Energy balance and acclimation to light and cold

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Changes in environmental conditions such as light intensity or temperature result in an imbalance between the light energy absorbed through photosynthesis versus the energy utilized through metabolism. Such an energy imbalance is sensed through alterations in photosystem II excitation pressure, which reflects the relative reduction state of the photosystem. Modulation of this novel, chloroplastic redox signal either by excess light, chilling or temperature signal may occur as a consequence of a reduction in PSI antenna size. These mechanisms result in adjustments in the functional absorption cross-sectional area of PSI (\( \sigma_{\text{PSI}} \)) which would reduce photosynthetic efficiency measured as either the quantum yield of CO\(_2\) assimilation (\( \phi_{\text{car}} \)) or the quantum yield of O\(_2\) evolution (\( \phi_{\text{O}_2} \)). In addition, some plants reduce leaf angle relative to the incident radiation, alter leaf optical properties or change their position in the water column in the case of algae to reduce the incident photon flux. Alternatively, photoautotrophs could acclimate by increasing the number of components acting as electron-consuming sinks by elevating the levels of Calvin cycle enzymes, which would increase the capacity for CO\(_2\) assimilation or photorespiration relative to electron transport. Clearly, in nature, photoautotrophs may exploit any one or a combination of these mechanisms to offset the potential ‘energy crisis’ during exposure to fluctuations in environmental conditions such as temperature and irradiance (Fig. 1). However, modulation of energy balance is not restricted to light and temperature. CO\(_2\) limitations associated with drought and deprivation of macronutrients such as nitrate might reduce sink capacity, which also leads to the over-reduction of PSI.

Changes in PSI excitation pressure are reflected in alterations in the redox state of PSI, which can be monitored in vivo by exploiting chlorophyll \( a \) fluorescence as a non-invasive probe. Photochemical fluorescence quenching can be used to estimate the proportion of PSI reaction centres that are ‘open’ (reflecting the relative oxidation state of \( Q_a \), the first stable quinone electron acceptor of PSI reaction centres) or ‘closed’ (thought to reflect the relative reduction state of PSI) (Fig. 2). Thus, an estimate can be made of the relative PSI excitation pressure to which photoautotrophs are exposed. Although not providing an exact estimate of the reduction state of PSI, this fluorescence parameter does provide a useful estimate of relative changes in PSI excitation pressure of organisms exposed to changing environmental conditions.
Fig. 1. Photosynthetic transformation of light energy and mechanisms to maintain an energy balance. Primary photochemistry involves the absorption of sunlight by the pigments of the light-harvesting protein complex (LHC) and the transfer of this energy to the reaction centres of photosystem II (PSII) and photosystem I (PSI) to induce charge separation in 10⁻¹⁵ to 10⁻¹⁶ s. Coupled biochemical redox reactions transfer electrons from PSII through the intersystem electron-transport chain, consisting of plastoquinone (PQ), the cytchrome f/b₆ complex (CYT f/b₆) and plastocyanin (PC), to PSI in about 10⁻³ s. Photosynthetic electron transport converts light into reducing power (NADPH) and, concomitantly, chemical potential energy (ATP), through chemiosmosis. These products are consumed through the reduction of C, N and S in the chloroplast, all of which occurs in seconds to minutes. These metabolic processes, in turn, are required for normal growth and development. Several mechanisms have evolved to ensure a balance between light energy absorbed versus energy utilized through electron transport and metabolism to protect PSII from over-excitation. Energy transfer from PSII to PSI (state transitions) and dissipation of excess absorbed energy as heat by non-photochemical quenching can occur in minutes. Over a longer time (hours to days), the following mechanisms occur: reduction of LHCII antenna size; alteration of PSII–PSI stoichiometry; stimulation of the rate of repair of the damaged D1 polypeptide of the PSII reaction centre; and stimulation of the capacity to utilize ATP and NADPH through metabolism to maintain high photochemical quenching of PSII excitation.

