Update on Chloroplast Biogenesis

Genetic Dissection of Chloroplast Biogenesis and Development: An Overview[1]

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The chloroplast is essential for photosynthesis and the production of hormones and metabolites. As a consequence, its biogenesis and development needs to be coordinated with seedling growth to ensure optimal rates of photosynthesis without oxidative damage upon seedling emergence. Studies into the processes of biogenesis and development of chloroplasts have shown how important chloroplast development during germination is for plant vitality, seed set, and growth. Indeed, even if chloroplast development is only impaired in cotyledons (the embryonic leaves of germinating seedlings) with normal chloroplast development in true leaves, plant growth and yield can be negatively impacted. Thus, it is necessary to understand the regulation and mechanism of chloroplast development.

This review will concentrate on how chloroplasts differentiate, giving emphasis to current insights on the different steps of chloroplast development as well as into regulatory factors and molecular requirements for chloroplast biogenesis and development gained from genetic and mutagenic studies. Given the breadth of the topic, we are restricted to citing examples of recent articles and reviews, with a strong emphasis on Arabidopsis (Arabidopsis thaliana), because the majority of the genetics studies have been performed on this model plant.

DESCRIPTION OF CHLOROPLAST BIOGENESIS AND DEVELOPMENT

Chloroplast biogenesis and development in seedlings can be described as the differentiation process from the plastid progenitor, a proplastid, to a mature chloroplast, whether direct or via the dark-grown intermediate form known as an etioplast (Fig. 1). In developed leaves, chloroplasts are further propagated by fission in a similar way as observed in bacteria. Chloroplasts are found in cotyledons, leaves, stems, fruits, and flowers, depending upon the species and stage of development. Due to the specialization of tissues the biogenesis and development of the chloroplasts differs between organs as well as between species. For example, we have to distinguish between seedlings with an epigaeic growth, having cotyledons that first serve as storage organs but become photosynthetically active, to the hypogaeic seedlings, where the cotyledons only serve as a storage organ and are not photosynthetic. Herein, we only refer to chloroplast biogenesis and development in epigaeic seedlings.

That chloroplast development proceeds differently in cotyledons and true leaves is demonstrated by genetic studies. That is, chloroplast mutants have been described that have the phenotype restricted to one leaf organ, such as chlorotic true leaves, but green cotyledons as in the variegated (var) and immutans mutants (Liu et al., 2010). Conversely, the snowy cotyledon (sco) mutant group has chlorotic or bleached cotyledons but green true leaves (Albrecht et al., 2006, 2008, 2010; Shimada et al., 2007). The sco and cyo1 (shiyō-u means cotyledon in Japanese) mutants are able to develop almost normally on soil, but their vitality is reduced compared with wild type as measured by reduced seed set. Since all of the SCO genes identified to date are unique and not only expressed in cotyledons but through the entire plant, we have to assume that there is no redundancy with other members of the gene family. Furthermore, the different SCO genes encode proteins involved in complete different processes such as chloroplast protein translation (SCO1), chloroplast protein folding (SCO2), or being associated with microtubules and the peroxisome (SCO3; Albrecht et al., 2006, 2008, 2010). Thus, the observed impairment in chloroplast development in cotyledons seems to be due to a differential chloroplast development process between the plant organs rather than redundancy of proteins in the true leaves. A third class of mutants have a white cotyledon phenotype, but require Suc to develop green true leaves. Since green leaves have developed the plants can grow without Suc and can be transferred to soil for propagation. To this group belongs, among others, plastid type I signal peptidase1, which will be discussed later (Shipman and Inoue, 2009).

So how do plastids develop in seedlings after germination? In dark-germinated seedlings, the largely undifferentiated proplastid can form an etioplast,
which exhibits a characteristic lattice-like membraneous structure known as the prolamellar body. The prolamellar body contains the precursor of chlorophyll, protochlorophyllide, bound to its reducing enzyme protochlorophyllide oxidoreductase (POR), NADPH, lipids, a few proteins, and typically two carotenoids, lutein and violaxanthin. From this lattice-like structure prothylakoids emanate into the plastid stroma. In angiosperms, light induces POR enzyme activity to convert protochlorophyllide into chlorophyllide a, which is subsequently converted into chlorophyll a and b. Gymnosperms additionally have a light-independent POR enzyme that catalyzes the same reaction (Forreiter and Apel, 1993). Together with the production of chlorophyll the internal membranous structure of the chloroplasts, the thylakoids, develop along with the newly assembled photosystems for assimilating light for photosynthesis. Alternatively, proplastid to chloroplast differentiation in the light proceeds directly. In some cases, chloroplasts can also develop from other plastids such as chromoplasts, though our focus here will be only on chloroplast differentiation from proplastids.

