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REVIEW PAPER

Photosynthesis, photorespiration, and light signalling in defence responses

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Abstract

Visible light is the basic energetic driver of plant biomass production through photosynthesis. The constantly fluctuating availability of light and other environmental factors means that the photosynthetic apparatus must be able to operate in a dynamic fashion appropriate to the prevailing conditions. Dynamic regulation is achieved through an array of homeostatic control mechanisms that both respond to and influence cellular energy and reductant status. In addition, light availability and quality are continuously monitored by plants through photoreceptors. Outside the laboratory growth room, it is within the context of complex changes in energy and signalling status that plants must regulate pathways to deal with biotic challenges, and this can be influenced by changes in the highly energetic photosynthetic pathways and in the turnover of the photosynthetic machinery. Because of this, defence responses are neither simple nor easily predictable, but rather conditioned by the nutritional and signalling status of the plant cell. This review discusses recent data and emerging concepts of how recognized defence pathways interact with and are influenced by light-dependent processes. Particular emphasis is placed on the potential roles of the chloroplast, photorespiration, and photoreceptor-associated pathways in regulating the outcome of interactions between plants and pathogenic organisms.

Key words: Photoreceptor, photorespiration, photosynthesis, plant immunity, reactive oxygen species, signalling.

Introduction

Plants are constantly challenged by fungal, bacterial, and viral pathogens that may cause enormous economic losses in agriculture and also have an ecological impact in nature. On a molecular level, disease resistance necessitates tight cross-communication between different signalling pathways in plants. It has become well known that recognition of pathogen-derived molecules is enabled by immune receptors, which elicit signalling cascades in which organelles carry out vital functions in determining appropriate immune reactions against a variety of biotic stress agents.

Chloroplasts have the potential to act as delicate environmental sensors, since they harbour numerous metabolic pathways that are readily unbalanced by environmental fluctuations. In photosynthetic light reactions, the thylakoid membrane protein complexes photosystem II (PSII), cytochrome b_6f complex, photosystem I (PSI), and ATP synthase harness solar energy into chemical form. The reducing equivalents and ATP produced are subsequently utilized in various metabolic and regulatory pathways in chloroplasts and other cellular compartments. Besides

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; CRY, cryptochrome; ET, ethylene; ETI, effector-triggered immunity; GPX, glutathione peroxidase; GR, glutathione reductase; H_2O_2, hydrogen peroxide; HR, hypersensitive response; JA, jasmonic acid; MAMP, microbe-associated molecular pattern; MAPK, mitogen-activated protein kinase; MTI, MAMP-triggered immunity; NDH, NADPH dehydrogenase; NDPK, nucleotide-diphophate kinase; NO, nitric oxide; $^1O_2$, singlet oxygen; $\cdot O_2^-$, superoxide; PCD, programmed cell death; PAL, phenylalanine ammonia lyase; PHOT, phototropin; PHY, phytochrome; PGR5, PROTON GRADIENT REGULATION 5; PRX, peroxiredoxin; PSI, photosystem I; PSII, photosystem II; RuBP, ribulose 1, *5-bisphosphate*; RCC, red chlorophyll catabolite; RCCR, RCC reductase, R:FR, red to far red light ratio; ROS, reactive oxygen species, SA, salicylic acid; TCV; *Turnip crinkle virus*, (TIR)-NBS-LRR, (toll/interleukin-1 receptor)-nucleotide-binding site leucine-rich repeat; TMV; *Tobacco mosaic virus*.

photosynthesis, chloroplasts also host crucial steps in the biosynthesis of amino acids, hormones, vitamins, secondary metabolites, and lipids, which comprise basic components of metabolic pathways but also carry out important functions in stress resistance and signalling in plant cells.

The photosynthetic electron transfer chain may undergo tremendous changes in redox potential due to metabolic variations caused by environmental cues. Pathways of cyclic electron flow, involving thylakoid NADPH dehydrogenase (NDH) and the PROTON GRADIENT REGULATION 5 (PGR5)-containing protein complexes, have a crucial role in balancing the function of linear electron transfer (Munekage *et al.*, 2002; DalCorso *et al.*, 2008; Suorsa *et al.*, 2009). Additionally, the ferredoxin–thioredoxin system is a well known mechanism that activates light-driven metabolic reactions in chloroplasts. Thioredoxins become reduced via PSI and ferredoxin–thioredoxin reductase in illuminated leaves, and provide a link between the activity of photosynthetic light reactions and activation of key enzymes of photosynthetic carbon fixation (Buchanan and Balmer, 2005).

Besides reduction of disulphide bridges through thioredoxin activity, oxidation of protein thiols by reactive oxygen species (ROS) may equally well induce alterations in the activity of enzymes and regulatory proteins. The chloroplast thiol redox state may thus also influence the 'retrograde signals' that originate from various processes in chloroplasts and regulate gene expression in the nucleus (Fernandez and Strand, 2008). Importantly, light-driven redox chemistry also provides plants with a mechanism for generation of ROS, which is a key player in the relay of stress signals in photosynthetic tissues (Foyer and Noctor, 2000; Gechev *et al.*, 2006). Indeed, transient increases in the levels of ROS in chloroplasts have vital signalling roles in the onset of immune reactions upon attempted infection in different cell types (Dat *et al.*, 2000; Joo *et al.*, 2005; Foyer and Noctor, 2009; Kangasjärvi *et al.*, 2009).

Since they are highly responsive to environmental cues, chloroplasts may carry out versatile functions in defence signalling, and are capable of inducing highly specific responses upon infection by various types of plant pathogens (Kachroo *et al.*, 2003; Kariola *et al.*, 2005; Muhlenbock *et al.*, 2008). The potential of chloroplasts to modulate immune reactions in plants has been evidenced by identification of a number of 'lesion-mimic mutants', which display spontaneous activation of defence pathways due to a malfunction in a chloroplastic process (Lorrain *et al.*, 2003).

Besides generation of ROS, formation of nitric oxide (NO) as well as reactions for biosynthesis of the plant hormones salicylic acid (SA) and jasmonic acid (JA) occur in chloroplasts, and contribute to the specificity of immune reactions (Kunkel and Brooks, 2002; Boller and He, 2009). However, as this review emphasizes, the final defence output results from extensive cross-communication between organellar and cytosolic components, including photoperiodic and hormonal signals, which together regulate cellular functions and modulate gene expression in the nucleus (Fedoroff, 2006; Roberts and Paul, 2006; Griebel and Zeier, 2008). Indeed, the specificity of defence reactions relies on the concerted action of organellar signals and cytosolic networks, which may have both synergistic and antagonistic interactions with each other.

Light could impact on plant defence responses in numerous ways. Based on current knowledge, three of the most important routes are likely to be (i) by influencing the general energy and reductant status of the cell (e.g. NADPH, ATP, and carbon skeletons) and thereby the 'fuel' available to launch and sustain the response against invaders; (ii) by effects on ROS production in the chloroplast or peroxisome, but perhaps also through more indirect effects on ROS generation by the mitochondrial electron transport chain or components such as NADPH oxidases; and (iii) signalling through photoreceptors, which are increasingly recognized as influencing the outcome of pathogenesis responses. The first two routes are obviously linked because both ROS production and removal depend at least partly on reductants, but emerging links between all three mechanisms point to a highly integrated system of regulation that acts to achieve outcomes appropriate to the prevailing environmental conditions. This review describes some of the recent advances in identifying components involved in this network, and discusses the physiology underlying some of the interactions.

Overview of defence strategies against different types of pathogens in plants

Plant pathogens are generally classified as biotrophs, hemibiotrophs, and necrotrophs depending on their lifestyle. Biotrophic and hemibiotrophic pathogens utilize plant-derived metabolites for growth, and therefore aim to maintain the cellular integrity of the host plant, at least during the early phases of infection. Necrotrophs, in contrast, often invade the plant tissue through wounded sites, and induce necrosis to utilize the cellular components of the collapsing host tissue. Therefore, plants deploy different strategies to combat different types of pathogens.

