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Photosystem II Excitation Pressure and Photosynthetic Carbon Metabolism in *Chlorella vulgaris*[1]

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***Chlorella vulgaris* grown at 5°C/150 μmol m^{-2} s^{-1} mimics cells grown under high irradiance (27°C/2200 μmol m^{-2} s^{-1}). This has been rationalized through the suggestion that both populations of cells were exposed to comparable photosystem II (PSII) excitation pressures measured as the chlorophyll *a* fluorescence quenching parameter, 1 − qP (D.P. Maxwell, S. Falk, N.P.A. Huner [1995] Plant Physiol 107: 687–694). To assess the possible role(s) of feedback mechanisms on PSII excitation pressure, stromal and cytosolic carbon metabolism were examined. Sucrose phosphate synthase and fructose-1,6-bisphosphatase activities as well as the ratios of fructose-1,6-bisphosphate/fructose-6-phosphate and sucrose/starch indicated that cells grown at 27°C/2200 μmol m^{-2} s^{-1} appeared to exhibit a restriction in starch metabolism. In contrast, cells grown at 5°C/150 μmol m^{-2} s^{-1} appeared to exhibit a restriction in the sucrose metabolism based on decreased cytosolic fructose-1,6-bisphosphatase and sucrose phosphate synthase activities as well as a low sucrose/starch ratio. These metabolic restrictions may feedback on photosynthetic electron transport and, thus, contribute to the observed PSII excitation pressure. We conclude that, although PSII excitation pressure may reflect redox regulation of photosynthetic acclimation to light and temperature in *C. vulgaris,* it cannot be considered the primary redox signal. Alternative metabolic sensing/signaling mechanisms are discussed.**

Recently, we reported that growth of *Chlorella vulgaris* at 5°C and 150 μmol m^{-2} s^{-1} results in the development of greater tolerance to high light at low temperature (2000 μmol m^{-2} s^{-1} at 5°C) than cells grown at 27°C and 150 μmol m^{-2} s^{-1} (Maxwell et al., 1994, 1995a). Photosynthetic adjustment at the level of pigment content and composition, Chl *a*/*b*, LHCII abundance, O_2 evolution, and increased tolerance to photoinhibition, which were assumed previously to be responses to low growth temperature, do not represent responses to growth temperature per se. Rather, these changes in the structure and composition of the photosynthetic apparatus reflect photosynthetic adjustment to growth under high PSII excitation. Excitation pressure is estimated as 1 − qP (Dietz et al., 1985; Demmig-Adams et al., 1990; Ögren, 1991), where qP is the photochemical quenching coefficient (Bradbury and Baker, 1981; van Kooten and Snel, 1990) and is an estimate of the proportion of Q_A in the reduced state $[(Q_A)_{red}/(Q_A)_{ox} + (Q_A)_{red}]$ in functional PSII units. Thus, photosynthetic adjustment in *C. vulgaris* reflects a response to changes in the redox poise of intersystem electron transport. We reported that *Chlorella* grown at 5°C and an irradiance of 150 μmol m^{-2} s^{-1} (5/150) were exposed to a predicted PSII excitation pressure that was equivalent to cells grown at 27°C and 2200 μmol m^{-2} s^{-1} (27/2200) (Maxwell et al., 1995a). Based on light response curves for CO_2-saturated O_2 evolution, room temperature Chl *a* fluorescence, pigment content and composition, LHCII abundance, and tolerance to photoinhibition, cells grown at 5°C and moderate irradiance were indeed equivalent to cells grown at high light and 27°C. Similarly, *Chlorella* grown at 5/20 were equivalent to cells grown at 27/150 (Maxwell et al., 1995a). Thus, we concluded that PSII excitation pressure, that is, the redox poise of intersystem electron transport, can be modulated to a similar extent either by reducing the temperature at a constant irradiance or by increasing irradiance at a constant temperature. Recently, we confirmed that higher plants (Gray et al., 1996) and cyanobacteria (S. Falk, D. Maxwell, N. Huner, and D. Laudenbach, unpublished data) also respond developmentally as well as photosynthetically to this altered redox poise. In addition, we have shown that regulation of *cab* mRNA abundance and LHCII apoprotein levels in *Dunaliella salina* are under similar redox control (Maxwell et al., 1995b).

