CHAPTER SIX

A Bacterial-Type Sensor Kinase Couples Electron Transport to Gene Expression in Chloroplasts

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Abstract Two-component systems, comprising sensor histidine kinases and response regulators, are ubiquitous signal transducers in bacteria. Chloroplasts, despite having a cyanobacterial ancestry, do not appear to possess two-component systems as signal transducers. Apart from a few reported cases of two-component systems of the red algal chloroplasts, it is generally believed that the two-component systems of the ancestral symbiont were lost or recruited in various locations of the host cell other than chloroplasts. Here we report a typical bacterial-type sensor kinase in chloroplasts. The gene for this kinase is found in cyanobacteria, from which chloroplasts evolved, and has moved, in evolution, to the nuclear genomes of algae and green plants. The gene encoding this Chloroplast Sensor Kinase (CSK), when inactivated in Arabidopsis, results in plants that are disabled in photosynthetic control of chloroplast gene transcription. This CSK-dependent process requires a sensor of electron transport between chloroplast photosystems I and II. Thus CSK is involved in a redox regulatory mechanism that couples photosynthesis to chloroplast gene expression. Sequence similarity searches find homologues of CSK to be present in many different lineages of algae and plants and to be related phylogenetically to the known plastid two-component systems of red algae. The persistence of this ancient signalling system of cyanobacteria in chloroplasts and its function in coupling photosynthesis to chloroplast gene expression bears directly on the premise that chloroplasts retain genes whose expression must be regulated by photosynthetic electron transport and that the mechanism of regulation has been conserved from the prokaryotic, ancestral endosymbiont.

Keywords Chloroplast, photosynthesis, two-component systems, redox, transcription, gene expression

Introduction

Photosynthesis is the conversion of radiant energy into chemical potential energy by plants, algae, and certain species of bacteria. These organisms

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thereby harness sunlight to drive the biogeochemical cycles of carbon and oxygen, sustaining life on Earth. The primary processes of photosynthesis are light absorption by chlorophyll and stabilised electrical charge separation, resulting in transfer of electrons along a chain of carriers (Blankenship 2002). In transfer of one or more electrons, each carrier undergoes a cycle of chemical reduction and oxidation, becoming reduced by accepting an electron from the previous carrier, and then oxidised by donating the electron to the next carrier in the chain. This reduction–oxidation or "redox" chemistry underpins energy conversion not just in photosynthesis but also in respiration – the re-mobilisation of the energy originally captured from sunlight and stored as the free energy of reactions between reductants and oxidants.

One type of photosynthesis uses water as the initial electron donor for the photosynthetic electron transport chain, and so produces free, molecular oxygen as the product of water oxidation. This "oxygenic" photosynthesis is now found in cyanobacteria and in their evolutionary descendants, the chloroplasts of eukaryotic plants and algae (Allen and Martin 2007). The ancient endosymbiosis that co-opted cyanobacteria as photosynthetic compartments of eukaryotic cells must have involved uptake and maintenance of the both the genetic and photosynthetic systems of the cyanobacterial endosymbiont (Gray 1992; McFadden 2001). Today, the chloroplasts of photosynthetic eukaryotes carry out oxygenic photosynthesis indistinguishable, except in fine detail, from that seen in cyanobacteria, while the chloroplast genome has been greatly decreased in size, as cyanobacterial genes have been lost or relocated to the eukaryotic cell nucleus (Martin et al. 2002).

Cyanobacterial genes retained in chloroplasts always include those encoding apoproteins of the photosynthetic reaction centres catalysing the primary, light-driven redox chemistry of photosynthesis. Why is this? One proposal is that expression of these special, structural genes must be subject to redox regulatory control, in order for the photosynthetic reaction centres to be synthesised in a precisely co-ordinated way, even when the light environment changes, or when metabolic supply and demand will otherwise alter the redox balance of the electron transport chain (Allen 1993a, 2003). Thus a co-location of gene with gene product within the same cellular compartment – the chloroplast – serves to ensure redox homeostasis, and a rapid and direct feedback control from photosynthesis to its genes.

Here we identify a bacterial sensor kinase (Allen 1993b; Ashby and Houmard 2006; Stock et al. 2000) in chloroplasts of higher plants, now encoded by a gene in the cell nucleus. Inactivation of this gene in *Arabidopsis thaliana* disrupts redox control of transcription of chloroplast. These results reveal a mechanism that couples photosynthesis to chloroplast genome function. This coupling has been conserved from the chloroplast's cyanobacterial ancestor, and therefore provides evidence for the function of cytoplasmic genetic systems in eukaryotes.

