Chapter 23

Biogenesis of Chloroplasts

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Summary

Chloroplasts belong to a diverse family of plant organelles called plastids that perform essential functions, including important steps in many biosynthetic pathways. All plastids differentiate from proplastids through a complex process, in which numerous events must be coordinated and integrated into the overall developmental pathway of the cell. Due to the overwhelming importance of chloroplasts as sites of oxygenic photosynthesis the differentiation of chloroplasts from proplastids has been most studied. Chloroplast biogenesis begins with the perception of light, which triggers the coordinated expression of genetic information contained in both the nuclear and plastid genomes. Subsequently the chloroplast protein import machinery plays a major role in organelle biogenesis, mediating the import of nuclear-encoded proteins into the organelle. This process is challenged by the complex organization of the chloroplast sub-compartments. The conversion of sunlight into chemical energy by the

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photosynthetic machinery requires thylakoid membranes, a specialized membrane system found in chloroplasts, and this process involving a complex cascade of biochemical and structural events. Here we will address the major molecular events following the initiation of chloroplast biogenesis, culminating in the formation of the mature chloroplast and the segregation of plastids to daughter cells during cell division.

I. Introduction

Chloroplasts are remnants of a cyanobacteria-like ancestor that was engulfed by a eukaryotic host cell approximately 1.2 billion years ago (McFadden 2001). During evolution chloroplasts developed specialized functions in different tissues and cell types, giving rise to a diverse group of other non-photosynthetic, interconvertible plastids (Fig. 23.1). Chloroplasts are the most prominent form of plastids, essential not only for photosynthesis, but also for nitrogen fixation, the synthesis of amino acids, fatty acids, purine and pyrimidine bases, isoprenoids and tetrapyrroles and for the synthesis of the lipid components for their own membrane structures (Tetlow et al. 2005).

Plastids are fully integrated into the life cycle of photosynthetic eukaryotes and have retained a semi-autonomous character, a minimal genetic machinery and genes for a small number of proteins. As a result of horizontal gene transfer, the coding capacity of the present-day plastid is reduced to only about 130 genes and the majority of plastid proteins are encoded in the nucleus (Sugita and Sugiura 1996). The transfer of genes from the endosymbiont to the nucleus of the host cell provided an opportunity for increased control of the plastid by the host cell, both at the level of cell-type specific expression of plastid genes directed by the nucleus and the control of the expression and import of nuclear encoded proteins into the plastids.

All plastids, including chloroplasts, develop from proplastids, small (0.5–1 μm in diameter) undifferentiated organelles present in meristematic cells of roots and shoots (Cran and Possingham 1972). As plant cells develop, plastids can undergo different patterns of differentiation depending on the committed developmental pathway within the cell. However, the differentiation pathways are dynamic and in response to cellular and environmental signals, plastid differentiation can be reversible where mature plastid types can redifferentiate from one vplastid type to another (Fig. 23.1). The majority of plastid research has focused on the chloroplast which will be the focus of this chapter.

Upon germination a seedling must establish an independent energy source before those stores in the seed are depleted. Attainment of this is dependent on the formation of photosynthetically active chloroplasts. Whilst proplastids are simple organelles, mature chloroplasts are large (5–10 μm in diameter), structurally complex organelles, which house the functional components required for both the light-driven and light-independent enzymatic reactions of photosynthesis. All plastids are bound by a double membrane called the envelope and chloroplasts have in addition a third internal membrane system, the thylakoid membrane, where the light-dependent reactions of photosynthesis take place. Consequently, the molecular mechanisms involved in the development of a proplastid to a mature chloroplast consist of a series of complex events

Abbreviations: ARC – Accumulation and replication of chloroplast; ATP – Adenosine-5'-triphosphate; Cry – Cryptochromes; GTP – Guanosine-5'-triphosphate; LHCII – Light harvesting complex II; NADPH – Nicotinamide adenine dinucleotide phosphate; NEP – Nuclear encoded polymerase; PDV – Plastid division proteins; PEP – Plastid encoded polymerase; PIFs – phytochrome-interacting factor; PSI – Photosystem I; PSII – Photosystem II; TIC – Translocon at the inner envelope membrane of chloroplasts; TOC – Translocon at the outer envelope membrane of chloroplasts; VIPP – Vesicle-inducing protein in plastids