The redox poise of intersystem electron transport will change as a consequence of any imbalance between light energy absorbed through photochemistry and energy used through intersystem electron transport, photosynthetic carbon metabolism, and photorespiration, as well as any other metabolic process that consumes ATP and/or NADPH.

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Abbreviations: CABP, 2-carboxyarabinitol 1,5-bisphosphate; Chl, chlorophyll; dH_2O, distilled H_2O; FBPase, fructose-1,6-bisphosphatase; Fru-1,6-BP, fructose-1,6-bisphosphate; GAPDH, glyceraldehydephosphate dehydrogenase; Glc-6PDH, glucose-6-phosphate dehydrogenase; HEP, high PSII excitation pressure; HK, hexokinase; k_{cat}, catalytic turnover number for Rubisco; LEP, low PSII excitation pressure; LHCII, light-harvesting complex II; PGA, 3-phosphoglycerate; PGI, phosphoglucoisomerase; Q_A, primary electron accepting plastoquinone of PSII; RuBP, ribulose 1,5-bisphosphate; SPS, sucrose phosphate synthase; triose-P, triose phosphate.

Low temperature imposes thermodynamic constraints on the rates of biochemical reactions and, as a result, plants exposed to low temperature also exhibit reduced rates of photosynthesis. Short-term effects of low temperature on photosynthesis and carbon metabolism have been examined in detail in higher plants (Leegood, 1985, 1995; Leegood and Furbank, 1986; Stitt et al., 1987; Stitt and Grosse, 1988; Labate et al., 1990; Paul et al., 1990, 1992; Holaday et al., 1992; Guy et al., 1992). The general consensus appears to be that low temperature inhibition of photosynthesis, in the short term, reflects feedback regulation and is the result of low temperature limitations in Suc synthesis, which results in Pi limitation of the chloroplast. This limits the rate at which ATP can be biosynthesized, which, in turn, limits the regeneration of RuBP. However, Sheen (1994) has suggested that feedback regulation of photosynthesis reflects metabolite regulation of gene expression.

During long-term exposure of cold-tolerant C_3 plants to growth at low temperature, the potential for imbalance between Suc synthesis and photosynthesis in the short term appears to be overcome as indicated by the recovery of the light-saturated rates of photosynthesis to control values (Hurry et al., 1995) or higher (Huner et al., 1993). This is coupled with the well-documented increases in Suc accumulation during cold hardening (Guy et al., 1992). This photosynthetic acclimation appears to be accomplished by increasing the availability of Pi (Hurry et al., 1993) as well as increasing the total activity and activation states of key regulatory enzymes of the Calvin-Benson cycle (Hurry et al., 1995). These results are consistent with recent suggestions that alterations in enzyme capacity, through modulation of gene expression by photosynthetic end products, are important in the acclimation of higher plants to environmental stress (Leegood, 1995; Stitt et al., 1990). Sheen (1994) has suggested that Glc is a universal signal, which regulates metabolite-mediated signal transduction in prokaryotes as well as eukaryotes.

In this report, we extend our previous studies on the response of green algae (*C. vulgaris* and *D. salina*) to PSII excitation pressure (Maxwell et al., 1994, 1995a, 1995b) to an examination of photosynthetic carbon metabolism in *C. vulgaris* grown at various PSII excitation pressures. Restrictions in photosynthetic carbon metabolism encountered as a consequence of growth of *C. vulgaris* at either low temperature or high irradiance may, through feedback mechanisms, induce the observed high PSII excitation pressures.

