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REVIEW

Novel connections in plant organellar signalling link different stress responses and signalling pathways

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Abstract

To coordinate growth, development and responses to environmental stimuli, plant cells need to communicate the metabolic state between different sub-compartments of the cell. This requires signalling pathways, including protein kinases, secondary messengers such as Ca^{2+} ions or reactive oxygen species (ROS) as well as metabolites and plant hormones. The signalling networks involved have been intensively studied over recent decades and have been elaborated more or less in detail. However, it has become evident that these signalling networks are also tightly interconnected and often merge at common targets such as a distinct group of transcription factors, most prominently ABI4, which are amenable to regulation by phosphorylation, potentially also in a Ca^{2+}- or ROS-dependent fashion. Moreover, the signalling pathways connect several organelles or subcellular compartments, not only in functional but also in physical terms, linking for example chloroplasts to the nucleus or peroxisomes to chloroplasts thereby enabling physical routes for signalling by metabolite exchange or even protein translocation. Here we briefly discuss these novel findings and try to connect them in order to point out the remaining questions and emerging developments in plant organellar signalling.

Key words: Acclimation to stress, calcium signal, chloroplast, mitochondria, phosphorylation, photosynthesis, reactive oxygen species, retrograde signalling, signalling, transcription factor.

Organelles and signalling

The compartmentalization of cellular domains in membrane-enclosed organelles is a hallmark of eukaryotic cells. On average, the membrane-enclosed compartments together occupy nearly half the volume of a cell (Alberts *et al.*, 2014). This allows separation of different cellular activities, such as metabolic pathways and their independent regulation, and facilitates certain biochemical reactions by increasing concentrations of metabolites in a smaller volume. On the other hand, this spatial separation requires also coordination of enzymatic activities and intense transport of metabolites as well as of proteins (Jarvis and Lopez-Juez, 2013; Murcha *et al.*, 2014; Rolland *et al.*, 2012; Sweetlove and Fernie, 2013).

Chloroplasts and mitochondria are organelles of endosymbiotic origin (Margulis, 1970), which means that they have transferred most of their genes to the nucleus of the eukaryotic host during evolution (Gould *et al.*, 2008; Martin *et al.*, 1998, 2002). However, at least for chloroplasts, most of these genes of true endosymbiotic origin that have been transferred to the host's nucleus do still function within the organelles (Bayer *et al.*, 2014). Therefore, different protein targeting mechanisms

Abbreviations: ABA, abscisic acid; ABI4, ABA INSENSITIVE4; AOX1, ALTERNATIVE OXIDASE1; CDPK, calcium-dependent protein kinase; ER, endoplasmic reticulum; flg22, flagellin 22; FLS2, FLAGELLIN SENSING2; GUN, GENOMES UNCOUPLED; JA, jasmonic acid; MAPK, mitogen-activated protein kinase; MEcPP, methylerythritol cyclodiphosphate; MeJa, methyl jasmonate; NPQ, non-photochemical quenching; NRIP1, nuclear receptor-interacting protein 1; PAP, 3′-phosphoadenosine 5′-phosphate; PET, photosynthetic electron transport; PS, photosystem; ROS, reactive oxygen species; SA, salicylic acid; TF, transcription factor.

had to be introduced in the course of evolution (Jarvis and Lopez-Juez, 2013; Murcha *et al.*, 2014). Nevertheless, the nucleus is not the only warehouse of genetic information, as chloroplasts and mitochondria kept a small portion of their own genome. This generated a need for bi-directional communication between organelles and the nucleus in order to coordinate gene expression and to ensure correct functioning of the overall cellular metabolism. The process by which the nucleus regulates organellar functions is referred to as anterograde signalling and is based on the delivery of (precursor) proteins to the organelle. Signalling in the opposite direction (i.e. organelle to nucleus) is referred to as retrograde signalling and is far less understood although its effects have been known for decades (Fig. 1). The first evidence for retrograde plastid-to-nucleus signalling was obtained in the *albostrians* barley mutant where impaired plastid protein synthesis also affected the cytoplasmic protein synthesis (Bradbeer *et al.*, 1979). Later, it was also observed that mutations in the mitochondrial genome affect the expression of nuclear-encoded genes such as cytochrome c or subunits of the ATP synthase in yeast (Parikh *et al.*, 1987). Further studies shedding light on the retrograde signalling pathways were then mostly based on chemical perturbation of plastid processes in plants (Woodson and Chory, 2008) or took advantage of yeast genetics for elucidating mechanisms of the mitochondrial retrograde signalling (Liu and Butow, 2006). Blocking plastidial translation with the antibiotic Lincomycin or photosynthesis with the herbicide Norflurazon results in a downregulation of nuclear-encoded photosynthetic genes, and was used for forward genetic screens. This resulted in the discovery of the *GENOMES UNCOUPLED* (*GUN*) mutants, in which the expression of the nuclear encoded *LIGHT-HARVESTING COMPLEX 1b* (*LHCB1*) gene is not repressed in response to Lincomycin treatment (Susek *et al.*, 1993). Today various retrograde signalling molecules have been described in plants, involving 'classical' retrograde signalling metabolites such as tetrapyrrol intermediates or heme (reviewed in Pogson *et al.*, 2008; Woodson and Chory, 2008), which are often linked to chloroplast development and thus classified as 'biogenic' control signals. Additionally, 'operational' signals from the plastid (Pogson *et al.*, 2008) are linked to plastid metabolism like the redox status of the chloroplast (Pfannschmidt, 2003; Pfalz *et al.*, 2012) or reactive oxygen species (ROS) (Kim and Apel, 2013); the role of ROS for organellar signalling is, for example, reviewed in Galvez-Valdivieso and Mullineaux (2010) and is also the topic of another review in this special issue (Mignolet-Spruyt *et al.*, 2016). More recently, secondary metabolites also have been discovered as retrograde plastid signals, such as the isoprenoid precursor methylerythritol cyclodiphosphate (MEcPP) (Xiao *et al.*, 2012), 3′-phosphoadenosine 5′-phosphate (PAP) (Estavillo *et al.*, 2011) and oxidation products of carotenes, such as the volatile β-cyclocitral (Ramel *et al.*, 2012). Last but not least, proteins have been identified as retrograde signalling molecules (Caplan *et al.*, 2008; Sun *et al.*, 2011; Isemer *et al.*, 2012). As a great number of comprehensive reviews is available on this topic (for recent reviews see for example Chi *et al.*, 2013*a*; Bobik and Burch-Smith, 2015; Chan *et al.*, 2016), we will not go further into detail here but rather try to indicate the remaining open questions or emerging novel trends.

