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Mitochondrial Energy Signaling and Its Role in the Low-Oxygen Stress Response of Plants[1[OPEN]]

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Cells of complex organisms typically rely on mitochondria for energy provision. The amount of energy required to sustain cellular activity can vary strongly depending on external conditions. Vice versa, constraints on respiratory activity due to metabolic status or stress insult require mitochondrial signaling to coordinate cellular physiology with the function of the organelle. In this update, we review recent insights into plant mitochondrial energy signaling in the light of their significance to stress acclimation. First, we focus on the characteristic adjustments of the nuclear transcriptome that occur after pharmacological inhibition of the mitochondrial electron transport chain as the output of mitochondrial retrograde signaling. Second, we discuss the proteins that have recently been identified as regulators of the transcript responses and the emerging picture of their action as a signaling network. We then pose the question of how well our current models of inducing mitochondrial dysfunction relate to conditions that plants face naturally. We reason that low-oxygen stress shows striking similarities with electron transport inhibitors with respect to their impact on mitochondrial energy physiology upstream, as well as the cellular transcriptomic response. Finally, we highlight and discuss changes in mitochondrial physiology that are common to both stimuli as candidates for upstream signals. The aim of this update is to better define the physiological context in which mitochondrial signaling operates to provide new directions for future research.

RESPONSES TO MITOCHONDRIAL ENERGY SIGNALING AT THE TRANSCRIPT LEVEL

The impact of mitochondrial-related stimuli on gene expression has been intensively studied using transcript abundance as a proxy, which has provided a rapidly growing number of genome-scale datasets in the recent years. The signals triggering organelle-related transcriptional changes are largely classified into *anterograde* and *retrograde*. While anterograde signaling describes the nuclear control of the

ADVANCES

- Several signaling proteins that mediate the response of nuclear gene expression to mitochondrial dysfunction have been identified through transcript analyses of pharmacological electron transport inhibition and genetic screens.
- An understanding of crosstalk between the transcriptional regulators of mitochondrial signaling is currently emerging, revealing interconnected regulatory networks that coordinate and integrate signaling originating from inside and outside the mitochondria.
- The impact of low oxygen stress on mitochondrial physiology is conceptually similar to pharmacological inhibition of mitochondrial electron transport. Common underlying mechanisms are reflected by overlapping transcriptomic profiles, pinpointing a natural stress situation in which mitochondrial signaling is activated.

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mitochondrion, retrograde signals originate from the organelle and induce nuclear transcriptional reprogramming, often referred to as *feedback* or *backward* flow of information.

Anterograde signals include programs of plant growth and development. As an example, the abundances of at least 45 nuclear-encoded mitochondrial proteins in photosynthetic Arabidopsis (*Arabidopsis thaliana*) tissue were shown to fluctuate with the diurnal cycle, probably to adjust organelle function between day and night. Individual tricarboxylic acid (TCA) cycle enzymes were more abundant and enzymatically more active early after the transition from light to dark (Lee et al., 2010). Most of the underlying genes were later shown to possess specific site II elements in their promoter region that bind to the *TEOSINTE BRANCHED 1, CYCLOIDEA, PCF1 (TCP)* family of transcription factors (Giraud et al., 2010), which are conserved among plants and control a wide range of developmental programs (Martín-Trillo and Cubas, 2010). Diurnal regulation of TCA cycle transcripts has also been demonstrated in unicellular photosynthetic diatoms (Chauton et al., 2013; Smith et al., 2016; Matthijs et al., 2017), green algae (Zones et al., 2015), and prokaryotic cyanobacteria (Stöckel et al., 2008; Welkie et al., 2014), suggesting conservation across photosynthetic organisms. The synthesis rates of mitochondrially encoded transcripts also show diurnal patterns, with lowest rates of transcription observed in Arabidopsis at the end of the day and early in the dark (Okada and Brennicke, 2006). Intriguingly, the steady-state levels of the mitochondrial transcripts remained constant during day and night time, despite fluctuating mRNA synthesis rates. How mitochondrial transcript levels maintained stable is not known, but diurnal changes in RNA decay rates appear to play a role (Okada and Brennicke, 2006). In line with the stability of mitochondrial transcript levels, polysome loading (the number of ribosomes actively translating the same mRNA molecule) of mitochondrial transcripts remained stable and at a relatively high level throughout day and night (Pal et al., 2013). This is in contrast to plastid polysome loading, which is much higher during the light period. Notably, more rapid changes of mitochondrial physiology caused by chemical perturbation of the electron transport chain were found to impact on mitochondrial transcript abundance (Zubo et al., 2014).

Retrograde signals have been widely studied in model systems that perturb mitochondrial function genetically or chemically to monitor the transcriptional response in the nucleus. The control of yeast citrate synthase was the initial model that opened up the field of retrograde signaling. Mitochondrial citrate synthase (CS1) is part of the TCA cycle in the mitochondrial matrix that supplies yeast cells with energy and intermediates required for growth. At *CS1* loss (genetic perturbation) or blockage of the electron transport chain through antimycin A (AA; chemical perturbation), the transcript of peroxisomal citrate synthase (*CIT2*) is induced, which has been interpreted as a mechanism to maintain the provision of biosynthetic intermediates required for cell growth in the absence of a functional TCA cycle (Liao et al., 1991). The authors later found that the up-regulation of *CIT2* strictly depends on the transcription factor *RTG1* and introduced the concept of *retrograde* communication (Liao and Butow, 1993).

AA treatment has become an intensively used model of respiratory inhibition and induction of a mitochondrial retrograde response (MRR) also in plants (Yu et al., 2001; Schwarzländer et al., 2012a; Umbach et al., 2012; De Clercq et al., 2013; Ng et al., 2013a; Van Aken et al., 2016a). AA inhibits the mitochondrial electron transport chain (mtETC) at the N site of complex III and leads to an increased rate of superoxide generation (Cadenas et al., 1977; Murphy, 2009). AA treatment of Arabidopsis tissues results in a characteristic transcriptomic response that includes the regulation of chaperones and heat-shock proteins, transporters, as well as the up-regulation of *ALTERNATIVE OXIDASE1a* (*AOX1a*) and other components of the alternative respiratory chain, such as *NAD(P)H DEHYDROGENASE B2* (*NDB2*). Alternative oxidases (AOXs) and NAD(P)H dehydrogenases (NDHs) represent an alternative pathway of the ETC that does not commonly exist in animals and can decrease or even fully bypass the production of ATP and reactive oxygen species (ROS) through the *classical* oxidoreductases of the *cyanide-sensitive pathway* (Schertl and Braun, 2014). In line with the function of their proteins, *AOX* and *NDH* genes are frequently coexpressed, for instance at inhibition of ETC complex I by rotenone or the ATP synthase (complex V) by oligomycin (Clifton et al., 2005, 2006). Like AA, these inhibitors stop electron flow down the respiratory chain, albeit at different steps with different physiological outcomes. The concerted action of transcriptionally elevated alternative electron transport components has been interpreted as an inducible safety valve to counterbalance ROS production independent of adenosine phosphorylation status (Vanlerberghe and McIntosh, 1992; Maxwell et al., 1999; Cvetkovska and Vanlerberghe, 2013). In the third section of this article, we will discuss how AA treatment compares with more physiological stimuli that induce the plant MRR. A general transcriptional overlap with different stress insults has clearly been indicated by multiple studies (Clifton et al., 2005; Van Aken et al., 2009; Umbach et al., 2012; Van Aken and Whelan, 2012). Overlaps with the transcriptomic fingerprints of mutants with functionally impaired mitochondria provide further support (Van Aken et al., 2007; Kühn et al., 2009), although mutants generally show far less differentially expressed genes than AA treatment. This may be explained by acclimation of mutants over their development to adopt a different steady state, while the AA treatment necessitates acute reprogramming (Van Aken and Pogson, 2017). Ease of the treatment and the apparent representative nature of the response for *mitochondrial stress* established AA treatment as the current standard in investigating the mitochondrial