MATERIALS AND METHODS

Growth Conditions

The unicellular green alga *Chlorella vulgaris* Beijer (University of Texas Culture Collection strain UTEX 265) was grown axenically in Bold's basal medium as previously described (Maxwell et al., 1994). All experiments were conducted using cells grown in 150-mL Pyrex glass tubes immersed in aquaria with the temperature maintained at either 27 ± 1°C or 5 ± 1°C. Cultures grown at 5°C were exposed to continuous irradiance of either 20 μmol m^{-2} s^{-1} (5/20) or 150 μmol m^{-2} s^{-1} (5/150). Cultures grown at 27°C were exposed to continuous irradiance of either 150 μmol m^{-2} s^{-1} (27/150) or 2200 μmol m^{-2} s^{-1} (27/2200) (Maxwell et al., 1995a). The irradiance was measured from the middle of each culture tube using a quantum sensor (Model 185A, Li-Cor, Lincoln, NE). Each tube was bubbled with sterile air (150 mL min^{-1}) and maintained at Chl concentrations of 2 to 5 μg mL^{-1} and in the exponential growth phase by dilution with fresh growth medium.

O_2 Evolution

CO_2-saturated O_2 evolution was measured polarographically using a temperature-controlled, aqueous phase, Clarke-type O_2 electrode (Hansatech Instruments, King's Lynn, UK). Measurements were conducted at the respective growth temperature as described in detail previously (Maxwell et al., 1994, 1995a).

PSII Excitation Pressure

All measurements were made using a pulse amplitude modulated fluorescence system (Heinz Walz, Effletrich, Germany) as described in detail by Maxwell et al. (1994). Excitation pressure was calculated as 1 − qP (Demmig-Adams et al., 1990; Ögren, 1991), where qP is the coefficient of photochemical quenching (Bradbury and Baker, 1981; van Kooten and Snel, 1990).

Enzyme Analyses

For all assays, exponentially growing cells were harvested by centrifugation and kept at −80°C until used. After thawing, cells were passed three times through a French pressure cell at 2 × 10^4 p.s.i. in the appropriate buffer, and aliquots of the cell extract were centrifuged at 1600*g* for 2 min. The supernatant was used immediately for enzyme assays.

FBPase was assayed spectrophotometrically according to the procedure of Sharkey et al. (1991b), with an extraction buffer of 20 mM Hepes-NaOH (pH 7.5), 125 mM NaCl, 400 μM EDTA, 0.02%(w/v) BSA, and 2% (w/v) polyvinylpolypyrrolidone. Cytosolic FBPase activity was determined in a buffer containing 100 mM Hepes-NaOH (pH 7.5), 100 mM KCl, 4 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM NADP, 2 units of PGI, and 1 unit of Glc-6-PDH. The reaction was initiated by the addition of 50 μM Fru-1,6-BP. Stromal FBPase activity was measured in a buffer containing 100 mM Bicine (pH 8.8), 0.5 mM EDTA, 50 mM DTT, 0.5 mM NADP, 2 units of PGI, and 1 unit of Glc-6-PDH. The reaction was initiated by the addition of 0.4 mM Fru-1,6-BP and 10 mM $MgCl_2$. The reduction of NADP was monitored at 340 nm, and the FBPase rates were determined 5 min after the start of the reaction.

SPS was assayed according to Vassey and Sharkey (1989). Cell samples were extracted with buffer containing 50 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM of freshly added DTT, and 0.1% (v/v) Triton X-100. After centrifugation, the supernatant was mixed with 250 mg of Sephadex G-25, incubated on ice for 10 min, and subsequently centrifuged for 5 min. SPS activity was measured in the supernatant as the time-dependent formation

of Suc plus Suc-P from UDP-Glc and Fru-6-P, according to Huber et al. (1991). SPS activity was determined either by the "limiting assay," which included limiting substrate concentrations in the presence of the SPS inhibitor, Pi, or by the V_{max} assay, which included saturating substrate concentrations in the absence of Pi.

