Review

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A structural phylogenetic map for chloroplast photosynthesis

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Chloroplasts are cytoplasmic organelles and the sites of photosynthesis in eukaryotic cells. Advances in structural biology and comparative genomics allow us to identify individual components of the photosynthetic apparatus precisely with respect to the subcellular location of their genes. Here we present outline maps of four energy-transducing thylakoid membranes. The maps for land plants and red and green algae distinguish protein subunits encoded in the nucleus from those encoded in the chloroplast. We find no defining structural feature that is common to all chloroplast gene products. Instead, conserved patterns of gene location are consistent with photosynthetic redox chemistry exerting gene regulatory control over its own rate-limiting steps. Chloroplast DNA carries genes whose expression is placed under this control.

Chloroplast as time capsule of microbial metabolism

At their evolutionary origin, eukaryotic plants and algae acquired a complete photosynthetic apparatus from incorporation of cyanobacteria, which are prokaryotes, as intracellular symbionts [1–12]. Cyanobacterial genes were subsequently copied to the cell nucleus of the host [13–18]. Many of these genes have survived and are still expressed [19,20]. Nuclear genes of cyanobacterial origin encode precursor proteins that are synthesised in the cytosol, on 80S ribosomes. Some of these precursor proteins are then imported, post-translationally, back into the chloroplasts into which the endosymbiotic cyanobacteria evolved [21–23].

However, the chloroplast photosynthetic apparatus is never completely encoded in the plant cell nucleus, because a few key proteins of photosynthesis are always synthesised entirely within chloroplasts themselves, on 70S bacterial-type ribosomes [24], using mRNA produced by transcription of their genetic templates in chloroplast DNA [25–28]. Chloroplast DNA is a discrete cytoplasmic genome [29,30]. Replicate copies are passed to daughter plastids, which arise from binary fission of pre-existing plastids [31,32]. Thus chloroplasts arise only from pre-existing chloroplasts, with a photosynthetic prokaryote as a remote, common progenitor.

Chloroplast DNA is replicated and transcribed, and the mRNA translated, by the chloroplast's own complete genetic apparatus. This genetic system is a legacy of the chloroplast's prokaryotic past. For both photosynthesis and the chloroplast genetic system that supports it, some components are synthesised *in situ*, whereas others are imported [1,6,25,33].

Here we draw on recent structural and genomic information to depict the energy-transducing chloroplast thylakoid membrane in such a way as to distinguish between the two locations – nucleus and chloroplast – of the genes for its constituent polypeptides. The major photosynthetic protein complexes carry out light capture, primary photochemistry, secondary electron transfer, ATP synthesis and CO_2 assimilation. These complexes are all genetic chimeras, because they contain subunits encoded and

Glossary

The major protein complexes are listed with Protein Data Bank (PDB) accession numbers for atomic co-ordinates.

Photosystem II (PS II): is a membrane-intrinsic, light-dependent water: plastoquinone oxidoreductase [94–97]. The primary electron donor chlorophyll *a*, P_{680}, passes electrons to Phe (for pheophytin), and from there to the bound plastoquinone molecules Q_A and Q_B. Lhcb refers to the outer, peripheral, light-harvesting, chlorophyll *a*– and *b*– and carotenoid–binding polypeptides of photosystem II [98,99]. Cyanobacterial reaction centre: 3ARC.pdb; 3BZ1.pdb; 3BZ2.pdb. Lhcb, Chloroplast LHC II: 1rwt.pdb; 2bhw.pdb.

Cytochrome b_6f complex (Cyt b_6f): is a membrane-intrinsic, light-independent, proton-translocating plastoquinol:plastocyanin oxidoreductase [100,101]. Cytochrome b_6f is a close structural and functional homologue of respiratory complex III, catalysing the proton-motive Q-cycle [81,82], in which each pair of electrons (e^-) moves four protons (H^+) from the N-phase (in chloroplasts, the stroma) to the P-phase (in chloroplasts, the thylakoid lumen). *Chlamydomonas*: 1Q90.pdb. Cyanobacteria, *Mastigocladus*: 1UM3.pdb. Cyanobacteria, *Nostoc*: 2ZT9.pdb.

Photosystem I (PS I): is a membrane-intrinsic, light-dependent plastocyanin:-ferredoxin oxidoreductase [102,103]. Ferredoxin transfers single electrons to the flavoprotein ferredoxin: $NADP^+$ reductase (FNR), while FNR transfers two electrons to $NADP^+$ and H^+ to give NADPH, terminating non-cyclic electron transport. Ferredoxin also transfers electrons to plastoquinone to give cyclic electron transport, and to oxygen, giving pseudocyclic electron transport. Cyanobacterial, *Thermosynechococcus elongatus*, reaction centre: 1JB0.pdb. Pea, *Pisum sativum*, chloroplast reaction centre: 3LW5.pdb; 2WSC.pdb.

ATP synthase: is a proton-translocating ATP hydrolase [104]. Photosynthetic electron transport establishes a transmembrane gradient of proton concentration, a proton-motive force, that serves to drive the ATPase reaction in the direction of ATP synthesis. The ATPase activity itself is in a membrane-anchored but water-soluble domain, CF_1. Proton translocation is by the hydrophobic, membrane-intrinsic CF_o. Spinach chloroplast CF_o has 14-fold rotational symmetry, corresponding to 14 copies of subunit III in the complete ring [105]. The corresponding number of F_o subunits in other systems can be 8, 10 [104,106], 11, 14, or 15 [107]. Bovine mitochondria. F_o: 1C17.pdb. F_1: 1E79.pdb; 2A7U.pdb; 1L2P.pdb.

Ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco): [108] catalyses carboxylation of ribulose-1,5-bisphosphate to give two molecules of 3-phosphoglycerate, the initial step of the reductive pentose phosphate pathway and autotrophic metabolism in plants, algae and many bacteria. The oxidase reaction gives, instead, one 3-phosphoglycerate and one 2-phosphoglycollate – no CO_2 is fixed, and light-dependent O_2 uptake gives 'photorespiration' [109]. Rubisco is a membrane-extrinsic and weakly water-soluble oligomeric protein consisting of eight large subunits (L) containing the catalytic site, and eight small subunits (S), giving L_8S_8, though L_2 forms are known [110]. Spinach, *Spinacea oleracea*: 1RCX.pdb. *Chlamydomonas*: 1GK8.pdb. Red alga *Galdieria partita*:1IWA.pdb. Cyanobacterium *Synechococcus* PCC 6301: 1RBL.pdb.

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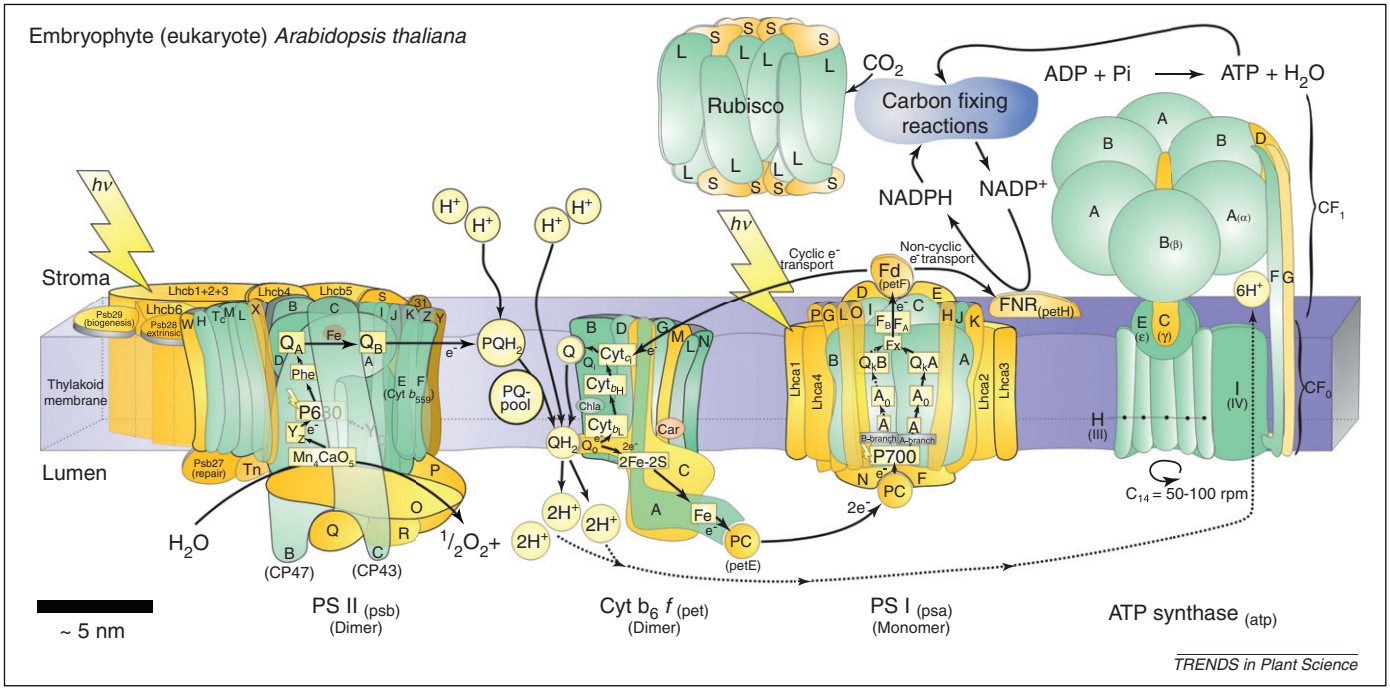


Figure 1. A high resolution version of this figure is available as Online Supplementary Figure S1. Major proteins and protein complexes of the chloroplast photosynthetic apparatus of a higher plant exemplified by *Arabidopsis thaliana*. Photosystem II (PS II); cytochrome b_6f (Cyt b_6f); photosystem I (PS I); ATP synthase; and Rubisco. Subunits are given single-letter names, omitting the three-letter prefix that denotes the complex of which each forms part. These prefixes are: psa for photosystem I; psb for photosystem II; pet (photosynthetic electron transport) for the cytochrome b_6f complex and secondary electron carriers; atp for the ATP synthase; and rbc for Rubisco. See Glossary for functions and major components. Polypeptide subunits encoded in the chloroplast are coloured green; polypeptide subunits encoded in the nucleus are coloured yellow.

made in the chloroplast itself that are nevertheless assembled into close proximity and intimate functional contact with subunits that are encoded on chromosomes in the nucleus and imported, instead, from the cytosol.

Thylakoids and the membrane protein complexes of photosynthetic electron transport

Figure 1 (see also Online Supplementary Figure S1) is a cartoon representation of the major protein complexes of the chloroplast thylakoid membrane of *Arabidopsis thaliana* (*Arabidopsis*), together with the enzyme Rubisco, which is extrinsic to the membrane and which catalyses the first step of the reductive pentose phosphate pathway of carbon dioxide assimilation. Colour-filled shapes represent individual polypeptides, with rectangles or boxes superimposed to denote notable co-factors or prosthetic groups. The shapes representing polypeptides are clustered to indicate their positions and functional interactions within the major complexes, as inferred from structural studies, mostly from X-ray crystallography. These major complexes are described briefly in the Glossary Box, where Protein Data Bank accession numbers are also given for atomic co-ordinates of 3-D structures.

Gene locations and sites of synthesis

In Figure 1, representations of chloroplast gene products are green, those of nuclear gene products, imported as precursors from the cytoplasm, are yellow [34–37]. A recurrent and obvious question is: what is the pattern behind the distribution of yellow and green? Why are some polypeptides imported, and others synthesised *in situ* within the chloroplast?