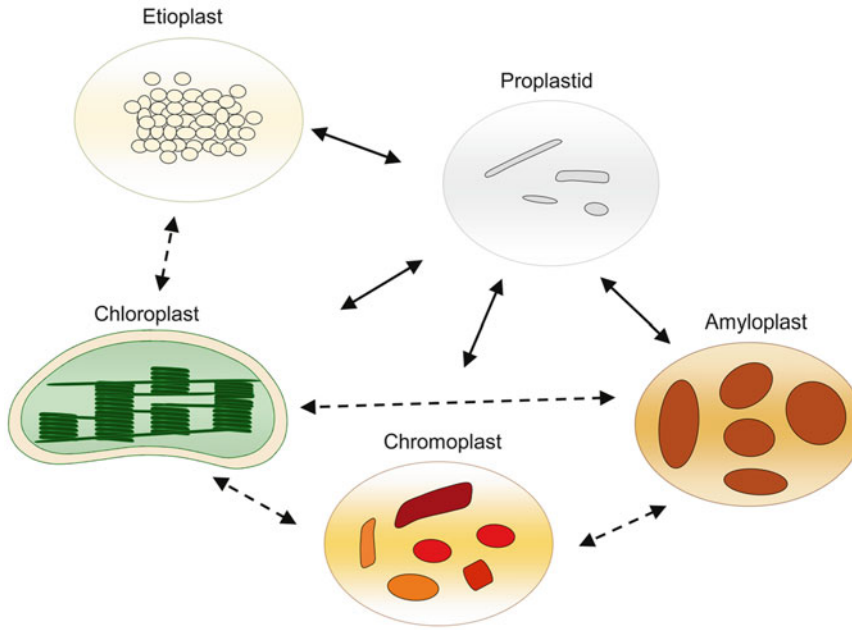

Fig. 23.1. The main forms of plastids in higher plants. All plastids develop from proplastids, undifferentiated organelles present in meristematic cells of roots and shoots. Plastids can undergo different patterns of differentiation depending on the developmental stage of the plant and environmental conditions. Plastids can store chlorophyll (chloroplast), carotenoids (chromoplasts) and starch (amyloplasts). *Solid lines* indicate conversions that occur under normal conditions; *dashed lines* specify conversions that are possible under a limited set of conditions or that are unusual.

and involve extensive structural and functional changes of the organelle.

Here we discuss progress in understanding the molecular mechanisms behind chloroplast biogenesis, including the initiation of photomorphogenesis, gene-expression, protein import and assembly of the thylakoid membrane system (Fig. 23.2). As well as considering molecular aspects of plastid differentiation, we will also address the replication of chloroplasts and fine tuning of the differentiation process.

II. Proplastid to Chloroplast Differentiation

The fundamental control point activating the proplastid-to-chloroplast differentiation programme is the perception of light. When light intensity is below a certain threshold level hypocotyl elongation is promoted, cotyledon opening is inhibited and the development of proplastids in leaves is arrested at an intermediate stage resulting in the formation of etioplasts (Von Arnim and Deng 1996) (Fig. 23.1). Once light has triggered the transition from dark growth (skotomorphogenesis) to light growth (photomorphogenesis) several molecular events are initiated including gene expression, protein import, and thylakoid membranes assembly (Fig. 23.2) ultimately culminating in the formation of mature chloroplasts.

A. Light Perception and the Initiation of Photomorphogenesis

Perception of light is mediated by a suite of photoreceptors, which detect specific wavelengths. Of primary importance in chloroplast biogenesis are the phytochromes (primarily PhyA and PhyB), which perceive red and far-red light, and the cryptochromes

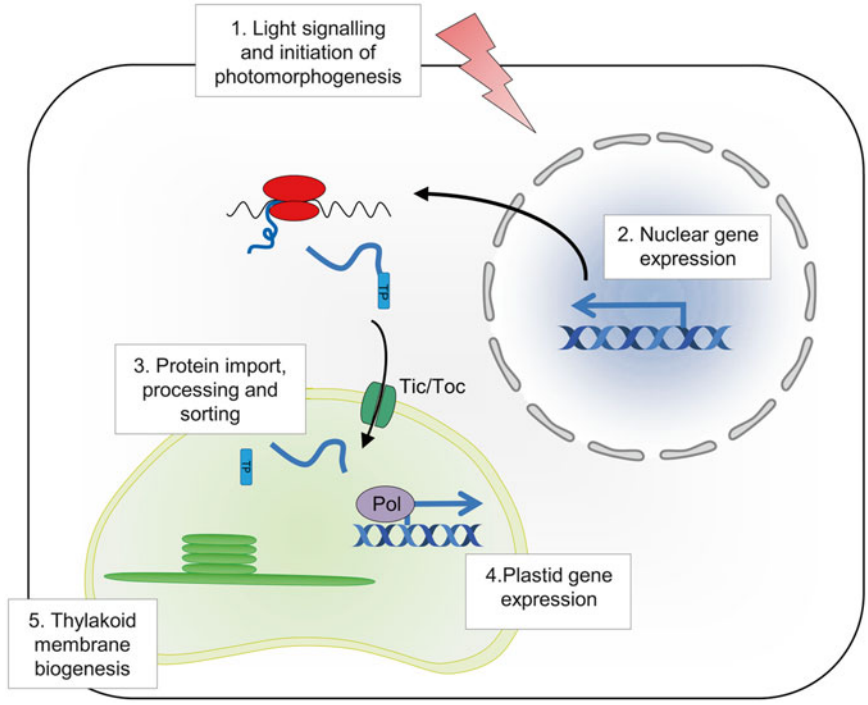

Fig. 23.2. Early events in chloroplast biogenesis. (1) The differentiation of proplastids to chloroplasts is triggered by the perception of light. (2) This in turn leads to the coordinated expression of nuclear genes. (3) Many nucleus-encoded proteins are translated in the cytoplasm and targeted for import into chloroplasts by a transit peptide (TP), which directs protein translocation through the Toc and Tic complex. After import many proteins are processed to remove the TP and proteins not destined for the stroma are directed to their proper location. (4) Plastid gene expression is regulated throughout differentiation by the activities of two classes of polymerase (pol); the nuclear encoded polymerase and plastid encoded polymerase. (5) The thylakoid network is assembled to generate a fully photosynthetically active chloroplast.

