



Plastid dynamism integrates development and environment

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ABSTRACT

In land plants plastid type differentiation occurs concomitantly with cellular differentiation and the transition from one type to another is under developmental and environmental control. Plastid dynamism is based on a bilateral communication between plastids and nucleus through anterograde and retrograde signaling. Signaling occurs through the interaction with specific phytohormones (abscisic acid, strigolactones, jasmonates, gibberellins, brassinosteroids, ethylene, salicylic acid, cytokinin and auxin). The review is focused on the modulation of plastid capabilities at both transcriptional and post-translational levels at the crossroad between development and stress, with a particular attention to the chloroplast, because the most studied plastid type. The role of plastid-encoded and nuclear-encoded proteins for plastid development and stress responses, and the changes of plastid fate through the activity of stromules and plastoglobules, are discussed. Examples of plastid dynamism in response to soil stress agents (salinity, lead, cadmium, arsenic, and chromium) are described. Albinism and root greening are described based on the modulation activities of auxin and cytokinin. The physiological and functional responses of the sensory epidermal and vascular plastids to abiotic and biotic stresses along with their specific roles in stress sensing are described together with their potential modulation of retrograde signaling pathways. Future research perspectives include an in-depth study of sensory plastids to explore their potential for establishing a transgenerational memory to stress. Suggestions about anterograde and retrograde pathways acting at interspecific level and on the lipids of plastoglobules as a novel class of plastid morphogenic agents are provided.

1. Introduction

Phytohormones are responsible for numerous plastid developmental changes and are involved in stress responses (Bittner et al., 2022). The relationship between plastid structure and type, phytohormones and stress responses is the topic of this review, with special emphasis on chloroplast, because the most extensively studied plastid type.

The signaling from the nucleus to the plastid is named anterograde communication. It determines plastid genesis and its homeostasis in strict coordination with the expression of the plastid DNA (the plastome). In turn, plastids signal their metabolic status and requirements back to the nucleus (referred to as retrograde communication) through various signaling compounds including reactive oxygen species (ROS), again in response to developmental and environmental hints. The bilateral communication between plastids and nucleus expresses the plastid plasticity and dynamism as essential cues for plant growth and survival. Plastid signaling contributes to major events of plant

development by interacting with specific phytohormones, which also interact with stress-related signals originating from plastids to detect environmental changes. For this reason, the main phytohormones related to plastids for synthesis and action, and their cross talk and interaction through retrograde and anterograde signaling pathways, are discussed in the review.

1.1. Aim

This review is focused on the modulation of plastid capabilities at the crossroad between development and stress. ‘Sensory plastids’ as specialized structures for sensing and signaling the anterograde and retrograde cellular communication by plastids during biogenesis and stress responses, and the cross talk with the main phytohormones involved, are discussed based on past important literature and, mainly, recent results. The message of the review is to show plastids as dynamic structures able to integrate development and environment.

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Abbreviations

ABA	abscisic acid	Hsp17.8	heat shock protein 17.8
ABI5	Abcisic acid Insensitive5	HY5	LONG HYPOCOTYL 5 bZIP factor
ACC	1-aminocyclopropane 1-carboxylate	IAA	Indole-3-acetic acid
<i>accD</i>	<i>acetyl-CoA carboxylase beta subunit gene</i>	ICS	isochorismate synthase
ACS	aminocyclopropane 1-carboxylate synthase	IPP	isopentenyl diphosphate
ACS1	Apocarotenoid Signal 1	IPT	isopentenyltransferase
aic1	arsenate induced chlorosis1 mutant	JA	jasmonic acid
AKR2	ankyrin repeat-containing protein 2	JA-Ile	JA-isoleucine
ARF2	Auxin Response Factor 2	JAR1	JASMONATE-RESISTANT 1
ARRs	ARABIDOPSIS RESPONSE REGULATORS	L	layer
Aux/IAAs	auxin/indole-3-acetic acid inducible proteins	LHC	light-harvesting complex
BL	brassinolide	L-Trp	L-tryptophan
BRs	brassinosteroids	LOG	5-monophosphate phosphoribohydrolase
BZR1	BRASSINAZOLE-RESISTANT1	MEP	methyl erythritol phosphate
<i>cad1-3</i>	cadmium-sensitive1-3 mutant	<i>MSH1</i>	<i>MutS Homolog1 gene</i>
CaSRs	Calcium-Sensing Receptors	MVA	mevalonic acid
<i>ccr2</i>	<i>carotenoid chloroplast regulation 2 mutant</i>	NEP	nuclear-encoded plastid RNA polymerase
<i>cis</i> -OPDA	12-oxophytodienoic acid	NTRC	NADPH-Thioredoxin Reductase
CGA1	GATA factor 1 zinc finger protein	PAL	phenylalanine ammonia-lyase
CK	cytokinin	PAP	3'-phosphoadenosine 5'-phosphate
CYP735A	CK trans-hydroxylase	PCs	phytochelatins
CL	carlactone	PEMT	phosphatidylethanolamine N-methyltransferase
CRASS	CHLOROPLAST RIBOSOME ASSOCIATED	PEP	PLASTID-ENCODED POLYMERASE
CRY	cryptochrome	PETE1	plastocyanin 1
CRF2	APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF)	PGs	plastoglobules
CUE1	CHLOROPHYLL A/B BINDING PROTEIN UNDEREXPRESSED	<i>PhANGs</i>	<i>Photosynthesis-Associated Nuclear Genes</i>
DAHPS	3-Deoxyd-Arabinose-7-Phosphate Synthase	PHY	phytochrome
<i>dn</i> OPDA	dinor-12-oxo-10,15(Z)-phytydienoic acid	PIF	basic helix-loop-helix Phytochrome-Interacting Factor
ECR	epidermal chloroplast response	PLB	prolamellar body
<i>EDS1</i> and <i>5</i>	<i>ENHANCED DISEASE SUSCEPTIBILITY 1</i> and <i>5</i>	<i>PRANGs</i>	plastid redox-associated nuclear genes
EIN3	ETHYLENE INSENSITIVE 3	<i>RFC3</i>	Regulator of Fatty acid Composition3 gene
ER	endoplasmic reticulum	ROS	reactive oxygen species
ET	ethylene	SA	salicylic acid
GARP	Glycoprotein A repetitions predominant	<i>SAG101</i> and <i>SAG102</i>	<i>Senescence-Associated Genes 101 and 102</i>
GAs	gibberellins	SAM	shoot apical meristem
GGPP	geranyl-geranyl diphosphate	SEPs	sensory epidermal plastids
GLK1 and GLK2	GOLDEN2-LIKE 1 and 2 proteins	<i>SIG2</i> and <i>SIG6</i>	<i>Sigma factor genes 2 and 6</i>
γ GCS	γ -glutamylcysteine synthetase	SK	shikimate
GNC	GATA-NITRATE-INDUCIBLE CARBON METABOLISM-INVOLVED transcription factor	<i>SKL1</i>	<i>SHIKIMATE KINASE-LIKE1 gene</i>
GNL	GNC-LIKE transcription factor	SLs	strigolactones
GUN1	Genomes Uncoupled 1	24-eBL	24-epibrassinolide
HP2	Hygh Pigment2	TIC	translocon at the inner envelope membrane of chloroplasts
		TOC	translocon at the outer envelope membrane of chloroplasts
		TGD5	TRIGALACTOSYLDIACYLGLYCEROL5
		XRN	5'→3'exoribonuclease
		<i>zds/clb5</i>	<i>zeta carotene desaturase/chloroplast biogenesis 5 mutant</i>

1.1.1. Plastid proteins are important not only for plastid development but also for plant stress response

Plastids arose through endosymbiotic events between a heterotrophic eukaryote and a cyanobacterium. Over the course of evolution, most of their genes were either lost or reallocated to the nucleus, with approximately only 80–250 genes retained in the plastome. Accordingly, protein synthesis in the plastid is carried out by bacterial type 70S ribosomes, composed of a 50S and a 30S subunits, containing ribosomal RNAs (rRNAs) and ribosomal proteins. In the 30S subunit of the chloroplast ribosome, only one rRNA species (16S rRNA) is present, whereas three rRNAs (23S rRNA, 5S rRNA, and 4.5S rRNA) are present in the 50S subunit. Twenty-one plastid proteins of the 30S subunit and 31 proteins of the 50S subunit descend from eubacterial orthologs. In addition, other proteins are present in the plastid ribosome, some of which recently identified by cryo-electron microscopy (Pulido et al., 2018). The plastomes of angiosperms show common features which include gene and

intron losses and sequence divergence in protein coding genes. Gene loss includes the *acetyl-CoA carboxylase beta subunit (accD)* gene, encoding a subunit of the acetyl-CoA carboxylase complex, *ycf1* and *ycf2*, and several other genes encoding ribosomal subunits. Plastome gene losses are frequently associated with functional transfer to the nucleus, as demonstrated for *accD* in legumes (Shrestha et al., 2019). Moreover, some plastome rearrangements are directly linked with repetitive sequences. Tandem repeated sequences are present in *accD* and *ycf1*, which are genes with accelerated substitution rates. It has been hypothesized that these repetitive sequences affect plastome stability (Wu et al., 2021).

Approximately 3000 proteins of the chloroplast are encoded by the nuclear DNA and synthesized in the cytoplasm as precursor proteins. The plastid translocon machinery for the import of such precursor proteins, comprises the TOC (translocon at the outer chloroplast envelope membrane) and TIC (translocon at the inner chloroplast envelope

membrane) complexes. Additionally, other noncanonical pathways are active (Sun and Jarvis, 2023). However, noncanonical pathways or pathways that also use intrinsic targeting information are involved in the plastid import of a low number of proteins. For example, the targeting information for proteins of the outer envelope resides within a transmembrane domain and the cytosolic sorting factors ankyrin repeat-containing protein 2 (AKR2) and heat shock protein 17.8 (Hsp17.8) guide these proteins to the plastid surface, even if their integration may require the main TOC channel (Sun and Jarvis, 2023). Another example of unusual pathway involves co-translational translocation into the endoplasmic reticulum (ER) and passage through the Golgi apparatus (Sun and Jarvis, 2023 and other references therein).

Moreover, plastid proteolysis widely occurs. It is a complex process and involves the concerted action of internal proteases of prokaryotic origin and the nucleus-cytosolic ubiquitin-proteasome system. With the aim of reconfiguring plastid functions in response to developmental and environmental signals, plastid proteolysis is in strict relation with protein import. For example, in the chloroplast, the ubiquitin-proteasome system targets the TOC complex to regulate protein import, as well as numerous internal proteins (Sun and Jarvis, 2023).