On first inspection, it might appear that the key, functional protein subunits are chloroplast-encoded, as if a green, stripped-down photosynthetic apparatus, representing a simple archetype, could in principle function without later evolutionary additions provided by the cell nucleus and cytoplasm. There are constraints on evolution of subsets of proteins of cyanobacteria [38]. Could these also have impeded transfer of endosymbiont genes to the nucleus? Closer inspection shows several clear counter-examples to this suggestion – in each case nuclear-encoded, imported, yellow subunits are necessary for the function of each whole complex. These include: psbO, required for stabilisation of the water-splitting complex of photosystem II; the Rieske iron–sulfur protein of cytochrome b_6f; ferredoxin on the acceptor side of photosystem I; CF_1-γ and the stator δ subunit of ATP synthase; and the Rubisco small subunit. Without these components, the parent complex could not work at all. Indeed, these components are found in modern cyanobacteria and must have been present in the chloroplast's endosymbiont progenitor. They are not additions at all.

An alternative attempt at pattern recognition might discern hydrophobic, membrane-intrinsic polypeptides as green and water-soluble polypeptides as yellow, suggesting a barrier to successful import of hydrophobic proteins, which thus have to be synthesised within the chloroplast. Again, there are conspicuous counter-examples: the light-harvesting, Lhc gene products of both photosystems I and II and the Rieske iron–sulfur protein of cytochrome b_6f are membrane-intrinsic and imported; while CF_1-α, CF_1-β, and the Rubisco large subunit are membrane-extrinsic and synthesised within the chloroplast [39]. Furthermore, analysis of the *Arabidopsis* thylakoid membrane proteome reveals no correlation between gene location and solubility of proteins in the organic solvents acetone and chloroform–methanol [40].

Perhaps there is a consistent pattern for gene location by combining these two initial suggestions, such that a hydrophobic protein must be chloroplast-encoded only if it originated in cyanobacteria and if it contains five or more transmembrane helices [41]? Again, this generalisation is confounded by green, single-helix (minor PS II subunits and cytochrome *f*) or double-helix (CF_o-III) polypeptides, and by the green, chloroplast-encoded subunits of the ATP synthase CF_1 and Rubisco.

We find no defining structural feature, chemical property or static functional consideration that is common to all chloroplast gene products, freeing residual subunits to be encoded by nuclear genes. Neither is there any apparent common denominator of imported polypeptides that requires nuclear encoding and that prohibits import of their remaining functional partners.

Comparative functional genomics: flowering plant, green alga, cyanobacterium and red alga

Figure 2 (see also Online Supplementary Figure S2) illustrates the photosynthetic membranes of *Arabidopsis thaliana*, *Chlamydomonas reinhardtii*, *Thermosynechococcus elongatus* and *Cyanidioschyzon merolae* – four species where complete nuclear and chloroplast genome sequences now allow us to itemise thylakoid polypeptide composition and corresponding gene location.

Chlorophytes

The chloroplast thylakoid membrane shown in Figure 1 is reproduced in simplified form in the top panel of Figure 2. The panel is labelled *Arabidopsis thaliana*, while the map is likely to be reasonably accurate for all photoautotrophic land plants [42,43]. The second panel from the top in Figure 2 depicts the thylakoid membrane of the unicellular green alga *Chlamydomonas reinhardtii* (*Chlamydomonas*), a long-established model organism for genetic studies of photosynthesis [44].

For the photosynthetic apparatus, comparison (Figure 2) reveals little difference between *Arabidopsis* and *Chlamydomonas* either in polypeptide composition or in gene location. *Chlamydomonas* has just one more electron carrier, cytochrome c_6, which serves to connect cytochrome *f* to P_{700} as an alternative to plastocyanin under conditions of copper limitation. Cytochrome c_6 is encoded by the nuclear gene petJ. *Arabidopsis* has a related cytochrome c_{6a} with, as yet, no clearly-resolved function [45,46].

Cyanobacteria

From the diverse and ecologically important cyanobacteria, the photosynthetic apparatus of one species, *Thermosynechococcus elongatus* [47], is shown as a reference in the bottom panel of Figure 2. Cyanobacteria, once known as blue-green algae or cyanophytes, are prokaryotes and have no subcellular organelles. All polypeptides are represented in green in Figure 2, since none are imported. In evolutionary terms chloroplasts *are* cyanobacteria.

