**Plant environmental sensing relies on specialized plastids**

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Highlight: Recently emerging literature reveals that plastids are more multifaceted in their spatial organization and environmental sensing and signaling than previously thought. This discovery opens the field to important new facets of how plants adjust to environmental change.

**Abstract**

In plants, plastids are thought to interconvert to various forms that are specialized for photosynthesis, starch and oil storage, and diverse pigment accumulation. Post-endosymbiotic evolution has led to adaptations and specializations within plastid populations that align organellar functions with different cellular properties in primary and secondary metabolism, plant growth, organ development and environmental sensing. Here, we review plastid biology literature in light of recent reports supporting a class of 'sensory plastids' that are specialized for stress sensing and signaling. Abundant literature indicates that epidermal and vascular parenchyma plastids display shared features of dynamic morphology, proteome composition and plastid-nuclear interaction that facilitate environmental sensing and signaling. These findings have the potential to reshape our understanding of plastid functional diversification.

**Key words**

Epidermis/abiotic stress/stromules/ROS/retrograde signaling

## Introduction

The evolution of multicellular eukaryotic organisms was sparked, in part, by successful endosymbiosis of a photosynthetic microbe, bringing about an entire divergent branch of autotrophic systems evolution (Marechal, 2018). Post-endosymbiotic expansion of the chloroplast has been punctuated by functional diversification, with many plastid types no longer fully photosynthetic. As an organellar group, distinct plastid forms can interconvert to provide specialized functions within a specific tissue type or developmental stage (Pyke, 2007). Yet, the vital importance of photosynthesis has inclined the majority of plastid studies toward the mesophyll chloroplast and its properties. The origin and specialized features of other classes of plastids are only now being elucidated, leading to better understanding of how plastid transitions are defined by changes in their nuclear-directed proteome composition (Christian et al. 2020; Chu et al. 2018; Melonek et al. 2016).

Earlier botanical literature referred to the "leucoplast", a collective term for plastids much smaller in size than the mesophyll chloroplast and non-photosynthetic (Pyke, 2007). Leucoplasts have been described in epidermal cells, vascular parenchyma, root tissues, meristem regions and reproductive tissues. However, the smaller plastids within the epidermal pavement cells of Arabidopsis do contain chlorophyll (Higa et al. 2014; Barton et al. 2016), and this appears to be the case in vascular parenchyma plastids as well (Virdi et al 2016), based on autofluorescence in laser confocal microscopy experiments. The abaxial epidermis "leucoplasts" of *Nicotiana benthamiana*, have a photosynthetic efficiency similar to that of mesophyll chloroplasts, indicating that different higher plant species may have a range of photosynthetic competencies in tissues such as epidermis, bundle sheath and vascular parenchyma (Fryer et al. 2003; Galvez-Valdivieso et al. 2009; Barton et al. 2016; Exposito-Rodriguez et al. 2017; Xiong et al. 2021). Studies have shown that the "leucoplast" proteome contains distinct proteins not found within the mesophyll chloroplast (Beltran et al. 2018). Still, to date this plastid type remains ill-defined in the literature, earlier described as participating in "synthesis and storage of starch, lipids and proteins" (Wise, 2006).

Leucoplasts, or a subset thereof, play important roles in a plant's interaction with, and response to, its environment. We focus on this plastid subtype here with primary emphasis on work conducted in the model species *Arabidopsis thaliana*. Based on outcomes of this recent research, which includes organelle dynamic morphology, proteome composition and genetic mutant studies, this organelle is referred to as the *sensory* plastid (Virdi et al. 2016; Beltran et al. 2018). For the purposes of this

review, we specifically limit mention of the guard cell plastids, which share several features with sensory plastids but carry out specialized functions that distinguish their particular cell type.

**Sensory plastids are specialized for environmental sensing/signaling**

Sensory plastids are characterized not only by a distinctively small size but by unusually dynamic properties, including the production of stromules. Stromules are plastid envelope tubular outgrowths that extend to various lengths and display highly dynamic properties (Hanson and Hines, 2018). The structures are thought to facilitate plastid intracellular positioning and increase in density in plastid relocation and division mutants (Caplan et al. 2015; Holzinger et al. 2018; Ishikawa et al. 2020). Stromules also increase when plastid outer envelope proteins are over-expressed (Machettira et al. 2012), reflecting interactions with other cellular components. Although most studies of stromule activity are conducted in leaf epidermal pavement cells, the structures are also abundant in vascular parenchyma and root hair plastids (Gray et al. 2012). Response to environmental stimuli can induce rapid and prolific stromule formation, and stromule numbers increase under conditions of high light and redox changes (Brunkard et al. 2015), increased glucose or sucrose levels (Schattat and Klosgen, 2011), and in response to abiotic stress-related hormone signaling and pathogen effector-triggered immunity (Gray et al. 2012; Caplan et al 2015; Kumar et al. 2018; Savage et al. 2021).

