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 photochemistry 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 or by low temperature initiates a signal transduction pathway. This appears to coordinate photosynthesis-related gene expression and to influence the nuclear expression of a specific cold-acclimation gene, plant morphology and differentiation in cyanobacteria. Thus, in addition to its traditional role in energy transduction, the photosynthetic apparatus might also be an environmental sensor.

Light-derived energy enters the biosphere through photosynthesis, and ultimately sustains virtually all living organisms. The rates of reactions involved in primary photochemistry, the subsequent transformation of the light into reducing power (NADPH) and chemical energy (ATP) and its ultimate utilization in metabolism and growth differ by at least 15 orders of magnitude (Fig. 1). Because the primary photochemical reactions of photosystem II (PSII) and PSI occur on a much faster time scale than electron transport and metabolism, exposure of plants to light energy that is in excess of that required for photosynthesis results in an energy imbalance that generally leads to photoinhibition[1]. Whenever the absorbed light energy exceeds the capacity of the organism either to use the trapped energy through photosynthesis (photochemical quenching) or to dissipate it as heat (non-photochemical quenching), damage to PSII may occur (Fig. 1). Furthermore, exposure to photoinhibitory conditions in oxygenic organisms can result in the formation of damaging reactive oxygen species either through the reduction of O_2 to yield the superoxide anion radical ($O_2^{-\cdot}$) or through energy transfer from excited triplet chlorophyll to ground-state O_2 to form singlet oxygen ($^1O_2$) (Ref. 2). If unchecked, irreversible photoinhibition can lead to significant decreases in plant productivity[1]. Thus, it is imperative that plants maintain a balance between the energy supplied through photochemistry and the energy consumed through photosynthetic CO_2 assimilation and contiguous metabolic pathways[3–6].

Cold acclimation: a potential 'energy crisis'

Plants can experience and must adjust to wide daily and seasonal fluctuations in temperature. Plant responses to these changes can be divided into two principal components. Adaptation is a genotypic response to long-term environmental changes. The alterations in the genome are stable and remain in the population over generations. By contrast, acclimation is a response induced by an environmental change that causes a phenotypic alteration with no change in genetic complement. However, acclimation is usually initiated by a stress response to an abrupt change in the environment that is characterized by transient, physiological, biochemical and molecular perturbations. The stress response subsequently leads to stable, long-term adjustments that reflect a developmental response to the new environmental condition. Thus, cold acclimation is a long-term developmental response to low temperature that results in the attainment of maximum freezing tolerance. This is estimated as the freezing temperature required to kill 50% of a plant population (LT50). The energy required to attain the cold-acclimated state is derived from photosynthesis.

The potential for an energy imbalance between photochemistry, electron transport and metabolism is exacerbated under conditions of either high light or cold temperatures, which lead to increased PSII excitation pressure (Box 1). On a time scale of minutes, organisms can acclimate in an attempt to compensate for exposure to high PSII excitation pressure by reducing energy transfer efficiency to PSII either by diverting energy from PSII to PSI through state transitions or by dissipating excess energy as heat by non-photochemical quenching (Fig. 1). On a longer time scale, photosynthetic acclimation to high PSII excitation pressure may occur as a consequence of a reduction in PSII antenna size. These mechanisms result in adjustments in the functional absorption cross-sectional area of PSII (σ_{PSII}), which would reduce photosynthetic efficiency measured as either the quantum yield of CO_2 assimilation (ΦCO_2) or the quantum yield of O_2 evolution (ΦO_2). In addition, some plants reduce leaf angle relative to the incident radiation, alter leaf optical properties[8] or change their position in the water column in the case of algae[9] 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 PSII.