In contrast to the chloroplast, where much progress has been made in the identification of retrograde signalling molecules, the nature of mitochondrial retrograde signalling is still not well understood in plants (Ng *et al.*, 2014; Welchen *et al.*, 2014). Notably, chemical perturbation of organellar functions was used to study retrograde signalling not only in chloroplasts, but also in mitochondria. Chemical inhibition of electron transport through the respiratory chain induces the expression of the *ALTERNATIVE OXIDASE 1a* (*AOX1a*) gene (Vanlerberghe, 2013), and a promoter analysis indicated the transcription factor (TF) ABA INSENSITIVE 4 (ABI4) as an important regulator of *AOX1a* (Giraud *et al.*, 2009). This is further supported by the observation that the respiratory chain inhibitor rotenone induces *AOX1a* expression in wild-type plants but not in *abi4* mutants. Also under certain stress conditions such as nutrient deficiency, cold, drought,

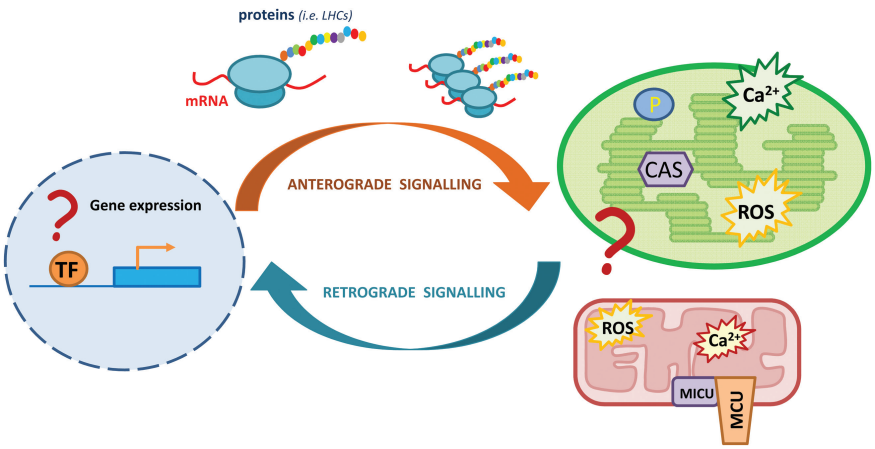

Fig. 1. Overview of cellular signalling pathways and open questions. This scheme illustrates the principle of anterograde and retrograde signalling in plant cells and depicts the two major questions of current research, which will be discussed in the text: (i) how are the retrograde signals generated by the organelles (i.e. chloroplasts and mitochondria), and (ii) how are the signals decoded in the nucleus? Additionally, some major players in these signalling processes are depicted. TF, transcription factor; CAS, chloroplast calcium sensor protein; MCU, mitochondrial uniporter; MICU, mitochondrial calcium uptake regulatory protein.

limiting oxygen availability and pathogen attack, mitochondrial functions are affected, which activates retrograde signalling to alter mitochondrial gene expression. In a recent transcriptomic analysis covering 27 different mitochondrial and chloroplast perturbations, a set of 12 nuclear-encoded mitochondrial genes that exclusively respond to mitochondrial perturbations was identified (Van Aken and Whelan, 2012). Mitochondrial stress responsive genes encode proteins of the mitochondrial dysfunction stimulon comprising parts of the alternative respiratory chain, heat shock proteins, and ROS marker genes. All those genes share a common motif in their promoter, called mitochondrial dysfunction motif (MDM), which is recognized by TFs of the ATAF1/2, NAC, NAM, and WRKY families (De Clercq *et al.*, 2013). Interestingly, among those, a group of NAC TFs seems to be bound to the ER-membrane and is released by the presence of ROS, suggesting this translocation of the TFs as a possible mechanism for mitochondria-to-nucleus communication (Ng *et al.*, 2014). Finally, there is also cross-talk between mitochondrial and chloroplast retrograde signalling: the impaired chloroplast development in the *albostrians* barley mutant affects mitochondrial transcripts (Hedtke *et al.*, 1999) and, vice versa, alterations in mitochondrial metabolism and physiology alter gene expression of nuclear-encoded photosynthetic genes (Schwarzländer *et al.*, 2012).

In general, the fundamental question in retrograde signalling is how the organelle connects its prokaryotic regulatory machinery to the eukaryotic signalling and gene regulation network of the host cell (Fig. 1). This question can be further subdivided into two key questions, which are still largely unanswered: (i) How is the signal generated in/by the organelle? (ii) How is the signal decoded by the nucleus of the host cell?

Retrograde signalling – where does the signal come from?

Carbohydrates are the end products of photosynthesis, and their accumulation has a negative effect on the expression of photosynthetic genes. Using a maize protoplast assay, Sheen (1990) showed that the transcriptional activity of seven photosynthetic genes was repressed by sucrose and glucose and indeed, sugar signals have been linked to plastidial signalling (Oswald *et al.*, 2001). This fits well to the proposal that metabolic signatures are recognized as retrograde signal (Pfannschmidt, 2010). This idea was further supported most recently by the observation that fast retrograde signals in response to high light mediated by the MITOGEN ACTIVATED PROTEIN KINASE 6 (MPK6) and AP2/ERF TFs depend on chloroplast metabolite export via the TRIOSE PHOSPHATE/PHOSPHATE TRANSLOCATOR (TPT) (Vogel *et al.*, 2014). A comprehensive analysis of transcriptional and metabolic acclimation of a TPT and ADP-GLUCOSE PYROPHOSPHORYLASE (AGPase) (*adg1-1 tpt-2*) double mutant in the high light acclimation uncovered several genes involved in retrograde control of nuclear gene expression (Schmitz *et al.*, 2014). Similarly, an accumulation of maltose in a maltose exporter mutant (*mex1*) causes chloroplast dysfunction, which may be signalled via retrograde signalling pathways (Stettler *et al.*, 2009).

The key role of the redox status in the regulation of all kinds of chloroplast processes has long been recognized (for a recent review see Balsera *et al.*, 2014) and soon after the initial discovery of thylakoid protein phosphorylation (Bennett, 1977), redox regulation was conceptually linked to these light-dependent phosphorylation events (Allen *et al.*, 1981). Furthermore, redox control has been proposed as the driving force for the maintenance of organellar genomes (Allen, 2015). The redox state of the chloroplast was shown to regulate nuclear-encoded plastid gene expression (Pfannschmidt *et al.*, 1999) and was linked to state transition, an acclimation of photosynthetic light-harvesting to different light qualities, mediated by phosphorylation of light-harvesting complex proteins. With the identification of the responsible state transition kinases, first in Chlamydomonas (Depege *et al.*, 2003) and later in Arabidopsis (Bellafiore *et al.*, 2005), the first molecular players in this process were defined. In Arabidopsis the state transition kinases STN7 and STN8 were shown to mediate short-term as well as long-term acclimation responses of chloroplasts to changing environmental signals (Bräutigam *et al.*, 2009; Rochaix, 2014). However, the signal that is sent from the chloroplast to the nucleus to mediate this response is still unknown.