Rubisco activity was determined according to Sharkey et al. (1991a). Cell samples were extracted into 100 mM Bicine (pH 7.8), 5 mM $MgCl_2$, 1 mM EDTA, 5 mM DTT, 1.5% (w/v) polyvinylpolypyrrolidone, and 0.02% (w/v) BSA. The extract was centrifuged at 1600*g* for 2 min at 5°C, and 5 μL of the supernatant was immediately used to determine initial Rubisco activity. The total Rubisco activity was determined after incubation of 1 mL of extract for 10 min in 20 mM $MgCl_2$ and 10 mM $NaHCO_3$. The carbamylation ratio was determined as initial Rubisco activity/total Rubisco activity. Total Rubisco protein was determined by using CABP to inhibit Rubisco activity in the extract. Rubisco activity was plotted against CABP concentration to estimate the total number of Rubisco sites to which CABP was bound. This value was divided by 8 to give the total amount of Rubisco protein. The k_{cat} was estimated by dividing the total activity by the total amount of enzyme.

Rubisco activity was determined in 50 mM Bicine (pH 8.0), 15 mM $MgCl_2$, 1 mM EDTA, 10 mM NaCl, 5 mM DTT, 10 mM $NaHCO_3$, 5 mM phosphocreatine, and 5 mM ATP in a final volume of 750 μL. The following were added: 10 μL of 20 mM RuBP, 10 μL of 10 mM NADH, 10 units of GAPDH/3-phosphoglucokinase, and 2 units of creatine phosphokinase. The reaction was initiated by the addition of 5 μL of appropriate extract, and the oxidation of NADH was monitored at 334 − 405 nm.

Metabolite Analyses

Metabolites were extracted from cells in 3.5% $HClO_4$. After centrifugation, the supernatant was neutralized with 2 N KOH in 0.15 mM Hepes and 10 mM KCl, and the precipitate was discarded after further centrifugation. The supernatant was used for the enzyme-linked photometric assay of metabolites as described by Loreto and Sharkey (1993).

Carbohydrate Analyses

Thawed cells were centrifuged at 12,000*g* for 10 min and then extracted in hot 80% ethanol until the cells were pigment free. After several passes through the French pressure cell, this extract was centrifuged at 1,600*g* for 10 min, and the pellet was washed twice with dH_2O. The pellet, containing starch, was resuspended in 1 mL of 0.2 N KOH and incubated in a boiling water bath for 30 min. After cooling, the pH was adjusted to 5.5 with 200 μL of 1 N acetic acid. For starch digestion, 1 mL of dialyzed amyloglucosidase (35 units of mL^{-1} in 50 mM Na-acetate buffer, pH 4.5) was added, and samples were incubated at 55°C for 30 min. After boiling for 1 min, the samples were centrifuged and Glc content in the supernatant was determined enzymatically (Rufty and Huber, 1983). The aqueous phase was dried at 30°C and then resuspended in 400 μL of dH_2O. This fraction was used to determine Glc, Fru, and Suc. The assay buffer contained 360 mM Tris-HCl (pH 6.9), 0.4 mM NADP, 1 mM ATP, 5 mM $MgCl_2$, 1 mM DTT, and 0.02% (w/v) BSA. In addition, the Glc assay mixture contained 1 unit of HK, the Fru assay contained 1 unit of HK and 5 units of PGI, the Suc assay contained 80 units of invertase in addition to the Fru reaction mixture. The reaction was started by adding 2 units of Glc-6-PDH, and the reduction of NADP was monitored at 340 nm.

Chl and Protein Determination

Chl concentration was calculated using the equations of Jeffrey and Humphrey (1975) after extraction into 90% (v/v) acetone, according to Maxwell et al. (1994). The cell extract was centrifuged at 100,000*g* for 1 h, and the soluble protein concentration of the supernatant was determined using the Bradford assay.