retrograde response as measured by specific marker transcripts (*AOX1a*, but also transcriptionally more strongly induced markers such as *UGT74E2* and *UP-REGULATED BY OXIDATIVE STRESS* (Ho et al., 2008; Tognetti et al., 2010; De Clercq et al., 2013)).

Not only inhibitors of the mtETC have been used to induce retrograde regulation in plants. Monofluoroacetate (MFA), an inhibitor of TCA cycle enzyme aconitase, also triggers a pronounced transcriptomic response that includes *AOX1a* and other marker transcripts and partly overlaps with the response to AA inhibition (Vanlerberghe and McLntosh, 1996; Umbach et al., 2012; Ng et al., 2013b; Van Aken et al., 2016b). Aconitase inhibition leads to a rearrangement of fluxes through the cellular metabolic network, and this is likely to include an accumulation of citrate, a candidate retrograde signaling molecule (Buffa and Peters, 1949). Indeed, addition of citrate led to *AOX* induction in soybean and tobacco (Vanlerberghe and McLntosh, 1996; Djajanegara et al., 2002; Gray et al., 2004). In Arabidopsis, induction of *AOX1a* by citrate treatments could not be confirmed, and the changes observed were either modest or absent (Ho et al., 2008; Finkemeier et al., 2013). Overall, the transcriptomic effects of externally supplied citrate in Arabidopsis are mild (Finkemeier et al., 2013) compared to those observed in tobacco and soybean (Djajanegara et al., 2002; Gray et al., 2004). In contrast, MFA leads to a very pronounced transcriptional effect in Arabidopsis, which peaks several hours later but is even stronger and longer-lasting than AA (Umbach et al., 2012; Van Aken et al., 2016b). This may indicate that the strong effects caused by MFA are not coupled to citrate accumulation directly but to more indirect metabolic and physiological rearrangements that are currently not fully understood. Citrate supplementation appears not to result in any pronounced changes in cellular ROS production (Djajanegara et al., 2002; Gray et al., 2004), and also MFA did not trigger a ROS response, as estimated using the unspecific probe H_2DCF-DA (see fourth section) in Arabidopsis leaves (Umbach et al., 2012). Addressing the mechanisms by which citrate, MFA, and ROS production cause different or overlapping responses will enhance our understanding of the mechanisms behind the mitochondrial retrograde response.

PROTEINS REGULATING NUCLEAR TRANSCRIPTION IN MITOCHONDRIAL SIGNALING

The detailed characterization of the mitochondrial retrograde response at transcriptional level has allowed the identification of upstream regulatory factors in recent years. Three studies conducted meta-analyses of upregulated transcripts at several conditions of mitochondrial dysfunction such as chemical treatments with AA, oligomycin, and rotenone or genetic modulations (Schwarzländer et al., 2012a; Van Aken and Whelan, 2012; De Clercq et al., 2013). Thirty transcripts were consistently induced, and a majority of those shared a cis-regulatory motif. These genes were defined as *MITOCHONDRIAL DYSFUNCTION STIMULON* (De Clercq et al., 2013), and their promotors were found to be targeted by five transcription factors of the NAC (NO APICAL MERISTEM/ARABIDOPSIS TRANSCRIPTION ACTIVATION FACTOR/CUP-SHAPED COTYLEDON) family (De Clercq et al., 2013), including ANAC013 and ANAC017/REGULATORS OF ALTERNATIVE OXIDASE 1a (RAO2). ANAC017 is an ER-membrane protein that is likely activated through endoproteolysis, supposedly through rhomboid protease activity. Its cleavage releases the N terminus of ANAC017 into the cytosol from where it translocates to the nucleus (Ng et al., 2013b). Also, ANAC013 and other Arabidopsis NACs have been shown to localize to the ER (De Clercq et al., 2013; Liang et al., 2015), and several, including ANAC017, contain well-defined nuclear localization sequences in the N-terminal region (Olsen et al., 2005; Ng et al., 2013b). ER localization, stress-induced proteolytic activation and translocation into the nucleus have been experimentally documented for a number of membrane-bound NACs from Arabidopsis (Kim et al., 2008; Yoon et al., 2008; Seo et al., 2010) and soybean (Li et al., 2016), suggestive of a more general signaling principle.

WRKY-type transcription factors (WRKY15/WRKY40/WRKY63; Vanderauwera et al., 2012; Van Aken et al., 2013) and ABI4 (ABSCISIC ACID INSENSITIVE4; Giraud et al., 2009) have been identified as additional downstream regulators in mitochondrial retrograde signaling. Notably, ANAC017, WRKY40 and ABI4 are also involved in retrograde signaling of the chloroplast (Koussevitzky et al., 2007; Shang et al., 2010; Van Aken et al., 2013; 2016a). Converging retrograde signaling cascades may allow for synchronization of the downstream response in both endosymbiotic energy organelles, but the level at which the integration occurs remains elusive (Finkemeier and Schwarzländer, 2017). ABI4 in the retrograde response of the chloroplast has recently been shown to be activated through phosphorylation by a MAP kinase cascade in a Ca^{2+}-dependent manner, suggesting potential molecular entry points of signal integration (Guo et al., 2016). ANAC017 appears also crucial for the transcriptional response to methyl viologen (MV; paraquat) that produces large amounts of superoxide using electrons from PSI under illuminated conditions and eventually leads to cell death (Van Aken and Van Breusegem, 2015; Van Aken et al., 2016a). Importantly, several MRR target genes (including *AOX1a*) are not induced by MV. Vice versa, MV induces additional transcripts (including *HSP17.6*) that are unaffected by AA treatment. ANAC017 appeared almost solely responsible for *HSP17.6* induction by MV, indicating that ANAC017 can relay signals originating from either chloroplasts or mitochondria yet regulate the expression of separate gene sets. This suggests that ANAC017 operates in concert with other factors that define its specificity and have not yet been identified. ABI4 further regulates plant

