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The making of a chloroplast

Mark T Waters and Jane A Langdale*

Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, UK

Since its endosymbiotic beginning, the chloroplast has become fully integrated into the biology of the host eukaryotic cell. The exchange of genetic information from the chloroplast to the nucleus has resulted in considerable co-ordination in the activities of these two organelles during all stages of plant development. Here, we give an overview of the mechanisms of light perception and the subsequent regulation of nuclear gene expression in the model plant *Arabidopsis thaliana*, and we cover the main events that take place when proplastids differentiate into chloroplasts. We also consider recent findings regarding signalling networks between the chloroplast and the nucleus during seedling development, and how these signals are modulated by light. In addition, we discuss the mechanisms through which chloroplasts develop in different cell types, namely cotyledons and the dimorphic chloroplasts of the C_4 plant maize. Finally, we discuss recent data that suggest the specific regulation of the light-dependent phases of photosynthesis, providing a means to optimize photosynthesis to varying light regimes.

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Introduction

As a defining feature of plants, the chloroplast represents a marvel of evolution. Since its origin as a cyanobacterial symbiont about 1 to 1.5 billion years ago (Douzery *et al*, 2004; Yoon *et al*, 2004), this organelle has become fully integrated into the life cycle of photosynthetic eukaryotes and has essentially underpinned global ecosystems. Photosynthesis comprises two conceptually distinct phases that occur entirely within the chloroplast. The light-dependent reactions take place on the thylakoid membrane, in which light energy drives electron transport between a series of multi-subunit protein complexes. In two of these complexes, photosystem I (PSI) and photosystem II (PSII), protein-bound chlorophyll pigments are excited by light and initiate electron flow, so generating ATP and reducing equivalents. This chemical energy is then used in the light-independent reactions that take place in the chloroplast stroma, in which CO_2 is fixed by Rubisco to generate sugars. Subsequently, this carbohydrate is either immediately exported to the cytosol or is stored within the chloroplast as starch. Beyond photosynthesis, the chloroplast is also the site of fatty acid biosynthesis, nitrate assimilation and amino-acid biosynthesis. Given the importance of plant products to human beings, photosynthetic development and the biogenesis of chloroplasts have received intense scrutiny. In seed plants, chloroplasts develop from a non-photosynthetic form called the proplastid, which is transmitted between generations through the ovule and is maintained in meristematic stem cells. How does a chloroplast develop from a proplastid? How is photosynthetic competence reached and sustained? These are certainly complex and open questions, but two central themes emerge. First, the co-ordination and integration of multiple parallel processes, none of which operates in isolation, are absolutely necessary. This theme is most clearly shown by the fact that mutations in single chloroplast components can have major ramifications beyond the immediate process in question. Second, constant interorganellar crosstalk occurs both during the initial construction of the chloroplast and to maintain form and function in mature tissues. Coupled with the need to respond to a constantly variable environment, this crosstalk reflects the existence of two genomes and the need to regulate dynamically the relative input from each towards constituent parts of the chloroplast. This review covers some of the major cellular and developmental aspects of chloroplast biogenesis that encompass the above themes.

Light signalling during photomorphogenesis

In seed plants, light is a prerequisite for the synthesis of chlorophyll, and chloroplasts do not develop in the dark. Photomorphogenesis describes the developmental programme undertaken by seedlings exposed to light, and is typified by the inhibition of hypocotyl growth, the development of chloroplasts and the opening of cotyledons (in eudicotyledonous species). Light is perceived by a suite

*Corresponding author. Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK.
Tel.: +44 1865 275099; Fax: +44 1865 275074;
E-mail: jane.langdale@plants.ox.ac.uk

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of wavelength-specific photoreceptor proteins that undergo conformational changes to allow interaction with downstream signalling partners. The phytochromes, which perceive red and far-red light, and the cryptochromes, which respond to blue and UVA light, are the two varieties of photoreceptor responsible for photomorphogenesis (Jiao *et al*, 2007). In *Arabidopsis*, there are five phytochromes (encoded by *PHYA* to *PHYE*), of which primarily phyA and phyB act during seedling photomorphogenesis (Quail, 2002; Tepperman *et al*, 2006). Phytochromes exist in the cytosol in an inactive Pr form that is activated by light and is converted into the biologically active Pfr form, which translocates into the nucleus to initiate signalling (Quail, 2002). The cryptochromes are represented by three proteins: cry1, which translocates from the nucleus to the cytosol on light activation; cry2, which is constitutively nuclear localized and cry3, which seems to be dual targeted to mitochondria and plastids (Kleine *et al*, 2003; Lin and Shalitin, 2003). A great deal of effort has been invested in clarifying the signalling and transcriptional networks that follow the perception of light, and the field has recently been reviewed thoroughly elsewhere (Jiao *et al*, 2007). Here, we offer a brief overview of the light signalling pathways that lead to the biogenesis of chloroplasts to provide a basis for introducing recent findings regarding signalling mechanisms.

A series of genetic screens to uncover regulators of light-dependent development revealed a class of loci that, when mutated, confer a partially constitutively photomorphogenic (*cop*) or de-etiolated (*det*) phenotype (reviewed by von Arnim and Deng, 1996). Collectively, these mutants define the *COP/DET/FUS* class of loci. When grown in the dark, these mutants resemble light-grown seedlings in many respects, typically with a short hypocotyl, open, expanded cotyledons and enhanced levels of photosynthetic gene expression. They do not show complete chloroplast development in the dark, because chlorophyll synthesis requires light, and photosystems cannot assemble without chlorophyll; however, plastids in dark-grown *cop1* and *cop9* seedlings, for example, contain a partially formed thylakoid network instead of normal etioplasts (see below) (Deng and Quail, 1992; Wei and Deng, 1992). Furthermore, *cop1* and *det1* are hyper-responsive to light, exhibiting ectopic chloroplast development in the roots (Chory and Peto, 1990; Deng and Quail, 1992). The recessive nature of these mutants suggests that the COP/DET/FUS proteins are light-inactivatable repressors of photomorphogenesis in the dark, and that they also have a function in suppressing chloroplast development in non-photosynthetic tissues. It is now known that several of these loci encode subunits of the COP9 signalosome (CSN), a nuclear-localized protein complex that functions as part of the ubiquitin-proteosome pathway, which regulates E3 ubiquitin ligases (Wei *et al*, 2008). *COP1* encodes such a ligase (Seo *et al*, 2003). COP1 activity is regulated in part at the level of nucleo-cytoplasmic partitioning: in the dark, it is preferentially localized to the nucleus, and it transfers to the cytoplasm in the light (von Arnim and Deng, 1994). COP1 functions together with three other components, COP10, DET1 and DNA damage-binding protein 1B (DDB1), to target specific proteins such as HY5 for proteasomal destruction by the CSN (Osterlund *et al*, 2000; Yanagawa *et al*, 2004).

