Chloroplast Redox Poise and Signaling

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Chloroplasts are membrane-bound compartments (organelles) in plant and algal cells. Chloroplasts perform all of the component reactions of photosynthesis, including absorption of light energy by chlorophyll; conversion of that energy into chemical potential; electron transfer; synthesis of ATP; and assimilation of carbon dioxide to give carbohydrates. Primary steps in photosynthesis are light-driven redox (reduction–oxidation) reactions; transfer of electrons or hydrogen atoms. These redox reactions function safely and efficiently only within a narrow range of degrees of reduction of their reactants and products. In electron transport pathways, each intermediate must be capable of acting as both an acceptor and a donor, and must therefore be present in both its oxidized and its reduced form. An optimal state of redox poise is achieved by a variety of regulatory mechanisms, operating on different stages of gene expression. Redox signaling occurs when a signal of redox imbalance initiates a change that corrects that imbalance, restoring poise. The response may be within or between cells, and may concern chloroplast or nuclear gene expression. Chloroplast redox signaling utilizes the chloroplast genome and apparatus of gene expression. Redox signaling may require, and be the reason for, both chloroplast and mitochondrial genomes.

The Redox Chemistry of Photosynthesis

Redox Reactions and Photosynthesis

A redox reaction involves an electron or hydrogen atom donor, and an electron or hydrogen atom acceptor. During the course of the redox reaction, as the electron or hydrogen atom is transferred, the donor reduces the acceptor, and the acceptor oxidizes the donor. Thus, an electron (or hydrogen) donor is a chemical reductant, and an electron (or hydrogen) acceptor is a chemical oxidant. The direction of electron (or hydrogen) transfer between two chemical species is determined by their relative activities and also by their relative electrochemical potentials, or redox potentials, as given by the Nernst equation. A species with a lower redox potential will tend to act as a reductant, or donor, when coupled in a redox reaction with a species with a higher redox potential, acting as the oxidant, or acceptor.

In photosynthesis, light-driven electron transport may be cyclic, or linear (also known as "noncyclic"). Linear electron transport requires an electron donor and an electron acceptor. In many bacteria the donor is an organic compound, or an inorganic reductant such as H_2S or H_2. In cyanobacteria and chloroplasts, the donor is water, and oxygen is released as a by-product. The electron acceptor is $NADP^+$. Together with ATP produced by photosynthetic phosphorylation, NADPH represents stored energy, which may be used to drive carbon dioxide assimilation and processes including synthesis (e.g., protein synthesis) and transport.

van Niel and Bacteria

Photosynthesis occurs in bacteria, and in most plants and algae. Even in eukaryotes, photosynthesis is localized in chloroplasts, which are bacterial in origin. The microbiologist Cornelius van Niel, working in Stanford, California, showed that photosynthetic bacteria use light as a source of energy to cause hydrogen atom transfer to carbon dioxide, from any one of a range of weak reducing agents, collectively designated "H_2A" in a reaction described by the van Niel equation:

$$2H_2A + CO_2 \xrightarrow{\text{light}} CH_2O + 2A + H_2O$$

In the special case of oxygenic photosynthesis in plant and algal chloroplasts and in cyanobacteria, the weak reducing agent (H_2A) is water (H_2O), and the van Niel equation then becomes:

$$2H_2O + CO_2 \xrightarrow{\text{light}} CH_2O + O_2 + H_2O$$

The Hill Reaction

Independently, Robert (Robin) Hill, in Cambridge, showed that chloroplasts isolated from leaves

produce oxygen in the light, provided a suitable electron acceptor, or "Hill oxidant" is available ("A," below):

$$2H_2O + 2A \xrightarrow{\text{light, chloroplasts}} 2AH_2 + O_2$$

Assimilation of CO_2 by reoxidation of AH_2 will give "complete" photosynthesis, and its overall formulation is identical to a simplified version of the oxygenic version of the van Niel equation:

$$H_2O + CO_2 \xrightarrow{\text{light, chloroplasts}} CH_2O + O_2$$

Reaction Centers

How does light drive electron or hydrogen atom transfer? Louis Duysens, in Leiden, and others demonstrated light-induced oxidation of chlorophyll (Chl) at photosynthetic reaction centers, and it became clear that some chlorophyll molecules themselves undergo photo-oxidation after absorbing a quantum of light and reaching an excited state (Chl^*). Photosynthetic electron transport is initiated when the chlorophyll excited state decays to a ground state by passing an electron on to an acceptor (A), and by taking one from a donor (D):

$$DChlA \xrightarrow{\text{light}} DChl^*A \rightarrow DChl^+A^- \rightarrow D^+ChlA^-$$

