Signalling crosstalk in light stress and immune reactions in plants

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The evolutionary history of plants is tightly connected with the evolution of microbial pathogens and herbivores, which use photosynthetic end products as a source of life. In these interactions, plants, as the stationary party, have evolved sophisticated mechanisms to sense, signal and respond to the presence of external stress agents. Chloroplasts are metabolically versatile organelles that carry out fundamental functions in determining appropriate immune reactions in plants. Besides photosynthesis, chloroplasts host key steps in the biosynthesis of amino acids, stress hormones and secondary metabolites, which have a great impact on resistance against pathogens and insect herbivores. Changes in chloroplast redox signalling pathways and reactive oxygen species metabolism also mediate local and systemic signals, which modulate plant resistance to light stress and disease. Moreover, interplay among chloroplast signalling networks and plasma membrane receptor kinases is emerging as a key mechanism that modulates stress responses in plants. This review highlights the central role of chloroplasts in the signalling crosstalk that essentially determines the outcome of plant–pathogen interactions in plants.

1. Introduction

Chloroplasts are metabolically versatile organelles that mediate key functions in the photochemical utilization of light, sensing and signalling of environmental stresses as well as elicitation and propagation of immune reactions against plant pathogens. Light as an environmental factor therefore has a key impact on the outcomes of biotic stress responses in plants [1,2]. Recent discoveries have started to uncover the sequence of events that starts with the recognition of a biotic stress agent in the extracellular space, proceeds through intracellular signalling interactions and culminates in physiological defence responses in infected tissues [3]. In this review, we elaborate on the role of chloroplasts as a central metabolic and regulatory hub, which largely specifies the extent and quality of defensive measures in plant cells.

2. Chloroplasts are targets for regulatory signals by both the defender and the invader in plant immunity

Plants can sense the presence of biotic stress factors by recognizing their conserved molecular patterns, which serve as ligands for plasma membrane receptor kinases (figure 1). One of the best-characterized examples for the recognition of pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) is the binding of flag22, a conserved peptide of bacterial flagellin, by the receptor-like kinase FLS2 [4]. The recognition of an external factor initiates a convergent sequence of events where the activation of the mitogen-activated protein kinase (MAPK) pathway is paralleled by changes in cytoplasmic calcium concentration, activation of...
calcium-dependent protein kinase (CDPK) cascades and rapid NADPH oxidase-driven burst of reactive oxygen species (ROS) in the apoplast. Together, these processes launch appropriate defence responses in infected tissues [5–8]. The transient early signalling events initiate mechanisms of PAMP-triggered immunity (PTI) [9] and are followed by more persisting changes occurring in organelar ROS metabolism, activation of hormonal signalling, sealing of infected cells by callose deposition into the cell wall and biosynthesis of antimicrobial secondary metabolites [10–14]. By combining different means of defence, plants attempt to prevent the pathogens and insect herbivores from colonizing the host tissue.

Two recent reports demonstrated that the recognition of external factors might also be directly signalled between the plasma membrane and chloroplasts. Nomura et al. [15] showed that the application of flg22 on Arabidopsis leaves promotes the formation of calcium transients in chloroplasts and that this response depends on the chloroplast CALCIUM-SENSING RECEPTOR (CAS). Analysis of gene expression in flg22-elicited leaves further revealed that CAS is required for the downregulation of photosynthesis-related genes and upregulation of defence genes in response to flg22 [15]. Subsequent work [16] reported that flg22 triggers the downregulation of non-photochemical energy quenching in chloroplasts, suggesting that controlled changes in the photo-protective mechanism represent an intrinsic component among plants' defence programmes. By using the air pollutant ozone as a tool to mimic pathogen-induced ROS signalling effects in guard cells, Vahisalu et al. [17] showed that a burst of ROS in guard cell chloroplasts coincided with a rapid decrease in stomatal conductance, a response that essentially limits pathogen entry sites upon infection. Evidently, plants have adopted an ability to deploy individual components of the photosynthetic apparatus in triggering pathogen-induced stomata closure and gene expression through light-dependent photosynthetic processes [3,15–17].

To combat the defence strategies of the plant, host-adapted pathogens secrete various effector molecules that suppress or circumvent plant immunity reactions, e.g. by directly targeting components of the PTI (figure 1). Plants in turn have evolved