Materials and methods

Sequence analysis. Sequence similarity searches were performed with blastp and tblastn programs. Sub-cellular localization prediction was done with TargetP, ChloroP, Predotar, PCLR and Wolf PSORT. Domains and motifs were identified with SMART database. Sequence alignment was generated with ClustalW and the alignment was edited with Jalview editor.

Immuno-localization of CSK in thylakoid fractions. A partial cDNA clone (U13211) encoding 450 residues from the carboxy-terminal of CSK was obtained from ABRC and expressed in a pGEX4T2 (Amersham) vector system as a GST fusion protein. Over-expressed CSK was purified by affinity chromatography (Glutathione Sepharose) and GST tail was removed with thrombin. A polyclonal Anti-CSK serum was generated in rabbit by injecting the purified CSK (Innovagen, Sweden). Standard western blotting procedures were followed for probing the Arabidopsis proteome with the Anti-CSK antibody.

Genotyping of the SALK T-DNA insertion lines. Two T-DNA lines (SALK_027360 and

SALK_018074) harbouring insertions in the gene (At1g67840) encoding the CSK protein were obtained from ABRC. Genomic DNA was isolated from these lines and genotyped for homozygous insertion lines by using genomic and T-DNA cassette primers. A confirmative reverse transcriptase PCR was also done to ensure that the expression from the locus At1g67840 is completely knocked-out. The genomic flanking sequence and the exact insertion site of T-DNA were determined by sequencing the PCR products.

Plant growth conditions. For the Light switch time-course experiment, Wild Type (Col-0) and CSK knockout mutant Arabidopsis lines were grown in white light (100 μE m^{-2} s^{-1}) for 12 days and then transferred to Light 1 or Light 2 cabinets and allowed to acclimatize in Light 1 or 2 conditions for 4 days. At the end of the fourth day, lights were switched. Leaves from —two to three plants were collected for RNA extraction before the light switch and at various time points extending to 32 h after the light switch. Chlorophyll a/b ratios were determined from plants, which were 2–4 weeks old and grown under 2 days of light 1, light 2 and white light conditions. Chlorophyll content was estimated by methods established by Porra et al. (2002).

RNA isolation and quantitative real time PCR. Total RNA was isolated from the leaves of 15–17-day old *Arabidopsis* plants with Qiagen RNeasy Plant mini kit. RNA was treated with RNase free DNase (Qiagen) to eliminate possible DNA contamination. Quantitative RT PCR was performed with Quantitech SYBR green kit from Qiagen, in a Chromo4 cycler (Bio-Rad).

Results

Figure 1 shows the predicted amino acid sequence of CSK, the At1g67840 gene product of *Arabidopsis thaliana*, aligned with sequences of three bacterial histidine sensor kinases and with sequences of selected homologous proteins from plant whole genomes or expressed sequence tags (EST). The bacterial sensor kinases ArcB, RegB and EnvZ all contain a histidine residue that has been characterised as the site, in the complete protein, of autophosphorylation by phosphoryl group transfer from ATP (Bauer et al. 2003; Duplessis et al. 2007; Iuchi and Lin 1992; Mizuno et al. 1982a, b). *Arabidopsis* and other plant CSKs contain an homologous "H-box" motif in which the histidine itself is replaced by a glutamic acid residue (Fig. 1a).

The predicted molecular mass of *Arabidopsis* CSK is 66 kDa for the cytosolically synthesised chloroplast thylakoid–targeted precursor and 57 kDa for the mature form of the protein. The mature 57 kDa CSK polypeptide is predicted by hydropathy analysis to contain one membrane-spanning α-helix with a short N-terminal segment exposed at the lumenal, P-phase surface of the chloroplast thylakoid membrane, according to the "positive inside" rule (von Heijne 1992). The extended, C-terminal domain is therefore exposed to the chloroplast stroma (N-phase) where its kinase domain is expected to interact with its substrate and to bind ATP. Figure 1b shows the result of a Western blot in which *Arabidopsis* and spinach thylakoid proteins were probed with a polyclonal antibody raised against the over-expressed and purified CSK. In Fig. 1b an immunoreactive band is seen in the thylakoid membrane fraction at an electrophoretic mobility corresponding to a molecular mass of 57 kDa, the predicted mass of the mature CSK. It is seen (Fig. 1c) that sequence motifs of the ATP-binding domain; N, G1, F, G2 and G3 are common to bacterial sensor kinases ArcB, RegB and EnvZ and to CSK.