The activities of plastid ribosomal proteins are important for plant development and survival. It is widely known that the chloroplast ribosomes are responsible for the synthesis of subunits of PSI, PSII, ATPase, and the large subunit of RuBisCO. It is less known that some ribosomal proteins are involved in stress responses. Among these proteins, CHLOROPLAST RIBOSOME ASSOCIATED (CRASS) protein, physically associated with the chloroplast ribosomal 30S subunit in *Arabidopsis*. While this stromal protein is not essential for plant survival under controlled growth conditions, it becomes limiting for chloroplast activities and plant growth under stress (Pulido et al., 2018).

2. Organellomic gradients change plastid fate

All organelles present in a biological system at a given moment constitute the cell organelle.

Plastids constitute the largest group of organelles in plant organelle and are central for the interaction between genotype and environment. Development and environment cause heterogeneity in the organelle components over time by changing the balance between their production and degradation and by changing their structure and metabolic status. These changes are collectively known as the organellomic gradients (Hickey et al., 2023).

Plastid interconversion is a not necessarily a tissue-specific phenomenon but it is affected by the environmental conditions. For example, the specialized plastids of *Begonia* called iridoplasts or lamelloplast differentiate from chloroplasts when the plants are grown under the cover of the tree canopy of very dense forests (Sierra et al., 2023). In these shade-adapted environments, iridoplasts absorb light more efficiently than chloroplasts and are located in the leaf epidermal cells (Pao et al., 2018), whereas chloroplasts are present in leaf chlorenchyma. In addition, the desiccoplasts, also known as xeroplasts, can be interconverted between proplastids and chloroplasts depending on the stress conditions. In fact, they appear in the leaves of the desiccation-tolerant plants and exhibit a dismantled thylakoid system, but, upon plant re-hydration, they re-differentiate into chloroplasts (Solymosi et al., 2013). As chloroplasts age, they become gerontoplasts. However, gerontoplasts may also differentiate under stress conditions. Even if gerontoplasts are not able to transit into any other plastid type, being end-products in plastid interconversion, chloroplasts may indeed change into gerontoplasts to cope with a variety of internal or external stresses. In this case, chloroplasts use senescence to avoid the accumulation of toxic chemical species, e.g., ROS (Nakamura and Izumi, 2018). Chloroplasts can also differentiate into chromoplasts as a stress response. Indeed, in *crtB*-induced artificial chromoplasts, which exhibit enhanced carotenoid production due to the activity of the bacterial protein *crtB*, the exposure to high light causes

chloroplast-to-chromoplast conversion to increase the photoprotection provided by carotenoids (Morelli et al., 2023).

There are also stresses that increase or decrease chloroplast number. For example, drought and heat stress cause an increased production of chloroplasts per cell, e.g. 20–40% more chloroplasts in wheat (Grigorova et al., 2012), whereas starvation reduces chloroplasts by about 25% per mesophyll cell in the same plant (Wardley et al., 1984).

All plant pathogens elicit effector proteins to suppress plant defense. Some of these effectors move into the chloroplast (Lu and Yao, 2018). These virulence factors alter chloroplast structure, number and functions, ultimately leading to gerontoplast transition and chlorosis. Thus, even if the chloroplasts also generate defense-related signaling molecules facilitating the interaction between photosynthesis and defense signaling (Lu and Yao, 2018), their number generally decreases under biotic stress.

In addition, the plastid infrastructures, such as thylakoids, stromules, and, mainly, plastoglobules (PGs), can change in abundance under stress. For example, the thylakoid number in *Triticum aestivum* chloroplasts is 10–15 per granum under unstressed conditions but decreases to 4–8 under drought stress (Grigorova et al., 2012). As reviewed by Hanson and Hines (2018), the stromule abundance increases in response to pathogen attacks, abiotic stresses, diurnal cycle, and the action of phytohormones. Similarly, an increase in the number and size of PGs, due to the accumulation of isoprenoids and lipids in their hydrophobic core, is observed under different stressful conditions, including oxidative stress, high light intensity, nitrate starvation, drought, high salinity, viral infection, chilling, and ozone (Morelli et al., 2023). In these conditions, PGs cluster together to form grape-like structures, which maintain their continuity through extensions of the lipid layer that surrounds them in continuity with the thylakoid stroma side. An example of PG blebbing from chromoplasts and chloroplasts has been described during lipid deposition in olive tree drupes under temperature lowering (Matteucci et al., 2011). Plastoglobules are also important in thylakoid maintenance during high light stress, with a photoprotection action due to an accumulation of carotenoids, prenylquinones and other compounds, e.g., vitamin E tocopherol cyclase, as examples of antioxidant molecules (Rottet et al., 2015).

2.1. Plastids in response to soil toxicity

The presence of salt in the soil may cause both osmotic and ionic stress, inducing chlorosis. Salinity alters chloroplast structure and metabolism by changing its organization and interfering with cross-membrane transport. Plants, such as some *Atriplex* species, undergo lipid deposition in the chloroplasts to counter the harmful effects of salt-induced toxicity, and others, e.g. some wheat cultivars and tobacco, accumulate starch as osmoticum for maintaining the structural integrity of the plastid (Hameed et al., 2021). In salt-sensitive plants (glycophytes) the salt stress-induced alterations in the chloroplasts include swelling of thylakoids (rice), increase in volume due to changes in the stroma ionic composition (wheat cultivars), decrease in volume with concomitant changes in light-scattering during electron transport (spinach), reduction in grana development (*Arabidopsis*), changes in thylakoid ultrastructure (potato and maize) (see Hameed et al., 2021 for further details). In the halophytes, i.e., plants able to complete their life cycle under high salinity, the photosynthetic machinery is adapted to salt. In fact, a stimulation of chlorophyll synthesis occurs in response to increasing NaCl concentrations and offers photoprotection to PSI and PSII functions and stability to thylakoid membranes. The mangroves are woody plants capable of tolerating a wide range of salinity. In the mangrove *Kandelia candel* the chloroplast ultrastructure changes with changing salt concentration. In 200mM NaCl-treated plants the ultrastructure analysis shows chloroplasts with a spindle shape and a typical granum structure, while in plants treated with 600 mM NaCl, the thylakoids become irregularly arranged and the PG number increases, suggesting that the treatment negatively affects chloroplast

functionality, at least at high salt concentrations. Interestingly, there are halophytes, such as *Sarcocornia frutescens*, which for mitigating salt stress employs the xanthophyll cycle for a non-photochemical quenching that dissipates excess excitation energy of PSII, thus preventing the formation of potentially cytotoxic ROS (Hameed et al., 2021).

Contamination of ecosystems by metals and metalloids is due to their bioaccumulation and persistence in the environment. Cadmium (Cd) and Arsenic (As), a non-essential heavy metal and a metalloid, respectively, either alone or combined, can induce severe toxicity to plants reducing their biomass and inhibiting chlorophyll biosynthesis (Fig. 1). The same as other heavy metals, Cd has destructive effects on chloroplasts and chromoplasts (Sanità di Toppi et al., 2012; Rottet et al., 2015). Indeed, upon Cd treatment, the disorganization of the photosynthetic apparatus is soon observed with the disassembly of PSI and its associated antenna proteins (Fagioni et al., 2009). Cadmium can replace other metal ions (Zn, Fe, Ca, Mg) in proteins and may affect the redox state by generating ROS. In spinach leaves Cd induces chlorosis consequently to chlorophyll decreases (Fig. 1), but concomitantly increases the xanthophyll content, with this overproduction, especially of lutein, as a defense mechanism against ROS (Fagioni et al., 2009). In the chloroplast, the effects of Cd toxicity resemble the typical symptoms of the senescence process. A notable change is a considerable increase in the number and size of PGs, and in the starch grain size, as observed in Cd-treated *Pisum sativum* plants (Sandalio et al., 2001).

Interestingly, the stressor level is also a modulating agent in the organelle fate. For example, during the process of chloroplast senescence in bean leaves, low concentrations of stress-inducing compounds like Cd salts stimulate chlorophyll synthesis and photosynthetic activity, causing rejuvenation and related increases in the amount of PSI, CO₂ fixation and starch accumulation (Nyitrai et al., 2004). In addition, the stress response may be biphasic, as shown by the chloroplast response to Cd stress. For instance, in carrot primary roots exposed to Cd, a two-phase response is evident. The first phase occurs within 4 days from the onset of treatment, during which there is an early acclimation to the pollutant aimed at reducing its movement across the root. Subsequently, a second phase occurs, which is instead characterized by extended cell damages, mainly due to chromoplast alterations by PG blebbing and intra-plastid accumulation of starch and Cd-particles (Sanità di Toppi

et al., 2012).

Arsenate [As(V)] and arsenite [As(III)] are the main inorganic As species present in soil. They are interconvertible depending on the environmental conditions. Plants take up both As species through different mechanisms, As(V) preferentially via phosphate transporters and As(III) through proteins of the aquaglyceroporin family. In the roots, As(V) is rapidly reduced to As(III), and firstly accumulates in this organ (Piacentini et al., 2024 and other references therein).

The translocation of As from the roots to the shoots is generally limited, but when As reaches the shoot tissues a common symptom of its toxicity becomes chlorosis, as in *Vigna mungo* leaves, suggesting chloroplasts as As targets. In fact, in this plant As can decrease chlorophyll content, suppress the PSII photochemistry reactions, and increase the oxidative stress (Srivastava et al., 2017) (Fig. 1). Similarly to Cd, As is detoxified primarily through complexation with phytochelatins (PCs) and other thiol compounds, however other mechanisms are also possible. An As(V)-sensitive mutant of Arabidopsis, arsenate induced chlorosis1 (*aic1*), has been isolated in the background of the PC synthase-defective mutant cadmium-sensitive1-3 (*cad1-3*), and the double mutant has been shown to be highly sensitive to As (Wang et al., 2018). *AIC1* is *TRANSLOCON AT THE OUTER ENVELOPE MEMBRANE OF CHLOROPLASTS132* (*TOC132*), encoding a protein for the import of preproteins from the cytoplasm to the chloroplast. Wang and coworkers (2018) demonstrated that the *AIC1/TOC132* translocon is required for protecting the chloroplast from As toxicity. In fact, with increasing As(V) concentration, the granum structure disappears, no starch is formed, and the chloroplast number highly decreases (Fig. 1). Among the less abundant proteins in *aic1 cad1-3*, a few are involved in chloroplast organization (RNA binding, translation initiation factor proteins, and thylakoid formation protein), others in photosynthesis (light reactions and carbon assimilation), and others are ribosomal proteins. This strengthens that also a correct chloroplast ribosome functioning is essential for the defense against As stress.

