

The Roles of Mitochondrial Reactive Oxygen Species in Cellular Signaling and Stress Response in Plants¹[OPEN]

Shaobai Huang, Olivier Van Aken, Markus Schwarzländer, Katharina Belt, and A. Harvey Millar*

ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia (S.H., O.V.A., K.B., A.H.M.); and Plant Energy Biology Lab, Institute of Crop Science and Resource Conservation (INRES), University of Bonn, Friedrich-Ebert-Allee 144, 53113 Bonn, Germany (M.S.)

ORCID IDs: 0000-0003-3667-611X (S.H.); 0000-0003-4024-968X (O.V.A.); 0000-0003-0796-8308 (M.S.); 0000-0002-3142-5656 (K.B.).

Mitochondria produce ATP via respiratory oxidation of organic acids and transfer of electrons to O₂ via the mitochondrial electron transport chain. This process produces reactive oxygen species (ROS) at various rates that can impact respiratory and cellular function, affecting a variety of signaling processes in the cell. Roles in redox signaling, retrograde signaling, plant hormone action, programmed cell death, and defense against pathogens have been attributed to ROS generated in plant mitochondria (mtROS). The shortcomings of the black box-idea of mtROS are discussed in the context of mechanistic considerations and the measurement of mtROS. The overall aim of this update is to better define our current understanding of mtROS and appraise their potential influence on cellular function in plants. Furthermore, directions for future research are provided, along with suggestions to increase reliability of mtROS measurements.

GENERATION OF MITOCHONDRIAL ROS

Molecules typically referred to as reactive oxygen species (ROS) in plant cells include ozone, singlet oxygen, superoxide, H₂O₂, and the hydroxyl radical. There is no reliable information on any significant generation of ozone and singlet oxygen by plant mitochondria, and the short half-life of the hydroxyl radical makes it incompatible with specific roles in signal transduction through selective modification of target molecules. This leaves superoxide and H₂O₂ as good candidates for ROS generated in plant mitochondria (mtROS) of regulatory significance. Superoxide is formed through

single electron reduction of O₂, and the respiratory Complexes I, II, and III have all been identified as major production sites but display fundamentally different rates and topologies of superoxide release (Murphy, 2009). The relative physiological contributions of each also remain notoriously hard to dissect. Plant-focused studies have provided some mechanistic insight (Rich and Bonner Jr, 1978; Møller, 2001; Gleason et al., 2011; Jardim-Messeder et al., 2015), but the bulk of the evidence is still derived from more detailed mammalian studies (Murphy, 2009). While superoxide production occurs during normal operation of the respiratory chain, its rate is strongly increased when respiratory rate is slowed down, e.g. by restricted ADP availability or respiratory chain inhibition, leading to a highly reduced state of mitochondrial electron transport chain (mtETC) components (Møller, 2001).

Superoxide production by the mtETC can be minimized by a variety of pathways in plant mitochondria that bypass the classical mtETC and oxidative phosphorylation. Uncoupling proteins promote proton leak across the membrane (Sweetlove et al., 2006), while NDs and AOXs bypass proton pumping by electron flow via Complex I, and Complex III and IV, respectively. The role of AOX in minimizing ROS production has been extensively reviewed (Rhoads et al., 2006; Vanlerberghe, 2013). Recently, overexpression of the Arabidopsis (*Arabidopsis thaliana*) uncoupler protein Uncoupling protein1 in tobacco was found to reduce the rate of ROS generation, induce the antioxidant defense system, and enhance resistance to multiple abiotic stresses (Barreto et al., 2014). Superoxide spontaneously disproportionates to H₂O₂ and O₂, but this can be strongly accelerated by superoxide dismutase (SOD; Møller, 2001; Morgan et al., 2008). Alternatively, superoxide readily

¹ This work was supported by the facilities of the Australian Research Council Centre of Excellence Program (CE140100008). M.S. thanks the Deutsche Forschungsgemeinschaft for support through the Emmy-Noether programme (SCHW1719/ 1-1), the Research Training Group GRK2064, and a grant (SCHW1719/5-1) as part of the package PAK918. S.H. and A.H.M. were funded as ARC Australian Future Fellows (FT130101338 and FT110100242, respectively). O.V.A. is supported in part by ARC Discovery Projects (DP130102918 and DP160103573). K.B. holds a Scholarship for International Research Fees and University International Stipend from the University of Western Australia.

* Address correspondence to harvey.millar@uwa.edu.au.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: A. Harvey Millar (harvey.millar@uwa.edu.au).

A.H.M. and S.H. conceived the review; S.H., O.V.A., M.S., and K.B. contributed draft sections in their areas of expertise; all authors were involved in assembling the review and discussed and revised the text based on their expertise.

[OPEN] Articles can be viewed without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.16.00166

reacts with other radicals such as NO or redox-active metal centers of other proteins (Tan et al., 2010). These reactions outcompete interactions with abundant biomolecules such as the amino acid residues of proteins, nucleic acids, lipids, and sugars. Suppression of the mitochondrial matrix manganese SOD in *Arabidopsis* disturbs thiol redox homeostasis of the matrix, retards root growth (Morgan et al., 2008), and leads to disruption of female gametogenesis (Martin et al., 2013). The H₂O₂ released by the matrix SOD can either be removed on site or exit the matrix. In both cases, efficient detoxification systems are in place and selective oxidation of regulatory targets may relay information on mitochondrial processes.

ROS SIGNALING WITHIN MITOCHONDRIA BY THIOL SWITCHING?

Specific protein thiols may be oxidized by H₂O₂. Several studies have found redox-active thiols on matrix proteins, some of which have been shown to adjust protein function *in vitro*, raising the possibility of intramitochondrial ROS-dependent thiol redox signaling to adjust functions at the protein level. Examples that have been specifically characterized include AOX (Yoshida et al., 2013) and several tricarboxylic acid (TCA)-cycle enzymes (Schmidtman et al., 2014; Yoshida and Hisabori, 2014; Daloso et al., 2015), with the TRX system as the likely endogenous reductant. Gel-based redox proteomics and TRX-trapping approaches have identified even more mitochondrial proteins that can, in principle, undergo thiol switching (Winger et al., 2007; Yoshida et al., 2013). For all of these proteins, it remains unresolved if the respective thiol switch can be oxidized by mtROS (directly or indirectly) and maintained in its oxidized state, considering the presence of a highly efficient rereduction system under physiological conditions. A switch would be a prerequisite for bona fide redox-regulation, as opposed to TRX-mediated ‘maintenance’ of a thiol in its reduced state. A particularly intriguing example of intramitochondrial thiol redox regulation has been studied in mammals where Complex I can be deactivated by reversible oxidation of a Cys-39 of ND3 (Chouchani et al., 2013) to avoid excessive superoxide release from reverse electron flow at reoxygenation after anoxia. The Cys motif at ND3 is well conserved in plants (Cys-45 in *Arabidopsis*; Braun et al., 2014), but evidence for reverse electron flow is currently lacking in plants. However, given the conservation it appears worthwhile to investigate its potential plant function, such as during the recovery from water-logging.