HY5 is a positive regulator of photomorphogenesis under a broad spectrum of light, suggesting that it acts downstream of phyA, phyB and the cryptochromes (Chory, 1992). It encodes a bZIP transcription factor that binds to a conserved G-box motif, CACGTG, in the promoters of many light-regulated genes including those related to photosynthesis (Oyama *et al*, 1997; Lee *et al*, 2007). HY5-binding targets account for some 60% of those genes regulated by phytochromes within 1 h of light exposure (Lee *et al*, 2007), suggesting that HY5 acts high up in the hierarchy of photomorphogenic regulation. Both phy- and cry-dependent signalling lead to an increase in HY5 levels (Osterlund *et al*, 2000), and while the phy-dependent mechanism for this observation is not fully understood, photo-activated cry1 inhibits COP1 in the nucleus, thus preventing HY5 degradation; this may be brought about through the translocation of COP1 into the cytoplasm (Figure 1) (Yang *et al*, 2001). Thus, cryptochrome-mediated chloroplast development is at least partly mediated through HY5. Phytochrome signalling, meanwhile, makes extensive use of a basic helix-loop-helix family of transcription factors called phytochrome-interacting factors or PIFs (Castillon *et al*, 2007). PIFs control distinct but overlapping sets of responses—again by binding to the G-box motif—and are mainly considered to be negative regulators of photomorphogenesis that act by blocking transcription (Castillon *et al*, 2007). The founding member of the PIF family, PIF3, has been characterized in some detail. On light exposure, phyB moves into the nucleus and binds to PIF3, triggering its phosphorylation and rendering it susceptible to degradation; however, this degradation is not mediated by COP1 (Bauer *et al*, 2004). Transcription from photomorphogenesis-related genes is then able to proceed (Figure 1). Recent evidence has shown that, in the dark, PIF3 negatively regulates the expression of *HEMA1* and *GUN5*, genes encoding two key regulatory enzymes in the chlorophyll biosynthetic pathway, and of *LHCA1* and *PsaE1*, two genes encoding PSI components (Shin *et al*, 2009). Consistent with this, dark-grown *pif3* mutants accumulate double the wild-type level of protochlorophyllide (Pchlide), a late chlorophyll intermediate, in the dark (Shin *et al*, 2009). PIF1 has also recently been shown to control chlorophyll biosynthesis, partly through direct interaction with the promoter of *PORC*, a gene that encodes Pchlide oxidoreductase (Moon *et al*, 2008). Significantly, *pif1 pif3 pif4 pif5* quadruple mutants are constitutively photomorphogenic with short hypocotyls and open cotyledons (Shin *et al*, 2009), revealing that this family of transcription factors strongly represses a suite of photomorphogenic attributes, especially chloroplast development.

Transition from proplastid to chloroplast

Once the seedling has become photoautotrophic, the next key stage in photomorphogenesis is activation of the shoot apical meristem (SAM) to produce leaves and chloroplasts therein. The *hy1* mutant is unable to synthesize phytochromobilin, the chromophore of phytochromes, and, therefore, lacks all phytochrome activity (Muramoto *et al*, 1999). Triple *hy1 cry1 cry2* mutants are highly defective in the release of SAM arrest, showing that phytochromes and cryptochromes act redundantly to initiate leaf production after emergence from the dark (Lopez-Juez *et al*, 2008). A careful transcriptome analysis of the SAM immediately after light exposure has revealed that the release of SAM arrest is accompanied by the upregulation of cytokinin and giberellin responses, and the

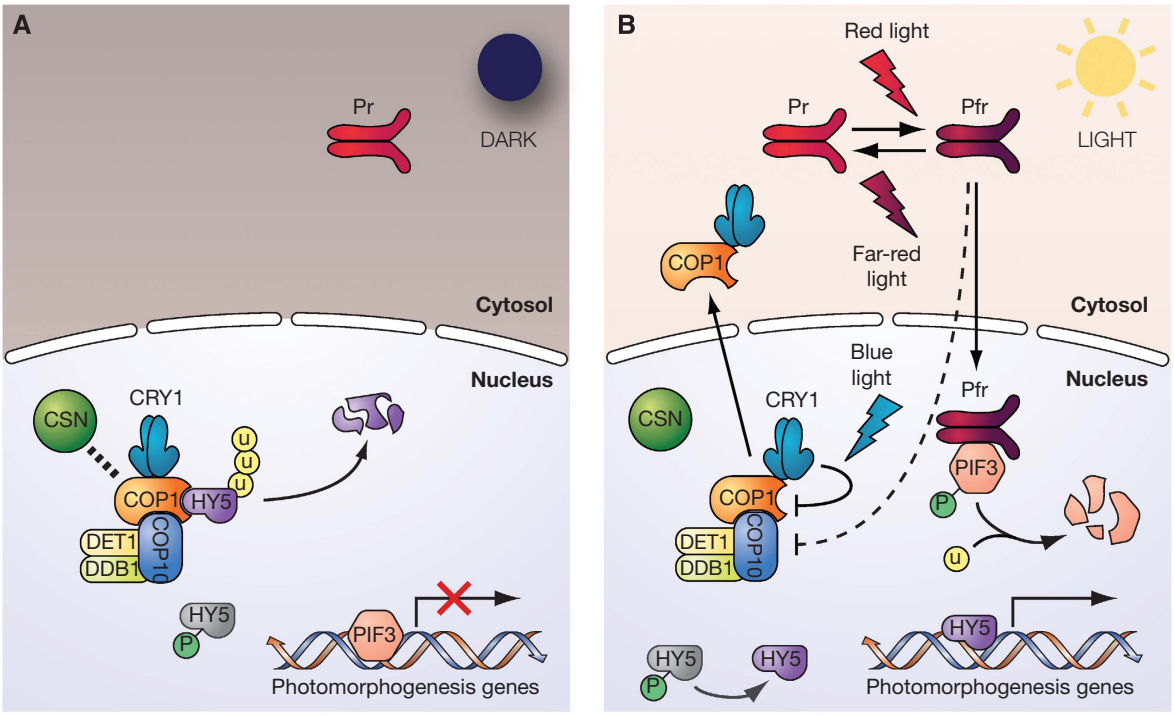

Figure 1 A simplified model of light signalling during photomorphogenesis. (**A**) In darkness, phytochrome dimers are in the inactive Pr state in the cytoplasm, and inactive CRY1 dimers are bound to COP1 in the nucleus. CSN, COP1 and the COP10/DET1/ DDB1 (CDD) complexes co-operate to promote the ubiquitination of photomorphogenesis-promoting transcription factors such as HY5. The CSN stabilizes the CDD complex and may regulate the activity of COP1. HY5 interacts with the WD40 repeat domain of COP1 and is ubiquitinated by the ubiquitin E3 ligase activity of COP1. Polyubiquitinated HY5 is subsequently degraded, presumably by the 26S proteasome. HY5 is mostly phosphorylated in the dark, a form that interacts poorly with target promoters; in addition, COP1 preferentially interacts with the unphosphorylated form of HY5, further suppressing levels of biologically active HY5 (Hardtke *et al*, 2000). In parallel, PIF3 is bound to G-box sequences in target promoters, inhibiting transcription of photomorphogenesis-related genes. (**B**) Blue light exposure triggers the photoactivation of CRY1, which leads to the exit of COP1 from the nucleus and thus allows HY5 levels to increase. HY5 is dephosphorylated, increasing its biological activity and further reducing its affinity for COP1; more HY5 is then available to bind to G-box motifs and promote transcription of genes such as *light-harvesting chlorophyll-binding1* (*Lhcb1/CAB1*), a major antenna protein of PSII. Note that HY5 can also negatively regulate transcription of target genes and is necessary, but insufficient to regulate transcription alone (Lee *et al*, 2007). Meanwhile, Pr is converted into the biologically active Pfr form by red light, which translocates into the nucleus and binds PIFs (such as PIF3). Phy-bound PIF3 is phosphorylated, rendering it susceptible to ubiquitination and subsequent degradation. As a result, transcription of genes such as those involved in chlorophyll biosynthesis can proceed. Phy-dependent repression of COP/DET/FUS proteins (revealed by epistasis) is depicted by a dashed arrow. Note that PIF3-regulated genes are not necessarily HY5 regulated, even though both transcription factors bind DNA through the G-box. In addition, there is some evidence that phyB may interact with COP1 (Yang *et al*, 2001). For abbreviations, see text.