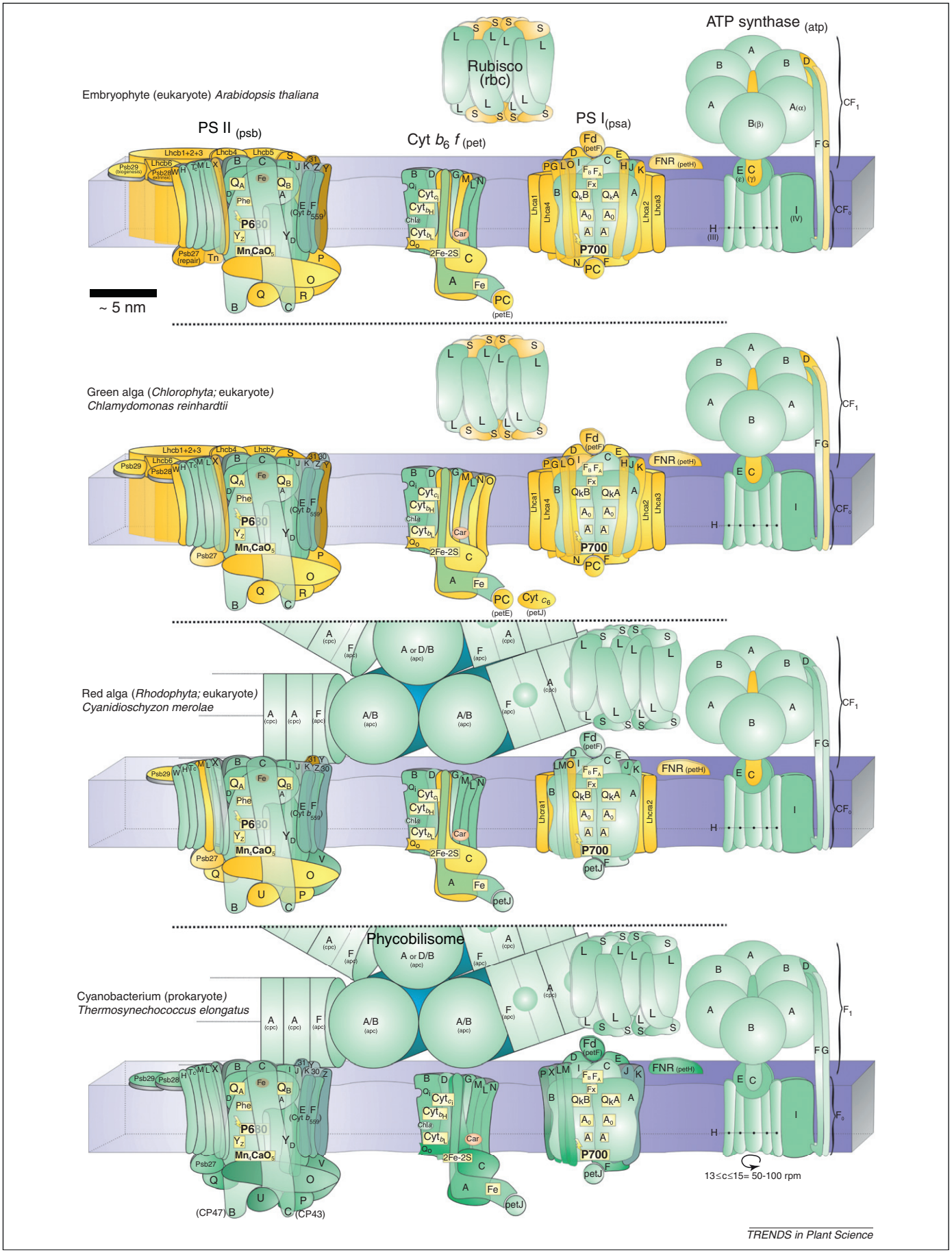
Although it is clear that the primary processes of photochemistry, electron transport and ATP synthesis are unchanged between cyanobacteria and green plants, their outer light-harvesting antennae of photosystems I and II are somewhat different. No member of the conventionally defined, chlorophyll-based Lhc (formerly cab) family of proteins [48] is present in any cyanobacterium known to date [39]. Instead, many cyanobacteria have water-soluble, light-harvesting phycobiliproteins, which are usually organised into large structures – phycobilisomes – that are extrinsic, although attached, to the cytoplasmic (stromal) surface of thylakoid membrane. The light-harvesting pigments give the blue-green or red colour from which the names of both the pigments and the phyla derive. These chromophores are phycobilins which are linear tetrapyrroles found also as the chromophores of phytochrome photoreceptors [49]. By contrast, cyclic tetrapyrroles coordinate metal atoms in the chlorin rings of chlorophylls and in the haems of cytochromes and haemoglobins. Light-harvesting phycobilins include phycocyanin, allophycocyanin and phycoerythrin. These, again unlike chlorophylls, are covalently bound to their apoproteins, to cysteine side chains of each respective polypeptide.

Although the phycobilisome was once thought to function exclusively as the antenna of photosystem II, it is now clear that the acclimation process known as state transitions involves its re-allocation between photosystems I and II [50–52] just as Lhcb phosphorylation reallocates chlorophylls in chlorophytes [50,53–55]. In order not to specify one photosystem, the phycobilisome is depicted as a looming presence in Figure 2, although excitation energy transfer requires precise docking and attachment to each photosystem.

Red algae

The third panel from the top in Figure 2 shows the coloured thylakoid map for the unicellular red alga *Cyanidioschyzon merolae* (*Cyanidioschyzon*) [56]. Red algae are eukaryotes with chloroplasts that contain phycobilisomes. It is interesting to ask whether green and red algae resulted from separate endosymbiotic events, or whether a single eukaryotic algal ancestor either lost phycobilisomes to give green algae, or gained them to give red algae [3,4,8,10,57–59]. As if to remove any doubt about hydrophobicity in relation to chloroplast gene location, the water-soluble and easily extracted phycobilisomes of red alga are entirely encoded within chloroplast DNA (Figure 2), whereas their functional analogues in chlorophytes, Lhc proteins, are hydrophobic and yet encoded in the nucleus and imported [48]. Red algae possess Lhc proteins that bind chlorophyll *a*, not *b*, and which function as part of the antenna system of photosystem I [39,60]. Red algal Lhca proteins are imported (Figure 2). It is possible to envisage that the entire Lhc family had a truly eukaryotic origin, later to acquire a light-harvesting function and to become adapted to work also with photosystem II [39]. While some cyanobacteria lack phycobilins and have chlorophyll *b*, which is otherwise characteristic of Lhcb in chlorophytes, chlorophyll *b* is then associated instead with proteins more closely related to CP43 and CP47 of photosystem II [61].

In general, red algae have ceded far fewer fundamental components of photosynthesis to the nucleus than have chlorophytes (Figure 2). Red algae thus yield a further example of a chimeric complex with components that function together despite having different gene locations