MEASURING MROS IN VITRO AND IN VIVO

The generation of mtROS is routinely studied using colorimetric and fluorescent detection systems. Probes such as 3,3'-diaminobenzidine, nitroblue tetrazolium (NBT), AmplexRed (for isolated mitochondria), 2',7'-dichlorofluorescein derivatives, and hydroethidine

have been reported to detect or even quantify H₂O₂, superoxide, or mtROS in plant studies (Gleason et al., 2011; Li and Xing, 2011; Martin et al., 2013). However, there are legitimate concerns about their specificity and subcellular accumulation and their interference with endogenous redox biology (Bonini et al., 2006; Zielonka and Kalyanaraman, 2010; Miwa et al., 2016), prompting the search for straightforward and applicable alternatives. Probes that can be specifically placed in mitochondria, either through electrical potential-driven accumulation (e.g. MitoSOX Red; Miller et al., 2009; Li and Xing, 2011; Martin et al., 2013; Liu et al., 2014) or through genetic targeting for fluorescent protein sensors (Schwarzländer et al., 2016) have addressed the localization issue. However, their chemical specificity in mitochondria also remains a concern. For example, cpYFP that was reported as responsive to superoxide and has been used as such also in plant mitochondria (He et al., 2012) has been conclusively shown not to react to superoxide, but to pH changes (Schwarzländer et al., 2014), while the H₂O₂-responsive HyPer family of fluorescent protein probes (Costa et al., 2010) also suffer from pH artifacts, making measurements problematic in the matrix where pH can change rapidly (Schwarzländer et al., 2012b). Dynamic measurements using such probes also rely on efficient probe regeneration after oxidation via the endogenous glutathione/glutaredoxin system. Yet, the only known matrix GRX (GRXS15) shows very low thiol exchange activity (Moseler et al., 2015; Stroehrer et al., 2016). Rigorous application and further optimization of HyPer probes as well as peroxidase-coupled, pH-insensitive roGFP sensors, like roGFP2-Orp1 (Gutscher et al., 2009), appear to offer the best potential to allow specific measurements of a defined mtROS (H₂O₂) and allow physiological interpretation. This may be complemented by mass spectrometry-based approaches that have been developed in mammalian mitochondria (Cochemé et al., 2011), but have not yet been used in plants.

Given the persisting methodological constraints, it is important to note that most historical reports of mtROS production in plants are only one interpretation of the chosen monitoring technique (i.e. chemical reaction product, optical probe, marker metabolite, or marker transcript) and are often based on assumptions that may or may not be justified in a particular case as discussed above. Future experiments should involve a transparent appraisal of the chosen technique or cellular mtROS indicator, which will allow accumulating evidence from orthogonal approaches across studies to paint a clearer picture.

CONSIDERATIONS FOR TRANSDUCTION OF MROS SIGNALS OUT TO THE CELL

Movement of mtROS signals out of mitochondria requires a direct and sensitive target and a means of transducing the signals from the various intramitochondrial locations to the cytosol. If there is a direct downstream target of a mitochondrial superoxide

signal, proteins with transition metal cofactors are likely to be the first candidates. An intriguing mechanism has been proposed in yeast and mammals by which the activity of cytosolic SOD1 controls mitochondrial respiration (Reddi and Culotta, 2013), but plants have not yet been investigated for comparable signaling. Efficient dismutation of superoxide inside mitochondria, however, makes H_2O_2 a more likely signal, aided by its much longer estimated lifetime and the ability for channel-mediated membrane passage through aquaporins (Bienert and Chaumont, 2014). At least one aquaporin (TIP5;1) can localize to the mitochondria in *Arabidopsis* (Soto et al., 2010). However, the reactivity of H_2O_2 is also remarkably limited by kinetic competition and most thiols are not particularly reactive for H_2O_2 within the cellular environment in the absence of catalysis. Exceptions, however, can be found in thiols of a small group of proteins that have been found enriched in mitochondria (Riemer et al., 2015). Particularly interesting are the thiol peroxidases, which have been recognized as among the most H_2O_2 -reactive thiols in cells (Perkins et al., 2015) and are key players in H_2O_2 detoxification in plants (Dietz, 2011). With PrxII F and a GPX-like protein, two thiol peroxidases have so far been found in plant mitochondria (Finkemeier et al., 2005; Navrot et al., 2006). Low K_d and high reactivity of the catalytic peroxiredoxin thiols can generate specific H_2O_2 drains that outcompete other thiols and ensure that a large part of H_2O_2 turnover flux will be focused to one specific thiol (Veal et al., 2007). This would provide kinetic control of H_2O_2 dynamics *in vivo* keeping ambient H_2O_2 levels low and protecting other biomolecules from oxidation. To serve as a signal, this information must be conserved, perhaps by dividing regeneration into a rapid-turnover, high-flux antioxidant pathway and a slow-turnover, low-flux signaling pathway, with their relative contributions set by distinct biochemical properties. Once translated, the H_2O_2 signal could then regulate downstream protein targets by a thiol switch or be further transduced by thiol-redox signaling (Riemer et al., 2015). This provides a means to quantify H_2O_2 flux by 'counting' molecules detoxified at a specific location. Maintaining information on the location of H_2O_2 sensing appears critical to discriminate between stimuli from distinct sources and to mount specific responses. Oxidation of mitochondrial proteins followed by proteolysis and export of the peptides has been proposed as a scenario of this general concept (Møller and Sweetlove, 2010). Indeed, oxidative modification has been observed for plant mitochondrial proteins (Kristensen et al., 2004; Tan et al., 2010), but further work will be required to clarify to what extent this occurs under physiological conditions *in vivo*.

Increasing experimental evidence supports the concept of colocalization of ROS signal and ROS sensing. Recently, mtETC-derived H_2O_2 were shown to be perceived in the intermembrane space (IMS) triggering a phosphorylation cascade in animals (Patterson et al., 2015). As the interface between the mitochondrion and the rest of the cell, the IMS hosts its own redox machinery,

which may make it well suited as the integration site to prepare mtETC-derived ROS signals for transduction across the cell before they get quenched by the strong antioxidant defenses of the cytosol. Placing the ROS-sensing system close to the mitochondrial surface, where H_2O_2 is thought to pass the outer mitochondrial membrane (OMM) via porins offers similar properties. Contact sites between endoplasmic reticulum (ER) and mitochondria may act as such hotspots (Jaipargas et al., 2015; Mueller and Reski, 2015). Although the exact mechanism remains to be determined, thiol switch-based sensing of mitochondrial-derived H_2O_2 at ER contact sites makes an attractive hypothesis. The reverse, i.e. H_2O_2 produced by the ER to be sensed within the mitochondrial matrix inducing glutathionylation of a Gly decarboxylase subunit, has been proposed recently (Hoffmann et al., 2013). A recent study suggests that the twin-Cys proteins At12cys1/2 can relocate from the mitochondria to cytosol and chloroplast upon Complex I dysfunction, providing a potential mechanism to signal mitochondrial dysfunction to the rest of the cell in plants (Wang et al., 2016).