developmental processes, such as seed germination (Finkelstein et al., 1998), flower development (Foyer et al., 2012; Shu et al., 2016), and lateral root formation (Shkolnik-Inbar and Bar-Zvi, 2010). It remains unclear if and how those programs are linked or even integrated with retrograde regulation. Similarly, WRKYs take part in developmental programs and in a variety of abiotic and biotic stresses (Rushton et al., 2010). WRKY40 was found to be associated with plastids by binding the envelope-spanning Mg-Chelatase H subunit and may be involved in seed development (Shang et al., 2010). WRKY40 and WRKY63 were further found to act in leaf senescence of plants carrying a mutated mitochondrial AAA-protease FILAMENTATION TEMPERATURE SENSITIVE H4 (FTSH4) (Zhang et al., 2017b). As both mutation in *FTSH4* (Zhang et al., 2014) and seed development (El-Maarouf-Bouteau and Bailly, 2008) have been associated with increased rates of mitochondrial reactive oxygen species (mtROS) production, the signal leading to developmental WRKY40/63 activation may be of mitochondrial (as opposed to nuclear) origin and provide a route to integrate mitochondrial signaling into regulation of plant growth and development.

A study using genetic mutants with dysfunctional mitochondria suggests that ANAC017 has a special role among the transcription factors identified so far in mitochondrial signaling. While loss of the PROHIBITIN3 scaffolding complex in the inner mitochondrial membrane (Van Aken et al., 2007) or of the dual-targeted RNA polymerase RPOTmp (Kühn et al., 2009) independently and constitutively induced marker transcripts of the mitochondrial retrograde response, their induction was drastically decreased in the absence *ANAC017* (Van Aken et al., 2016b). A similar pattern was observed when mitochondrial dysfunction was induced by AA. The *AOX1a* transcript was unresponsive in the *anac017* null mutant but regulated similar to wild type in the absence of other *NAC*s, *ABI4*, and *WRKY40/63* (Van Aken et al., 2016a). A central role for ANAC017 is further supported by a pronounced reduction of primary root growth induced by AA, rotenone, or MV in *anac017*. This effect was absent in lines lacking individual *ANAC013/053/078* or *WRKY40/63* transcripts, demonstrating the functional significance of ANAC017 for acclimation of the plant to mitochondrial dysfunction. Acclimation was even improved beyond wild-type capacity by overexpression of ANAC017, which led to longer primary roots in the presence of the inhibitors. Particularly high expression as compared to the other transcription factors of the NAC family is characteristic for ANAC017. This may provide an explanation for the phenotypes associated with its loss, implying that functional backup may be available for less abundant family members (Van Aken et al., 2016a).

Recently, Zhang et al. (2017a) identified MYB DOMAIN PROTEIN29/RAO7 as an additional transcription factor that has a negative, albeit indirect, regulatory impact on downstream genes related to mitochondrial stress, including *AOX1a*, but notably also *WRKY40* and *ANAC053*. Since WRKY40 in turn regulates expression of *ABI4* (Liu et al., 2012b) it appears that the transcription factors in retrograde signaling form expression networks. Being part of such a network, MYB DOMAIN PROTEIN29 acts as a positive regulator of plant growth, development, and energy metabolism and appears to balance needs under stressed and nonstressed conditions.

The CYCLIN-DEPENDENT KINASE E1 (CDKE1/RAO1) was identified in a screen for *AOX1a* regulators in response to AA and proposed to be a hub of retrograde signaling from mitochondria as well as chloroplasts (Ng et al., 2013a; Blanco et al., 2014). CDKE1 is predicted to be a subunit of the Arabidopsis mediator complex, which acts as a scaffold that bridges DNA-bound transcription factors to RNA polymerase II, linking signaling input to transcription (Mathur et al., 2011; Poss et al., 2013). Nuclear-localized CDKE1 interacts with the SNF1-Related Kinase1 (SnRK1) catalytic subunit KIN10, which integrates transcription networks of plant stress signaling, sugar metabolism, and developmental programs (Baena-González et al., 2007; Cho et al., 2012). KIN10 is conserved across plants and has been shown to interact with several transcription factors (Kleinow et al., 2009; Tsai and Gazzarrini, 2012; Zhai et al., 2017). The integrative role of KIN10 is further discussed by Wurzinger et al. (2018) as part of this Focus Issue. In the mitochondrial retrograde response, CDKE1 has been proposed to act independently of *ABI4* (Blanco et al., 2014). In principle, the mediator complex would provide a mechanism for CDKE1 to operate upstream of different transcription factors, but systematic experimental validation is currently lacking.

LOW-OXYGEN STRESS: LINKING OUR INSIGHTS FROM MODELS OF MITOCHONDRIAL ENERGY SIGNALING TO THE NATURAL CONTEXT

The progress that has been made in measuring the transcriptomic output and identifying regulatory proteins of mitochondrial energy signaling prompts the question of under what natural conditions their action is relevant for the plant. This deserves a closer look given that many of our insights rely on ETC inhibitors, like AA, as model stimuli. Only natural conditions that involve a rapid transition (minutes to hours) and require an acute response need considering because the inhibitors are typically applied in an abrupt fashion (e.g. sudden spraying rather than long-term supplementation of a medium). AA has been widely used with the rationale of triggering an increase in the production of ROS by the mitochondria and *mitochondrial dysfunction* in a sense that has not been further defined. Hence, natural conditions that lead to an increase in mtROS production may also activate the identified signaling responses. Indeed, several biotic and abiotic stresses have been associated with mtROS production (Rhoads et al., 2006; Yao and Greenberg, 2006; Chang et al., 2012;