Root epidermal plastids bear striking resemblance to the above-ground sensory plastid in size and behavior, and root epidermal cells are also notable for dynamic stromule formation (Pyke, 2007). Root plastids influence interactions between root cells with symbiotic microbes, for example, so that sites where fungal interactions occur within arbuscule cells produce extensive stromule networks (Fester et al. 2001; Hans et al. 2004). These points of interaction undergo measurable changes in plastid metabolic activity during establishment of symbiosis (Lohse et al. 2005).

Most studies that focus on plastid stromule activity have been conducted in epidermal pavement cells. This reliance on epidermis may be one of convenience, facilitating live cell imaging. Yet, mesophyll chloroplasts show markedly reduced stromule numbers, average length and activity relative to epidermal cell plastids (Waters et al. 2004; Higa et al. 2014). This disparity in plastid stromule activity could be a function of differences in plastid size and density in mesophyll and epidermal cells, with greater stromule activity in plastids that can move more freely within the cell

(Waters et al. 2004). Sensory plastid stromule activity also appears to be an important specialized feature of environmental sensing and signaling activities concentrated in particular cell types and activated under any condition that limits photosynthesis (Mullineaux et al. 2020; Breeze and Mullineaux, 2022).The epidermal sensory plastid is also a system of choice for investigating plastid perinuclear associations. These plastids can position in close nuclear proximity, with movement controlled by the actin cytoskeleton (Sheahan et al. 2004). During cell division, this association can serve to ensure distribution of sufficient plastids to each daughter cell. Epidermal plastid-nuclear associations can involve physical connection, with plastids able to surround the nucleus and attach to the nuclear membrane *via* membrane contact sites; this activity is influenced by trafficking through the perinuclear space, which is contiguous with the endoplasmic reticulum (Kwok and Hanson, 2004; Higa et al. 2014; Breeze and Mullineaux 2022). Recent evidence shows that plastid perinuclear association in epidermal cells occurs in response to plant environmental stress (Savage et al. 2021). Actin-mediated attachment of plastids to the nucleus is necessary for the high light avoidance response, in which blue light-induced movement of the nucleus requires attachment to plastids (Higa et al. 2014). This plastid-nuclear attachment relies on plastid division components that include *PLASTID DIVISION1* and *2* (*PDV1, PDV2*) and *PARALOG OF ARC6* (*PARC6*) (Higa et al. 2014; Itoh et al. 2018).

Mutant studies of plastid morphology show that PARC6, a factor that positions the plastid for division in vascular plants, functions predominantly to regulate non-mesophyll plastid morphology and stromule activity (Itoh et al. 2018; Ishikawa et al. 2020). Additional genetic factors differentially regulate non-mesophyll plastid development, such as *TGD5*, a component of lipid metabolism that influences sensory plastid but not mesophyll chloroplast development (Itoh et al. 2021). TGD5 is a protein that participates in endoplasmic reticulum-to-plastid transport for thylakoid lipid assembly. This alternative pathway for lipid trafficking appears to be preferred for epidermal and also root plastid development (Obata et al. 2021).

Transcription factors GATA NITRATE-INDUCIBLE CARBON-METABOLISM-INVOLVED (GNC) and CYTOKININ-RESPONSIVE GATA1 (CGA1) regulate plastid development such that ectopic overexpression induces enhanced chlorophyll production and chloroplast-like thylakoid development in epidermal and vascular parenchyma cell plastids (Chiang et al. 2012). These various observations are evidence of nuclear-directed plastid functional differentiation between adjacent

cell types, and incorporation of this type of mutant analysis helps to define plant tissues that share plastid types and developmental programs.

