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Review

Insights into chloroplast biogenesis and development☆

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ABSTRACT

In recent years many advances have been made to obtain insight into chloroplast biogenesis and development. In plants several plastids types exist such as the proplastid (which is the progenitor of all plastids), leucoplasts (group of colourless plastids important for storage including elaioplasts (lipids), amyloplasts (starch) or proteinoplasts (proteins)), chromoplasts (yellow to orange-coloured due to carotenoids, in flowers or in old leaves as gerontoplasts), and the green chloroplasts. Chloroplasts are indispensable for plant development; not only by performing photosynthesis and thus rendering the plant photoautotrophic, but also for biochemical processes (which in some instances can also take place in other plastids types), such as the synthesis of pigments, lipids, and plant hormones and sensing environmental stimuli. Although we understand many aspects of these processes there are gaps in our understanding of the establishment of functional chloroplasts and their regulation. Why is that so? Even though chloroplast function is comparable in all plants and most of the algae, ferns and moss, detailed analyses have revealed many differences, specifically with respect to its biogenesis. As an update to our prior review on the genetic analysis of chloroplast biogenesis and development [1] herein we will focus on recent advances in Angiosperms (monocotyledonous and dicotyledonous plants) that provide novel insights and highlight the challenges and prospects for unravelling the regulation of chloroplast biogenesis specifically during the establishment of the young plants. This article is part of a Special Issue entitled: Chloroplast Biogenesis.

1. Molecular mechanisms of chloroplast biogenesis

The process of chloroplast biogenesis is highly complex and the molecular intricacies have not been fully characterised. The complexity of this process is not surprising in light of its ancestry, having originated through endosymbiosis with species of cyanobacteria [2]. Thus, the chloroplast itself can be considered a separate, but dependent, entity within the plant cell, including its own separate genome, which introduces the challenge of coordination between genomes. Indeed, a lot of signalling occurs between the nucleus and chloroplast to relay information between the two genomes in order to guide proper formation and assembly of the molecules required to properly form a functional and photosynthetically active chloroplast [3]. This intricate signalling—or what is known so far—will be discussed below.

Generally, the chloroplast develops from undeveloped proplastids, which contain vesicles but no differentiated structures. During this differentiation thylakoids are formed and stacked into defined grana. The thylakoids are the internal lipid membranes interlaced with protein complexes, which provide the platform for the light reactions of photosynthesis and thus could be considered as one of the most important structures in the chloroplast [4]. Indeed, the thylakoid itself is an intriguing and complicated structure, and the mechanism for its formation is not fully characterised, but several hypotheses are presented in a recent review [5]. Under specific circumstances, the dark-intermediate etioplast develops from the proplastid, which is defined by the prominent prolamelar body (PLB); a lattice-like membranous structure and a few metabolites and proteins required for photosynthesis. From this lattice-like structure prothylakoids emanate into the plastid stroma and the PLB disassembles and reforms into thylakoids upon exposure to light (Fig. 1). In some cases, chloroplasts can also develop from other plastids such as chromoplasts.

It is more and more evident that chloroplast biogenesis and development differ between dicotyledonous and monocotyledonous plants.

In monocotyledonous plants the process of chloroplast development from the proplastid to functional chloroplasts can be observed as a gradient along the leaf blade (Fig. 2). Interestingly, despite this gradient of differentiation, genetic studies have revealed differential regulation of chloroplast biogenesis at the adaxial and abaxial side, respectively between the midrib of the leaf and the rest of the leaf blade. In a rice (*Oryza sativa*) mutant *chr4*, containing a lesion in a chromatin-remodelling factor, only the adaxial side of the leaves demonstrates an albino phenotype due to a selective down-regulation of chloroplast-development genes in cells of the adaxial side [6]. On the other hand, the rice *albino midrib1* (*am1*) mutation of a chloroplast potassium efflux transporter, results in young leaves displaying a variegated leaf phenotype and the older leaves have a pale phenotype restricted to the midrib of the leaf blade [7]. In maize leaves C4 photosynthesis results in

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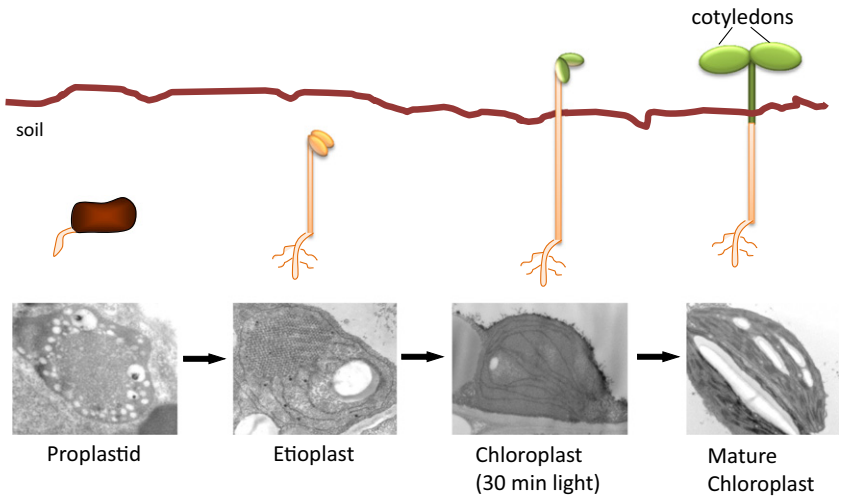

