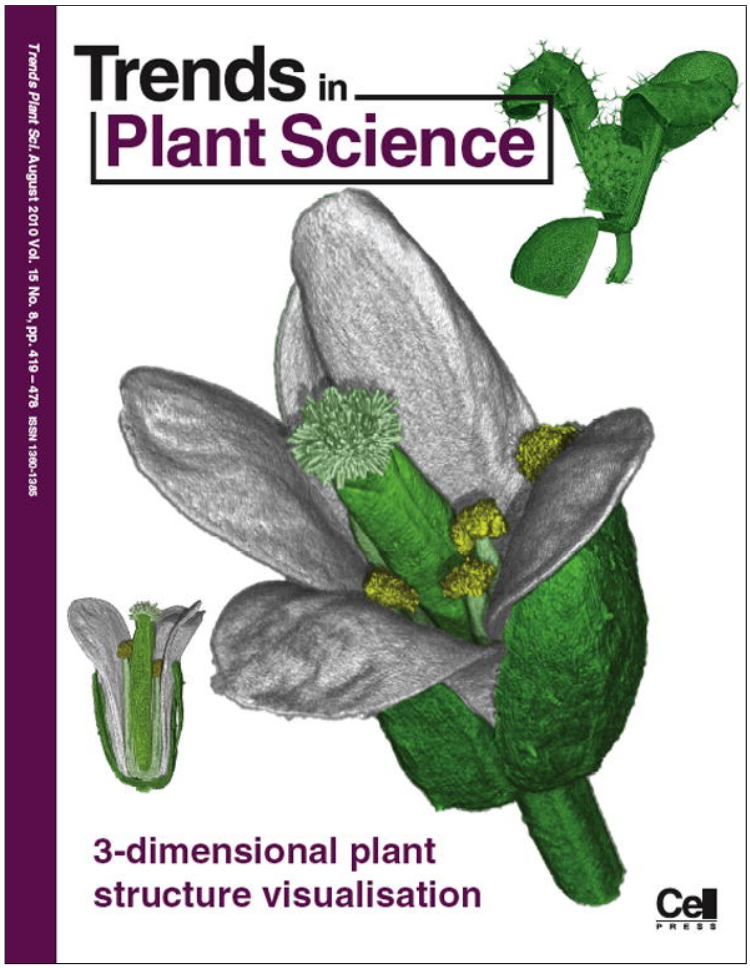

Opinion

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Plastidial retrograde signalling – a true "plastid factor" or just metabolite signatures?

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The genetic compartments of plant cells, nuclei, plastids and mitochondria exchange information by anterograde (nucleus-to-organelle) and retrograde (organelle-to-nucleus) signalling. These avenues of communication coordinate activities during the organelles' development and function. Despite extensive research retrograde signalling remains poorly understood. The proposed cytosolic signalling pathways and the putative organellar signalling molecules remain elusive, and a clear functional distinction from the signalling cascades of other cellular perception systems (i.e. photoreceptors or phytohormones) is difficult to obtain. Notwithstanding the stagnant progress, some basic assumptions about the process have remained virtually unchanged for many years, potentially obstructing the view on alternative routes for retrograde communication. Here, I critically assess the current models of retrograde signalling and discuss novel ideas and potential connections.

Necessity for signalling between the genetic compartments of plant cells

Plant cells possess three genetic compartments, with the nucleus carrying the largest part of genomic information on chromosomes and the plastids and mitochondria, in *Arabidopsis* encoding 128 [1] and 57 [2] genes, respectively, on highly polyploid organelle-own genomes. Plastids and mitochondria, however, contain several thousand different proteins, and thereby the great majority of their proteome is encoded in the nuclear genome and must be translated in and imported from the cytosol. Therefore, these organelles are regarded as genetically semi-autonomous because they depend on a permanent "forward" flow of information from the nucleus to the organelle, so-called anterograde signalling. By contrast, retrograde signalling can be best described as a "backward" flow of information from chloroplasts and mitochondria to the nucleus, transmitting information on the developmental or functional state of the organelles. Different stages of development, age or metabolic activities will be translated into the up- and downregulation of nuclear-encoded plastidial or mitochondrial proteins, which adapt organellar functions to the respective conditions. Therefore, organelles function as sensors for the individual stage of tissue and plant development as well as for environmental changes and initiate appropriate responses at the cellular level. This topic has attracted much interest among plant scientists over the years and accordingly has been extensively reviewed (see [3–38] for a comprehensive if not exhaustive list). Why then add one more paper? Because 30 years after its first description in plastids [39] the process of retrograde signalling is still poorly understood and it is time to think about novel approaches and ideas to study it. For details on the history and current knowledge of plastidial signalling I refer the interested reader to the reviews cited above. Instead, I will take a critical look at what we really know about this phenomenon.