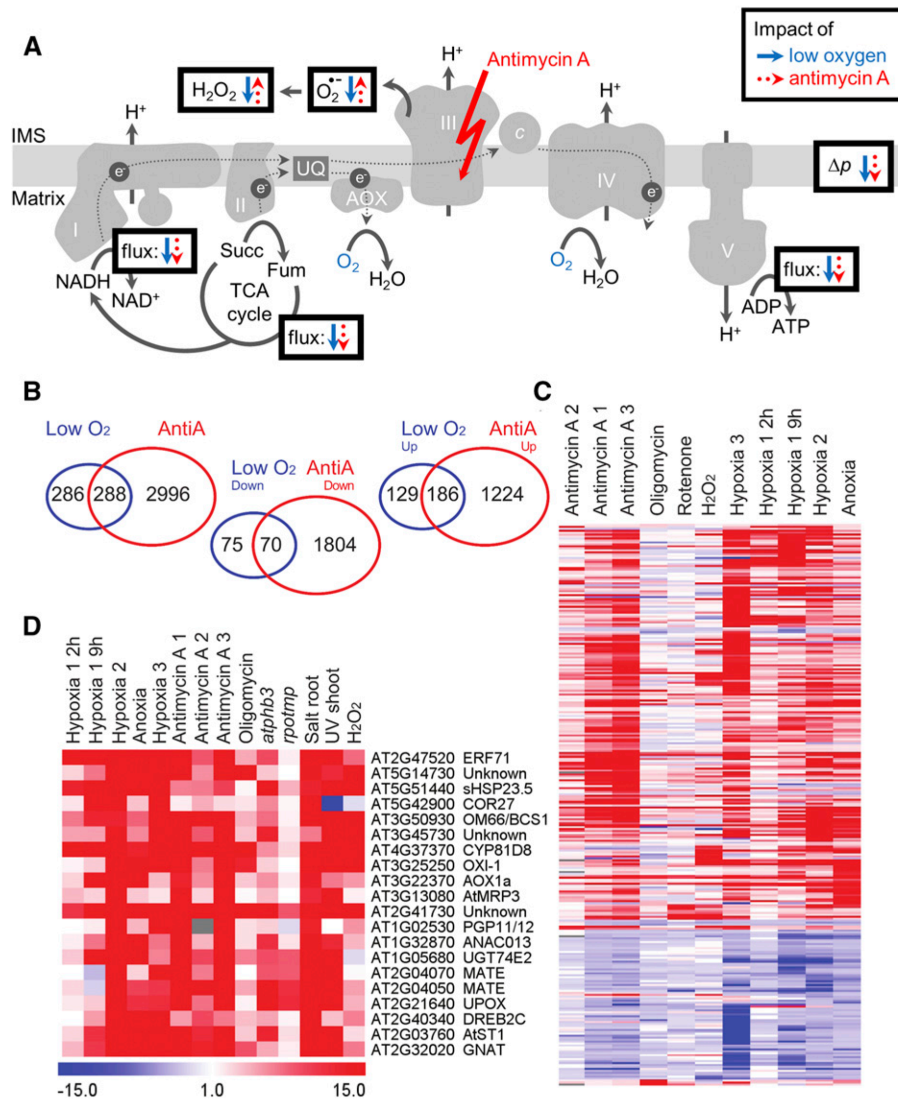

Figure 1. Effects of low-oxygen and antimycin A treatments on mitochondrial energy physiology and the nuclear transcriptome. A, Central respiration-associated processes at the inner mitochondrial membrane are outlined. Parameters highlighted in boxes are further discussed in the text as candidate signaling molecules in mitochondrial energy signaling. Arrows in solid blue (low oxygen) and dotted red (antimycin A) indicate increases and decreases. The decrease in superoxide ($O_2^{\bullet-}$) under low-oxygen stress represents a theoretical consideration; the transient increase in superoxide production has been proposed and is discussed in the text. B, To define the low-oxygen response at the transcript level, publicly available datasets (GSE accessions GSE9719 [hypoxia #1], GSE21504 [hypoxia #2], GSE16222 [anoxia], and GSE14420 [hypoxia #3]) were analyzed, and 574 genes were identified as commonly differentially regulated by low oxygen (using Cyber-T; Kayala and Baldi, 2012); 2-fold change, cum.ppde.$P > 0.95$). The antimycin A response was analyzed analogously using GSE accessions GSE41136 (antimycin A #1) and GSE36011 (antimycin A #2), identifying 3,284 genes differently regulated by AA. The Venn diagram shows the overall overlaps between the extracted genes, as well as the gene transcripts that were commonly up- or down-regulated. C, Normalized expression values for all 288 genes overlapping between low-oxygen and antimycin A treatment after hierarchical clustering (Pearson correlation and average linkage). For comparison, expression values from Arabidopsis cell culture responses to oligomycin (complex V inhibitor), rotenone (complex I inhibitor), and H_2O_2 are shown (GES3709). Expression values are fold changes normalized to the respective control experiments (set to 1). D, Normalized expression values for common differentially expressed genes at AA treatment (as defined in B) and in *atphb3* mutant plants lacking the abundant PROHIBITIN3 scaffolding complex in the inner mitochondrial membrane (Van Aken et al., 2007). Expression values for salt (E-GEOD-5623) and UV (E-GEOD-5626) treatment are also shown.

Cvetkovska and Vanlerberghe, 2013) as well as with the induction of *AOX1a* and other retrograde marker transcripts (Van Aken and Whelan, 2012). Yet, the link between mtROS and induction of signaling remains correlative, which is partly due to the difficulty to trigger increased mtROS production without causing gross perturbation of respiratory physiology. In addition to mtROS, mitochondrial physiology also shows

changes in several other stimuli that represent equally good candidates as upstream signals. Complex III inhibition by AA enables the prediction of several changes in mitochondrial energy physiology (Fig. 1A): block of the cyanide-sensitive pathway (while AOX remaining active), drop in oxygen consumption rate, drop in proton pumping rate (with likely impact on membrane potential and pH gradient and acidification of the mitochondrial matrix affecting supercomplex composition; Ramírez-Aguilar et al., 2011), drop in ATP synthase activity, drop in adenosine energy charge, increase of superoxide release rate into the intermembrane space, increase in reduction of the ubiquinone pool, decrease of oxidation rate of matrix NADH, decrease of the rate of matrix catabolic pathways (including the TCA cycle) with impact on extramitochondrial pathways via modified rates of transport across the mitochondrial membranes. Drought and acute ozone exposure have both been shown to inhibit mitochondrial respiration by strongly decreasing the capacity of the classical electron transport pathway, while inducing the alternative pathway (Ederli et al., 2006; Dahal and Vanlerberghe, 2017). In that respect, both stresses are likely to include the response that is triggered by AA-induced signaling. Yet their primary impact on the mitochondria is mechanistically poorly defined and probably involves a combination of several changes. More specific inhibition of the electron transport with remarkable mechanistic resemblance to that of AA can be predicted for oxygen limitation (Fig. 1A). Lack of oxygen as the final mitochondrial electron acceptor of the respiratory chain may be conceptualized as a reasonably specific block of electron transport at the terminal oxidase (complex IV, AOX). Given that the mitochondrion is by far the main consumer of oxygen in the cell, the expected impact on mitochondrial energy physiology is likely to be particularly similar to that of AA. Otto Warburg made a similar assumption 60 years ago when studying the metabolism of animal cancer cells. "From the standpoint of energy", he wrote, "no matter whether oxygen is withdrawn from the cell or whether the oxygen is prevented from reacting by a poison, the result is the same in both cases—namely, impairment of respiration from lack of energy" (Warburg, 1956). Although this consideration has not been made for plant mitochondrial energy signaling, it is indeed well supported by similarities of the transcriptomic response between anoxia/hypoxia and AA (Fig. 1, B and C): half of about 600 genes consistently differently regulated under anoxic or hypoxic conditions overlapped with the genomic response to AA. The majority of the *MITOCHONDRIAL DYSFUNCTIN STIMULON* (see above; De Clercq et al., 2013) is part of that overlap (Fig. 1D), suggesting that NAC-dependent signaling makes a substantial contribution to the response to low-oxygen stress.