Fig. 1. Chloroplast biogenesis and development in dicotyledonous seedlings alongside germination. Here illustrating epigaeic seedlings.

differences in chloroplast biogenesis, since the mesophyll cells function in CO_2 capture and bundle sheath cells function in CO_2 reduction: plasmodesmata facilitate the exchange the metabolites between such cells [8].

In dicotyledonous plants the development of chloroplasts differs between the developmental stage of the plant, different plant organs, and the plant tissue. It is not as in monocotyledonous plants, where there is an observable gradient along the leaf blade, but is restricted to a short time frame, as will be discussed later. Additionally, in dicots, the proplastid develops into a dark-adapted intermediate, the etioplast, prior to the formation of the mature chloroplast (Fig. 1) [6]. The formation of the chloroplast is light-dependent, timing alongside the emergence of the seedling from the soil, and requires many different processes, such as the biosynthesis and import of proteins, as well as the import of lipids and metabolites, required for the formation of the thylakoids and the biosynthesis of pigments such as chlorophyll and carotenoids [1,9,10]. Genetic studies revealed that chloroplast development differs between the cotyledons and the true leaves. This was shown in many mutant lines exhibiting phenotypes restricted to one organ only, such as chlorotic true leaves but green cotyledons as observed in the *variegated* (*var*) and *immutans* (*im*) mutants [11]. Conversely, other mutant lines demonstrate a bleached or chlorotic phenotype restricted to the cotyledons as described e.g. for the *snowy cotyledon* (*sco*) mutants [12–15] and many other mutant lines which will be discussed in more detail below.

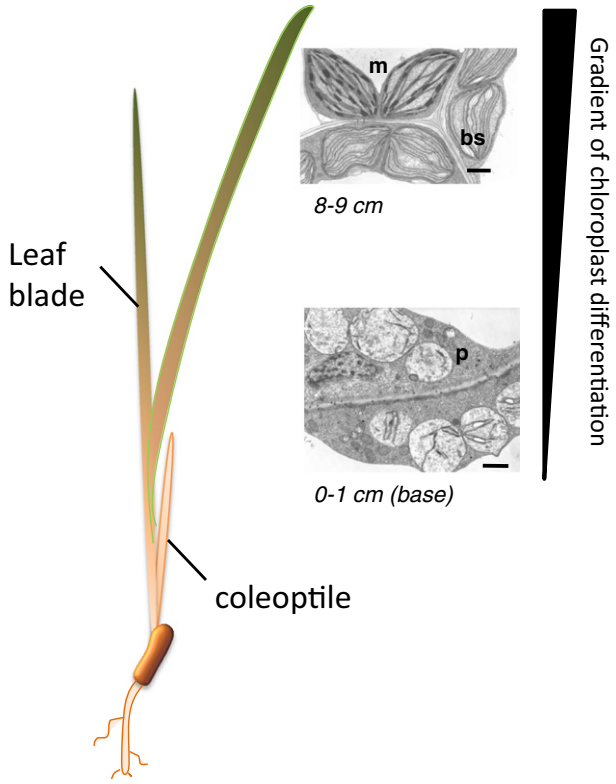

Fig. 2. Chloroplast biogenesis and development in monocotyledonous seedlings. Example shown for a maize plant. P: proplastid; m: mesophyll cell; bs: bundle sheath cell (TEM pictures generously provided by Klaas van Wijk).

Proplastids in the cotyledons are present in all cells and chloroplasts develop immediately at the time of illumination via the process of photomorphogenesis (light-mediated development), which is regulated by a sophisticated network of photoreceptors, among which the phytochromes are considered the most important regulators, and plant hormones, such as brassinosteroids [16,17]. This process is discussed further below. In contrast to the cotyledons, chloroplast development in true leaves primarily occurs at the shoot apical meristem (SAM) and primordia of the leaves, and subsequent multiplication is by chloroplast division as opposed to de novo assembly.