Box 1. Energy balance and photosystem II excitation pressure during acclimation to light and temperature

The rate of excitation of photosystem II (PSII) under light-limiting conditions can be estimated as:

\[ \sigma_{\text{psii}} \times I \]

where \( \sigma_{\text{psii}} \) is the functional absorption cross-sectional area of PSII and \( I \) is the incident photon flux (units \( \text{mol photons m}^{-2} \text{s}^{-1} \)). Under light-saturating conditions, the rate of utilization of this excitation energy through electron transport and metabolism can be estimated as:

\[ n \times \frac{1}{t} \]

where \( n \) is the number of components acting as sinks that consume electrons, \( t \) is the life-time and, hence, \( \frac{1}{t} \) is the turnover rate of these sinks. A balance between energy absorbed and energy utilized is attained when:

\[ \sigma_{\text{psii}} \times I = n \times \frac{1}{t} \]

Consequently, any environmental condition that satisfies the following inequality would result in over-excitation of PSII (i.e. PSII excitation pressure):

\[ \sigma_{\text{psii}} \times I > n \times \frac{1}{t} \]

Thus, an energy imbalance can result from exposure to excessive irradiance, \( I \), at constant temperature. Alternatively, an energy imbalance can occur as a consequence of exposure to low temperature at constant irradiance. Low temperature causes a reduction in \( \frac{1}{t} \). Thus, PSII excitation pressure can be created either by modulating irradiance, which affects temperature-insensitive photochemical processes (\( Q_{10} = 1 \)), or by modulating temperature, which affects temperature-dependent, biochemical processes (\( Q_{10} = 2 \)).
The photo-oxidation of P680 to \( P_{680}^{+} \) (electron acceptor). The transfer of absorbed light energy to 'open' PSII reaction centres causes

\[ \text{Z. Concomitantly, } Q_A \rightarrow Q_B \]

The arrow indicates a layer of epidermal cells that contains anthocyanin.

**Fig. 3.** Cross-section of a needle of cold-acclimated *Pinus banksiana*. The arrow indicates a layer of epidermal cells that contains anthocyanin.

**Photosynthetic adjustment during cold acclimation**

It appears that nature has provided several alternative solutions to the common problem of stress-induced energy imbalance (Fig. 1). The mechanisms employed are integrally related to the time scale of exposure and to the developmental strategy of the species in question.

**Evergreen plants**

In the Northern hemisphere, the leaves of evergreen plants typically develop and expand during the warm spring and summer months and are subsequently retained and maintained during the winter months when all growth ceases. The reversible interconversion of the light-harvesting xanthophyll, violaxanthin, to the energy quencher, zeaxanthin, occurs over periods of minutes upon exposure to excessive light whereas an increase in the total xanthophyll pool size and an accumulation of zeaxanthin can occur over a period of days or months in response to extensive periods of low, winter temperatures, when light-saturated photosynthetic rates as well as photosynthetic efficiency are depressed. This results in a sustained capacity to dissipate excess light as heat by non-photochemical quenching mechanisms (Fig. 1). However, in *Pinus sylvestris*, LHCII is reorganized into large pigment-protein aggregates containing both chlorophyll and zeaxanthin that appear to dissipate excess energy as heat.

Because non-photochemical quenching appears to play a major role in the protection of the photosynthetic apparatus from severe photodamage, evergreens appear to adjust \( q_P \) in response to the imbalance in energy budget that occurs during the winter months. Other temperate conifers such as *P. banksiana* exhibit 'purpling' caused by the accumulation of anthocyanin in epidermal cells (Fig. 3) in response to a complex interaction of photoperiod, low temperature and irradiance. Accumulation of anthocyanins appears to protect the needles against photoinhibition of PSII when exposed to light and cold temperatures through a simple screening effect that reduces the absorbed photon flux. This is consistent with the suggestion that anthocyanins are a natural sun screen against both UV-B radiation and high visible irradiance.