The Z-Scheme and Two Photosystems

In 1960, Robert Hill and Fay Bendall proposed a "Z-scheme" for photosynthetic electron transport, in which two separate photosystems, termed I and II, each with a core reaction center, act as intermediates in the chloroplast electron transport chain. The two photosystems are connected, in series, by electron carriers (Figure 1).

Chloroplast Function

Intrinsic to internal membranes called thylakoids, chloroplasts contain chlorophyll and other pigments (such as carotenoids) that harvest and convert light energy, an electron transport chain that intersects with light-harvesting pigments at photosynthetic reaction centers, and an ATPase that couples electron transport with synthesis of ATP. The soluble phase of the chloroplast usually contains the enzymes and intermediates of the Benson–Calvin cycle of assimilation of carbon dioxide. In development, chloroplasts originate from plastids in parallel with nonphotosynthetic plastids with other specific functions in metabolism. Although most plastid proteins are imported from the cytosol, as precursors, all plastids also contain DNA, RNA, ribosomes, and a genetic system, which is responsible for synthesis of some of their components.

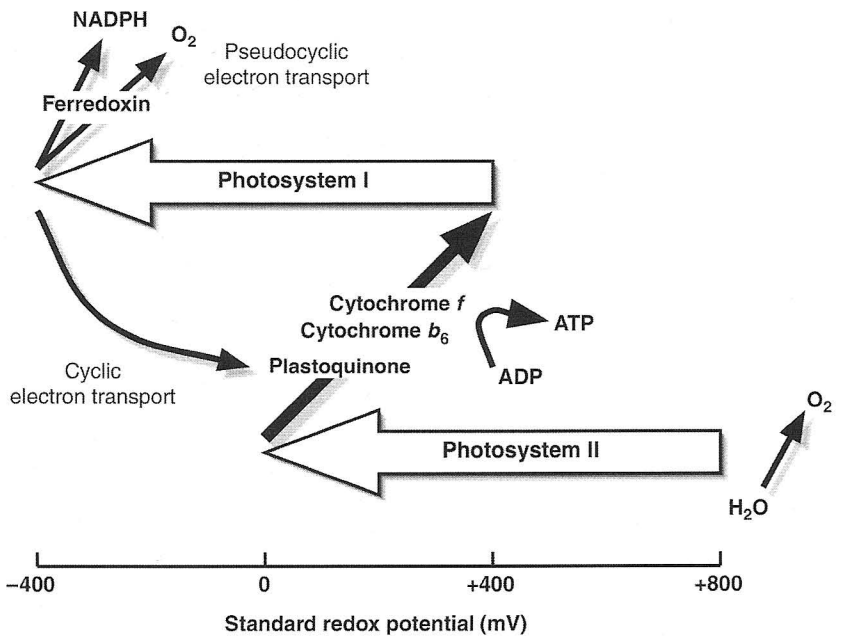

FIGURE 1 The two-light reaction model, or "Z-scheme," for photosynthetic, noncyclic electron transport and photophosphorylation (ATP synthesis) in chloroplasts. Noncyclic electron transport involves photosystems I and II, which are connected in series. The cyclic and pseudocyclic pathways are superimposed on the noncyclic pathway.

Chloroplasts, in particular, still bear a striking resemblance to prokaryotic cyanobacteria, from which all plastids evolved.