Classes of plastidial signals

Plastidial signals are currently classified into five major groups depending on where the signals originate from: (i) plastidial gene expression (PGE) including transcription and translation; (ii) pigment biosynthesis i.e. intermediates of carotenoid and tetrapyrrole biosynthesis; (iii) reactive oxygen species (ROS) generation and ROS-related processes; (iv) redox processes in photosynthesis and (v) metabolite pool changes. However, this classification mainly represents the experimental systems and approaches under which the action of plastidial signals has been observed rather than describing the signal itself. So far no true signalling molecule leaving the plastid has been identified. It was long proposed that PGE provides a "plastid factor" that leaves the plastid and represses nuclear photosynthesis genes [40]. However, no experimental evidence for protein or RNA export from the plastid has been obtained. The chlorophyll precursor magnesium protoporphyrin IX (Mg Proto IX), which has long been regarded as the top candidate acting as a signalling molecule, can also be excluded from the list. Recent reports have clearly demonstrated that changes in its accumulation do not correlate with changes in *Lhcb* expression and, instead, it has been proposed that metabolite fluxes through synthesising enzyme complexes might generate the signal [41–43]. A critical re-evaluation of tetrapyrrole-mediated signalling has recently been performed [35,44]. Most ROS (maybe with the exception of H_2O_2) are very short-lived and, therefore, dissociate before they can cross the chloroplast envelope and serve as a direct signal. Furthermore, ROS are rather unspecific signalling molecules because many other stress-related processes such as pathogen defence or wounding responses also involve the

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action of ROS. Therefore, plastid-generated ROS initiate signalling cascades within the organelle, which then pass the envelope by unknown means [45]. Photosynthetic redox signals from the plastoquinone pool seem to be converted into a phosphorylation cascade [46,47] but no substrates have so far been identified. An earlier study implicated a protein dissociating from the plastidial envelope and binding the *Lhcb* promoter in response to such a signal [48]; however, this protein has never been identified. Finally, many metabolites have been suggested to pass the plastidial envelope via several more or less specialised transporters. However, the metabolites leaving the plastids play an essential and intrinsic part in the cell's metabolism [49] and it is difficult to assign a specific signalling role to a particular molecule. So, what data actually support the idea of retrograde signalling?

Basic assumptions about retrograde signalling

Plastidial signals can be disrupted genetically

Assumption 1: A signal and/or factor from the plastid repress nuclear genes when plastid development or function is impaired by chemical treatment or genetic defects.

Historically, this was the conclusion from the first observations that nuclear genes *Lhcb1* and *RbcS* were repressed when the plastids were dysfunctional after photo-oxidation [50,51]. Later, this observation was used to establish a successful screen for isolating mutants with genetic defects which disrupt the downregulation of the *Lhcb* promoter in the presence of norflurazon, a compound inducing strong photo-oxidation and subsequent plastid dysfunction [52]. In this screen, six so-called *genomes uncoupled* (*gun*) mutants were identified that displayed *Lhcb* gene expression despite their impaired plastids. *gun1* encodes a PPR protein implicated in plastid gene expression, whereas *gun2 to gun5* encode components of the tetrapyrrole biosynthesis pathway [30]. These mutants demonstrated for the first time that plastidial signals can be disrupted genetically, supporting the idea of a true signalling pathway. However, despite many years of research, including the identification of all proteins encoded by the *gun1 to gun5* genes, it is still not understood how retrograde signalling is disturbed in these mutants. A simple explanation would be that the genetic defects define conceptual negative elements, which impair the processes sending the repressive signal. Thus, *Lhcb* and *RbcS* expression is derepressed in these mutants [52] (Figure 1a). However, the *gun* mutant screen is based on a molecular phenotype [52] and other interpretations are also possible. From the physical point of view, organelles and nuclei can be regarded as combined sensor–emitter units within the cell that perceive and respond to environmental cues as well as intra- and intercellular signals. These units are connected by channels or a network of channels that allow the targeted transport of information between them. If inhibitor treatments or genetic defects lead to a block in the development and/or function of the plastids the plastidial sensor–emitter unit is destroyed and can no longer send. Thus, instead of sending a repressive signal it could be simply the inability of sending a positive signal, which in healthy plants normally results in the activation of the nuclear *Lhcb* and *RbcS* genes