In general terms, resistance against biotrophic pathogens mainly involves SA-dependent signalling pathways, and culminates in a localized cell death termed the hypersensitive response (HR) in the vicinity of the pathogen entry site. Pathways utilizing JA and ethylene (ET), on the other hand, act against necrotrophic pathogens (Kunkel and Brooks, 2002). SA and JA/ET signalling pathways have been considered mutually antagonistic, but a growing number of reports have also demonstrated the existence of synergistic interactions between the two pathways in plants (Shah *et al.*, 1999; Devadas *et al.*, 2002). Moreover, increasing evidence points to a multifaceted role for the phytohormone abscisic acid (ABA) in determining the extent of SA- and JA/ET-dependent responses (for recent reviews, see Asselbergh *et al.*, 2008; Cao *et al.*, 2011; Robert-Seilaniatz *et al.*, 2011).

Recognition of an invading pathogen promotes a massive reprogramming of gene expression to elicit specific defence reactions in the infected plant. Perception of conserved microbial features, termed microbe-associated molecular

patterns (MAMPs), by plasma membrane-spanning pattern recognition receptors induces a repertoire of immune responses including closure of stomata to limit pathogen entry sites, changes in host gene expression, proteome, and metabolome, and fortification of the cell wall. Such mechanisms are known as MAMP-triggered immunity (MTI; Boller and He, 2009), and are sufficient to prevent a range of microbes from colonizing the host tissue. However, host-adapted pathogens have evolved effector molecules that target the mechanisms of MTI in attempts to enhance pathogen growth. Plants, in turn, have co-evolved intracellular nucleotide-binding site leucine-rich repeat (NBS-LRR) resistance proteins, which specifically recognize the presence of effector molecules, and elicit a second layer of immune reactions, which may be highly specific and often accompany the HR at the attempted site of infection (Jones and Dangl, 2006). This second layer of induced resistance is termed effector-triggered immunity (ETI; Boller and He, 2009).

Allocation of recourses for the onset of immune reactions and biosynthesis of protective compounds causes a demand for energy in the infected tissue. However, several studies have reported that photosynthesis becomes down-regulated, and that plants shift towards non-assimilatory metabolism in response to various types of pathogens or phytophagous insects (reviewed by Roberts *et al.*, 2006; Bolton, 2009; Major *et al.*, 2010; Kerchev *et al.*, 2011). Such metabolic shifts are plant driven and tightly coordinated, and, depending on the type of plant–pathogen interaction, the extent of the response may vary from a single cell at the site of infection (Scharte *et al.*, 2005) to an entire leaf that contains uninfected areas as well (Meyer *et al.*, 2001). For example, when source leaves of tobacco were infected with *Phytophthora nicotianae*, full activation of defence responses and the HR was preceded by interruption of photosynthetic electron transfer and down-regulation of photosynthetic activity during the first hours after the inoculation (Scharte *et al.*, 2005). Infection of bean (*Phaseolus vulgaris*) leaves with *Colletotrichum lindemuthianum*, in contrast, led to necrosis and successive down-regulation of photosynthesis at later stages of infection (Meyer *et al.*, 2001). Collapse of photosynthetic activity inevitably leads to a metabolic transition from source to sink in infected tissues. The resulting demand for carbohydrates and energy becomes compensated through increased activities of cell wall invertases, hexose transporters, the oxidative pentose phosphate pathway, and respiratory metabolism (Essmann *et al.*, 2008; Scharte *et al.*, 2009). Such reprogramming of primary carbon metabolism may further enhance the expression of defence-related genes, and favour the production of secondary compounds with antimicrobial activity (Bolton, 2009).

Since chloroplasts host a number of defence-related pathways, it is not surprising that host-targeted pathogen effector proteins may target chloroplastic functions, presumably to inhibit chloroplast-derived defence signals. The bacterial effector HopI1 of *Pseudomonas syringae*, a hemibiotrophic bacterial pathogen, localizes to chloroplasts, where it disrupts the structural organization of the thylakoid membrane and suppresses SA production, presumably to aid in successful host colonization by the pathogen (Jelenska *et al.*, 2007). HopU1, on the other hand, is a mono-ADP-ribosyltransferase that targets chloroplast RNA-binding proteins (Fu *et al.*, 2007). Whether and how chloroplasts recognize such bacterial effectors is a matter of increasing interest in the research field.

The role of chloroplasts in triggering ROS/redox-dependent events in defence signalling

Perception of an invading pathogen commonly promotes transient increases in the generation of ROS, which act as secondary messengers and may elicit an HR in infected tissues. The specificity of ROS signals is determined by multiple interacting factors, including the localization, chemical identity, and abundance of ROS. Within the cell, ROS abundance is determined by ROS lifetime, itself determined by a multilayered antioxidant network. It has also become clear that ROS do not act as damaging agents that cause cell death merely through excessive oxidation of cellular components, but rather they elicit active cell death programmes, whereby ROS are perceived by as yet unidentified receptor molecules.

In early stages of infection, ROS accumulation is triggered by plasma membrane-bound NADPH oxidases and typically occurs in the apoplast (Torres and Dangl, 2005), but the contribution of chloroplastic and peroxisomal ROS production to plant immunity has also been described (Karpinski *et al.*, 1999; Vandenabeele *et al.*, 2004). Intracellular ROS produced as hydrogen peroxide (H_2O_2) in the peroxisomes can interact with specific NADPH oxidases to govern SA-dependent defence metabolism and resistance (Chaouch *et al.*, 2011). The relationship between organelle-derived ROS and induction of defence gene expression or the HR, however, is not straightforward and strongly depends on the interacting pathogen and host (Belhaj *et al.*, 2009; Zurbriggen *et al.*, 2009). Moreover, it seems that besides initiating ROS signals, chloroplasts also perceive, mediate, and even amplify ROS signals that originate from the apoplast (Joo *et al.*, 2005). Indeed, the role of chloroplastic ROS production in coordinating cell death or modulating defence outputs appears to be highly specific in targeting various types of invading pathogens. Presumably, the action of chloroplast-derived ROS depends on the activation state of other defence signalling components as well as antioxidant agents inside and outside of the organelles.

Besides the fact that immune reactions employ ROS generated in different cellular compartments, the emerging picture on oxidative signalling is further complicated by differential ROS-dependent functions in infected and neighbouring cells. Whereas oxidative bursts in the apoplast and chloroplast often elicit an HR in infected cells, NADPH oxidase activity and consequent accumulation of extracellular ROS appear to contain the HR in neighbouring uninfected cells (Torres and Dangl, 2005). Moreover, vascular tissues seem to respond specifically to light-induced

ROS signals in disease resistance (Lorrain *et al.*, 2004). Such cell-specific functions are accompanied by specific metabolic properties and unique characteristics of ROS tolerance in bundle sheath cells (Fryer *et al.*, 2003; Kangasjärvi *et al.*, 2009).

High light, ROS, and defence signalling through chloroplasts

Photosynthetic electron transfer reactions comprise a significant source of ROS due to formation of highly reactive intermediates in light-exposed green tissues. In the reaction centre of PSII, singlet oxygen ($^1O_2$) is produced via reactions between excited triplet reaction centre chlorophyll P680 and molecular oxygen, especially when plants are exposed to high irradiance levels inducing PSII photoinhibition (Hideg *et al.*, 2002; Aro *et al.*, 2005). Upon overreduction of the electron transfer chain, molecular oxygen may also drain electrons from several sites, notably the highly reducing components in and after PSI, which results in formation of superoxide ($\cdot O_2^-$) and H_2O_2 in the chloroplast stroma (Asada, 1999). Production of ROS and photodamage to PSII are linked to the high turnover rate of the D1 reaction centre protein, which becomes degraded and replaced by *de novo* protein synthesis in the so-called PSII repair cycle (Mulo *et al.*, 2008). By this means, plants avoid more extensive oxidative damage to other components of the photosynthetic electron transfer chain.

FtsH protease is one of the multiple components that mediate the coordinated turnover of D1, and analysis of *Arabidopsis thaliana variegated* mutants deficient in FtsH has indisputably demonstrated its importance for maintenance of chloroplast integrity (Miura *et al.*, 2007; Yu *et al.*, 2008). It is therefore notable that infection of tobacco (*Nicotiana benthamiana*) leaves with *Tobacco mosaic virus* (TMV) diminished the level of the FtsH protease called DS9, which presumably disturbed the PSII repair cycle and therefore caused inhibition of photosynthetic electron transport (Seo *et al.*, 2000). This, in turn, was accompanied by HR-like cell death in TMV-infected tobacco leaves (Seo *et al.*, 2000). Moreover, transgenic tobacco plants containing increased amounts of the DS9 protein showed delayed onset of TMV-induced HR (Seo *et al.*, 2000). These observations suggest that programmed inhibition of the PSII repair cycle through specific down-regulation of protease activity may provide plants with a mechanism to elicit ROS production and cell death upon infection.