Changes in PSII excitation pressure are reflected in alterations in the redox state of PSII, which can be monitored *in vivo* by exploiting chlorophyll *a* fluorescence as a non-invasive probe[10]. Photochemical fluorescence quenching can be used to estimate the proportion of PSII reaction centres that are 'open' (reflecting the relative oxidation state of Q_A, the first stable quinone electron acceptor of PSII reaction centres) or 'closed' (thought to reflect the relative reduction state of PSII) (Fig. 2). Thus, an estimate can be made of the relative PSII excitation pressure to which photoautotrophs are exposed[1,6,11]. Although not providing an exact estimate of the reduction state of PSII, this fluorescence parameter does provide a useful estimate of relative changes in PSII excitation pressure of organisms exposed to changing environmental conditions[12].
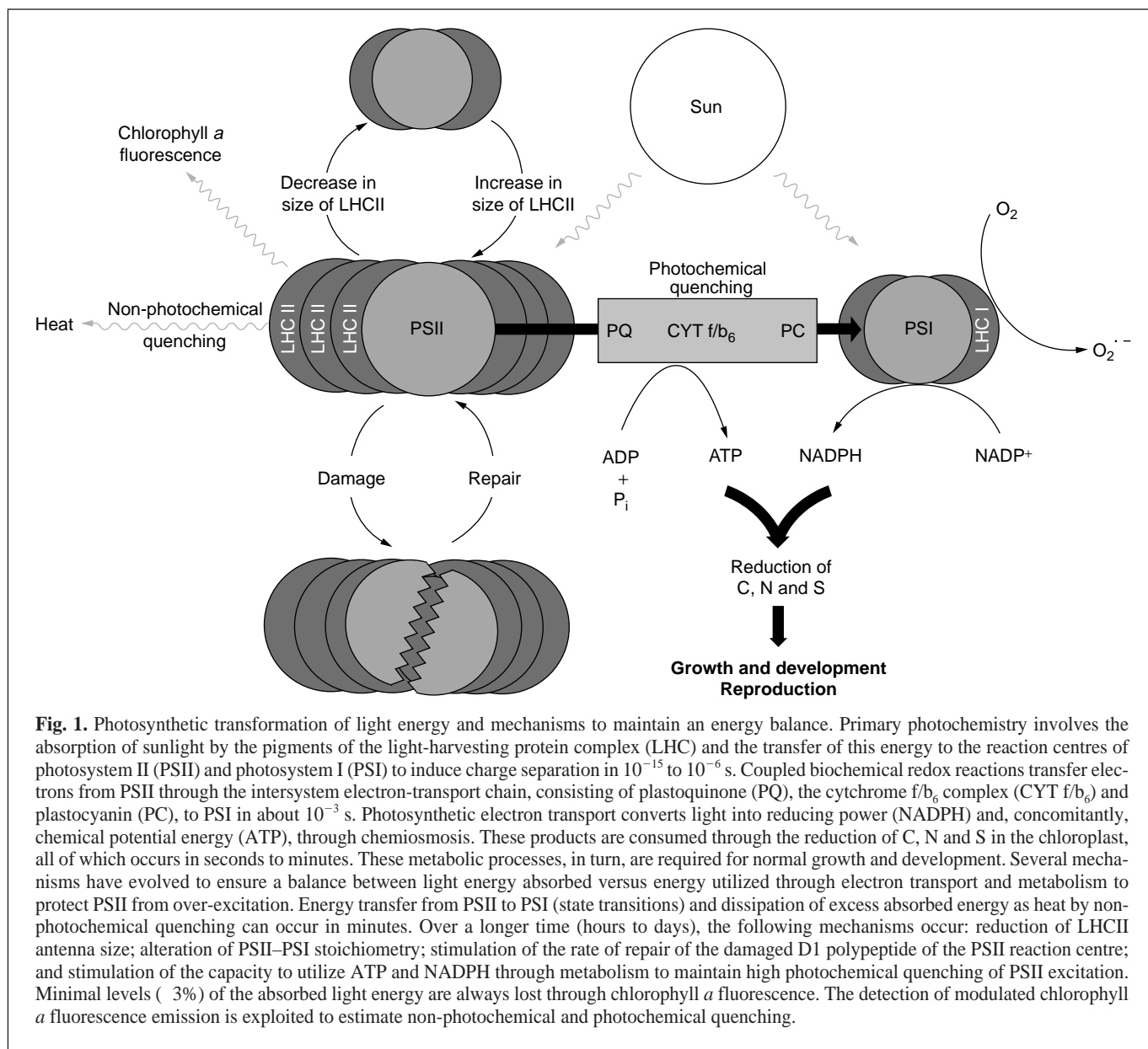
Fig. 1. Photosynthetic transformation of light energy and mechanisms to maintain an energy balance. Primary photochemistry involves the absorption of sunlight by the pigments of the light-harvesting protein complex (LHC) and the transfer of this energy to the reaction centres of photosystem II (PSII) and photosystem I (PSI) to induce charge separation in 10^{-15} to 10^{-6} s. Coupled biochemical redox reactions transfer electrons from PSII through the intersystem electron-transport chain, consisting of plastoquinone (PQ), the cytchrome f/b_6 complex (CYT f/b_6) 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. Minimal levels (3%) of the absorbed light energy are always lost through chlorophyll *a* fluorescence. The detection of modulated chlorophyll *a* fluorescence emission is exploited to estimate non-photochemical and photochemical quenching.