**Identifying likely components of the sensory plastid proteome**

Several components of the sensory plastid proteome are shared with the mesophyll chloroplast in a manner that permits for specialized functions in the two plastid types. These plastid targeted proteins are often encoded by duplicate genes where assignment to a putative sensory plastid versus mesophyll chloroplast function can be made by *in silico* analysis of publicly available expression data. With ePlant (Waese et al. 2017), for example, incorporation of *MutS HOMOLOG 1* (*MSH1)* as a standard for sensory plastid-specific (Virdi et al. 2016; Beltran et al. 2018) and *PSBO1*, an extrinsic subunit of Photosystem II, as a standard for mesophyll chloroplast-localized proteins can facilitate initial assignment of expression for other candidates to one plastid type or the other. Discrimination is based largely on comparison of leaf and root expression patterns in response to environmental change, with *MSH1* expressing moderately throughout the plant and in epidermal and root vascular tissues and *PSBO1* showing strong leaf and above-ground expression and nearly undetectable root signal under most conditions tested (Figure 1).

Putative protein assignment to sensory plastid can be followed by genetic and biochemical analyses for further confirmation, often producing interesting observations that further elaborate sensory plastid function in environmental sensing. for example, the Arabidopsis *CHLOROPHYLL A/B BINDING PROTEIN UNDEREXPRESSED* (*CUE1*, a.k.a. *PPT1*) gene encodes a phosphoenolpyruvate/phosphate translocator protein located on the inner membrane of the sensory plastid, and a *cue1* mutation produces a reticulate (dark-green leaf venation) phenotype (Li et al. 1995; Voll et al. 2003). This mutant phenotype reflects vascular tissue-associated expression and can be complemented by supplementation with aromatic amino acids, indicative of shikimate pathway influence (Voll et al. 2003). The CUE1 protein is enriched in sensory plastid proteome studies (Beltran et al. 2018) and *CUE1* shows sensory plastid-associated expression *in silico* (Fig. 1). The homologous locus, *PPT2*, encodes the mesophyll chloroplast PEP translocator protein (Hilgers, et al., 2018) and shows mesophyll chloroplast-associated expression *in silico*. *cue1* mutant serves as second site suppressor of *ros1* mutations (Shen et al. 2009); ROS1 encodes a demethylase that modulates nuclear cytosine methylation genome-wide, serving as a rheostat for dynamic methylome adjustment (Williams et al. 2015).

The shikimate pathway gives rise to numerous products vital to plant defense and stress response (Maeda and Dudareva, 2012). The first enzyme of the shikimate pathway is 3-deoxy- d-arabino-heptulosonate-7-phosphate synthase (DAHPS), which converts PEP to 3- dehydroquaianate. The enzyme is encoded by two genes in Arabidopsis, *DAHPS1* and *DAHPS2* (Tzin & Galili, 2010). *In silico* expression profiling indicates that DAHPS1 is likely localized to the sensory plastid, and DAHPS2 to the mesophyll chloroplast (Fig. 1); DAHPS1 also localizes to the sensory plastid by proteome analysis (Beltran et al 2018). *DAHPS1* is induced in response to wounding and pathogen infection (Keith et al. 1991;Gorlach et al. 1995) while *DAHPS2* is constitutively expressed (Maeda and Dudareva, 2012). These observations suggest that gene duplication and differential accumulation in the two plastid types further specialize this enzyme for environmentally-responsive shikimate-related functions.

Other genetic components of the shikimate pathway are similarly configured. For example, shikimate kinase catalyzes a regulatory step in the pathway, converting shikimate to shikimate 3-phosphate (Tzin & Galili, 2010). Again, Arabidopsis encodes two SK isoforms, *SK1* and *SK2* with SK1 predicted to accumulate in the sensory plastid and SK2 in the mesophyll chloroplast, based on *in silico* data. The shikimate pathway controls aromatic amino acid biosynthesis, and the branch point between phenylalanine and tyrosine biosynthesis lies at conversion of arogenate to tyrosine by arogenate dehydrogenase (TyrA). In Arabidopsis, TyrA is encoded by two genes, *TyrA1* and *TyrA2* with TyrA2 protein enriched in the sensory plastid proteome (Beltran et al. 2018). For tryptophan biosynthesis, a first step is catalyzed by anthranilate synthase (AS), which functions as a heterotetramer of two alpha and two beta subunits (Niyogi et al., 1993; Poulsen et al., 1993). Two genes encode the AS alpha subunit in Arabidopsis, *ASa1* and *ASa2* with one *ASb1*. *In silico* analysis indicates ASa1 to be a candidate for sensory plastid localization and ASa2 for mesophyll chloroplast, while *ASb1* encompasses both patterns of expression. Consistent with this prediction, *Asa1* is regulated in response to wounding or pathogen stress while *Asa2* is constitutively expressed, hinting at how TRP pathway product synthesis is regulated for plant defense (Bohlmann et al. 1995; Nyogi and Fink, 1992). Similarly, *ICS1* and *ICS2* are duplicate genes encoding isochorismate synthase, which converts chorismate to isochorismate in phylloquinone biosynthesis. Both enzymes also participate in salicylic acid biosynthesis, important to plant defense (Catinot et al. 2008) and responsive to sensory plastid perturbation (Shao et al. 2017; Yang et al. 2020). These genes are differentially