MtROS AND RETROGRADE SIGNALING

Mitochondria can signal information about their functional status to the nucleus, leading to targeted transcriptional responses, a process often described as mitochondrial retrograde regulation (MRR; Rhoads and Subbaiah, 2007; Ng et al., 2014). Many factors involved in the transcriptional regulation of MRR have been identified and belong to a range of functional classes, including transcription factors (NAC, WRKY, ABI4) and cyclin-dependent kinases (Ng et al., 2014). Also, an antagonistic relationship between auxin and retrograde signaling has been shown in a variety of mutant backgrounds (including PIN1, BIG, and AS1; Ivanova et al., 2014). Surprisingly, MRR mediators ANAC013 and ANAC017 are bound to the ER (De Clercq et al., 2013; Ng et al., 2013). Upon stress, they are thought to be released and relocate to the nucleus to exert transcriptional regulation. This identified the ER as a key signaling intermediate in MRR, and based on inhibitor studies, the proteolytic release of NAC transcription factors (TFs) may be mediated by rhomboid-class proteases (Ng et al., 2013).

The identity of the signal(s) that relays information from a dysfunctional mitochondrion via the ER to the downstream transcription factor cascades currently remains unknown. Evidence so far suggests that TCA cycle intermediates like citrate have relatively mild transcriptional effects, and TCA-intermediate signaling does not appear to occur via the NAC pathway, as the affected genes are very different (Finkemeier et al., 2013). However, several individual findings point toward a role for mtROS, and taken together they make a strong case. One of the most commonly used ways of triggering MRR in plants has been treatment with complex III inhibitor antimycin A (AA). AA treatment is well established to induce retrograde marker genes

such as *AOX1a* in Arabidopsis (Dojcinovic et al., 2005; Ng et al., 2013). AA application to plant cells induced increased ROS production as detected by 2',7'-dichlorofluorescein accumulation in the mitochondria (Maxwell et al., 1999), suggesting that the ROS produced by AA could act as signaling intermediates. Further in line with a signaling role for mtROS, AA-induced retrograde signaling was suppressed when ROS production was reduced by overexpression of AOX in tobacco (Maxwell et al., 1999).

Other studies also indirectly suggest a correlation between mitochondrial defects, (mt)ROS formation, and retrograde signaling. For example, Complex I subunit *ndufs4* mutants have a higher basal superoxide production rate as measured by NBT staining as well as constitutive retrograde marker gene expression (Meyer et al., 2009). Other studies have looked at nuclear transcription in backgrounds with mtROS-related enzymes, such as manganese SOD *msd1* knock-down plants, and AA treatment in peroxiredoxin II *F prxII F* mutants compared to wild type (Schwarzländer et al., 2012a). However, both studies showed relatively weak transcriptional changes in common MRR marker genes, so it is difficult to draw clear conclusions for the role of mtROS in retrograde signaling from these experiments.

More evidence for a role of mtROS in retrograde signaling comes from the observation that MRR responses show remarkable overlaps with transcriptional responses to different ROS triggers and abiotic stress, e.g. H₂O₂ and salt stress. This is the case both for chemically induced MRR as well as MRR caused by genetic defects in mitochondrial components (Van Aken et al., 2007; Meyer et al., 2009; Van Aken and Whelan, 2012). Furthermore, many of the core ANAC017-dependent transcripts affected by AA also showed altered responses to H₂O₂ application (Ng et al., 2013). Remarkably, 87% of transcript changes caused by H₂O₂ were affected in *anac017* mutants. This indicates that ANAC017 is a key regulator of H₂O₂ responses and that at least one avenue for ROS production may be via mitochondria. Future work will be required to understand how mtROS signals would trigger such MRR response in a mechanistic way.

MtROS AND PLANT HORMONE SIGNALING

Plant hormones play a key role in regulating growth and development and in response to stresses. Hormone-ROS interactions have recently been reviewed (Del Río, 2015) as well as links between MRR regulation and hormone signaling (Berkowitz et al., 2016). Both connections have recently been brought together by accumulating evidence indicating that mitochondria play a role in regulation of plant hormone signaling, such as abscisic acid (ABA) and auxin, and that mtROS may be key intermediates.

Evidence for an interplay between ABA and mitochondria via mtROS has been found in lines with different sensitivity to ABA (Laluk et al., 2011; He et al., 2012; Murayama et al., 2012; Sechet et al., 2015).

Collectively, these reports show that the underlying causes of increased ABA sensitivity in a range of mutants are mitochondrial defects. In some of these mutants, increases in ROS were reported, while others report decrease, using a variety of different detection methods. Given that mitochondria are crucial during early germination and seedling establishment (both are repressed by ABA; Howell et al., 2006; Wang et al., 2014), it appears conceivable that mitochondrial mutants are particularly susceptible to unfavorable germination conditions. While more evidence is required to test if mtROS have a direct role in ABA signaling, transcription of a range of genes encoding mitochondrial proteins is clearly responsive to ABA (Van Aken et al., 2009; Wang et al., 2014).

Regulation of auxin homeostasis has been linked to increased ROS levels (Tognetti et al., 2012) and mitochondria have been suggested to be involved. By screening for *more axillary shoot* mutants, mitochondrial AAA-protease *AtFtSH4* was identified. Loss of *FtSH4* function resulted in a reduction in protein stability in the mitochondria and increased H₂O₂ levels, which may be used for peroxidase-mediated oxidation of indole-3-acetic acid and cause excessive axillary branches and a dwarf phenotype (Zhang et al., 2014b). Also overexpression of mitochondrial *AtPHB3* or MRR/ROS-signaling target gene *UGT74E2* (an auxin glycosyltransferase) results in profuse shoot branching (Van Aken et al., 2007; Tognetti et al., 2010). It was previously shown that *ftsh4* mutants show induction of a number of MRR/ROS marker genes, providing further links between mtROS signaling and auxin homeostasis (Gibala et al., 2009).

Treatment with AA resulted in decreased indole-3-acetic acid levels and changes in other genes related to auxin conjugation, transportation, and receptors (Ivanova et al., 2014). In reverse, treatments or mutations that block polar auxin transport (e.g. *pin1*) result in a hyperactivated MRR response to AA (Ivanova et al., 2014; Kerchev et al., 2014). This antagonistic relationship between auxin and MRR could provide the plant with a switching mechanism between growth under optimal conditions (no MRR signals, auxin-stimulated growth) and growth-arrest during stress conditions (MRR signaling negatively affects auxin-induced growth). Furthermore, it may allow the plant to reverse MRR-induced growth arrests when the mitochondrial dysfunction has been resolved.

MtROS IN PROGRAMMED CELL DEATH AND PATHOGEN RESPONSE

mtROS production in plants has been implicated in the execution of programmed cell death (PCD; Van Aken and Van Breusegem, 2015). Great efforts have been made to validate relatively well-described yeast or animal PCD pathways in plants; yet with limited success. For instance, cytochrome *c* release, which is also observed in plants during hypersensitive response (HR)-induced PCD, may merely reflect a final phase of

mitochondrial dysfunction, rather than an initial step of the process (Yao et al., 2004). mtROS production is often increased during stress and PCD, likely due to mtETC inhibition. Several studies support this by showing that mtETC enzymes, such as AOX, protect against PCD (Li and Xing, 2011; Liu et al., 2014; Wu et al., 2015). A recent study showed that mitochondrial Complex I is responsible for the ROS production that leads to PCD in the *Arabidopsis mosaic death 1 (mod1)* mutant, even though the MOD1 protein is active in chloroplasts (Wu et al., 2015). A critical role for mtROS, but not apoplasmic or chloroplast ROS, has also been suggested in harpin-induced PCD (Garmier et al., 2007).