Chloroplasts also comprise an early target for a specific mitogen-activated protein kinase (MAPK) cascade, which promotes ROS production and the HR upon viral infection (Liu *et al.*, 2007). This MAPK cascade was first identified in tobacco leaves, and consists of a MAPK kinase, *Nt*MEK2, and its downstream MAPKs SIPK, Ntf4, and WIPK (Liu *et al.*, 2007). In transgenic tobacco, inducible overexpression of *Nt*MEK2 resulted in a rapid and drastic decline in photosynthetic carbon assimilation, which was proposed to mimic excess light conditions and therefore lead to production of $\cdot O_2^-$ and H_2O_2 in chloroplasts. Notably, these metabolic disturbances preceded the light-dependent onset of HR in tobacco leaves (Liu *et al.*, 2007).

Studies of mutants have also revealed the contribution of photosynthetic electron transport and light-induced ROS production to the onset of cell death in response to bacterial pathogens (Bechtold *et al.*, 2008). Much of this understanding has been obtained by utilizing lesion-mimic mutants that show enhanced HR-like cell death under high irradiance levels (Lorrain *et al.*, 2003). One of the best known examples is the *lesion simulating disease 1* (*lsd1*) mutant, which fails to limit the spread of the HR, and undergoes runaway cell death when infected by avirulent pathogens or upon exposure to excess irradiance levels (Dietrich *et al.*, 1994; Mateo *et al.*, 2004). This has been linked to failure of *lsd1* to up-regulate genes encoding Cu/Zn superoxide dismutase and catalase 1 (CAT1), which act as antioxidant enzymes in chloroplasts and peroxisomes, respectively (Mateo *et al.*, 2004). The significance of changes in *CAT1* transcripts remains unclear, as this catalase is not highly expressed in leaf tissues, and knockout mutants show virtually unchanged leaf catalase activity (Mhamdi *et al.*, 2010*a*). Lesion formation becomes diminished in a double mutant combination of *lsd1* and *chlorophyll a/b binding harvesting organelle specific* (*cao*), which displays reduced PSII antenna size due to deficient folding of the light-harvesting antenna proteins (Mateo *et al.*, 2004; Klimyuk *et al.*, 1999). This indicates that the HR-eliciting redox signal that evidently promotes cell death in *lsd1* involves PSII electron transport (Mateo *et al.*, 2004). Further analysis provided evidence that the light-dependent defence and death signals most probably originate from reduction of the plastoquinone pool, and are relayed to the nuclear genome through the cytosolic components LSD1, ENHANCED DISEASE RESISTANCE 1 (EDS1), and PHYTOALEXIN DEFICIENT4 (PAD4), which form central regulatory nodes in plant immunity (Muhlenbock *et al.*, 2008).

Chloroplast defence signalling may also involve an *S*-sulphocysteine synthase, CS26, which may contribute to the maintenance of redox balance in chloroplasts (Bermudez *et al.*, 2010). This protein catalyses the incorporation of thiosulphate into *O*-acetyl serine to form *S*-sulphocysteine, which can then be converted to cysteine (Bermudez *et al.*, 2010). A mutation in CS26 results in severely stunted growth, accumulation of ROS, and transcriptional activation of both SA- and JA-responsive defence genes (Bermudez *et al.*, 2010). Even though the molecular targets for *S*-sulphocysteine remain to be demonstrated, it is clear that the function of CS26 is indispensable for light acclimation and appropriate defence signalling in plants. CS26 is predicted to localize to the chloroplast lumen, where its reaction product *S*-sulphocysteine has been hypothesized to mediate redox regulation of the thylakoid protein kinase, STN7 (Bermudez *et al.*, 2010). Intriguingly, STN7 is a strictly redox-regulated protein kinase responsible for phosphorylation of the PSII light-harvesting antenna proteins, although its precise physiological role in light acclimation and chloroplast signalling has been a matter of

intensive debate (Bellafiore *et al.*, 2005; Rochaix, 2007; Tikkanen *et al.*, 2011).

The impact of chloroplast ROS on immune reactions was also explored by a different approach, using transgenic tobacco plants that overexpress a plastid-targeted cyanobacterial flavodoxin, and are therefore unable to generate high levels of ROS in chloroplasts (Zurbriggen *et al.*, 2009). These mutant plants displayed attenuated cell death upon infection by a non-host pathogen *Xanthomonas campestris*, whereas neither the synthesis of SA or JA nor the expression of defence-related genes was affected by the presence of the flavodoxin in chloroplasts (Zurbriggen *et al.*, 2009). In this particular plant–pathogen interaction, ROS formation in chloroplasts is evidently not required to induce defence gene expression in the nucleus.

Mutant screens have also identified chloroplastic components that might represent targets for microbial effector molecules. A highly conserved *Arabidopsis* chloroplast protein RESISTANCE TO PHYTOPHTORA (RPH1) is required for activation of specific immune reactions against *Phytophthora brassiceae*, an oomycete postulated to excrete a chloroplast-targeted effector molecule that may interact with RPH1 (Belhaj *et al.*, 2009). RPH1 is a putative membrane protein with three predicted transmembrane helixes and an unknown molecular function (Belhaj *et al.*, 2009). Notably, *rph1* mutant plants display runaway cell death but reduced oxidative burst by plasma membrane NADPH oxidase when exposed to *Phytophthora* (Belhaj *et al.*, 2009). These observations suggest that chloroplasts communicate through RPH1 to elicit ROS production in the apoplast, presumably to contain the spread of the lesion. Intriguingly, *rph1* plants show wild-type resistance to another oomycete *Hyaloperonospora arabidopsidis*, as well as to *P. syringae* and the necrotrophic fungal pathogen *Botrytis cinerea* (Belhaj *et al.*, 2009), thus elegantly demonstrating the specificity of signalling effects mediated by a chloroplastic component.

Chlorophyll metabolism as a source of chloroplast ROS signals

Besides photosynthetic electron transfer reactions, the photoactive nature of chlorophyll provides a mechanism for ROS formation in chloroplasts. Uncontrolled accumulation of phototoxic intermediates of chlorophyll biosynthesis or degradation may lead to generation of ROS in chloroplasts, as evidenced by lesion-mimic phenotypes of mutants deficient in distinct steps in the biosynthesis or degradation of chlorophyll (Lorrain *et al.*, 2003). Detailed analysis of these mutants has revealed that even though many of them share the common property of a light-dependent HR, cell death is not simply a consequence of ROS accumulation in chloroplasts. Rather, defects in individual components of chlorophyll metabolism elicit immune responses in a highly specific manner.

The chlorophyll biosynthesis mutant *fluorescent* (*flu*) has provided an elegant model to study singlet oxygen signalling in chloroplasts. Dark treatment of *flu* results in accumulation of protochlorophyllide, which causes a massive release of $^1O_2$ upon re-illumination (op den Camp *et al.*, 2003). The accumulation of $^1O_2$ leads to rapid and selective transcriptional reprogramming, and finally induces programmed cell death (PCD) in *flu* plants (op den Camp *et al.*, 2003). The induced genes include both SA and JA defence marker genes (Danon *et al.*, 2005), suggesting a role for chloroplast singlet oxygen in synergistic interactions between the different hormonal pathways.

It is notable that light-dependent release of $^1O_2$ alone is not sufficient to induce the PCD response in *flu* seedlings, and suppressor screens have resulted in identification of components that are required for induction of cell death in re-illuminated *flu* plants. These include the chloroplastic EXECUTER proteins (Wagner *et al.*, 2004) as well as EDS1 (Ochsenbein *et al.*, 2006) and the blue light receptor cryptochrome (CRY1; Danon *et al.*, 2006). Blue light was also found to be required to trigger cell death in *flu* plants (Danon *et al.*, 2006). Transcript profiling of *flu* single and *flu cry1* double mutants indicated, however, that only a subset of $^1O_2$-induced genes require CRY1activity (Danon *et al.*, 2006). Thus, blue light has the ability to influence chloroplast $^1O_2$ signalling in a highly specific manner.