Differences in the transcriptomic profiles may be accounted for by mechanistic differences between the consequences of AA and anoxia/hypoxia in mitochondrial energy physiology: low-oxygen stress will block additional oxygen-dependent pathways in the cell (e.g. cytosolic oxygenases, ER oxidoreductins that drive oxidative protein folding) that are not inhibited by AA. The concept of signal integration from various cellular compartments in low-oxygen signaling is reviewed by Schmidt et al. (2018) as part of this Focus Issue. In the mitochondria, lack of oxygen will lead to efficient inhibition of electron transport flux without the backup by AOX, as in the case of AA. Indeed, the direct impact of a gradual decline in oxygen on AOX activity is expected before that on complex IV, due to a higher K_m of AOX for oxygen (Gupta et al., 2009). A comparable block of ETC flux at both terminal oxidases may be achieved pharmacologically by combined treatments of e.g. AA and the AOX inhibitor n-propyl gallate or salicylhydroxamic acid. Likewise, low-oxygen stress treatments of plant tissues can be varied to obtain different physiological outcomes. Typically, experiments are performed in the dark and involve a rapid transition to hypoxic or anoxic conditions, a scenario that naturally equals flash flooding at night. When the water retreats, reoxygenation can occur rapidly or may also occur internally once oxygen evolution by photosynthesis starts in the morning. Those scenarios show parallels to the ischemia-reperfusion situation that has been extensively studied in mammalian cells, predominately cardiac myocytes (Granger and Kvietys, 2015). In mammals, it appears that the severity of the stress impact is determined less by the hypoxic period itself, but rather by the restart of respiration that can involve excessive mtROS production at complex I through reverse electron transport leading to cell death (Chouchani et al., 2016). In analogy, tight regulation of the restart of respiration is likely to be critical to limit damage in plant cells, requiring specific mitochondrial signals. Mitochondrial signaling at the restart of mtETC after inhibition has not been studied, however, since removal of inhibitors such as AA is not feasible experimentally. Hence, low-oxygen stress may be considered a particularly relevant natural stress to study mitochondrial energy signaling. Its mode of biochemical action is well defined and comparable to that of the widely used ETC inhibitors, while carrying physiological meaning. In the following section, we will discuss physiological parameters and molecules that are affected when mtETC flux is inhibited, either in response to low oxygen or AA. In the absence of knowledge of any endogenous mitochondrial energy sensors, those are strong candidates to act upstream in mitochondrial signaling as primary stimuli or second messengers.

WHICH MOLECULES QUALIFY AS UPSTREAM MITOCHONDRIAL SIGNALS?

Reactive Oxygen Species

A candidate signal favored in several studies of mitochondrial energy signaling is an elevated rate of mtROS production. Although changes in plant mitochondrial

ROS production have rarely been shown with any certainty and specificity in planta (Huang et al., 2016), ROS have been generally recognized for their signaling properties in a wide range of biological situations. Those include plant mitochondrial signaling based on indirect evidence (Ng et al., 2014; Huang et al., 2016), although a concept of where and by what mechanism different ROS may interact with a specific player downstream in the cascade is lacking. On the one hand, individual species, e.g. superoxide, the hydroxyl radical and hydrogen peroxide, differ vastly in their chemical properties, which may allow for distinct and specific cellular signals in principle (Schwarzländer and Finkemeier, 2013). On the other hand, their rapid interconversion may hamper exploitation of their distinct properties (Møller and Sweetlove, 2010).

Mitochondria produce superoxide as the primary species at complex I (NADH dehydrogenase) and complex III (cytochrome bc_1 complex) through one-electron reduction of molecular oxygen. In animal and plant tissues, typically between 1% and 2% of consumed oxygen is considered to drive ROS production and, intuitively, the rate of ROS production is positively correlated with the availability of oxygen (Chance et al., 1979; Turrens et al., 1982; Puntarulo et al., 1988). At first glance, and paradoxically, rapid transition to low-oxygen conditions has also been found to elevate ROS production in cultured human cells (Chandel et al., 1998). The authors used the fluorescent dye 2′,7′-dichlorofluorescin (DCF), which indicated pronounced ROS production when cells were shifted from normoxic (21% O_2) to hypoxic (2% O_2) conditions, which they attributed to superoxide production at complex III using mETC inhibitors. While controversies persist, mtROS are now widely considered a potential early trigger in the mammalian transcriptional response to low oxygen (Bailey-Serres and Chang, 2005; Waypa et al., 2016). Whether, however, superoxide itself has a role as trigger remains challenging to answer. Superoxide is efficiently converted to H_2O_2 by superoxide dismutase (SOD) and most, if not all, probes that have been used in the relevant ROS quantitation experiments show poor specificity for an individual species. Despite this limitation, Chang et al. (2012) used H_2DCF-DA in Arabidopsis seedlings to monitor ROS production when plants were deprived of oxygen. Intriguingly, they observed an increase in DCF fluorescence that colocalized with mitochondria, suggesting low-oxygen-induced ROS production in or near mitochondria. The interpretation of localized ROS production is, however, complicated by the fact that DCF, like a number of other fluorescent dyes, tends to accumulate in mitochondria (Rashid and Horobin, 1991; Huang et al., 2016). Similar to the observations of Chandel et al. (1998), AA treatment mimicked this effect, while myxothiazol, a chemically distinct complex III inhibitor that does not elevate superoxide production at the N site, did not. Increased production of superoxide at mitochondrial complex III in response to low oxygen thus appears conserved in animals and plants. In a plant that is subjected to submergence, this elevation is likely to occur in a transient fashion; however, as ROS formation from oxygen becomes chemically implausible when oxygen availability is strongly reduced and even impossible under bona fide anoxia, i.e. the complete absence of oxygen (Sasidharan et al., 2017). A sustained increase in ROS production as observed in different plant species (Yan et al., 1996; Liu et al., 2012a; Vergara et al., 2012) is unlikely to originate from the mitochondria and more likely the result of respiratory burst NADPH oxidase activity at the plasma membrane. Alternative mechanisms of increasing in steady-state ROS levels, e.g. due to progressing failure of NADPH-dependent ROS detoxification systems or as a result of other metabolic changes, such as the drop in adenylate energy charge and acidification of the cytosol (Blokhina et al., 2003), have rarely been considered. Li et al. (2013) proposed that superoxide in rice triggers a mitochondrial retrograde response. They induced superoxide production through MV treatment and found that the induction of *OsAOX1a* as a retrograde marker was much more pronounced than following H_2O_2 treatment. The induction through MV was independent of light, suggesting that superoxide formation at complex I of mitochondria and not chloroplast was responsible for *OsAOX1a* elevation. Further, the overexpression of SOD in mitochondria, but neither in the chloroplasts nor cytosol, and application of TEMPOL (an SOD mimetic) dampened the transcriptional response to drought, cold, and salinity. The effect of mtSOD was, however, not tested in response to direct MRR triggers such as AA. In Arabidopsis, the transcriptional landscape in a SOD knockout mutant did not obviously overlap with AA-responsive genes such as *AOX1a*, questioning a conserved function throughout plants (Schwarzländer et al., 2012a).