regulated and ICS2 localizes to vascular tissue, implying sensory plastid association ( Macaulay et al. 2017).

As evidenced by *cue1*, the reticulate leaf mutant phenotype is characteristic of sensory plastid dysfunction. As many as 14 *reticulata* phenotype mutants have been associated with the sensory plastid by protein enrichment in the sensory plastid proteome (Beltran et al. 2018). Most show some version of a darker green venation with pale interveinal leaf tissue. Several of these mutants are related to the shikimate pathway, display vascular parenchyma-specific or vascular enriched expression patterns, and can be complemented by aromatic amino acid supplementation (Lundquist et al. 2014). Aromatic amino acid metabolism can affect plant defense. For example, phenylalanine pools and phenylpropanoid metabolism influence effector-triggered immunity (Yoo et al 2020). The *Reticulata*-related proteins also serve to interconnect photoperiodic growth, amino acid biosynthesis and ROS metabolism during Arabidopsis leaf development (Perez-Perez et al. 2013).

Blue-native gel analysis of a *reticulata* mutant reveals changes in lipid remodeling, amino acid metabolism and plastid division components (Lundquist et al. 2017). Sensory plastids, while overlapping with mesophyll chloroplasts in several proteome factors, appear to implement these factors to confer novel capabilities for environmental responsiveness and development.

Investigation of stress-responsive plastid proteins can sometimes identify root counterparts to above-ground pathways, again differentiating sensory plastid from mesophyll chloroplast functions. The genes encoding $NADP^+$ oxidoreductases are distinguished by their function in photosynthetic chloroplasts (*LFNR1* and *LFNR2*) versus non-photosynthetic plastids (*RFNR1* and *RFNR2*) (Grabsztunowicz et al. 2021). RFNR types respond to low temperature stress in the root (*RFNR2*) and ozone treatment in the leaf (*RFNR1*), where these oxidoreductases accumulate primarily within vascular and epidermal tissues. Recognizing these differential patterns of localization, expression and environmental responsiveness will likely reveal additional sensory plastid features in future studies.

**Assigning some retrograde signaling components to putative sensory plastid-specific responses**

The sensory plastid proteome is characterized by particular proteins specific to this plastid type. Putative sensory plastid-specific proteins include *SAL1(ALX8*, *FRY1*), a component of organellar retrograde signaling, and *MutS HOMOLOG 1* (*MSH1*), an organellar DNA binding protein.

The redox-regulated phosphatase SAL1 is predominantly expressed within vascular tissue and localizes to both mitochondria and plastids to regulate levels of 3'-phosphoadenosine 5'-phosphate (PAP) by dephosphorylation to AMP (Estavillo et al., 2011). PAP is a byproduct of sulfur metabolism and is transferred to the nucleus, where it inhibits XRN type exoribonucleases (Estavillo et al., 2011; Litthauer & Jones, 2018). These exoribonucleases target miRNAs, comprising a means for broad influence on plant stress responses. Tocopherols derived from tyrosine in the sensory plastid (via the shikimate pathway) serve to upregulate PAP (Fang et al., 2019). This process is dependent on CUE1 (PPT1) (Fang et al, 2019) on the sensory plastid inner envelope (Beltran et al. 2018; Lundquist et al. 2014).

Plastid-derived 3'-phosphoadenosine 5'-phosphosulfate (PAPS) is a high-energy sulfate donor for sulfation reactions that must be transported to the cytosol and Golgi apparatus for sulfotransferase reactions. A thylakoid ADP/ATP carrier, PAPST1 (TAAC), has been shown to transport PAPS across the plastid envelope and to favor PAP and ATP as substrates (Gigolashvili et al. 2012). Expression analysis of the PAPST1 protein indicates its accumulation in epidermis, vascular tissues, meristem and reproductive tissues, but not mesophyll (Gigolashvili et al. 2012).