(Cry1 and Cry2), which respond to blue and UVA light. Extensive research efforts have identified a complex and interconnecting signalling network downstream of these photoreceptors (Reviewed in Chory 2010) and key events in triggering photomorphogenesis will be summarized only briefly.

Downstream of the photoreceptors are two main light signalling branches, the COP1-HY5 pathway and the PIF pathway (Fig. 23.3). COP1, along with the COP10, DET1, DDB1 (CDD) complex, is a central repressor of photomorphogenesis, repressing light signalling in darkness (Osterlund et al. 2000; Schroeder et al. 2002; Yanagawa et al. 2004). COP1 is an E3 ubiquitin ligase which tags light response activators, such as the bZIP transcription factor HY5, for targeting to the 26S proteasome for subsequent degradation (Osterlund et al. 2000). In darkness, inactive Cry dimers are localized to the nucleus where they bind COP1. Blue light leads to the photoactivation of CRY1 and triggers CRY1 relocation to the cytoplasm. Consequently, in the light COP1 is mostly absent from the nucleus and HY5 is no longer ubiquitinated and degraded, ultimately leading to an increase in the expression of photomorphogenesis genes (Saijo et al. 2003) (Fig. 23.3).

In parallel to this, phytochrome signalling acts through a family of basic helix–loop–helix (bHLH) transcription factors called PIFs (for phytochrome-interacting factor). Several PIFs have been identified in *Arabidopsis* and function mainly as repressors of photomorphogenesis (Castillon et al. 2007) (Fig. 23.3). For example, PIF3

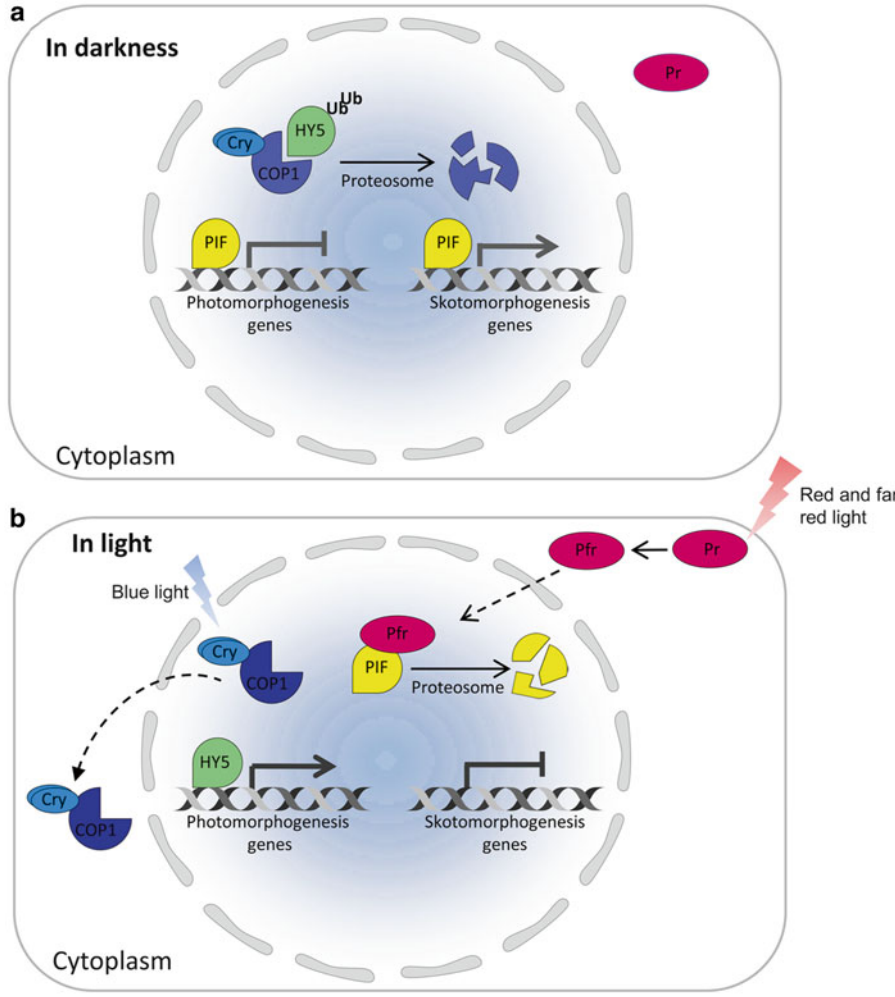

Fig. 23.3. A simplified model of the initiation of light signaling during photomorphogenesis. (**a**) In darkness, inactive CRY1 dimers are bound to COP1 in the nucleus. COP1 is an E3 ligase and promotes the ubiquitiation (Ub) of photomorphogenesis-promoting transcription factors, such as HY5. Polyubiquitinated HY5 is subsequently degraded by the 26S proteasome. Phytochrome dimers are in their inactive (Pr) form and localize to the cytoplasm. Skotomorphogenesis promoting transcription factors, such as PIF3, are bound to target promoters and also inhibit the transcription of photomorphogenesis-related genes. (**b**) In light, *blue light* triggers the activation of CRY1 dimers, leading to their translocation to the cytoplasm. This leads to accumulation of HY5 in the nucleus, which in turn activates transcription of photomorphogenesis genes. In parallel, red and far red light cause the conversion of phytochrome dimers from their inactive Pr form to their active Pfr form. Active Pfr dimers translocate to the nucleus where they bind to PIF3, ultimately leading to its degradation. This de-represses the expression of photomorphogenesis related genes. *Dashed lines* indicate the translocation of photoreceptors in response to light.