The chloroplast is also a major site of ROS production in plants (Noctor *et al.*, 2002), and ROS were increasingly recognized as signalling molecules (Apel and Hirt, 2004; Shapiguzov *et al.*, 2012). For some time ROS have been discussed as retrograde signals in NF-treated plants, but their function as retrograde signals depend on the developmental stage. Only when seedlings were exposed to NF after the light-dependent chloroplast formation had been completed, could enhanced ROS production be detected and retrograde signalling occur (Kim and Apel, 2013). Notably, cytoplasmic ROS signals were shown to activate MPK3/MPK6 (Kovtun *et al.*, 2000). Recently it was shown that in Arabidopsis mutants lacking a functional AtRBOHD (subunit of plasma membrane NADPH oxidase), the flagellin-induced ROS burst was completely blocked but the activation of MPK3/MPK6 was not affected. This indicated that the rapid ROS burst and MAP kinase activation occur independently downstream of flagellin receptor FLS2 (Xu *et al.*, 2014). This could therefore point to chloroplasts as a source of the ROS that activate the MPKs. The role of chloroplasts for plant immune signalling has recently been reviewed (Stael *et al.*, 2015) and is also covered in another review of this special issue (Serrano *et al.*, 2016).

Another signal coming from the chloroplast in response to stress involves the secondary messenger calcium. The concentration of free Ca^{2+} ions in cellular compartments is tightly regulated and the differences in Ca^{2+} levels allow generation of calcium fluxes upon perception of environmental stimuli (Dodd *et al.*, 2010; Stael *et al.*, 2012*b*). Biotic and abiotic stress conditions also evoke calcium transients in chloroplasts, mitochondria and the nucleus (Ma *et al.*, 2009; Manzoor *et al.*, 2012; Nomura *et al.*, 2012; Sano *et al.*, 2014) but their role in

activation of responses is still unclear. Although the role of calcium in chloroplasts for a number of different processes (e.g. water splitting at PSII, Calvin-Benson cycle) has been known for ~30 years (reviewed in Stael *et al.*, 2012*b*), chloroplast calcium signals were only reported in 2002 after light-dark transitions (Sai and Johnson, 2002). A functional link to plant immunity was reported in 2012 when Nomura and colleagues found that flg22-induced cytosolic Ca^{2+} signatures are rapidly translated into calcium transients in chloroplasts (Nomura *et al.*, 2012). The chloroplast calcium sensor protein CAS was identified as an important regulator for the generation of the calcium signature in the plastid and the cytosol as well as in the induction of salicylic acid (SA) accumulation. Additionally, CAS was found to be phosphorylated in a Ca^{2+}-dependent manner (Stael *et al.*, 2012*a*). Evidence for a crucial role of CAS in the generation and/or regulation of cytoplasmic Ca^{2+} responses was provided already before (Nomura *et al.*, 2008; Weinl *et al.*, 2008). While the molecular function of the CAS protein is still enigmatic in higher plants, CAS has been implicated in light acclimation of photosynthesis in the algae *Chlamydomonas rheinhardtii*. Chlamydomonas CAS knockdown lines were unable to induce the expression of the LHCSR3 protein, which is required for non-photochemical quenching (NPQ), a high-light protection response, as well as cyclic electron flow (CEF). Importantly, this inhibition was rescued by increasing the extracellular Ca^{2+} concentration (Petroutsos *et al.*, 2011; Terashima *et al.*, 2012). CEF has been described as an acclimation response to adverse environmental conditions (Eberhard *et al.*, 2008) and it has been suggested that CAS and Ca^{2+} (via CEF) could activate $^1O_2$-mediated retrograde signalling (Terashima *et al.*, 2012). The role of chloroplast calcium signals in the regulation of photosynthesis and plant innate immunity was recently reviewed (Hochmal *et al.*, 2015; Stael *et al.*, 2015). Moreover, in a quantitative proteomics analysis of responses governing acclimation to iron deprivation and regulation associated with photosynthesis-dependent growth in Chlamydomonas (Hohner *et al.*, 2013), Hippler and co-workers identified a calcium-binding type of thioredoxin, which they called calredoxin. This protein would present a novel link between redox and Ca^{2+} regulation, but notably no homologues seem to exist in higher plants (Michael Hippler, pers. comm.).

In addition to rather small metabolites, for which either transporters facilitate their transport across organellar membranes such as PAP (Estavillo *et al.*, 2012) or which might even be membrane-permeable themselves (i.e. β-cyclocitral), proteins also were implicated in mediating retrograde signalling. As protein translocation into chloroplasts (or mitochondria) is evidently a one-way road, this raises the question how these proteins could act in the nucleus. Here a number of novel mechanisms have been discovered, which could provide an answer to this question. The TF PTM presents such an example of direct communication of the chloroplast state to the nucleus in order to change gene expression. PTM is normally localized at the chloroplast envelope and, upon a stimulus triggering *GUN1*-mediated retrograde signalling, PTM translocates into the nucleus where it enhances the expression of *ABI4* leading to the down-regulation of nuclear-encoded photosynthesis genes (Sun *et al.*, 2011). Another example is the TF WHIRLY1 that translocates from the chloroplast to the nucleus where it regulates the expression of pathogenesis related (PR) genes (Isemer *et al.*, 2012). A model has been proposed in which, upon sensing of SA-mediated changes in the redox state of the plastid, WHIRLY1 is released from a complex at the thylakoid membrane and is relocated into the nucleus to regulate transcription (Foyer *et al.*, 2014). Furthermore, the NUCLEAR RECEPTOR INTERACTING PROTEIN1 (NRIP1) that is normally localized in chloroplasts is recruited to the cytoplasm and the nucleus upon interaction with the 50-kDa helicase (p50) domain of *Tobacco mosaic virus* (TMV) (Caplan *et al.*, 2008). Recently, it was shown that stromules are involved in this translocation to the nucleus as well as the concomitant nuclear ROS production (Caplan *et al.*, 2015). Finally, an indirect but also chloroplast-mediated translocation of a transcriptional regulator has been reported downstream of abscisic acid (ABA) signal perception. The MAGNESIUM-PROTOPORPHYRIN IX CHELATASE H subunit (CHLH/ABAR) was proposed as ABA receptor spanning the chloroplast envelope. The cytosolic C terminus of ABAR interacts with a group of WRKY TFs (WRKY40, WRKY18 and WRKY60), that function as negative regulators of ABA signalling and repress the expression of ABA-responsive genes. High levels of ABA promoted the ABAR-WRKY40 interaction and resulted in the recruitment of WRKY40 from the nucleus to the cytosol, leading to a relief of *ABI5*-dependent repression of ABA target genes (Shang *et al.*, 2010). Protein translocation was found to occur also in mitochondrial retrograde signalling. The NAC domain-containing TFs ANAC013 and ANAC013 are targeted to the endoplasmic reticulum (ER) via a C-terminal transmembrane (TM) domain. Upon protease cleavage the N-terminal part is released and migrates to the nucleus in the mitochondrial retrograde response (De Clercq *et al.* 2013; Ng *et al.*, 2013). Strikingly, also here the WRKY TFs WRKY40 and WRKY63 play a major role in the regulation of the target genes (Van Aken *et al.*, 2013).

Retrograde signalling – many pathways but how is the signal decoded in the nucleus?