Lead (Pb) is a heavy metal pollutant common in the soil. Once absorbed by the soil, most of the Pb ions remain in the roots, but a part, even if small, is transferred to stems and leaves, and may be deleterious for the chloroplasts. Lead ions cause direct damages to the photosynthetic systems, as occurs in *Robinia pseudoacacia* (Yang et al., 2015). In

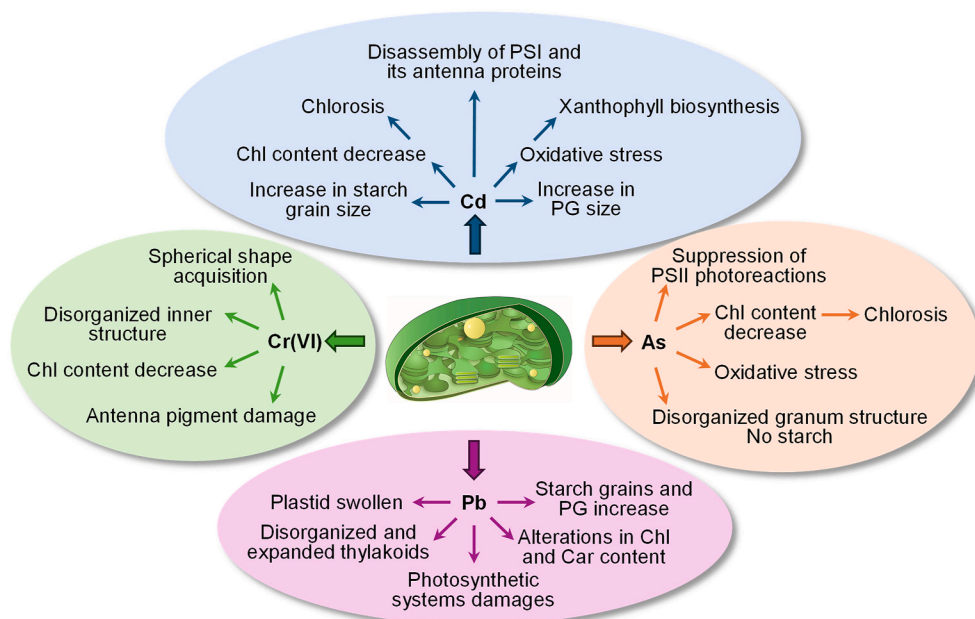


Fig. 1. Schematic representation of the main changes induced by Cd, As, Pb, and Cr(VI) soil pollutants on the chloroplast structure and functions. Damages to the plastid morphology and the photosynthetic apparatus are summarized for each pollutant. However, the effects can be very different depending on the species and concentration of the pollutant. Further details in the text.

ryegrass, the levels of carotene, chlorophyll *a*, chlorophyll *b* and photosynthesis rate are lowered when plants are exposed to 500 μM Pb, supporting negative effects of lead on chloroplast pigments and activity (Bai et al., 2015) (Fig. 1). By contrast, in lead-stressed privet seedlings, carotene levels increase as Pb concentration increases. However, chloroplast shape and sub-organelle organization change gradually with increasing Pb concentration, with the plastid becoming progressively swollen, rich in starch grains and lipid globules, and with a disorganization of thylakoid lamellae (Zhou et al., 2018) (Fig. 1).

Most of the chromium (Cr) in the soil occurs in its trivalent state [Cr(III)], which is less toxic and hardly mobile as compared to the hexavalent one [Cr(VI)], that is readily soluble and more toxic. Chloroplasts are important targets of Cr toxicity, contracting and becoming spherical in the presence of Cr(VI), as shown by various cultivars of *Brassica napus* (Gill et al., 2015). Also proplastids can be affected by Cr(VI). In fact, in the cells of the root tips of Arabidopsis, the proplastids not only exhibit a spherical shape with cytoplasmic invaginations but also contain starch grains and dense droplets scattered in the stroma or accumulated around the starch grains (Eleftheriou et al., 2015). In rice seedlings, Cr(VI) deposits gradually increase in the chloroplasts as the concentration of Cr(VI) increases from 25 to 200 μM . Concurrently, Chl *a* content progressively decreases, antenna pigments are damaged, and chloroplasts become globular and with a disorganized inner structure (Ma et al., 2016) (Fig. 1). The Chl content decreases under Cr toxicity also in numerous other plants (*Citrus aurantium*, *Najas indica*, *Vallisneria spiralis*, *Alternanthera sessilis* and *Triticum aestivum*), with chloroplast ribosomal recycling factors involved in the plastid alterations through post-translational modifications (Saud et al., 2022).

2.2. Plastid anterograde and retrograde signaling as a feed-back loop for development and environmental responses

The majority of the proteins required for the plastid pathways are encoded in the nucleus. Thus, the differentiation and functioning of the different plastid types rely on the supply of nuclear-encoded proteins by an anterograde signaling working in strict coordination with the expression of the plastome genes (Chu et al., 2020). The retrograde signaling is instead the way by which plastids signal their metabolic status and requirements in response to developmental and environmental cues to the nucleus. The anterograde signaling cascades are referred to as biogenic control pathways because signals are produced during the differentiation process of the plastids for adjusting the expression of nuclear genes required for their biogenesis in coordination with the progress of plant development (de Souza et al., 2017). The retrograde signal cascades are defined as operational control pathways, as they operate in fully differentiated plastids in response to environmental fluctuations and adjust the organelle activities, not only in relation to the nucleus but also in relation to other cellular compartments (Sierra et al., 2022).

Anterograde signaling is the necessary consequence of the ancestral endosymbiotic event that gave rise to the chloroplast. Even if transferred from the plastome to the nuclear genome, many of these genes remained functionally linked to the chloroplast, because, after transcription in the nucleus and translation in the cytoplasm, their protein products became active in the chloroplast (Griffin and Toledo-Ortiz, 2022).

The anterograde signaling pathways are also involved in circadian responses (Atkins and Dodd, 2014). Both phytochromes (PHYs) and cryptochromes (CRYs) orchestrate the gene expression changes for photomorphogenesis and for the beginning of the development of the chloroplast, including its greening. In fact, PHYs and CRYs are involved in the response to light of the *Photosynthesis-Associated Nuclear Genes* (PhANGs) activating or repressing numerous transcription factors, e.g., *LONG HYPOCOTYL 5* (HY5) bZIP factor (Toledo-Ortiz et al., 2014), *basic helix-loop-helix Phytochrome-Interacting Factors* (PIFs) (Leivar and Quail, 2011), and *GOLDEN2-LIKE 1* and *2* (*GLK1* and *GLK2*) genes coding for Glycoprotein A Repetitions Predominant (GARP) proteins (Leister and

Kleine, 2016) (Fig. 2). In addition, at least part of the anterograde signaling is involved in transmitting information to the plastome for tuning it with the environmental changes, forming a feed-back loop with the retrograde signaling pathways (Griffin and Toledo-Ortiz, 2022).

The retrograde signaling holds significance primarily because multiprotein complexes within plastids, like photosystems and RuBisCO, consist of subunits encoded by both the nuclear DNA and the plastid DNA. Therefore, the expression of each subunit, governed by these two distinct genomes, must be synchronized. Secondly, retrograde signaling is important because plastids are also dependent on the external environmental stimuli, firstly light conditions. Thus, retrograde signaling pathways are essential for shaping chloroplast responses. During germination or early seedling development, the transition from etioplasts (or proplastids) to chloroplasts occurs. This involves the retrograde signaling which tunes-up and -down multitude of nuclear-encoded genes whose proteins target the chloroplast. Signals emitted by the plastid to deliver information to the nucleus include carotenoid derivatives, isoprenes, phosphoadenosines, tetrapyrroles, and ROS, among the others (Chan et al., 2016) (Fig. 2). The importance of the retrograde signaling for seedling survival has been also determined by using mutants with impaired retrograde signaling, and by the application of stress-inducing compounds in the forming chloroplasts (Chan et al., 2016). To adapt to the environment, the plastid must adjust nuclear gene expression and protein flow by retrograde signaling pathways. These operational signals coming from the plastid build a cellular communication network decoding the languages of the chloroplast (Chan et al., 2016; Griffin and Toledo-Ortiz, 2022), with PHYs and CRYs, and the light-modulated transcription factors, e.g., HY5 and PIFs, involved. Interestingly, also during flowering, HY5, in conjunction with CRYs, plays a role in coordinating light and retrograde signals to regulate the accumulation of anthocyanins and flavonoids (Richter et al., 2020).

High light and shaded conditions also represent scenarios in which photoreceptors adjust growth responses based on chloroplast functions. Interestingly, there is a convergence of photoreceptors and retrograde signals in modulating proteins that have dual localization (in both chloroplasts and nucleus), including WHIRLY and HEMERA, regulators of chloroplast biogenesis and of the nuclear control of PLASTID-ENCODED POLYMERASE (PEP) activity. These proteins play crucial roles in optimizing photosynthetic activities in changing environmental conditions (Griffin and Toledo-Ortiz, 2022; Sierra et al., 2022) (Fig. 2).

The interplay of PEP with the nuclear-encoded plastid RNA polymerase (NEP) is necessary for plastid development and functioning. Both PEP and NEP function in all plastid types. Their transcriptional activity is under the control of endogenous and exogenous factors and the link between their activity and the Genomes Uncoupled 1 (GUN1) protein is essential for chloroplast-to-nucleus retrograde signaling. GUN1 localizes to plastids and seems to be a central node in the retrograde signaling pathways regulating PhANG gene expression (Tadini et al., 2020).

Some of the best characterized retrograde signals are those derived from the tetrapyrrole biosynthetic pathway (Terry and Smith, 2013). Many metabolic processes in plastids generate ROS, such as singlet oxygen ($^1\text{O}_2$) and hydrogen peroxide (H_2O_2), each of which plays diverse signaling roles in plant development and stress responses, either by direct diffusion of ROS from the plastid or indirectly by interposed molecules (Exposito-Rodriguez et al., 2017). The best-characterized ROS signaling pathway is the $^1\text{O}_2$ -induced cell death pathway. When an Arabidopsis mutant that hyperaccumulates the chlorophyll precursor in the dark is shifted to light conditions, $^1\text{O}_2$ accumulates rapidly in the chloroplasts, leading to growth arrest and cell death, with the pathway mediated by the plastid-localized proteins EXECUTER1 and EXECUTER2 (Kim and Apel, 2013). The retrograde signal may also change the fate of the chloroplast, as is the case of the carotenoid phytoene. In fact, the ectopic differentiation of chloroplasts to chromoplasts in leaves results from phytoene accumulation causing an initial drop in the PSII