Hydrogen peroxide (H_2O_2) has a comparatively long half-life in the cell with 1 ms as a very rough estimate (Dat et al., 2000). As such, steady-state concentrations of H_2O_2 are about three orders of magnitude higher than those of superoxide. Aquaporin-facilitated diffusion of uncharged H_2O_2 across membranes (Bienert and Chaumont, 2014) may add to its role as a cellular signaling molecule. Recently, H_2O_2 has been proposed as a mobile signal in the chloroplast retrograde response, where it originates from the organelle, moves to the nucleus, and might directly act on transcription factors (Exposito-Rodriguez et al., 2017). Exogenous application of H_2O_2 in cell suspensions of Arabidopsis and *Chlamydomonas* has been shown to induce mitochondrial retrograde marker transcripts such as *AOX1a* and *NDB2* (Vanlerberghe and McLntosh, 1996; Ho et al., 2008; Blaby et al., 2015). A large overlap exists also between the transcriptional footprints of H_2O_2 and AA in Arabidopsis (Ng et al., 2013b; Huang et al., 2016), making H_2O_2 a strong candidate for transmitting plant MRR signals. While the cascade from H_2O_2 production to transcriptional reprogramming via the nucleus requires further clarification, organelle function can also be more directly adjusted by posttranslational

modifications. Mitochondrial citrate synthase, for instance, can be directly inactivated by H_2O_2 and is activated through interaction with and reduction by thioredoxins (Schmidtmann et al., 2014). Redox regulation was also demonstrated for other plant TCA cycle enzymes (Daloso et al., 2015) and previously for the AOX (Vanlerberghe et al., 1995). While oxidation of the enzyme thiols directly by H_2O_2 is kinetically unlikely in vivo, H_2O_2 may selectively react with the high-reactivity thiols of the mitochondrial thiol peroxidase system (e.g. Prx IIF), transferring the oxidation via the thioredoxin system to the different enzyme targets. Thiol-switching of mitochondrial enzymes could act as a direct signal to readjust organellar function on site even before and not necessarily linked directly to transcriptional programs.

Nitric Oxide

The plant hypoxic response is centrally regulated through the transcription factor RELATED TO APETALA (RAP) 2.12 that interacts with a hypoxia responsive promoter element conserved across plant species (Gasch et al., 2016). It controls mitochondrial respiration and TCA cycle enzymes, offering a genetic handle to adjust mitochondrial function when oxygen becomes limiting (Paul et al., 2016). RAP2.12 undergoes proteasome-mediated proteolysis through the N-end rule pathway under normoxic conditions and is protected from degradation under hypoxia (Gibbs et al., 2011; Licausi et al., 2011). Mitochondria could provide stability to RAP2.12 through nitric oxide (NO) production. NO production by the plant mtETC is elevated under hypoxia (reviewed by Hebelstrup and Møller, 2015). Alber et al. (2017) recently proposed that NO generation happens at complex III, while AOX dampens NO generation rate. Gibbs et al. (2014) further showed that NO acts in the stabilization of RAP2.12, and this requires cytosolic nitrate reductase activity in vivo. As nitrate reductase in the cytosol could theoretically fuel mitochondrial NO production (via nitrite), this finding does not rule out that mitochondrially produced NO acts in RAP2.12-mediated hypoxic signaling. Isolated pea root mitochondria were found to generate NO under hypoxic conditions when nitrite is present, which had stabilizing impact on mitochondrial function (Gupta et al., 2017). A key protective mechanism of NO was recently identified in mouse, where a specific Cys of complex I subunit ND3 is S-nitrosated to prevent reverse electron flow and excess mtROS production at reoxygenation (Chouchani et al., 2013). This Cys is highly conserved, suggesting a similar mechanism to operate also in plants (Braun et al., 2014). A nitrate reductase mutant was also used by Gupta et al. (2012) to suggest that NO inhibits activity of the TCA cycle enzyme aconitase. This block resulted in citrate accumulation as a potential retrograde and NO-mediated energy signal in hypoxic plants. Aconitase activity is further decreased through superoxide and H_2O_2 and may be considered an integration point of plant oxidative stress (Sweetlove et al., 2002).

Posttranslational Modification of Proteins

More direct regulation could be achieved through protein succinylation. In animals and plants, succinate accumulates under hypoxia (Rocha et al., 2010; Sweetlove et al., 2010; Narsai et al., 2011; Chouchani et al., 2014; António et al., 2016). In animals, succinate can indirectly stabilize the 1α subunit of the transcriptional regulator hypoxia-inducible factor, and succinylation, which relies on succinyl-CoA as substrate, regulates TCA cycle and glycolytic enzymes (Mills and O'Neill, 2014). Interestingly, proteins active in the TCA cycle and glycolysis have also been found succinylated in tomato (*Solanum lycopersicum*), wheat (*Triticum durum*), rice (*Oryza sativa*), and *Brachypodium*, suggesting a conserved role of succinate and succinyl-CoA in the regulation of metabolic processes (He et al., 2016; Jin and Wu, 2016; Zhen et al., 2016; Zhang et al., 2017c). Other posttranslational modifications such as protein phosphorylation (e.g. through SnRK1, as discussed by Wurzinger et al. [2018] in this Focus Issue) and protein Lys acetylation also critically rely on substrates that can be derived from mitochondrial energy metabolism, i.e. ATP and acetyl-CoA. Both classes have been discussed as signals in the retrograde response (Hartl and Finkemeier, 2012), but their contributions are currently largely unknown (Ng et al., 2013a; Blanco et al., 2014).