blocks the transcription of genes involved in chlorophyll biosynthesis and photosystem I (PSI) biogenesis (Ni et al. 1998). In the dark, PIFs are active and regulate gene expression to promote skotomorphogenic growth. In the light phytochrome dimers are converted from their inactive Pr form to their active Pfr form and translocate from the cytoplasm to the nucleus, where they localize to foci called phytochrome nuclear bodies (Quail 2002). Here the phytochromes bind to PIFs, ultimately leading to their

phosphorylation and subsequent degradation. The block in the transcription of the photomorphogensis-related genes is thus released (Al-Sady et al. 2006; Ni et al. 1999) (Fig. 23.3). Recently it has been discovered that PIF1 and PIF3 protein accumulation is also regulated by HEMERA (*HMR*; pTAC12), which localizes to both the periphery of phytochrome nuclear bodies and to chloroplasts. Loss of HMR leads to a block in chloroplast development in response to light and it is proposed that HMR influences gene expression directly in both the nucleus and chloroplasts (Chen et al. 2010; Pfalz et al. 2006).

Shortly after light exposure, even when light-stimulated photomorphogenesis is barely observable, families of transcription factors are rapidly and differentially expressed (Lopez-Juez et al. 2008). The switch from dark to light growth leads to the differential regulation of up to one third of all nuclear *Arabidopsis* genes in a coordinated manor: Initially genes involved in ribosome biogenesis, protein translation and cell proliferation are upregulated whilst genes involved in plastid biogenesis and photosynthesis are largely induced at least 6 h after light exposure (Lopez-Juez et al. 2008; Ma et al. 2001).

B. Chloroplast Protein Import

Approximately 3,000 different nuclear-encoded proteins must be imported into chloroplasts during chloroplast biogenesis to fulfil the variety of cellular processes involving chloroplasts (Martin et al. 2002). Most chloroplast proteins are synthesized as precursor proteins (preproteins) in the cytosol and are imported post-translationally into the organelle. Preproteins bear an N-terminal transit peptide, responsible for the specificity of targeting which is proteolytically degraded after successful import. Once the preproteins have been guided to the chloroplast by chaperones, they interact with receptors on the membrane surface and are transported through the membranes in a GTP- and ATP-dependent manner.

The major pathway for protein import into chloroplasts is mediated by two multiprotein complexes or translocons of the outer membrane (TOC) and the inner membrane (TIC) of chloroplasts (Fig. 23.4). The first committed step in chloroplast protein translocation involves the preprotein recognizing part of the TOC complex on the outer envelope membrane. The core of the TOC complex consists of the proteins Toc159, Toc34 and Toc75. Toc159 and Toc34 are likely to recognize the precursor proteins directly and Toc75 (a β-barrel protein) acts as a translocation channel across the outer membrane (Hinnah et al. 2002; Kessler et al. 1994). In *Arabidopsis* two isoforms of Toc34 (atToc33 and atToc34) and four isoforms of Toc159 (Toc159, Toc132, Toc120, Toc90) have been identified (Fig. 23.4). *Arabidopsis* deletion mutants of the major isoforms have severe phenotypes suggesting their essential roles in chloroplast biogenesis. In particular, the *Arabidopsis* Toc159 mutant (*ppi2*) is albino, does not accumulate photosynthetic proteins and does not survive past the cotyledon stage (Bauer et al. 2000). Fascinatingly, it has been demonstrated that receptor absence in *ppi2* is not the only reason for the reduction in protein accumulation in plastids but also the downregulation of a suite of photosynthetic genes indicating tight regulation through retrograde signaling (Kakizaki and Inaba 2010). The *AtToc33* knockout mutant (*ppi1*) shows a pale green phenotype and is defective in the import and accumulation of several photosynthetic proteins (Kubis et al. 2003). It has been suggested that Toc complexes with different isoforms are able to regulate protein import of different classes of preproteins important for chloroplast differentiation (Ivanova et al. 2004).

Once the preproteins have crossed the intermembrane space the preproteins encounter the Tic translocon. Tic110, Tic40 and Hsp93 represent a minimal functional Tic unit (Fig. 23.4), where regulatory subunits are dynamically associated. Tic110 is most abundant mediating precursor binding, chaperone recruitment on the stromal