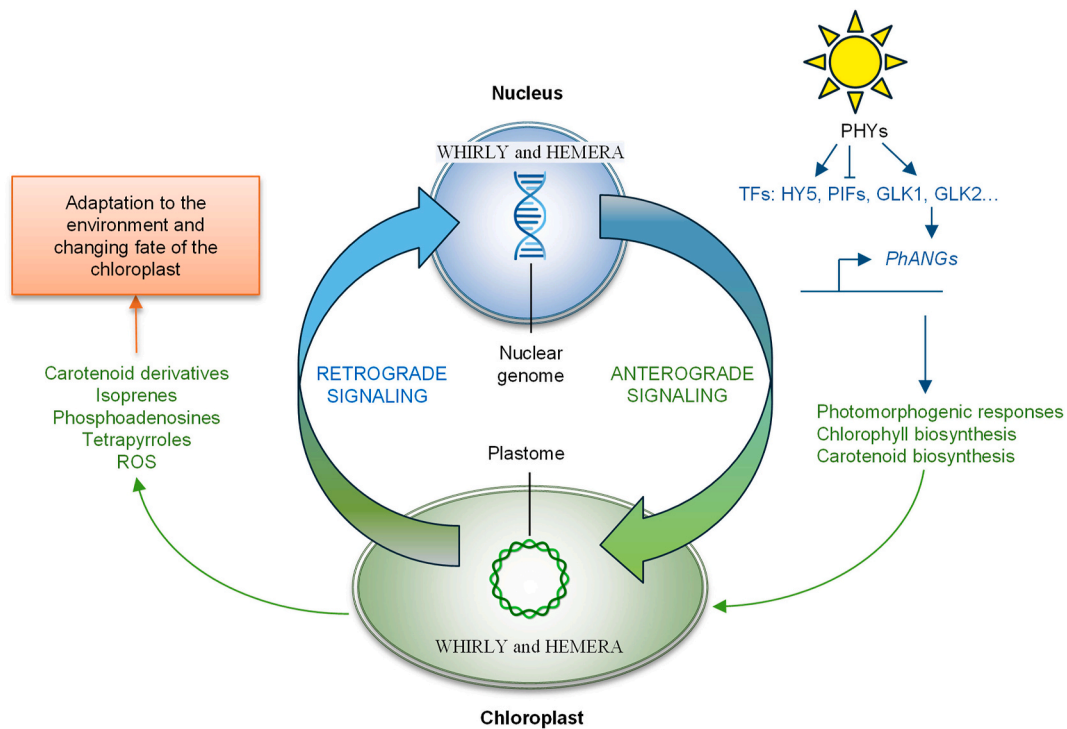


Fig. 2. Schematic representation of the connection between anterograde and retrograde signaling pathways between chloroplast and nucleus. The anterograde signaling is orchestrated, for example, by PHYs which induce gene expression changes for photomorphogenesis and chloroplast development/greening through the light-induced *PhANG* genes. Part of the anterograde signaling transmits information to the plastome for tuning it with the environmental changes. The retrograde signaling is essential for chloroplast responses and modulates the expression of nuclear-encoded genes whose proteins target the chloroplast. Signals from the plastid include carotenoid derivatives, isoprenes, phosphoadenosines, tetrapyrroles, and ROS. WHIRLY and HEMERA are examples of proteins localized in both nucleus and chloroplast, with regulatory activities in chloroplast biogenesis and in optimizing photosynthesis in changing environments. The retrograde signal is also involved in changing the chloroplast fate.

quantum yield in the chloroplasts, followed by a transcriptional reprogramming of nuclear-encoded genes associated with carotenogenesis and chromoplast differentiation (Llorente et al., 2020). The retrograde signal may also perturb the conversion of the etioplast into chloroplast. For instance, the analysis of the Arabidopsis *carotenoid chloroplast regulation 2* (*ccr2*) mutant demonstrates that the accumulation of specific *cis*-carotenes disrupts etioplast during leaf greening thus altering its conversion into chloroplast (Cazzonelli et al., 2020).

Connection by stromules of chloroplasts and other plastid types has been observed with nuclei, but also with plasma membranes and ER to facilitate communication. It induces responses to alterations in photosynthetic redox state and ROS, as in chloroplasts, or in sugar metabolism, as in leucoplasts (Hanson and Hines, 2018).

Stromules surrounding the nucleus and the ER also originate in chloroplasts developing into gerontoplasts. In this case, stromules function in the retrograde signaling involved in leaf senescence by channeling and speeding up signal transduction directly to the nucleus, avoiding the cytoplasmic scavenging systems (Exposito-Rodriguez et al., 2017). A stromule role in chloroplast-to-nucleus retrograde signaling during innate immunity has also been suggested (Hanson and Hines, 2018).

Based on data summarized by Chan and coworkers (2016), important considerations must be made on retrograde signaling. The retrograde signaling requires a spatial coordination in different cell types/plastid types/sub-plastidial compartments. Moreover, different compounds may function in signaling depending on the developmental stage of the plant and even in response to the same stress (Kim and Apel, 2013). Anterograde and retrograde signals may be also affected by phytohormonal signaling networks, with different phytohormones acting at different times. Adding to the complexity, it should be emphasized that the feed-back loop between anterograde and retrograde

signaling pathways may be also linked to the cell/tissue type and the organ to which plastids belong. An important example is provided by the shoot apical meristem (SAM). Dalal et al., 2018 found that in Arabidopsis during SAM differentiation, plastid biogenesis varies depending on the cell layer. Plastids with few thylakoids, that during their progression towards the leaf primordia will differentiate into non-photosynthetic epidermal plastids (pavement/sensory plastids, as described below), are contained in the epidermal layer (L1) at the SAM center (Fig. 3). In contrast, proplastids that will differentiate into chloroplasts in the maturing leaf primordia are contained in the cells of the mesophyll-generating layer (L2) at the center of the same meristem

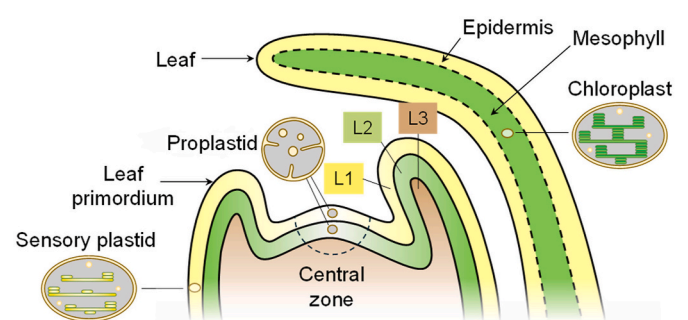


Fig. 3. Scheme showing that plastid biogenesis varies depending on the cell layer in the shoot apical meristem (SAM). Proplastids fated to become epidermal plastids (sensory plastids) are contained in the L1 epidermal layer at the SAM central zone. Proplastids that will differentiate into chloroplasts in the maturing leaf primordia are contained in the cells of the L2 mesophyll-generating layer at the SAM central zone. Further details in the text.

PAP, a 3'-phosphorylated-nucleotide, which contributes to the response pathway of ABA during the closure of stomata, is kept at low levels by the activity of SAL1, a dinucleotide phosphatase/inositol phosphate phosphatase. However, under an excess of light, photo-oxidative stress is induced and SAL1 is made nonfunctional by oxidation. This event highly increases PAP levels and primes numerous stress avoidance signals in the chloroplast (Pornsiriwong et al., 2017).

In addition to the direct association between chloroplast functions and ABA biosynthesis, as well as the indirect link between ABA and functional retrograde signals, the phytohormone can influence the development and the characteristics of the plastid through an additional level of control, again involving both retrograde and anterograde signaling pathways, but also stromule formation. Indeed, the transitory expression of redox biosensors in the chloroplast stroma, cytosol, and nucleus reveals that H₂O₂ generated during photosynthesis in the chloroplast impacts the H₂O₂ level in the nucleus, with the H₂O₂ transfer occurring as a stromule-mediated retrograde signal (Exposito-Rodriguez et al., 2017). In accordance, ABA induces a stromule-mediated formation of chloroplast–nucleus complexes in tobacco and wheat seedlings, and treatments with ABA biosynthesis inhibitors prevent the formation of stromules under stress (Bittner et al., 2022, and references therein).

2.3.2. Strigolactones

Strigolactones (SLs) are terpenoid lactones derived from carotenoids, exhibiting many roles in photomorphogenesis and plant development (Müller and Munné-Bosch, 2021; Altamura et al., 2023). Their biosynthesis starts in the chloroplast where the all-trans- β -carotene is converted into carlactone (CL) by the activity of three enzymes. Later, CL is converted into both canonical and non-canonical SLs by the activity of the CYP711A subfamily of cytochrome P450 oxygenases (Mashiguchi et al., 2021).

In Arabidopsis, the application of GR24, a synthetic analog of SLs, triggers the upregulation of genes associated with light responses, and the same genes are repressed in mutants lacking SLs. Furthermore, tomato mutants with compromised SL biosynthesis (*SI-ORT1*) exhibit diminished chlorophyll content and decreased expression of *light-harvesting complex (LHC)* genes in comparison to wild-type plants. However, treatments with GR24 enhance chlorophyll levels and the expression of *LHC* genes, thereby promoting photosynthesis (Müller and Munné-Bosch, 2021 and references therein). Research with SL mutants and SL biosynthesis inhibitors also shows that ABA-induced stromule formation is positively linked to SL biosynthesis in the plastids (Fig. 4), indicating a cross talk between the two phytohormones during this plastid morphogenic process (Hanson and Hines, 2018 and references therein). Nevertheless, further investigation is required to elucidate the precise manner by which ABA and SLs take part in the regulatory mechanisms governing chloroplast morphogenesis and activities.

2.3.3. Jasmonates

The functions and the expression of organellar proteins encoded by the nucleus are known to be affected by transcriptional changes mediated by jasmonates, i.e. jasmonic acid (JA), its precursors and related compounds (Bittner et al., 2022). It is well known that the first precursors of JA, i.e., 12-oxophytodienoic acid (cis-OPDA) and dinor-12-oxo-10,15(Z)-phytyldienoic acid (dnOPDA), are synthesized in the chloroplast through the octadecanoid signaling pathway and the hexadecanoid pathway, respectively. These JA-precursors rapidly increase upon biotic stresses and growth processes, suggesting a role for them in plant defense and development (Liu and Park, 2021). However, other plastid proteins, located in the PGs, are involved in JA synthesis, for example ABC1K3 and ABC1K1, governing the metabolism of prenylquinones. In fact, the Arabidopsis PG proteome reveals a surprisingly high number of ABC1-like kinases, including ABC1K3 and ABC1K1, and the *abc1k1abc1k3* double mutant displays increased sensitivity to light stress, resulting in a fast leaf bleaching and an incapacity to accumulate anthocyanin (Lundquist et al., 2013).

After initiation in the chloroplast, OPDAs travel to the peroxisomes through proteins of the plastid envelope and/or peroxisomal ATP-binding transporters where the formation of JA is continued (Zhao et al., 2020, and references therein).