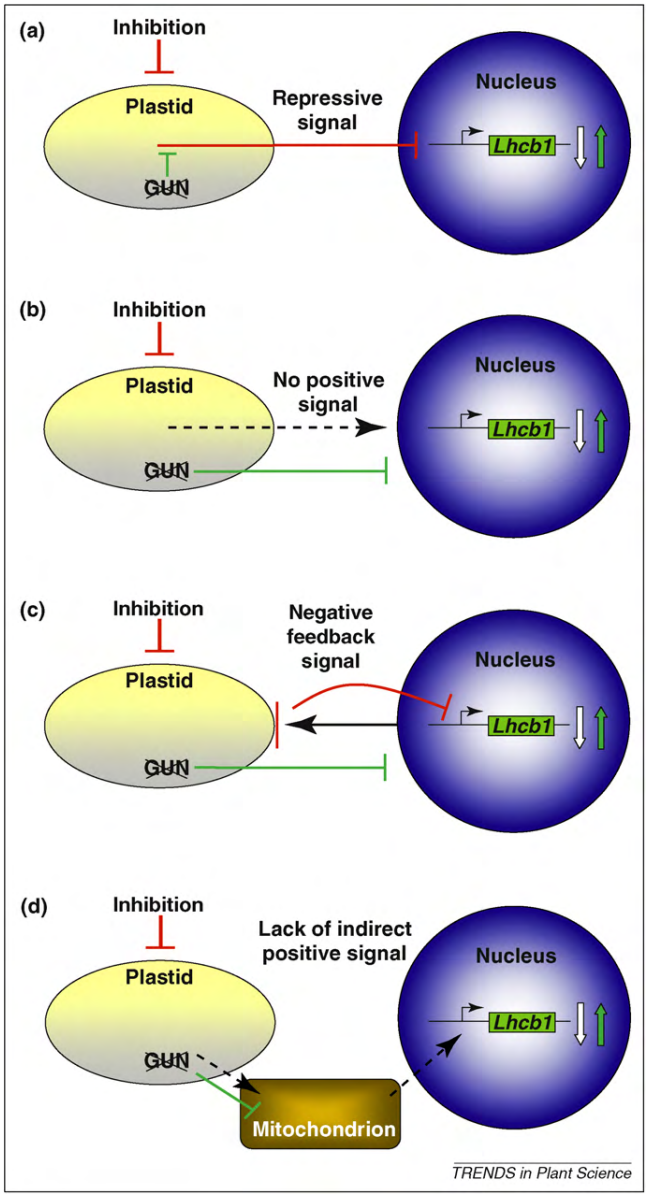

Figure 1. Potential alternatives to a repressive plastidial signal. Different models for communication between a dysfunctional plastid (yellow/grey oval) and a nucleus (blue circle) in a wild type cell are depicted. The models show how the genetic defects in *gun* mutants, which lead to the lack of GUN components (indicated by crossing out), would need to affect the respective situation to derepress nuclear gene expression. Note that this differs from typical genetic diagrams usually giving the function of the wild type products. The expression of the *Lhcb1* gene (green box) is indicated by a thin arrow, whereas a downward directed white arrow indicates low expression of the gene and a green upward directed arrow indicates high expression, which is caused by the *gun* mutations. Red lines represent repression, black arrows active, positive signals and green lines the repressive effects of the lack of the GUN component. **(a)** Depicts the classical model based on repressive signals from plastids if the organelle's proper development is inhibited. *gun* mutations repress the repressive signal, leading to the derepression of *Lhcb1* expression. **(b)** A model based on the lack of a positive signal (dotted arrow). *gun* mutations repress the sensing of the lacking positive signal. **(c)** A model based on feedback inhibition by the accumulation of *Lhcb* precursor proteins in the cytosol. *gun* mutations might interfere with the sensing mechanism for Lhcb protein accumulation. **(d)** A model based on the interaction of plastids with mitochondria (brown rectangle) and an indirect signalling to the nucleus via mitochondrial function. Here, *gun* mutations might induce the transition from heterotrophic to autotrophic metabolism in mitochondria.

(Figure 1b). Support for such a possibility comes from recent observations on *cab underexpressed* (*cue*) mutants [53]. Alternatively or concomitantly, the destroyed plastidial sensor–emitter unit is not able to perceive an anterograde signal (e.g. it cannot import Lhcb and RbcS precursor subunits), which might lead to an accumulation of these proteins in the cytosol, which then in turn causes a feedback inhibition of their own expression (Figure 1c). The principle of feedback inhibition by the accumulation of pathway intermediates is common in biosynthetic pathways (e.g. feedback inhibition of glutamyl-tRNA-reductase through the accumulation of decisive intermediates in tetrapyrrole biosynthesis such as haem or Mg Proto IX [54]) and thereby also represents a possible scenario in the context of retrograde signalling [29]. A recent study demonstrated that defects in plastid protein import repress the expression of nuclear-encoded photosynthesis genes [55], providing experimental support for this idea. In these alternative models, the *gun* mutations need to restore either the positive signal or the import capability to allow for normal *Lhcb* expression in the nucleus. However, it seems rather unlikely that the defects in GUN1 or tetrapyrrole biosynthesis components, which conceptually represent a negative or repressive signal, act here in a positive way and compensate for the adverse effects of plastid dysfunction. Thus, it is easier to assume that the *gun* mutations affect the nucleus in a way that impairs its sensing capacity for the functional state of the plastid. A fourth possibility involves the action of mitochondria (Figure 1d). During the first steps of seedling development the plant depends solely on storage compounds, and mitochondria are essential in providing the necessary ATP during this heterotrophic growth phase. In later stages of plant growth, this dependency still applies to the developing meristematic tissues that represent a metabolic sink. With the light-induced initiation of chloroplast development the seedling or young tissues change to an autotrophic lifestyle; however, this requires the establishment of metabolic interactions between plastids and mitochondria, which is disturbed if plastidial development is prevented. The shift from heterotrophic to autotrophic metabolism might create a mitochondrial signal that is sensed by the nucleus and indirectly communicates the developmental stage of the plastids. *gun* mutations might interfere with mitochondrial processes during the early heterotrophic stage, which usually results in a shift of the nuclear response towards autotrophic growth including a derepression of *Lhcb* expression. Finally, combinations of the scenarios given above cannot be ruled out (e.g. a combination of scenario (a) (Figure 1a) and scenario (b) (Figure 1b) in which both negative and positive signals act in parallel and the *gun* mutations affect the balance between them). Testing such alternative models will be an interesting field of future research.