Tight stoichiometric assembly of nuclear-encoded and plastidic-encoded proteins together with chlorophylls and carotenoids is essential for photosynthesis, both with respect to limiting oxidative damage and ensuring optimal rates for protein synthesis. This requires coordination between the two organelles at the level of transcription, translation, import, protein turnover, and metabolite flux—all of which is largely controlled by plastid and nuclear factors (Fig. 2; see “Plastidic and Nuclear Factors Required for Chloroplast Biogenesis and Development”). However, photosynthesis proceeds within the context of the environment, cell, and leaf development, so environmental and cytosolic processes also influence chloroplast biogenesis and development, which will be discussed in “Nonplastidic Factors Required for Chloroplast Biogenesis and Development” and outlined in Figure 3.

PLASTIDIC AND NUCLEAR FACTORS REQUIRED FOR CHLOROPLAST BIOGENESIS AND DEVELOPMENT

Here, we provide a brief overview of different processes identified by mutations in chloroplastic and nuclear proteins that specifically affect aspects of chloroplast biogenesis and development as shown in Figure 2 (see also Supplemental Table S1 for further information about the genes and corresponding citations).

Nuclear Gene Transcription

Upon illumination, one-third of the nuclear gene transcription changes (Chen et al., 2010). Among the genes dramatically up-regulated in the light are most of the genes encoding chloroplast-targeted proteins, reflecting that the vast majority of the several thousand chloroplast proteins are nuclear encoded. The perception of light has been shown to require the activation of the primary photoreceptors known as phytochromes, such as phyA and phyB. Protein interaction partners of the phytochromes, PIFs or phytochrome interacting factors, are transcription factors involved in this light-mediated transcription. Indeed, the loss of either PIF1 or PIF3 results in delayed development of the chloroplasts (Moon et al., 2008). However, other transcription factors are also required for chloroplast development. Indeed, transcription factors that are directly involved in the transcription of photosynthesis genes have been identified, such as
the Golden2-like proteins (GLKs). In Arabidopsis, loss of both GLK transcription factors leads to a pale mutant phenotype that can be complemented by over-expression of either of the two GLK proteins (Waters et al., 2009).

**Chloroplast Protein Import and Processing**

Considerable progress has been made in deciphering the mode of protein import through the outer and inner envelope (membranes) of the chloroplasts. The majority of chloroplast-targeted proteins enter the plastids via the translocon at outer envelope of chloroplast (TOC) and translocon at inner envelope of chloroplast (TIC) translocon complexes at the outer and inner envelope, respectively; others are targeted via the endoplasmic reticulum (Kessler and Schnell, 2009). The proteins are directed to the complex by their chloroplast targeting peptide by heat shock protein 90 (HSP90) and TOC159. Mutant analyses of the different components of this complex demonstrate that chloroplast biogenesis is dramatically impaired if the major component of the outer complex, TOC159, is knocked out, though they survive if minor components such as Toc33 or Toc34 are missing (Kessler and Schnell, 2009; Inoue et al., 2010; Shi and Theg, 2010). The complex mechanism of protein import into the chloroplast via the TOC/TIC complex is reviewed elsewhere (Kessler and Schnell, 2009).

Imported proteins, either into the chloroplast or into the thylakoids of the chloroplasts, need to be processed by removal of their N-terminal targeting signal. For the TOC/TIC complex a stromal processing peptidase as well as heat shock proteins have been described (Cline and Dabney-Smith, 2008).

Within the chloroplast, there is also a targeting system to integrate proteins into the thylakoid membranes and/or lumen. Integration of proteins into the membrane can either be spontaneous or via the chloroplast signal recognition particle pathway that has been described for light-harvesting chlorophyll-binding proteins (Falk and Sinning, 2010). Part of this mechanistic pathway is the ALBINO3 protein, which by loss of function—as the name already suggests—leads to a white seedling phenotype. Transport of proteins into the thylakoid lumen is either ATP dependent via the chloroplast Sec pathway or ΔpH dependent using the chloroplast twin arginyl translocation mechanism. For both pathways, mutants have been described as being either seedling lethal or affecting thylakoid formation (Cline and Dabney-Smith, 2008). A thylakoidal processing peptidase, PISP1, is required for removing the thylakoid-targeting sequence from thylakoidal proteins and has been shown to be involved in processing PsbO and PsbP proteins. Loss of the protein to be seedling lethal if no exogenous carbon source is provided (Shipman-Roston et al., 2010).