Isoleucine (Ile) too is synthesized in chloroplasts. JASMONATE-RESISTANT 1 (*JAR1*) protein is the first responsible for conjugating JA with Ile to form JA-Ile. Many studies indicate that JA-Ile is the most biologically active JA-derivative, particularly important in stress responses. In accordance, increased levels of JA-Ile, but also of JA and OPDA, have been found in plants exposed to high light and heat stress in combination (Balfagón et al., 2019). Interestingly, in Arabidopsis, JA-treatments and *JAR1* overexpression enhance the expression of many chloroplast-encoded genes for priming the plant to better combat drought stress (Mahmud et al., 2022). However, in addition to these positive JA roles on chloroplast gene expression, JA is also known to be positively involved in chlorophyll degradation and downregulation of *RUBISCO ACTIVASE* (Shan et al., 2011) (Fig. 4). These effects and those of JA signaling on leaf senescence by the induction of *SENESCENCE-ASSOCIATED GENES (SAGs)* and of genes involved in chlorophyll catabolism (Shan et al., 2011), collectively suggest a role for JAs also in chloroplast to gerontoplast transition (Fig. 4).

2.3.4. Gibberellins

Gibberellins (GAs) are phytohormones that positively affect the growth of most organs. They belong to the family of diterpenoids. Their biosynthesis starts from geranyl-geranyl diphosphate (GGPP) via isopentenyl diphosphate (IPP). In the green tissues, IPP is generated either through the mevalonic acid (MVA) pathway in the cytoplasm or the methyl erythritol phosphate (MEP) pathway in the plastids. The biosynthesis pathway includes the production of the *ent*-kaurene occurring in the proplastids, and its further oxidation to GA₁₂-aldehyde by the catalyzing activity of cytochrome P-450 mono-oxygenases at the ER. The last phase of the pathway is catalyzed by 2-oxoglutarate-dependent di-oxygenases in the cytosol (Salazar-Cerezo et al., 2018).

During the de-etiolation process induced by light, a reduction in the concentrations of GAs, due to the activity of the catabolic enzyme GA-A2-OXIDASE2, and a concomitant accumulation of DELLA proteins are observed. DELLA proteins amplify the levels of HY5 and provoke the biosynthesis of chlorophylls and carotenoids (Müller and Munné-Bosch, 2021 and references therein). Jasmonates retard DELLA degradation carried out by GAs, showing that the cross talk between JAs and GAs signaling pathways regulates photomorphogenesis (Fig. 4). Moreover, the GATA-NITRATE-INDUCIBLE, CARBON METABOLISM-INVOLVED (GNC), and GNC-LIKE (GNL) transcription factors act downstream of DELLA to suppress the GAs signaling, thus regulating the metabolism of protochlorophyllide and the biosynthesis of chlorophyll (Richter et al., 2010).

In contrast with these negative effects, GAs stimulate RuBisCO (Yuan and Xu, 2001). Moreover, GAs also affect chloroplast replication. In fact, *ga1-3* and *d18-AD*, GA-deficient mutants of Arabidopsis and rice respectively, exhibit a reduction in the expression of genes related to chloroplast division (Jiang et al., 2012).

2.3.5. Brassinosteroids

Brassinosteroids (BRs) are other phytohormones affecting plant growth and development. They are involved in the control of events such as cell division and elongation, photomorphogenesis, and senescence (Altamura et al., 2023, and references therein). These compounds are characterized by polyhydroxylated sterol structures. Based on carbons number, C₂₇, C₂₈, and C₂₉-type of BRs were identified. Independently of carbon number, the early steps of their synthesis are common and occur via either MVA or non-MVA pathways. The C₂₈-BR biosynthesis pathway is the best studied and it leads to the biosynthesis of campesterol. Up to now, all steps of BR biosynthesis and the role of plant organellome still needs investigation (Bajguz et al., 2020). The Brassinolide (BL) is the most active BR and derives from the campesterol.

Many studies have reported the positive roles of exogenous BRs on photosynthesis. In fact, in *Brassica juncea*, treatments with 28-homobrasinolid and 24-epibrassinolid (24-eBL) up-regulate photosynthesis by enhancing photochemical/non-photochemical quenching, PSII efficiency, electron transport rate, stomata movement and conductance, photosynthetic rate and carbohydrate synthesis (Siddiqui et al., 2018).

While BRs themselves are not influenced by light, their signaling pathway, mediated by the transcription factor BRASSINAZOLE-RESISTANT1 (BZR1), plays a role in regulating light signaling elements. This transcription factor forms a complex with PIF4, an enhancer of etiolation. Both the degradation of PIF by light signaling, and the inactivation of BZR1 by reduced BR signaling, decrease the BZR1-PIF dimer formation, promoting photomorphogenesis. Moreover, BZR1 not only directly cooperates with the negative regulators (PIFs) but also transcriptionally represses the positive regulators of photomorphogenesis (Fig. 4; Müller and Munné-Bosch, 2021 and references therein). In etiolated *det2* mutant seedlings of Arabidopsis, characterized by a disturbed BR biosynthesis and consequent low content of BRs, a de-etiolation phenotype was observed in comparison with the wild type. This phenotype was associated with increases in the levels of chlorophylls *a* and *b* and carotenoids and an accumulation of the mRNAs of several nuclear and plastid genes encoding proteins of the photosynthetic machinery (Efimova et al., 2012), demonstrating the importance of specific levels of BRs for the activation of the transcription of chloroplast genes necessary for photomorphogenesis.

Brassinosteroids have a pivotal function also in enhancing drought resistance, in accordance with their role as stress avoidance compounds (Betti et al., 2021). In this role on stress, BRs also affect the photosynthetic apparatus. Indeed, drought-sensitive and drought-tolerant genotypes of maize show differences in the endogenous levels of BRs, with the drought-tolerant genotype exhibiting higher levels of BRs, chlorophyll and carotenoids (Fig. 4) (Tümová et al., 2018). In accordance, in cowpea exogenous 24-eBL improves the efficiency of the photosynthetic process and plant growth under water deficiency conditions (Lima and Lobato, 2017). Also, in numerous other plants increased chlorophyll and carotenoid concentrations and PSII efficiency are among the positive effects on photosynthesis caused by 24-eBL, collectively supporting the role of these phytohormones in favoring photoprotection (Lima and Lobato, 2017; Zhao et al., 2017).

2.3.6. Ethylene

Ethylene (ET) plays a central role in the plant life cycle but is also an important mediator of stress signaling, interacting with plastid signaling.

It is well known that ET biosynthesis occurs in the cytosol where the aminocyclopropane 1-carboxylate synthase (ACS) converts S-adenosyl-methionine to 1-aminocyclopropane 1-carboxylate (ACC) and 5'-methylthioadenosine. Subsequently, in the presence of oxygen, the 1-aminocyclopropane 1-carboxylate oxidase converts ACC to ET. However, the homeostasis of S-adenosyl-methionine is linked to the chloroplast via the methionine synthesis. In fact, as demonstrated in Arabidopsis, the synthesis of methionine occurs in a process involving the activities of cystathionine γ -synthase and cystathionine β -lyase, which are exclusively plastidial compounds (Watanabe et al., 2021). Thus, there is an important link between ET and chloroplast, as the plastid is the site of ET precursor biosynthesis. Data from mutants in tomato and Arabidopsis further support the role of the plastid in the production of the ET precursors. For example, in tomato, the *lutescent 2* mutant is characterized by the down regulation of *LUTESCENS 2*, encoding a plastid thylakoid membrane-localized zinc-dependent metalloprotease. The mutant shows slower chloroplast development in the leaves, and altered ripening, reduced photosynthetic rate and ET levels in the fruits compared with the wild type (Barry et al., 2012). In the same plant, the *Orr^{Ds}* mutation, causing deficiency in a subunit of the plastidial NADH dehydrogenase, also delays the onset of the fruit ripening and reduces the ET levels emitted from the fruits (Bittner et al., 2022, and references

therein).

In addition, the signaling via the ET-responsive nuclear transcription factor ETHYLENE INSENSITIVE 3 (EIN3) affects chloroplast biogenesis in an anterograde way. In fact, under darkness, the expression of many *LHC* genes is inhibited by the interaction of EIN3 with PIF3. However, when exposed to light, the PHY B-mediated degradation of both EIN3 and PIF3 facilitates the transcription of *LHC* genes, positively affecting the development of the chloroplast (Liu et al., 2017).

2.3.7. Salicylic acid

Salicylic acid (SA) is involved in mitigating abiotic stresses, e.g., heating, chilling, salinity, metal toxicity, drought, mainly working as a signaling molecule for the regulation of ROS production (Liu et al., 2022).

It is known that plants synthesize SA both by an isochorismate synthase (ICS) pathway and a phenylalanine ammonia-lyase (PAL) pathway. Both pathways start from the chorismate, and both affect, alone or together, SA synthesis. In the ICS pathway, the only pathway already characterized for the first biosynthetic step, isochorismate is synthesized from chorismate in the chloroplast and is then translocated into the cytosol, where SA is produced (Lefevre et al., 2020).

Both under normal and stressed conditions, SA affects the expression of nuclear-encoded proteins that are translocated to chloroplasts. Moreover, the hormone directly interacts with several proteins and ROS in different cell compartments, including the chloroplasts (Bittner et al., 2022). The thermal stability of the electron donor and reaction centers of PSII are maintained by SA applications, which improves the antioxidant system and chlorophyll fluorescence (Fig. 4), as described in alfalfa (Liu et al., 2022). Moreover, SA regulates photosynthesis also through RuBisCO. For example, treatments with SA protect tomato plants from drought stress, mainly by maintaining the activities of the carbonic anhydrase that directly affects the rate of CO₂ fixation (Liu et al., 2022 and references therein). In contrast, SA deficiency aggravates Cd-induced damage on chlorophyll and upregulates *phosphatidylethanolamine N-methyltransferase (PEMT)* and *plastocyanin 1 (PETE1)*, which are genes related to photosynthetic electron transport (Liu et al., 2022).

The SA signaling pathway is interconnected with the ROS pathway. The ROS are by-products of electron transfer in the chloroplast. In peculiar conditions, e.g., during reactions to pathogens and through retrograde signaling, ROS regulate genes working upstream of SA, such as *ENHANCED DISEASE SUSCEPTIBILITY 1* and *5 (EDS1* and *5)* (Bittner et al., 2022). Besides ROS, other plant hormones are involved in the SA signal transduction pathway (Fig. 4), with a relationship between ABA and SA levels occurring under stress, in particular.

2.3.8. Cytokinin

The developmental processes regulated by cytokinin (CK) include cell cycle control, root and shoot meristem activity, shoot and root branching, floral transition and leaf senescence, among the others. The phytohormone also participates in the response to numerous environmental stresses, including drought, cold, osmotic stress, and high light conditions (Brenner et al., 2017 and references therein).

The role of CK is determinant for the chloroplast, being central for its differentiation and functioning, and for the biosynthesis of chlorophyll, with about 26% of the CK-induced proteins involved (Cortleven and Schmölling, 2015). Among the multitude of genes regulated by CK and associated with photosynthesis there are genes encoding for the RuBisCO subunits and the light-harvesting chlorophyll *a/b*-binding proteins of PSII (Fig. 4) (Brenner et al., 2017 and references therein).