A direct signalling pathway from the plastid to the nucleus

Assumption 2: For signal transduction a component must leave the plastid, activating a signalling cascade that passes the cytosol, enters the nucleus and finally affects gene expression.

This assumption proposes a direct signalling pathway from the plastid to the nucleus and several reports have identified protein components that might mediate the signal. However, all protein candidates found to be involved (EX1 and EX2 [56], SOLDAT10 [57], GUN1–5 [52] and STN7 [47]) reside within the plastid, whereas no protein has been identified as part of the assumed cytosolic part of the pathway. A few proteins have been identified that seem to act as general or pleiotropic regulators coordinating the nuclear responses to plastid signals, for instance ABI4 (ABSCISIC ACID INSENSITIVE4), PRL1 (Pleiotropic Response Locus 1) or GLK (GOLDEN2-LIKE) [58–60]. However, components acting further upstream of these regulators are unknown. This suggests that either (i) all screens performed so far have been unable to hit such cytosolic components or (ii) such components do not exist and the plastidial signals are fed into other existing signalling networks such as the interactions shown with ABI4 [58] and photoreceptor-mediated pathways [61]. Should the second conclusion be true, it still begs the question of how the information leaves the plastid. This scenario would also compromise the idea of a direct signalling pathway. Instead, one must assume that plastidial signals provide an input into the overall signalling network of the plant cell where they are integrated with other internal and/or external stimuli originating from the various perception systems, for instance from the photoreceptors, phytohormone receptors or plasma membrane-located receptors. Because a complex network is difficult to block by single mutations this would explain the failure to identify important cytosolic mediators of plastid signals. Thus, a detailed dissection of the network by multiple mutations will be necessary to uncover whether distinct routes for plastidial signals exist in the cytosol.

Multiple plastidial signals exist

Assumption 3: Multiple plastidial signals exist.

As mentioned above, the classification of identified plastidial signals mainly relies on the functional context in which its action is observed or concluded. However, when analysed in detail it becomes obvious that the different kinds of signals are dependent on each other, simply because the respective functional processes are connected. For instance, genetic analyses of the *gun* mutants suggest the existence of separate pathways for signals from tetrapyrrole biosynthesis and plastidial translation [62,63]. However, PGE is required to express the *trnE* gene. This particular tRNA is a precursor for aminolevulinic acid, which itself is the precursor for the tetrapyrrole biosynthesis pathway [46] linking PGE and tetrapyrrole-based signals. Another example for signal overlap is the norflurazon treatment that blocks phytoene desaturase, an important enzyme in carotenoid biosynthesis, which finally leads to the oxidative destruction of the plastid [64]. This interferes, on the one hand, with ROS-mediated signalling and, on the other hand, with the synthesis of carotenoid precursors for abscisic acid (ABA), an important hormone in stress signalling that might well be involved in plastidial signalling [29]. Furthermore, common experimental approaches to studying signals from functional chloroplasts often include treatments with different light

intensities. These tend to create overlaps between photosynthetic redox signalling, ROS signalling and metabolite pool changes that exacerbate conclusive interpretation of results [65]. Thus, many experimental setups result in the generation of several parallel plastidial signals, making it difficult to conclude on clearly defined causal relationships. Although the signal classification is useful for our understanding, it is important to be aware of these interdependencies between the different classes of signals.

Plastidial signals and light signals cannot be separated

Assumption 4: Plastidial signals and light signals cannot be separated.

Many previous studies have used *Lhcb1* and *RbcS* as reporter genes for plastidial signalling. However, these genes are also influenced by photoreceptor signalling, circadian rhythms and hormones; consequently, light and plastidial signals are difficult to distinguish at the level of promoter element usage [61,66–68]. One explanation for this is that photosynthesis genes possess a highly complex arrangement of light-responsive elements (LREs) in their promoters that exhibit strong gene-specific and species-specific diversity [69]. In general, photosynthesis genes (often summarised under the term "photosynthesis-associated nuclear genes" [PhANGs] [69]) are regarded as the primary targets of plastidial signals. However, many studies referring to PhANGs in fact only investigate the expression patterns of *Lhcb1* and/or *RbcS*. Because of the diversity in the LREs of PhANGs it is questionable if these two genes really reflect the expression characteristics of the entire group. Furthermore, it has been shown that not only PhANGs but also metabolism genes are controlled by plastidial signals [70,71]. Conversely, macroarray studies have revealed that PhANGs represent a very special regulon that under many conditions display distinct and differential expression patterns compared with other gene groups [72]. More differential approaches with a variety of additional reporter genes are therefore necessary to reveal the interactions or distinctions between light and plastidial signals.