Photophosphorylation

In 1954, F. R. (Bob) Whatley, Mary Belle Allen, and Daniel I. Arnon, in Berkeley, California showed that isolated chloroplasts carry out ATP synthesis in the light, a process also demonstrated for isolated bacterial membranes by Albert Frenkel:

$$ADP + P_i \xrightarrow{\text{light, chloroplasts}} ATP + H_2O$$

Cyclic

The original chloroplast photophosphorylation of Whatley, Allen, and Arnon was not accompanied by net redox changes, and is coupled to cyclic electron transport. In the Z-scheme, this cyclic electron transport drives cyclic photophosphorylation, and requires photosystem I alone (Figure 1), although photosystem II activity and other factors greatly affect its onset.

Noncyclic

The Arnon group later showed that ATP synthesis is also coupled to the Hill reaction, and the yield of ATP occurs in a fixed stoichiometry with reduction of a Hill oxidant. There is thus a distinction between cyclic and noncyclic photophosphorylation. After 1960 it was realized that noncyclic photophosphorylation normally requires both photosystems I and II (Figure 1).

Pseudocyclic

In 1952, Alan Mehler discovered that molecular oxygen will act as a Hill oxidant. Noncyclic electron transport (Figure 1) with oxygen as the terminal acceptor is coupled to ATP synthesis, and requires photosystems I and II. However, oxygen is both consumed and produced in this reaction, no net oxidation–reduction is observed, and so the ATP synthesis resembles cyclic one, and is known as pseudocyclic phosphorylation.

Kinetics and Maintenance of Redox Poise

The interplay between noncyclic, pseudocyclic and true cyclic photosphosphorylation first gave rise to the concept of "redox poise" in chloroplast photosynthesis. If the photosystem I of the Z-scheme (Figure 1) is the same for all three forms of phosphorylation, then there is competition between paths of electron transport.

An optimal state for cyclic electron tranport is one of maximal "redox poise," when the total pool of each component gives a redox state that is 50% oxidized and 50% reduced. The rapid, primary photochemical reactions at photosynthetic reaction centers use light energy to move electrons in the direction opposite to that predicted by their redox potentials: the donor has a much higher redox potential than the acceptor, and light is therefore used to generate a redox couple that is far from redox equilibrium. Secondary electron transport nevertheless moves the electrons in the predicted direction, towards equilibrium. "Redox poise" can then still be applied to linear or noncyclic electron transport, and especially to any of its components. When applied to noncyclic electron transport, "redox poise" indicates a position of optimal redox state where the activities of components are such that their effective redox potentials favor physiologically useful electron transfer. Two extreme departures from redox poise exist in the form of states of over-reduction and over-oxidation (Figure 1). A cyclic chain is said to be over-reduced when all components are in their reduced forms; there are no electron acceptors. The same chain is said to be overoxidized when all components are in their oxidized forms; there are no electrons to cycle. When applied to noncyclic electron transport, "over-reduced" and "overoxidized" can be applied to indicate the preponderence of one or other redox state, giving the tendency of a component to engage in redox chemistry with donors or acceptors that are presumed to be nonphysiological, especially where reactive and toxic chemical species are produced in consequence. Oxygen, for example, readily accepts electrons from most components of the photosynthetic chain, a product is the superoxide anion radical, and redox poise may ensure that the "correct" electron transfer competes kinetically with reduction of oxygen.

Cyclic Electron Transport and Photophosphorylation

As first identified by Whatley and Bruce Grant, the onset of cyclic photophosphorylation is delayed, sometimes indefinitely, under anaerobic conditions. The delay can be avoided by addition of an inhibitor of electron transport in photosystem II, or by choice of a wavelength of illumination that is selective for photosystem I. It was concluded that the cyclic chain can become over-reduced when photosystem II is active, and no electron acceptor is available. Over-reduction could also be reversed, and redox poise restored, if a pulse of an oxidant, such as potassium ferricyanide or oxygen, is added to remove excess electrons from the cyclic chain.