Electron Microscopy

Cells were prepared for transmission electron microscopy by filtration on 0.45-μm Millipore membranes and subsequently were resuspended in 2% (w/v) agar at 45°C. Small agar blocks (1 mm^3) were fixed on ice in freshly prepared 1.5% (w/v) aqueous $KMnO_4$ for 45 min. Agar blocks containing cell samples were rinsed with dH_2O and dehydrated in an ethanol series, infiltrated, and embedded in Epon-Araldite (EF Fullam, New York, NY). Samples were poststained with 5% (w/v) uranyl acetate for 20 min. Transmission electron microscopy was conducted on a Philips (Eindhoven, The Netherlands) CM10 at 80 kV.

RESULTS

Cell Ultrastructure

Previously, we reported that *Chlorella* grown at either 27/150 or 5/20 are exposed to comparable LEP (Maxwell et al., 1995a). Figure 1 illustrates that *Chlorella* grown at LEP at either 27/150 (Fig. 1A) or 5/20 (Fig. 1B) exhibited normal, stacked thylakoid membranes. In contrast, cells exposed to HEP by growth at either 27/2200 (Fig. 1C) or 5/150 (Fig. 1D) exhibited significant disorganization in thylakoid membrane structure and an abundance of starch granules. Total Chl per cell for *Chlorella* grown at HEP was 4- to 5-fold lower than for cells grown at LEP (Table I). This was accompanied by a 2-fold higher Chl *a/b* in cells grown at HEP than those grown at LEP (Table I). Furthermore, growth at HEP at either 5 or 27°C resulted in comparable tolerance to photoinhibition but significantly greater tolerance than cells grown at LEP (Table I). Thus, these data indicate that *Chlorella* grown at either 5/150 or 27/2200, that is, HEP, are not only comparable in their susceptibility to photoinhibition, they are similar ultrastructurally. In addition, cells grown at either 5/20 or 27/150, that is LEP, are also similarly susceptible to photoinhibition and are ultrastructurally comparable (Fig. 1) (Maxwell et al., 1994, 1995).