Classification of plastidial signals based on the developmental stage of plastids

The previous sections demonstrate that the current classification of plastidial signals can be imprecise, leading to ambiguities in interpreting which signal is really active. An alternative classification of plastidial signals could be based on the developmental stage of the plastids and the cell in which they reside [31]. The authors of [31] referred to this as 'biogenic control' in early plastidial development and 'operational control' in mature plastids. Biogenic control covers all signals described to be connected to PGE and pigment biosynthesis. These signals inform the nucleus about the progress of the developmental programme in the plastid. Operational control occurs in fully functional chloroplasts that perform photosynthesis and many essential biosynthetic pathways. Environmental changes affect the operation of these processes and generate signals from photosynthetic electron transport, ROS accumulation, glutathione biosynthesis and others, which are then transduced to the nucleus with the aim to adapt nuclear gene expression to the changed functions and demands of the plastid. In addition, one could propose a third stage named "degradational control", which covers signals generated by chloroplasts in old tissues or at the end of the vegetative phase. Here, the plastids initiate processes that lead to senescence during which valuable resources such as nitrogen are collected and reallocated to other parts of the plant e.g. the developing seeds. The dominant process is the degradation of Rubisco as the major source of reduced nitrogen. This is exported from the plastids by vesicles and then takes different routes for further degradation, thereby providing a potential means for retrograde signalling [73,74]. This development-dependent classification provides the advantage that the defining parameter is not the experimental approach, but the biological process affected. This can be best explained using biogenic control as an example. Different inhibitors have been used to stop plastidial development and "induce" a plastidial signal, but most were active only when applied in early stages of plastidial development. This suggests that the inhibitors induce the plastidial signal via the same principle. In variegation mutants, the decision if a plastid becomes white or green is also taken very early in development, whereas the variegation pattern of the leaves develops during the growth of the leaf blade depending on the arranging of the plastids during cell division [75,76]. After this decision is made a white plastid will never later turn into a green one and its progeny will also only be white, which suggests that a decisive checkpoint during cell development that cannot be regained has been missed. This irreversibility points to an incompatibility between the plastidial developmental stage and the cell developmental programme (Figure 2). A likely explanation is that the internal cell programme has a fixed time schedule that cannot be stopped or slowed down because it is a fixed cascade. If plastidial development is retarded, blocked or partially inhibited by mutations or inhibitors it will fall behind the developmental programme of the cell. As a consequence of this de-synchronisation plastidial development becomes incompatible from the (protein) products delivered. This could be either because an essential developmental step in the plastids for its acceptance has not (yet) been performed or a specific essential component has no longer been produced. This happens if the required protein is expressed in the nucleus only at an early and very brief stage, which is defined by the developmental programme of the plant cell. This might involve the action of mitochondria as discussed above.

A consequence of this view is that we can clearly separate the experimental setups into those that study plastidial signals in development (by blocking plastid biogenesis via chemical treatments or mutations in seedlings) and those investigating plastidial signals in environmental acclimation (by the manipulation of the physiology of adult plants). Because during the course of evolution plastid development has been embedded in the process of photomorphogenesis, this explains the close connection with photoreceptor-controlled networks. By contrast, mature chloroplasts function as environmental sensors, which together with mitochondria control stress responses and environmental acclimation in a largely photoreceptor-independent manner.