Mitochondria have also been proposed to be linked with PCD via increased ROS production during pathogen defense, such as during the HR (Lam et al., 2001; Mur et al., 2008; Van Aken and Van Breusegem, 2015). In agreement, a wide variety of mutants in mitochondrial functions have been reported to have altered PCD or pathogen defense phenotypes. Upon infection with the bacterial pathogen *Pseudomonas syringae* and PPIX treatment, *Arabidopsis* protein ACD2 (Accelerated Cell Death 2) changed its location from chloroplastic to both chloroplastic and mitochondrial (Yao and Greenberg, 2006). More recent work showed that the ACD2-substrate red chlorophyll catabolite, a toxic chlorophyll-breakdown product that can trigger ROS production, can shift to mitochondria to cause ROS-induced PCD (Pattanayak et al., 2012). This suggests that mitochondrial ROS formation may be the key cause of PCD induction in *acd2* mutants, further supported by the observation that ectopic targeting of antioxidant enzymes like ascorbate peroxidase and catalase to the mitochondria can suppress PCD (Yao and Greenberg, 2006).

Perturbations in the mtETC that are linked to ROS metabolism can result in altered pathogen susceptibility. A well-known example came from analysis of *Nicotiana sylvestris* CMSII line with complex I deficiency (Garmier et al., 2002; Dutilleul et al., 2003b). There are dramatic differences in antioxidant and defense gene expression between wild-type and CMSII leaves inoculated with a bacterial elicitor (Garmier et al., 2002). CMSII(N) plants have higher resistance to tobacco mosaic virus than wild-type plants, mainly due to the inhibition of the virus by the high level of leaf antioxidant enzymes (Dutilleul et al., 2003b). During *Erwinia amylovora* hairpin-induced HR, this CMSII line showed impaired AOX engagement, but an increase in mitochondrial SOD activity and antioxidant capacity (Vidal et al., 2007).

Further evidence for a role of mtROS in PCD comes from a recent finding that spontaneous PCD lesion formation in the *mod1* mutant with deficiency in fatty acid biosynthesis can be suppressed by genetic impairment of Complex I (Wu et al., 2015). Also, treatment with Complex I inhibitor rotenone could partially suppress PCD. This work indicates that deficiencies in Complex I can result in decreased ROS production and compromise HR and resistance against bacteria (Wu et al., 2015). To address whether mtROS or ATP status

regulate PCD, the authors showed that overexpression of cytosolic Cu/Zn SOD could suppress PCD, suggesting that ROS is indeed causative (at least potentially mitochondrial ROS that reaches the cytosol). This study also indicated that the ROS generated by the plasma membrane NADPH oxidase is not directly involved in *mod1* PCD, emphasizing that formation of ROS through the mtETC likely initiates the PCD process (Wu et al., 2015).

Complex II subunit SDH1-1 was found to act as intermediate in a signal transduction pathway that induces promoter activity of salicylic acid (SA)-responsive plant defense genes (Gleason et al., 2011). Moreover, the effect of the mutation could be overcome by triggering promoter activity with exogenous H₂O₂, suggesting that the effect of SDH1-1 lies upstream of ROS presence. Indeed, this mutant showed less mtROS production and was more susceptible to several pathogens (Gleason et al., 2011), indicating the importance of mitochondrial function in pathogen defenses via ROS production. The *slo2* mutant, for which reductions in Complexes I, III, and IV have been reported along with increased ROS levels, is also more susceptible to the necrotrophic pathogen *Botrytis cinerea* (Zhu et al., 2014).

AOX has also been reported to be involved in plant defense against bacterial, viral, and fungal pathogens via regulation of ROS metabolism (Cheng et al., 2011; Lee et al., 2011; Cvetkovska and Vanlerberghe, 2012, 2013; Zhang et al., 2012). Differential expression of AOX and *MnSOD* transcripts at pathogen exposure and HR indicate a complex response pattern (Cvetkovska and Vanlerberghe, 2012, 2013). Silencing of AOX resulted in an increase of detectable matrix superoxide (Cvetkovska and Vanlerberghe, 2012). Furthermore, in AOX RNAi lines the threshold for cell death at inhibition of the cytochrome pathway by AA was lowered (Cvetkovska and Vanlerberghe, 2012, 2013), while AOX overexpression prevented HR and plant cell death (Vanlerberghe et al., 2002).

Non-mtETC proteins have reported roles in plant pathogen defense. A gene encoding mitochondrial inner membrane protein, *PAM16*, connected to the TIM23 translocase complex was identified as negative regulator of R-protein (Huang et al., 2013). The *atpam16* mutant has increased levels of *PR1* and *PR2* transcript, increased ROS production as measured by a luminol-based assay and 3,3'-diaminobenzidine staining, smaller rosette size, and enhanced resistance to virulent pathogen attack (Huang et al., 2013). AtPAM16 may be involved in the import of a negative regulator of plant immunity to the mitochondrial matrix, which could function as a modulator of ROS generation (Huang et al., 2013). Plants overexpressing the mitochondrial outer membrane AAA ATPase *AtOM66* also have increased basal ROS production (NBT staining) and expression of SA marker gene *PR1* (Zhang et al., 2014a). AtOM66 overexpression plants show increased SA content and accelerated cell death rates and have altered pathogen resistance (Zhang et al., 2014a). The *Arabidopsis* defense protein PEN2 has recently been

found to hyper-accumulate on the outer membrane of mitochondria that cluster around infection sites of leaf epidermal cells (Fuchs et al., 2015). Redox sensing revealed an oxidative shift in the matrix glutathione pool of mitochondria at the infection site in relation to other parts of the same cell or neighboring cells. This observation is suggestive of specialized functions of mitochondrial subpopulations within a cell, which may include strictly localized ROS-linked immune responses.

In tomato, mitochondrial alpha-ketoglutarate dehydrogenase (*Sla*-kGDH) E2 binds SA, and *Sla*-kGDH E2 silencing increased resistance to tobacco mosaic virus in an AOX-dependent way (Liao et al., 2015). Finally, a role of mitochondrial Pro metabolism in mtROS production and PCD has been emerging. Pro levels increase during HR, and the transcripts of *Pro dehydrogenase* (*ProDH1* and *ProDH2*) are induced by SA in Arabidopsis (Cecchini et al., 2011). Furthermore, silencing of *ProDH1* and *ProDH2* in Arabidopsis reduces ROS levels after pathogen infection but makes plants more susceptible to avirulent pathogens (Cecchini et al., 2011; Senthil-Kumar and Mysore, 2012). Similarly, silencing of mitochondrial Orn delta-aminotransferase and Pro dehydrogenases in *N. benthamiana* delayed occurrence of HR and favored nonhost pathogen growth (Senthil-Kumar and Mysore, 2012). How ROS is produced by Pro metabolism is not entirely clear, with some suggestion that pyrroline-5-carboxylate (P5C) to Pro cycling is indirectly responsible or that P5C itself can cause ROS and PCD (Qamar et al., 2015). For instance, overexpression of P5C dehydrogenase increases the resistance of plants to P5C-induced HR-like PCD and ROS production (Miller et al., 2009).