expression of genes involved in ribosome production, protein translation and cell proliferation before visible leaf emergence (Lopez-Juez *et al*, 2008). Genes involved in chloroplast biogenesis—primarily photosynthesis genes—are expressed subsequently, 6 to 24 h after light exposure (Lopez-Juez *et al*, 2008). Within the leaf primordium, phytochromes and cryptochromes bring about a myriad of changes that initiate chloroplast biogenesis, and a series of subsequent molecular events must occur in parallel to complete the process successfully. Obvious activities include the import of nuclear-encoded proteins, the ramping up of chlorophyll levels and the establishment of a thylakoid network complete with photosynthetic electron transport (PET) complexes. Table I and Figure 2 summarize the main functional processes that occur in making a chloroplast, along with examples of chloroplast components that perform those processes. Below, we discuss some aspects of this process in more detail.

Protein import

The biogenesis of chloroplasts requires substantial protein import from the cytosol. Most chloroplast proteins are imported through the Toc/Tic complex, which both recognizes and transports nascent proteins across both envelope membranes (for a review, see Soll and Schleiff, 2004). Major components of the Toc/Tic complex are upregulated by light and even provide substrate specificity. For example, the *Arabidopsis* Toc33 knockout mutant, *ppi1*, is defective in the import and accumulation of photosynthetic proteins, but not of most non-photosynthetic proteins, and *AtTOC33* is most strongly expressed in young, light-grown seedlings (Kubis *et al*, 2003). Toc159, a GTP-dependent molecular motor that drives translocation, is also required for precursor protein recognition. The Toc159 subunits are encoded by four genes in *Arabidopsis*: *AtTOC159*, *AtTOC132*, *AtTOC120* and *AtTOC90*. The *atToc159* mutant is albino and does not survive past the cotyledon stage, implying that the other Toc159 family members cannot compensate for this defect (Bauer *et al*, 2000). Furthermore, overexpression of *AtTOC159* is unable to complement the pale green *atToc132 atToc120* phenotype (Kubis *et al*, 2004). Together, these findings imply that each Toc159 isoform exhibits substrate selectivity. Expression of such different isoforms may provide an

Table I Examples of nuclear-encoded, chloroplast-localized components necessary for chloroplast biogenesis, grouped by functional class

Protein	Molecular function	Mutant phenotype[a]	Remarks	Reference
Protein import and suborganellar targeting				
AtTOC33	Protein translocation across outer envelope	Pale green, especially juvenile plants (*ppi1*)	Involved in import of photosynthetic proteins	Kubis *et al* (2003)
cpSRP43	Subunit of stromal signal recognition particle	Pale green with reduced levels of thylakoid protein complexes (*chaos*)	Mediates insertion of proteins into thylakoid membrane	Klimyuk *et al* (1999), Amin *et al* (1999)
RNA processing				
PPR4	Splicing of plastid *rps12* transcript	Embryo lethal (*ppr4*)	PPR family member required for plastid ribosome biogenesis	Schmitz-Linneweber *et al* (2006)
CRR2	PPR-like protein; regulates RNA splicing between *rps7* and *ndhB* transcripts	Impaired accumulation of NDH complex (*crr2-1* and *crr2-2*)	NDH complex is involved in cyclic electron flow around PSI	Hashimoto *et al* (2003)
SVR1	Pseudouridine synthase, RNA editing	Yellow-green; reduced stature (*svr1-2*)	*svr1* is also a suppressor of *var2*	Yu *et al* (2008)
Protein maturation and degradation				
BSD2	DnaJ-like protein chaperone	Pale green due to abnormal BS cell chloroplasts (*Zea mays*)	Required for post-transcriptional regulation of Rubisco large subunit (LSU)	Brutnell *et al* (1999)
FtsH2 (VAR2)	ATP-dependent metalloprotease	Variegated yellow-green leaves; cotyledons normal (*var2*)	Likely function in D1 protein turnover in photodamaged PSII	Chen *et al* (2000), Lindahl *et al* (2000)
ClpP6	Stromal ATP-dependent Clp protease	RNAi lines exhibit chlorosis of younger leaves	Degrades a variety of stromal proteins	Sjögren *et al* (2006)
Plastid gene expression				
SIG6	Sigma factor conferring promoter specificity to RNA polymerase	Delayed greening in cotyledons (*sig6-1*)	One of many sigma factors required for plastid gene transcription	Ishizaki *et al* (2005)
FUG1	Plastid translation initiation factor	*fug1-2* is embryo lethal	*fug1* alleles suppress *var2*	Miura *et al* (2007)
Thylakoid biogenesis and lipid biosynthesis				
AtTerC	Unknown; required for early thylakoid biogenesis	Seedling lethal on light exposure	Similar to bacterial tellurite resistance proteins	Kwon and Cho (2008)
FZL	Dynamin-like GTPase; membrane fusion	Pale green; disorganized granal thylakoids	May be involved in thylakoid remodelling	Gao *et al* (2006)
MGDG synthase	Catalyses final step in MGDG biosynthesis	Sucrose required for germination; albino; frequent inner envelope invaginations	Mutant phenotype supports budding hypothesis for thylakoid biogenesis	Kobayashi *et al* (2007)
VIPP1	Possible function in membrane budding from inner chloroplast envelope	Viable with exogenous sucrose	Protein located on inner envelope and thylakoid membrane	Kroll *et al* (2001), Aseeva *et al* (2007)
Chlorophyll biosynthesis				
GUN4	Enhances Mg-cheletase activity	Pale green (*gun4-1*, weak); yellow-white (*gun4-2*, null)	Essential under normal growth conditions	Larkin *et al* (2003)
CHLM	Mg-protoporphyrin methyltransferase	*chlm* null mutants are albino and lack thylakoid protein complexes	Essential under normal growth conditions	Pontier *et al* (2007)
Metabolite transport				
CUE1 (AtPPT1)	Imports phospho*enol*pyruvate (PEP) into chloroplast stroma	Reticulate pale green leaves with dark green BS cells; perturbed M cell differentiation	PEP is required for fatty acid, amino acid and isoprenoid biosynthesis through the shikimate pathway	Li *et al* (1995), Streatfield *et al* (1999)
Photosystem assembly				
LPA2	Required for stability/ assembly of PSII core	Pale green (*lpa2*); reduced PSII levels	Intrinsic thylakoid protein	Ma *et al* (2007)

PPR, pentatricopeptide repeat protein; NDH, nicotinamide dinucleotide (phosphate) dehydrogenase; MGDG, monogalactosyldiacylglycerol, a non-phosphorous glycolipid of thylakoid membranes.
[a]*Arabidopsis* unless otherwise specified.

efficient strategy for enhancing the rate of photosynthetic protein import over that of non-photosynthetic proteins during early chloroplast development.