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:

$$\sigma_{PSII} \cdot I$$

where σ_{PSII} is the functional absorption cross-sectional area of PSII and I is the incident photon flux (units μmol photons m^{-2} s^{-1}) (Ref. 7). Under light-saturating conditions, the rate of utilization of this excitation energy through electron transport and metabolism can be estimated as:
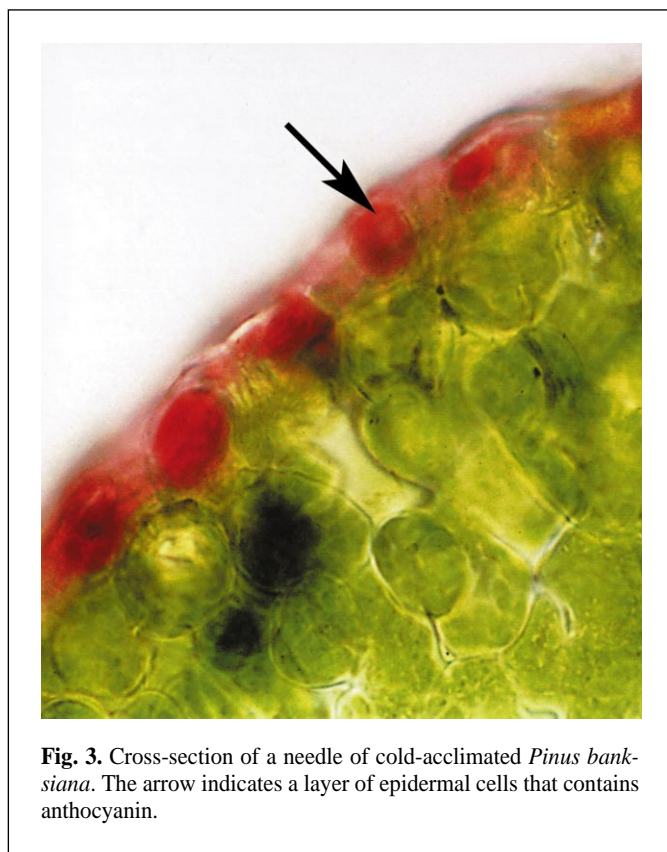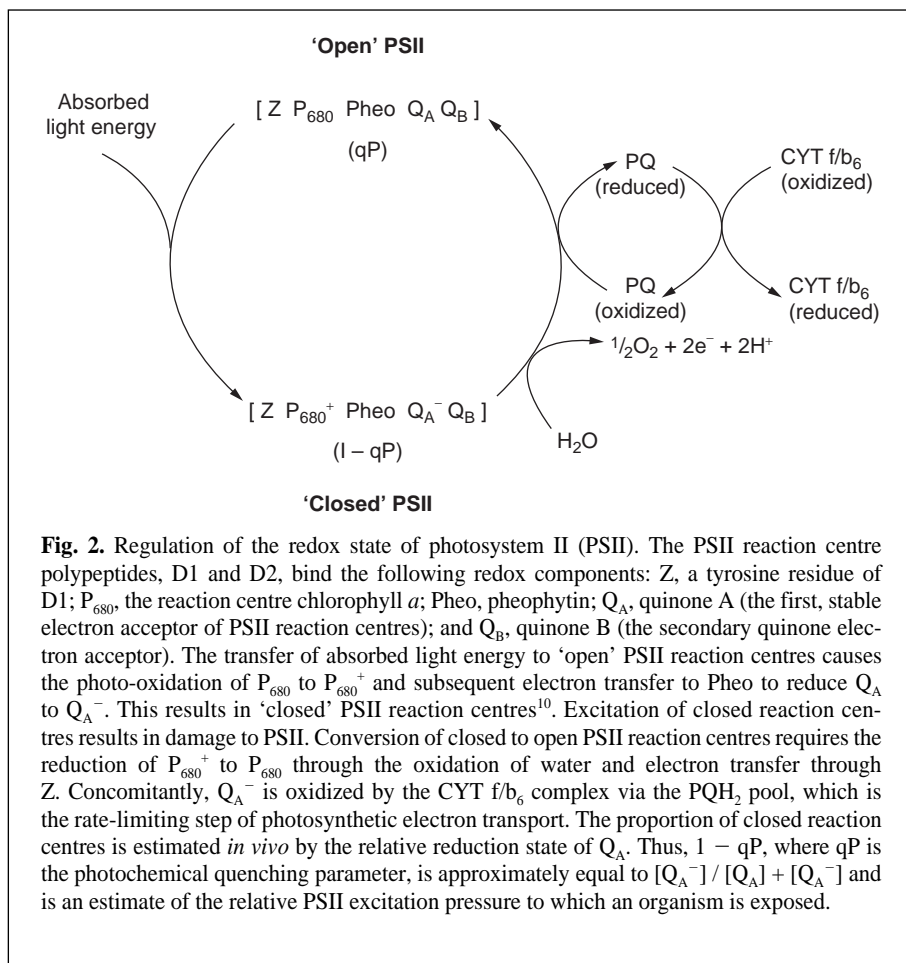
$$n \cdot 1/\tau$$