Embryophyte (eukaryote) *Arabidopsis thaliana*
PS II (psb)
Cyt b_6 f (pet)
PS I (psa)
ATP synthase (atp)
Rubisco (rbc)
~ 5 nm
Green alga (*Chlorophyta;* eukaryote)
Chlamydomonas reinhardtii
Red alga (*Rhodophyta;* eukaryote)
Cyanidioschyzon merolae
Phycobilisome
Cyanobacterium (prokaryote)
Thermosynechococcus elongatus
13≤c≤15= 50-100 rpm
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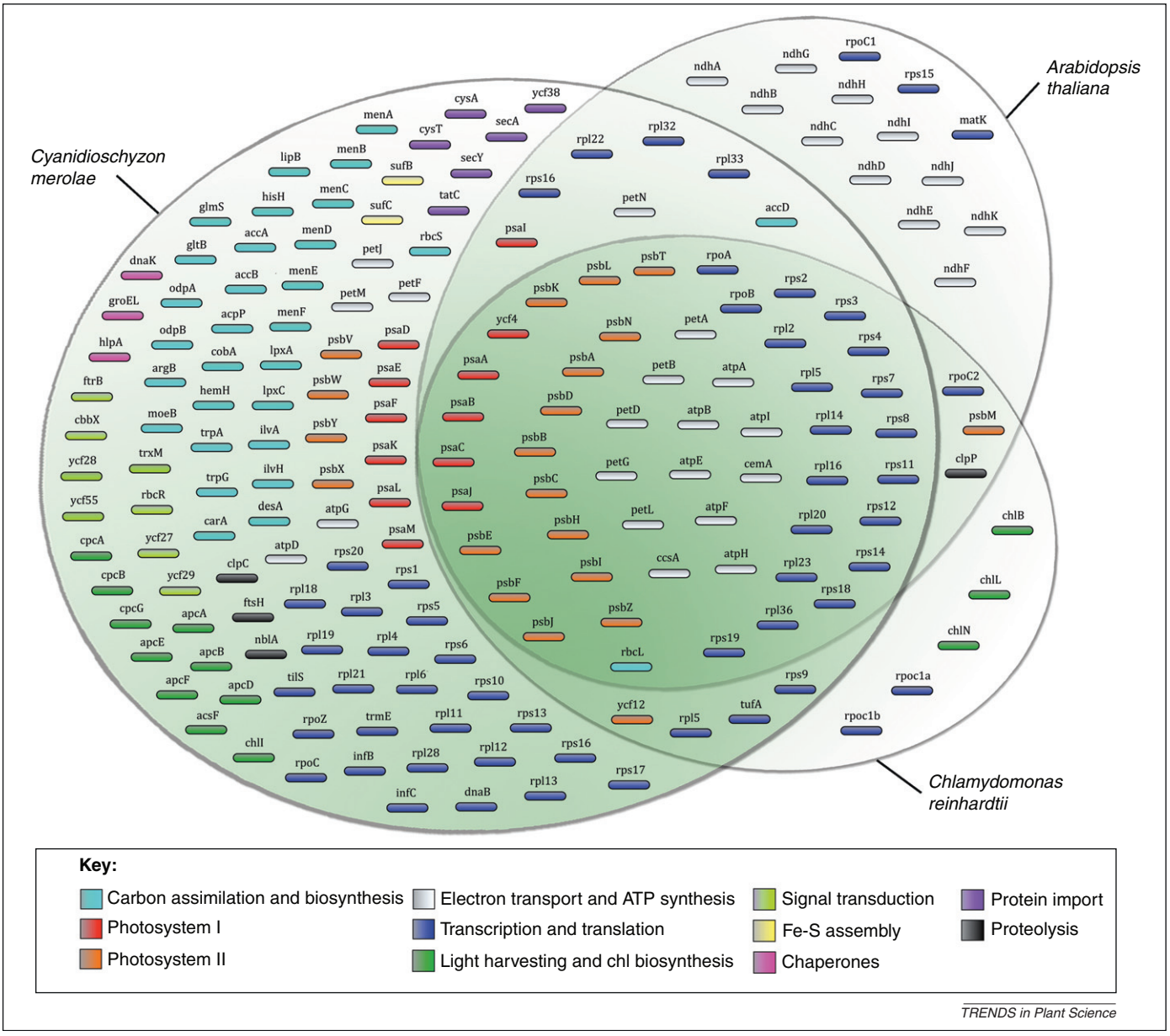

Figure 3. A high resolution version of this figure is available as Online Supplementary Figure S3. A Venn diagram of gene content of the chloroplast (plastid) genomes of the higher plant *Arabidopsis thaliana,* the green alga *Chlamydomonas reinhardtii,* and the red alga *Cyanidioschyzon merolae.*

– ferredoxin has a chloroplast gene while its close interaction partner, FNR, is encoded in the nucleus and then imported. Both Rubisco subunits are products of red algal chloroplast DNA. Figure 2 allows direct comparison of *Cyanidioschyzon* with *Chlamydomonas*. Chloroplast encoding is retained in most *Cyanidioschyzon* polypeptides normally thought of as nuclear gene products, with the exception of FNR, the Rieske protein, and six polypeptides with secondary roles in photosystem II.

A Venn diagram of chloroplast genes

Figure 3 (see also Online Supplementary Figure S3) shows all genes found in the true chloroplast genomes of the three eukaryotic species – *Arabidopsis*, *Chlamydomonas* and *Cyanidioschizon* – as chosen for the thylakoid maps in Figure 2 and listed in Table 1 (see also online Supplementary Table S1). In Figure 3, subsets of genes are bounded by ellipses denoting the species in which each component of the subset is found. Functional classification is provided by 11 colours, one for each class, and the classes are identified in the key given as part of Figure 3. This diagram follows one presented for mitochondrial genes and genomes by Gray *et al.* [62]. All chloroplast genes are identified in Figure 3, not just those whose products form part of the photosynthetic apparatus, as in Figures 1 and 2 and Table 1.

There are 180 chloroplast genes represented in Figure 3: 158 are present in *Cyanidioschyzon*, 71 are

Figure 2. A high resolution version of this figure is available as Online Supplementary Figure S2. Major proteins and protein complexes of the photosynthetic apparatus of *Arabidopsis thaliana* (Figure 1), the green alga *Chlamydomonas reinhardtii,* the red alga *Cyanidioschyzon merolae,* and the cyanobacterium *Thermosynechococcus elongatus.* See Glossary. Polypeptide subunits encoded in the chloroplast or cyanobacterium are coloured green; polypeptide subunits encoded in the nucleus are coloured yellow.