Thylakoid biogenesis

Thylakoid membranes are rich in galactolipids, which are synthesized in the chloroplast envelope membranes (Kelly

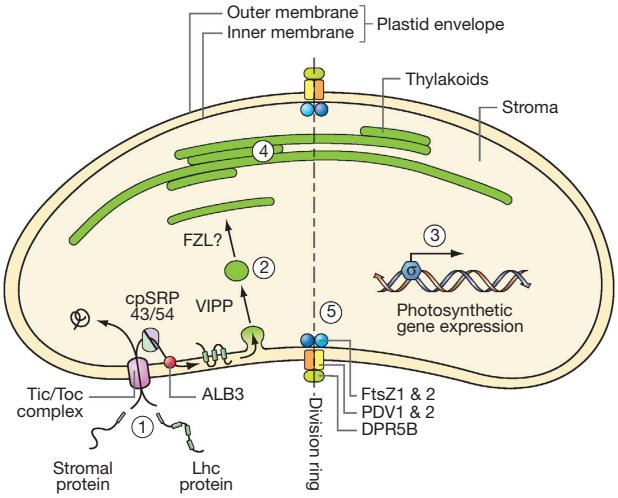

Figure 2 Early events during the transition from proplastid to chloroplast. (1) Import of nuclear-encoded proteins through the Tic/Toc complex. Stromal proteins fold directly in the stroma with the assistance of chaperone proteins. Some thylakoid-targeted proteins, such as Lhc, are recognized by the stromal chloroplast signal recognition particle (cpSRP43/54), which mediates insertion of the protein into the inner envelope (IE) membrane (Amin *et al*, 1999; Klimyuk *et al*, 1999). Complete insertion of Lhc requires the membrane-resident protein ALB3 (Bellafiore *et al*, 2002), and the binding of chlorophyll and carotenoids that are synthesized on the IE membrane. Note that the targeting of proteins to the thylakoid membrane is highly simplified here; the cpSRP- and ALB3-dependent route is only true for certain thylakoid-resident proteins such as Lhc, which may also insert directly into the thylakoid network, bypassing the IE membrane. (2) The thylakoid network is generated from Lhc/chlorophyll-laden vesicles derived from the IE membrane in a budding process dependent on factors such as VIPP1. GTPases such as FZL may perform further remodelling of thylakoid membranes into a reticulate network. (3) Concurrently, light activates PGE through nuclear-encoded sigma factors (σ), resulting in the synthesis of core proteins of the photosystem reaction centres, such as PsbD. Extensive additional regulation takes place at the levels of RNA processing and ribosome assembly. (4) Assembly of the photosystems and other electron transport components leads to further elaboration of the thylakoid network, forming stacked regions (grana) and unstacked stromal lamellae. (5) PDV involves the assembly of an inner PDV ring, consisting of FtsZ proteins, and an outer PDV ring that is partly comprised of DRP5B, which is recruited and anchored to the outer envelope membrane by the PDV proteins. The division rings form around the middle of the chloroplast, yielding two chloroplasts through binary fission.

and Dörmann, 2004), and galactolipid biosynthesis is essential for thylakoid formation (Kobayashi *et al*, 2007). Proplastids contain a limited amount of internal membranes, called prothylakoids, which form the starting point for the biogenesis of *bona fide* thylakoids. Many of the enzymes in the later stages of carotenoid and chlorophyll biosynthesis are also present on the plastid envelope, as these lipid-soluble pigments must be incorporated into light-harvesting chlorophyll (Lhc)-binding proteins that are being inserted into the inner envelope membrane as a continuation of the protein import process (Hoober *et al*, 2007). These hydrophobic components must reach the prothylakoids by crossing the aqueous stroma. Several lines of evidence suggest that vesicles bud from the inner envelope membrane, most likely carrying a cargo of chlorophyll, enzymes and photosynthetic proteins, and migrate across the stroma to fuse with the developing thylakoids. First, when leaves are cooled to 12°C, vesicle-like structures contiguous with the inner envelope membrane accumulate in the chloroplast stroma (Morre *et al*, 1991). Second, direct connections between the inner envelope and thylakoid membranes have been reported, implying that the two compartments represent a partly contiguous, dynamic continuum (Shimoni *et al*, 2005). Third, the *vipp1* mutant is defective in thylakoid formation and does not form cold-induced vesicles (Kroll *et al*, 2001; Aseeva *et al*, 2007). Another mutant, *thf1*, exhibits a variegated phenotype, and affected chloroplasts contain profuse vesicles with no thylakoid membrane (Wang *et al*, 2004). VIPP1 is associated with both the thylakoids and inner envelope, whereas THF1 is found in the stroma and thylakoids; the presence of two suborganellar locations is consistent with a trafficking function for these proteins. Finally, chloroplast bioinformatics has revealed the presence of homologues of small GTPases with putative membrane fusion functions similar to those in the eukaryotic secretory pathway, such as ARF1 and Sar1 (Andersson and Sandelius, 2004). Recently, a dynamin-like GTPase called FZL has been identified that specifically affects thylakoid membrane structure in *Arabidopsis*. Again, FZL is localized to both the inner envelope and the thylakoid membranes (Gao *et al*, 2006). Although *fzl* mutant plants are not deficient in thylakoid formation *per se*, *fzl* chloroplasts are large and unusually shaped, they contain abnormal proportions of stromal and granal lamellae and they frequently accumulate small vesicles (Gao *et al*, 2006). These findings imply that FZL is a membrane-remodelling factor that is required for maintaining a dynamic thylakoid network, but the basis for abnormal chloroplast division is unclear.

Chloroplast division

Once chloroplast biogenesis is underway, the chloroplasts must proliferate to match cell division and expansion: *Arabidopsis* mesophyll (M) cells can contain over 100 individual chloroplasts and the final count is tightly correlated with cell size (Pyke and Leech, 1994). The molecular nature of chloroplast division has been covered extensively in recent reviews (Maple and Moller, 2007; Yang *et al*, 2008), but one particular development is worth discussing here. As leaf development progresses, chloroplasts become progressively larger and dumb-bell-shaped plastids become less common, suggesting that division occurs early in chloroplast biogenesis (Pyke, 1999; Okazaki *et al*, 2009). Chloroplasts divide by binary fission, driven by two contractile protein rings that form on each side of the chloroplast envelope. The inner division ring forms first and is composed of the FtsZ1 and FtsZ2 proteins, which are homologous to bacterial fission proteins (Osteryoung and McAndrew, 2001). The constituents of the outer ring are not fully known, but the plastid division1 (PDV1) and PDV2 proteins in the outer envelope membrane recruit a cytosolic dynamin-like component, DRP5B, around the chloroplast exterior in alignment with the inner ring (Miyagishima *et al*, 2006). It has recently been shown that PDV1 and PDV2 are determinants of the rate and extent of chloroplast division, a question that has remained open for some time (Okazaki *et al*, 2009). *pdv1* and *pdv2* mutants had earlier been shown to contain large, deformed chloroplasts (Miyagishima *et al*, 2006), but when both PDV1 and PDV2 are overexpressed together, *Arabidopsis* M cells contain small chloroplasts that are twice as numerous as in wild type (Okazaki *et al*, 2009). PDV promoter activity is highest

around the meristem, in which proplastids are differentiating into chloroplasts. Crucially, the levels of PDV protein decrease in concert with the rates of chloroplast division as leaves aged, but FtsZ2 and DRPB5 levels remain at similar levels throughout development (Okazaki *et al*, 2009), tying in neatly with observed developmental patterns of chloroplast division and size. Constitutive expression of the cytokinin responsive transcription factor *CRF2* and application of exogenous cytokinins both increase the activity of PDV2, linking cell division and chloroplast division and implying that the PDV proteins are primary mechanistic components in determining the cell's chloroplast complement. This PDV-dependent mechanism seems to be evolutionarily conserved, holding true in the moss *Physcomitrella patens*, in common with other components of the PDV machinery such as FtsZ (Okazaki *et al*, 2009).