A recent detailed study on chloroplast development in the SAM used chloroplast structure and the presence of essential photosynthetic proteins within the chloroplasts to analyse the different cell layers of the SAM, namely L1, L2 and L3 and the leaf primordia (LP), to unravel the differentiation process of the chloroplasts within these cells [18]. The authors observed in the L1 layer, LP and for most of the L3 layer that the plastids contain a developed thylakoid network as well as photosynthetically active proteins (Fig. 3). Contrastingly, in the central zone of the L2 layer, only proplastid-like structures could be observed with no photosynthetic activity. As the epidermis of true leaves develops from the L1 layer, one has to assume that these thylakoids are being degraded during the development of the true leaves as most of the plastids within the epidermis cell are not photosynthetically active [18]; however, this assumption requires testing. In the last decade a lot of research has focused on signalling between the layers of the SAM, focusing on proteins such as WUSCHEL (WUS) and related proteins [19]. But how the signalling or related regulation involved in defining chloroplast development between the layers of the SAM still requires detailed future research.

As described above for monocotyledonous plants with a chloroplast-deficient phenotype restricted to the midrib, a similar phenotype was observed in *Arabidopsis thaliana* (Heyn.), *cue1 (CAB underexpressed 1)*, affected in the phosphoenolpyruvate/phosphate transporter resulting in

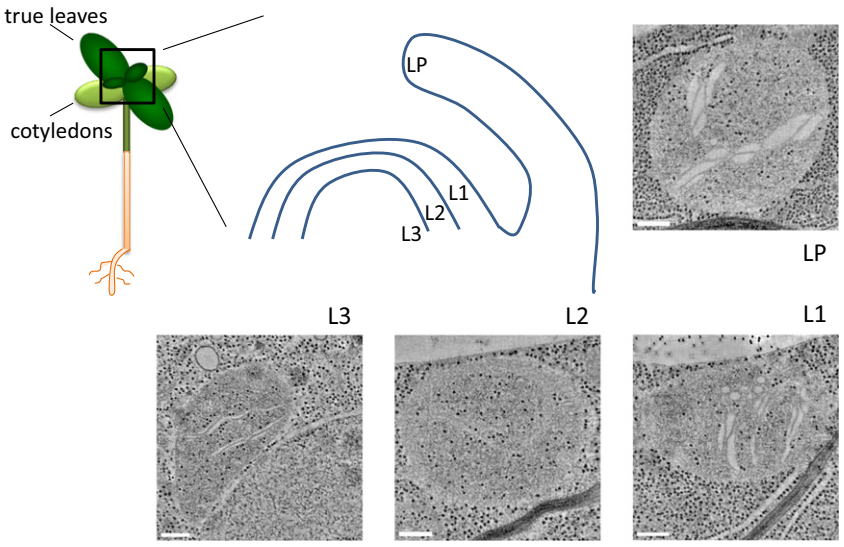

Fig. 3. Chloroplast development in the shoot apical meristem. L1: layer 1, L2: layer 2, L3: layer 3, LP: leaf primordia. Scale bar indicates 200 nm (tomographic pictures of 15 nm thick slices generously provided by Ziv Reich).

dark green vasculature but pale mesophyll cells [20]. In fact, such phenotypes have been observed mostly with mutants either affected in metabolite transport or in signalling pathways and were reviewed in detail [21].

When considering dicotyledonous chloroplast development, it has to be taken into account that seedling development differs between species. That is, there are epigaeic seedlings, with their cotyledons emerging from the soil and undergoing photomorphogenesis, and hypogaeic seedlings where the cotyledons do not emerge from the seed and the greening process only occurs in true leaves. To the latter belong pea (*Pisum sativum L.*) and bean (*Phaseolus vulgaris L.*). Yet even within these two hypogaeic species there are differences in chloroplast development with respect to the timing and staging of chloroplast protein accumulation [10]. In bean the first proteins to detect after 6 h of illumination are from the photosystem II (PS II), such as Lhcb2 (Light harvesting chlorophyll a/b binding), whereas pea first assembles PS I, as Lhca2 and PsaA are already present in etiolated seedlings and one of the first proteins detected after 2 h of illumination is Lhca3 [10]. The differential sequence of protein synthesis together with variances in chloroplast development in different tissues highlights the complexity that researchers face when trying to decipher the regulation of chloroplast development.

2. Regulation of chloroplast development

Across tissues and species there are commonalities in the regulation of chloroplast biogenesis. Firstly, for protein transcription, translation, import and turnover, coordination between the nucleus and chloroplasts is indispensable (Fig. 4). This facilitates stoichiometric assembly of nuclear-encoded and plastidic-encoded proteins with chlorophylls and carotenoids; which is essential to limit oxidative damage caused by free photoreactive pigments and to ensure optimal rates for protein synthesis. Secondly, metabolite import and metabolite synthesis within chloroplasts are required for processes such as the formation of thylakoids. Thirdly, signalling is required for this level of coordination. This