Cytokinin synthesis involves an enzymatic cascade driven by genes for isopentenyltransferase (IPT), CK trans-hydroxylase (CYP735A), and the CK nucleoside 5-monophosphate phosphoribohydrolase (LOG). The CK signal is perceived by histidine kinases and transmitted from the receptors to type-B response regulators (type-B ARRs), which affect the CK-response genes, including those linking the hormone activity to

chloroplast functions (Cortleven and Schmölling, 2015, and references therein).

It has been suggested that the CK genes were transferred from the plastid to the nuclear genome by the endosymbiotic pathway, and that in the nucleus the CK genes were adapted to regulate the development and function of the plastid, through anterograde and retrograde signaling pathways (Cortleven and Schmölling, 2015 and references therein). However, at least in Arabidopsis, the chloroplasts contain numerous IPT enzymes. The presence of these IPT proteins and of numerous CK metabolites in the plastid has suggested a specific chloroplast function for maintaining the cellular CK homeostasis (Cortleven and Schmölling, 2015).

Cytokinin is also determinant for proplastid and etioplast transition to chloroplast. In fact, it was shown that during the dark-to-light transition, CK accelerated the differentiation of the prolamellar body (PLB) into the thylakoid lamellar structures, accompanied by an increase in chlorophyll biosynthesis. This result has been reinforced by the observation that Arabidopsis seedlings grown in a CK-free medium display small etioplasts with PLBs, while seedlings exposed to CK exhibit larger lens-shaped etioplasts which are converting into chloroplasts because of the appearance of bi-thylakoid membrane structures and disappearance of PLBs (Chory et al., 1994). During de-etiolation, CK also regulates the synthesis of Pchl_{ide} and the photoreduction of Pchl_{ide} to Chl_{ide} (Fig. 4) and alters the transcript levels of specific genes of the tetrapyrrole biosynthesis pathway (Cortleven and Schmölling, 2015).

It has been repeatedly demonstrated that also in light-grown plants the endogenous CK content is positively correlated with the chlorophyll content, with a fully functional CK signaling system important to reach a normal chlorophyll content. However, in accordance with numerous Authors it is to be stressed that CK is not necessary for chlorophyll biosynthesis but rather modulates its concentration.

Another role of CK is its protective role for the photosynthetic apparatus under light stress. Under this stress, CK enhances the functions of numerous antioxidants, aiding in the scavenging of ROS and facilitating the repair of D1, a crucial PSII subunit (Müller and Munné-Bosch, 2021).

This phytohormone also regulates nuclear genes which encode plastid-related proteins, as documented by genome-wide transcript analyses performed in numerous plants. Among these genes, there are *CGA1* and *CRF2* transcription factor genes. GATA factor 1 (*CGA1*) is a type IV zinc finger protein whose expression correlates with that of genes involved in light responses, including *HY5*, and its overexpression promotes the differentiation of proplastids to etioplasts in the dark, and to chloroplasts in the light, and the ectopic production of chloroplasts in roots (Fig. 4) (Cortleven and Schmölling, 2015 and references therein). Moreover, *CGA1* is also involved in GA and auxin signaling (Fig. 4; Richter et al., 2010). *CRF2*, belonging to the APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) class of transcription factors, is implicated in controlling chloroplast division by CK (Cortleven and Schmölling, 2015).

Moreover, using CK-insensitive mutants, it has been demonstrated that CK positively regulates the nuclear-encoded *SIGMA FACTOR* genes *SIG2* and *SIG6*. Sigma factors are involved in the transcription of PEP, which is essential for transcribing genes related to photosynthesis. Consequently, Sigma Factors could be among the initial elements influenced by CK for modulating the gene expression of the plastid in strict dependency from developmental and environmental signals (Müller and Munné-Bosch, 2021).

The antagonism of ABA and CK in greening and chloroplast development has been well established (Fig. 4). Cytokinin antagonizes the inhibition of greening in cotyledons caused by ABA through the degradation of Abscisic Acid Insensitive5 (*ABI5*) (Guan et al., 2014). This action is dependent on several proteins of the CK signaling pathway, namely receptors, phospho-transmitter proteins, and the B-type ARR12 transcription factor (Guan et al., 2014).

Synergistic relationships exist between CK and other

phytohormones. For example, a positive regulation of the expression of photosynthesis-related genes by CKs and SLs has been demonstrated (Fig. 4; Brenner et al., 2017 and references therein). Cytokinin induces ET biosynthesis in etiolated seedlings by increasing the stability of ACC synthase, but a relationship between CK and ET also in the control of photomorphogenesis has been suggested (Fig. 4; Hansen et al., 2009).

A relationship is also present between GA and CK. In fact, as already shown, the two hormones regulate *HY5* stability and activity. Moreover, the transcription factor *CGA1* is an important target downstream of GA, DELLA, and PIFs, regulating greening, among the other processes, and acting as a point of convergence for GA and CK signaling (Fig. 4; Richter et al., 2010).

Data suggest that *CGA1* and *HY5* are important players in the cross talk of CK and auxin in the regulation of greening (Fig. 4). In fact, *HY5* enhances the expression of negative regulator genes of auxin signaling and the CK-induced *CGA1* expression meets with the expression of *HY5* (Cluis et al., 2004). Moreover, the Auxin Response Factor *ARF2*, a putative target of *HY5* (Cluis et al., 2004), represses *CGA1* gene expression and mutations in *ARF2* result in phenotypes similar to those caused by the *CGA1* overexpression, including greening (Kobayashi et al., 2017).

2.3.9. Auxin

Indole-3-acetic acid (IAA) is the plant most common auxin and is another phytohormone very important for chloroplast development and functions. Numerous auxin responses are mediated by the ARFs, which bind to promoters of auxin responsive genes to regulate transcription, and by the auxin/indole-3-acetic acid inducible (Aux/IAA) proteins that repress ARF function (Ljung, 2013). The phytohormone can be synthesized from its precursor L-tryptophan (L-Trp) via the shikimate pathway. The first step occurs in the chloroplast, whereas the subsequent steps are located in the cytosol (Ljung, 2013). Numerous studies confirm that auxin biosynthesis can start within the plastids and in accordance, the presence of IAA within the chloroplast has been found in numerous species including Arabidopsis, barley, tobacco, and coffee (Salazar-Irbe and De-la-Peña, 2020).

Auxin affects several essential components of the chloroplast involving anterograde and retrograde signaling pathways. In accordance, a high number of auxin-related genes is expressed in the chloroplast, e.g., *NADPH-Thioredoxin Reductase (NTRC)* gene necessary for the correct functioning of the SK pathway, *IAA/AUX*, *ARF*, *GH3*, *SAUR*, and *TIR* genes for signaling pathways, biosynthesis-related genes (e.g. *YUCCAs*) and auxin transporter genes, such as *PINs*, as reviewed by Salazar-Irbe and De-la-Peña (2020). Several studies also show a positive involvement of auxin in the production of photosynthetic pigments and in the development of the chloroplast, e.g., by modifying the expression of *GLK2*, *HY5* or *SHIKIMATE KINASE-LIKE1 (SKL1)* genes (Lupi et al., 2019).

The first proteins discovered to connect auxin to chloroplast development include chl a–b binding proteins and PSI proteins (Fig. 4) (Salazar-Irbe and De-la-Peña, 2020 and references therein). Moreover, the plastid rRNA exhibits an important function in the control of auxin activities. In fact, a chloroplast methyltransferase rRNA regulates the expression of various auxin response genes, such as *AUX/IAAs*, *SAUR* and *GH3* (Zou et al., 2020). A low expression of these genes characterizes the Arabidopsis chloroplast methyltransferase rRNA mutants, as well as an impaired thylakoid development and the down regulation of *YUCCA8*, *CYP71A27* and *CYP79B3* (putative P450 monooxygenases), and of the auxin polar transport *PIN* genes (Zou et al., 2020).

Auxin signaling promotes chloroplast biogenesis also in *Solanum lycopersicum* fruits, where the transcription factor *SIGLK2* is predominantly expressed and positively affects the expression of genes for photosynthesis, chloroplast differentiation and sugar storage (Fig. 4) (Lupi et al., 2019).

Auxin is also important for the process of plastid interconversion. In fact, some *ARF* genes have been supposed to be at the convergence point with carotenoid biosynthesis, affecting the conversion of chloroplast to

chromoplast during fruit ripening (Salazar-Irbe and De-la-Peña, 2020 and references therein). In *S. lycopersicum*, the *high pigment2* (*hp2*) mutant is characterized by an over-accumulation of chlorophyll and of chloroplasts in leaves and immature fruits, but also by an intense red fruit pigmentation. In this mutant, the loss of the HP2 function drastically alters auxin signaling, resulting in a higher accumulation of auxin, severe downregulation of *AUX/IAA* genes and altered accumulation of *ARF2a* and *ARF2b* transcripts (Cruz et al., 2018).

Moreover, in white clover, IAA treatments inhibit the transcription of the *Senescence-Associated (SAGs)* *SAG101* and *SAG102* genes, involved in the regulation of leaf senescence through chlorophyll degradation (Fig. 4). This suggests that IAA treatments contrast the conversion of chloroplasts into gerontoplasts by preventing chlorophyll degradation and photoinhibition (Zhang et al., 2020).

Despite numerous reports on the positive effects of IAA, it is important to underline that the action of this phytohormone on chloroplast development may be negative during the early phases of development or in different contexts, depending on the endogenous levels. For instance, auxin negatively regulates the root greening by inhibiting chloroplast development, thus promoting the differentiation of proplastids into amyloplasts. However, when the shoot is removed, chlorophyll accumulation and chloroplast development occur in the roots (Fig. 5) (Cackett et al., 2022).

2.3.9.1. *Root greening is promoted by cytokinin and inhibited by auxin.* It is well known that chloroplasts are usually present in the photosynthetic tissues of leaves and young stems, but not in roots and their tissues, however this is not always true.

In Arabidopsis, the phenomenon known as root greening occurs when roots are detached from shoots. In this case, the root plastids synthesize chlorophyll and differentiate a functional photosynthetic apparatus. This ectopic chloroplast differentiation is mediated by an increase in CK content and a deficiency in auxin content, as widely described by Kobayashi and coworkers (2017).

Upon CK perception, ARRs up-regulate the B-GATA GNC and the GNL transcription factors, and the two induce root greening by up-regulating chlorophyll biosynthesis and *PhANG* genes, with this usually occurring in the root cortical parenchyma and pericycle (Kobayashi et al., 2017).

It has been proposed that Hy5 and CGA1 may be the interactors between CK and IAA in the regulation of root greening (Fig. 5). In fact, the GNC- and GNL-mediated root plastid differentiation is controlled by HY5 and the inhibition of root greening by auxin occurs by the IAA signaling protein IAA14 and the auxin responsive transcription factors ARF7 and ARF19 which repress HY5 and GNC/CGA1 expression (Fig. 5; Kobayashi et al., 2017).

