MEDP genes involves complex cross-talk between ROS signalling, calcium signalling, hormone signalling and possibly small RNAs (Fig. 5).

CONCLUSIONS AND PERSPECTIVES

There is a strong evidence to support the role of ROS in influencing the status of cellular energy in plant development. Thus, the co-ordination of ROS production and their activities as well as cellular energy and/or redox balance are emerging as important themes in understanding how plant growth and development and stress responses are integrated. The involvement of members of the MEDP in lowering ROS production as well as in the regulation of cellular energy may, to some extent,

provide a mechanism by which the developmental programme of plants is integrated with their cellular energy needs. However, the regulation of MEDP members is complex and occurs at the transcriptional level, translational level, post-translational level and metabolic level. How this regulation is linked to actual fluxes through the AOXs/UCPs *in vivo* remains elusive; for instance, the correlation between allosteric activation of AOX by organic acids and the *in vivo* electron flux to AOX has not been fully understood. A detailed analysis on the relationship between AOX activation state and electron flow to AOX is required.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxford-journals.org and consist of Tables S1–S4: gene sets co-expressed with *AOX* and *UCP* under perturbation (with positive and negative correlations). Table S5: differentially expressed gene sets between Col and hy5, Col and hyh, Col and the hy5-hyh double mutant, and control and tunicamycin-treated wild-type plants.

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