where n is the number of components acting as sinks that consume electrons, τ is the life-time and, hence, $1/\tau$ is the turnover rate of these sinks. A balance between energy absorbed and energy utilized is attained when:

$$\sigma_{PSII} \cdot I = n \cdot 1/\tau$$

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

$$\sigma_{PSII} \cdot I > n \cdot 1/\tau$$

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 $1/\tau$. 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 ($Q_{10} = 2$).

'Open' PSII

$[Z \ P_{680} \ Pheo \ Q_A \ Q_B]$

(qP)

Absorbed light energy

PQ (reduced)

CYT f/b$_6$ (oxidized)

PQ (oxidized)

CYT f/b$_6$ (reduced)

$\frac{1}{2}O_2 + 2e^- + 2H^+$

$[Z \ P_{680}^+ \ Pheo \ Q_A^- \ Q_B]$

(l – qP)

H_2O

'Closed' PSII

Fig. 2. Regulation of the redox state of photosystem II (PSII). The PSII reaction centre polypeptides, D1 and D2, bind the following redox components: Z, a tyrosine residue of D1; P_{680}, the reaction centre chlorophyll *a*; Pheo, pheophytin; Q_A, quinone A (the first, stable electron acceptor of PSII reaction centres); and Q_B, quinone B (the secondary quinone electron acceptor). The transfer of absorbed light energy to 'open' PSII reaction centres causes the photo-oxidation of P_{680} to P_{680}^+ and subsequent electron transfer to Pheo to reduce Q_A to Q_A^-. This results in 'closed' PSII reaction centres[10]. Excitation of closed reaction centres results in damage to PSII. Conversion of closed to open PSII reaction centres requires the reduction of P_{680}^+ to P_{680} through the oxidation of water and electron transfer through Z. Concomitantly, Q_A^- is oxidized by the CYT f/b$_6$ complex via the PQH$_2$ pool, which is the rate-limiting step of photosynthetic electron transport. The proportion of closed reaction centres is estimated *in vivo* by the relative reduction state of Q_A. Thus, $1 - qP$, where qP is the photochemical quenching parameter, is approximately equal to $[Q_A^-] / [Q_A] + [Q_A^-]$ and is an estimate of the relative PSII excitation pressure to which an organism is exposed.

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[12] (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[13]. Because non-photochemical quenching appears to play a major role in the protection of the photosynthetic apparatus from severe photodamage, evergreens appear to adjust σ_{PSII} 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[14]. 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[15].

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[16] 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[17]. 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[17]. In contrast to the alpine arctic species *Oxyria digyna*[18], 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_A oxidized (i.e. high photochemical quenching) due to an elevated photosynthetic capacity with no change in photosynthetic efficiency. The potential to keep Q_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[17].