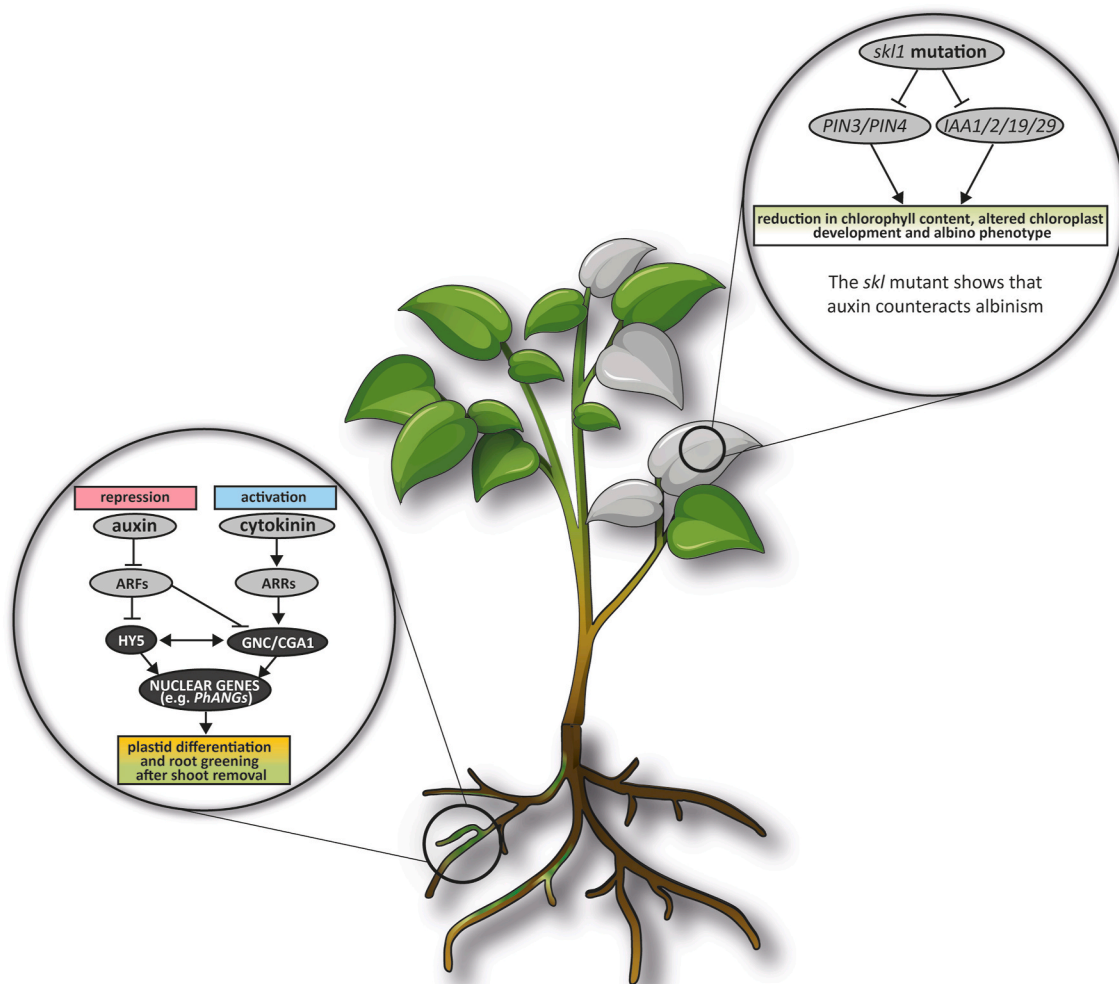


Fig. 5. Left side: schematic representation of the contrasting roles of auxin and cytokinin on the activation/repression of genes for chloroplast differentiation necessary to root greening after shoot removal. Right side: example of albinism in leaves of the *skl* mutant of *Arabidopsis thaliana* negatively affected in Trp biosynthesis and in the expression of genes for auxin response and transport. The mutant phenotype demonstrates the positive role of auxin in counteracting albinism. Further details on the text.

2.3.9.2. Auxin counteracts albinism. The absence of chloroplasts in tissues that are typically green is the rare phenomenon known as albinism. Albinism has been reported in several plant species exposed to different growth or stress conditions. For example, in *Delonix regia*, the chloroplasts of albino plants are poorly developed, the internal membrane system is poorly defined and numerous PGs are present (Silva et al., 2020). Molecular studies have shown that a decrease in the expression of genes related to the photosynthesis occurs in albino plants. For example, in *Agave angustifolia*, albinism causes low levels of photosynthetic pigments and low expression of chlorophyll and carotenoid biosynthetic genes (Us-Camasa et al., 2017). In most cases, albinism is a lethal condition, but it may be also a periodic event, as in the case of tea mutants. In these plants, albinism occurs in the leaves at very low temperatures, but the plants re-green when temperatures become high, with the phenomenon controlled by a regulatory network based on specific miRNAs (Xu et al., 2023).

Albinism is related to auxin responses, with numerous studies collectively supporting a role for the hormone in counteracting the albino phenotype (Fig. 5). For example, in *Arabidopsis*, the *shikimate kinase-like1* mutant (*skl1*), negatively affected in Trp biosynthesis, exhibits a dramatic reduction in chlorophyll content and in the expression of a lot of photosynthesis-related genes. In accordance this mutant shows albino phenotype with a blockage in chloroplast development. Moreover, in the same mutant, genes related to the response to auxin such as *IAA1*, *IAA2*, *IAA19* and *IAA29* show low levels in their transcripts, and the auxin transporters PIN3 and PIN4 are also deregulated (Xu et al., 2018).

2.3.9.3. Auxin cross talk with other phytohormones. A cross talk among phytohormones is necessary to regulate photosynthesis during plant growth and to optimize the vigor of the plant in changing environments. As also commented before for CK and the other main phytohormones involved in plastid differentiation and activities, the interactions may be direct/indirect, positive/negative and may involve all the major classes of phytohormones (Fig. 4). For example, SLs, JAs, CKs, and DELLA proteins contribute to the promotion of photomorphogenesis by influencing the activity of HY5 (Müller and Munné-Bosch, 2021 and other references therein). On the contrary, ET, GAs and BRs repress photomorphogenesis either by interacting with PIFs or by inhibiting B-GATAs (Yang et al., 2012; Liu et al., 2017). The interaction between JAs and GAs influences photomorphogenesis by slowing down the degradation of GAs-induced DELLA proteins via JAs and affecting the interplay between DELLA proteins and PIFs (Yang et al., 2012). Furthermore, the action of phytohormones on photomorphogenesis can vary in opposing directions depending on their levels within the plant, as shown by BRs.

It is well known that auxin participates with other phytohormones in the cross talk that controls chloroplast biogenesis, functioning and stress responses (Fig. 4). For example, in clover, treatments with IAA increase, and treatments with a strong IAA inhibitor decrease chlorophyll, ABA and JA levels, by that means enhancing or reducing, respectively, drought tolerance (Zhang et al., 2020). Moreover, photosynthesis in the fruit is regulated by the interplay between IAA and CK (Bianchetti et al., 2017), and prolonged photosynthesis and delayed fruit ripening are due to the cross talk between auxin and ET which leads to a repression of ET biosynthesis (Fig. 4; Yuan et al., 2019).

However, the role of auxin seems to be more complex than those of the other phytohormones because auxin exhibits a dual effect on photosynthesis and photomorphogenesis. For example, IAA inhibits stoma formation, negatively impacting photosynthesis, while simultaneously enhances photosynthesis through the development of leaf venation (Müller and Munné-Bosch, 2021 and references therein). Moreover, chlorophyll accumulation is inhibited in the seedlings of *Arabidopsis* wild type and *yuc2 yuc6* double mutant under exogenous IAA application, and mainly in the mutant. This suggests that, at least during de-etiolation, endogenous and exogenous auxin work together as negative regulators of chlorophyll accumulation (Luo et al., 2023).

Although the interactions between IAA and CK are often considered antagonistic, as in the case of root greening (Fig. 5), the truth is that their cross talk involves both antagonistic and synergistic roles, often working in a cell and/or tissue-specific way, and at a specific time and environmental context (Zubo and Schaller, 2020).

3. Sensory plastids

3.1. Epidermal sensory plastids

Initially described as leucoplasts (Brunkard et al., 2015), through a merging of transcriptomics and metabolomics analyses, cell/tissue proteomics, super-resolution microscopy, and mutant characterization, further analyses have shown that the *Arabidopsis* epidermal pavement cells contain plastids which accumulate chlorophyll and display light avoidance responses. Despite morphological similarities with chloroplasts, these organelles differ from the latter ones, because they are few per cell, but mainly because they show smaller dimensions, a low chlorophyll content, and impaired grana development compared to chloroplasts (Barton et al., 2016). Due to these distinctions, these plastids have been called as pavement cell chloroplasts, epidermal chloroplasts, or more recently, sensory epidermal plastids (SEPs) (Barton et al., 2016; Viridi et al., 2016; Beltrán et al., 2018; Sierra et al., 2023). Differently from the chloroplasts present in the stoma cells of the epidermis, which, while sharing some features with sensory plastids, perform their own specialized functions (Mackenzie and Mullineaux, 2022), SEPs display dynamic morphology, proteome composition, and plastid-nuclear interactions to facilitate environment and pathogen sensing and signaling, as their name suggests. These characteristics are also shared with the vascular parenchyma plastids (vascular sensory plastids) (Viridi et al., 2016) that will be discussed below.

Sensory epidermal plastids show physiological and functional responses to abiotic and biotic stimuli, which had been previously attributed to mesophyll chloroplasts. For instance, in *Arabidopsis*, these small-sized organelles may be considered as defense plastids because able to motion for positioning immune components in the epidermis (Irieda and Takano, 2021).

Interestingly, the sensory functions of SEPs supply a new understanding of the epidermal roles of the aerial organs, not only as a physical barrier of the plant but also as a strategic sensory tissue of external conditions. In fact, the response to environmental stimuli, such as high light or increased glucose or sucrose levels, can induce rapid and prolific stromule formation in SEPs (Brunkard et al., 2015). Stromule formation also occurs in response to the abiotic stress which links the hormone signaling to the pathogen effector-triggered immunity (Savage et al., 2021). However, there is a disparity between SEPs and mesophyll chloroplasts in stromule formation/activity which could be a function of differences in plastid size and density between mesophyll and epidermal cells. The activity of stromules in SEPs emerges as a key specialized trait for environmental perception and signaling, triggered when photosynthesis is restricted under various conditions (Breeze and Mullineaux, 2022; Mackenzie and Mullineaux, 2022). Sensory epidermal plastids are also known to position in proximity to the nucleus, particularly in response to plant environmental stress (Savage et al., 2021) and in *in vitro* cultured tobacco thin cell layers explants (Altamura, personal communication). This perinuclear positioning is controlled by the actin cytoskeleton. Consequently, SEPs surround the nucleus and attach to the nuclear membrane via membrane contact sites. This activity is influenced by trafficking through the perinuclear space contiguous with the ER (Breeze and Mullineaux, 2022). TRIGALACTOSYLDIACYLGLYCEROL5 (TGD5), a component of the lipid metabolism that influences the SEPs, but not the mesophyll chloroplast development, participates in ER-to-plastid transport for thylakoid lipid assembly (Mackenzie and Mullineaux, 2022 and references therein).