The vast majority of genes activated by $^1O_2$ in *flu* were different from those induced by treating plants with methyl viologen, a herbicide that induces generation of $\cdot O_2^-$ through PSI activity in chloroplasts (op den Camp *et al.*, 2003). Nevertheless, H_2O_2 signalling seems to interact with signals that originate from $^1O_2$ in chloroplasts. Overexpression of the H_2O_2-metabolizing enzyme, thylakoid ascorbate peroxidase (tAPX), in the *flu* background led to enhanced $^1O_2$-dependent gene expression and cell death as compared with the parental *flu* plants (Laloi *et al.*, 2007). These findings lead to the conclusion that $^1O_2$ signalling is fine-tuned by antagonistic effects of H_2O_2 in chloroplasts.

Components that participate in the degradation of chlorophyll may also promote highly specific signalling effects in leaves. A specific *Arabidopsis* CHLOROPHYLLASE 1 (*At*CHL1) operates at the initial step of the chlorophyll degradation pathway and becomes transcriptionally induced upon treatment by necrotrophic pathogens, JA, or wounding, presumably to control the release of ROS by chlorophyll molecules that become released from the thylakoid membrane upon tissue damage (Kariola *et al.*, 2005). Silencing of *AtCHL1* rendered the mutant resistant against the necrotrophic bacterial pathogen *Erwinia carotovora* but susceptible to the necrotrophic fungal pathogen *Alternaria brassicicola* (Kariola *et al.*, 2005). Even though both of these pathogens have a necrotrophic lifestyle, resistance against *Erwinia* employs both JA/ET and SA pathways (Kariola *et al.*, 2005), whereas resistance against *Alternaria* depends on JA signalling. *At*CHL1 was suggested to fine-tune the balance between SA- and JA-dependent signalling pathways, and thus the tolerance of plants to these different types of plant pathogens, by modulating ROS levels (Kariola *et al.*, 2005). Even though it remains unclear how the extent of ROS accumulation determines the extent of SA/JA-dependent signals, it is clear

that signals elicited by ROS cross-communicate with other signalling components to determine the final outcome of the defence reaction. Recent data suggest that the glutathione system may be an important modulator of both SA and JA signalling in response to ROS production. In *gr1* mutants lacking expression of one of the two *Arabidopsis* genes encoding glutathione reductase (GR), a suite of JA-associated genes was repressed, including genes involved in JA synthesis and signalling, but also downstream genes such as *AtCHL1* (Mhamdi *et al.*, 2010*b*).

Chloroplast degeneration, senescence, and cell death regulation under low light

Lesion-mimic mutants that show premature yellowing conditionally under moderate light intensity and become rescued upon growth under high light have also been identified in *Arabidopsis* mutant screens (Mateo *et al.*, 2006; Trotta *et al.*, 2011). These include *constitutive expression of PR genes 5* (*cpr5*), *defence no death 1* (*dnd1*), and the recently identified *pp2a-b'γ*, which displays reduced expression of a regulatory subunit B'γ of the heterotrimeric protein phosphatase 2A (PP2A). In *cpr5*, the mutation lies in a gene coding for a membrane protein of unknown function, whereas *dnd1* is deficient in cyclic nucleotide-gated cation channel 2 (CNGC2) (Clough *et al.*, 2000; Ali *et al.*, 2007). The low-light-enhanced phenotypes of *cpr5* and *dnd1* were discussed in terms of increased foliar SA levels, but no functional connection between CPR5 and DND1 has been reported (Mateo *et al.*, 2006). PP2A-B'γ and CPR5, instead, appear to be functionally connected (Trotta *et al.*, 2011).

Knock-down *pp2a-b'γ* plants show senescence-like symptoms including premature yellowing and eventually cell death in leaves, which is accompanied by accumulation of H_2O_2 through a pathway that requires functional CPR5 (Trotta *et al.*, 2011). Similarly to *cpr5*, the *pp2a-b'γ* mutant shows constitutive activation of both SA- and JA-dependent defence pathways. In contrast to *cpr5*, however, *pp2a-b'γ* leaves do not contain increased levels of SA or JA. Rather, the constitutive defence response is associated with hypomethylation of DNA and increased levels of methionine salvage pathway components in *pp2a-b'γ* leaves (Trotta *et al.*, 2011).

The slow degeneration of cells in *pp2a-b'γ* leaves is accompanied by disintegration of chloroplasts, which contain peculiar thylakoid-deficient extrusions (Trotta *et al.*, 2011). Similar swollen chloroplasts with homogenous protrusions were also observed in wounded leaves of the *lethal leaf spot 1* (*lls1*) mutant of maize (*Zea mays*) (Gray *et al.*, 2002). Subsequent work demonstrated that *LLS1* in an orthologue for *Arabidopsis ACCELERATED CELL DEATH 1* (*ACD1*), which encodes a pheophorbide *a* oxygenase (PaO) that functions in chlorophyll degradation and performs a reaction that yields red chlorophyll catabolite (RCC) (Yang *et al.*, 2004). Also mutants lacking RCC reductase (RCCR) develop a light-dependent lesion-mimic phenotype and are designated *acd2* (Mach *et al.*, 2001). Besides the proposed role as a photosensitizer, pheophorbide *a* may also promote the onset of cell death through an as yet unidentified light-independent mechanism. This became evident upon induction of senescence during a prolonged 5 d dark treatment of *Arabidopsis* wild-type and antisense-ACD1 plants (Hirashima *et al.*, 2009). Under these experimental conditions, antisense-ACD1 plants showed accumulation of H_2O_2 and enhanced cell death (Hirashima *et al.*, 2009). Whether accumulation of pheophorbide *a* leads to perturbation of cellular homeostasis and thus induces a general alarm signal or whether pheophorbide *a* itself acts as a signalling molecule has not yet been resolved. Even so, senescence-associated components that ensure controlled degradation of chlorophyll in ageing leaves indisputably also hold the potential to elicit defence reactions upon infection. Moreover, enhanced senescence seems to represent a mechanism for induction of cell death under low irradiance and in the dark.

Antioxidant systems in defence signalling

In parallel with the recognition of ROS as key signalling molecules, the function of antioxidant enzymes and ROS scavenging in the fine-tuning of defence reactions has also become widely accepted. Plants possess versatile antioxidant systems to ensure that H_2O_2 is maintained at low levels during basic leaf metabolism (Pastori and Foyer, 2002; Mittler *et al.*, 2004). In chloroplasts, the low molecular weight antioxidants ascorbate and glutathione contribute chemically to the quenching of ROS, and comprise a key redox buffer in plant cells (Mittler *et al.*, 2004; Foyer and Noctor, 2009). H_2O_2 can also be enzymatically detoxified by APX, peroxiredoxin (PRXs), or glutathione peroxidase (GPX) activities. It should be noted that the last class of enzymes is misnamed and probably mainly uses thioredoxin rather than glutathione as its *in vivo* reductant (Noctor *et al.*, 2011, and references cited therein). The extent of the functional overlap between these systems still remains unclear, though one difference is that APX is H_2O_2 specific while the other peroxidases can also use small organic peroxides. A peculiar characteristic of chloroplast APXs and PRXs is that they are prone to inactivation when H_2O_2 accumulates in excess (Asada, 1999; König *et al.*, 2002; Kitajima *et al.*, 2006; Kitajima, 2008). While the physiological significance of this phenomenon has not been experimentally demonstrated, one can assume that such ROS-mediated inactivation of antioxidant enzymes could provide plants with a mechanism to amplify further the ROS burst in chloroplasts (Kitajima, 2008). In leaf peroxisomes, a specific catalase, annotated CAT2 in some species (e.g. *Arabidopsis*) but differently in other species (e.g. CAT1 in tobacco), is considered to act as the major H_2O_2 detoxifying enzyme (Mhamdi *et al.*, 2010*a*).