The increased capacity of rye and wheat to keep Q_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[19]. 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[17]. Thus, the elevated sucrose levels normally associated with cold acclimation do not result in the repression of photosynthetic gene expression in *Arabidopsis*[20], rye or wheat[6,17,19], 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[20]. 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_2 assimilation and photosynthetic efficiency, calculated on a per cell basis, concomitant with an increase 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[6]. 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 *Lhcb* mRNA as well as Lhcb polypeptides; and an increased level of the carotenoid-binding protein, Cbr (Fig. 4a: 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[6]. As a consequence, algal cultures grown at low temperature exhibited a distinctive yellow colour (Fig. 4a). The repression in the accumulation of *Lhcb* mRNA and Lhcb polypeptides is not due to sucrose suppression, since the capacity for sucrose accumulation is depressed upon cold acclimation of *Chlorella vugaris*.

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[21], which results in a decrease in σ_{PSII}.

Cyanobacteria
In contrast to chloroplasts, the cyanobacterium *Synechococcus* sp. PCC 7942 possesses three homologous genes for the D1 protein of PSII reaction centres[22], designated *psbAI*, *psbAII* and *psbAIII*. The *psbAI* gene encodes form 1, designated D1:1; *psbAII* and
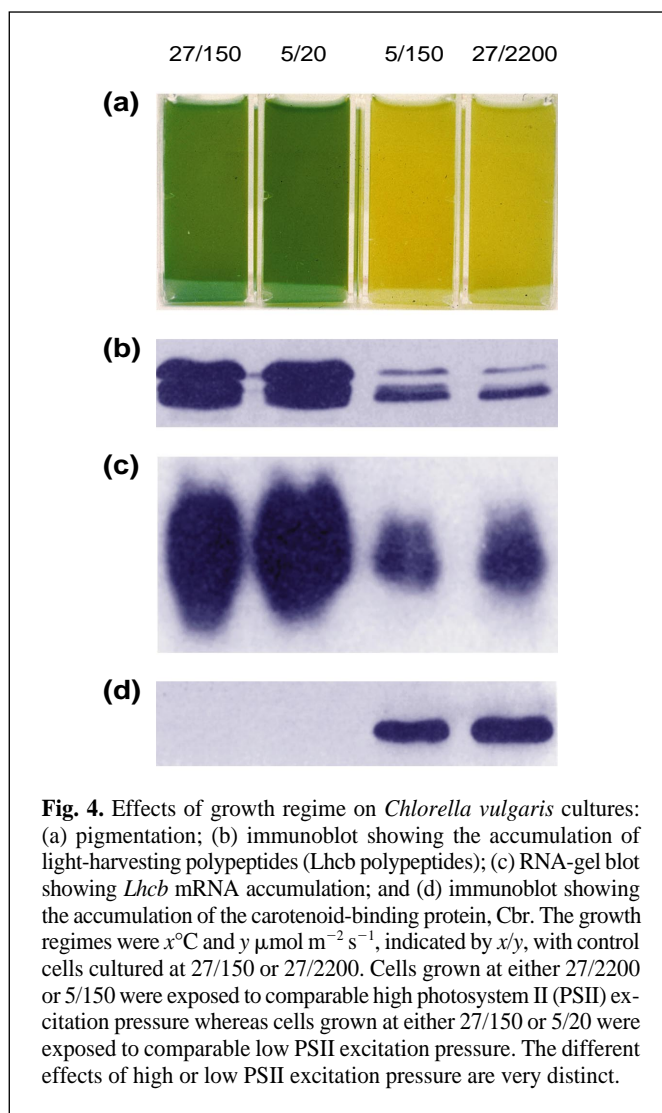

Fig. 4. Effects of growth regime on *Chlorella vulgaris* cultures: (a) pigmentation; (b) immunoblot showing the accumulation of light-harvesting polypeptides (Lhcb polypeptides); (c) RNA-gel blot showing *Lhcb* mRNA accumulation; and (d) immunoblot showing the accumulation of the carotenoid-binding protein, Cbr. The growth regimes were x°C and y μmol m^{-2} s^{-1}, indicated by x/y, with control cells cultured at 27/150 or 27/2200. Cells grown at either 27/2200 or 5/150 were exposed to comparable high photosystem II (PSII) excitation pressure whereas cells grown at either 27/150 or 5/20 were exposed to comparable low PSII excitation pressure. The different effects of high or low PSII excitation pressure are very distinct.

psbAIII 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[22].