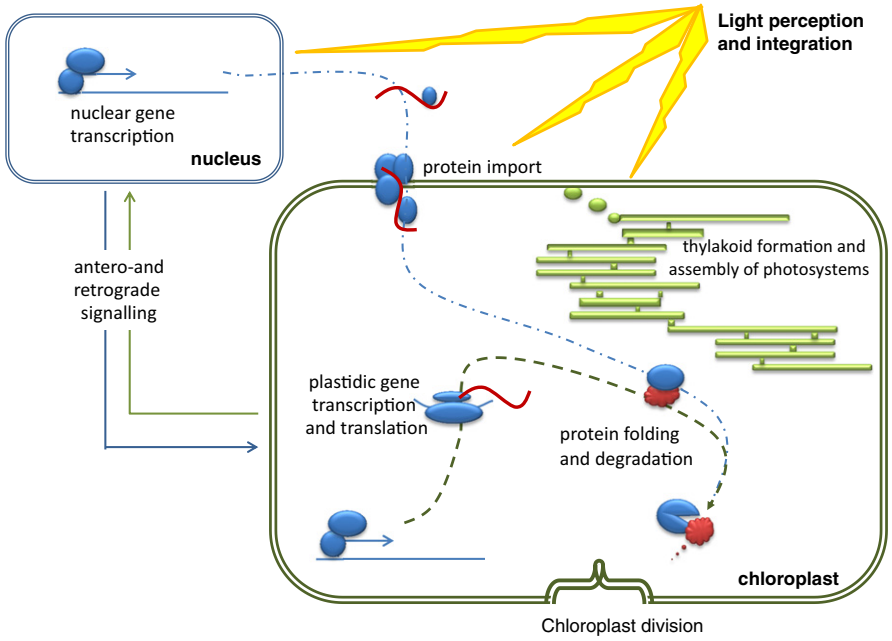

Fig. 4. Communication and transport between the etioplast/chloroplast and nucleus is crucial for proper chloroplast development.

includes inter-organelle communication between the nucleus and chloroplast for coordination of protein synthesis and between chloroplasts, peroxisomes and mitochondria for metabolite exchange. Intercellular communication between mesophyll and bundle sheaths cells is also crucial for proper chloroplast development and function.

As mentioned above, signalling between plastids and the nucleus is crucial for proper development but so, too, is the transport of various molecules, including metabolites and proteins, to the chloroplast. Previous estimates suggest that approximately 3000 nuclear-encoded proteins are localised to the chloroplast [22]. Fully-assembled protein complexes are not transported to the chloroplast. Rather they are synthesised by cytoplasmic ribosomes as preproteins, containing an amino-terminal cleavable targeting signal that direct their import to chloroplasts through interaction with chloroplast membrane complexes, such as the TOC and TIC complexes (translocon at the outer/inner envelope membrane of chloroplasts) [23,24]. The importance of protein import into chloroplasts will be discussed further below.

Factors influencing chloroplast development can be divided into those affecting the biogenic process, those involved in the perception and regulation of environmental and temporal factors and the interaction between organ development and plastid differentiation.

3. Biogenic and environmental control

Upon illumination one third of the nuclear transcriptome changes [25], including many transcripts encoding chloroplast-targeted proteins. The perception of light requires the activation of the phytochrome photoreceptors, such as PhyA and PhyB. Upon perception of light, these Phy proteins undergo conformational changes from an inactive to active form, upon which they are transferred into the nucleus where they regulate the activity of transcription factors that regulate the transition to light-mediated growth (photomorphogenesis) [26,27]. For example, one group of transcription factors, the Phytochrome Interacting Factors (PIFs), are regulated by activated Phy proteins and play an important role during seedling establishment and photomorphogenesis by regulating important genes that encode proteins for gibberellic acid biosynthesis and signalling or chlorophyll biosynthesis [28].

Many transcription factors are also regulated by plant hormones. One of these are the DELLA transcription factors involved in the response to the plant hormone gibberelic acid (GA). Characterisation of DELLA mutants uncovered the complex regulation of chloroplast development as it has been shown that GA prevents the greening of seedlings in the dark. GA-regulated DELLA proteins are involved in regulating the levels of the POR protein (protochlorophyllide oxidoreductase) as well as the pigments protochlorophyllide and carotenoids during deetiolation [29–31]. Besides controlling chloroplast biogenesis GA is also involved in influencing chloroplast division and the grana stacking of the thylakoids, a process discussed to be linked to optimise photosynthetic efficiency in plants [30].

Brassinosteroids (BR) also impact chloroplast development as inhibition of BR increased accumulation of chlorophyll. The *bpg3-1D* (*Brz-insensitive-pale green3-1D*) mutant is insensitive to the inhibitor Brassinazole resulting in a pale plant phenotype due to impaired regulation of photosynthesis [32]. In addition, a genome-wide analysis of protein–DNA interactions revealed that the BZR1 family of transcription factors (Brassinazole-resistant), which are activated upon BR-perception by the BRI1 cell surface receptor, are involved in regulating a diverse range of developmental processes including photomorphogenesis [16]. More specifically, BZR1 was found to repress photomorphogenesis. This is achieved through repression of light-signalling components, including phytochrome B and phototropin 1, and activating the expression of other negative regulators, such as COP1 and SPA1 that interact to mediate ubiquitination and proteolysis of downstream light-activated transcription factors [16] (Fig. 2 of this review provides an illustration of the brassinosteroid signalling network). But it is considered that the impact of GA or BR (or that of other plant hormones) on chloroplast biogenesis might be collateral effects rather than direct involvement in chloroplast development.