It is clear that molecular-genetic approaches have been incredibly powerful tools in establishing what events are critical for chloroplast biogenesis. A notable point is that mutations in genes required for any one particular molecular process, such as chloroplast RNA processing, severely hamper the establishment of photosynthetic competence in general, as many mutants are pale green, albino or even embryo lethal (Table I). As such, many chloroplast processes are in some way interdependent: for example, the light-harvesting complex of PSII (LHCII) is comprised of several Lhc-binding proteins, which are only imported into the chloroplast and properly folded in the presence of chlorophyll synthesized on the inner envelope membrane (Espineda *et al*, 1999; Reinbothe *et al*, 2006). Similarly, defects in lipid biosynthesis severely compromise chlorophyll levels and PET complex assembly because thylakoid membranes cannot be generated (Kroll *et al*, 2001; Kobayashi *et al*, 2007). Such tight mutual dependence requires exquisite co-ordination between the chloroplast, in which the events are happening, and the nucleus, in which many of the protein components are encoded.

Retrograde chloroplast-to-nucleus signalling

In many respects, chloroplast biogenesis is rather nucleocentric: the early events in light signalling dominate in the nucleus, and in *Arabidopsis*, the nucleus encodes about 2100 chloroplast proteins, compared with just 117 originating from the chloroplast genome (Richly and Leister, 2004; Cui *et al*, 2006). This forward, or anterograde, communication to the chloroplast is balanced by retrograde signals passing in the opposite direction. The existence of such signals has been well documented by a number of experimental approaches over the past 30 years (reviewed by Nott *et al*, 2006). When seedlings are treated with chemical inhibitors of chloroplast biogenesis, transcript levels of nuclear genes encoding photosynthetic proteins are reduced, implying the existence of a plastid-derived retrograde signal that can repress nuclear gene expression when chloroplasts are damaged. A genetic screen for mutants defective in such repression led to the isolation of five non-allelic nuclear loci called *genomes uncoupled* (*gun*) (Susek *et al*, 1993). All five of these loci have since been identified, of which four (*gun2* to *gun5*) encode plastid-localized proteins that function in tetrapyrrole biosynthesis, a pathway that culminates in heme and chlorophyll production (Nott *et al*, 2006). This observation led to a substantial body of evidence that implicated a chlorophyll intermediate—specifically Mg protoporphyrin IX (MgProtoIX)—as the identity of a negative signal emanating from defective plastids to repress gene expression in the nucleus (Mochizuki *et al*, 2001; Strand *et al*, 2003; Ankele *et al*, 2007). However, two recent landmark papers have shown that there is no correlation between the steady-state levels of MgProtoIX, or indeed any of the chlorophyll biosynthetic intermediates, and the degree to which nuclear photosynthetic gene expression is repressed (Mochizuki *et al*, 2008; Moulin *et al*, 2008). Instead, it is suggested that the destruction of chloroplasts may trigger the generation of short-lived reactive oxygen species (ROS) from limited amounts of tetrapyrrole intermediates, several of which are phototoxic (Mochizuki *et al*, 2008; Moulin *et al*, 2008). This is consistent with the sensitivity of nuclear gene transcripts to singlet oxygen that results from increased levels of Pchlide (op den Camp *et al*, 2003). However, a direct link between ROS and photosynthesis-related transcripts has yet to be shown.

Plastid gene expression pathway

Among all the original *gun* mutants, *gun1* is unique because it is the only one to respond similarly to both norflurazon, which inhibits carotenoid biosynthesis and induces photooxidative damage, and lincomycin, an inhibitor of plastid protein synthesis (Gray *et al*, 2003; Nott *et al*, 2006). Double mutant analyses have shown that *GUN1* and *GUN2–GUN5* define two distinct, but partially redundant signalling pathways that regulate overlapping groups of nuclear genes (Mochizuki *et al*, 2001; Strand *et al*, 2003). GUN1 is, therefore, required for a second signal that is triggered by defects in plastid gene expression (PGE). GUN1 was recently identified as a plastid-localized pentatricopeptide repeat protein that is associated with nucleoids, which are transcriptionally active complexes of plastid DNA, RNA and ribsomes (Koussevitzky *et al*, 2007). An abscisic acid-insensitive mutant, *abi4*, also exhibits a *gun* phenotype, showing that ABI4 is a further component of the PGE pathway (Koussevitzky *et al*, 2007). ABI4 is a nuclear transcription factor that binds to a sequence adjacent to or overlapping the G-box motif. GUN1 is also required for transmitting the 'MgProtoIX' signal described above, and for glucose-mediated repression of photosynthetic gene expression. As such, GUN1 acts as an integrator of several signals within the plastid (Koussevitzky *et al*, 2007). A model has, therefore, been proposed in which GUN1 is a master switch that generates or transmits an unknown signal, which in turn induces ABI4 to bind to promoter sequences and block photosynthetic gene expression in the nucleus, perhaps by inhibiting access of transcription factors such as HY5 to the G-box (Koussevitzky *et al*, 2007; Larkin and Ruckle, 2008). Key questions that remain are the mechanism by which GUN1 integrates multiple signals, one of which does not seem to be a chlorophyll intermediate after all, and the nature of the secondary signal that is transmitted subsequently.

Remodelling of retrograde signals by light

It is clear that during photomorphogenesis, developing chloroplasts are subject to a combination of positive and negative signals resulting from light and plastid status. How might these conflicting signals be integrated into an appropriate

gene expression response? It has recently been shown that light and plastid signals can modulate one another. In a genetic screen to identify new components in plastid signalling, Ruckle *et al* (2007) recovered four mutants with a subtle *gun* phenotype, all of which turned out to be *cry1* mutant alleles. This is surprising, because cry1 is usually considered to be a positive regulator of *Lhcb* expression. An even more surprising result was that *cry1 gun1* double mutants showed much stronger derepression of *Lhcb* when grown on lincomycin than either single mutant. This suggests that cry1 and GUN1 act synergistically to effect most, if not all, of *Lhcb* repression under blue light when chloroplast biogenesis is blocked, and that a plastid signal can convert cry1 from a positive into a negative regulator of *Lhcb*. This observation is true in blue light and white light, but not in red light, suggesting that when chloroplast biogenesis is blocked, maximum repression of *Lhcb* expression requires photo-activated cry1. Consistent with the function of cry1 acting through COP1 to regulate HY5, *cop1-4* is epistatic to *cry1* (i.e. *cry1 cop1-4* double mutants do not exhibit derepression of *Lhcb* expression when treated with lincomycin), and *hy5* is a subtle *gun* mutant (Ruckle *et al*, 2007). Furthermore, a *hy5 cry1* double mutant is indistinguishable from either single mutant in blue light, suggesting that both cry1 and HY5 operate in the same pathway. This implies that in healthy seedlings, cry1 acts through HY5 to promote *Lhcb* expression, but in the presence of dysfunctional plastids, a GUN1-independent signal converts HY5 into a negative regulator (Larkin and Ruckle, 2008). This is in agreement with additional data suggesting that HY5 alone is insufficient to regulate transcription, and that HY5 can act as both a positive and a negative regulator (Lee *et al*, 2007).