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[23]. 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[23]. 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[24].

Acclimation 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 exhange and sensitivity to
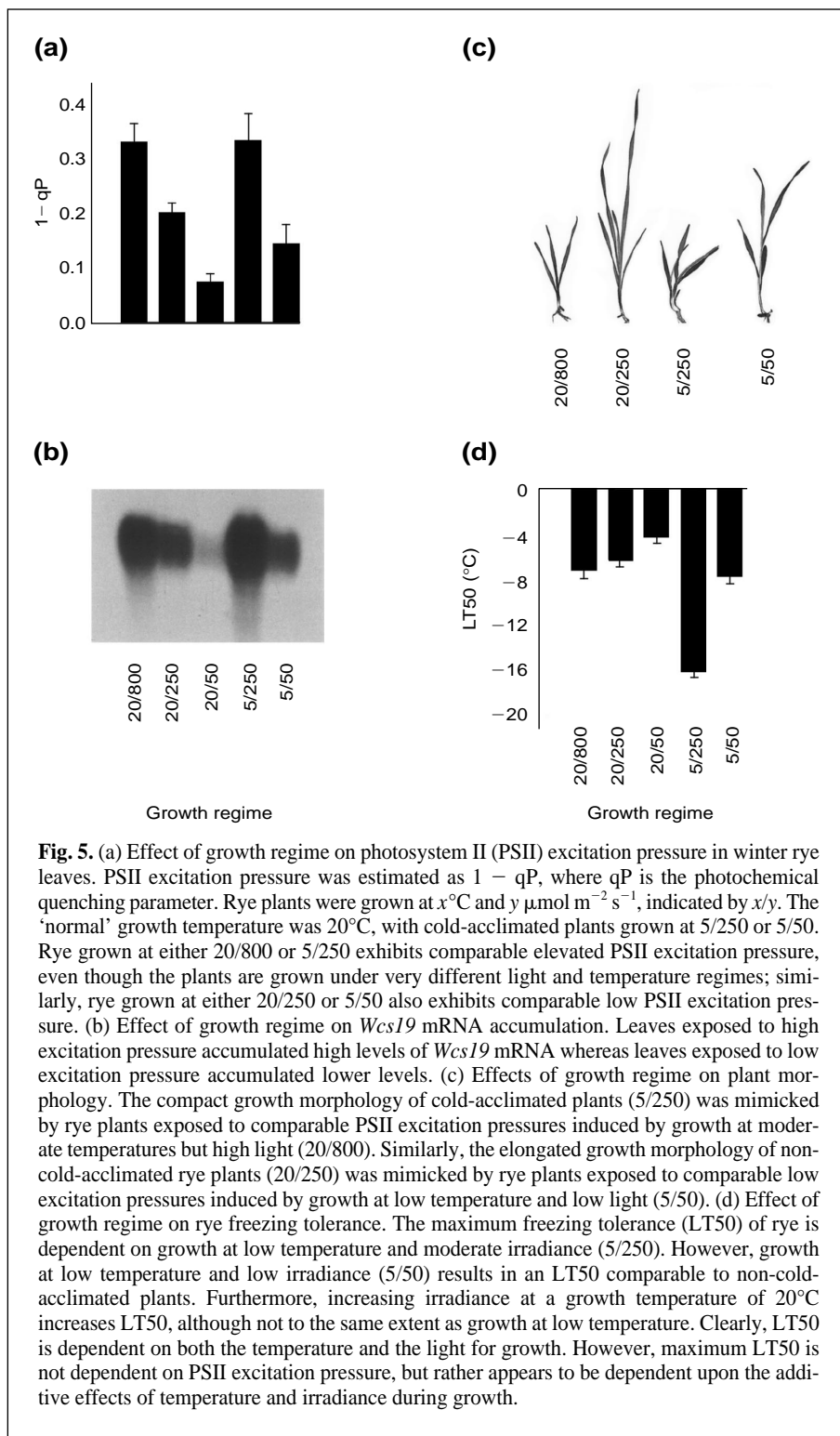
Fig. 5. (a) Effect of growth regime on photosystem II (PSII) excitation pressure in winter rye leaves. PSII excitation pressure was estimated as $1 - qP$, where qP is the photochemical quenching parameter. Rye plants were grown at x°C and y μmol m^{-2} s^{-1}, indicated by x/y. The 'normal' growth temperature was 20°C, with cold-acclimated plants grown at 5/250 or 5/50. Rye grown at either 20/800 or 5/250 exhibits comparable elevated 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 exhibits comparable low PSII excitation pressure. (b) Effect of growth regime on *Wcs19* mRNA accumulation. Leaves exposed to high excitation pressure accumulated high levels of *Wcs19* mRNA whereas leaves exposed to low excitation pressure accumulated lower levels. (c) Effects 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 excitation pressures induced by growth at low temperature and low light (5/50). (d) Effect of growth regime on rye freezing tolerance. The maximum freezing tolerance (LT50) of rye is dependent on growth at low temperature and moderate irradiance (5/250). However, growth at low temperature and low irradiance (5/50) results in an LT50 comparable to non-cold-acclimated plants. Furthermore, increasing irradiance at a growth temperature of 20°C increases LT50, although not to the same extent as growth at low temperature. Clearly, LT50 is dependent on both the temperature and the light for growth. However, maximum LT50 is not dependent on PSII excitation pressure, but rather appears to be dependent upon the additive effects of temperature and irradiance during growth.