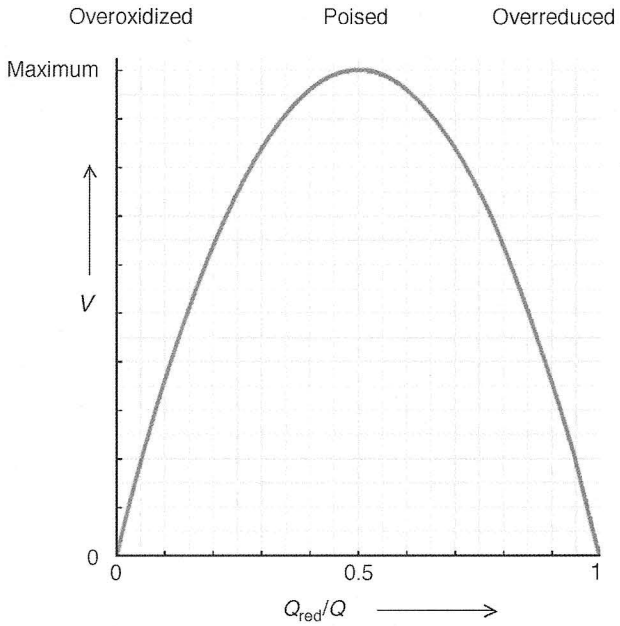

FIGURE 2 The ideal rate, v, of cyclic electron transport as a function of the redox ratio of one of its components, Q. Q must be present in both its oxidized and reduced forms: in cyclic electron flow, Q must be both a donor and acceptor of electrons.

In contrast, under aerobic conditions, Grant and Whatley found that the photosystem II inhibitor, just like a choice of wavelength specific to photosystem I, actually inhibits photosystem I-cyclic photophosphorylation. This is explained by the ability of oxygen to accept electrons from photosystem I, so that, in the absence of photosystem II activity, the cyclic chain becomes drained of electrons, or overoxidized (see Figure 2).

Photosystem 1 Cyclic Electron Flow

The contribution of cyclic photophosphorylation to photosynthesis (Figure 1) depends on maintaining redox poise, and avoiding over-reduction and overoxidation (Figure 2). There is evidence that oxygen acts as a poising oxidant under physiological conditions. Ulrich Heber and colleagues in Wurzburg showed that intact, functional chloroplasts are held in an indefinite lag phase under anaerobic conditions. However, if a pulse of oxygen is given at any time, sufficient ATP is produced to start the Benson–Calvin pathway, and regeneration of the electron acceptor $NADP^+$ then allows whole-chain electron transport to generate oxygen, maintaining a poised cyclic chain, ATP synthesis, and complete photosynthesis. *In vivo*, down-regulation of photosystem II may also counteract over-reduction, and chloroplast NAD(P)H dehydrogenase activity may counteract overoxidation.

The Q-Cycle

In the electron transport chain between photosystems I and II, the cytochrome b_6f complex catalyzes a cycle of electrons through two cytochromes b, involving intermediate states of the two-electron carrier plastoquinone. Redox poise is likely to be essential in the Q-cycle, where the plastosemiquinone participates in one-electron transfer with cytochrome b, despite its tendency to transfer its single electron to oxygen, generating superoxide.

Noncyclic Electron Transport

In intact chloroplasts, continued operation of noncyclic electron transport seems to depend on production of some additional ATP by cyclic electron transport. The ATP is required to drive the Benson–Calvin cycle, and therefore for regeneration of the physiological electron acceptor, $NADP^+$. Insufficient ATP therefore "stalls" photosynthesis by causing over-reduction of the cyclic chain.

Oxygen and Poise

Oxygen is an effective electron acceptor for noncyclic electron transport, and also acts as a poising oxidant for cyclic electron transport. However, the products of oxygen reduction are superoxide and hydrogen peroxide. Reduction of oxygen by the iron–sulfur protein and electron transport intermediate, ferredoxin, may proceed by a physiologically safe route of two one-electron transfers, and also lead to creation of "anti-oxidant" defenses against the toxicity of reactive oxygen species.

Posttranslational Modification of Pre-Existing Proteins

Regulatory devices that maintain redox poise also extend to gene expression at different levels. Feedback control loops involving components of electron transfer chains and stages in gene expression are seen in all bioenergetic systems. Close association between bioenergetic coupling membranes and genes for their protein components is conspicuous in chloroplasts and mitochondria. The need for direct redox signaling has been proposed as the primary reason for the persistence, in evolution, of chloroplast and mitochondrial genomes.