**The MSH1 system triggers sensory plastid-specific signaling**

The plant-specific gene *MSH1* encodes a dual-targeted mitochondrial and plastid DNA binding protein that localizes to the sensory plastid but not the mesophyll chloroplast (Xu et al., 2011). *MSH1* participates in stabilizing the mitochondrial and plastid genomes by suppressing illegitimate recombination (Davila et al. 2011; Xu et al. 2011). The *msh1* mutant has a variable and pleiotropic phenotype that reprograms development (Xu et al. 2012) , involving altered plastoquinone pool, delayed growth and maturation, altered circadian clock effects and enhanced abiotic and biotic

stress response (Shao, et al., 2017; Virdi et al., 2016; Xu et al., 2011; Xu et al., 2012). *MSH1* RNAi-suppressed plants produce progeny showing *msh1* stress memory effects that are heritable indefinitely (Virdi et al., 2015; Xu et al., 2012). Transition to this epigenetic memory state is dependent on the RNA-directed DNA methylation (RdDM) pathway (Yang et al. 2020).

Nuclear epigenetic changes are triggered by plastid perturbation in the *msh1* mutant (Xu et al. 2012; Virdi et al. 2016). These unusual effects can be further studied through grafting of *msh1* as rootstock with wild type scion. Next-generation graft progeny from these experiments are measurably enhanced in growth vigor and yield over wild type, effects that are also RdDM-dependent (Kundariya et al. 2020). The effects of MSH1 depletion on nuclear DNA methylation repatterning and gene expression reveal gene networks that contribute to the unusual phenotypic changes in plant growth and stress response (Kundariya et al. 2020; Yang et al. 2020).

It is unclear how disruption of a plastid DNA binding protein triggers nuclear epigenetic effects. Studies suggest that instability of the plastid genome triggers nuclear genome responses. For example, plastid genome instability induced by treatment with DNA gyrase inhibitors such as ciprofloxacin (CIP) can impact cell cycle and manifest in endoreduplication and plastid DNA fragmentation (LePage et al. 2013; Zampini et al. 2015; Duan et al. 2020). Likewise, the Arabidopsis triple mutants *whirly1* (*why1*) / *why3* / *type1-polymerase* (*polb1*) and *why1*/*why3*/*plastid DNA recombinase1* (*recA1*) display plastid genome instability. These mutants and treatments result in a wide range of stress-responsive, growth and developmental phenotypes (LePage et al. 2013; Duan et al. 2020), some similar to those of *msh1* plants (Xu et al. 2011; Beltran et al. 2018). Different monocot and dicot *msh1* mutant plant species show a leaf variegated phenotype, which is strongly associated with sensory plastid genome instability in cells of the vasculature and epidermis but also impact on chloroplast function in mesophyll tissue. The *why1/why3/polb1*, *why1/why3/recA1* and *msh1* mutants have a lowered photosynthetic efficiency in green parts of variegated tissues and show increased reactive oxygen species (ROS) levels by dye staining (Xu et al 2011; LePage et al. 2013; Duan et al 2020). Elevated ROS lead, in turn, to a reconfiguration of nuclear gene expression associated with responses to excess light intensity, which affects a range of cellular functions (LePage et al. 2013; Beltran et al 2018; Duan et al 2020). A failure of such plants to induce anthocyanin pigmentation in their leaves upon exposure to excess light intensities was taken as evidence for increased photo-oxidative stress tolerance associated with improved seed yield and/or

increased shoot biomass (LePage et al 2013; Xu et al. 2011). However, at least the *why1/why3/polb1* plants are hypersensitive to paraquat (methyl viologen; LePage et al 2013), an observation that has not so far been reconciled with the above explanation.

The CIP-triggered phenomenon requires *SUPPRESSOR OF GAMMA RADIATION1* (*SOG1*), a gene that encodes a putative nuclear transcription factor responsive to DNA damage. The gene is thought to participate in a ROS-activated retrograde signaling pathway that regulates the expression of cell cycle genes (Duan et al. 2020). Epidermal and vascular parenchyma sensory plastids are enriched for proteins involved in responses to cadmium ion compared with mesophyll chloroplasts (Beltran et al. 2018) and *why1/why3/polb1* plants are enriched for altered expression of genes coding for glutathione redox processes (LePage et al 2013). SOG1 also regulates the induction of tolerance to cadmium toxicity and the accompanying oxidative stress (Hendrix et al. 2020).