saccharina[25] and the expression of early light-inducible proteins (ELIPs) in barley[26].

It may be that exposure to high excitation pressure initiates a signal transduction pathway from the chloroplast to the nucleus that represses *Lhcb* and concomitantly derepresses *Cbr* gene expression. This leads to low levels of Lhcb, but high levels of Cbr precursor polypeptides synthesized in the cytoplasm – these are subsequently processed during transport into the chloroplast and inserted into the thylakoid membrane.

Thus, photoautotrophs sense imbalances in the energy budget through changes in the relative reduction state of PSII (i.e. changes in PSII excitation pressure). However, PSII cannot be the primary redox sensor that regulates nuclear *Lhcb* and *cbr* expression in *Chlorella* and *Dunaliella*[6]. Rather, the changes in the relative reduction state of PSII probably reflect the redox status of a component further downstream of PSII, which is consistent with the proposal that either the thylakoid plastoquinone pool[27,28] or the CYT b$_6$/f complex acts as the chloroplast redox sensor for the regulation of photosynthesis-related genes[29].

Cold, light and freezing tolerance
The photosynthetic response and tolerance to photoinhibition of cold-acclimated wheat and rye are also due to growth at elevated PSII excitation pressure[30]. However, does the modulation of PSII excitation pressure by either irradiance or temperature influence events beyond photosynthesis and sensitivity to photoinhibition? In contrast to the *Wcs120* family of cold-acclimation genes[31], 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 PSII excitation pressure[30] (Fig. 5). The sensing and signalling events in photomorphogenic development are typically associated with photoreceptors such as phytochrome as well as blue light and UV-light photoreceptors[32]. However, the results for the compact growth morphology cannot be due to phytochrome, because the compact growth morphology is induced simply by lowering the temperature with no change

photoinhibition, as well as the accumulation of Lhcb polypeptides and cbr, 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[21]. Similar conclusions regarding the role of PSII excitation pressure have been reported for thermal and photoacclimation of *Laminaria*

in either light quality or photoperiod (Fig. 5). Thus, processes as diverse as photosynthesis, morphogenesis and cold acclimation are not only regulated by photoreceptors, but also appear to be very sensitive to the overall metabolic energy balance[3–6,33].

Photosynthesis provides the energy necessary for the attainment of a cold-acclimated state and, hence, maximum freezing tolerance. Thus, any environmental factor that has 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

fact, supersede the genetic potential of the organism to exhibit its maximum freezing tolerance. This has important implications not only for genetic engineering of cold tolerant and freezing-resistant plants but also for research focused on the elucidation of stress-tolerance mechanisms.

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 manner[30] (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 pathways associated with each of these signals probably interact not only with each other but also synergistically with other important signal transduction pathways – involving phytochrome[32], sugar sensing[34], protein phosphorylation and dephosphorylation, Ca^{2+} and plant growth regulators[31] – 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 morphology[31]. 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.

Photosynthesis: a dual role?