State Transitions

Control of gene expression by posttranslational modification underpins a well-known process, namely, state transitions. Photosystems I and II have different

light-harvesting pigment systems, and different absorption and action spectra. Thus, any randomly chosen wavelength of light is likely to favor either photosystem I or photosytem II. Yet their connection in series requires a fixed ratio of rates of electron transport through their reaction centers, 1:1 for noncyclic electron transport alone, but incrementally more (estimated at 20%) to photosystem I to account for additional, cyclic electron transport. Cecilia Bonaventura and Jack Myers in Austin, Texas, and Norio Murata in Tokyo independently showed that different unicellular algae redistribute absorbed excitation energy between photosystems I and II, as if to achieve balanced delivery of energy to the two reaction centers. Light delivered to one reaction center whose rate is limited by another will be wasted as heat or fluorescence. Redistribution therefore achieves maximal efficiency despite changing wavelengths of light that otherwise favor one photosystem or the other. Photosystem II works effectively at wavelengths up to ~660–670 nm; photosystem I can utilize light beyond this "red drop" in photosynthetic yield. A beam of light with a spectrum centered at, say, 700 nm, will be selective for photosystem I, and is termed "light 1." A beam centered below 660 nm will drive both photosystems, but is required for photosystem II, and is termed "light 2." Switching beams from light 2 to light 1, or superimposing light 1 onto light 2, induces a change in the light-harvesting apparatus, redistributing energy to photosystem II at the expense of photosystem I. The state arrived at is called the "light 1 state" or "state 1." Conversely, switching from light 1 to light 2 induces a "light 2 state," or "state 2."

Chloroplast Protein Phosphorylation

John Bennett at Warwick University discovered chloroplast protein phosphorylation and showed that one conspicuous phosphoprotein was a light-harvesting chlorophyll *a*/*b*-binding protein forming part of chloroplast light-harvesting complex II (LHC II). LHC II is an intrinsic membrane protein which becomes phosphorylated on a threonine residue by the action of a membrane-associated LHC II kinase.

Plastoquinone Redox Control of the LHC II Kinase

The LHC II kinase was originally thought to be activated by light. However, in Urbana, Illinois, in a collaboration with Bennett, John F. Allen and Charles J. Arntzen showed that LHC II becomes phosphorylated in darkness, provided the electron carrier plastoquinone is chemically reduced. The apparent light activation is sufficiently explained as redox-activation, with electrons being supplied to plastoquinone from photosystem II. In addition, LHC II phosphorylation is accompanied by changes in chlorophyll fluorescence and in electron transport, demonstrating that absorbed light energy simultaneously became redistributed to photosystem I at the expense of photosystem II.

Distribution of Absorbed Excitation Energy

The state-2 transition, an apparently purposeful response increasing efficiency of energy conversion, is the result of activation of the LHC II kinase by reduced plastoquinone. In light 1, the state-1 transition results from oxidation of plastoquinone by photosystem I: the LHC II kinase becomes inactivated, a light- and redox-dependent phospho-LHC II phosphatase catalyzes dephosphorylation of phospho-LHC II, and light energy absorbed by chlorophyll molecules of LHC II is returned to photosystem II, at the expense of photosystem I (Figure 3). Phosphorylation affects the three-dimensional structure of LHC II, and the movement of LHC II between photosystems I and II proceeds because its two structural forms differ in their capacity to bind and interact functionally with the two photosystems – a change in molecular recognition. An LHC II kinase has been identified by Jean–David Rochaix and co-workers in Geneva.

Thioredoxin

A number of enzymes of the Benson–Calvin cycle, and the coupling ATPase of chloroplast membranes, are activated by the reduced form of a soluble iron–sulfur protein, thioredoxin. This example of chloroplast redox signaling was discovered by Robert (Bob) Buchanan and co-workers in Berkeley, California. Thioredoxin accepts electron from ferredoxin, an electron carrier on the acceptor side of photosystem I, and light activation therefore depends on the activity of both photosystems. The function of thioredoxin redox signaling seems to be to ensure that CO_2 assimilation and ATP synthesis are inactivated in darkness, so that energy stored in photosynthesis is not subsequently dissipated.