Table 1. Genes for polypeptide subunits of protein complexes of the photosynthetic apparatus[a]

Gene	Product	Eukaryote			Prokaryote
		Arabidopsis thaliana	*Chlamydomonas reinhardtii*	*Cyanidioschyzon merolae*	*Thermosynechococcus elongatus*
Photosystem II					
psbA	D1	●[b]	●	●	●
psbB	CP47	●	●	●	●
psbC	CP43	●	●	●	●
psbD	D2	●	●	●	●
psbE	Cytochrome b_{559} α	●	●	●	●
psbF	Cytochrome b_{559} β	●	●	●	●
psbH	PsbH	●	●	●	●
psbI	PsbI	●	●	●	●
psbJ	PsbJ	●	●	●	●
psbK	PsbK	●	●	●	●
psbL	PsbL	●	●	●	●
psbM	PsbM	●	●	◐	●
psbN	PsbN	●	●	●	●
psbO	OEC33	◐	◐	◐	●
psbP	OEC23	◐	◐	◐	●
psbQ	OEC16	◐	◐	◐	●
psbR	PsbR	◐	◐	○	○
psbS	PsbS	◐	◐	○	○
psbTc	PsbTc (Ycf8)	●	●	●	●
psbTn	PsbTn	◐	○	○	○
psbU	PsbU	○	○	◐	●
psbV	Cytochrome c_{550}	○	○	●	●
psbW	PsbW	◐	◐	●	○
psbX	PsbX	◐	◐	●	●
psbY	PsbY	◐	◐	●	●
psbZ	PsbZ (Ycf9)	●	●	●	●
psb27	Psb27	◐	◐	◐	●
psb28	Psb28	◐	◐	○	●
psb29	Psb29	◐	◐	◐	●
psb30	Psb30	○	●	●	●
psb31	Psb31	◐	◐	◐	●
lhcb	LHC II	◐	◐	○	○
Cytochrome b_6f					
petA	Cytochrome *f*	●	●	●	●
petB	Cytochrome b_6	●	●	●	●
petC	Rieske Fe-S	◐	◐	◐	●
petD	Subunit IV	●	●	●	●
petG	Subunit G	●	●	●	●
petL	Subunit L	●	●	●	●
petM	Subunit M	◐	◐	●	●
petN	Subunit N	●	◐	●	●
petO	Subunit V	○	◐	○	○
Photosystem I					
psaA	PsaA	●	●	●	●
psaB	PsaB	●	●	●	●
psaC	PsaC	●	●	●	●
psaD	PsaD	◐	◐	●	●
psaE	PsaE	◐	◐	●	●
psaF	PsaF	◐	◐	●	●
psaG	PsaG	◐	◐	○	○
psaH	PsaH	◐	◐	○	○
psaI	PsaI	●	◐	●	●
psaJ	PsaJ	●	●	●	●
psaK	PsaK	◐	◐	●	●
psaL	PsaL	◐	◐	●	●
psaM	PsaM	○	○	●	●
psaN	PsaN	◐	◐	○	○
psaO	PsaO	◐	◐	◐	○

Table 1 (*Continued*)

Gene	Product	Eukaryote			Prokaryote
		Arabidopsis thaliana	*Chlamydomonas reinhardtii*	*Cyanidioschyzon merolae*	*Thermosynechococcus elongatus*
psaP	PsaP	●	●	○	●
psaX	PsaX	○	○	○	●
lhca	LHC I	●	●	●	○
ATP synthase					
atpA	α-Subunit	●	●	●	●
atpB	β-Subunit	●	●	●	●
atpC	γ-Subunit	●	●	●	●
atpD	δ-Subunit	●	●	●	●
atpE	ε-Subunit	●	●	●	●
atpF	Subunit I	●	●	●	●
atpG	Subunit II	●	●	●	●
atpH	Subunit III	●	●	●	●
atpI	Subunit IV	●	●	●	●
Phycobiliproteins					
apcA	Allophycocyanin α	○	○	●	●
apcB	Allophycocyanin β	○	○	●	●
apcD	Allophycocyanin α-B	○	○	●	●
apcF	Allophycocyanin β-18	○	○	●	●
cpcA	Phycocyanin α	○	○	●	●
cpcB	Phycocyanin β	○	○	●	●
Rubisco					
rbcL	Large subunit	●	●	●	●
rbcS	Small subunit	●	●	●	●
PC					
petE	Plastocyanin	●	●	○	○
Cytochrome c_6					
petJ	Cytochrome c_6	●	●	●	●
FRX					
petF	Ferredoxin	●	●	●	●
FNR					
petH	FNR	●	●	●	●

[a]A high resolution version of this table is available as Online Supplementary material Table S1.

[b]Green indicates that the gene concerned is located in the chloroplast or cyanobacterium, and yellow indicates that the gene concerned is located in the nucleus. Grey indicates absence of a gene, as inferred from the complete nuclear and chloroplast genome sequences of the species represented.

present in *Arabidopsis*, and 59 are present in *Chlamydomonas*. 101 chloroplast genes are present only in *Cyanidioschyzon*, 5 are present only in *Chlamydomonas*, and 14 are present only in *Arabidopsis*. *Cyanidioschizon* shares 54 genes with *Arabidopsis* and 51 genes with *Chlamydomonas*; *Chlamydomonas* shares 51 genes with *Arabidopsis*.

Of the 14 genes unique to flowering plant chloroplasts, assuming for simplicity that *Arabidopsis* stands for all, 11 are for subunits of a chloroplast NADP(H) dehydrogenase of unknown function. *Ndh* genes persist in colourless plastids of parasitic plants that have lost photosynthesis [63,64], suggesting that 'chloroplast complex I' participates there in a respiratory electron transport chain with an imported plastid terminal oxidase resembling the alternative oxidase of plant mitochondria. It is also important to note that chloroplasts share their genomes with other plastids found within individual land plants, such as amyloplasts, chromoplasts and leucoplasts [32]. Different plastids, derived from proplastids, may have bioenergetic functions and redox chemistry even in the absence of photosynthesis, in which case the NAD(P)H dehydrogenase genes may be retained for the same reason as respiratory complex I in mitochondria of both plant and animal cells.

Of the 101 genes seen in Figure 3 as unique to *Cyanidioschyzon* chloroplasts, eight encode light-harvesting phycocyanobiliproteins. A larger number of light-harvesting genes is present in the chloroplasts of the majority of red algal species, which also contain phycoerythrin. Other notable red-algal chloroplast genes (Figure 3) encode molecular chaperones, protein assembly and turnover factors, additional ribosomal proteins, signal transduction components including response regulators of two-component regulatory systems, and transcription factors.