The chloroplast Cu/Zn superoxide dismutase, CSD2, seems to have a particularly important role in controlling ROS levels in infected tissues (Mateo *et al.*, 2004). In *pp2a-b'γ* mutant leaves, the constitutive defence responses and elevated ROS levels associate with an increased level of CSD2 (Trotta *et al.*, 2011). This is paralleled by an elevated

level of aconitase, which is a classical enzyme in the mitochondrial citric acid cycle, but has also been assigned a function in the regulation of CSD2 by binding to the 5′-untranslated region of CSD2 mRNA in the cytosol (Moeder *et al.*, 2007). Tobacco plants with reduced aconitase levels displayed increased tolerance against methyl viologen induced photo-oxidative stress, which further implies that aconitase operates in the antioxidant network in plants (Moeder *et al.*, 2007). Moreover, aconitase was found to promote cell death at early phases of infection, but to restrict the spread of the lesions at later time points (Moeder *et al.*, 2007). Thus, CSD2 and aconitase seem to mediate complex interactions in defence signalling in plants.

Of the chloroplast H_2O_2-scavenging enzymes, GPX7 and PRXQ seem to be specifically involved in the fine-tuning of defence reactions according to internal and external cues. *Arabidopsis* plants lacking GPX7 become vulnerable to photo-oxidative stress, but at the same time acquire resistance against infection by *Pseudomonas* strains (Chang *et al.*, 2009). Another study indicated a role for PRXQ in mediating responses against *Botrytis*, a necrotrophic fungus (Kiba *et al.*, 2005). In knock-down *pp2a-b'γ* mutant leaves, both GPX7 and PRXQ accumulate less than in the wild type, and these adjustments are associated with slight resistance against both *Pseudomonas* and *Botrytis* strains (Trotta *et al.*, 2011).

A key outstanding point concerns the extent to which defence-related ROS signalling is mediated—as well as controlled—by the antioxidative system. One long discussed possibility is that ROS-induced perturbations of the glutathione pool trigger changes in protein thiol status, thereby transmitting ROS signals (Foyer and Noctor, 2009; Noctor *et al.*, 2011, and references cited therein). There is a close relationship between expected intracellular H_2O_2 availability (which is not easy to quantify directly) and the redox state of the glutathione pool (Mhamdi *et al.*, 2010*a*). Pathogen responses triggered by catalase deficiency in *Arabidopsis cat2* mutants are dependent on GR activity (Mhamdi *et al.*, 2010*b*), while knocking out a specific NADPH oxidase activity in the *cat2* background largely annuls both *cat2*-triggered SA signalling and *cat2*-triggered changes in glutathione (Chaouch *et al.*, 2011). Together, these observations point to a crucial role for glutathione as a modulator of H_2O_2 signals during SA-dependent defence responses, in addition to its role as an antioxidant. Interestingly, the chloroplast is one of the major sites in which oxidized glutathione accumulates in response to increased intracellular H_2O_2, even when this oxidant is produced in the peroxisomes (Smith *et al.*, 1985; Queval *et al.*, 2011*a*). Oxidant-induced accumulation of glutathione is associated with induction and activation of enzymes involved in sulphur assimilation (Bick *et al.*, 2001; Queval *et al.*, 2009), and could contribute to links between sulphur nutrition and pathogen resistance that have been described (Bloem *et al.*, 2007; Zechmann *et al.*, 2007). Moreover, since the GR/glutathione system can affect pathogen resistance, including genes involved in both JA and SA signalling (Ball *et al.*, 2004; Parisy *et al.*, 2007; Mhamdi *et al.*, 2010*b*), this chloroplast response could be functionally significant in determining how intracellular ROS activate the expression of defence hormone signalling. Changes in ascorbate content have also been well documented to modulate pathogenesis responses, with ascorbate-deficient mutants showing constitutive activation of *PR* genes and related effects (Pastori *et al.*, 2003; Conklin and Barth, 2004; Pavet *et al.*, 2005).

Photorespiratory metabolism and defence: the physiology of photorespiration

Despite the focus on production of ROS at the plasmalemma, it is clear that the plant cell contains numerous intracellular sources of ROS, notably located in the chloroplasts, but also in peroxisomes and mitochondria. Redox states in all these compartments can be modified by photosynthesis, with photorespiration in particular involving complex intercompartmental cycling through redox shuttles (Hanning and Heldt, 1993; Igamberdiev and Gardeström, 2003). Thus, factors that alter the rate of photorespiration could impact on the probability of ROS accumulation in several organelles. Photorespiration most obviously affects ROS production in the peroxisome, where glycollate oxidation can produce abundant amounts of H_2O_2 as part of the photorespiratory carbon recycling pathway (Noctor *et al.*, 2002; Foyer and Noctor, 2003). However, photorespiration-linked changes in redox cycling could also alter NAD(P) redox states in the chloroplast and mitochondrion, and thus the rate of ROS production in these compartments (Scheibe *et al.*, 2005; Foyer *et al.*, 2009).

Peroxisomes are a rich source of oxidative and related signals (Nyathi and Baker, 2006). An important role for peroxisomal metabolism in some biotic interactions is supported by the observation that these organelles congregate at the site of invasion during exposure of cells to fungi (Lipka *et al.*, 2005). Studies on catalase-deficient plants, in which ROS signals are conditional on increased photorespiration, have demonstrated the potential of this pathway to trigger pathogenesis-linked reactions (Chamnongpol *et al.*, 1996, 1998; Du and Klessig, 1997; Takahashi *et al.*, 1997; Chaouch *et al.*, 2010, 2011). Impaired stomatal function has been shown to trigger cell death and pathogenesis responses under high light conditions (Mateo *et al.*, 2004), an effect that is most probably linked to increased photorespiration. Other evidence pointing to a role for photorespiratory metabolism in defence comes from studies of plants with altered peroxisomal serine:glyoxylate aminotransferase activity (Taler *et al.*, 2004).

Higher irradiance should favour increased photorespiratory flux. Because the rate of photorespiration is inextricably linked to photosynthetic metabolism, both photosynthesis and photorespiration should show a similar dependence on irradiance. Thus, supersaturating irradiances should not drive photorespiratory metabolism at much higher rates than those observed at saturating light (unless associated with increased leaf temperature or decreased CO_2

diffusion to the chloroplast from outside the leaf). The irradiance required to saturate photosynthesis is both species specific and influenced by the history of a given leaf or plant, as well as other factors such as temperature. For example, photosynthesis generally reaches its ceiling rate at ~500 μmol m^{-2} s^{-1} in *Arabidopsis* grown under typical controlled conditions, but higher irradiances (1000–1500 μmol m^{-2} s^{-1}) may be required to saturate photosynthesis in wheat leaves (e.g. Veljovic-Jovanovic *et al.*, 2001; Novitskaya *et al.*, 2002). For sun-exposed leaves of plants growing in the field, saturation of photosynthesis (and, therefore, photorespiration) can require even higher irradiances. It is important to note that many of the advances in dissecting pathogenesis responses (e.g. in *Arabidopsis*) have been obtained on plants grown at lower (sometimes much lower) irradiances than those required to saturate photosynthesis, and it is possible that responses are somewhat different when photosynthesis and photorespiration are more rapid.

Higher temperatures will favour increased photorespiratory flux, even if the irradiance remains constant. This is because the ribulose 1,5-*bis*phosphate (RuBP) carboxylation:oxygenation ratio (C:O) depends on (i) the intrinsic preference of Rubisco for CO_2 compared with O_2 (specificity factor) and (ii) the stromal concentration of CO_2 relative to that of O_2 (Keys, 1999; Von Caemmerer, 2000). Both of these factors decrease with increasing temperature, thus favouring photorespiration relative to overall photosynthetic rates and providing a satisfying ecophysiological explanation of the geographical distribution of C_3 and C_4 species. At low temperatures, photorespiration is likely to be slow, both because photosynthetic metabolism is slow and because C:O is relatively high.

Decreased stomatal conductance will promote photorespiration as the stromal CO_2 concentration drops, thus decreasing the C:O ratio. Among the factors triggering stomatal closure in the light, drought and salt/osmotic stress are prominent. However, many bacterial pathogens that gain entry into the leaf through the stomata, such as the well studied *P. syringae*, can also trigger this response (Melotto *et al.*, 2008).