**Chloroplast Gene Transcription and Translation**

Chloroplast gene transcription, RNA maturation, protein translation, and modification have a great impact on chloroplast biogenesis and development. This includes both nuclear-encoded polymerase and plastid-encoded polymerase machinery. Plastidic RNA polymerase sigma (sig) factors are required as transcription factors for chloroplast genes with the loss of sig2 and sig6 delaying chloroplast biogenesis (Ishizaki et al., 2005; Chi et al., 2010) and nuclear-encoded RNA polymerases, such as rpoT, has been shown to affect chloroplast biogenesis (Azevedo et al., 2008).

Interestingly, a protein that is required for plastid transcription, pTAC12/HEMERA, has been shown to be dual targeted to the chloroplast and nucleus. In the
latter it regulates phytochrome-mediated transcription, as it is required for the regulation of PIF1 and PIF3 protein accumulation. Loss of this protein results in an albino phenotype that can only be rescued when both the chloroplast and the nuclear-targeting signals are accessible (Chen et al., 2010).

While the chloroplast genome only encodes about 100 genes, mutations that affect transcription and RNA processing, such as RNA editing, lead to impairment of photosynthetic function (Delannoy et al., 2009). Recently, a large class of nuclear-encoded proteins known as PPRs (pentatricopeptide repeats) have been demonstrated to be critical for RNA processing, splicing, editing, stability, maturation, and translation in the chloroplast. In most cases the phenotypes of PPR mutations are seedling lethal and typically each PPR protein has a specific role, be it RNA editing or transcript stability, for a particular chloroplast-encoded gene (Chateigner-Boutin et al., 2008; Pfalz et al., 2009; Chi et al., 2010).

Maturated and/or edited chloroplast mRNA is then translated into proteins by the ribosomal complex. Complete loss of essential proteins of the plastid ribosomal complex results in an embryo-lethal phenotype. Phenotypes associated with a reduced functionality due to leaky point mutations in proteins of the ribosomal complex can be described as virescent, or somewhat green, as is the case for sco1 or the rice (Oryza sativa) mutant virensent2 (Osvisr2; Albrecht et al., 2006; Sugimoto et al., 2007). Such mutations either result in a reduced protein translation, as has been shown for sco1 mutant that impairs the binding of chloroplast elongation factor G to the ribosomal complex, or a reduced content of the ribosomal complex, as described for the loss of the DNA- and RNA-binding protein Whirly1 in maize (Zea mays; Prikryl et al., 2008).

Protein Folding and Degradation

Transcribed proteins need to be folded and further processed or even degraded if nonfunctional. Chloroplast protein folding is mediated by chaperones, such as HSP70 and Cpn60, with the hsp70-1 mutation leading to variegated cotyledons (Latijnhouwers et al., 2010). Also, protein disulfide isomerases have been shown to be required for protein folding, with, for example, sco2/cyo1 lesions in a protein disulfide isomerase, resulting in a cotyledon-restricted pale-green phenotype (Shimada et al., 2007; Albrecht et al., 2008). The rates of protein translation and degradation are important for chloroplast development with mutations in the family of FtsH proteases, such as var2, impairing PSII D1 protein degradation causing a white-patched phenotype in true leaves, but not cotyledons. This phenotype can be overcome by decelerating protein biosynthesis by second site mutations in genes involved in protein translation (Miura et al., 2007; Zhang et al., 2010). The multisubunit ClpPR protease complex has a central role in degrading proteins and is required for chloroplast protein homeostasis. Loss of ClpP proteins are either embryo or seedling lethal (Kim et al., 2009b).

Thylakoid Formation and Pigment Biosynthesis

The thylakoids are formed mainly by mono- and digalactosyldiacylglycerol that are either produced within the chloroplasts or at the endoplasmatic reticulum. The biosynthetic pathway of the lipids as well as proteins involved in their integration and formation of thylakoids has been described elsewhere (Benning, 2009). Many mutations have been reported to affect thylakoid formation, although a direct involvement of the corresponding protein in thylakoid formation could not be attributed to their protein function, rather than that they are essential in particular thylakoid protein complexes.