Obviously, all retrograde signalling pathways have to somehow end up in the nucleus thus making TFs self-evident targets. However, one of the major obstacles in identifying specific targets was based on the fact that almost all studies of retrograde signalling were carried out using either inhibitors of organellar functions such as Lincomycin or Norflurazon, or constitutive mutations affecting organellar functions, thereby focusing on the endpoints of perturbations (Pesaresi *et al.*, 2006). This made it very challenging to disentangle the primary and secondary mechanisms involved. To overcome this problem, Leister *et al.* (2014) used an inducible RNAi approach to demonstrate that changes in organellar gene expression (OGE) directly trigger retrograde signalling. The authors perturbed OGE in adult Arabidopsis plants by specifically down-regulating the chloroplast and mitochondrial

PROLYL-tRNA SYNTHETASE (PRORS1) and found that >1000 genes responded to this treatment within 2–3 d after starting the induction of repression. Given that it will take some time before the repression will lead to a significant effect, this indicates a direct coordination of chloroplast and nuclear photosynthetic gene expression rather than a developmental response. The authors identified two promoter sequences in the affected genes, which are also present in light-regulated genes thus indicating a common signalling pathway between OGE- and light-dependent retrograde signalling. This has also been suggested previously based on the isolation of a new cryptochrome allele (*cry1*) from a screen for *gun* mutants (Ruckle *et al.*, 2007) and the observation that plastid signals and light operate via the same *cis*-acting elements (Kusnetsov *et al.*, 1996). Finally, the authors deduced TFs binding to the identified *cis*-elements of the genes responding to OGE perturbation, which uncovered members of the NAC family, bZIP, zinc-finger, Myb, CCAAT-binding, homeobox, AP2/ERF and WRKY TFs as putative primary targets.

Already some years ago, Koussevitzky *et al.* (2007) showed that three plastid-generated signals, namely Mg-protoporphyrin IX, plastid gene expression (PGE) and photosynthetic electron transport (PET), converge on the protein GUN1. *GUN1* encodes a chloroplast-localized pentatricopeptide-repeat (PPR) protein, which transmits chloroplast signals to the ERF/AP2 TF ABI4. Ultimately, ABI4 binds to the promoter of photosynthetic genes, regulating their expression. This work already implicated a common retrograde signalling pathway and indeed tetrapyrrole synthesis has been suggested as the primary signal for plastid-to-nucleus signalling (Terry and Smith, 2013). However, the nature of the signal that carries information between the chloroplast and nucleus remains unclear. Strikingly, over the past few years ABI4 turned out to be a key factor in signalling pathways downstream of ABA perception, SA perception, GUN1-dependent chloroplast retrograde signalling, the redox state of chloroplast, sugar signalling and mitochondrial retrograde signalling (Shang *et al.*, 2010; Kerchev *et al.*, 2011; Reeves *et al.*, 2011; Foyer *et al.*, 2012; Leon *et al.*, 2012). ABI4 was shown to be involved in crosstalk between ABA and sugar signalling (Rook *et al.*, 2006), and the carbon/nitrogen balance (Lu *et al.*, 2015). Finally, ABI4 was recently found to repress two key genes for ethylene synthesis (Dong *et al.*, 2016) and has now emerged as one of the major regulators of retrograde signals in the nucleus.

However, ABI4 is not the only TF implied in retrograde signalling. Many nuclear genes encoding organellar proteins that are involved in acclimation to environmental stress have been found to have a common putative WRKY TF-binding site. The Arabidopsis genome encodes >70 different WRKY TFs and many of them seem to play multiple roles in divergent stress responses (Bakshi and Oelmuller, 2014). WRKY40 and WRKY63 were found to modulate the expression of stress-responsive nuclear genes that encode mitochondrial and chloroplast proteins (Van Aken *et al.*, 2013). WRKY40, together with WRKY18 and WRKY60, regulates the expression of *ABI4* in ABA signalling (Shang *et al.*, 2010), and they are involved in the enhanced tolerance to biotic stresses as well as in regulation of crosstalk between SA- and JA- dependent pathways (Bakshi and Oelmuller, 2014). Additionally, WRKY15 functions in osmotic stress tolerance by affecting the mitochondrial retrograde signalling (Vanderauwera *et al.*, 2012). This broad regulation by the WRKY proteins may be possible through the formation of multimeric protein complexes where different WRKY combinations bind to different promoters, leading to distinct effects (Bakshi and Oelmuller, 2014).

The activity of WRKYs as well as other TFs is often regulated post-translationally, for example by phosphorylation or Ca^{2+} signals mediated by calmodulin (Van Aken *et al.*, 2013). Indeed, several WRKY TFs were identified as direct targets of MAPK pathways (Popescu *et al.*, 2009; Ishihama and Yoshioka, 2012) or CDPK pathways (Gao *et al.*, 2013), and modulation of WRKY activity by binding to calmodulin and calmodulin-binding proteins has been shown (Chi *et al.*, 2013*b*). Therefore, we exploited the PhosPhAT 4.0 database (http://phosphat.uni-hohenheim.de/) (Heazlewood *et al.*, 2008) to search for experimentally verified phosphorylation sites – or at least the presence of known consensus protein kinase target sites – in those TFs that are known to be involved in retrograde signalling. Strikingly, ten out of the twelve TFs we analysed (ABI4, ANAC029, ANAC032, ANAC44, MYB44, WRKY15, WRKY18, WRKY33, WRKY40, WRKY60, ZAT6, ZAT10) were found to be phosphorylated *in planta*, and 11 of them harboured MAPK consensus sites. For many of them phosphorylation by a MAPK – in most cases MPK6 – has even been shown experimentally (Table 1). However, sites other than MAPK sites were additionally found to be phosphorylated, which indicates that other protein kinases are involved in their regulation also, and in fact TFs can be targeted by several different kinases simultaneously (see, for example, Mair *et al.*, 2015). Thus, a set of distinct TFs involved in retrograde signalling, which are regulated by the eukaryotic signalling network of the 'host' cell (i.e. MAP kinases or Ca^{2+}-dependent events such as CDPKs), could represent one mechanism to link the 'prokaryotic' organellar functions to the 'eukaryotic' host cell.

Organellar signalling involves responses to different stresses

As discussed previously, in the past retrograde signalling was mostly studied using quite artificial experimental conditions such as treatments with antibiotics or herbicides. However, more recently it became clear that 'natural' environmental conditions such as different stresses trigger retrograde signalling also.

Photosynthetic efficiency can be negatively affected by abiotic stresses, such as heat, drought, cold, salinity, and high light. Changes in light quality and quantity require a rearrangement of the photosynthetic machinery in order to balance the excitation energy between PHOTOSYSTEM I (PSI) and PSII and maximize the photosynthetic yield and to avoid damage and ROS production as discussed before. To date, the responsible kinase for this process, the STATE TRANSITION

Table 1. *Transcription factors involved in retrograde signalling as potential phosphorylation targets*

Summary of phosphorylation sites of transcription factors (TFs) implicated in retrograde signalling according to the PhsphAT 4.0 database (Heazlewood *et al.*, 2008). 'Peptide' column refers either to known consensus sites (i.e. for MAP kinases) or to experimentally verified sites (in bold). 'Exp. data' column indicates if phosphorylation has been shown, and 'Kinase (pred.)' column specifies the kinase responsible or at least the predicted class of kinase. * Multiple MAPK sites present in sequence, in which case the attribution of individual sites is uncertain.