Because of the above factors, the rate of H_2O_2 production through the peroxisomal glycollate oxidase reaction will, up to a limit, increase with increased irradiance, temperature, or stomatal closure. The last could also favour chloroplast ROS production if RuBP oxygenation is not able to sustain metabolism by completely replacing CO_2 fixation. In this case, the regeneration of $NADP^+$, the main acceptor for the electron transport chain, could be slowed, possibly favouring ROS production in the chloroplast (Fig. 1).

Catalase down-regulation and defence responses to enhanced photorespiratory H_2O_2

Other than the rate of photorespiration itself, a key player determining whether ROS associated with this pathway contribute to defence responses is likely to be catalase activity. ROS are distinguished by their high reactivity and by their ongoing metabolism through an active antioxidant system. While the first property makes them suitable as signal molecules, the second means that cells can potentially control the probability that ROS interact with signalling components by regulating key antioxidative systems, independently of the rate of ROS generation. In catalase-deficient tobacco lines, enhanced peroxisomal H_2O_2 availability can trigger SA-related pathogenesis responses (Chamnongpol *et al.*, 1996, 1998; Du and Klessig, 1997; Takahashi *et al.*, 1997). This includes cell death, though this does not occur through simple generalized oxidative damage but rather through a PCD-like phenomenon (Dat *et al.*, 2003). Recent characterization of *Arabidopsis* gene-specific *cat2* knockouts has opened up the possibility of genetic studies to analyse the relationship between enhanced peroxisomal H_2O_2 and defence responses. These have established that lesion formation in this line is daylength dependent (Queval *et al.*, 2007) and conditional on SA synthesis through the isochorismate pathway that is activated during the response to biotrophic pathogens (Chaouch and Noctor, 2010; Chaouch *et al.*, 2010). Thus, the *sid2* mutation, which blocks isochorismate synthesis, also blocks a range of pathogenesis responses that are otherwise activated in *cat2* (Chaouch *et al.*, 2010). Using targeted and non-targeted metabolite analysis, it was shown that metabolic signatures triggered by the *cat2* mutation were highly similar to those that follow challenge with virulent and avirulent bacteria (Chaouch *et al.*, 2011). Further, the *atrbohF* mutation specifically affected metabolic signatures triggered by the *cat2* mutation and by bacterial challenge in a similar manner (Chaouch *et al.*, 2011). Together, these findings show that H_2O_2 produced inside the cell can contribute strongly to the activation of the isochorismate-dependent SA synthesis pathway and, therefore, downstream reactions (Fig. 1).

While these studies have unequivocally demonstrated that genetically engineered catalase deficiency can act similarly to pathogen challenge to trigger defence pathways, it is not yet established that catalase down-regulation is an important part of pathogenesis responses. Nevertheless, literature studies have described several possible levels at which such regulation could occur. These include down-regulation of expression of the major leaf catalase in tobacco exposed to pathogens or SA (Dorey *et al.*, 1998) and more direct inhibition of enzyme activity by SA, NO, or inhibitors that are yet to be fully characterized (Beffagna and Lutzu, 2007; Vlot *et al.*, 2009). Other mechanisms regulating catalase include a G-box binding factor (GBF1) that interacts with the *CAT2* promoter, and this mechanism has been implicated in regulating leaf senescence (Smykowski *et al.*, 2010). In mammalian cells, programmed degradation of catalase may trigger autophagic cell death (Yu *et al.*, 2006). Studies on catalase turnover in several plant species have identified the protein as one of the most labile in leaf cells. The fast turnover of catalase is light dependent, whereas resynthesis to replenish the catalase pool may be negatively affected by stresses such as cold and salt (Volk and Feierabend, 1989; Hertwig *et al.*, 1992; Streb and Feierabend, 1996; Schmidt

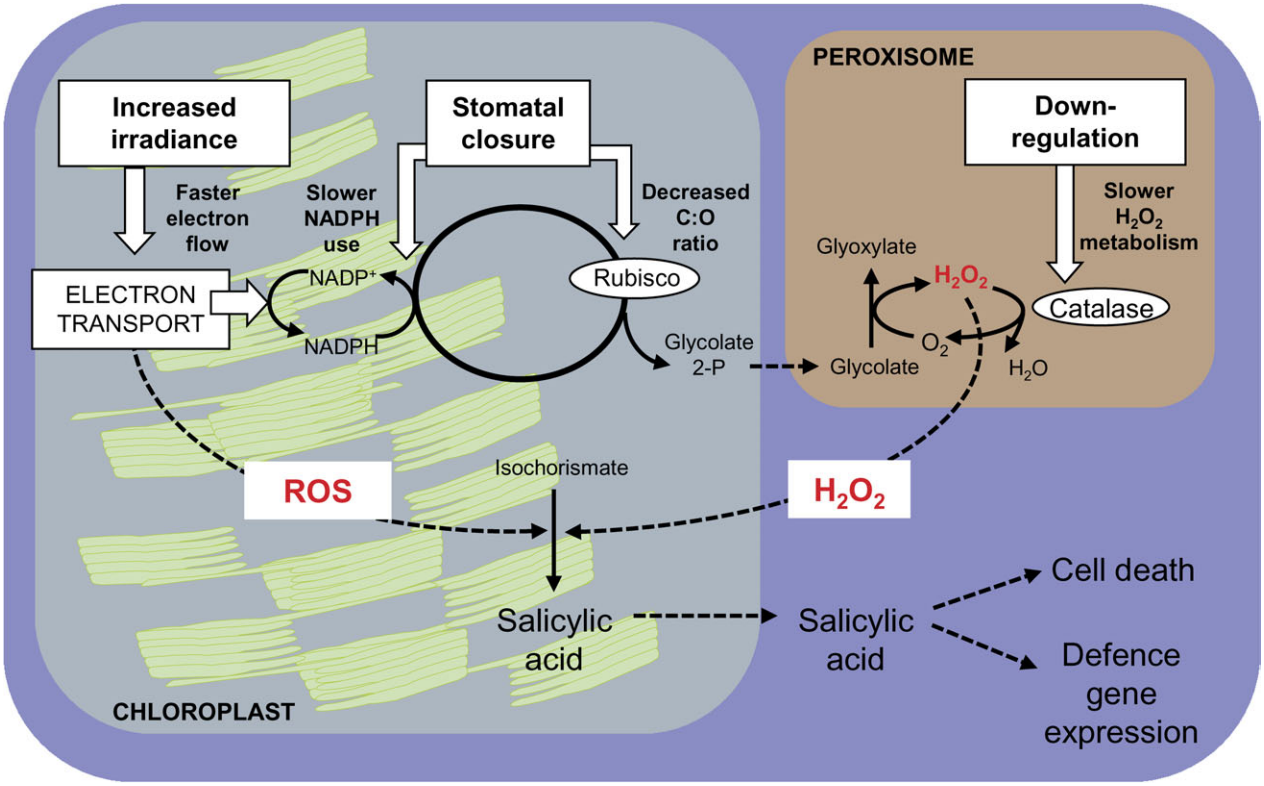

Fig. 1. Photosynthetic and photorespiratory ROS production and some of the factors that may promote their contribution to salicylic acid-related defence responses. C:O ratio, relative rates of carboxylation and oxygenation catalysed by Rubisco; glycolate 2-P, glycolate 2-phosphate; ROS, reactive oxygen species.

et al., 2006). Finally, although catalase is considered to be mainly peroxisomal, the details of its import mechanisms and their regulation remain to be definitively elucidated (Mhamdi *et al.*, 2010*a*). It is possible that mechanisms that act to down-regulate leaf catalase may be part of events contributing to the general increase in intracellular ROS that are necessary to activate pathways such as SA synthesis (Fig. 1).

Light perception in pathogen defence

In addition to effects of light quantity on redox status, light quality is important in pathogen defence. For example, in light conditions such as shading, where the red:far red light ratio (R:FR) is altered, the response to pathogens is decreased. This has been observed in the *sav3* (SHADE AVOIDANCE 3) mutant (Moreno *et al.*, 2009). It has been proposed that shading, characterized by a low R:FR, reduces plant sensitivity to jasmonates (Moreno *et al.*, 2009). Thus, in addition to the effects of light on redox and energetic processes, interactions with light quality and photoreceptor signalling are influential in the plant defence response.