Chloroplast genes in common

The 48 genes found in all three of these diverse chloroplast genomes fall into just 5 of the 11 functional classes identified in Figure 3. These five classes are: photosystem I (4); photosystem II (11); secondary electron transport and ATP synthesis (13); Rubisco (1); and ribosomal subunits and RNA polymerase (19). Therefore, in total, all three genomes share genes that fall into only two broad groups: 29 genes for the photosynthetic apparatus; and 19 genes for components of the genetic system itself.

Why aren't plastid genes obsolete? The CoRR hypothesis

There are estimated to be over 25,000 genes in *Arabidopsis thaliana* [42], which means that its chloroplast contains at most 71/25,000 (or 0.284%) with chloroplast photosynthesis genes accounting for no more than 34/25,000 (or 0.136%). What is it about the protein subunits coloured green in Figures 1 and 2 that has led evolution to retain this tiny minority of genes in chloroplasts, at the cost of maintaining an entire cytoplasmic genetic system, separate from that in the nucleus and cytosol? As described earlier, hydrophobicity is irrelevant. Prokaryotic ancestry certainly is relevant, but about 4,500 protein-coding *Arabidopsis* genes (~18%) have cyanobacterial ancestry [19]. Thus, for each chloroplast gene from cyanobacteria, there are approximately 63 (4500/71) other formerly cyanobacterial genes now found in the nucleus. In addition, the *Arabidopsis* nuclear-cytosolic system successfully supplies in the range of 3000 proteins (~12% of the total) to chloroplasts [65], therefore for each chloroplast-encoded protein there are about 42 nuclear-encoded proteins whose precursors are processed for assembly after having accurately been targeted for import into chloroplasts from the cytosol. Why, then are there any genes at all in the chloroplast [37,66]?

In answer to this question we favour the hypothesis that the stoichiometry (quantity relative to each other) of the following components of the photosynthetic apparatus is required to be regulated rapidly and unconditionally when environmental conditions change, and in direct response to attendant changes in reduction–oxidation (redox) state of at least one component of the electron transport chain: photosystem II reaction centre, cytochrome b_6 and cytochrome f, photosystem I reaction centre, Rubisco catalytic large subunit, ATP synthase $CF_1\,\alpha$ and β subunits, and CF_o subunit III. Each of these components may suddenly and unpredictably become rate-limiting for photosynthetic electron transport. When one does, it must be increased in activity and relative quantity. When another becomes rate-limiting, instead, then the first begins to be present at over-capacity, and its activity and relative quantity must decrease.

This hypothesis for the evolutionary retention of chloroplast genomes is termed Co-location for Redox Regulation, or CoRR [34,67]. CoRR states that the benefit of retaining rapid and unconditional redox control of expression of the genes for the key components listed above is so great that, on its own, it repays the cost of maintaining a separate genetic system in close proximity to the electron transport chain. This co-location of gene and gene product applies to the subset of photosynthesis genes (Figure 3) both to receive the redox signal and to exert control over the stoichiometry of the gene products, relative to each other.

The redox regulatory system at the heart of this control is ancient, found in prokaryotes, and continues to be indispensable for safe and efficient photosynthesis in chloroplasts [68–70]. It is a genetic level of control working in parallel to post-translational modification, as seen in state transitions [53,71], and alongside redox and metabolic regulation. Post-translational and metabolic adjustments can together achieve a 'quick fix' but not change the underlying architecture of the photosynthetic apparatus [72–74].

Redox regulation at the level of gene transcription and translation is ancient and found even in anaerobic prokaryotes. However, the advent of molecular oxygen at least 2.3 billion years ago [75–77] introduced an additional, severe penalty for failure of precise reconstruction of electron transport chains in response to altered environmental conditions. This penalty is univalent (single-electron) reduction of oxygen to give the toxic and mutagenic superoxide anion radical and its free-radical products [78–80]. Semiquinones, including plastosemiquinone, are necessary intermediates in the proton-motive Q-cycle [81,82], and readily transfer single electrons oxygen [80]. The redox state of quinone pools, including the plastoquinone pool of photosynthesis, must therefore be maintained for reasons of safety as well as for efficiency in energy transduction [79,83].

We do not propose, and CoRR does not require, that every chloroplast gene is redox-regulated; nor does CoRR predict that every redox-regulated gene is found in the chloroplast. Each of the components of the photosynthetic apparatus listed above works together with other subunits and is likely to be the dominant and rate-limiting factor in assembly of the respective whole, functional complex. In terms of their assembly properties, two classes of proteins exist among chloroplast-encoded core subunits of the electron transport complexes. The first class defines Dominant Assembly Factors (DAFs), and the second defines CES (Control by Epistasy of Synthesis) subunits [41]. The assembly of CES subunits depends on the presence of DAFs in the thylakoid membrane. In the absence of DAFs, the synthesis of CES subunits is inhibited by an autoregulatory translational feedback loop. Therefore, for regulatory control of assembly to occur, the genes encoding CES subunits need to be present in the same compartment where the assembly takes place. For the assembly of whole complexes, this means that DAFs are synthesised and inserted in the thylakoid membrane first, followed by synthesis and assembly of CES subunits. The nuclear-encoded subunits, which comprise mostly the peripheral subunits of the complexes, assemble around the core made by the chloroplast-encoded subunits.