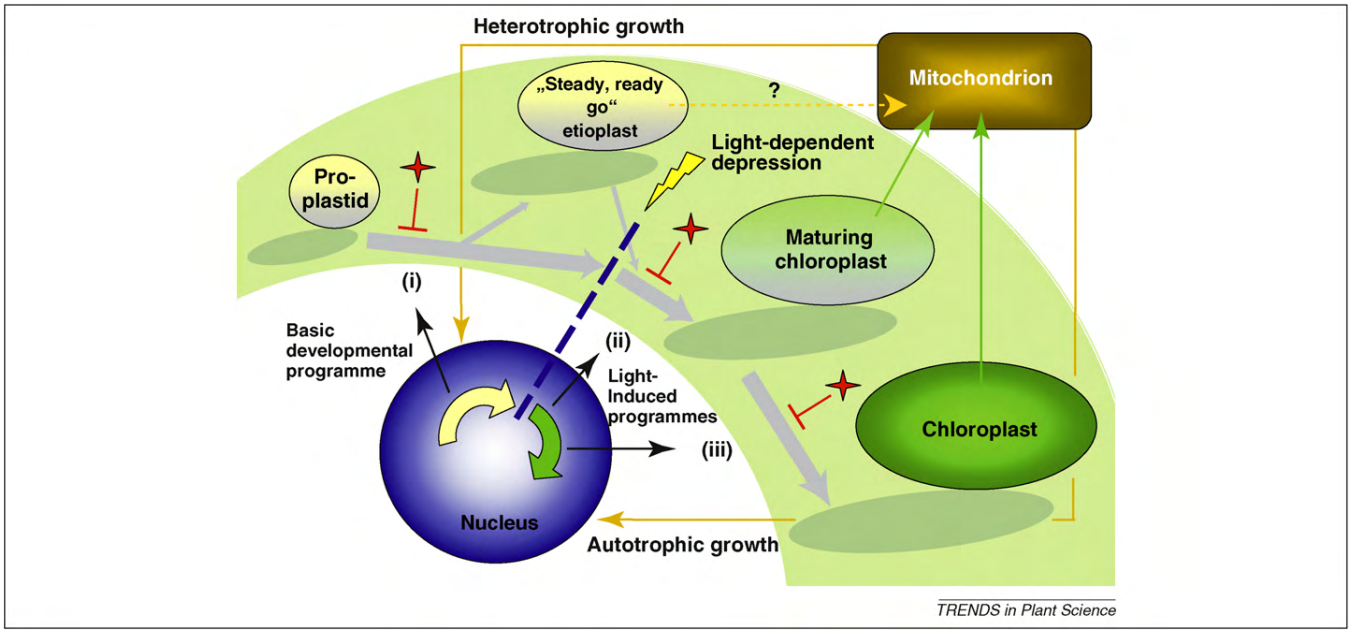

Figure 2. Decisive checkpoints in light-dependent plastid maturation. The model describes the progress in plastid structure and function during seedling photomorphogenesis, explaining the susceptibility to translation inhibitors or norflurazon. In later stages of plant development, these processes are likely to be limited to the undifferentiated, non-green cells of meristems. Red lines represent repression and black arrows active, positive signals. Chloroplasts develop from undifferentiated precursor organelles termed the proplastids. The developmental programme of chloroplasts (depicted as grey shadows on a green background) is integrated and synchronised with the basic developmental programme of the cell, which is controlled by a fixed cascade of gene expression events in the nucleus (curved arrows). This programme delivers regulatory factors and components for the photosynthetic function to the plastids in a timely, adjusted manner. At a certain time point these programmes require light. If this is not supplied, both plastid and cellular development do not exceed this checkpoint (blue barrier), and proplastids develop into etioplasts instead of chloroplasts. Recent proteomic analyses have shown that etioplasts (and especially their prolamellar bodies) accumulate many more different components of the photosynthetic apparatus than anticipated, indicating a waiting position until the photomorphogenic programme is initiated (defined as ''steady, ready, go'') [104,105]. As soon as a light signal (yellow flash) is perceived, the programmes proceed toward fully developed chloroplasts. During the course of development, plastids pass further checkpoints such as the assembly of the additional subunits of the plastidial-encoded RNA polymerase, which must be finished before further steps of maturation can be performed (transition from step 2 to step 3). The inhibition of plastid development at any one of these programme steps (red stars) either by genetic lesions or inhibitor treatments results in the inhibition of further plastid development and de-synchronisation from nuclear developmental programmes, giving rise to pale or white phenotypes [106]. In parallel, the functional interaction between chloroplasts and mitochondria (green arrows) has to be established. It is unclear if metabolic interactions between etioplasts and mitochondria (orange dotted arrow) exist. Mitochondrial communication to the nucleus is defined by the metabolic activities of this organelle during seedling development.

The involvement of mitochondria

The integration of mitochondria into our current experimental approaches is urgently required because plastids and mitochondria are biochemically connected by manifold metabolic pathways and cannot be regarded as isolated compartments [77,78] (Figure 3). Although this concept has already been implemented in several earlier models [6,24,28,30], experimental data about retrograde signals from mitochondria in plants are still rare [79]. Much more is known about such signals in yeast and mammals [80]; however, this knowledge is only of limited use because plant mitochondria possess several unique features that are absent from heterotrophic organisms. For instance, plant mitochondria possess a glycine decarboxylase complex that is involved in photorespiration. In addition, non-phosphorylating pathways exist in the respiratory chain [including the cyanide-resistant alternative oxidase (AOX)], which help to dissipate excess reductants from chloroplasts generated under high light [77,78]. These features probably evolved because of the multiple interactions with the plastids, giving rise to a high metabolic flexibility of plant mitochondria. Because of these differences, mitochondrial retrograde signalling in plants can also be expected to work differently. In an *Arabidopsis* cell culture, it has been demonstrated that during sugar starvation the expression of the mitochondrial and nuclear genomes are strongly coordinated [81]. Even though whether such a system reflects an *in vivo* situation is doubtful; however, it clearly demonstrates the possibility of such coordination. Recent mutant analyses have demonstrated that the downregulation of plastidial and mitochondrial translation downregulated PhANG expression in a synergistic manner [82], demonstrating an interaction of both organelles at the level of gene expression. The best-known target for mitochondrial retrograde signals is the gene for AOX, which is upregulated under chemical or physiological conditions that limit mitochondrial electron transport [79]. It has been used as a reporter to identify mutants defective in retrograde signalling [83], but so far no signalling components have been isolated. However, a recent study could demonstrate that the transcription factor ABI4 is involved in the regulation of AOX1a, providing an interesting link to plastidial signals [84]. This suggests that mitochondrial and plastidial signalling might converge under some circumstances, generating a common organellar signal. Clearly more experimental work is needed to uncover how close the interactions of the organelles are in this context.

Do novel observations suggest alternative pathways?