**Cereals**

In contrast to evergreens, cold-tolerant winter rye and wheat must grow and develop at low temperatures for maximum cold tolerance and successful winter survival in temperate climates. Thus, these plant species must maintain the capacity for active photosynthesis during prolonged exposure to low, non-freezing temperatures during the cold acclimation period with minimal changes in pigment composition. Somersalo and Krause were the first to report that cold acclimation results in an increased tolerance to photoinhibition, a result that was subsequently confirmed for winter rye and wheat. This was not because of changes in leaf optical properties, but was associated with minimal changes in thylakoid membrane microviscosity based on differential scanning calorimetry and electron spin resonance measurements. In contrast to the alpine arctic species *Oxyria digyna*, the increased tolerance to photoinhibition...
in wheat and rye is not caused by an increased capacity to repair damaged PSII reaction centres or increased non-photochemical quenching, but rather an increased capacity to keep the quinone Q\text{A} oxidized (i.e. high photochemical quenching) due to an elevated photosynthetic capacity with no change in photosynthetic efficiency. The potential to keep Q\text{A} oxidized and the potential to increase the photosynthetic capacity as a consequence of cold acclimation are correlated with both tolerance to photoinhibition and the capacity of winter rye and wheat cultivars to develop maximum freezing tolerance.33

The increased capacity of rye and wheat to keep Q\text{A} oxidized appears to be a consequence of a cold acclimation-induced stimulation of mRNA and protein levels associated with the major regulatory enzymes of photosynthetic carbon metabolism: Rubisco, stromal and cytosolic fructose bisphosphatase and sucrose phosphate synthase. This is reflected in an increased enzyme activity as well as an increased activation state of these important regulatory enzymes. This is translated into increased growth rates under potentially photoinhibitory conditions. Thus, the elevated sucrose levels normally associated with cold acclimation do not result in the repression of photosynthetic gene expression in Arabidopsis,40 rye or wheat,67 contrary to current models related to sucrose regulation of plant gene expression. It appears that increased cold tolerance and tolerance to photoinhibition may be the result of a ‘reprogramming’ of carbon metabolism in these species. Thus, winter wheat and rye appear to adjust the number of electron-consuming sinks in response to growth at low temperatures. However, this response appears to be species specific.

Green algae

Similar to the evergreens, low-temperature acclimation of the unicellular green algae, Chlorella vulgaris and Dunaliella salina, results in a depression of the capacity for CO\textsubscript{2} assimilation and photosynthetic efficiency, calculated on a per cell basis, concomitant with a decrease in the total xanthophyll pool size as well as a lower epoxidation state of the xanthophyll cycle pigments due to the conversion of violaxanthin to zeaxanthin. However, in contrast to evergreens and both winter rye and wheat, cold acclimation of these green algae is associated with a six-fold lower chlorophyll content per cell; a lower abundance of Lhc\textsubscript{b} mRNA as well as Lhc\textsubscript{b} polypeptides; and an increased level of the carotenoid-binding protein, ChlB (Fig. 4c: compare 27/150 and 5/150). However, these algae appear unable to up-regulate carbon metabolism and thus are unable to adjust the number of electron-consuming sinks during growth and development at low temperature. As a consequence, algal cultures grown at low temperature exhibited a distinctive yellow colour (Fig. 4a). The repression in the accumulation of Lhc\textsubscript{b} mRNA and Lhc\textsubscript{b} polypeptides is not due to sucrose suppression, since the capacity for sucrose accumulation is depressed upon cold acclimation of Chlorella vulgaris.

Cultures grown at low temperature exhibited a three- to four-fold increased tolerance to photoinhibition. Thus, in contrast to either evergreens or wheat and rye, these green algae alter their pigmentation significantly in response to growth at low growth temperature or high growth irradiance. This reflects a reduction in light-harvesting capacity coupled with an increased capacity to dissipate excess light non-photochemically as heat through zeaxanthin and possibly lutein, which results in a decrease in \( \sigma_{\text{PQ}} \).