Plants respond to cadmium exposure by substantially increasing their glutathione content as the biosynthetic precursor of phytochelatins and to combat increased oxidative stress (Cobbett and Goldsbrough, 2002; Semane et al 2006; Hendrix et al. 2020). Glutathione (as the reduced form GSH) could play a central role in signaling of sensory plastid genome instability for several reasons. First, GSH levels and redox state are critical for viability of root apical meristems and progression through the cell cycle (Vernoux et al 2000; Maughan and Foyer, 2006). The mutant *root meristemless1* (*rml1*) encodes a defective plastid-targeted γ-glutamylcysteine synthase (GSH1) that catalyzes the first step in glutathione biosynthesis. The *rml1* mutant may provide the link between glutathione content and consequently its redox state to the way in which retrograde signaling from sensory plastids with compromised genomes could initiate an epigenetic response. A further linkage is that glutathione-S-transferases (GSTs) catalyze conjugation of GSH to anthocyanins to allow accumulation in epidermal cell vacuoles (Marrs 1996; Kytridis et al. 2006; Li et al. 2011). Diminution of GSH content, as in *msh1* plants, would indirectly inhibit anthocyanin accumulation and a consequent lower pigmentation of their leaves. Third, a light intensity -dependent spreading variegation in tobacco over-expressing *E.coli* GSH1 in dysfunctional plastids might be similar to phenotypes displayed by *msh1* plants of various species (Creissen et al. 1999; Xu et al. 2011).

**Plastid-associated stress signaling**

Plastids participate in plant stress response but the retrograde signaling pathways that function in sensory plastid signaling have not been defined. Aspects of plastid-nuclear stress signaling originally attributed to mesophyll chloroplasts may, in some cases, be properties of sensory plastid stress response behaviors. In Arabidopsis, chloroplast-induced programmed cell death (PCD) pathways are initiated by the ROS singlet oxygen ($^1O_2$) that is generated during severe photoinhibition (Dogra et al. 2018; Dogra and Kim 2020). The *flu1* (*fluorescent in blue light1*) mutant, which accumulates photo-toxic porphyrin biosynthetic intermediates that generate $^1O_2$ (Op den Camp et al. 2003), shows a transcriptome profile that overlaps significantly with that of *why1/why3/polb1* plants (LePage et al. 2013). The oxidative stress tolerance of plastid genome instability mutants, accompanied by susceptibility to photoinhibition, could point to $^1O_2$ being involved in their phenotypes. But whether these properties belong to mesophyll or sensory plastids is not known.

Three retrograde signaling pathways initiated by $^1O_2$ have been identified thus far and could provide both intracellular and systemic components in sensory plastid signaling. The *EXECUTOR* genes *EX1* and *EX2* participate in $^1O_2$ signaling by promoting and suppressing respectively the formation of specific oxidized chloroplast FtsH2 peptides that could be mobile signal transducers from the chloroplast (Dogra et al. 2019; Dogra and Kim 2020). Recently, EX1/EX2 have been shown to suppress the endoplasmic reticulum unfolded protein response (UPR), which potentially links chloroplast-directed PCD to a wide range of environmental stresses, including pathogen infection, heavy metal toxicity and heat stress (Beaugelin et al 2020; Breeze and Mullineaux 2022).

However, *EX1*and *EX2* expression, deduced *in silico*, localizes predominantly within photosynthetic tissues of the plant, and there is no indication of any specific function or expression in sensory plastids. The *ex1/ex2* double mutant does not suppress a lesion mimic phenotype that is produced by the *myoinositol phosphate synthase1(mips1*) mutant commonly used in the study of PCD. The *mips1* mutant is dependent on light and chlorophyll biosynthesis for lesion formation (Meng et al. 2009) and the MIPS gene is, likewise, expressed in photosynthetic chloroplast-containing tissues based on *in silico* evaluation. However, the *mips1* phenotype is suppressed by mutants of *SAL1* (Bruggeman et al. 2016), the nuclear-encoded gene that regulates levels of 3'phosphadenosine-5-phosphate (PAP) and expresses in sensory plastid-containing cells (Estavillo et al. 2011). PAP movement from the plastid to the nucleus alters activity of 5'-3' exoribonucleases that target micro-

RNAs, increasing abiotic stress responsive gene expression (Estavillo et al., 2011; Litthauer & Jones, 2018; Fang et al. 2019). Whether these observations reflect distinct mesophyll (EX1/EX2) versus sensory plastid (SAL1) properties is not yet clear.