Transcription factor	AGI code	Peptide	Exp. data	Kinase (pred.)	Reference
ABI4*	At2g40220	NL**TP**S**SP**SSVSS	no	MAPK	PhosPhAT
ANAC029	At1g69490	RPNRAAVSG**Y**WK	yes	-	PhosPhAT
		NEWXYFF**SP**RER	no	MAPK	
ANAC032	At1g77450	PNRAAGTG**Y**WKA	yes	-	PhosPhAT
		KEWYFF**SP**RDRK	no	MAPK	
ANAC044*	At3g01600	NC**TY**RIDN**S**NVL	yes	-	PhosPhAT
		TVQAYGTGQRKR	yes	-	PhosPhAT
MYB44*	At5g67300	LRWCNQL**SP**QVE	yes	MPK3,6	(Nguyen *et al.*, 2012*a*)
		LYMSPG**SP**TGSD			
WRKY15*	At2g23320	RKCN**S**ENLLTGK	yes	-	PhosPhAT
		EEPKT**TP**FQ**SP**L	no	MAPK	
WRKY18	At4g31800	Q**SP**EIEQTDIPI	yes	MAPK	PhosPhAT
		LQSRQ**SP**EIEQT	no	MAPK	
WRKY33*	At2g38470	STS**S**LEDLEIPK	yes	-	PhosPhAT
		ISI**SP**SLV**SP**ST	Yes	MPK3,6	(Mao *et al.*, 2011)
		F**SP**SLFLD**SP**AF	yes	MPK4	(Qiu *et al.*, 2008)
WRKY40*	At1g80840	KVT**SP**TSR	yes	MAPK	PhosPhAT
		DQI**SP**PKK	yes	MAPK	PhosPhAT
		QI**SP**PKKRK**SP**A	no	MAPK	
WRKY60*	At2g2500	ELQSRK**SP**ESVN	no	MAPK	
		SPRAYFRCSF**SP**	no	MAPK	
ZAT6*	At5g04340	LETLT**SP**RLS	yes	MPK6	(Liu *et al.*, 2013)
		EEVM**SP**MPAKK	yes	MPK6	(Liu *et al.*, 2013)
ZAT10*	At1g27730	LEALT**SP**RLA	yes	MPK3,6	(Nguyen *et al.*, 2012*b*)
		EVM**SP**MPAKKP	yes	MPK3,6	(Nguyen *et al.*, 2012*b*)

KINASE 7 (STN7), has mostly been implicated in light stress responses. However, there is now emerging evidence that STN7 can be also regulated by other stresses like salinity and oxidative stress (Chen and Hoehenwarter, 2015). Particularly in monocots, phosphorylation events at thylakoid membrane proteins and related NPQ have recently been suggested as acclimation strategies of chloroplasts to environmental stress (Chen *et al.*, 2013; Marutani *et al.*, 2014).

Treatments of Arabidopsis with high light-intensity repressed genes involved in photosynthesis that were also downregulated by perception of the 22 amino acid bacterial elicitor peptide flagellin 22 (flg22) (Sano *et al.*, 2014). Interestingly, a subset of flg22-regulated genes was also induced/repressed by other abiotic stresses (Jung *et al.*, 2013; Sano *et al.*, 2014) implicating a central role for chloroplast signals in the biotic and abiotic stress response (Stael *et al.*, 2015). Both stresses lead to changes in the redox balance of chloroplasts and could generate ROS molecules as retrograde signals, to induce gene expression required for stress acclimation (Jung *et al.*, 2013; Sano *et al.*, 2014). In parallel, these changes of the redox state in the chloroplast also lead to the differential regulation of a wide range of secondary metabolites acting as ROS-scavengers, or osmolytes, thus protecting chloroplasts/cells from damage (Krasensky and Jonak, 2012; Krasensky *et al.*, 2014).

The studies of chloroplast-localized mechanosensitive channels and their role in osmotic stress response uncovered another interesting finding, placing plastids in a central position in the osmotic stress response (Veley *et al.*, 2012; Wilson *et al.*, 2014). The knock-out of two plastid-localized homologues of the bacteria mechanosensitive channel MscS (MSL2 and MSL3) that ensure osmotic balance – most likely by the efflux of osmolytes when the plastid inner envelope is under tension – leads to constitutive hyperosmotic stress of the organelle (Veley *et al.*, 2012). This causes a cellular response to osmotic stress including proline accumulation, ABA accumulation, and stomata closure – even in the absence of an external stimulus (Wilson *et al.*, 2014). Together, this indicates that the perturbation of chloroplast homeostasis/function mimicking the presence of stress is relayed to the nucleus and leads to reprogramming of the cellular functions. Additionally, the importance of chloroplasts in the response to abiotic and biotic stress is underlined by the fact that plastids are the source of the stress-related hormones jasmonate (JA), ABA, and SA, which will be discussed later.

In order to obtain a more quantitative picture of the involvement of chloroplast functions in different stress responses, we analysed published microarray datasets from Arabidopsis (Table 2). We retrieved lists of differentially expressed Arabidopsis genes [differentially expressed genes

Table 2. *Chloroplast-localized differentially expressed genes responding to stress or hormones*

Overview of differentially expressed genes (DEGs, fold change >2, *P*-value <0.05) from different microarray studies of Arabidopsis plants in response to different treatments, and number of chloroplast localized proteins among these DEGs according to a confirmed plastid localization from the PPDB database (Sun *et al.*, 2009). The % of chloroplast-localized DEGs of total DEGs changing in response to the respective treatment is also shown.

Treatment	DEGs	Chloroplast DEGs	%	Reference
Drought stress	1837	354	19.3	(Huang *et al.*, 2008)
Osmotic stress	3832	634	16.5	(Sham *et al.*, 2015)
Salt stress	1640	194	11.8	(Sham *et al.*, 2015)
Cold stress	1258	132	10.5	(Kreps *et al.*, 2002)
BTH (SA analogue)	2383	335	14.1	(Wang *et al.*, 2006)
Herbivore	2778	190	6.8	(Appel *et al.*, 2014)
MeJa	2057	125	6.1	(Goda *et al.*, 2008)
ABA	3893	181	4.6	(Goda *et al.*, 2008)