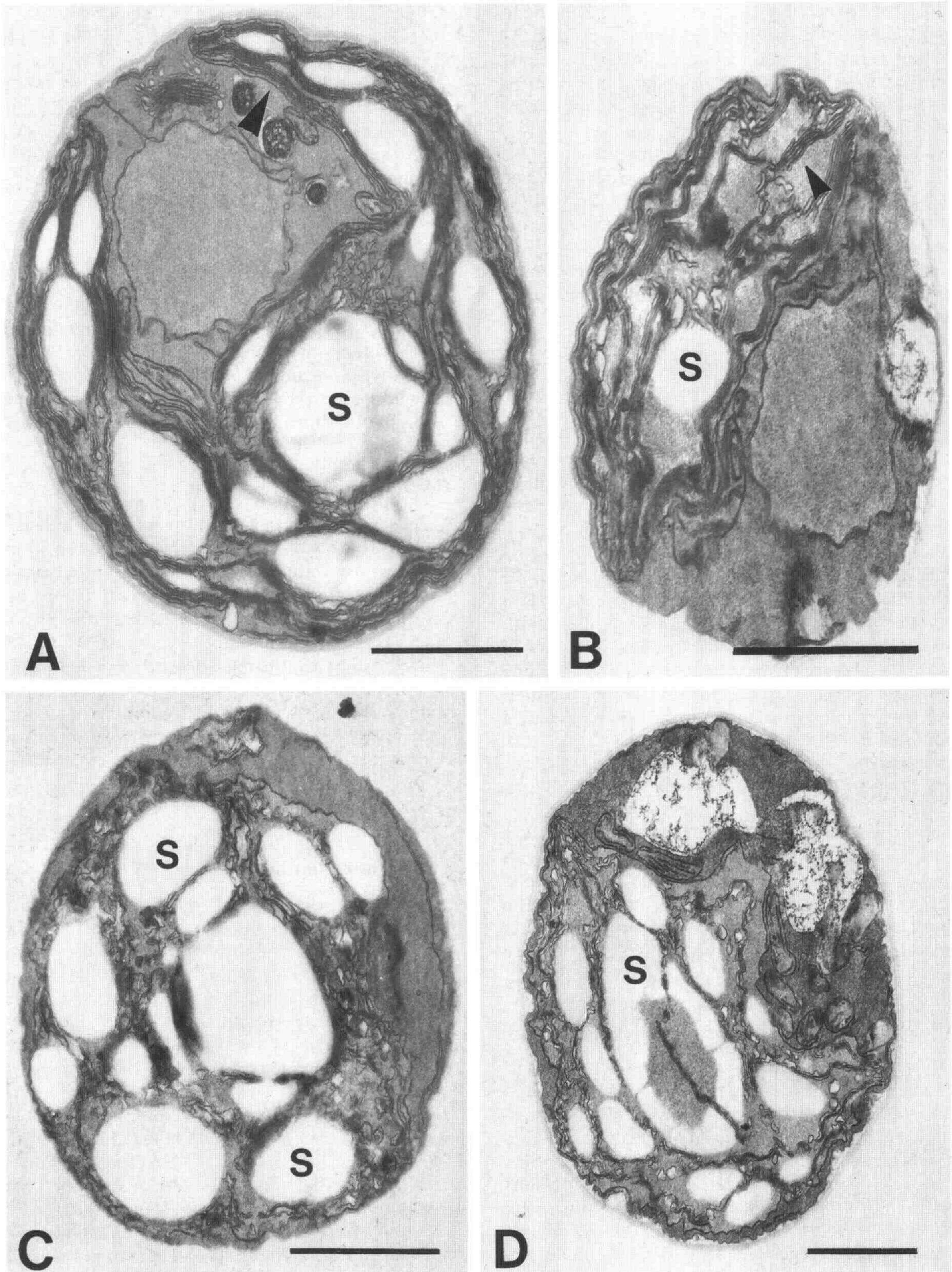

Figure 1. Electron micrographs of *C. vulgaris* grown at 27/150 (A), 5/20 (B), 27/2200 (C), and 5/150 (D). Bars represent 1 μm. Arrowheads in A and B indicate thylakoid stacks. S, Starch.

Table I. *Characteristics of C. vulgaris grown under two conditions of HEP and LEP*

Data represent mean ± SE, $n = 4$. F_v, Variable Chl *a* fluorescence with all PSII traps open; F_m, maximal Chl *a* fluorescence with all PSII traps open.

Parameters	Growth Conditions			
	27/150 LEP	27/2200 HEP	5/20 LEP	5/150 HEP
	°C/μmol $m^{-2}s^{-1}$			
Chl (*a* + *b*) (pg $cell^{-1}$)	0.73 ± 0.05	0.18 ± 0.06	0.71 ± 0.03	0.13 ± 0.01
Chl *a*/Chl *b*	3.59 ± 0.08	7.92 ± 0.58	3.61 ± 0.11	8.47 ± 0.24
Oxygen evolution, Pg (nmol $[10^7 \text{ cells}]^{-1}$ h^{-1})	701 ± 97	1159 ± 54	115 ± 16	98 ± 11
Dark respiration (% from Pg)	77	18	52	36
F_v/F_m (control)	0.74 ± 0.01	0.61 ± 0.02	0.75 ± 0.01	0.70 ± 0.02
F_v/F_m (after photoinhibition)	0.07 ± 0.02	0.32 ± 0.02	0.15 ± 0.03	0.46 ± 0.02
F_v/F_m (% of control)	9.5	52.5	20	65.7

O_2 Evolution

Previously, we reported that *Chlorella* grown at 5/150 exhibited similar rates of CO_2-saturated O_2 evolution as cells grown at 27/2200 when calculated on a Chl basis and when both were compared at the same measuring temperature of either 27°C or 5°C (Maxwell et al., 1994, 1995a). However, when the photosynthetic light response curves were measured at the prevailing growth temperature (Fig. 2), cells grown and measured at 5°C, as expected, exhibited lower light-saturated rates of O_2 evolution than those grown and measured at 27°C, regardless of whether photosynthesis was calculated on a per cell or on a Chl basis (Fig. 2, A and B). Furthermore, cells grown at LEP exhibited higher initial slopes than those grown at HEP (Fig. 2B). However, *Chlorella* grown at 27/2200 were grown under light-saturated conditions in contrast to cells grown at either 5/150 or 5/20.