It is now well established that in addition to its influence on plant growth and development, light signalling is required to establish an efficient response in several plant–pathogen interactions (Genoud *et al.*, 2002; Zeier *et al.*, 2004; Chandra-Shekara *et al.*, 2006; Griebel and Zeier, 2008). When *Arabidopsis* plants are inoculated in the dark with an avirulent strain of *Pseudomonas syringae*, they are not able to accumulate SA and this is accompanied by the failure to induce expression of the phenylpropanoid pathway enzyme, phenylalanine ammonia lyase (PAL) (Zeier *et al.*, 2004). Not only SA biosynthesis, but also SA perception is controlled by light. When treatment of *Arabidopsis* leaves with exogenous SA is performed in dim light or in the dark, expression of the SA-induced defence gene *PR-1* is compromised (Genoud *et al.*, 2002). Light regulation of defence responses is relevant not only during artificial darkening but also within light/dark cycles that naturally occur. However, a daytime-dependent difference in *P. syringae*-induced plant defences did not result from the circadian rhythm (Griebel and Zeier, 2008). Light availability is particularly important during the first hours after inoculation, as the absence of light during the early plant–pathogen interaction negatively affects development of an HR at later stages of the interaction (Griebel and Zeier, 2008).

Plant photoreceptors and defence

At least four classes of photoreceptors have been identified in *Arabidopsis*. The phytochromes are now known to be a family of five genes in *Arabidopsis* (PHYA–PHYE) and are most important in sensing red and far-red light (Rockwell *et al.*, 2006; Franklin and Quail, 2010). Three distinct classes of specific UV-A/blue light sensors are known: cryptochromes (CRY1 and CRY2), phototropins (PHOT1 and PHOT2),

and Zeitlupes (ZTL, FKF1, and LKP2) (Imaizumi *et al.*, 2003; Lin and Shalitin, 2003; Christie, 2007; Demarsy and Fankhauser, 2009; Kami *et al.*, 2010). A third member of the cryptochromes related to DNA photolyases, known as CRY3 (or cry-DASH), has been observed in *Arabidopsis*. The search for other photoreceptors is still ongoing in higher plants. First, higher plants possess a UV-B receptor with broad roles in photomorphogenesis, but its molecular nature is still elusive (Jenkins, 2009). Secondly, the putative photoreceptor role of zeaxanthin in stomatal opening remains to be resolved (Talbott *et al.*, 2003). Finally, a novel photoreceptor might be responsible for green light-mediated rapid stem elongation (Folta and Maruhnich, 2007).

Several studies have shown that specific photoreceptors can influence defence responses (Fig. 2). Systemic acquired resistance (SAR) usually requires molecular recognition events such as gene-for-gene-based resistance, in which disease resistance (R) genes notably include the large NBS-LRR class. The *constitutive shade-avoidance 1* mutant (*csa1*) carries a mutation in a defence response-related protein (TIR-NBS-LRR), resulting in a dominant negative effect on phytochrome signalling. Moreover, this mutant shows decreased resistance against pathogenic *Pseudomonas*. Thus, *csa1* provides one of several pieces of evidence that phytochrome and defence signalling interact (Faigon-Soverna *et al.*, 2006). It is also demonstrated that CRY1 positively regulates R protein-mediated resistance to avirulent *P. syringae* RPT2 in incompatible plant–pathogen interactions (Wu and Yang, 2010).

Genoud *et al.* (2002) demonstrated that phytochrome signalling pathways can activate both SA perception and HR development triggered by avirulent *P. syringae*. In particular, protein phosphatase 7 (*At*PP7) has been identified as a modulator of phytochrome signals and has been found to interact with nucleotide-diphosphate kinase 2 (NDPK2), an upstream element involved in the modulation of the SA-dependent defence pathway by light (Genoud *et al.*, 2008). However, the use of *Arabidopsis* photoreceptor double mutants has shown that the induction of defence responses at inoculation sites is not or only slightly modulated when cryptochrome, phototropin, or phytochrome photoreception is diminished. This contrasts with SAR, which depends on phytochrome photoreception, but can be established without functional cryptochrome or phototropin signalling pathways (Griebel and Zeier, 2008).

Chandra-Shekara *et al.* (2006) reported that the HR triggered by *Turnip crinkle virus* (TCV) and resistance to viral infection is influenced by light, but independent of the photoreceptors phytochrome A and phytochrome B. When Di-17, which is a TCV-resistant line when inoculated in the light, was inoculated with TCV or TMV following extended darkness before the regular day/night rhythm, development of HR was absent and the virus spread systemically. HRT is a putative resistance protein which confers the HR and resistance to TCV. When this protein was overexpressed in *phyA* or *phyB* mutant backgrounds, neither phytochrome was required for development of an HR resembling that seen in Di-17 (Chandra-Shekara *et al.*, 2006). The absence of light does not affect the induction of SA by TCV, although SA applied in the dark was unable to induce SA-mediated signalling leading to resistance or *PR-1* gene expression. Thus, both light and SA are key players in host–virus interactions (Chandra-Shekara *et al.*, 2006). Additionally, the blue-light photoreceptors CRY2 and

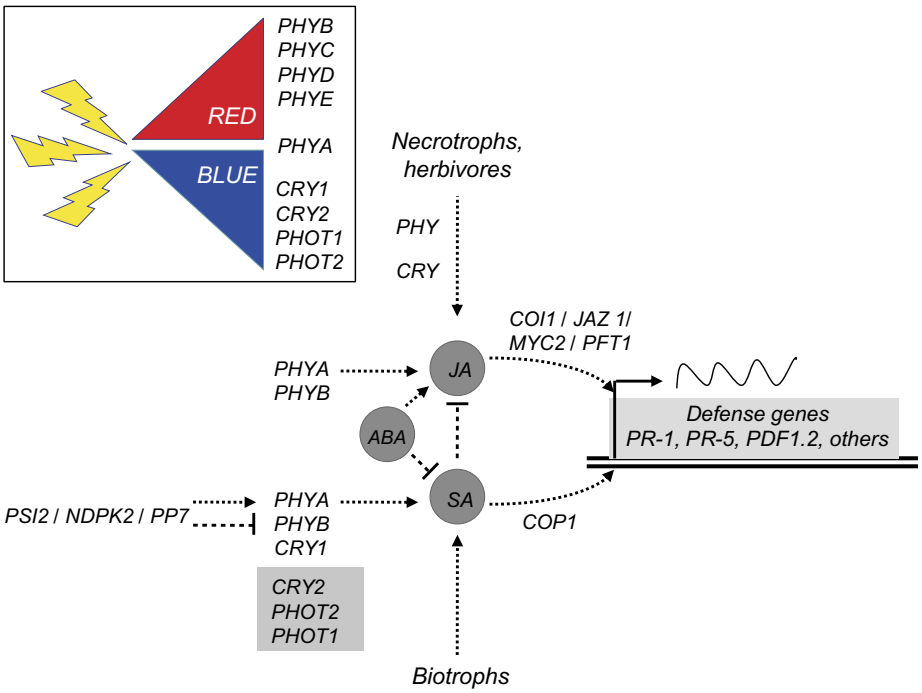

Fig. 2. Possible roles of photoreceptors in salicylic acid (SA) and jasmonic acid (JA)-related signalling pathways. CRY, cryptochrome; PHOT, phototropin; PHY, phytochrome. For discussion, see text.

PHOT2 are specifically required for maintaining the stability of the HRT protein (and thereby resistance to TCV) by interacting with and negatively regulating the activity of COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC 1). COP1 is an E3 ubiquitin ligase, which is known to target proteins for 26S proteasome-mediated degradation. CRY1 and PHOT1, in contrast, influence HRT-mediated resistance without affecting the stability of the R protein (Jeong *et al.*, 2010).