$^1O_2$-production in chloroplasts produces a cocktail of lipid peroxides and oxidized carotenoid derivatives that, in turn, give rise to a mix of reactive carbonyl species, molecules that can trigger PCD and are blocked by detoxifying enzymes, most notably GSTs (Marrs 1996; Mano et al. 2019; D'Alessandro et al. 2018; Muñoz and Munné-Bosch 2020). Lipid peroxidation products include oxylipins leading to jasmonic acid (JA) production. This process sets up a broad defensive response against stress conditions, especially in mutants such as *chlorina1,* which is highly susceptible to photoinhibition and production of $^1O_2$ (Ramel et al. 2012; Ramel et al. 2013). Prominent among the oxidized carotenoid products is β-cyclocitral, which can be used to elicit $^1O_2$-induced oxidative stress resistance and drive expression and activation of the transcription factor (TF) SCARECROW LIKE14 (SCL14). This factor, in turn, is proposed to drive a network of NAC TFs that regulate the expression of genes coding for detoxifying enzymes (D'Alessandro et al 2018). SOG1 is not among these NAC TFs, but linkage to a β-cyclocitral-directed route remains a possibility for transmitting signals from sensory plastids.

Based on the phenotypes of unstable plastid genome mutants, $^1O_2$-mediated signaling from compromised or dysfunctional plastids is a distinct possibility. However, other retrograde signaling molecules and pathways that have been described should be considered. The plastid isoprenoid biosynthetic intermediate methylerythritol cyclodiphosphate (MEcPP) is a retrograde signal that drives UPR and SA-mediated signaling in response to pathogen infection, wounding and high light intensities (Xiao et al. 2012; Walley et al. 2015). However, the *ceh1* mutant, which accumulates high levels of MEcPP, does not display the phenotypes associated with unstable plastid genome mutants, suggesting that this pathway may not be prominent in considerations here.

Hydrogen peroxide ($H_2O_2$) signaling in response to high light intensities provides possibilities for transmitting plastid-sourced signals within cells, between cells and systemically across the plant (Exposito-Rodriguez et al. 2017; Galvez-Valdivieso et al. 2009; Karpinski et al. 1999; Rossel et al. 2007; Miller et al. 2007; Mittler and Berkowitz 2001). *In vivo*, $H_2O_2$ is sufficiently stable to be mobile

(D'Autreaux and Toledano 2007; Exposito-Rodriguez et al. 2017), and several of the plastid genome unstable mutants appear to have higher levels based on dye staining methods and induction of antioxidant protection (Beltran et al. 2018; Duan et al. 2020; LePage et al. 2013). Therefore, $H_2O_2$ has attracted interest as a signal transducer or one that participates in cascades of cell-to-cell transmission to propagate a signal systemically (Karpinski et al. 1999; Miller et al. 2007). Questions remain about how signaling specificity involving the ubiquitous $H_2O_2$ molecule might operate (Mullineaux et al. 2020), but invoking a role for $Ca^{2+}$ and calcium-dependent and mitogen-activated protein kinases for retrograde signaling in the *msh1* mutant is consistent with a role for $H_2O_2$ in transmitting signals from sensory plastids (Beltran et al 2018). A further point to consider is that not all sensory plastids may engage in retrograde signaling to the nucleus (Exposito-Rodriguez et al. 2017; Mullineaux et al. 2020) and this raises the question of whether, in any particular cell type, all plastids engage in driving environmental sensing and signal transmission to the rest of the cell and the plant. For example, abscisic acid (ABA) is produced in vascular parenchyma during high light intensity exposure, when it occurs under low humidity conditions that trigger a transitory drop in leaf water status (Fryer et al., 2003; Galvez-Valdivieso et al., 2009). The ABA is secreted into bundle sheath cells (BSCs) where it activates two distinct signaling routes; a SNF1-RELATED PROTEIN KINASE2.6 (SnRK2.6) -PROTEIN PHOSPHATASE2C (PP2C ) module and a G-PROTEIN ALPHA SUBUNIT1 (GPA1)-directed signaling pathway (Galvez-Valdivieso et al., 2009; Gorecka et al., 2014). The action of SNRK2.6-PP2C is further enhanced in its induction of genes coding for antioxidant defenses by $H_2O_2$-directed retrograde signaling, most likely by inhibiting the bundle sheath cell protein phosphatase isoforms. This vascular parenchyma-directed ABA signaling pathway appears to be confined to bundle sheath cells, playing little, if any, role in the response of mesophyll tissues (Galvez-Valdivieso et al. 2009; Gorecka et al. 2014). Since vascular parenchyma cells are deemed to have sensory plastids, then here we have one example of how sensory plastids transmit a signal (in this case activation of ABA biosynthesis) and consequently integrate a second environmental component (a transient change in leaf water status) to modulate a retrograde signaling response to a distinct environmental challenge in a neighboring cell type.