Figure 2a–d shows kinetics of *psaA* and *psbA* transcript accumulation in wild type and CSK mutant *Arabidopsis* plants in response to shifts in spectral quality of incident light. When light 1 is replaced by light 2, transcripts of the chloroplast *psaA* gene for a photosystem I reaction centre protein accumulate up to 11-fold in 26 h for the Wild Type (Fig. 2a), a functional response of up-regulation of genes for the rate-limiting reaction centre. In contrast, the CSK-mutant plants show only a fivefold increase in *psaA* gene transcription under the same conditions (Fig. 2a). This change represents a decrease of 55% in the *psaA* transcriptional response in the mutant. The reverse light switch,

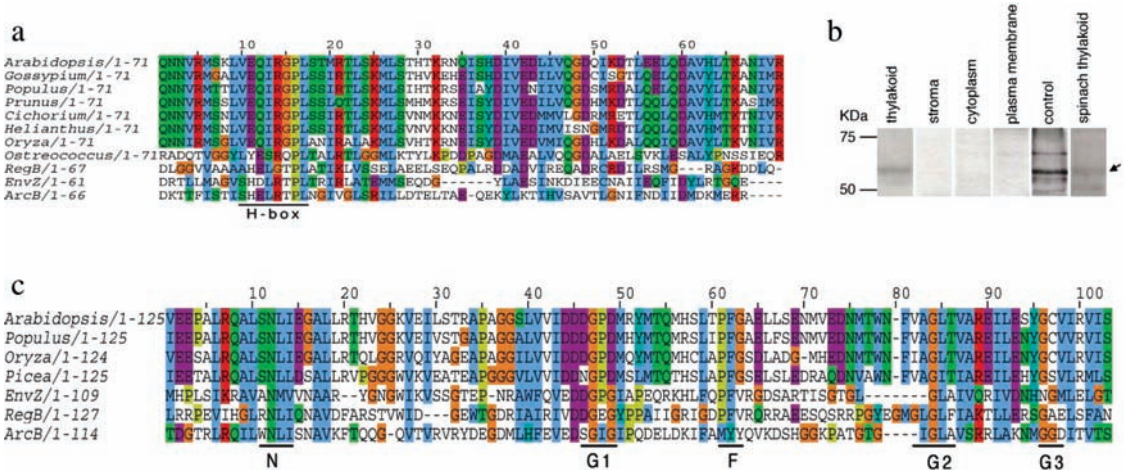

Fig. 1 CSK is modified histidine kinase that is targeted to chloroplasts. **a**. The HisKA domain (dimerization and phosphoacceptor domain as defined by SMART database) of CSK and its homologues along with that of three canonical histidine kinases, ArcB, RegB and EnvZ, are aligned. The site of auto-phosphorylation, H-box, is shown at the bottom. The auto-phosphorylating histidine is replaced by glutamate in CSK of higher plants, while a tyrosine replaces histidine in the Ostreococcus protein. **b**. Immuno-localization of CSK in thylakoids. Western blot of Arabidopsis proteome, probed with a polyclonal antibody raised against over-expressed CSK. An immunoreactive band is seen in the thylakoid fraction with an electrophoretic mobility corresponding to a protein of 57 KDa, the predicted molecular weight of mature CSK. A fainter band is also seen around 52 kDa, which is likely to be a cleavage product from the CSK. In the control, the polyclonal serum is tested against the over-expressed and affinity purified CSK protein. Spinach thylakoid shows similar immuno-reactive bands when probed with the CSK polyclonal serum. The arrow indicates the position of the major immunoreactive band. **c**. Sequence alignment of the ATP-binding domain of CSK and its homologues along with that of ArcB, RegB and EnvZ from bacteria. All the signature motifs (shown as N, G1, F, G2 and G3) characteristic of ATP binding domain of histidine kinases are conserved in CSK and its homologues