ATP

Lack of oxygen as the terminal electron acceptor in oxidative phosphorylation results in a rapid drop of MgATP concentration in the cytosol, which is otherwise comparatively well buffered (De Col et al., 2017). As alternative ATP sources activated under hypoxic conditions are comparatively inefficient, this ultimately results in an energy crisis of the cell (van Dongen and Licausi, 2015). Notably, the adenylate energy state is already changed very early at the onset of hypoxia, when oxygen concentrations are still considerably above the K_m of cytochrome *c* oxidase (complex IV) for oxygen, suggesting that in principle oxidative phosphorylation at the mETC could be maintained even under relatively strong hypoxic conditions. The kinetic properties of complex IV, however, have been shown to directly depend on molecular oxygen, and in vivo flux through the cytochrome *c* oxidase is expected to gradually decrease at oxygen concentrations above a theoretically achievable K_m (Chandel et al., 1996). Adenylate changes at the onset of low-oxygen stress have been additionally explained by *proactive* regulation that rely on proteins other than complex IV as oxygen sensors, and recent studies have helped to gain mechanistic insights (Geigenberger, 2003; Weits et al., 2014; António et al., 2016). In the retrograde response of budding yeast (*Saccharomyces cerevisiae*), ATP can act as a signaling

trigger to adjust metabolic processes according to energy availability. The transcription factors RTG1 and RTG3, responsible for transcriptomic reprogramming, require activation through RTG2. RTG2 has an N-terminal ATP-binding domain, and ATP-binding in yeast releases RTG2 from another protein, MKS1P, that is a cytosolic dephosphorylase and acts on RTG3 to make the RTG1/RTG3 complex translocate to the nucleus for genomic control (Zhang et al., 2013). In Arabidopsis, inhibition of the ATP synthase through oligomycin transcriptionally activates retrograde markers (e.g. *AOX1a* and *NDB2*) and overlaps markedly with the transcript responses to different abiotic and biotic stresses (Geisler et al., 2012; Fig. 1, C and D). Whether ATP and/or the ATD/ADP/AMP ratios are directly involved in activating those responses is, however, not known.

Phosphoadenosine Phosphate

PAP (3′-phosphoadenosine 5′-phosphate) has been proposed as an active signal in the retrograde response of chloroplast and mitochondria. PAP is dephosphorylated by SAL1 to form AMP and SAL1 is a dual-localized protein that resides in both chloroplasts and mitochondria (Estavillo et al., 2011). In yeast, PAP inhibits nuclear 5′ to 3′ exoribonucleases (XRNs), thereby modulating RNA catabolism and transcript abundances of XRN targets. Accordingly, Arabidopsis *sal1* mutants that accumulate PAP show transcriptional changes largely overlapping with *xrn2 xrn3* double-mutant plants (Gregory et al., 2008; Estavillo et al., 2011). A significant number of these transcripts is also changed under specific *AOX1a*-inducing conditions including high light, drought, and ABA treatment, pointing to a function in retrograde stress signaling. The idea of PAP acting as a direct signal in this response is supported by the fact that loss of *SAL1* in both plastids and mitochondria can be compensated by artificial expression of SAL1 in the nucleus (Estavillo et al., 2011). Furthermore, there is significant overlap between the transcriptional signatures of PAP-related mutants *sal1* and *xrn2 xrn3* with MRR-inducing conditions such as AA, *atphb3*, and *rpotmp* mutants (Van Aken and Whelan, 2012; Van Aken and Pogson, 2017). As many of these commonly regulated genes are ANAC017 dependent, it was recently suggested that the PAP and ANAC017 pathways are mechanistically linked (Van Aken and Pogson, 2017). At least part of this common pathway appears capable of suppressing cell death and lesion formation, potentially by suppressing, e.g., extrusion of toxic compounds such as chlorophyll degradation products (Bruggeman et al., 2016; Van Aken and Pogson, 2017).

Mitochondrial Membrane Potential

In yeast, the mitochondrial membrane potential has been proposed to be central in triggering the mitochondrial retrograde response. Miceli et al. (2012) modulated the membrane potential of *Saccharomyces cerevisiae* strain rho^0 lacking mitochondrial DNA by expressing either a mutated F1-ATPase that results in an elevated membrane potential or by deleting *COX4* to reduce the membrane potential. Hyperactivity of the F1-ATPase suppressed translocation of RTG3 into the nucleus and thus dampened the MRR. Reduction of the membrane potential, conversely, elevated the response. This was independent of increasing concentrations of a free radical scavenger, indicating that indeed the membrane potential and not ROS formation downstream of that, triggered up-regulation of retrograde marker genes such as *CIT2*. The interplay between ROS formation, the mitochondrial membrane potential, and ATP production complicates making statements about cause and effect in mitochondrial retrograde signaling, also in plants. This was nicely illustrated by Krause and Durner (2004), who treated Arabidopsis cells in suspension with a bacterial elicitor. The activated defense response decreased the mitochondrial membrane potential and total ATP levels and led to an accumulation of H_2O_2 and, ultimately, *AOX1a* transcript. A better understanding of the sequence of events, which we can similarly assume to happen in hypoxia, is still required.

Calcium Ions

Ca^{2+} is involved in the mammalian mitochondrial retrograde response (Butow and Avadhani, 2004), where Ca^{2+} concentrations are linked to ATP production (Griffiths and Rutter, 2009). In both mammals and plants, a uniporter complex is involved in mitochondrial Ca^{2+} import (Kamer and Mootha, 2015; Wagner et al., 2015; Teardo et al., 2017), which makes use of the mitochondrial membrane potential. Ca^{2+} transients in the mitochondrial matrix have been shown to occur in response to various abiotic stresses (Logan and Knight, 2003; Loro et al., 2012) that also activate an MRR. Treatment of tobacco cells with Ca^{2+} channel blockers (ruthenium red and $LaCl_3$) suppressed the induction of AOX capacity at AA treatment, suggesting a contribution of Ca^{2+} to mitochondrial stress signaling (Vanlerberghe et al., 2002). Ruthenium red also partially suppressed *AOX1* induction in rice and Arabidopsis at more general stress such as cold, drought, salinity, and hypoxia (Tsuji et al., 2000; Vanderauwera et al., 2012; Li et al., 2013). Conversely, addition of cyclopiazonic acid (an ER calcium channel inhibitor that results in increased cytosolic Ca^{2+} concentration in animal cells) intensified *AOX1a* gene expression induction by salt (Vanderauwera et al., 2012). Hypoxia has been suggested to drive Ca^{2+} extrusion from mitochondrial stores in cultured maize cells (Subbaiah et al., 1998), but supporting experimental data are scarce. Conceptually, the existence of such Ca^{2+} stores in the matrix under nonpathological conditions requires further scrutiny, considering a minor gradient in free Ca^{2+} concentration (about 100 nM in the cytosol versus

200 nM in the matrix at baseline; Wagner et al., 2015). Considering the highly negative potential across the inner mitochondrial membrane, this strongly favors uptake rather than release of Ca^{2+}. A breakdown of membrane potential due to sustained hypoxia may allow for Ca^{2+} release and mobilize the considerable total amounts of bound Ca^{2+} in the matrix. Even if such a scenario could be empirically confirmed, the small volume of mitochondria, as compared to the vacuole or the extracellular space, appear not optimal for mitochondria to act as stores for cytosolic Ca^{2+} signaling. Ca^{2+} release from mitochondria may rather represent a hallmark of cellular energy crisis and loss of homeostasis than contribute to a specific signal. Yet, the cytosolic increase in Ca^{2+} that has been consistently observed at hypoxia appears to be conserved throughout plants and chemical suppression hampers transcriptional induction of hypoxia-responsive genes such as *ALCOHOL DEHYDROGENASE* (Subbaiah et al., 1994; Sedbrook et al., 1996; Yemelyanov et al., 2011). The mechanism by which Ca^{2+} fluxes modulate nuclear transcription is currently unknown.