(DEGs), fold change >2, *P*-value<0.05] under abiotic stress conditions or hormone treatments, and determined the number of chloroplast-localized proteins among the DEGs using the Plastid Proteome Database (PPDB, http://ppdb.tc.cornell.edu/; Sun *et al.*, 2009). The results clearly illustrate that chloroplasts are strongly affected by abiotic stresses and hormone treatments (Table 2). Similar results were obtained in a comparison of proteome datasets from abiotic stress-treated Arabidopsis plants showing that the abundance of 56% of proteins is changing in response to stress and 28% of significantly changing proteins are localized in chloroplasts (Taylor *et al.*, 2009). The importance of chloroplast functions for stress tolerance is further underpinned by the fact that out of 30 different proteins improving abiotic stress tolerance in Arabidopsis, rice and tobacco, most were localized in the nucleus (42%), followed by a chloroplast localization (23%) (Nouri and Komatsu, 2013). To obtain a broader picture on the role of chloroplasts in different stress responses, we set out to analyse a greater number of published microarrays using ExPath (http://expath.itps.ncku.edu.tw/), a publicly available database comprising 1057 samples of Arabidopsis treated with biotic and abiotic stresses as well as hormones based on the AtGenExpress and NASCArrays (Chien *et al.*, 2015). We generated lists of DEGs for cold, drought, salt, oxidative and osmotic stress, treatments with ABA, MeJa, wounding and *Pseudomonas* infection. The fold change was set to >2, with a *P*-value of <0.1. The lists were then used to generate Venn diagrams using a web tool (http://bioinformatics.psb.ugent.be/webtools/Venn/), provided by the VIB Ghent in order to determine the overlap between the treatments. The genes encoding JAZ1, ZAT10, ANAC019, ANAC029, and At3G18560 were regulated by at least seven out of nine treatments. When only the hormone treatments were taken into account, six genes were overlapping: *ZAT10, AERD2, JAZ1, DRT100, PNC2*, and At1G02360. Eleven genes were co-regulated by all abiotic stresses used for the analysis including *JAZ1, JAZ5, GID1B, GOLS1, FMO OX4, GBF3, LTI30, HSP90*, At4G20860, At2G20560, and At3G18560. Strikingly, the genes co-regulated in most of the conditions encode for TFs that are responsive to stress but have also been connected with retrograde signalling (Rossel *et al.*, 2007; Leister *et al.*, 2014). This quick unbiased search illustrates that both abiotic stress-related and hormone-related signalling involves many chloroplast functions and most likely also retrograde signalling. A much deeper analysis of chloroplast responses to stress generated by disturbing chloroplast functions by chemical treatments or mutation has been carried out by Gläßer *et al.* (2014). They performed a meta-analysis of transcriptome and protein interaction data of retrograde signalling pathways responding to six different treatments and identified a core response module composed of 39 genes involved in sugar, ROS, ABA, and auxin signalling pathways.

The role of plant hormones in organellar signalling

Phytohormones have been studied for decades as key regulators of plant development and environmental responses (i.e. abiotic and biotic stress). However, more recently, crosstalk and interactions among plant hormones have been increasingly recognized (Pieterse *et al.*, 2012). Moreover, roles in retrograde signalling were reported for different plant hormones, which will be discussed below.

Abscisic acid has always been associated with responses to drought, salt, cold and osmotic stress. Accordingly, the genes encoding enzymes involved in its biosynthesis are up-regulated under these conditions (Finkelstein, 2013). Notably, most steps of ABA biosynthesis take place in the chloroplasts, starting with the degradation of β-carotene up to generation of xanthoxin that is exported into the cytosol where the two last steps of synthesis are performed (Danquah *et al.*, 2014). ABA-mediated stress responses involve changes in transcription as well as calcium-dependent and -independent signalling, regulating anion channels for stomatal closure under drought stress conditions (Danquah *et al.*, 2014; Munemasa *et al.*, 2015). However, this is a two-sided response: it avoids water loss, but it also limits the CO_2 availability leading to reduced photosynthetic performance and eventually also to increased ROS production (Apel and Hirt, 2004). It is therefore not surprising that many nuclear genes encoding plastid proteins contain ABA-responsive regulatory elements in their promoters to coordinate these responses (Rook *et al.*, 2006). This is in agreement with the fact that one of the downstream targets of ABA-signalling is the TF ABI4. ABI4 acts as repressor of nuclear-encoded plastid genes including those encoding components of photosynthetic machinery (Shen *et al.*, 2006; Leon *et al.*, 2012), and thereby connects ABA-signalling and chloroplast retrograde signalling, especially with the model that ABAR (GUN5, CHLH) presents a chloroplast envelope-localized ABA-receptor (Shen *et al.*, 2006; Shang *et al.*, 2010; Liu *et al.*, 2012; Mei *et al.*, 2014; Liang *et al.*, 2015). It was also suggested that defects in ABAR cause the suppression of Ca^{2+} mobilization from internal stores

affecting the Ca^{2+}-dependent branch of ABA-mediated stomata closure (Tsuzuki *et al.*, 2011).

Jasmonic acid is another chloroplast-derived plant hormone. Its biosynthesis starts at the chloroplast membrane from linoleic acid that is also a substrate for biosynthesis of other oxylipins (Wasternack and Hause, 2013; Savchenko *et al.*, 2014). The chloroplastic branch of JA synthesis finishes with 12-oxo-phytodienoic acid (12-OPDA) that is further processed in peroxisomes (Wasternack and Hause, 2013). It is still unknown how OPDA is exported from chloroplasts to peroxisomes, but the peroxisomal ABC transporter PED3 with a broad range of substrate specificity was identified to participate in the JA biosynthesis (Theodoulou *et al.*, 2005). However, *ped3* mutants show only partial depletion of JA, thus suggesting that either other transporters or other mechanisms of transport through the membranes are involved (Theodoulou *et al.*, 2005). JA is best known for its role in the herbivore and wounding response (Reymond *et al.*, 2000), but it is also part of the cold-, heat-, salt- and drought-stress response (Kazan, 2015). JA downstream signalling includes down-regulation of photosynthetic genes (Attaran *et al.*, 2014) and there is clear evidence that stress-induced JA signalling shifts the cell priority from growth to response. Genetic studies showed that the oxylipins are also important for the acclimation to drought and salt stress (Savchenko *et al.*, 2014; Wasternack and Strnad, 2015). Additionally, proteomic studies in Arabidopsis and Physcomitrella revealed 12-OPDA as influential component of stress response shifting the energy investment from growth to defence, thus underpinning the central role of chloroplasts in energy management under stress conditions (Dueckershoff *et al.*, 2008; Toshima *et al.*, 2014).