Some recent observations seem to open up further novel avenues for the nature of possible plastidial signals. Again

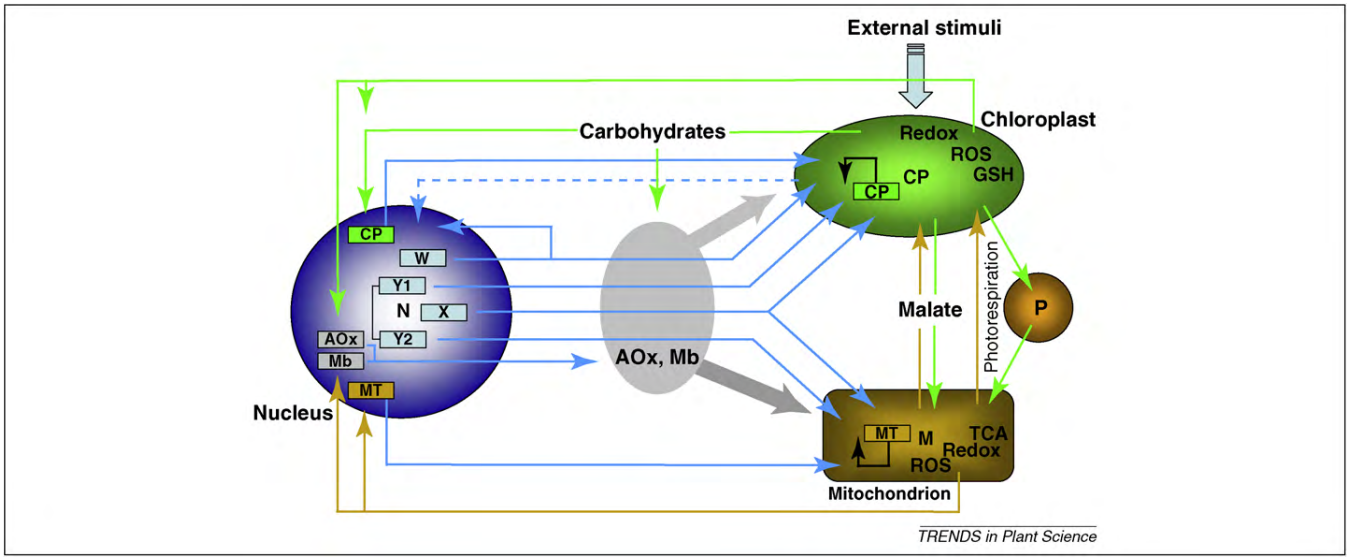

Figure 3. The interaction of the three genetic compartments in cellular communication. The scheme depicts operational control in a fully developed plant cell. The arrows represent the flow of information. The nucleus expresses genes (boxes) whose products are transported to plastids and mitochondria (blue arrows) where they are assembled with the organelle-encoded gene products. Nuclear delivery involves the dual targeting of single products (X) and the parallel targeting of orthologues (Y1, Y2) as well as organelle-specific targeting (CP, MT). Dual targeting to chloroplast and nucleus is also given (W), including a potential retargeting of W to the nucleus after plastid destruction (dotted blue arrow). Components of AOX and the metabolism (Mb) are delivered to the cytosol where they are functioning (grey oval), whereas the chloroplasts (green arrows) and mitochondria (brown arrows) provide metabolites that affect nuclear gene expression and/or are fed into cytosolic syntheses. Plastidial functions are dominated by external stimuli from the environment (especially light), whereas mitochondrial functions are dominated by internal stimuli mainly from metabolism or diverse stresses (dark grey arrow). Operational organellar signals toward the nucleus are indicated (ROS: reactive oxygen species, redox: disturbances in electron transport chains, GSH: glutathione, TCA: tricarboxylic acid cycle). Furthermore, the functions of chloroplasts and mitochondria are tightly connected, for instance, by the malate/oxaloacetate shuttle (the ''malate valve'' [107]) for the transfer of the reducing power or photorespiration, which also involves the action of peroxisomes. The functional balance of the two organelles affects the metabolites transported to the cytosol and nucleus, providing a means for ''organellar signalling''. The scheme is designed to indicate the multiplicity of relationships rather than being complete; thus, for clarity several metabolic interactions are omitted.

the tetrapyrrole biosynthesis pathway seems to be involved. First, the Chl H subunit of the Mg chelatase in *Arabidopsis* (*A. thaliana*) has been reported to be able to bind ABA, which would provide a link between tetrapyrrole and carotenoid biosynthesis [85,86]. However, this observation has been challenged by a comparable study in barley (*Hordeum vulgare*) in which no interaction between Chl H and ABA was found [87]. A putative function of the Chl H subunit as a plastidial ABA receptor is currently under debate [88] but seems unlikely because a more convincing ABA receptor has been identified [89,90]. Nevertheless, the ability to bind ABA could be a side effect of the Mg Proto IX binding properties of the protein and would still provide a molecular link between the Chl H subunit and ABA.

Another report has demonstrated that haem, an intermediate of the tetrapyrrole biosynthesis branch leading to phytochromobilin, affects the nuclear gene expression of HSP70 and enzymes of haem production in *Chlamydomonas reinhardtii* [91]. In the green alga, haem is exclusively produced in the chloroplast [92] and is thereby a component that leaves the plastid to move to all compartments that require haem. Thus, it fulfils one major requirement for the elusive plastidial signal. Whether this movement also applies to higher plants has to be tested because it is still a matter of debate whether haem is synthesised exclusively in plastids or not [93].