Posttranscriptional Control

Within chloroplasts, reduced thioredoxin also activates ribosomal translation and processing of RNA, and has been studied for the *psbA* gene product (the D1 reaction center apoprotein of photosystem II) by Steven Mayfield at the Scripps Institute in San Diego, California.

Transcriptional Control

Redox and other signal transduction pathways in bacteria often exert parallel effects at posttranslational

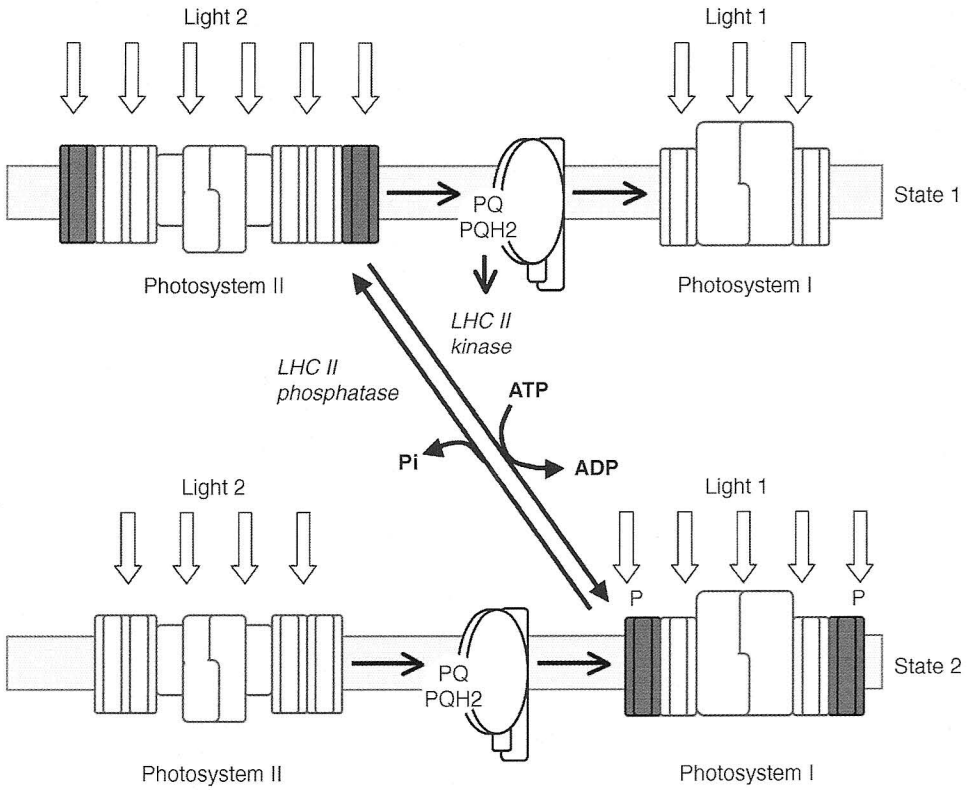

FIGURE 3 Plastoquinone redox control of LHC II kinase activity and its role in balancing distribution of excitation energy between photosystems I and II.

and transcriptional levels of gene expression. Chloroplasts are no exception.

Chloroplast Genes

Thomas Pfannschmidt and John F. Allen, working at Lund University, showed that transcription of chloroplast genes for reaction center apoproteins is regulated by the redox state of plastoquinone. As with phosphorylation of LHC II, the direction of control is functionally intelligible. Reduced plastoquinone is a signal that photosystem I is rate-limiting and photosystem II activity is in excess. Reduction of plastoquinone turns off transcription of photosystem II reaction center genes, and turns on transcription of photosystem I reaction center genes. Conversely, oxidized plastoquinone is a signal of imbalance with photosystem II rate-limiting, and photosystem I in excess: oxidized plastoquinone turns off photosystem I transcription, and turns on photosystem II transcription. Thus, the stoichiometry of photosystems I and II will tend to adjust itself to match changes in the prevailing light regime, as well as changes in metabolic demand for ATP relative to NADPH. Plastoquinone redox control of reaction center gene transcription maintains redox poise of plastoquinone (Figure 4) and of other components linked to it, including components of the cyclic electron transport pathway of photosystem I (Figure 1).