Regarding the involvement of SEPs in pathogenic responses, it has been observed that *Arabidopsis* SEPs display relocation movements,

named epidermal chloroplast responses (ECRs) in response to fungal interaction. These ECR movements are activated by cell wall-degrading enzymes that accumulate during fungal formation when the immunity system becomes ineffective (Irieda and Takano, 2021). Multiple immune components such as γ -glutamylcysteine synthetase (γ GCS), EDS5, and Calcium-Sensing Receptors (CaSRs), that contribute to non-host resistance responses, have been specifically detected in the stromules of the SEPs in infected plants, supporting that this accumulation is a key mechanism in the defense response against fungal infections (Irieda and Takano, 2021).

Numerous elements of SEP proteome are common with those of mesophyll chloroplasts, but in a way that allows for distinct roles in each of the two plastid types, and some plastid-targeted proteins are encoded by duplicate genes. For example, the Arabidopsis CHLOROPHYLL A/B BINDING PROTEIN UNDEREXPRESSED (CUE1), also known as PPT1, is enriched in the SEP proteome (Beltrán et al., 2018) and shows SEP-associated expression in-silico, whereas the homologous PPT2 is a mesophyll chloroplast PEP translocator protein showing mesophyll chloroplast-associated expression in-silico (Mackenzie and Mullineaux, 2022). Moreover, the in-silico expression profiling of the 3-Deoxyd-Arabinose-Heptulosonate-7-Phosphate Synthase (DAHPS), the first enzyme of the SK pathway, encoded by two genes (*DAHPS1* and *DAHPS2*) in Arabidopsis, shows that *DAHPS1* is localized in the SEPs, while *DAHPS2* in the mesophyll chloroplasts, with only *DAHPS1* expressed in response to wounding and pathogen infection (Mackenzie and Mullineaux, 2022 and references therein).

However, the SEP proteome is also characterized by proteins specific to this plastid type, e.g., *SAL1*, a component of organellar retrograde signaling, and MutS Homolog1 (*MSH1*), an organellar-DNA binding protein. Differently from the redox-regulated phosphatase *SAL1* which is predominantly expressed within the vascular tissue, and for this will be described below, *MSH1* encodes a dual-targeted mitochondrial and plastid DNA binding protein that localizes to the SEP but not to the mesophyll chloroplast (Xu et al., 2011). *MSH1* participates in stabilizing the plastid genome (Xu et al., 2011). The *msh1* mutant has a variable and pleiotropic phenotype, involving defects in leaf variegation (Sierra et al., 2023), alterations to the plastoquinone pool, delayed growth, and maturation, altered circadian clock effects, and enhanced abiotic and biotic stress responses (Xu et al., 2011, 2012; Viridi et al., 2016).

Moreover, both SEPs and vascular parenchyma sensory plastids (see below) are enriched for proteins involved in responses to Cd ions compared with mesophyll chloroplasts (Beltrán et al., 2018). This suggests a role for both in sensing heavy metal toxicity.

The retrograde signaling pathways that function in the SEP signaling have not been defined (Sierra et al., 2023).

3.2. Vascular sensory plastids

It is still unclear whether the SEPs and the plastids located in the vascular parenchyma belong to the same type. As reviewed by Sierra et al. (2023), the reticulate phenotype that affects mesophyll development and chloroplast differentiation specifically in the interveinal tissue of the *cue* reticulated mutants seems to show characteristics of a sensory plastid vascular dysfunction. This suggests sensory functions also in the plastids of the vascular tissues, recently named vascular sensory plastids. In addition, several *reticulata* mutants are related to the SK pathway and display vascular parenchyma-specific or vascular enriched expression patterns and can be complemented by aromatic amino acid supplementation (Lundquist et al., 2014). Interestingly, *SAL1* is predominantly expressed within the vascular parenchyma cells and is confined in mitochondria and plastids to regulate levels of PAP (Estavillo et al., 2011). Under stress conditions and altered circadian rhythms, *SAL1* activity is inhibited, and this causes an accumulation of PAP in the plastids. This accumulation affects the expression of plastid redox-associated nuclear genes (*PRANGs*) through the inhibition of the activity of the 5'→3'exoribonucleases (XRNs) (Estavillo et al., 2011;

Litthauer and Jones, 2018). Tocopherols, derived from tyrosine in the vascular sensory plastids (via the SK pathway), serve to up-regulate PAP in a process dependent on *CUE1*, also active in SEPs. The upregulation of PAP protects primary miRNAs from degradation and promotes the production of mature miRNAs (Fang et al., 2019; Mackenzie and Mullineaux, 2022). In this way tocopherols take part in the modulation of the pathway of *SAL1*-PAP-XRN retrograde signaling under stress conditions and in circadian systems (Litthauer and Jones, 2018).

In *A. thaliana*, *MSH1* is found not only in SEPs, but also in plastids of the vascular parenchyma cells, further supporting that these plastids function as environmental sensors (Beltrán et al., 2018). In accordance, various monocot and dicot *msh1* mutants show a leaf variegated phenotype, in epidermal cells, but also in cells of the vasculature, and the transcriptomic analyses of such mutants also show altered expression of genes for ABA biosynthesis, cold, oxidative, osmotic and salt stress, calcium signaling, and auxin responses (Sierra et al., 2023 and references therein). It has been hypothesized by Mackenzie and Mullineaux (2022) that the presence of sensory plastids in the vascular parenchyma cells represents an example of how they transmit a signal, e.g., the activation of ABA biosynthesis, that, in turn, modulates a retrograde signaling pathway. However, it must be noted that the retrograde signaling pathways involved in vascular sensory plastid signaling remain still undefined.

4. Future outlooks and concluding remarks

4.1. Outlooks

There is still much to explore regarding the functions and interactions of the different plastid types, particularly at the tissue and cell type levels, and in non-model plants. Apart from the case of gerontoplasts, which represent the irreversibly determined fate of the plastid, the other plastid types express a continuous dynamism, therefore a modulable fate, certainly influenced by numerous hormones, according to anterograde and retrograde pathways and by environmental parameters, but also possibly affected by the relationship with the tissue type. An example is the ectopic chloroplast differentiation in specific root tissues, but general information is limited and needs further investigation. The discovery of sensory plastids with epidermal or vascular locations, and their influence on plastids with other tissue locations could provide a possible line of intervention. The relationship is also organotypic. In fact, plastid signals which affect leafy morphology are also required for the establishment of lateral root primordia and for the maintenance of their root apical meristem. This suggests a long-distance, not organ-specific, plastid-signaling. Although this long-distance signaling system involving sensory plastids remains to be fully investigated, it is noteworthy that a gene coding for a chloroplast ribosomal protein has already been discovered to be involved, at least in Arabidopsis. This suggests that another line of intervention could concern an in-depth study of the activity of the ribosomal proteins. These proteins are important not only for development but also for stress response. In fact, in the present review it has been mentioned that a correct chloroplast ribosome functioning is essential for defense, e.g., against As stress. This is just one example on how the investigation into the activity of ribosomal proteins could also be an interesting and essential defense strategy against environmental toxic agents for many plants.

It is also possible to inquire whether there are anterograde and retrograde signaling pathways that act at an interspecific level. The cytoplasmic hybrid is produced by the fusion of protoplasts from organisms of different species. In cybrids, the nuclear genome of one species comes into contact with the cytoplasmic genome of another species, including the plastome. Cybridization may be reciprocal, i.e. the nuclear and cytoplasmic DNAs of the two parental lines may be exchanged. This allows to identify phenotypic effects of the cytoplasmic or nuclear genomes, or of their interactions (Flood et al., 2020). It is

therefore possible to use the biotechnology of cybridization to study the expression of plastome genes and their activities in the anterograde and retrograde ways outside the context of the parental nuclear genome. This information might be useful for understanding autonomous and non-autonomous control factors of the plastid, including ribosomal proteins, and possibly for applying this information in plant breeding experiments.

The multiple roles of PGs in the plastid development and response to environmental stress, along with their relationship with the hormonal multi-control, have been discussed in the review. Given that lipids are the main components of PGs, a promise avenue for future research lies in investigating the role of these structures in relation to the dynamism of the plastidial transition and the response to stress. For example, a hypothesis regarding the last (and irreversible) step of plastid differentiation, i.e. the gerontoplast formation, could bring the PG role in chloroplast dismantling closer to the mechanism of microlipophagy described for the degradation of lipid droplets during starvation-induced stress (Fan et al., 2019). This would give the lipids of PG a very important role that needs further investigation.

Last but not least, we mentioned MSH1, an organellar-DNA binding protein which participates in stabilizing the plastid genome (Xu et al., 2011). Interestingly, the MSH1 RNAi-suppressed plants produce progeny showing *msh1* stress-memory effects that are heritable indefinitely (Xu et al., 2012; Viridi et al., 2015), with the transition to this epigenetic memory state dependent on the RNA-directed DNA methylation pathway (Mackenzie and Mullineaux, 2022). The pleiotropic responses linked to the inhibition of *MSH1* arise from SEPs (Viridi et al., 2016), but MSH1 is also found in the plastids of the vascular parenchyma cells (Beltrán et al., 2018). It is therefore possible to hypothesize that future research could also be aimed at an in-depth study of sensory plastids as a means of creating a transgenerational memory to stress, in plastids and in the plant.

4.2. Concluding remarks

The present review integrates relevant recent and past information about plastid dynamism during development and in response to external cues. This leads to specific changes in organelle proteome and plastid morphology finally leading to plant adaptation/environmental reaction. It is tempting to speculate that the contrasting developmental processes involving plastid plasticity produce specific signals able to play determinant roles during developmental transitions. These signals may be interpreted as “endogenous” (developmental) stress responses which are affected by exogenous (environmental) stress signals, with phytohormones as important mediators.

As also recently discussed by other Authors (Hickey et al., 2023), but also based on the recent investigation on sensory plastids, it may be concluded that measuring the plastid type specific parameters under different developmental/stress conditions is essential for exploiting plant organellome responses to each cue in the context of interactions between genotype and environment and for investigating the possibility of plant capacity for a transgenerational stress memory.

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Maria Maddalena Altamura: Writing – review & editing, Investigation, Conceptualization. **Diego Piacentini:** Writing – original draft. **Federica Della Rovere:** Writing – original draft. **Laura Fattorini:** Writing – original draft. **Alessio Valletta:** Writing – review & editing, Writing – original draft. **Giuseppina Falasca:** Writing – review &

editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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