During thylakoid formation the photosynthetic complexes are formed, which requires a coordinated import of pigment-binding proteins such as chlorophyll a/b-binding proteins (CAB/light-harvesting chlorophyll-binding) and the biosynthesis of the pigments. Thus, carotenoid and chlorophyll biosynthesis is tightly regulated during chloroplast development as

**Figure 3.** Model of environmental, cellular, and temporal factors that influence chloroplast biogenesis and development in seedlings. Environmental factors involved are, among others, light, the cellular factors include the communication between the organelles, and the embryo development is one of the temporal factors that influences chloroplast biogenesis in germinating seedlings.
they are essential for the assembly of photosynthetic complexes and photoprotection (Cazzonelli and Pogson, 2010). Therefore, it is not surprising that screens for chloroplast development mutants have identified steps in these pathways, such as chloroplast biogenesis4 (clb4) and clb6, which are lesions in enzymes in the chloroplast 2-C-methyl-d-erythritol 4-P pathway (Gutiérrez-Nava Mde et al., 2004; Guevara-García et al., 2005). The chlorophyll precursors, tetrapyrroles, have been implicated in coordination of chloroplast biogenesis (Zhang et al., 2006) as well as the ratio of different carotenoids in photosynthetic tissues that is regulated by nuclear gene transcription and a histone methyl transferase, SDG8 (Cazzonelli et al., 2009).

Retrograde Signaling

The fact that most of the proteins needed for chloroplast function are encoded in the nucleus necessitates a tight regulation between the chloroplast functional status and nuclear gene transcription. Thus, signaling from the chloroplast is involved in regulating nuclear gene transcription, a process called retrograde signaling. Many factors have been suggested to be involved in the retrograde signaling control, ranging from reactive oxygen species to metabolites and proteins that regulate or transduce the signals (Pogson et al., 2008; Pfannschmidt, 2010). Of particular interest in the context of this article are signals related to biogenic control, that is, those that affect nuclear transcription during plastid development (Pogson et al., 2008). The PSII-associated proteins, EXECUTER1 and EXECUTER2, mediate singlet oxygen signaling pathways (Kim et al., 2009a). Interestingly, the double mutant ex1ex2 exhibit white cotyledon regions that contain undifferentiated plastids that resemble proplastids. This chloroplast biogenesis defect can be overcome by growth of the seedlings on abscisic acid-containing media (Kim et al., 2009a).

A series of mutants that affect nuclear transcription in response to inhibitors of pigment biosynthesis and/or plastid transcription and translation are the genomes uncoupled (gun) mutants (Pfannschmidt, 2010). This series of mutants demonstrate a role for tetrapyrrole biosynthesis and the PPR protein, GUN1, in retrograde signaling, but the nature of the signaling cascade is still debated and although the gun1 mutation affects the signaling from chloroplast to nucleus, chloroplast biogenesis proceeds normally in the absence of inhibitors. This and other findings raise the question as to how much the different retrograde signaling mutants directly affect signaling or result in a perturbation of plastidic processes and metabolic signatures that alters nuclear transcription (Pfannschmidt, 2010).

Chloroplast Division

A typical higher plant cell contains between 100 and 300 chloroplasts, which necessitates organelle division that has been shown to be largely indepen-
chloroplast development, that is, even though proplastids are present in the seed prior to germination and are largely undifferentiated, the way in which they are formed during embryogenesis affects chloroplast biogenesis. Mutants affected in chloroplast biogenesis in the embryonic leaves (cotyledons) of germinating seedlings, such as sco and ex1ex2, are not affected in chloroplast development during embryogenesis in the developing silique (Albrecht et al., 2006; Kim et al., 2009a). Indeed, it could be shown that these mutants exhibit normal green cotyledons if the mature green embryo was extracted from green seed and germinated precociously. On the other hand, if siliques of the ex1ex2 double mutant were covered with aluminum foil, so that the embryo matured instead of continuous light prior to seed desiccation and dormancy, then seedlings derived from this dark-matured embryo do not exhibit impaired chloroplast biogenesis (Kim et al., 2009a). Thus, some as-yet-unknown process(es) during embryogenesis influences the differentiation of proplastids to chloroplasts in germinating seedlings.

SUMMARY AND FUTURE PROSPECTS

The process of chloroplast biogenesis and development requires a network of environmental, cellular, and temporal factors. The environmental factors are influencing gene transcription and protein degradation processes largely regulated via light signaling or oxidative stress. Cellular factors can be divided into those within the chloroplast and those outside of the plastid. To date most studies have identified chloroplast-localized proteins involved in import, chloroplast transcription/RNA maturation/protein translation, assembly, and signaling. Temporal factors have to be taken into account and insights into the effects of embryogenesis on photomorphogenesis reveal new fields to analyze the regulation of chloroplast biogenesis. Key questions that remain include: (1) what is the interaction between chloroplast development and photomorphogenesis; (2) how do interactions with different organelles influence the biogenesis of the chloroplast; (3) what are the components in retrograde signaling that mediate chloroplast development; and (4) what are the check points in chloroplast biogenesis and development?

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Table S1. List of mutants and genes affected and involved in chloroplast biogenesis.

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LITERATURE CITED


motors and regulates early chloroplast development in cotyledons. Plant J 42: 133–144

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