CONCLUSION AND PERSPECTIVES

Plant mtROS are primarily produced, and their rate of production is controlled, by the kinetics of the respiratory electron transport chain. However, mtROS biology still relies on an eclectic collection of indirect detection techniques, many of which have known shortcomings. A large literature based on mutant studies and transcriptional profiling argues that mtROS represent a hub in a highly interconnected network associated with abiotic stress, defense responses, hormone signaling, intracellular signal transduction, cell death, and development in plants. Significant progress has been made in identifying nonmitochondrial components involved in perpetuating putatively mtROS-dependent processes. However, the mitochondrion itself has remained a black box in terms of identifying a sensor or signaling component that can transmit mtROS signals into the cellular ROS network. Future studies will need to consolidate the mtROS literature by using the most appropriate ROS measurement method(s) to answer a specific biological question and provide additional forms of evidence to support the proposed role for mtROS in specific scenarios. These can include: measurement of concomitant alterations in indicators of

oxidation such as the redox potential of mitochondrial thiols, physiological parameters associated with mitochondrial respiration such as matrix pH, metabolic products, or oxidative modifications of cellular biomolecules; genetic evidence based on targeting appropriate antioxidants or limiting ROS generation systems; and/or analysis of transcriptional responses that overlap with other reports or meta-analyses of oxidative challenge.

Key biological questions for the future include:

- In which cell types does mtROS-mediated oxidation occur in vivo and what molecules exactly are oxidized by which reactive species?
- How do biochemical oxidation mechanisms account for the specificity required for mtROS signaling to be involved in many distinct processes?
- What is the physiological and evolutionary significance of the interactions between mtROS and plant hormone signaling?
- Do mtROS have a specific role in PCD regulation and pathogen responses in plants?

Received February 12, 2016; accepted March 21, 2016; published March 28, 2016.

LITERATURE CITED

- Barreto P, Okura VK, Neshich IAP, Maia IdeG, Arruda P (2014) Overexpression of UCP1 in tobacco induces mitochondrial biogenesis and amplifies a broad stress response. *BMC Plant Biol* **14**: 144
- Berkowitz O, De Clercq I, Van Breusegem F, Whelan J (2016) Interaction between hormonal and mitochondrial signalling during growth, development and in plant defence responses. *Plant Cell Environ* (in press)
- Bienert GP, Chaumont F (2014) Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide. *Biochim Biophys Acta* **1840**: 1596–1604
- Bonini MG, Rota C, Tomasi A, Mason RP (2006) The oxidation of 2',7'-dichlorofluorescein to reactive oxygen species: a self-fulfilling prophesy? *Free Radic Biol Med* **40**: 968–975
- Braun HP, Binder S, Brennicke A, Eubel H, Fernie AR, Finkemeier I, Klodmann J, König AC, Kuhn K, Meyer E, et al (2014) The life of plant mitochondrial complex I. *Mitochondrion* **19 Pt B**: 295–313
- Cecchini NM, Monteoliva MI, Alvarez ME (2011) Proline dehydrogenase contributes to pathogen defense in Arabidopsis. *Plant Physiol* **155**: 1947–1959
- Cheng D-D, Jia Y-J, Gao H-Y, Zhang L-T, Zhang Z-S, Xue Z-C, Meng Q-W (2011) Characterization of the programmed cell death induced by metabolic products of *Alternaria alternata* in tobacco BY-2 cells. *Physiol Plant* **141**: 117–129
- Chouchani ET, Methner C, Nadtochiy SM, Logan A, Pell VR, Ding S, James AM, Cochemé HM, Reinhold J, Lilley KS, et al (2013) Cardioprotection by S-nitrosation of a cysteine switch on mitochondrial complex I. *Nat Med* **19**: 753–759
- Cochemé HM, Quin C, McQuaker SJ, Cabreiro F, Logan A, Prime TA, Abakumova I, Patel JV, Fearnley IM, James AM, et al (2011) Measurement of H₂O₂ within living *Drosophila* during aging using a ratiometric mass spectrometry probe targeted to the mitochondrial matrix. *Cell Metab* **13**: 340–350
- Costa A, Drago I, Behera S, Zottini M, Pizzo P, Schroeder JI, Pozzan T, Lo Schiavo F (2010) H₂O₂ in plant peroxisomes: an in vivo analysis uncovers a Ca²⁺-dependent scavenging system. *Plant J* **62**: 760–772
- Cvetkovska M, Vanlerberghe GC (2012) Coordination of a mitochondrial superoxide burst during the hypersensitive response to bacterial pathogen in *Nicotiana tabacum*. *Plant Cell Environ* **35**: 1121–1136
- Cvetkovska M, Vanlerberghe GC (2013) Alternative oxidase impacts the plant response to biotic stress by influencing the mitochondrial generation of reactive oxygen species. *Plant Cell Environ* **36**: 721–732