Nuclear Genes

Redox signals from the chloroplast also affect transcription of nuclear genes. In the case of light 1–light 2 effects and redox control at the level of plastoquinone, nuclear genes for photosystems I and II are regulated in the same functionally intelligible way, suggesting a backup which serves the same goal of maintaining redox poise within the chloroplast.

Mechanisms and Evolutionary Implications

Mechanisms and components in chloroplast redox signaling are not fully resolved, especially as regards transcription and translation. Based on bacterial redox signaling, candidate pathways are proposed as follows, and preliminary evidence is consistent with these.

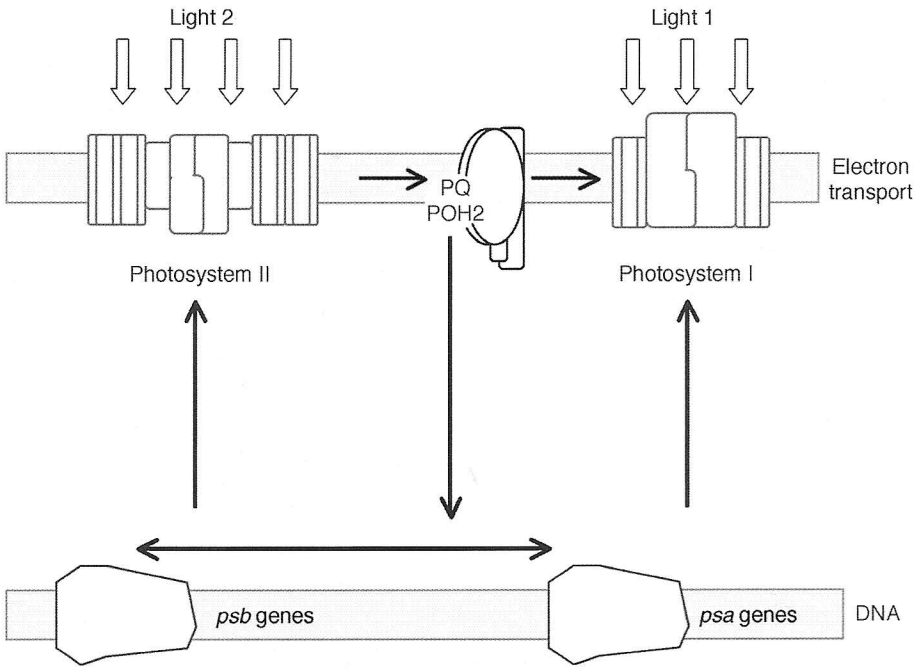

FIGURE 4 Plastoquinone redox control of chloroplast photosynthetic reaction center gene transcription and its role in adjusting the stoichiometry of photosystem I to photosystem II.

Two-Component Systems

A bacterial two-component redox regulatory pathway utilizes a membrane-intrinsic histidine sensor kinase which becomes phosphorylated when ubiquinone in the respiratory chain is reduced. The phosphate from the sensor is then transferred to an aspartate of a soluble response regulator. The response regulator is a sequence-specific DNA-binding protein, and the effect is to switch on transcription of genes for proteins required for anaerobic respiration. This aerobic respiratory control (Arc) system senses redox state. Photosynthetic bacteria, including cyanobacteria, possess cognate systems. Chloroplast genomes of some eukaryotic, red and brown algae have genes for similar components (sensors and response regulators), and green algae and plants have nuclear genes for these components, including transit sequences required for chloroplast import.

FNR and Redox Activators

At the level of thioredoxin, accepting electrons from ferredoxins, bacteria have DNA-binding iron–sulfur proteins. The best-studied example is "fumarate and nitrate reductase" of *Escherichia coli* (FNR: by coincidence, the abbreviation also for ferredoxin–NADP reductase of photosystem I). Since reduced ferredoxin sends redox signals to gene expression, it is likely that a cognate redox activator system is involved.