Curiously, *RbcS* expression is not derepressed in lincomycin-treated *cry1* or *cry1 gun1* mutants, suggesting that cry1 only induces *RbcS* expression and cannot repress it (Ruckle *et al*, 2007). Furthermore, under the conditions used by Ruckle *et al* (2007), HY5 does not induce *RbcS* in blue light because *hy5* mutants accumulate similar levels of *RbcS* transcripts as wild type. This implies the existence of another cry1/COP1-regulated transcription factor that does promote *RbcS* transcription. However, HY5 can still *repress RbcS* when chloroplast biogenesis is blocked as it does for *Lhcb*, provided the GUN1-mediated plastid signal is inactivated (i.e. in a *gun1* mutant background) (Ruckle *et al*, 2007). This observation fits neatly with the model in which the GUN1 signal induces ABI4 to bind immediately upstream of the G-box of photosynthesis-related genes, thus preventing access by HY5 (Koussevitzky *et al*, 2007). There is also good evidence that phyB contributes to the repression of *Lhcb*, but not *RbcS*, when chloroplast development is blocked (Ruckle *et al*, 2007). Overall, these data imply that genes such as *Lhcb* and *RbcS* are regulated by complex and distinct mechanisms, incorporating a GUN1-independent plastid signal that can convert positive regulators into negative ones when plastids are damaged. Importantly, it is clear that this signalling network is crucial for efficient chloroplast biogenesis. *gun1*, *cry1* and *hy5* mutants are all more susceptible than wild type to photo-oxidative damage induced by high intensity light, with *gun1 cry1* and *gun1 hy5* double mutants being particularly badly affected (Ruckle *et al*, 2007). Finally, plastid signals dependent on cry1 and GUN1 influence several other aspects of seedling development, including anthocyanin biosynthesis, cotyledon expansion and inhibition of hypocotyl elongation (Ruckle and Larkin, 2009).

Cell-specific chloroplast development

During evolution, cell specialization within the photosynthetic organs of angiosperms has resulted in distinct chloroplast subtypes with varying functions. In *Arabidopsis* and tobacco, the plastids of epidermal pavement cells contain chlorophyll, but are small and underdeveloped in comparison to those in M cells, reflecting the function of the leaf epidermis as a protective, transparent cell layer (Dupree *et al*, 1991; Pyke and Page, 1998). However, the chloroplasts of stomatal guard cells are fully developed, suggesting that photosynthetic activity is necessary for efficient stomatal function (Lawson, 2009). Such cell-specific plastid development implies distinct developmental programmes that may result from a combination of positive and inhibitory cell-autonomous factors. Besides the function of the COP/DET/FUS family of photomorphogenic regulators inhibiting chloroplast development in roots, little is known about cell-specific chloroplast biogenesis. Here, we discuss some progress on this front using two examples.

Chloroplast biogenesis in cotyledons

In epigeous seedlings, germination takes place beneath the soil surface, and hypocotyl elongation pushes the cotyledons into the light. The cotyledons initially act as storage organs to support seedling growth, and only later become photoautotrophic. Although true leaves are generated post-embryonically from the shoot apex, development of the cotyledons largely takes place during embryogenesis when tissue types and growth axes are specified (Aida *et al*, 1999; Stoynova-Bakalova *et al*, 2004). Likewise, the chloroplasts in leaves develop from meristematic proplastids as the leaf primordia emerge, but chloroplasts in cotyledons develop from etioplasts that are already present in M tissue within the embryo. These etioplasts are primed for rapid conversion to chloroplasts on light exposure. The prolamellar body, a crystalline agglomeration of Pchlide, Pchlide oxidoreductase and fragments of prothylakoid membranes, provides the structural framework for the incipient photosynthetic apparatus (Sundqvist and Dahlin, 1997). The etioplast state does not normally occur in leaves because the shoot apex and leaf primordia are routinely exposed to light. Consistent with their different cytological origins, several lines of genetic evidence suggest that chloroplasts in cotyledons develop through distinct mechanisms from those in leaves.

Genetic screens have revealed mutants in which the greening of cotyledons and leaves are differentially affected. The *snowy cotyledon* (*sco*) mutants exhibit pale green or white cotyledons, but normal, green leaves. In *sco1-1* mutants, germination is delayed and the cotyledons are initially white, but occasionally the cotyledons eventually are green and the seedlings survive if provided with exogenous sucrose (Albrecht *et al*, 2006; Ruppel and Hangarter, 2007). Immature *Arabidopsis* embryos contain photosynthetically active chloroplasts (Ruuska *et al*, 2004), but during seed dehydration and maturation, the chlorophyll and thylakoid membranes are lost, resulting in white embryos. Immature green *sco1-1* embryos dissected from siliques germinate normally and do not exhibit white cotyledons, suggesting that *sco1-1* mutants

are defective in the re-greening process (Ruppel and Hangarter, 2007). However, it is unknown if etioplast development is normal in *sco1-1* mutants, so the defect could occur before or during seed maturation. Indeed, the *sco1-2* and *sco1-3* null alleles confer embryo lethality, suggesting that SCO1 is essential for early embryogenesis as well as de-etiolation (Ruppel and Hangarter, 2007). *SCO1* encodes a chloroplast-localized elongation factor G (EF-G) and is thought to bind to the ribosomal complex to support plastid translation in a manner similar to that in *Escherichia coli* (Mohr *et al*, 2002). The *Arabidopsis* genome encodes just two other EF-Gs, which appear to be weakly expressed compared with SCO1 and may be dual targeted to the plastid and mitochondrion (Ruppel and Hangarter, 2007), and it is possible that these EF-Gs support plastid translation in other tissue types. The *sco1* embryo-lethal phenotype emphasizes the importance of plastid translation on all stages of plant development (Ahlert *et al*, 2003).

Another mutant specifically defective in cotyledon chloroplast biogenesis is *cyo1*. This mutant has recently been characterized in detail and is allelic to *sco2* (Shimada *et al*, 2007; Albrecht *et al*, 2008). Unlike *sco1*, null *cyo1* mutants complete embryogenesis and germinate normally, and etioplasts resemble those of wild type (Shimada *et al*, 2007). However, similar to *sco1*, precocious germination of *cyo1* mutants rescues the pale green cotyledon phenotype (Albrecht *et al*, 2008). Thus, the defect is specific to the generation of chloroplasts from etioplasts in cotyledons. The CYO1 protein includes a predicted zinc-finger domain similar to *E. coli* DnaJ, possesses disulphide isomerase activity and is localized to the thylakoid membrane (Shimada *et al*, 2007). CYO1 may be required for the folding of cysteine-rich thylakoid-resident proteins, such as those comprising the photosystems, during de-etiolation when rapid arrangement of the photosynthetic apparatus is critical. Presumably, this function is either unnecessary in leaf chloroplasts or is performed by another unidentified chaperone. It is notable that DnaJ-like proteins have been shown to have several functions in plastid biogenesis: in maize, BSD2 is required for the assembly of the Rubisco holoenzyme in bundle sheath (BS) cells (Brutnell *et al*, 1999), and in cauliflower, the gain-of-function *or* mutation triggers the accumulation of carotenoids in the plastids of otherwise colourless tissues (Lu *et al*, 2006).