Abscisic Acid

Levels of the phytohormone abscisic acid (ABA) are typically elevated under various stress conditions, including hypoxia, and induce *AOX1a* and other typical transcripts of the mitochondrial retrograde response (Giraud et al., 2009; Van Aken et al., 2009). ABA, although not ultimately synthesized in the plant organelles, has been proposed to act as an integrator of retrograde signals. A mitochondrial protein (RRL; RETARDED ROOT GROWTH-LIKE) has been suggested to mediate between ABA and the transcription factor ABI4. The overexpression of RRL markedly increased the transcriptional up-regulation of *ABI4* and *AOX1a* in response to ABA, while both transcripts were not ABA inducible in an *rrl* mutant (Yao et al., 2015). The link between ABA and ABI4 in that scenario might further involve WRKY40 that represses *ABI4* in the absence of ABA (Shang et al., 2010). ABA also suppresses biosynthesis of ethylene through ABI4-mediated transcriptional changes (Dong et al., 2016) and the interplay between ABA, ethylene, and also auxin is supposed to adjust plant growth under hypoxic conditions (Xu et al., 2013).

Auxin

Auxin, in contrast to ABA, represses *AOX1a*. Mutants compromised in auxin transport show elevated levels of *AOX1a* transcript at stress treatment. Conversely, treating plants with auxin represses the induction of *AOX1a*, e.g. upon AA-induced mitochondrial dysfunction (Ivanova et al., 2014; Kerchev et al., 2014). He et al. (2012) characterized a mitochondrial DEXH RNA helicase (ABO6) that is necessary for proper splicing of multiple mitochondrial complex I subunits. Upon *ABO6* loss, mitochondria were proposed to produce ROS at elevated levels that were further modulated by exogenous application of ABA and auxin. The authors proposed that both hormones act as mitochondrial signals through ROS formation. Those conclusions will require future reassessment because the employed ROS-detection methodology based on the cpYFP sensor protein has since proven inappropriate (Schwarzländer et al., 2014; Demaurex and Schwarzländer, 2016). A potential link between mitochondrial ROS and auxin is independently supported by Arabidopsis plants lacking the mitochondrial FTSH4 AAA-protease that is required for the assembly and/or stability of complex I and, even more, complex V. Similar to *abo6* mutants, auxin accumulation or responsiveness was reduced in these plants, and external application of auxin could revert the phenotypic symptoms of *ftsh4* mutant plants (Zhang et al., 2014). Interestingly, *ftsh4* plants also accumulated salicylic acid, suggesting plant mitochondrial signaling is linked to hormonal regulation at

OUTSTANDING QUESTIONS

- What are the upstream stimuli and second messengers of mitochondrial signaling? Which of them are shared between retrograde signaling from the chloroplasts and the mitochondria, considering that several regulators of nuclear gene expression are shared by the pathways from both organelles?
- How can retrograde signaling responses from both organelles be integrated and coordinated to act concertedly under stress situations, e.g. in flooded leaves between light and dark?
- How is specificity achieved considering the extensive crosstalk between different layers of mitochondrial energy signaling (e.g. phytohormones and transcription factors)?
- What are the stress conditions under which the mitochondrion acts as a primary site of stress perception and what is the contribution of mitochondrial energy signaling to the cellular stress response?
- What are the mechanisms by which plants integrate the response to multiple stresses with primary effects on mitochondrial function, such as low oxygen and high salinity, e.g. in sea water flooding?
- What is the causal and temporal relationship between the changes in mitochondrial energy physiology and signaling that occur at respiratory inhibition and low oxygen stress?
- To what extent can mitochondria as primary oxygen consumers contribute to cellular oxygen sensing? How can a gradual decrease of oxygen be mechanistically perceived at the organelle?

several levels. It appears that the constant balancing act between fast growth during optimal conditions and reduced growth/stress response during challenging conditions may be achieved by antagonistic relationships between for instance auxin (growth promoting) and MRR (stress-response promoting; Ivanova et al., 2014; Kerchev et al., 2014).

CONCLUSION

The understanding of mitochondrial signaling has seen major advances in recent years. More and more protein players of mitochondrial dysfunction signaling are being identified, and a complex signaling network is emerging that integrates and coordinates a wide range of seemingly distinct cellular function (Ng et al., 2014). Yet, little is known about the natural circumstances under which mitochondrial signaling operates to the advantage of the plant. Comparative transcriptomics provide first insights and hint to a role of mitochondrial energy signaling in different stress scenarios and hypoxia in particular. Yet, the complex signaling network that separates primary stimuli from gene expression response can hamper unambiguous deduction of a natural condition from a transcriptome response. Hence, the upstream stimuli in response to a naturally occurring transition deserve focused investigation. The capture of those changes at the right time and in the correct place, including the mitochondria, has been limiting, which is why the primary events of mitochondrial signaling remain unknown, similarly to those of most stress-signaling pathways (Zhu, 2016). The usage of genetically encoded biosensors has the potential to overcome several critical hurdles and deliver information from the upstream end of signaling, similarly to what transcript analysis achieves at the downstream end. Sensors for some of the promising candidates of mitochondrial signaling stimuli have been created or adapted for use in plants and an understanding of the cell-compartment-specific in vivo dynamics of several of the molecules discussed here has moved into reach, including H_2O_2, ATP, and Ca^{2+} (Costa et al., 2010; Loro et al., 2012, 2016; Bonza et al., 2013; De Col et al., 2017). A next major step forward is likely to come from measuring the dynamics of a large array of chemical species in concert. *Multiplexing* approaches will resolve the order of events and help to dissect cause from coincidence as well as the interconnectivity of the physiological network that feeds into the signaling network through specific messengers. Recent in vivo sensing approaches have unearthed first links between membrane potential, pH, Ca^{2+}, ABA, and GA, although more than two or three parameters have rarely been assessed at a time (Schwarzländer et al., 2012b; Rizza et al., 2017; Waadt et al., 2017). Breaking those limitations and driving sensor multiplexing toward the *omics* scale of small molecular signal candidates would enable us to get direct and specific fingerprints of any transition, may it be a stress stimulus or the effect of a specific inhibitor. This would not only elucidate upstream triggers of signaling but also discriminate different scenarios of mitochondrial signaling. Eventually, this opens the door to reliably match different mitochondrial energy signaling modes with the natural conditions they have evolved for in the plant.

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