Another recent study described Mg Proto IX as a cell cycle coordinator in the primitive red alga *Cyanidioschyzon merolae,* which harmonises DNA replication and gene expression in the nucleus and plastid [94]. This coordination process is barely understood and the proposed role of Mg Proto IX as a mediator would fit with the assumptions made in Figures 1 and 2. If this role of Mg Proto IX can be confirmed by other studies in green algae and higher plants, it would provide an interesting mechanism for the *gun* mutants to control the nucleus as proposed for the models in Figures 1b and c. However, the observations in *C. merolae* are based on feeding experiments, which bear the risk that the cells of the red alga react in an unpredicted way because the "signal" originates from the outside and not the inside of the cell. Thus, further experimental evidence under *in vivo* conditions for this potential pathway is required.

Ca^{2+} is an important secondary messenger in many plant signalling networks. Recently, a Ca^{2+} sensing receptor (CAS) protein was identified that is localised to the chloroplast thylakoid membrane in *Arabidopsis*. CAS knockout lines impaired cytosolic Ca^{2+} transients, stomatal responses and plant growth and development, indicating that this protein has a strong impact on Ca^{2+}-dependent processes outside of the chloroplast [95–97]. A Ca^{2+}-based signal from the chloroplast would be an interesting novel player in the models of organellar signals. Intriguingly, the CAS protein has been reported to be a phosphorylation target of the thylakoid kinase STN8, providing a potential functional link between photosynthetic redox regulation and Ca^{2+} signalling. Further experiments will show if and how such a connection could work.

A further novel idea has emerged from studies on the transcription factor Whirly1 (Why1), a protein originally described as a telomere-binding protein in the nucleus [98,99]. Recent studies have shown that this protein is also imported into plastids by dual targeting [100,101]. It

has been hypothesised that Why1 is set free into the cytosol if plastids are damaged by stress or senescence, providing a means to transport a stress signal directly to the nucleus without further elements, a mechanism which would solve all problems of transfer and transport. The idea is supported by the finding that dual targeting to nuclei and plastids might apply to several proteins [102]; however, more work is required to confirm this hypothesis.

Future prospects

For future advances we need additional novel concepts similar to those mentioned above to investigate retrograde signalling and its underlying mechanisms and functions. We should also not be afraid to scrutinise even the most basic assumptions of the whole process.

First, further progress requires the identification of novel target genes [35,65]. *Lhcb* and *RbcS* were good candidates for initial studies because they are highly expressed and thereby easy to detect. However, for more detailed studies these genes are unsuitable because their expression depends on too many different signalling pathways. Therefore, to analyse various types of plastidial signals it is mandatory to identify novel genes that can serve as distinct reporters without any or at least less crosstalk between different retrograde signals. To date it is unclear whether such genes exist. Therefore, more specific physiological analyses combined with array technologies are required to help obtain a complete catalogue of nuclear genes that are controlled or influenced by plastidial signals. Most important here is to define "true" primary target genes. Kinetic analyses have revealed that, for instance, plastidial redox signals affect nuclear gene expression in a fast and dynamic manner [71]. The identity of genes affected shortly after the induction of the plastidial signal varies largely from those at the end of the response. Therefore, it can be assumed that many genes reported to be affected by plastidial signals might only represent tertiary targets regulated very indirectly. Therefore, the identification of primary target genes requires inducible systems that allow us to set a signal at a given time point (either by physiological and genetic or molecular means) and to follow the effect on nuclear gene expression [35,65,103]. This will help distinguish between primary effects initiated from mitochondria and plastids and the more pleiotropic effects described in end point analyses of mutants grown under stable conditions. This also includes effects on genes that do not encode components of the organelle itself. Should such genes be identified they might provide novel tools to build up screens for *trans*-acting regulators, which in a second step might provide insights into the underlying regulatory network by using molecular interaction techniques.

We should also ask whether the term retrograde signalling always applies to the different observed processes. During biogenic control nuclear activities clearly dominate and regulate plastidial development and the organelle responds to it. Under operational control, however, plastids sense environmental changes, report them to the nucleus and initiate nuclear responses. This represents a reversal of the signalling direction defined in the introduction. Thus "forward" and "backward" are relative descriptions and despite the general acceptance of the term "retrograde" the initial terminology "plastidial signalling" would be more correct. Furthermore, if we want to also include mitochondria it would be best to talk about "organellar signalling" because this provides the possibility to expand the term to comparable signalling events in heterotrophic eukaryotes.

Finally, let us also ask a heretical question. The basic idea of plastidial signalling assumes that "something" leaves the plastid, which then informs the nucleus about developmental status or function. It is not yet clear if this something is a protein or a signal of a different nature. All genetically and physiologically identified protein components involved in plastidial signalling are located within the plastid and no single cytosolic component has been identified so far. Do we really need a signal that leaves the plastid to explain the coupling of nuclear gene expression to plastidial function? What if this "factor" does not exist and we are just chasing a ghost? Plastids and mitochondria are involved in most biosynthetic pathways of the cell and specific carriers transport many different metabolites into and out of them (Figure 3). Maybe a single metabolite is not sufficient to work as a signal, but what about a metabolite signature? Changes in organellar function are known to result in corresponding changes of at least some metabolite pool sizes, which define distinct metabolic states or metabolite signatures [71]. Variations in the functional balance of plastids and mitochondria thereby create differences between these signatures, which could represent a candidate for an "organellar signal" that does not require any further components than those already known. Future research will show whether one or several of the models discussed above holds true.

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