- Daloso DM, Müller K, Obata T, Florian A, Tohge T, Bottcher A, Riondet C, Bariat L, Carrari F, Nunes-Nesi A, et al (2015) Thioredoxin, a master regulator of the tricarboxylic acid cycle in plant mitochondria. *Proc Natl Acad Sci USA* **112**: E1392–E1400
- De Clercq I, Vermeirssen V, Van Aken O, Vandepoele K, Murcha MW, Law SR, Inzé A, Ng S, Ivanova A, Rombaut D, et al (2013) The membrane-bound NAC transcription factor ANAC013 functions in mitochondrial retrograde regulation of the oxidative stress response in *Arabidopsis*. *Plant Cell* **25**: 3472–3490
- Del Río LA (2015) ROS and RNS in plant physiology: an overview. *J Exp Bot* **66**: 2827–2837
- Dietz KJ (2011) Peroxiredoxins in plants and cyanobacteria. *Antioxid Redox Signal* **15**: 1129–1159
- Dojcinovic D, Krosting J, Harris AJ, Wagner DJ, Rhoads DM (2005) Identification of a region of the *Arabidopsis* AtAOX1a promoter necessary for mitochondrial retrograde regulation of expression. *Plant Mol Biol* **58**: 159–175
- Dutilleul C, Garmier M, Noctor G, Mathieu C, Chétrit P, Foyer CH, de Paeppe R (2003b) Leaf mitochondria modulate whole cell redox homeostasis, set antioxidant capacity, and determine stress resistance through altered signaling and diurnal regulation. *Plant Cell* **15**: 1212–1226
- Finkeimer I, Goodman M, Lamkemeyer P, Kandlbinder A, Sweetlove LJ, Dietz K-J (2005) The mitochondrial type II peroxiredoxin F is essential for redox homeostasis and root growth of *Arabidopsis thaliana* under stress. *J Biol Chem* **280**: 12168–12180
- Finkeimer I, König AC, Heard W, Nunes-Nesi A, Pham PA, Leister D, Fernie AR, Sweetlove LJ (2013) Transcriptomic analysis of the role of carboxylic acids in metabolite signaling in *Arabidopsis* leaves. *Plant Physiol* **162**: 239–253
- Fuchs R, Kopschke M, Klapprodt C, Hause G, Meyer AJ, Schwarzlander M, Fricker MD, Lipka V (2015) Immobilized subpopulations of leaf epidermal mitochondria mediate PEN2-dependent pathogen entry control in *Arabidopsis*. *Plant Cell* **28**: 130–145
- Garmier M, Dutilleul C, Mathieu C, Chétrit PP, Boccara M, de-Paeppe R (2002) Changes in antioxidant expression and harpin-induced hypersensitive response in a *Nicotiana sylvestris* mitochondrial mutant. *Plant Physiol Biochem* **40**: 561–566
- Garmier M, Priault P, Vidal G, Driscoll S, Djebbar R, Boccara M, Mathieu C, Foyer CH, De Paeppe R (2007) Light and oxygen are not required for harpin-induced cell death. *J Biol Chem* **282**: 37556–37566
- Gibala M, Kicia M, Sakamoto W, Gola EM, Kubrakiewicz J, Smakowska E, Janska H (2009) The lack of mitochondrial AtFtsH4 protease alters *Arabidopsis* leaf morphology at the late stage of rosette development under short-day photoperiod. *Plant J* **59**: 685–699
- Gleason C, Huang S, Thatcher LF, Foley RC, Anderson CR, Carroll AJ, Millar AH, Singh KB (2011) Mitochondrial complex II has a key role in mitochondrial-derived reactive oxygen species influence on plant stress gene regulation and defense. *Proc Natl Acad Sci USA* **108**: 10768–10773
- Gutschner M, Sobotta MC, Wabnitz GH, Ballikaya S, Meyer AJ, Samstag Y, Dick TP (2009) Proximity-based protein thiol oxidation by H₂O₂-scavenging peroxidases. *J Biol Chem* **284**: 31532–31540
- He J, Duan Y, Hua D, Fan G, Wang L, Liu Y, Chen Z, Han L, Qu LJ, Gong Z (2012) DEXH box RNA helicase-mediated mitochondrial reactive oxygen species production in *Arabidopsis* mediates crosstalk between abscisic acid and auxin signaling. *Plant Cell* **24**: 1815–1833
- Hoffmann C, Plocharski B, Haferkamp I, Leroch M, Ewald R, Bauwe H, Riemer J, Herrmann JM, Neuhaus HE (2013) From endoplasmic reticulum to mitochondria: absence of the *Arabidopsis* ATP antiporter endoplasmic Reticulum Adenylate Transporter1 perturbs photorespiration. *Plant Cell* **25**: 2647–2660
- Howell KA, Millar AH, Whelan J (2006) Ordered assembly of mitochondria during rice germination begins with pro-mitochondrial structures rich in components of the protein import apparatus. *Plant Mol Biol* **60**: 201–223
- Huang Y, Chen X, Liu Y, Roth C, Copeland C, McFarlane HE, Huang S, Lipka V, Wiermer M, Li X (2013) Mitochondrial AtPAM16 is required for plant survival and the negative regulation of plant immunity. *Nat Commun* **4**: 2558
- Ivanova A, Law SR, Narsai R, Duncan O, Lee J-H, Zhang B, Van Aken O, Radomiljac JD, van der Merwe M, Yi K, et al (2014) A functional antagonistic relationship between auxin and mitochondrial retrograde signaling regulates alternative oxidase1a expression in *Arabidopsis*. *Plant Physiol* **165**: 1233–1254
- Jaipargas EA, Barton KA, Mathur N, Mathur J (2015) Mitochondrial pleomorphy in plant cells is driven by contiguous ER dynamics. *Front Plant Sci* **6**: 783
- Jardim-Messeder D, Caverzan A, Rauber R, de Souza Ferreira E, Margis-Pinheiro M, Galina A (2015) Succinate dehydrogenase (mitochondrial complex II) is a source of reactive oxygen species in plants and regulates development and stress responses. *New Phytol* **208**: 776–789
- Kerchev PI, De Clercq I, Denecker J, Mühlenbock P, Kumpf R, Nguyen L, Audenaert D, Dejonghe W, Van Breusegem F (2014) Mitochondrial perturbation negatively affects auxin signaling. *Mol Plant* **7**: 1138–1150
- Kristensen BK, Askerlund P, Bykova NV, Egsgaard H, Møller IM (2004) Identification of oxidised proteins in the matrix of rice leaf mitochondria by immunoprecipitation and two-dimensional liquid chromatography-tandem mass spectrometry. *Phytochemistry* **65**: 1839–1851
- Laluk K, Abuqamar S, Mengiste T (2011) The *Arabidopsis* mitochondria-localized pentatricopeptide repeat protein PGN functions in defense against necrotrophic fungi and abiotic stress tolerance. *Plant Physiol* **156**: 2053–2068
- Lam E, Kato N, Lawton M (2001) Programmed cell death, mitochondria and the plant hypersensitive response. *Nature* **411**: 848–853
- Lee W-S, Fu S-F, Verchot-Lubicz J, Carr JP (2011) Genetic modification of alternative respiration in *Nicotiana benthamiana* affects basal and salicylic acid-induced resistance to potato virus X. *BMC Plant Biol* **11**: 41
- Li Z, Xing D (2011) Mechanistic study of mitochondria-dependent programmed cell death induced by aluminium phytotoxicity using fluorescence techniques. *J Exp Bot* **62**: 331–343
- Liao Y, Tian M, Zhang H, Li X, Wang Y, Xia X, Zhou J, Zhou Y, Yu J, Shi K, Klessig DF (2015) Salicylic acid binding of mitochondrial alpha-ketoglutarate dehydrogenase E2 affects mitochondrial oxidative phosphorylation and electron transport chain components and plays a role in basal defense against tobacco mosaic virus in tomato. *New Phytologist* **205**: 1296–1307
- Liu J, Li Z, Wang Y, Xing D (2014) Overexpression of ALTERNATIVE OXIDASE1a alleviates mitochondria-dependent programmed cell death induced by aluminium phytotoxicity in *Arabidopsis*. *J Exp Bot* **65**: 4465–4478
- Martin MV, Fiol DF, Sundaresan V, Zabaleta EJ, Pagnussat GC (2013) oiwa, a female gametophytic mutant impaired in a mitochondrial manganese-superoxide dismutase, reveals crucial roles for reactive oxygen species during embryo sac development and fertilization in *Arabidopsis*. *Plant Cell* **25**: 1573–1591
- Maxwell DP, Wang Y, McIntosh L (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. *Proc Natl Acad Sci USA* **96**: 8271–8276
- Meyer EH, Tomaz T, Carroll AJ, Estavillo G, Delannoy E, Tanz SK, Small ID, Pogson BJ, Millar AH (2009) Remodeled respiration in *ndufs4* with low phosphorylation efficiency suppresses *Arabidopsis* germination and growth and alters control of metabolism at night. *Plant Physiol* **151**: 603–619
- Miller G, Honig A, Stein H, Suzuki N, Mittler R, Zilberstein A (2009) Unraveling delta1-pyrroline-5-carboxylate-proline cycle in plants by uncoupled expression of proline oxidation enzymes. *J Biol Chem* **284**: 26482–26492
- Miwa S, Treumann A, Bell A, Vistoli G, Nelson G, Hay S, von Zglinicki T (2016) Carboxylesterase converts Amplex red to resorufin: Implications for mitochondrial H₂O₂ release assays. *Free Radic Biol Med* **90**: 173–183
- Møller IM (2001) Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. *Annu Rev Plant Physiol Plant Mol Biol* **52**: 561–591
- Møller IM, Sweetlove LJ (2010) ROS signalling—specificity is required. *Trends Plant Sci* **15**: 370–374
- Morgan MJ, Lehmann M, Schwarzländer M, Baxter CJ, Sienkiewicz-Porzucek A, Williams TC, Schauer N, Fernie AR, Fricker MD, Ratcliffe RG, et al (2008) Decrease in manganese superoxide dismutase leads to reduced root growth and affects tricarboxylic acid cycle flux and mitochondrial redox homeostasis. *Plant Physiol* **147**: 101–114
- Moseler A, Aller I, Wagner S, Nietzel T, Przybyla-Toscano J, Mühlenhoff U, Lill R, Berndt C, Rouhier N, Schwarzländer M, et al (2015) The mitochondrial monothiol glutaredoxin S15 is essential for iron-sulfur protein maturation in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* **112**: 13735–13740
- Mueller SJ, Reski R (2015) Mitochondrial dynamics and the ER: the plant perspective. *Front Cell Dev Biol* **3**: 78