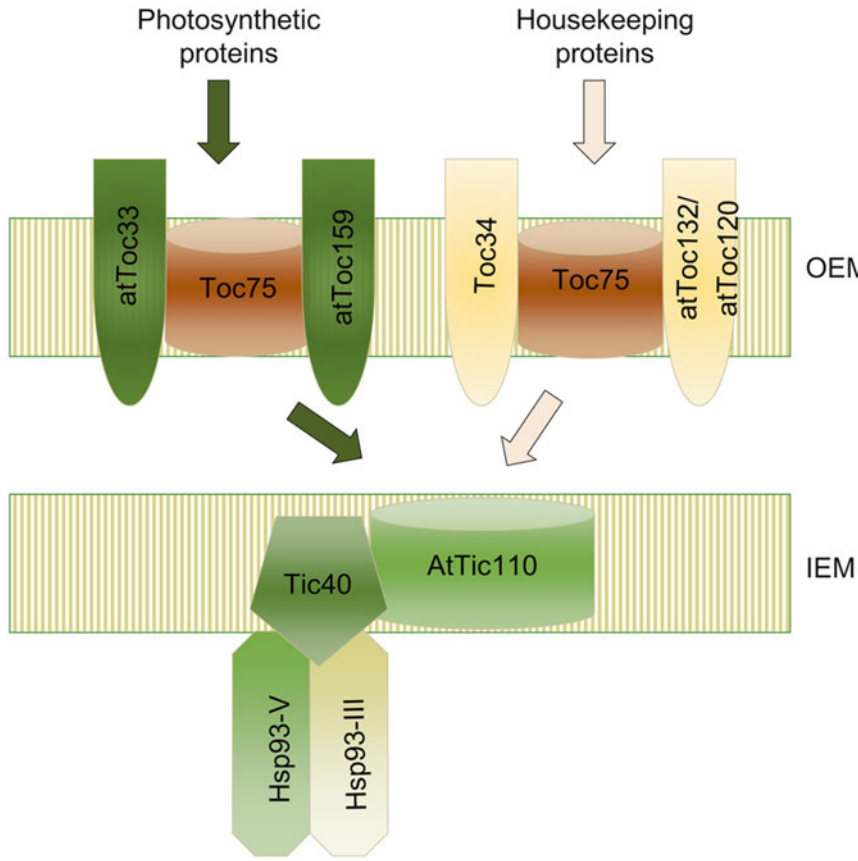

Fig. 23.4. A simplified illustration of the main components of chloroplast import apparatus. Nuclear-encoded chloroplast proteins are first translated on cytosolic ribosomes and then transported to the chloroplast outer envelope membrane (OEM). The TOC complex on the outer envelope membrane recognizes the preproteins. Once the preproteins have crossed the intermembrane space they encounter the Tic translocon on the inner envelope membrane (IEM). Green color indicates proteins demonstrated to be involved in the import of photosynthetic proteins; *yellow* indicates the ones involved in the import of housekeeping proteins.

side of the inner membrane as well as constituting a protein translocation channel (Lubeck et al. 1996). Reduction in *atTic110* expression results in a pale green phenotype, inappropriate plant growth and a marked decrease in thylakoid membranes (Inaba et al. 2003). In *Arabidopsis* there are two nuclear-encoded *hsp93* genes: *hsp93-III* (*clpC2*) and *hsp93-V* (*clpC1*). Whilst the *hsp93-III* mutant displays no phenotype the *hsp93-V* mutant contains chloroplasts with reduced thylakoid membranes, decreased amounts of PSI and photosystem II (PSII) proteins and reduced import efficiency (Constan et al. 2004). Similarly, null mutants of *AtTic40* display a pale green phenotype, slow growth and have less grana stacks in the thylakoids (Chou et al. 2003).

Stromal proteins may also be targeted to the thylakoid lumen which requires a second transit peptide involving either the Sec machinery-dependent or the twin-arginine translocation (Tat) protein translocation pathways (Robinson et al. 2001). Added to this is the fact that not all plastid proteins are imported through the conventional TOC and TIC complexes. Indeed research has shown that proteins can also be imported into chloroplasts through alternative pathways (Miras et al. 2007).

C. Plastid Gene Expression

The expression of plastid-encoded genes is mediated by two types of RNA polymerases of different origin, the nuclear-encoded polymerase (NEP; named RPOTp and RPOTmp), which probably derives from the mitochondrial RNA polymerase, and the multimeric plastid-encoded RNA polymerase (PEP), which has been retained from the ancestral endosymbiont (Hajdukiewicz et al. 1997; Shiina et al. 2005). Most plastid genes or operons are preceded by multiple promoters allowing transcription by NEP as well as by PEP. Plastid genes can generally be ascribed to three categories: those with PEP promoters (Class I; primarily genes encoding components of the photosynthetic apparatus), NEP- and PEP- promoters (Class II; non-photosynthesis-related genes), or NEP-promoters only (Class III; a small group of genes) (Fig. 23.5) (Hajdukiewicz et al. 1997).

Selective transcription of the groups of genes by the NEP and PEP provides a mechanism of differential gene expression during chloroplast development. For example, genes with NEP promoters are preferentially transcribed early in chloroplast development (Fig. 23.5). These provide mainly housekeeping functions and components of the plastid genetic machinery, including *rrn*, *atpB*, *atpI* and *clpP* and subunits of the PEP. This leads to a dramatic increase in the transcription and translation activity in the chloroplast. Once the NEP function has led to the translation of the PEP chloroplast-encoded subunits, the PEP becomes more important in the expression of photosynthesis-related genes, such as *rbcL*, *psbA* and *psbD* (Fig. 23.5) (Hajdukiewicz et al. 1997), ultimately leading to the synthesis and assembly of the photosynthetic machineries.