Salicylic acid is the third plant hormone originating primarily from chloroplast metabolism (Seyfferth and Tsuda, 2014). Although the role of SA is most pronounced in the regulation of plant immunity to biotrophic pathogens (Caarls *et al.*, 2015), several reports indicated also an important role in response to abiotic stress conditions (Miura and Tada, 2014; Khan *et al.*, 2015). SA biosynthesis occurs via two pathways, both utilizing chorismate as a substrate, but the chloroplast-localized ISOCHORISMATE SYNTHASE1 (ICS1)-dependent pathway is the major source of SA biosynthesis in response to biotic/abiotic stress (Dempsey *et al.*, 2011). Both biotic and abiotic stress conditions promote the accumulation of SA as well as up-regulation of ICS1 (Dempsey *et al.*, 2011). The positive effect of exogenous application of SA on plant fitness under abiotic stress is concentration dependent: low concentrations of SA allow proper maintenance of redox balance, ion homeostasis, regulation of osmolyte production, stomata closure and protection of the photosynthetic machinery. In contrast, high concentrations of SA lead to oxidative stress, damage of photosystems and membranes, and consequently to cell death (Miura and Tada, 2014; Khan *et al.*, 2015). In general, abiotic stress-induced elevation of SA activates production of ROS signalling molecules that induce MAPK signalling, leading to downstream transcriptional reprogramming that renders plants tolerant (Miura and Tada, 2014). SA signalling involves redox-mediated de-oligomerization of NONEXPRESSER OF PR GENES 1 (PR1) and its translocation to the nucleus, where it interacts with TGA TFs that induce stress-responsive genes (Foyer *et al.*, 2014; Caarls *et al.*, 2015). A recent study reports that NPR1-dependent SA signalling is crucial for salt and oxidative stress tolerance (Jayakannan *et al.*, 2015). The regulation of SA biosynthesis is maintained mostly at the transcriptional level via fine-tuned expression of *ICS1*. The promoter region of *ICS1* contains several TF-binding sites. EIN3, NAC and EIL1 are negative regulators of *ICS1* expression, whereas CBP60g, WRKY28, TCP8 and TCP9 are direct positive regulators of SA biosynthesis (Dempsey *et al.*, 2011; Wang *et al.*, 2015). CBP60g was shown to bind calmodulin and might directly integrate calcium signatures generated upon external stimuli into the enhanced production of SA (Wang *et al*, 2009; Wan *et al*, 2012).

Last but not least, the crosstalk between hormones has also a crucial role in successful response to environmental changes, especially during the acclimation to combinatorial stresses. The positive regulator of SA biosynthesis ICS1 and the TFs TCP9 and TCP20 were shown to have an inhibitory effect on the expression of the LIPOXYGENASE 2 (LOX2) (Danisman *et al*, 2012; Wang *et al*, 2015), suggesting that the TCPs might present a communication point in regulation of SA-JA crosstalk (Wang *et al*, 2015). Additionally, WRKY39 was reported to down-regulate JA signalling by up-regulation of SA signalling under heat stress. On the other hand, WRKY33 was necessary for down-regulation of SA signalling and up-regulation of JA signalling upon treatment with necrotrophic fungi (Bakshi and Oelmuller, 2014). Although it is still unclear how JA and SA signalling interact under abiotic stress, a dominant role of SA- over JA-mediated signalling was proposed in plant innate immunity (Caarls *et al.*, 2015). ABA negatively regulates ethylene production through ABI4-mediated transcriptional repression of the ethylene biosynthesis genes (Dong *et al.*, 2016) and ABI4 was also found to mediate antagonistic effects of ABA and gibberellins by regulation of key biosynthetic genes (Shu *et al.*, 2016).

Physical connections between organelles – routes for metabolite exchange and signalling?

The biosynthetic pathways of the plant hormones discussed before do already illustrate the crucial need for an efficient exchange of metabolites (and signals) between different organelles. A close proximity of the involved membranes would therefore clearly facilitate this exchange, and indeed such regions in which the membranes from different organelles come quite close together have been observed and were defined as membrane contact sites (MCS). MCS with a typical intermembrane distance in the order of the size of a single protein (10–30 nm), were first observed in ultrastructural studies (Elbaz and Schuldiner, 2011) and are highly conserved throughout evolution. Initially, they have been discovered as an association between mitochondria and the ER in animal cells (Copeland and Dalton, 1959). However, despite more

than half a century of research, their functional role is still not understood and research on MCS seems so far restricted to animals. Here, they seem to be important in three cellular functions: signalling, passage of ions across membranes (i.e. Ca^{2+}), and non-vesicular lipid trafficking from one cellular compartment to another (Helle *et al.*, 2013). MCS may be particularly important in the function of the endoplasmic reticulum (ER) since this is the major site of lipid synthesis within cells. MCS can form between the ER and many organelles, including mitochondria, Golgi, endosomes, lysosomes, peroxisomes, chloroplasts, and the plasma membrane. For a recent review, see Shai *et al.* (2015).

In plants, the most obvious example for intense exchange of metabolites is photorespiration, which involves three organelles: chloroplasts, peroxisomes, and mitochondria. Also during JA biosynthesis chloroplasts and peroxisomes exchange the metabolite OPDA, as described before. Accordingly, it is not surprising that these organelles have always been observed in close proximity in electron micrographs, which has already been correlated to photosynthetic activity in very early studies (Trelease *et al.*, 1971). Even a certain dynamic aspect was observed in these studies, which was been refined in recent work that took advantage of modern *in situ* laser scanning microscopy and femtosecond laser technology to analyse adhesion between chloroplasts and peroxisomes (Oikawa *et al.*, 2015; Gao *et al.*, 2016). Oikawa and colleagues even observed a change in shape of peroxisomes from spherical to elliptical, concomitant with an increased interaction area between the organelles in the dark-light transition. Interestingly, this switch in shape was independent of the cryptochrome and phytochrome photoreceptors but could be inhibited by DCMU, an inhibitor of photosynthetic electron transport, or DCCD, an inhibitor of the chloroplast ATP-synthase. At the peroxisomal site, mutants with defects in peroxisomal protein import (*ped2*) or photorespiratory metabolism (*shmt1*) did not show a clear difference to the wild type, thus leaving photosynthetic activity as sole regulatory mechanism and the mechanism involved completely open. Nevertheless, two earlier studies revealed two peroxisomal membrane proteins that seem to be involved in peroxisome-chloroplast interactions: (i) the expression of the peroxin (essential protein for peroxisomal biogenesis) PEX10 with inactivated RING finger domain in a wild-type background resulted in a reduced attachment of peroxisomes to chloroplasts and photorespiratory metabolite exchange (Schumann *et al.*, 2007), and (ii) a mutation in the SNOWY COTYLEDON 3 protein, which is also localized at the peroxisomal membrane, impairs chloroplast development and results in photoinhibition, even under extreme CO_2 concentrations (Albrecht *et al.*, 2010). Hence, at least from the peroxisomal site, first contact points for the association with chloroplasts might be identified.

Much better studied are mitochondrial ER interactions – at least in animal cells. Here, the exchange of Ca^{2+} signals between the ER as major storage compartment and mitochondria is well known. Mitochondrial Ca^{2+} uptake had already been established in the 1960s, around when it was also found that Ca^{2+} is required for activities of TCA cycle enzymes and dehydrogenases in mitochondria (reviewed in Glancy and Balaban, 2012; Rizzuto *et al.*, 2012). Contact sites between mitochondria and the ER have also been observed on electron micrographs as well as the exchange of Ca^{2+} among them (Rizzuto *et al.*, 1998), and more recently MITOFUSIN2 (MFN2), a component of the mitochondrial fusion and fission machinery, has been identified as mediator of this interaction (de Brito and Scorrano, 2008). The uptake of Ca^{2+} into mitochondria occurs via the MITOCHONDRIAL UNIPORTER (MCU), which is regulated by the MITOCHONDRIAL CALCIUM UPTAKE 1 (MICU1) protein. MICU1 contains two canonical EF hands that are essential for its activity (Perocchi *et al.*, 2010). Recently, a functional orthologue has also been identified in plants (Wagner *et al.*, 2015). For a more detailed discussion of plant and animal mitochondrial Ca^{2+} signalling we refer the reader to the review of Wagner *et al.* (2016) in this special issue.