- Mur LAJ, Kenton P, Lloyd AJ, Ougham H, Prats E (2008) The hypersensitive response; the centenary is upon us but how much do we know? *J Exp Bot* **59**: 501–520
- Murayama M, Hayashi S, Nishimura N, Ishide M, Kobayashi K, Yagi Y, Asami T, Nakamura T, Shinozaki K, Hirayama T (2012) Isolation of *Arabidopsis* ahg11, a weak ABA hypersensitive mutant defective in nad4 RNA editing. *J Exp Bot* **63**: 5301–5310
- Murphy MP (2009) How mitochondria produce reactive oxygen species. *Biochem J* **417**: 1–13
- Navrot N, Collin V, Gualberto J, Gelhaye E, Hirasawa M, Rey P, Knaff DB, Issakidis E, Jacquot JP, Rouhier N (2006) Plant glutathione peroxidases are functional peroxidoredoxins distributed in several subcellular compartments and regulated during biotic and abiotic stresses. *Plant Physiol* **142**: 1364–1379
- Ng S, De Clercq I, Van Aken O, Law SR, Ivanova A, Willems P, Giraud E, Van Breusegem F, Whelan J (2014) Anterograde and retrograde regulation of nuclear genes encoding mitochondrial proteins during growth, development, and stress. *Mol Plant* **7**: 1075–1093
- Ng S, Ivanova A, Duncan O, Law SR, Van Aken O, De Clercq I, Wang Y, Carrie C, Xu L, Kmiec B, et al (2013) A membrane-bound NAC transcription factor, ANAC017, mediates mitochondrial retrograde signaling in *Arabidopsis*. *Plant Cell* **25**: 3450–3471
- Pattanayak GK, Venkataramani S, Hortensteiner S, Kunz L, Christ B, Moulin M, Smith AG, Okamoto Y, Tamiaki H, Sugishima M, et al (2012) Accelerated cell death 2 suppresses mitochondrial oxidative bursts and modulates cell death in *Arabidopsis*. *Plant J* **69**: 589–600
- Patterson HC, Gerbeth C, Thiru P, Vögtle NF, Knoll M, Shahsafaei A, Samocha KE, Huang CX, Harden MM, Song R, et al (2015) A respiratory chain controlled signal transduction cascade in the mitochondrial intermembrane space mediates hydrogen peroxide signaling. *Proc Natl Acad Sci USA* **112**: E5679–E5688
- Perkins A, Nelson KJ, Parsonage D, Poole LB, Karplus PA (2015) Peroxidoredoxins: guardians against oxidative stress and modulators of peroxide signaling. *Trends Biochem Sci* **40**: 435–445
- Qamar A, Mysore KS, Senthil-Kumar M (2015) Role of proline and pyrroline-5-carboxylate metabolism in plant defense against invading pathogens. *Front Plant Sci* **6**: 503
- Reddi AR, Culotta VC (2013) SOD1 integrates signals from oxygen and glucose to repress respiration. *Cell* **152**: 224–235
- Rhoads DM, Subbaiah CC (2007) Mitochondrial retrograde regulation in plants. *Mitochondrion* **7**: 177–194
- Rhoads DM, Umbach AL, Subbaiah CC, Siedow JN (2006) Mitochondrial reactive oxygen species. Contribution to oxidative stress and inter-organellar signaling. *Plant Physiol* **141**: 357–366
- Rich PR, Bonner WD Jr (1978) The sites of superoxide anion generation in higher plant mitochondria. *Arch Biochem Biophys* **188**: 206–213
- Riemer J, Schwarzländer M, Conrad M, Herrmann JM (2015) Thiol switches in mitochondria: operation and physiological relevance. *Biol Chem* **396**: 465–482
- Schmidtman E, König AC, Orwat A, Leister D, Hartl M, Finkemeier I (2014) Redox regulation of *Arabidopsis* mitochondrial citrate synthase. *Mol Plant* **7**: 156–169
- Schwarzländer M, Dick TP, Meyer AJ, Morgan B (2016) Dissecting redox biology using fluorescent protein sensors. *Antioxid Redox Signal* (in press)
- Schwarzländer M, König AC, Sweetlove LJ, Finkemeier I (2012a) The impact of impaired mitochondrial function on retrograde signalling: a meta-analysis of transcriptomic responses. *J Exp Bot* **63**: 1735–1750
- Schwarzländer M, Logan DC, Johnston IG, Jones NS, Meyer AJ, Fricker MD, Sweetlove LJ (2012b) Pulsing of membrane potential in individual mitochondria: a stress-induced mechanism to regulate respiratory bioenergetics in *Arabidopsis*. *Plant Cell* **24**: 1188–1201
- Schwarzländer M, Wagner S, Ermakova YG, Belousov VV, Radi R, Beckman JS, Buettner GR, Demareux N, Duchon MR, Forman HJ, et al (2014) The 'mitoflash' probe cpYFP does not respond to superoxide. *Nature* **514**: E12–E14
- Sechet J, Roux C, Plessis A, Effroy D, Frey A, Perreau F, Biniek C, Krieger-Liszakay A, Macherel D, North HM, et al (2015) The ABA-deficiency suppressor locus HAS2 encodes the PPR protein LOI1/MEF11 involved in mitochondrial RNA editing. *Mol Plant* **8**: 644–656
- Senthil-Kumar M, Mysore KS (2012) Ornithine-delta-aminotransferase and proline dehydrogenase genes play a role in non-host disease resistance by regulating pyrroline-5-carboxylate metabolism-induced hypersensitive response. *Plant Cell Environ* **35**: 1329–1343
- Soto G, Fox R, Ayub N, Alleva K, Guaimas F, Erijman EJ, Mazzella A, Amodeo G, Muschietti J (2010) TIP5;1 is an aquaporin specifically targeted to pollen mitochondria and is probably involved in nitrogen remobilization in *Arabidopsis thaliana*. *Plant J* **64**: 1038–1047
- Stroehrer E, Grassl J, Carrie C, Fenske R, Whelan J, Millar AH (2016) Glutaredoxin S15 is involved in Fe-S cluster transfer in mitochondria influencing lipoic acid-dependent enzymes, plant growth and arsenic tolerance in *Arabidopsis*. *Plant Physiol* **170**: 1284–1299
- Sweetlove LJ, Lytovchenko A, Morgan M, Nunes-Nesi A, Taylor NL, Baxter CJ, Eickmeier I, Fernie AR (2006) Mitochondrial uncoupling protein is required for efficient photosynthesis. *Proc Natl Acad Sci USA* **103**: 19587–19592
- Tan Y-F, O'Toole N, Taylor NL, Millar AH (2010) Divalent metal ions in plant mitochondria and their role in interactions with proteins and oxidative stress-induced damage to respiratory function. *Plant Physiol* **152**: 747–761
- Tognetti VB, Mühlenbock P, Van Breusegem F (2012) Stress homeostasis: the redox and auxin perspective. *Plant Cell Environ* **35**: 321–333
- Tognetti VB, Van Aken O, Morreel K, Vandenbroucke K, van de Cotte B, De Clercq I, Chiwocha S, Fenske R, et al (2010) Perturbation of indole-3-butyric acid homeostasis by the UDP-glucosyltransferase UGT74E2 modulates *Arabidopsis* architecture and water stress tolerance. *Plant Cell* **22**: 2660–2679
- Van Aken O, Pecenková T, van de Cotte B, De Rycke R, Eeckhout D, Fromm H, De Jaeger G, Witters E, Beeemster GT, Inzé D, et al (2007) Mitochondrial type-I prohibitins of *Arabidopsis thaliana* are required for supporting proficient meristem development. *Plant J* **52**: 850–864
- Van Aken O, Van Breusegem F (2015) Licensed to kill: mitochondria, chloroplasts, and cell death. *Trends Plant Sci* **20**: 754–766
- Van Aken O, Whelan J (2012) Comparison of transcriptional changes to chloroplast and mitochondrial perturbations reveals common and specific responses in *Arabidopsis*. *Front Plant Sci* **3**: 281
- Van Aken O, Zhang B, Carrie C, Uggalla V, Paynter E, Giraud E, Whelan J (2009) Defining the mitochondrial stress response in *Arabidopsis thaliana*. *Mol Plant* **2**: 1310–1324
- Vanlerberghe GC (2013) Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. *Int J Mol Sci* **14**: 6805–6847
- Vanlerberghe GC, Robson CA, Yip JYH (2002) Induction of mitochondrial alternative oxidase in response to a cell signal pathway down-regulating the cytochrome pathway prevents programmed cell death. *Plant Physiol* **129**: 1829–1842
- Veal EA, Day AM, Morgan BA (2007) Hydrogen peroxide sensing and signaling. *Mol Cell* **26**: 1–14
- Vidal G, Ribas-Carbo M, Garmier M, Dubertret G, Rasmusson AG, Mathieu C, Foyer CH, De Paepe R (2007) Lack of respiratory chain complex I impairs alternative oxidase engagement and modulates redox signaling during elicitor-induced cell death in tobacco. *Plant Cell* **19**: 640–655
- Wang Y, Law SR, Ivanova A, van Aken O, Kubiszewski-Jakubiak S, Uggalla V, van der Merwe M, Duncan O, Narsai R, Whelan J, et al (2014) The mitochondrial protein import component, TRANSLOCASE OF THE INNER MEMBRANE17-1, plays a role in defining the timing of germination in *Arabidopsis*. *Plant Physiol* **166**: 1420–1435
- Wang Y, Lyu W, Berkowitz O, Radomiljac JD, Law SR, Murcha MW, Carrie C, Teixeira PF, Kmiec B, Duncan O, et al (2016) Inactivation of mitochondrial complex I induces the expression of a twin-cysteine protein that targets and affects cytosolic, chloroplastidic and mitochondrial function. *Mol Plant* (in press)
- Winger AM, Taylor NL, Heazlewood JL, Day DA, Millar AH (2007) Identification of intra- and intermolecular disulphide bonding in the plant mitochondrial proteome by diagonal gel electrophoresis. *Proteomics* **7**: 4158–4170
- Wu J, Sun Y, Zhao Y, Zhang J, Luo L, Li M, Wang J, Yu H, Liu G, Yang L, et al (2015) Deficient plastidic fatty acid synthesis triggers cell death by modulating mitochondrial reactive oxygen species. *Cell Res* **25**: 621–633
- Yao N, Eisfelder BJ, Marvin J, Greenberg JT (2004) The mitochondrion—an organelle commonly involved in programmed cell death in *Arabidopsis thaliana*. *Plant J* **40**: 596–610
- Yao N, Greenberg JT (2006) *Arabidopsis* ACCELERATED CELL DEATH2 modulates programmed cell death. *Plant Cell* **18**: 397–411
- Yoshida K, Hisabori T (2014) Mitochondrial isocitrate dehydrogenase is inactivated upon oxidation and reactivated by thioredoxin-dependent reduction in *Arabidopsis*. *Front Environ Sci* **2**: 38