A recent study on transcriptional regulation of Phytochrome-dependent genes revealed the early up-regulation of the known nuclear transcription factors *GLK1* and *GLK2* (*Golden2 like*) required for transcription of genes encoding chloroplast proteins, and of *Sig 2* and *Sig 6* (*Sigma factor*), which regulate the transcription of chloroplast genes [33]. The regulation of these transcription factors is providing the anterograde signal from the nucleus to the chloroplast to signal for the induction of photomorphogenesis. Interestingly, though the Sigma factors are involved in regulating the transcription of many of the chloroplast-encoded genes they also provide a signal back from the chloroplast to the nucleus, a so-called retrograde signal [34]. HEMERA/pTAC12 was described to be localised both in the chloroplast transcriptional apparatus as well as in the nucleus [25]. In the nucleus it seems to be involved in the regulation of PIFs and PhyA protein degradation. The dual localisation and differential role of HEMERA/pTAC12 make this protein an interesting candidate for retrograde signalling control.

Of particular interest in the context of chloroplast development are signals related to biogenic control, that is, those that regulate nuclear gene transcription during plastid biogenesis [35]. This has been described above for the Sigma factors and pTAC12, proteins directly involved in transcriptional regulation. Additionally, the PSII associated proteins EXECUTER1 and 2 (EX1, EX2), mediate singlet oxygen signalling in response to excess light and are involved in biogenic control as the double mutant *ex1ex2* exhibits white cotyledon regions, which contain undifferentiated plastids that resemble proplastids. Loss of Ex1 and Ex2 function results in a dramatic change in transcription as many genes are altered in their transcript abundance [36]. In contrast to the thousands of genes misregulated in *ex1ex2* only a few genes are differentially regulated in the bleached cotyledons of *sco3* compared to wild type [15]. Intriguingly, the SCO3/QWRF1 protein is neither located to the chloroplast nor to the nucleus but to the periphery of the peroxisome: and, as proteins of the QWRF family are demonstrated to have microtubule interaction properties (demonstrated for Embryo defective 1/QWRF5 and Augmin 8/QWRF8) [37,38] this suggests that the *sco3* mutation might interfere with metabolic exchange or impair signalling pathways yet to be described.

Stress and nutrient limitation, such as iron deficiency, can cause defects in chloroplast development and function, possibly by the excess production of reactive oxygen species (ROS). Iron is indispensable for photosynthesis as it provides for chlorophyll and heme biosynthesis, assembly of Fe-S clusters, and the electron transport chain [39]. A response mechanism to regulate transcription, chloroplast development and leaf development under iron deficiency was identified in the characterisation of mutant lines affected in the helix loop helix transcription factors bHLH39, bHLH100, and bHLH101 [39]. Double and triple mutants display reduced growth and chlorotic leaves when grown under iron deficient conditions [39].

Another interesting observation is that some proteins identified as being involved in abiotic stress response also influence chloroplast development. An example is the MDA1 gene (*MTERF DEFECTIVE IN Arabidopsis1*), the mutant of which (*mda1*) was described as having defects in their chloroplast structure among other phenotypic alterations [22]. *mda1* mutants have an altered steady-state level of chloroplast gene transcripts due to up-regulated plastid RNA Polymerase RpoTp/SCA3. Furthermore, the *mda1* mutant also exhibited altered response to abiotic stresses including the perception in elevation of stress-associated hormones such as a reduced sensitivity to ABA (abscisic acid), which happens to be a negative transcriptional regulator of chloroplast development [22,40]. This suggests an interaction between components of both stress response and chloroplast development, with the possibility of more undiscovered interactions.

Chloroplast development is modulated by a variety of different kinds of abiotic stresses, such as temperature that was identified as a major

modulator with variations in temperature causing alterations in membrane fluidity that can result in changes in the position of protein complexes [41]. Chilling stress results in the accumulation of ROS within the chloroplast, which activates ROS protective mechanisms, of which carotenoid molecules play a pivotal role, as well as inducing a number of cold responsive genes that protect thylakoid membrane integrity [65]. Conversely, heat stress on chloroplasts induces a different response through the activation of heat-shock elements [41,42]. HSP21 was recently identified as a chloroplast heat shock protein, involved in plastid-encoded RNA polymerase dependent transcription, that is essential for chloroplast development under heat stress. A mutation in this factor did not alter ROS accumulation; however, it resulted in abnormal thylakoid membrane formation and a decrease in plastid-encoded proteins, possibly caused by the reduction in the activity of a plastid-encoded RNA polymerase [42].