Carbohydrate Accumulation

Chl $cell^{-1}$ varied dramatically upon growth at HEP or LEP (Table I), so all photosynthetic end products and metabolite data are presented on a per cell basis for comparative purposes. We initiated our study of the effects of PSII excitation pressure on carbon metabolism by measuring the levels of the major end products of photosynthesis, Suc, and starch (Fig. 3). Irrespective of PSII excitation pressure, cells grown at 27°C generally exhibited higher levels of Glc, Fru, Suc, and starch compared with cells grown at 5°C (Fig. 3). However, when PSII excitation pressure was increased at 27°C, the Suc/starch ratio increased 25-fold (Fig. 3E). This was due to an 18-fold increase in Suc (Fig. 3C) associated with a concomitant 25% decrease in starch content (Fig. 3D). In contrast, the Suc/starch ratio decreased by 23-fold when excitation pressure was increased at 5°C (Fig. 3E), which occurred as a consequence of a 7-fold decrease in Suc (Fig. 3C) with a concomitant doubling in the starch content (Fig. 3D).

Although cells grown at 5/20 and 27/150 exhibited similar PSII excitation pressures (Table I), the former exhibited a lower abundance of both Suc and starch (Fig. 3, C and D) and a 3-fold higher Suc/starch ratio than the latter (Fig. 3E). This indicates that *Chlorella* grown at 5°C and in light-limiting conditions experienced some limitation in carbon metabolism relative to cells grown at 27/150, which is supported by the fact that the gross photosynthetic rate in *Chlorella* grown at 5°C was independent of irradiance when measured at the growth temperature, whereas the gross photosynthetic rate of *Chlorella* grown at 27°C exhibited the expected increase with increased irradiance (Table I).

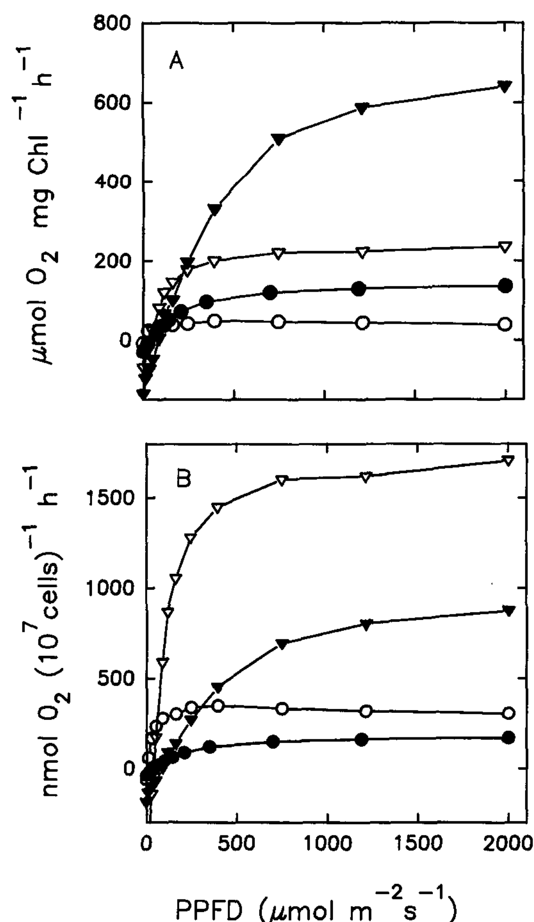

Figure 2. Irradiance response curves for CO_2-saturated O_2 evolution. O_2 evolution calculated on a Chl basis (A) and on a per cell basis (B). *Chlorella* was grown at either LEP (27/150 [△], 5/20 [○]), or HEP (27/2200 [▲], 5/150 [●]). All measurements were made at the respective growth temperatures. Data are averages of three to five experiments with error bars equal to or smaller than the symbols.