Cyanobacteria

In contrast to chloroplasts, the cyanobacterium Synechococcus sp. PCC 7942 possesses three homologous genes for the D1 protein of PSII reaction centres42, designated psb\text{AI}, psb\text{AII} and psb\text{AIII}. The psb\text{AI} gene encodes form 1, designated D1:1; psb\text{AII} and psb\text{AIII} encode form 2, designated D1:2. Mutants expressing D1:2 exhibit greater tolerance to photoinhibition than wild-type cells that express D1:1. Recently, it has been shown that exposure of wild-type Synechococcus sp. PCC 7942 to low temperature induces a transient exchange of the D1:1 form for the D1:2 form of the PSII reaction centre polypeptide, resulting in an increased tolerance to photoinhibition.43

There is a consensus that a rapid cycle of damage and repair of the D1 polypeptide during photoinhibition is an intrinsic feature of PSII (Ref. 1). A decrease in membrane lipid unsaturation inhibits subsequent recovery from photoinhibition through an impairment of the D1 repair process in cyanobacteria.44 This appears to be due to an inability to process the newly synthesized D1 protein, resulting in the accumulation of inactive PSII reaction centres. Moreover, exposure to cold enhances thylakoid fatty acid unsaturation, and increases the tolerance of cyanobacteria to low-temperature photoinhibition and chilling injury.44,45 Thus, cyanobacteria appear to respond to photoinhibition by adjusting the capacity to repair PSII (Fig. 1). It may be that changes in membrane fluidity act as a primary signal for changes in temperature in cyanobacteria.46

Accumulation to cold and high light intensity

Photosynthetic adjustment of Chlorella to growth at low temperature and moderate irradiance is comparable to cells grown at high light with respect to pigmentation, gas exchange and sensitivity to...
Cold, light and freezing tolerance
The photosynthetic response and tolerance to photoinhibition of cold-acclimated wheat and rye may be due to growth at elevated PSII excitation pressure. However, does the modulation of PSII excitation pressure by low temperature or irradiance influence events beyond photosynthesis and sensitivity to photoinhibition? In contrast to the Wcs120 family of cold-acclimation genes, expression of the nuclear-encoded cold acclimation gene Wcs19 as well as the induction of the short, compact growth habit associated with cold hardening are not direct responses to cold acclimation, but are rather responses to changes in PSI excitation pressure.

Fig. 5. (a) Effect of growth regime on photosystem II (PSII) excitation pressure in winter rye leaves. PSII excitation pressure was estimated as $qP$, where $qP$ is the photochemical quenching parameter. Rye plants grown at 5°C and 5 μmol m$^{-2}$ s$^{-1}$ exhibited low PSII excitation pressure, even though the plants are grown under very different light and temperature regimes. Similarly, rye grown at either 20/250 or 5/50 also exhibited lower levels. (b) Effect of growth regime on plant morphology. The compact growth morphology of cold-acclimated plants (5/250) was mimicked by rye plants exposed to comparable PSII excitation pressures induced by growth at moderate temperatures but high light (20/800). Similarly, the elongated growth morphology of non-cold-acclimated rye plants (20/250) was mimicked by rye plants exposed to comparable low PSII excitation pressures induced by growth at low temperature and low light (5/50).

Photoinhibition, as well as the accumulation of Lhc polypeptides and ctr, the carotenoid-binding protein (Fig. 4). This cannot be explained as either a simple growth temperature effect or a simple irradiance response. Cultures grown at either 5°C and low light intensity or 27°C and high light intensity are photosynthetically adjusted to growth at high PSII excitation pressures; cultures grown at either 27°C and low light or 5°C and very low light are photosynthetically adjusted to growth at low PSII excitation pressures. Similar conclusions regarding the role of PSII excitation pressure have been reported for thermal and photoacclimation of Laminaria saccharina and the expression of early light-inducible proteins (ELIPs) in barley.

Photosynthesis provides the energy necessary for the attainment of a cold-acclimated state and, hence, maximum freezing tolerance. Thus, any environmental factors that have a negative impact on photosynthesis may ultimately influence the induction of freezing tolerance. Since maximum LT50 is dependent upon both light and temperature, the physiology of the plant may, in
Although maximum freezing tolerance (LT50) is altered by changing either the growth temperature or the growth light (Fig. 5d), LT50 is not modulated by PSII excitation pressure. It is dependent on both light and temperature in an independent but additive manner29 (Fig. 5d). Thus, cold acclimation and freezing tolerance may well be the result of complex interactions of low temperature, light and chloroplastic redox poise, as reflected by the sensitivity to PSII excitation pressure. The transduction pathway is initiated in response to one or more of these signals, which might interact not only with each other but also synergistically with other important signal transduction pathways — involving phytochrome32, sugar sensing33, protein phosphorylation and dephosphorylation, Ca2+ and growth regulators34,35 to elicit the appropriate physiological response.