The importance of protein synthesis, import and turnover of proteins for chloroplast biogenesis and the balancing of these processes was demonstrated in many publications for which only a few will be mentioned here as they are already indicated above and reviewed recently [1]. The process of transport into the chloroplast requires the function of various chaperone molecules. Recently, the function of a chloroplast heat shock protein (Hsp90C) was identified as a component that interacts with intermediates of nuclear-encoded pre-proteins during post-translational import into chloroplasts. Hsp90C co-precipitated in a complex of protein import components, including various TIC and TOC components and stromal chaperones [23]. Mutations in the *Hsp90C* gene were also found to be embryo lethal [23], demonstrating the importance of chaperone function for proper regulation of chloroplast development and reinforcing the link between impaired chloroplast biogenesis and embryo lethality. The *variegated 2* (*var2*) mutant has been an interesting genetic tool to investigate chloroplast development in true leaves by identification of suppressor mutants with normal green leaves. *var2* impairs the function of the metalloprotease FTSH2, involved in the turnover of photosynthetic proteins such as the PSII core protein D1 [43], and many of the mutations that suppress the variegated phenotype are involved in regulating protein translation or folding [43–45]. Recently another suppressor was identified, *svr4* (*suppressor of variegation*) which was described independently in another analysis as *Atecb1/mrl7* (Early chloroplast biogenesis 7/MESOPHYLL-CELL RNAI LIBRARY LINE 7) [46,47]. SUV4 appeared to be associated with the PEP (Plastid-encoded polymerase) complex and might be involved in its regulation by its thioredoxin-activity [47]. Mutations in this gene impact the structure or function of thylakoids, thus impairing NPQ (non-photochemical quenching) [46]. Indeed, the formation of thylakoids is one of the important aspects of the biogenic control on chloroplast biogenesis [5].

The formation and stacking of thylakoids is regulated by proteins that are either involved in lipid biosynthesis and transport, vesicle formation, thylakoid stacking, and photosystem assembly. Phosphatidylglycerol (PG) is one of the major phospholipids in the thylakoid membrane and also plays an important role in photosynthesis. Thus, it is not surprising that *pgp1 (phosphatidylglycerophosphate synthase 1)* mutant lines exhibit severely compromised thylakoid membranes, and complete disruption of PG biosynthesis in the *pgp1pgp2* double mutant is embryo-lethal [48]. Transport vesicles are required for the formation of thylakoids as several mutants were identified affecting the formation or fusion of the vesicles to the thylakoid membrane. This revealed roles for VIPP1 (vesicle inducing plastid protein) and its interacting protein partner Hsp90.5, as well as for cpSAR1 (chloroplastic SAR1), and SCO2 [49–52]. The chloroplast secretory pathway protein SAR1, containing a GTPase activity, is proposed to be directly involved in the formation of the vesicles as bioinformatics analysis revealed co-expression of cpSAR1 with other COPII chloroplast vesicle cargo proteins [49,53]. In contrast, SCO2 was shown to directly interact with LHCB proteins which suggests a role in loading the vesicles with the photosynthesis-related proteins for transport to the thylakoids [51]. The function of the VIPP1 protein seems to be diverse and will be discussed elsewhere in this issue. Not only the formation but also the stacking of grana proves to be important for chloroplast biogenesis, as in the *angulata 10* (*anu10*) mutant that lacks grana due to reduced levels of trimeric light-harvesting complex II (LHCII) that results in a pale leaf phenotype [54]. The function of ANU10 is yet unknown. Similarly, no grana formation due to missing assembly of trimeric LHCII complexes (light harvesting complex) was observed in the seedling lethal *gdc1* mutant (*grana deficient chloroplast 1*) [55].

A link between the stacking of grana as well as the formation of transport vesicles and plastoglobules was demonstrated in the characterisation of the *cpRABA5e* mutant which is delayed in germination and is not able to grow under oxidative stress [56]. Under such conditions the mutants display more vesicles, larger plastoglobules but less grana structures compared to wild type indicating that cpRABA5e is involved in the fusion/fission/docking process of the vesicles to the membranes and the plastoglobuli resulting in reduced exchange of materials between these chloroplast compartments [56].