Redox Signaling and the Function of Cytoplasmic Genomes

Redox signaling is unique neither to chloroplasts nor to photosynthesis. Close coupling between primary redox chemistry and gene expression is seen in bacteria and chloroplasts, with some preliminary evidence also in mitochondria. Inspection of the genes contained in chloroplast and mitochondrial DNA reveals no clear correlation with the hydrophobicity of the gene product, as sometimes supposed. Rather, the core, membrane-intrinsic proteins of primary electron transfer are always encoded in chloroplasts and mitochondria, and synthesized on organelle ribosomes. This rule is especially clear for chloroplast reaction center genes, whose transcription is known to be controlled by the redox state of the plastoquinone pool. It has been proposed that retention, in evolution, of chloroplast and mitochondrial genetic systems gives a co-location of gene with gene product, and that this co-location is essential for redox regulation. Chloroplast redox poise and signaling therefore have important implications for cell evolution, and may account for the distribution of genes between nucleus and cytoplasm in eukaryotic cells.

See Also the Following Articles

Chlorophylls and Carotenoids • Chloroplasts • Ferredoxin • Ferredoxin-$NADP^+$ Reductase • Photosynthesis

Glossary

chloroplast (from Greek for "green box") A subcellular organelle of plants and algae. Chloroplasts are the location for the process of photosynthesis in eukaryotes.

photosynthesis The capture of light energy and its utilization to produce reduced carbon compounds whose subsequent oxidation in respiration releases the free energy originally obtained from light.

poise A state of balance. "Redox poise" in electron transport occurs when each electron-carrying intermediate is present in both its oxidized state and its reduced state, in order for that component both to accept and to donate electrons or hydrogen atoms.

redox An adjective derived from "reduction–oxidation," and describing a class of chemical reactions that involve transfer of electrons or hydrogen atoms.

redox signaling Coupling between biological electron transfer and gene expression. Regulatory control is exerted in both directions.

Further Reading

Allen, J. F. (1992). Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta* **1098**(3), 275–335.

Allen, J. F. (2002). Photosynthesis of ATP-electrons, proton pumps, rotors, and poise. *Cell* **110**(3), 273–276.

Allen, J. F. (2003). The function of genomes in bioenergetic organelles. *Philos. Trans. Roy. Soc. London Series B-Biological Sci.* **358**(1429), 19–38.

Allen, J. F. (2004). Chromosome b6f: Structure for signalling and vectorial metabolism. *Trends Plant Sci.* **9**(3), 130–137.

Bauer, C. E., Elsen, S., and Bird, T. H. (1999). Mechanisms for redox control of gene expression. *Annu. Rev. Microbiol.* **53**, 495–523.

Blankenship, R. E. (2002). *Molecular Mechanisms of Photosynthesis.* Blackwell Science, Oxford.

Heathcote, P., Fyfe, P. K. and Jones, M. R. (2002). Reaction centres: The structure and evolution of biological solar power. *Trends Biochem. Sci.* **27**(2), 79–87.

Pfannschmidt, T. (2003). Chloroplast redox signals: How photosynthesis controls its own genes. *Trends Plant Sci.* **8**(1), 33–41.

Race, H. L., Herrmann, R. G. and Martin, W. (1999). Why have organelles retained genomes? *Trends Genet.* **15**(9), 364–370.

Wollman, F. A. (2001). State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. *EMBO J.* **20**(14), 3623–3630.

Biography

John F. Allen is Professor of Plant Cell Biology in Lund University, Sweden. He was educated in Newport, Monmouthshire, UK, and then at King's College in London University, obtaining his Ph.D. with David O. Hall. Dr. Allen carried out postdoctoral work in Oxford and Warwick Universities and in the University of Illinois at Urbana, subsequently working in Leeds University, and, on sabbatical, in University of California, Berkeley. He was Professor of Plant Physiology in Oslo for two years before moving to his present position in 1992. Allen demonstrated superoxide production by isolated chloroplasts and, later, plastoquinone redox control of chloroplast protein phosphorylation. His work on redox regulation of photosynthesis contributed to his theory that chloroplast and mitochondrial genomes allow genetic control of redox poise.