Finally, a specific peculiarity of chloroplasts with emerging implications for inter-organellar signalling is their ability to form stromules. Stromules are stroma-filled tubular extensions from chloroplasts, which had already been observed at the end of the 19th century in *Selaginella* and *Acetabularia*, and in higher plants in the 1930s (reviewed in Gray *et al.*, 2001). Nevertheless, their function remained unknown until Kohler *et al.* (1997) observed that stromules could facilitate the exchange of proteins between chloroplasts. This was subsequently much debated and eventually falsified using photoconversion of mEosFP-labelled plastids. This indicated that stromules do not function in exchange of proteins or small molecules from one plastid to another (Schattat *et al.*, 2012). Moreover, it was observed that stromule formation is induced by different stresses (Gray *et al.*, 2012), which supported the idea that they mediate the exchange of signals or metabolites between plastids and other subcellular compartments (Hanson and Sattarzadeh, 2013). More recently, this idea gained significant support with the finding that stromules form in response to light-sensitive redox signals within the chloroplast, which was solely dependent on chloroplast factors as even isolated chloroplasts could still form stromules (Brunkard *et al.*, 2015). Notably, these effects are not restricted to Arabidopsis and were also found in the alpine plant *Ranunculus glacialis* under low CO_2 conditions, which triggers also ROS formation in chloroplasts (Buchner *et al.*, 2015). In terms of organellar signalling the most striking observation was the role of stromules during innate immunity. Caplan *et al.* (2015) showed that stromules, which are induced in response to recognition of the TMV p50 effector protein, form dynamic connections to the nucleus and mediate ROS formation in the nucleus. This work demonstrated that stromules can indeed transfer protein (as well as small molecules) from the chloroplast to the nucleus. More details on the role of chloroplasts, stromules and plant innate immunity can be found in the review of Serrano *et al.* (2016) in this issue. Together with the reported induction of stromules also under abiotic stress conditions, this might indicate a more general role of stromules in chloroplast retrograde signalling involving ROS signals and maybe even other signals, which still remain to be identified. Strikingly, ROS signals are also

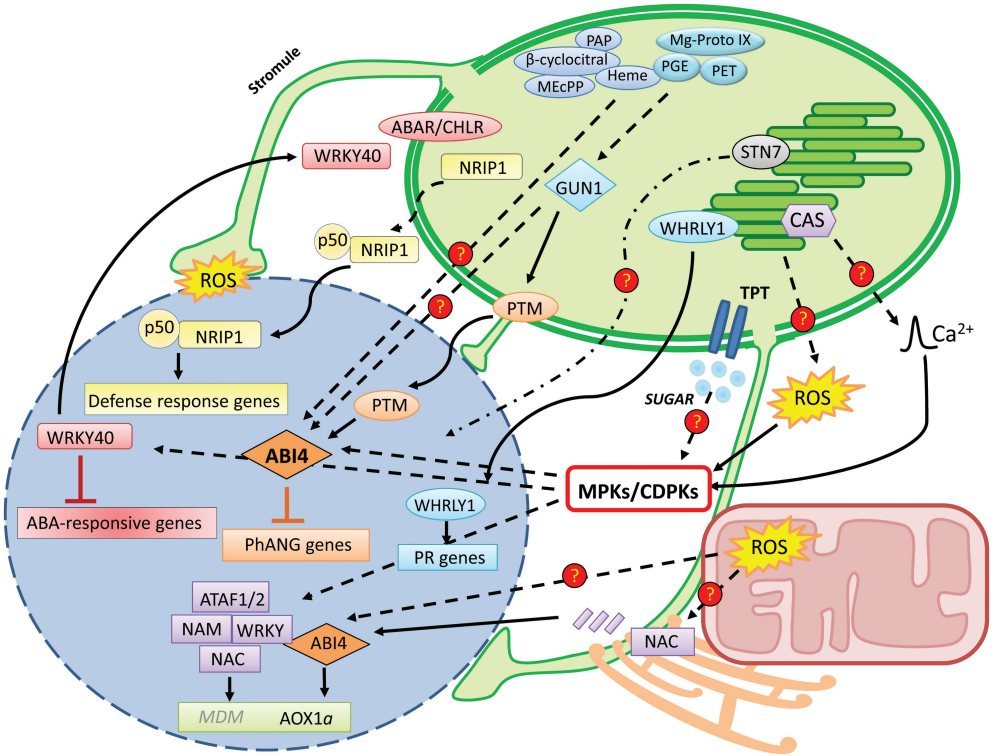

Fig. 2. Novel connections in plant organellar signalling pathways. This scheme illustrates the major factors involved in the generation of signals from the organelles (i.e. ROS and Ca^{2+}) and the complex interconnections of different signalling pathways merging at common TFs in the nucleus (i.e. ABI4, NACs, WRKYs) as described in the text. Cytosolic protein kinases (MPKs and CDPKs; highlighted in the red box) act by translating the organellar signals into a cellular response by modification of the TFs. Additionally, ROS signals are transported from chloroplasts to the nucleus via stromules and some TFs also translocate from the chloroplast or the ER to the nucleus.

directly transferred from mitochondria to the nucleus in animal cells, but here the entire organelle is moving. Exposure of endothelial cells to hypoxia triggered microtubule and dynein-dependent retrograde mitochondrial movements resulting in a perinuclear clustering of mitochondria. This subcellular redistribution of mitochondria was accompanied by the accumulation of ROS in the nucleus (Al-Mehdi *et al.*, 2012).

Conclusions

Over the past few years a number of reports supported the view that many different signals (i.e. metabolites, ROS, Ca^{2+}) mediate retrograde signalling and a set of distinct TFs (ABI4, NACs, WRKYs etc.) emerge as integration points, eventually transmitting the signal in the nucleus and regulating gene expression. Independently, various reports showed also that the activity of these TFs can be modified by phosphorylation, for example via MAPKs, CDPKs or Ca^{2+} signals. On the other hand, it was also reported that chloroplasts can generate Ca^{2+} signals, ROS and other small molecules in response to certain stimuli, which could then activate the kinases regulating these TFs. This offers now the interesting opportunity to generate and transduce retrograde signals, and shows how the organelles of prokaryotic origin could be connected to the eukaryotic signalling machinery of the host cell (Fig. 2). Intriguingly, direct contacts between particular membrane sites emerge as novel signalling routes. However, for this latter aspect, the molecular players involved are mostly still unknown, although it is tempting to speculate that they will include proteins that are also amenable in response to stimuli, for example by Ca^{2+} binding or phosphorylation, in order to keep the system dynamic. A possible scenario could be transmembrane proteins or transporters with EF-hands, which have already been described for chloroplasts as well as for mitochondria (Stael *et al.*, 2011).

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