As discussed earlier, in true leaves chloroplasts are propagated by chloroplast division. A typical higher plant cell contains between 80 and 120 chloroplasts. Organelle division is largely independent of cell division. Chloroplast division is mediated by tubulin-like proteins known as FtsZ and dynamin-like proteins, such as ARC5 (accumulation and replication of chloroplasts 5) that form concentric rings within and outside the chloroplast envelope, respectively [57]. Only recently a link between chloroplast development and chloroplast division was published demonstrating that the ARC1/FtsHi1 protein couples the processes of chloroplast development and division: mutant seedlings are pale containing smaller but numerous chloroplasts [58]. The link between chloroplast development and chloroplast division was also observed in the rice mutant *osdg2* (*Oriza sativa delayed greening 2*) as the mutation results in the suppression of the gene expression of the chloroplast division proteins FtsZ and OSPOLP1, which delayed the greening process before the four-leaf stage [59]. Similar phenotypes as observed in the *arc1* mutant are also described in mutants with altered plastidic lipid composition leading to chloroplast division defects and abnormal thylakoid ultrastructure [58].

4. Chloroplast development and embryos

As chloroplast biogenesis is fundamental for plant development it is not surprising that many mutations in genes essential for chloroplast gene transcription and translation, protein import, or thylakoid formation and photosynthesis result either in an embryo-lethal or seedling-lethal phenotype. Interestingly, all the mutations affecting embryo development via impacting plastid biology result in the premature arrest at the globular-to-heart stage of embryo development [60,61]. This is an indication that the time-point of onset of chloroplast biogenesis begins at this very early stage of plant development. Functional embryonic chloroplasts then degenerate in the developing seed to so-called eoplasts [62]. In some mutant lines with mutations in essential chloroplast-development genes the embryos mature to seeds and subsequently can germinate but are then not able to develop functional chloroplasts in the seedling stage. Most of these seedlings are not able to grow further and are thus seedling-lethal. Some can overcome the deficiency by growth on sucrose-containing media. An interesting observation was that some mutations impairing chloroplast biogenesis only affected seedling development, but did not affect the embryo, as observed in some *sco* mutants, the *ex1ex2* double mutant or the *spd1* mutant (*seedling plastid development 1*) [13,15,62,63]. That is, even though the seedlings are affected in chloroplast biogenesis, chloroplast development occurs normally within the embryos. Prematurely dissected embryos were allowed to germinate precociously on media and developed normal green cotyledons [13,15]. Furthermore, an ultrastructural analysis of cotyledon cells of the *sco* mutant lines revealed that within the same cells both normal and aberrant chloroplasts were present. This suggests that there is a certain "threshold" of impaired development,

after which the chloroplasts are able to develop normally [15,51]. These indications about a link between chloroplast development and degeneration in the embryo and chloroplast development in the germinating seedling, as well as the mechanism responsible for the "gradient" of impaired chloroplast development in chloroplast biogenesis mutants might provide, through future research, novel insights into the regulatory processes.

In addition to chloroplast development influencing embryo maturation the converse also occurs, namely, the type of plastids present during embryogenesis affects chloroplast biogenesis during germination. For example, *sco3* and *ex1ex2* seedlings have bleached cotyledons in seedlings if the embryos mature in the light. However, when the embryos matured in the dark, the pale cotyledon phenotype of *sco3* and *ex1ex2* is reverted to an almost wildtype green [36,64]. The nature of this embryonic control on plastid development in germinating seedlings is as yet unknown, although ABA is proposed to be involved [36]. Another interesting possibility could be the involvement of DNA methylation. A burgeoning area of research, there are now examples where DNA methylation is influenced by environmental cues, such as during abiotic stress, leading to gene expression changes [65,66]. Furthermore, changes in DNA methylation are observed in nature between populations living under different conditions [67]. Therefore, the perception of light, or lack thereof, could be influencing changes in DNA methylation of nuclear encoded chloroplast biogenesis genes, or their regulators, thus influencing chloroplast development. Whether or not DNA methylation is truly involved in this process requires intensive further investigations. Since the chloroplast genome itself is insensitive to methylation changes, a focus has to be on methylation changes within the nucleus [68].

5. Chloroplasts and plant development

Quite obviously chloroplast development influences plant development because of its function in providing energy, amino acids, lipids, and other essential metabolites for normal growth. Impairment results in reduced plant growth, alters the flowering time and as a consequence seed set is diminished (Fig. 5A) [12]. Changes in chloroplast development can also impact on leaf development and shape. The mutation in *sco1-2* is embryo-lethal [61] and complementation with constitutively expressed SCO1 resulted in plant lines with a differential degree of greening, the extent of which appeared to correlate with the intensity of serration of leaf blades (Fig. 5B, personal observation). Furthermore, while PhyB complements impaired chloroplast biogenesis in *sco3*, the double mutant has fewer leaves with altered shape [64]. This adds a further level of complexity as it suggests a link between chloroplast development and leaf morphology and number. Additionally, a recent study indicated the existence of genetic interaction between SCO3, which is still unknown, and the PhyB, involved in light signalling [46]. This is unsurprising on one hand as one would expect the perception of light to play an important role in inducing chloroplast biogenesis; however, the intricacies of this interaction are not well understood. Also changes in thylakoid formation as described in *angulata 10* influences leaf shape [52]. But also derivatives of carotenoids are influencing leaf shape and plastid development as shown in the *zds/clb5* (*ζ-carotene desaturase/chloroplast biogenesis 5*), a carotenoid biosynthesis mutant, where a yet unknown carotenoid accumulated resulting in the altered phenotype [69]. The extent of these emerging relationships between carotenoid biosynthesis, plant development and chloroplast development requires more exploration.