These assembly properties ensure sequential and coordinated assembly of multisubunit, genetically chimeric electron transport complexes in chloroplasts [84]. They also mean that rapid redox regulation of genes encoding DAFs alone, or in some cases both DAFs and CES subunits, can make or break complexes and thereby maintain redox homeostasis in chloroplasts in changing environmental conditions. CoRR and CES properties of chloroplast genes, acting together, may produce species-specific differences in the assembly of electron transport complexes and, as a result, species-specific differences in the gene content of chloroplasts. The observation that PS I genes *psaD*, *E*, and *F* are chloroplast encoded in *Cyanidioschyzon* (Figure 2), but not in *Chlamydomonas* or in *Arabidopsis* may be consistent with this idea. Additionally, when chloroplasts lose their photosynthetic function and redox regulation is no longer a requirement, CES properties may account for

the chloroplast location of genes encoding nonphotosynthetic enzymatic complexes such as the fatty acid synthase (*accD* gene), as suggested for the chloroplasts of the parasitic orchid *Rhizanthella gardneri* [85]. The same assembly principles could be true for ribosomal proteins and other components of the chloroplast genetic system itself. It is also seen that gene transfer to the nucleus is infrequent in organisms with just one organelle per cell [86]. A plausible explanation is that DNA transfer results from organellar damage and lysis, thus nuclear transformation from single organelles occurs only in cells that leave no progeny [63,87,88]. Notwithstanding CES and restrictions on nuclear transfer in special cases, the possibility of any protein being chloroplast-encoded exists only because of the selective value of there being a chloroplast genetic system in the first place.

The CoRR hypothesis is put forward to explain the coincidence of a sequestered genetic system with a redox-coupled energy transduction system in eukaryotic bioenergetic organelles generally [34,66]. Thus CoRR applies to mitochondria, oxidative phosphorylation, and maternally-inherited mitochondrial genomes [89]. CoRR appears to be the only hypothesis that provides a consistent single explanation of the existence of genomes in both chloroplasts and mitochondria. The explanation is that chloroplasts and mitochondria stem from prokaryotic endosymbionts, which themselves have genomes. CoRR offers an account of why chloroplasts and mitochondria held on to these genomes during evolution.

Evidence is available to demonstrate redox control of transcription of some of the essential sub-set of genes encoding proteins listed above, and depicted in green in Figure 1 [90–93]. As summarised in Box 1, there is also progress in resolving the redox signalling pathway that couples chloroplast transcription with photosynthetic electron transport. As predicted by the CoRR hypothesis, a conserved pathway of cyanobacterial origin is certainly involved [69,74].

There is mounting support for the proposal that rapid and unconditional redox regulation of gene expression is the single reason for the retention of the whole chloroplast genetic system. It follows that a close regulatory coupling of electron transfer with gene expression may be the reason why any gene at all is retained in chloroplast DNA.

Box 1. Connecting photosynthetic electron transport to chloroplast DNA transcription

Direct control of chloroplast transcription by photosynthetic electron transport was first demonstrated by Pfannschmidt *et al.* [90,91,93,111]. It was found that transcription of chloroplast genes for reaction centre apoproteins of photosystem I and photosystem II is under the control of the redox state of plastoquinone. When photosystem I electron transport is rate-limiting, plastoquinone becomes reduced. Reduced plastoquinone induces photosytem I and/or represses photosystem II. When photosystem II electron transport is rate-limiting, plastoquinone becomes oxidised. Oxidised plastoquinone induces photosystem II and/or represses photosystem I. As a result of this plastoquinone redox control of transcription, the stoichiometry of the two photosystems relative to each other adjusts itself. Photosystem stoichiometry adjustment is seen in cyanobacteria [112] and involves a typically prokaryotic two-component regulatory system [113]. Two-component systems require a histidine sensor kinase and a response regulator [114]. Chloroplast Sensor Kinase (CSK), first reported by Puthiyaveetil *et al.* [70] is required for photosystem stoichiometry adjustment in eukaryotic plants and algae, as summarised in Figure I. There seems to be no cognate response regulator in *Arabidopsis*, where the output from CSK controls transcription by its interaction with a bacterial-type Sigma factor, Sig-1 [115], and a plastid transcription kinase, PTK [69,74]. Algal chloroplasts employ recognisably bacterial sensor kinases and response regulators, the latter receiving a phosphate group onto an aspartate side-chain from a histidine of the sensor kinase [68,116,117]. Plastoquinone redox control of reaction centre gene transcription has clearly persisted throughout the transition from free-living prokaryote, through endosymbiont, to chloroplast.

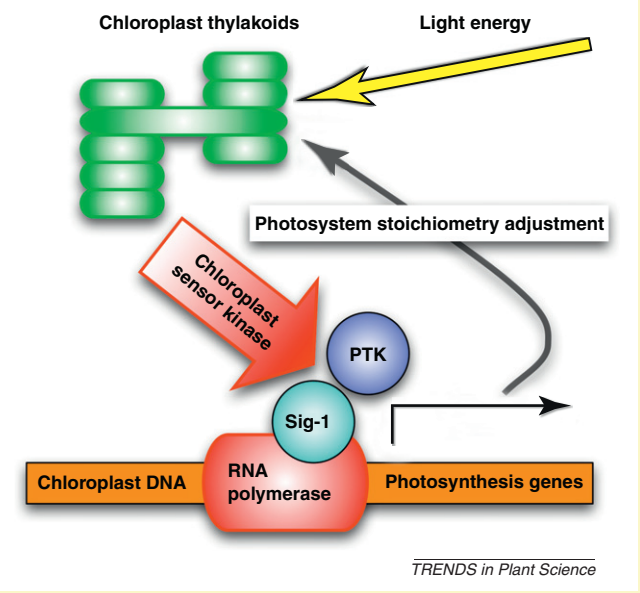

Figure I. Chloroplast Sensor Kinase acts on the transcriptional regulators Plastid Transcription Kinase (PTK) and Sigma Factor 1 (Sig-1). Changes in incident light energy produce photosystem stoichiometry adjustment in the chloroplast thylakoid membrane. Plastoquinone carries electrons between the photosystems, and its redox state depends on the rate of each photosystem relative to the other. Plastoquinone redox state is information. This information is communicated to the RNA polymerase at the promoter regions of genes for the reaction centres of the two photosystems, where their relative rates of transcription are determined by the phosphorylation state of Chloroplast Sensor Kinase. Chloroplasts inherit a typically bacterial mode of gene expression that must act on a typically bacterial genome. Thus, in photosynthetic eukaryotes, chloroplasts retain genomes, and genes for photosynthetic reaction centres are always found only in chloroplast DNA.

Disclosure statement

We declare complete absence of conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tplants.2011.10.004.

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