Chloroplast maturation is accompanied by the repression of NEP activity. The replacement of NEP activity by PEP activity in chloroplasts defines a switch in differential gene expression and a commitment to photosynthetic development. The molecular mechanism underlying the switch is however questionable; it has been hypothesized that glutamyl-tRNA, a product of PEP transcription, binds to and inhibits the transcriptional activity of NEP, however, the specificity of this interaction is unclear (Bohne et al. 2009; Hanaoka et al. 2005). Alternatively, thylakoid sequestration may down-regulate NEP activity; however, given that the plastid transcription machinery is membrane-associated

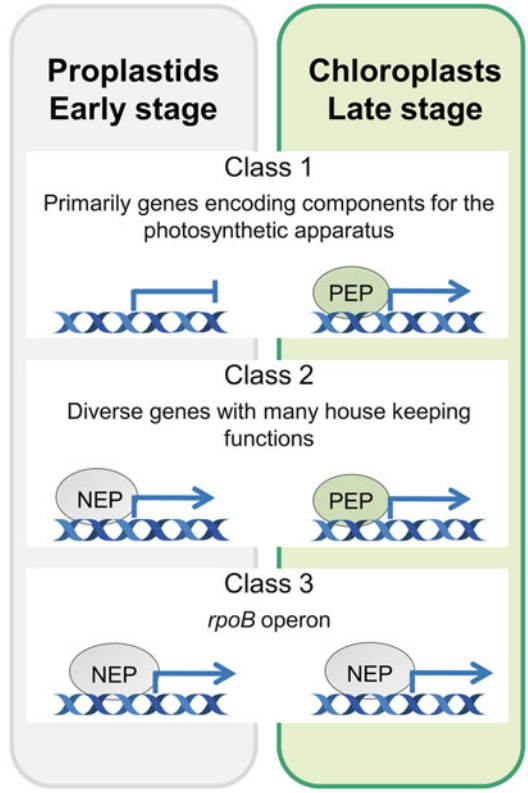

Fig. 23.5. Transcription from NEP- or PEP-dependent promoters in proplastids and chloroplasts. Differential gene expression during chloroplast development is partially based on recognition of distinct classes of promoters by NEP and PEP. In proplastids the NEP is most active and responsible for the transcription of housekeeping genes (Class 2), as well as a small class of genes including the *rpoB* operon, which encodes for subunits of the PEP (Class 3). In the proplastid the photosynthetic genes are not transcribed. During chloroplast biogenesis the subunits of the PEP are transcribed and the activity of the PEP becomes more important as the chloroplast develops. In the mature chloroplast the photosynthetic genes (Class 1) and the housekeeping genes (Class 2) are transcribed by the PEP.

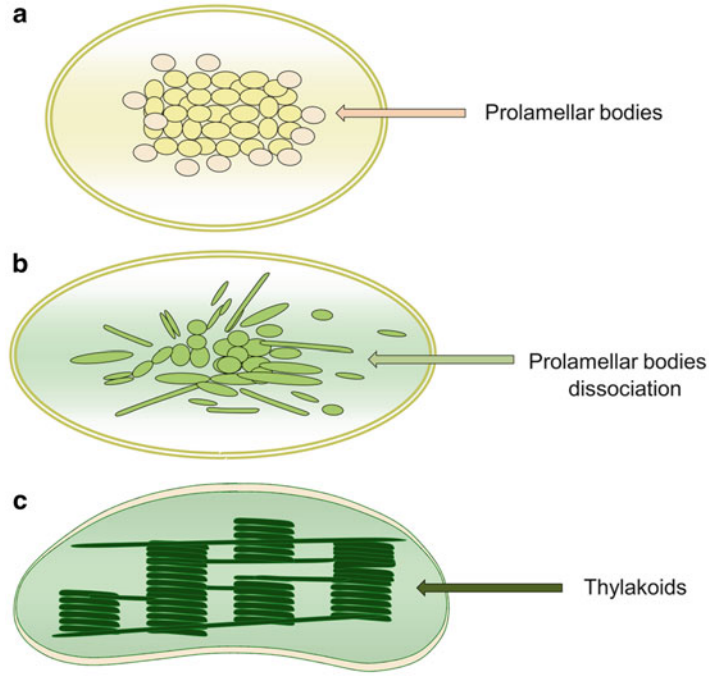

Fig. 23.6. Thylakoid formation in the presence of light. (**a**) Etioplast are characterized by the presence of a semicrystalline structure called prolamellar bodies (PLBs). (**b**) Upon illumination the PLBs dissociate and lamellar structures begin to be assemble. (**c**) Fully developed thylakoid networks are observed after several days depending on the species and light conditions.

more work is required to ascertain whether thylakoid association affects NEP activity (Azevedo et al. 2008).

After the switch to PEP activity in chloroplasts, the transcription of PEP-transcribed genes remains under nuclear control through the action of nuclear encoded sigma factors, which provide promoter specificity to PEP. The *Arabidopsis* genome encodes six sigma factors, which are expressed in a developmental- and light-dependent manner. Several of the sigma factors are plastid-localized and have overlapping functions and the functional role of sigma factors during chloroplast biogenesis is yet to be fully understood (Fujiwara et al. 2000; Isono et al. 1997).

D. Thylakoid Membrane Biogenesis

Thylakoid membranes are found exclusively in organisms performing photosynthesis. In plants the thylakoid membranes are arranged into highly interconnected lamellar networks by the presence of cylindrical stacks called grana, which are linked to each other by unstacked membrane regions called stroma lamellae (Adam et al. 2010).

Thylakoid ontogeny in plant is highly dependent on the development of the chloroplasts from undifferentiated proplastids. Proplastids contain very few internal membranes, often found as vesicles containing little or no photosyntethic complexes. In the absence of light proplastids differentiate into etioplasts, which contain prolamellar bodies and perforated lamellae (Fig. 23.6). The prolamellar body is a paracrystalline structure consisting of lipids, NADPH, the precursor of chlorophyll α (protochlorophyllide) and the enzyme that calalyzes its photoreduction to chlorophyllide (Vothknecht and Westhoff 2001).