Carotenoids are pure isoprenoid compounds, synthesised in coordination with chlorophyll [70], that perform a variety of essential roles for all photosynthetic organisms. In addition to stabilising membranes, including the thylakoidal membrane, and accelerating photomorphogenesis [71], a number of carotenoid molecules act as accessory pigments, assisting chlorophyll, to capture light energy for delivery to the photosystems [70] and diverting excess energy away from the photosystems to alleviate oxidative stress [70]. However, despite their importance for the photosystems, there is still limited understanding of the mechanisms that coordinate both these processes. A transcriptional study was performed to uncover the transcriptional regulation of the coordinated synthesis of pigments [72]. The authors found that the phytoene synthase gene, encoding the first dedicated and rate-limiting enzyme of carotenoid biosynthesis, is highly co-expressed with many photosynthesis-related genes, providing evidence that the coordinated transcriptional regulation of these pathways plays a crucial role in proper chloroplast

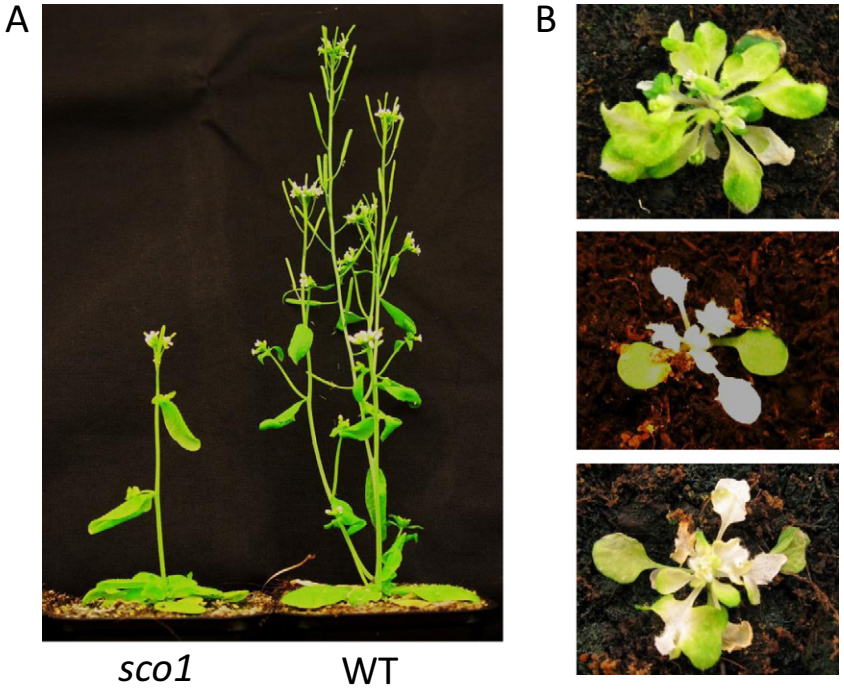

Fig. 5. Example of the *sco1* mutant with impaired chloroplast development and its impact on (A) plant growth and seed set as well as (B) on leaf shape after transformation with constitutively expressed SCO1. Shown are different independent transformed lines.

development. They also found that many of the co-expressed genes activity was dependent on gibberrelic acid and BR signalling [72] further reinforcing the importance of hormone signalling and revealing the complex nature of chloroplast development bringing together many different processes together to direct, arguably, the most important biological reaction: photosynthesis.

6. Conclusion

The analysis of chloroplast biogenesis and development turns out to be a more complex challenge than anticipated. Now that many different aspects in this network of processes are identified there is potential to answer long-standing questions. As chloroplast development requires a complex interaction between organelles, sensory mechanisms for environmental cues, and is influenced by other developmental stages an integrated strategy is required. Various unknowns have been identified in this review and the future challenge is to combine the threads identified in the past using the different mutant lines or treatments to unravel the complex network required for chloroplast development in plants. This will require a series of strategies. 1. The analysis of genome-wide modifications during chloroplast biogenesis, be it methylation or cysteine modifications. 2. The combination of intra- and inter-organellar communication and signalling within the cells. 3. The exploration of embryo-derived involvement on chloroplast development in seedlings and plants. 4. The investigation of different stress responses on chloroplast development but also chloroplast development on stress responses. 5. Further characterisation of the carotenoid biosynthesis pathway and its regulation along with the coordination with chloroplast development.

Conflict of interest

There is no conflict of interest with neither the co-authors nor with other scientists.

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