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**Figure 1.** Chloroplast as a central component in plant immunity. Recognition of conserved microbial patterns (PAMPs) by plasma membrane receptor kinases launches the activation of mitogen-activated protein kinase (MAPK) cascades, formation of calcium transients, calcium-dependent protein kinases (CDPKs) and apoplastic reactive oxygen species (ROS) burst through NADPH oxidase activity. These initial signals trigger defence mechanisms collectively termed PAMP-triggered immunity (PTI). Host-adapted pathogens secrete various effector molecules to suppress PTI, while plants can specifically recognize effectors and activate effector-triggered immunity (ETI), which culminates in the onset of the hypersensitivity response (HR). Biosynthesis of salicylic acid (SA) and ROS signalling in chloroplasts are key components in plant immunity signalling. Photosynthetic production of ROS, controlled by peroxisomal catalase 2 (CAT2), further promotes these signalling effects. Some pathogen effectors, such as Hop1 and HopL1, aim to suppress SA and/or ROS signalling by targeting specific chloroplastic functions.
systems to recognize the activities of pathogen effectors, which trigger a process termed effector-triggered immunity (ETI) [9]. The hypersensitive response (HR), a hallmark of ETI characterized by cell death in the vicinity of the pathogen entry site, relies on a burst of ROS in chloroplasts. It is, therefore, not surprising that bacterial effectors may directly target chloroplastic functions. For example, the bacterial pathogen *Pseudomonas syringae* secretes an effector molecule, HopI1, which alters the structural organization of the thylakoid membranes and suppresses the accumulation of the stress hormone salicylic acid (SA), thus negatively regulating SA-mediated defences [18].

Another *Pseudomonas* effector molecule, HopN1, annihilates ROS production in chloroplasts, thereby inhibiting callose deposition and cell death in infected leaves [19]. HopN1 is a cysteine protease that targets the PsbQ protein of the oxygen-evolving complex and is therefore likely to mediate its effects by deteriorating electron transport in photosystem II (PSII) [19]. Requirement of intact PsbQ for full resistance against *Pseudomonas* infection also speaks for the importance of functional chloroplast electron transfer chain in defence responses [19]. In this respect, it is intriguing that the PsbO1 and PsbP proteins rapidly increased in abundance upon infection by avirulent (ETI-triggering) *P. syringae* expressing the effector AvrRpm1 [20]. Thus, the oxygen-evolving complex seems to form a central component that modulates ROS metabolism and cell death during an ETI.

### 3. Biosynthetic pathways of chloroplasts contribute to defensive measures of plants

Organelles contribute to plant immunity also by hosting diverse biosynthetic pathways, the reaction products of which carry out important functions in stress resistance and signalling in plant cells (figure 2). The isochorismate branch of the shikimate pathway, for example, is the main source of SA in infected leaves. The gene encoding *ISOCHORISMA S YNTHASE 1* (ICS1), the rate-limiting step in the biosynthesis of SA, is
transcriptionally regulated and becomes activated in response to infection [21,22]. The shikimate pathway also serves the biosynthesis of aromatic amino acids, which are precursors for the biosynthesis of indolic and phenolic secondary metabolites, and has been estimated to consume at least 30% of photosynthetically fixed carbon [23]. Indolic glucosinolates (GLS) derive from tryptophan and provide an intricate example of metabolic connections between chloroplast signalling and defence mechanisms in plants (figure 2). In Brassicaceae, the biosynthesis of the sulfur-rich GLS is linked to the universal sulfate donor phosphoethenodine 5'-phosphosulphate (PAPS), whose by-product phosphoethenodine 5'-phosphate (PAP) has been shown to mediate retrograde signals from chloroplast to nucleus under high light stress [24–26]. As sulfur metabolism is regulated by photosynthesis-driven redox chemistry [27], a link between the biosynthesis of these secondary compounds and light-mediated signalling seems evident [28]. Moreover, degradation products of the indolic GLS have also been shown to act in parallel with SA signalling to promote callose deposition to the cell wall [14].

4. Light-induced reactive oxygen species signals trigger a genetically encoded cell death pathway in plant immunity

Photosynthesis is a key modulator of cellular redox metabolism and has far-reaching effects on the metabolic status and stress tolerance of plants [3,29]. Photosynthetic activity provides NADPH, ATP and carbon skeletons, which fuel the initiation and maintenance of responses against external stress factors. Sudden exposure of plants to high light intensity may cause different levels of ‘light stress’, which may vary from the transient over-reduction of the photosynthetic electron transfer chain and photoinhibition of PSI to irreversible ROS-induced damage to photosynthetic components. As discussed below, these differentially harmful effects may have completely different stress signalling effects in plant cells. Formation of ROS, including superoxide and hydrogen peroxide (O2⁻ and H2O2, respectively), is in principle unavoidable in photosynthesis owing to the generation of strongly reactive species during primary photochemistry. It should be noted, however, that although the formation of O2⁻ and H2O2 is commonly associated with photosystem I (PSI) activity, recent reports have questioned the role of PSI electron transfer to molecular oxygen as a main source of ROS in higher plants [30,31]. Imbalanced electron transfer reactions may also lead to the formation of singlet oxygen \( ^1O_2 \) in PSI [32,33]. However, chloroplasts are well equipped with a multitude of overlapping antioxidant systems, which maintain ROS below dangerous levels for the cells.