Any change in environmental conditions such as temperature, light, nutrient status, CO_2 concentrations or water availability can potentially cause a metabolic energy imbalance which, through metabolic feedback loops, will affect chloroplast 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 temperature[24] 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 Fuijita and co-workers[29] and subsequently supported by others[6,7,27,28], 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 differentiation[35]. Evidence for an intrachloroplastic redox signalling pathway has recently been provided with the identification of the ferredoxin–thioredoxin system as a critical component of redox regulation of chloroplast translation[36]. Although signalling between the chloroplast and the nucleus has been investigated for some time[37], 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 genes[38]. 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'[5,39].

Dedication

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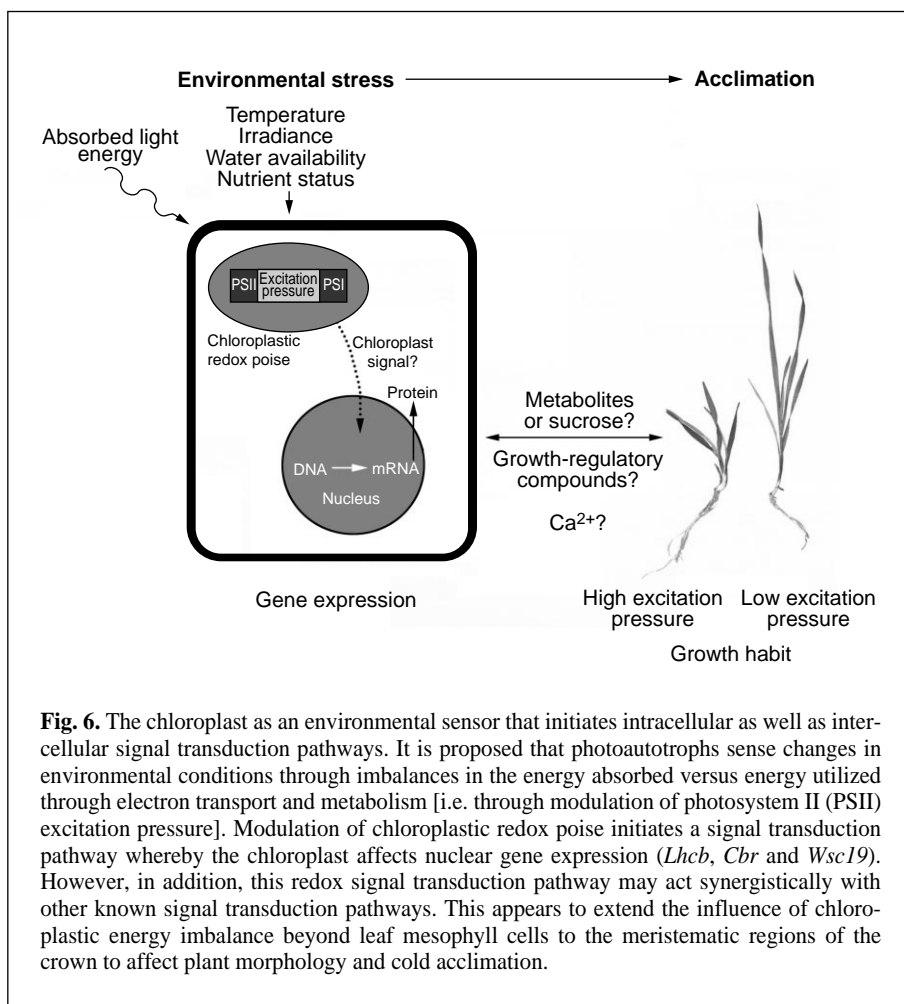
Acknowledgements

Fig. 6. The chloroplast as an environmental sensor that initiates intracellular as well as intercellular signal transduction pathways. It is proposed that photoautotrophs sense changes in environmental conditions through imbalances in the energy absorbed versus energy utilized through electron transport and metabolism [i.e. through modulation of photosystem II (PSII) excitation pressure]. Modulation of chloroplastic redox poise initiates a signal transduction pathway whereby the chloroplast affects nuclear gene expression (*Lhcb*, *Cbr* and *Wsc19*). However, in addition, this redox signal transduction pathway may act synergistically with other known signal transduction pathways. This appears to extend the influence of chloroplastic energy imbalance beyond leaf mesophyll cells to the meristematic regions of the crown to affect plant morphology and cold acclimation.

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