In the presence of light, proplastids develop into mature chloroplast (Fig. 23.6). Under normal conditions, membrane formation and protein complex assembly occur in a coordinated manner. First, prolamellar bodies start to disassemble and the formation of long, unconnected lamellae is observed (Sperling et al. 1998). How this transition occurs is still unclear. Next, lamellar regions begin to overlap and the assembly and incorporation of photosynthetic complexes into the membrane leads to the formation of the

grana. The establishment of granal domains is likely driven by the tendency of PSII and light harvesting complex II (LHCII) to assemble into super-complexes (Horton et al. 1991). Fully developed networks are completed within one to several days, depending on the plant species and light conditions.

The formation of any membrane structure requires lipid molecules. The thylakoid membranes have a unique lipid composition different from other cell membranes. The thylakoid lipids consist of galactosyl diglycerides and both monogalactosyl diacylglycerol and digalactosyl diacylglycerol are exclusively found in plastid membranes (Block et al. 1983). Galactolipids, due to two highly unsaturated fatty acyl chains, have a poor tendency to form bilayers in aqueous solution. This feature makes the lipid bilayer assembly process in thylakoid membranes rather complex. The formation of bilayer structures is an energetic process that requires LHCII and membrane proteins that have to be combined with lipids. It has indeed been shown that increasing the protein to lipid ratio induces the formation of ordered lamellar structures (Simidjiev et al. 2000).

How the lipid trafficking is carried out is controversially discussed. The vesicular transport mechanism theory is supported by the observation of membrane vesicles in leaves exposed to normal and low temperatures (Morre et al. 1991). Analysis of *Arabidopsis* mutants led to the identification of VIPP1 (vesicle-inducing protein in plastids 1), a protein conserved from cyanobacteria to plants (Kroll et al. 2001; Westphal et al. 2001), that was found to be associated with both the inner envelope and the thylakoid membranes. At low temperatures a the *vipp1* mutant does not accumulate vesicles, suggesting that VIPP1 may be involved in the budding of vesicles from the plastid envelope membrane (Kroll et al. 2001). Alternatively, lipids may be shuttled to the thylakoid membranes through direct connections with the inner envelope membrane (Shimoni et al. 2005).

III. Regulation and Maintenance of Chloroplast Populations

A. Chloroplast Division

Once chloroplasts have differentiated from proplastids, chloroplast must divide in order to match cell division and expansion and to provide the full complement of chloroplast in mature mesophyll cells. *Arabidopsis* leaf mesophyll cells contain over 100 chloroplasts (Marrison et al. 1999). Proplastid and chloroplast division is regulated and coordinated with mesophyll cell division and expansion, which is supposed to assure the size and photosynthetic competence of the mature chloroplast. During leaf development chloroplasts become larger, whereas dumbbell-shaped plastids became less common, suggesting that division occurs early in chloroplast biogenesis (Pyke 1999).

Chloroplasts divide by binary fission whereby constriction of the inner and outer membranes occurs at the division site (Fig. 23.7). This process is driven by the coordinated action of two distinct machineries located on the inner and outer chloroplast envelopes. The division machinery involves proteins derived from the cytokinetic machinery of cyanobacteria and proteins originating from the eukaryotic host cell. FtsZ is thought to be one of the first proteins of the inner machinery to arrive at the division site where it acts as a scaffold for other proteins (Osteryoung et al. 1998). It is believed that polymerization of the FtsZ proteins into a ring structure (Z-ring) is the initiating event in plastid division. The *Arabidopsis* plastid division component ARC6 is required for the stability and the maintenance of the Z-ring whilst its paralogue (PARC6) appears to destabilize the Z-ring (Glynn et al. 2009; Vitha et al. 2003). Moreover, the Min proteins regulate the exact placement of the Z-ring in order to ensure the generation of two equally sized daughter plastids (Colletti et al. 2000; Maple et al. 2002). Once the stromal ring is formed and fixed to the inner membrane the plastid division proteins (PDV) PDV1 and