Even though harsh redox modifications may have deleterious effects on photosynthetic activity [34], the accumulation of redox-active intermediates and/or ROS becomes beneficial in terms of signalling effects against different types of stress, including high light and pathogens [35]. Recent work done with green algae has suggested a possible mechanism explaining this tight relationship. Expression of a nuclear-encoded reporter gene coupled to a H2O2 sensitive promoter was shown to respond not only to the levels of exogenously added H2O2 but also to light-induced photosynthetic electron transfer, its transcriptional activation correlating with a lower intracellular H2O2 scavenging activity [36]. This suggests that enhanced H2O2 production in the light should reflect a transient inactivation of the ROS scavenging processes rather than an increased H2O2 production, possibly to trigger specific ROS signalling responses within the cell.

How are ROS signals translated into physiological responses? Using Arabidopsis as a model organism, Lee et al. [37] have demonstrated the existence of genetic determinants that control ROS signalling. Starting from the Arabidopsis flu mutant, which accumulates the chlorophyll precursor protochlorophyllide in the dark (and therefore shows enhanced \( ^1O_2 \) accumulation upon re-illumination), the authors selected and characterized a second site suppressors. This led to the isolation of EXECUTERS 1 and 2, two chloroplast-targeted and thylakoid-bound proteins, which control \( ^1O_2 \) responses [37]. A comparative global analysis of transcript changes upon light exposure of dark-adapted Arabidopsis wild-type and mutant plants showed that the FLU-mediated \(^1O_2\) signalling pathway is similar to the general stress response induced by pathogen attack, wounding or high light and drought stress. Moreover, the fact that the flu phenotype is conditioned by the presence of EXECUTER proteins demonstrates that the pathway is genetically determined. More recently, another essential piece of information on ROS signal transduction has been provided by the finding that ROS-related pathogenesis responses occur via Ca\(^{2+}\) signalling, via the activity of the chloroplast CAS [15]. However, the molecular mechanisms relating ROS, pathogen defence response and CAS are still largely undetermined.

In contrast to the large number of Arabidopsis mutants that display enhanced cell death upon increasing irradiance levels [38], a knockdown mutant deficient in a specific cytosolic regulatory B\(\gamma\) subunit of the trimeric protein phosphatase 2A (PP2A-B\(\gamma\)) shows a constitutive pathogenesis response and premature leaf yellowing under moderate light intensity, whereas high light partially rescues the phenotype [39]. The pp2a\(-b\gamma\) mutant shows disintegration of chloroplasts and accumulation of H2O2 in the leaf mesophyll cells even in visually healthy leaves [39]. The disintegration of chloroplasts in pp2a\(-b\gamma\) precedes the degradation of other cellular components and in this respect resembles the early steps of singlet oxygen-induced programmed cell death of the flu mutant [40]. PP2A-B\(\gamma\) therefore has a role in the crosstalk between defence and light acclimation, and is likely to control cytoplasmic components that respond to organellar signals [39].

5. Interplay between apoplastic and chloroplastic reactive oxygen species signalling impacts on light acclimation, disease resistance and plant development

Light-induced ROS signalling effects may also arise as a result of crosstalk between different cellular compartments. In the plasma membrane, NADPH oxidases transfer electrons from NADPH to oxygen, leading to the generation of superoxide and hydrogen peroxide in the apoplasm [41]. The activation of the NADPH oxidase, which mediates the progression of ROS signals from cell to cell in a ‘ROS wave’, is now known to be mediated by CDPK 5 [42,43]. Through yet unknown mechanisms, NADPH oxidase-dependent apoplastic ROS burst also triggers ROS production in chloroplasts [15,44]. This is structurally feasible, because under high light chloroplasts adopt...
a position adjacent to the plasma membrane, thus allowing efficient communication.

A key role for the chloroplast envelope in cellular signalling has also recently become evident [45,46]. For example, Arabidopsis reticulata (re) mutants, deficient in a transmembrane protein of the chloroplast inner envelope, exhibit reticulated leaf pigmentation and constitutive accumulation of ROS along leaf veins conditionally under long day conditions [47,48]. The re mutants also show enhanced cell death in response to apoplastic ROS signals when exposed to ozone fumigation under moderately high light intensity [48,49]. Thus, the interplay between apoplastic and chloroplastic ROS signalling cascades seems to enhance cell death initiation in re leaves.

Despite their imbalanced ROS metabolism, re mutants display a decreased expression of SA signalling markers, a response attributed to imbalanced amino acid homeoceaostasis and a consequent inability to synthesize SA through the ICS1-dependent pathway [48]. These characteristics position RE in a regulatory network, which seems to functionally interconnect photoperiodic growth, amino acid homeoceaostasis and ROS signalling in Arabidopsis leaves [48,50,51]. Indeed, the photoperiod is emerging as a key environmental factor that modulates plant responses to organellar ROS signals [2,22,48,52–56].

In conclusion, plant–biotic interactions are under multifaceted influence of light, which exerts its effects through light-intensity-dependent photosynthetic processes, as well as mechanisms that may respond to the quality and duration of light [2]. Understanding the complex molecular crosstalk in environmental light signalling will be highly relevant in efforts to enhance the stress tolerance of plants for sustainable productivity in the future.

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