**Summary comments**

We contend that sensory plastids and mesophyll chloroplasts are distinct, raising new questions important to our understanding of plant metabolism, environmental sensing and land plant evolution (Figure 2). Addressing these questions will present considerable experimental challenges, but recognizes that the entire nature, distribution and importance of specialized plastids may have

been underestimated. While there are, as yet, no definitive descriptions of how plastid-to-nucleus intracellular signaling and transduction occur cell-to-cell and systemically, the above brief descriptions provide characteristics and components worthy of investigation and models to be developed further. The sensory plastid was first described in studies of the *msh1* mutant, with a defining part of its impact on transgenerational epigenetic processes (Beltran et al 2018; Dopp et al. 2021). Similarly, SAL1 signaling effects on miRNA stability and nuclear stress-associated gene expression comprises another distinctive system for understanding sensory plastid-induced nuclear response to environmental change as does the signaling between vascular parenchyma and bundle sheath cells. These emerging systems, and others, serve to establish new benchmarks for testing the impact of any plastid retrograde signaling process or mutant. With increasing awareness of the partitioning of functions across different plastid types, future studies will inevitably serve to enrich our understanding of distinct plastid proteome compositions, functional features, and influence on plant growth and defense phenotypes.

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**Conflict of Interest Statement.** The authors have no conflicts of interest.

**Figure legend**

**Figure 1. Heatmap-based visualization of gene expression variation for selected nuclear genes encoding plastid proteins**. The heatmap viewer function within ePlant (Waese et al. 2017; Bio-Analytical Resource for Plant Biology) was used to visually compare transcriptome data across more than 350 samples, with corresponding subcellular localization of the gene products. Each line on the heat map corresponds to a different data point, with band intensity proportional to expression level. Data are shown for *MSH1* (At3G24320), *PsbO1* (At5G66570), *CUE1/PPT1* (At5G33320), *PPT2* (At3G01550), *DAHPS1* (At4G39980) and *DAHPS2* (At4G33510). This type of *in silico* analysis permits preliminary assignment of *MSH1*-like or *PSBO1*-like expression patterning as a first indicator of sensory plastid or mesophyll chloroplast localization, respectively. From data shown, we tentatively classify *MSH1*, *CUE1* and *DAHPS1* as sensory plastid-localized, with *PSBO1*, *PPT2* and *DAHPS2* as mesophyll chloroplast candidates.

**Figure 2. Modeling sensory plastid biology**. A model of sensory plastid behavior in an epidermal or vascular perenchyma cell adjacent to mesophyll photosynthetic cells. Important questions remain regarding the regulation of protein cell-specific accumulation and cell type-specific signaling. Sensory plastid response to environmental change can trigger nuclear epigenomic and miRNA-directed changes via cell-type specific signaling processes that elicit changes in stress response, metabolism and growth. A photosynthetic mesophyll cell in this model is expected to be distinct in its signaling repertoire, responding primarily to changes in light quality, quantity, and stress effects to trigger changes in nuclear and plastid photosynthetic gene expression.

Figure 1

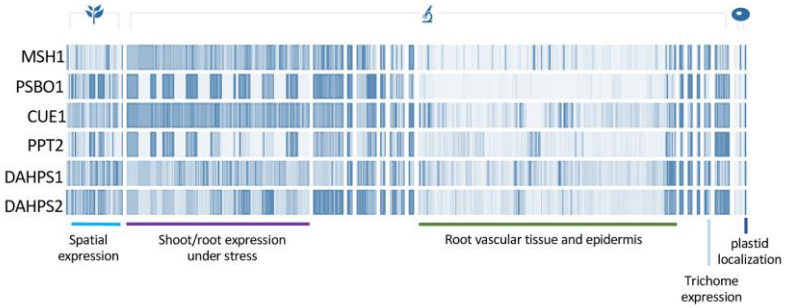

Figure 2