The plastid genome is transcribed by both a nuclear-encoded RNA polymerase and a plastid-encoded, eubacterial-like RNA polymerase (PEP). Promoter specificity of PEP is mediated by nuclear-encoded sigma factors, of which there are six in *Arabidopsis* (Allison, 2000). Of these, SIG2 and SIG6 are thought to have a specific function in the de-etiolation of cotyledon chloroplasts. Although the cotyledons of *SIG2* antisense plants are chlorophyll deficient, the leaves are dark green (Privat *et al*, 2003); similarly, *sig6-1* null mutants exhibit delayed greening of cotyledons, but otherwise normal leaves (Ishizaki *et al*, 2005). *In vitro*, SIG2 strongly binds to *rbcL* and *PsbA* promoters, whereas SIG1 does not, even though both sigma factors are expressed in young seedlings (Privat *et al*, 2003). This observation suggests that differential promoter recognition may be partly responsible for different paths of chloroplast biogenesis. The degree of redundancy in the SIG family has yet to be determined; complementation tests, promoter swaps and analysis of mutant combinations will help elucidate tissue specificities and functional divergence.

Chloroplast dimorphism in C_4 photosynthesis

In C_4 plants such as maize, photosynthesis is spatially divided between two cell types in the leaf: the M and the BS. Carbon is initially fixed in M cells before being shuttled to the BS cells in which the Calvin cycle operates. The chloroplasts of M and BS cells of maize are morphologically and biochemically distinct: M cell chloroplasts contain grana, accumulate PSII and lack starch, whereas BS cell chloroplasts lack grana, accumulate Rubisco and contain multiple starch granules (Nelson and Langdale, 1992). A proteomic analysis of the stromal proteins of each chloroplast subtype has revealed in detail the degree to which each chloroplast type is specialized (Majeran *et al*, 2005). One of the more interesting findings of this study was that homologues within gene families are differentially expressed in each chloroplast type, consistent with models predicting functional divergence after selection at gene regulatory regions (Sage, 2004). Nevertheless, relatively few maize mutants have been characterized with defects specific to M and BS cells. One is *BS defective2*, which lacks the BSD2 DnaJ-like protein discussed above and, therefore, exhibits perturbed BS cell chloroplasts (Brutnell *et al*, 1999). Another mutation, *golden2* (*g2*), also leads to aberrant BS chloroplasts, but in this case the mutated gene encodes an Myb family transcription factor that now defines a family of *golden2-like* (*GLK*) genes present in diverse groups of land plants (Rossini *et al*, 2001; Yasumura *et al*, 2005). Each species contains at least two *GLK* genes, and in *Arabidopsis* and moss, each gene acts largely redundantly to promote nuclear photosynthetic gene expression in all photosynthetic cell types (Fitter *et al*, 2002; Yasumura *et al*, 2005; Waters *et al*, 2009). This redundancy also seems to be true in rice, as a pale green phenotype only results once the activity of both *GLK* genes is knocked down (P Wang and JAL, 2009, unpublished data). In maize, however, *g2* specifically disrupts photosynthetic development in BS cells, leaving M cell chloroplasts unaffected (Langdale and Kidner, 1994). Accordingly, *G2* is expressed in BS cells, whereas its homologue *ZmGLK1* is expressed most strongly in M cells (Rossini *et al*, 2001). This has led to the intriguing speculation that, as transcriptional activators, *G2* and *ZmGLK1* each has a central function in the series of events that leads to BS and M cell differentiation. However, attempts to recover a *zmglk1* mutant to test this hypothesis have been unfruitful, which may indicate an essential function for *ZmGLK1* in early photosynthetic development of the maize seedling.

A recently characterized maize mutant that is specifically deficient in M cell function has provided some insight into how differential gene expression across the two cell types might be achieved. *Zmhcf136* is a seedling lethal mutation, which leads to loss of PSII activity and the absence of grana in M cell chloroplasts, whereas those in BS cells are unaffected (Covshoff *et al*, 2008). This phenotype is consistent with the earlier assigned function of HCF136 in *Arabidopsis* as a PSII stability or assembly factor (Plücken *et al*, 2002). Accordingly, *ZmHCF136* transcripts accumulate only in M cells. Analysis of BS and M transcriptomes revealed that each cell type responded independently to the *Zmhcf136* mutation: generally, genes that are normally differentially expressed in the BS became less so, and M-enriched transcripts became

more abundant than in wild type (Covshoff *et al*, 2008). Such transcripts include those encoding the C_4 enzymes carbonic anhydrase (relatively more M-enriched in *Zmhcf136* compared with wild type) and phospho*enol*pyruvate carboxykinase (relatively less BS-enriched). In the light of the extensive feedback signalling between the plastid and nucleus as described above, Covshoff *et al* (2008) suggest that much of the differential gene expression associated with the C_4 state may result from modifications of the cell environment—such as plastid redox profiles and energy metabolite gradients—without invoking the need for extensive innovation at multiple gene regulatory regions. This might help explain how C_4 photosynthesis has evolved independently from the basal C_3 state at least 45 times in the angiosperms (Kellogg, 1999).

Photosynthetic acclimation to the light environment

Plants must balance the energy required for growth with that obtained through photosynthesis. However, the light levels experienced by different leaves and even different cells within a leaf vary substantially over time. To cope with these fluctuations, plants acclimate to their environment by dynamically adjusting the proportion of light energy used to drive photosynthesis (Walters, 2005). Under conditions in which light availability limits photosynthetic rate, *Arabidopsis*, like most plants, invests a greater proportion of resources into the light-capture stages of photosynthesis relative to carbon fixation (Walters and Horton, 1994) and grows broader, thinner leaves to maximize light interception (Anderson *et al*, 1995; Weston *et al*, 2000). In addition, low-light-grown plants decrease the relative ratio of the two different chlorophyll pigments (Chl *a* to Chl *b*) and possess larger grana (Weston *et al*, 2000). Plants accustomed to high light intensities exhibit the opposite characteristics. Although much research has focused on short-term photosynthetic acclimation brought about by state transitions (Allen, 2005; Bellafiore *et al*, 2005), relatively little is known about the regulation of the longer-term adaptations described above, all of which require substantial developmental changes. Long-term acclimation is likely brought about by redox signals from the chloroplast (Pfannschmidt *et al*, 2009). When harvested light energy consistently does not match metabolic requirements—such as the ATP and NADPH demands of the Calvin cycle—the overall redox state of the PET chain is altered. By an unknown pathway, these redox signals are