Despite these observations, not all inducible local plant defences require the presence of light. In *Arabidopsis* Col-0 leaves inoculated with *P. syringae avrRPM1*, continuous darkness did not affect biosynthesis of the *Arabidopsis* phytoalexin, camalexin, JA accumulation, or expression of *GST1*, a ROS-induced glutathione *S*-transferase (Zeier *et al.*, 2004). JAs are plant hormones that regulate many physiological processes, including pathogen defence. The characterization of several mutant lines deficient in JA biosynthesis and signalling has provided evidence of links between JA and phytochrome signalling. *JASMONATE INSENSITIVE 1* (*jar1*) has the same locus as *FAR-RED INSENSITIVE219* (*fin219*), which has been demonstrated to interact with *GSTU20* in response to light (Chen *et al.*, 2007). The JA receptor *COI1* (*CORONATINE INSENSITIVE1*), a central component of JA signalling, is necessary for a number of high irradiance responses in far-red light, and this requires stability of another important JA signalling component, *JAZ1* (*JASMONATE ZIM DOMAIN*; Robson *et al.*, 2010). It has also been recently shown that the *PHYTOCHROME AND FLOWERING TIME1* (*PFT1*) gene, which encodes the mediator 25 subunit of the plant Mediator complex, is a key regulator of JA-regulated transcription and is required for resistance to leaf-infecting necrotrophic fungal pathogens (Kidd *et al.*, 2009).

As well as pathogen responses, defence against the attack of insect herbivores is influenced by light. There is evidence that plant responses to herbivores and shading are in competition with each other, which may become crucial when, for example, plants under a canopy face such an attack. This is known as the plant dilemma, in which the plant must prioritize expression of shade avoidance responses or induction of chemical defences (Ballare, 2009). It has been shown that shade can down-regulate plant defences and so increase the leaf area eaten by herbivores (Izaguirre *et al.*, 2006; Moreno *et al.*, 2009). Thus, in shade conditions, priority will be given to reallocation of carbon resources to minimize the risk of competition (Kami *et al.*, 2010). As discussed above, shading, which is characterized by low R:FRs, decreases plant sensitivity to jasmonates (Moreno *et al.*, 2009). Thus, shade may weaken the defence response by repressing JA synthesis and signalling.

Circadian rhythms, growth daylength, and ROS

The plant circadian clock controls several elements of plant biochemistry and physiology and spans a period close to 24 h. An outcome of circadian control is gating, implying that equal stimuli applied at different times of the day can lead to different intensities of a specific plant response (Hotta *et al.*, 2007). A link between defence and circadian signalling has been based on the fact that PCC1 (PATHOGEN AND CIRCADIAN CONTROLLED1) and PAL1 follow a circadian expression pattern, but the functional significance of this is not yet clear. So far, the expression of these rhythmically expressed pathogen/defence-related genes has also been found to be inducible by pathogens, signalling molecules, and abiotic stresses (Weyman *et al.*, 2006). However, the effect of infections at different times of the day on the induction of gene expression or the pattern of expression in circadian clock-defective mutants has not yet been investigated (Roden and Ingle, 2009).

Although the role of the circadian clock remains unclear, recent findings suggest that signalling pathways related to daylength may be important in governing the outcome of ROS-triggered signalling. In the *Arabidopsis cat2* mutant, SA accumulation and associated responses do not occur in short days (8 h light/16 h dark). The failure to up-regulate these defences in these conditions does not seem to be trivially linked to an insufficiently severe oxidative stress (Queval *et al.*, 2007). Furthermore, responses in other *Arabidopsis* lesion-mimic mutants such as *lsd1* and *mips1* have also been shown to be influenced by the light regime (Dietrich *et al.*, 1994; Meng *et al.*, 2009).

The phenotypic differences in the response to H_2O_2 in *cat2* growing in short and long days are preceded and accompanied by daylength-specific *cat2*-dependent changes in gene expression. Daylength-specific patterns include oxidative stress-associated genes, which are generally more strongly induced in short days, and pathogenesis-related gene expression, which is more evident in long days (Queval *et al.*, 2007, 2011*b*; Chaouch *et al.*, 2010). Interestingly, the effect of daylength is not confined to oxidative stress, but also influences transcriptomic responses to the CO_2 level (Queval *et al.*, 2011*b*). Neither is the oxidative stress–daylength interaction confined to the *cat2* background, because the outcome of equal time exposure to ozone can also be influenced by the growth photoperiod context (Vollsnes *et al.*, 2009). Further evidence that daylength modulates redox regulation of defence-linked gene expression is supported by analysis of *gr1* mutants lacking expression of the cytosolic/peroxisomal isoform of GR. Although these mutants show neither phenotypic evidence of oxidative stress nor increased ROS signals, their relatively oxidized leaf glutathione status affects JA-associated gene expression in a manner dependent on growth daylength (Mhamdi *et al.*, 2010*b*). As noted above, links have been described between ROS, SA, JA, photoreceptors, flowering, and defence reactions (Genoud *et al.*, 2002; Martinez *et al.*, 2004; Danon *et al.*, 2005; Robson *et al.*, 2010).

Conclusions and perspectives

Although it is well established that plant defence is under genetic control, the outcome of defence signalling is also

influenced by environmental conditions and nutritional status. Understandably, much of the focus on defence signalling has been on cytosol–nuclear interactions (e.g. Mou *et al.*, 2003; Kaminaka *et al.*, 2006), but the chloroplast, as the engine of plant growth, also plays a crucial role. This organelle houses several important steps in the synthesis of phytohormones involved in defence, such as SA, JA, and ABA. As the ultimate source of photoassimilate, the chloroplast also contributes to sugar status, which can influence the SA pathway and interact with signalling through other phytohormones such as ABA that are involved in biotic challenge (Finkelstein and Gibson, 2002; Roitsch *et al.*, 2003; Asselbergh *et al.*, 2008). Moreover, several recent studies have shown that chloroplast-located proteins are involved in cross-talk with the cytosol and nucleus to govern the outcome of defence signalling. Further important information in this area is likely to be generated by the continued use of genetic studies in amenable species such as *Arabidopsis*.

The chloroplast is potentially a major source of ROS. It is the most important cellular player in production of $^1O_2$, and is also traditionally considered to be the major intracellular producer of partially reduced oxygen species such as $\cdot O_2^-$ and H_2O_2. However, these latter molecules can also be produced in substantial amounts by other organelles, notably peroxisomes and mitochondria (Foyer and Noctor, 2003), and a key outstanding question concerns the importance of different subcellular compartments in ROS production during plant responses to pathogens. Full resolution of this issue has been hampered by the absence of techniques able to generate quantitative information with sufficient resolution. Because of the reactivity of ROS and the complex redox matrix of plant tissues, most techniques used to detect intracellular ROS have hitherto been semi-quantitative (Queval *et al.*, 2008). As well as the question of spatial differences, the role of different ROS and their interactions (Gadjev *et al.*, 2006) remain to be fully elucidated. More insight into these questions is likely to be provided following the emergence of *in vivo* sensors that are able to report on specific ROS in a reliable, quantitative, and compartment-specific manner.

The most important redox parameter in defence responses might not be ROS titre *per se*. While the plastoquinone and TRX pools are key players in generating signals from the photosynthetic electron transport chain, an increasing number of studies are also providing insight into the important role of antioxidants, such as ascorbate and glutathione, in redox regulation. It is a striking but often overlooked fact that plants with decreased amounts of major antioxidative enzymes (APX and catalase) show clear evidence of oxidative stress despite a failure to display sustained increases in detectable ROS (e.g. Rizhsky *et al.*, 2002; Chaouch *et al.*, 2010, 2011). This probably reflects the potency of the intracellular antioxidative system in ROS homeostasis, and several observations suggest that ROS-triggered modulation of components such as glutathione may be one route by which oxidative signals are perceived by the plant cell (Mhamdi *et al.*, 2010a, *b*; Noctor *et al.*, 2011). Comprehensive high-throughput proteomics technologies are likely to be particularly important in elucidating the network of post-translational modifications involved in redox regulation.

Intriguing information is accumulating on the role of photoreceptor-mediated light signalling, circadian rhythms, and daylength in determining or toning the outcome of defence responses. Such effects are clearly of potential relevance to horticulture and agriculture, as they could contribute to seasonal variations in plant susceptibility to disease and other stresses. Photoreceptor pathways could be important, for example, in determining the daylength dependence of responses to intracellular H_2O_2. However, light modulation of oxidative stress responses could be dependent on chloroplast pathways such as those discussed in the first part of this review. Future studies should continue to throw further light on the complexity of the integrated circuitry that governs how plants cope with the attempts of microorganisms and herbivores to gain access to their resources.

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