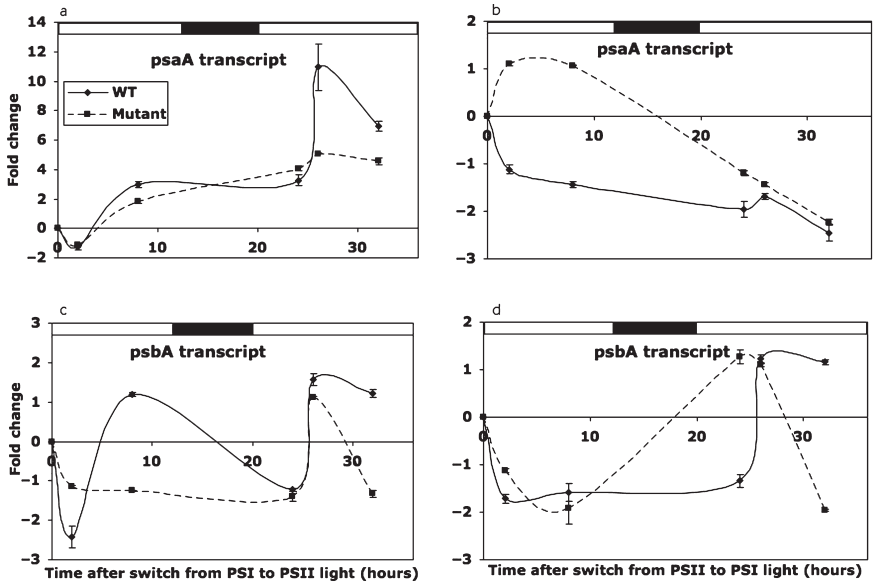

Fig. 2 *psaA* and *psbA* gene transcription kinetics in Wild Type (solid lines) and CSK knockout mutant (broken lines) as quantified with real time RT PCR. Changes in gene expression are shown as fold change plotted against time. Experimental conditions are PS1 to 2 and PS2 to 1 light switches. The time point at which the lights are switched is taken as zero time and the fold change thereupon (up or down regulation) is calculated by taking the expression at the time of light switch (zero time) as baseline. Error bars represent ±SE from three replicates. An 8-h dark photoperiod is shown as dark shaded rectangle on the X-axis

from light 2 to light 1, produces a 2.5-fold decrease in *psaA* expression in the wild type (Fig. 2b), while the *psaA* transcript quantity does not fall under the same conditions in the CSK-mutant. Instead, *psaA* transcript quantity increases for 8 h in the mutant, eventually falling in the same way as in the wild type between 26 and 32 h (Fig 2b).

Transcription of the chloroplast gene *psbA*, which encodes a reaction centre protein of photosystem II, follows comparable kinetics to *psaA* in response to light switches (Pfannschmidt et al. 1999a, b), but the with the opposite sign. When photosystem I becomes rate-limiting, *psbA* for the photosystem II protein is switched off (Fig. 2c). Thus transcript quantity of *psbA* in the wild-type plants decreases by 2.5-fold in the first 2 h following the switch from light 1 to light 2, while the decrease is smaller in the mutant (Fig. 2c). Conversely, when light 2 is replaced by light 1, *psbA* transcript quantity increases in the wild type after 24 h, but decreases in the CSK-mutant (Fig. 2d).

Figure 3 shows a phylogenetic tree constructed from CSK sequences of two cyanobacteria, one red alga, one green alga, and four green plants. All CSKs have a common ancestor in the cyanobacterial histidine kinase, Hik2. This a maximum likelihood tree generated by the PHYML program.

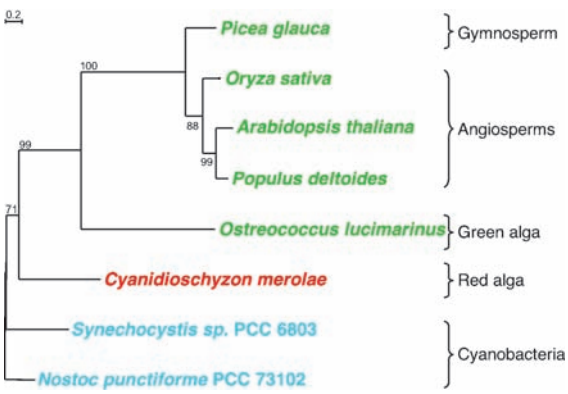

Fig. 3 CSK evolved from a hik2 like cyanobacterial protein. A phylogenetic tree of CSK and its homologues generated using PHYML program. Major taxonomic groups are bracketed. Bootstrap value for each split is also shown

Discussion

In green plants, CSK appears as a cyanobacterial sensor kinase (Fig. 1a, c) that is targeted to chloroplasts (Fig. 1b) and encoded by a gene that has moved from the chloroplast to the cell nucleus. Inactivation of this gene in *Arabidopsis thaliana* disrupts functional redox control of transcription of chloroplast genes (Fig. 2a–d). These results suggest that a two-component system underlies the mechanism that couples photosynthesis to chloroplast genome function. This coupling has evidently been inherited, and maintained, from the chloroplast's cyanobacterial ancestor, and may illustrate a general function for cytoplasmic genetic systems in eukaryotes (Allen 1993a, 2003). Local, redox control of gene expression by energy transduction may explain the persistence, in evolution, of both chloroplast and mitochondrial genetic systems as extra-nuclear elements (Kirk and Tilney-Bassett 1978) responsible for non-Mendelian inheritance of characters connected with photosynthesis and respiration (Allen et al. 2005).

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