- Yoshida K, Noguchi K, Motohashi K, Hisabori T (2013) Systematic exploration of thioredoxin target proteins in plant mitochondria. *Plant Cell Physiol* **54**: 875–892
- Zhang B, Van Aken O, Thatcher L, De Clercq I, Duncan O, Law SR, Murcha MW, van der Merwe M, Seifi HS, Carrie C, et al (2014a) The mitochondrial outer membrane AAA ATPase AtOM66 affects cell death and pathogen resistance in *Arabidopsis thaliana*. *Plant J* **80**: 709–727
- Zhang L, Oh Y, Li H, Baldwin IT, Galis I (2012) Alternative oxidase in resistance to biotic stresses: *Nicotiana attenuata* AOX contributes to resistance to a pathogen and a piercing-sucking insect but not *Manduca sexta* larvae. *Plant Physiol* **160**: 1453–1467
- Zhang S, Wu J, Yuan D, Zhang D, Huang Z, Xiao L, Yang C (2014b) Perturbation of auxin homeostasis caused by mitochondrial FtSH4 gene-mediated peroxidase accumulation regulates *Arabidopsis* architecture. *Mol Plant* **7**: 856–873
- Zhu Q, Dugardeyn J, Zhang C, Mühlenbock P, Eastmond PJ, Valcke R, De Coninck B, Öden S, Karampelias M, Cammue BPA, et al (2014) The *Arabidopsis thaliana* RNA editing factor SLO2, which affects the mitochondrial electron transport chain, participates in multiple stress and hormone responses. *Mol Plant* **7**: 290–310
- Zielonka J, Kalyanaraman B (2010) Hydroethidine- and MitoSOX-derived red fluorescence is not a reliable indicator of intracellular superoxide formation: another inconvenient truth. *Free Radic Biol Med* **48**: 983–1001