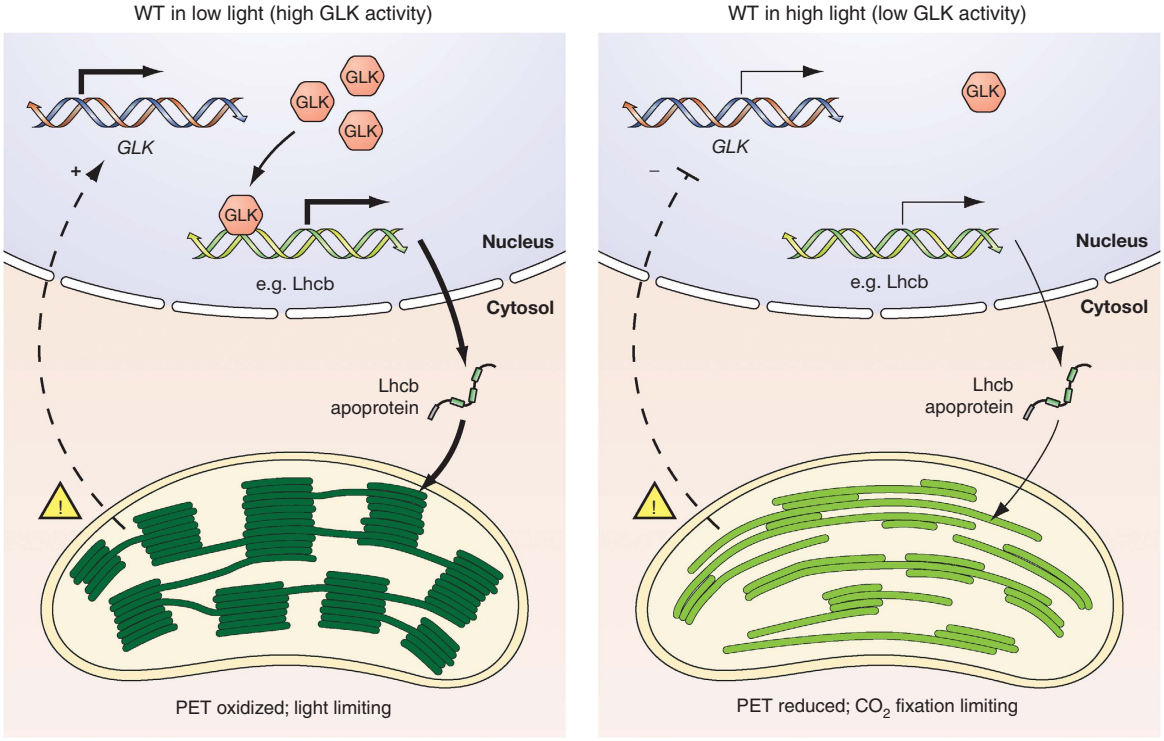

Figure 3 A model for long-term photosynthetic regulation by GLK proteins. Under light-limiting conditions (left), the PET chain cannot supply sufficient ATP and reducing equivalents to the Calvin cycle, and, therefore, tends to be in an oxidized state. This prompts a chloroplast-derived signal to the nucleus (dashed arrow) that upregulates transcription of *GLK* genes. GLK proteins in turn bind to promoter sequences of genes that function in light harvesting, such as *Lhcb* and key chlorophyll biosynthetic genes. Transcript levels of these GLK target genes increase, leading to higher levels of the corresponding protein (Lhcb in this case), as depicted by the thicker arrow. Upregulation of chlorophyll biosynthesis and LHC assembly leads to higher specific chlorophyll levels, a lower Chl *a*/*b* ratio and more abundant grana (stacked discs of thylakoids), as observed in *35S:GLK* transgenic plants. Increased grana abundance is associated with LHC trimers forming highly organized photosystem supercomplexes (Allen and Forsberg, 2001; Kovacs *et al*, 2006). When light is plentiful or even at inhibitory levels (right), the rate of CO_2 fixation is insufficient to use all of the output of the light-harvesting reactions, resulting in an overly reduced PET. This triggers a negative signal (and/or absence of a positive signal) that leads to lower rates of *GLK* transcription. The accompanying decrease in *Lhcb* and chlorophyll-related gene transcripts eventually results in a fall in the light-harvesting components in the thylakoid membrane and lower chlorophyll levels. In turn, there are fewer, less stacked grana and a higher proportion of non-stacked, stromal lamellae, as observed in *glk1 glk2* mutants. Together, these changes help to redress the imbalance between light absorption and CO_2 fixation. Note that *glk1 glk2* mutants are always paler than WT plants, suggesting that some degree of GLK activity is required under all conditions.

transduced to the cell nucleus in which gene expression is modified accordingly through transcription factors (Bonardi *et al*, 2005). Recent work has shown that GLK transcription factors are prime candidates for modifying the capacity of the light-dependent stages of photosynthesis.

Arabidopsis glk1 glk2 double mutants are pale green and contain chloroplasts with non-stacked thylakoids and reduced levels of PET complexes (Fitter *et al*, 2002). Furthermore, they have an unusually high ratio of Chl *a* to Chl *b*: grown under identical conditions, the ratio in wild-type plants is ~3.5 and in mutants ~5.5 (Waters *et al*, 2009). This alteration is likely to result partly from reduced levels of LHC proteins, to which Chl *b* is exclusively bound (Green and Durnford, 1996). When *GLK* genes are overexpressed in a mutant background, the total chlorophyll content is greater than in comparable wild-type plants, and the Chl *a*/*b* ratio is reduced to wild-type levels or lower, suggesting that GLK proteins act to promote chlorophyll synthesis and LHC assembly (Waters *et al*, 2008, 2009). The GLK1 transcription factor acts directly on the promoters of genes encoding LHC proteins, especially those of LHCII, and key enzymes of the chlorophyll biosynthetic pathway (Waters *et al*, 2009). Accordingly, in *GLK*-overexpressing plants, transcript levels of these genes are significantly higher than in the wild type; crucially, however, genes encoding enzymes of the Calvin cycle are unaffected. Together, these findings imply that GLK proteins may be responsible for regulating the balance between the light-dependent stages of photosynthesis and carbon fixation. As GLK proteins regulate a large suite of genes involved in light-harvesting and thylakoid protein complexes, they represent a potent control point in the nucleus. Consistent with this notion, levels of *GLK* transcripts are sensitive to plastid-derived retrograde signals, at least one of which is GUN1 independent (Waters *et al*, 2009). In addition, GLK proteins act as cell autonomously, providing a means by which the specific photosynthetic requirements of each cell across the leaf can be regulated independently (Waters *et al*, 2008). Although it has yet to be established that whether redox-dependent retrograde signals affect *GLK* expression in mature plants, we propose a model in which GLK proteins act as key photosynthetic regulators as part of plant acclimation to variable environmental circumstances (Figure 3).

Concluding remarks

Clearly, chloroplast development is a complex and highly regulated process. The data reviewed here have placed a strong emphasis on the function of PGE—incorporating transcription, mRNA editing, translation and protein complex assembly—in mediating the critical early steps of chloroplast biogenesis. Nevertheless, given the swathe of information on the molecular biology of plastids obtained from recent studies, it is surprising that broader aspects of chloroplast biogenesis remain largely unaddressed. For example, considering that etiolation is an evolutionarily derived state, is chloroplast development the default pathway that is, therefore, continually repressed in non-photosynthetic tissues? What factors determine such cell-specific plastid development? Why do some cell types contain hundreds of plastids, and others very few? In light of the clarification of PDV proteins in regulating PDV, addressing this latter question will now be much easier, and it will be particularly interesting to see whether PDV overexpression leads to excess PDV in normally sparsely populated cells. Further mechanistic factors may be uncovered with suppressor/enhancer screens, screens for PDV-interacting protein partners and microarray mining to discover regulatory pathways. In addition, the basis for the developmental changes induced during long-term photosynthetic acclimation are still poorly understood. Even the developmental changes beyond the photosynthetic apparatus—such as palisade cell elongation—are likely to be driven by chloroplast-derived signals, given the influence plastids have on cell and organ development (Lopez-Juez and Pyke, 2005). It will be interesting to determine whether photosynthetic mutants and chloroplast signalling mutants exhibit defects in the different aspects of acclimation. Elucidation of the sources and nature of chloroplast redox signals will be paramount to moving forward our understanding of this aspect of plant biology.

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Conflict of interest

The authors declare that they have no conflict of interest.

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