Recent results indicate that chromosome 5A of wheat carries the regulatory gene or genes that not only control the expression of the gene families correlated with freezing tolerance but also genes controlling plant morphology36. Perhaps a locus or loci on chromosome 5A represents a master regulator that co-ordinates the expression of genes associated with cold acclimation, freezing tolerance and plant morphology. If this is the case, clearly this nuclear-encoded, master regulator must be sensitive to PSII excitation pressure and hence chloroplastic redox poise.

Pathway that coordinates photosynthesis-related gene expression and, hence, photosynthetic acclimation. In addition, this photosynthetic redox sensing and signalling mechanism might also influence such diverse processes as cold acclimation, plant morphology (Fig. 6) and cyanobacterial differentiation37. Evidence for an intrachloroplastic redox signaling pathway has recently been provided with the identification of the ferredoxin-thioredoxin system as a critical component of redox regulation of chloroplast translation38. Although signalling between the plastid and the nucleus has been investigated for some time39, the nature of the chloroplastic signal (the elusive "plastid factor") and the signal transduction pathway have yet to be elucidated. However, it has recently been shown that Mg-protoporphyrin IX acts as a plastid factor in the signal transduction pathway involved in the light induction of nuclear heat-shock genes39. Thus, the process of photosynthesis appears to exert a broad influence on diverse molecular, physiological and developmental processes, consistent with the notion of a "grand design of photosynthesis"39,40.

Dedication

This article is dedicated to Dr Fergus D.H. Macdowall, mentor and friend.

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**Photosynthesis: a dual role?**

Any change in environmental conditions such as temperature, light, nutrient status, CO2 concentrations or water availability can potentially cause a metabolic energy imbalance which, through metabolic feedback loops, will affect chloroplastic metabolism and hence modulate PSII excitation pressure (Fig. 6). However, plants do not sense environmental changes only through modulation of PSII excitation pressure. Clearly, all organisms must be able to sense specific changes in temperature32 as well as perturbations of other environmental conditions. Nevertheless, in nature, changes in environmental parameters such as temperature rarely occur independently of other factors such as irradiance. Thus, caution must be exercised in interpreting data obtained from an experimental design that does not include controls to allow one to distinguish between responses caused by the specific environmental perturbation of interest from those due to changes in PSII excitation pressure.

In addition to the traditional role of photosynthesis in energy transduction, the redox state of the photosynthetic apparatus might also act as an environmental sensor by detecting energy imbalances between photochemistry and biochemistry (see Box 1). As initially proposed by Fujita and co-workers40 and subsequently supported by others41,42,43, a putative photosynthetic redox signal might initiate a transduction pathway that coordinates photosynthesis-related gene expression and, hence, photosynthetic acclimation. In addition, this photosynthetic redox sensing and signalling mechanism might also influence such diverse processes as cold acclimation, plant morphology (Fig. 6) and cyanobacterial differentiation44. Evidence for an intrachloroplastic redox signaling pathway has recently been provided with the identification of the ferredoxin-thioredoxin system as a critical component of redox regulation of chloroplast translation45. Although signalling between the plastid and the nucleus has been investigated for some time46, the nature of the chloroplastic signal (the elusive "plastid factor") and the signal transduction pathway have yet to be elucidated. However, it has recently been shown that Mg-protoporphyrin IX acts as a plastid factor in the signal transduction pathway involved in the light induction of nuclear heat-shock genes46. Thus, the process of photosynthesis appears to exert a broad influence on diverse molecular, physiological and developmental processes, consistent with the notion of a "grand design of photosynthesis"46,47.

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