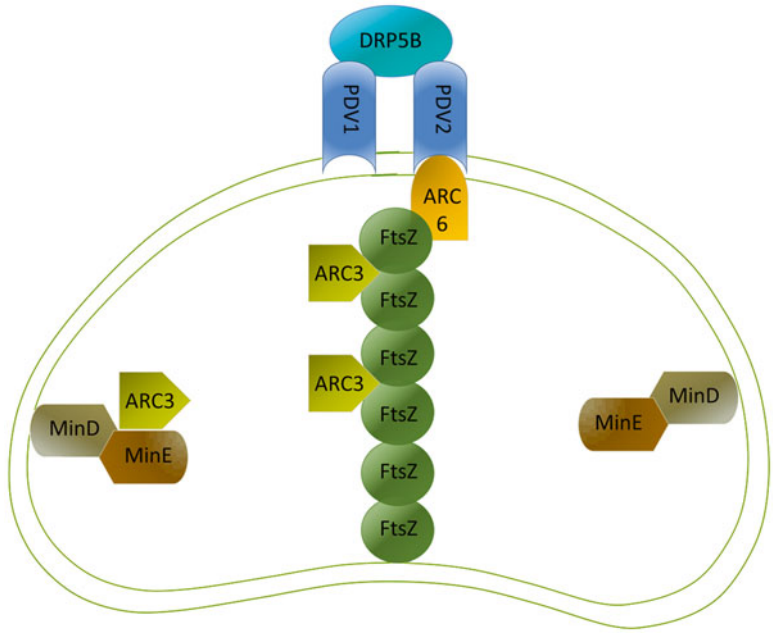

Fig. 23.7. Model of the plastid division machinery. Chloroplasts divide by binary fission whereby constriction of the inner and outer membranes occurs at the division site. Initially the FtsZ proteins (AtFtsS1-1, AtFtsZ2-1 and AtFtsZ2-2) form a Z-ring at the centre of chloroplasts. ARC6 and ARC3 are recruited to the Z-ring through specific interactions with AtFtsZ2-1 and AtFtsZ1-1, respectively. ARC6 is required for the stability and the maintenance of the Z-ring while ARC3 and Min proteins regulate the exact placement of the Z-ring. PDV1 and PDV2 localize to ring-like structures on the cytosolic surface of the outer envelope membrane and recruit DRP5B to the division site to constitute the cytosolic division machinery. Coordination and signalling between the two division machineries may occur through a direct interaction between ARC6 and PDV2.

PDV2, in the outer membrane, recruit DRP5B (cytosolic dynamin-like protein) around the chloroplast in alignment with the inner ring (Gao et al. 2003; Miyagishima et al. 2006). It has been shown that when the interaction between PDV2 and ARC6 is disrupted PDV2 is unable to recognize the chloroplast division site and subsequently unable to recruit DRP5B to the correct place (Glynn et al. 2008). This demonstrates that the two division machineries are coordinated with each other. Recently, it has been found that the PDV levels determine the rate of chloroplast division (Okazaki et al. 2009). Since the level of PDV2 has been shown to decrease during leaf development, whilst the levels of FtsZ and DRB5 remain similar, it suggests that PDV proteins may play a regulatory role in development. Furthermore, application of exogenous cytokinin leads to an increase in PDV2 levels, accompanied by an increase in chloroplast number in cotelydons, supporting a role of PDV proteins during development.

The rate of chloroplast development is an important determinant of the photosynthetic capacity of leaves. The control of chloroplast division and maintenance of chloroplast numbers is of fundamental importance. *Arabidopsis* accumulation and replication of chloroplast mutants (*arc* mutants) show severely decreased numbers of chloroplasts and have a significantly reduced photosynthetic capacity and altered thylakoid architecture. This in turn leads to a different light harvesting capacity that seems also to affect their ability to adapt to changes in growth conditions and light intensity (Austin II and Webber 2005).

B. Fine Tuning of the Differentiation Pathway

Whilst all green tissues contain chloroplasts, different cells within the tissue can harbor different chloroplast complements, which can further vary in terms of size and levels of membrane development and chlorophyll accumulation. This demonstrates that the process of chloroplast biogenesis is controlled in a cell-specific manner. The mechanisms in place to fine tune the process are not well understood. However, a number of mutants have been identified with defects in both chloroplast biogenesis and the cell cycle, which represent potential candidates to link plastid differentiation, plastid division and cell division. For example, CRUMPLED LEAF (CRL) is targeted to the plastid envelope and *crl* mutants shows defects in not only plastid division and expansion, but also in overall plant development (Asano et al. 2004; Chen et al. 2009). Additionally, deficiencies in the two *CDT1* genes (*AtCDT1a* and *AtCDT1b*) *lead to cell cycle abnormalities and large*, unevenly divided chloroplasts (Raynaud et al. 2005). *AtCDT1a* and *AtCDT1b* are key components needed to initiate nuclear DNA replication and are also targeted to plastids where they interact with ARC6, suggesting that they play a major role in regulating events in both the nucleus and plastid (Raynaud et al. 2005).

In addition to cell-specific variations in chloroplast biogenesis, chloroplasts must also sense and adapt to environmental fluctuations that the cell and tissue responds to. For example, within a leaf, chloroplasts experience different levels of light and in order to adapt to these fluctuations in light levels chloroplast acclimatize by adjusting the proportion of light energy used to drive photosynthesis. Recent work has highlighted the GLK transcription factors, which regulate a large suite of genes involved in light-harvesting and thylakoid protein complex formation in a cell-autonomous fashion, thereby enabling the photosynthetic capacity of each cell in a leaf to be regulated independently (Waters et al. 2008).

IV. Conclusions

Chloroplast biogenesis is a complex and highly regulated process and our understanding of the molecular mechanisms involved during proplastid to chloroplast differentiation has improved dramatically in the last decade. Although the molecular framework of chloroplast biogenesis is starting to be unraveled, it remains a major challenge to dissect the precise control mechanisms, which integrate environmental, cellular and temporal factors that affect chloroplast biogenesis and development. It is also intriguing to consider that differentiation from proplastids to chloroplasts is probably a default developmental pathway, with non-photosynthetic forms of plastids arising after the evolution of chloroplasts. It will be fascinating to discover, what mechanisms are in place to prevent chloroplast biogenesis in certain tissues and instead promote the vastly different pathways of plastid differentiation, which lead to the development non-photosynthetic plastids. Recent years have also seen an increase in our understanding of the components essential for chloroplast division, but the mechanisms in place to control the number of plastids in a cell-specific manner remains unclear. Recent identification of the role of both the PDV proteins and hormones will add new impetus to this field of research.

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