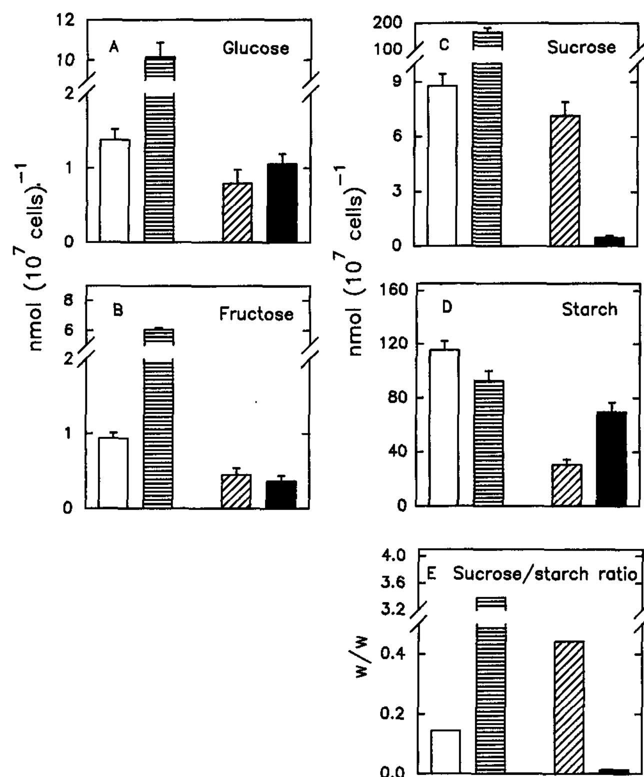

Figure 3. Abundance of the major carbohydrates in *C. vulgaris.* Cells were grown at 27/150 (open bars), 27/2200 (horizontally hatched bars), 5/20 (diagonally hatched bars), and 5/150 (solid bars). All data represent the means of four measurements ± SE.

Enzyme Activities

We extended our investigation by examining several stromal as well as cytosolic regulatory enzyme activities. At 27°C, cells grown at HEP exhibited a 23% lower Rubisco content than cells grown at LEP. However, total Rubisco activity, on a per cell basis, was not significantly different between cells grown at either 27/2200 or 27/150 (Table II). Both cytosolic and stromal FBPase activities were 40% lower in cells grown at 27/2200 than those grown at 27/150 (Table II). SPS activity was 2.6-fold higher in *Chlorella* grown at 27/2200 than in cells grown at 27/150 (Table II).

Cells grown at LEP at 5°C exhibited a 2.8-fold higher amount of Rubisco, a 2-fold higher Rubisco activity, and an enhanced potential for Suc synthesis as a consequence of a 1.7-fold higher SPS activity relative to cells grown at LEP but at 27°C (Table II). No limitations at the level of stromal FBPase were evident upon growth at LEP at either 5 or 27°C. However, cytosolic FBPase activity was 34% lower in cells grown at 5°C than 27°C and LEP, which indicates a potential restriction in Suc biosynthesis that could be further exacerbated by increased irradiance at 5°C. This is consistent with the data for *Chlorella* grown at 5°C and HEP, for which the total Rubisco activity was 38% lower, the relative cytosolic FBPase activity was 45% lower, and the SPS activity was 87% lower than cells grown at 5°C and LEP (Table II). Thus, it appears that increasing excitation pressure at 27°C results in a significant limitation at the level of stromal FBPase, whereas increasing excitation pressure at 5°C results in major limitations in cytosolic FBPase and SPS activity. Given that the carbamylation ratios for Rubisco and the activation states for SPS were comparable (Table II), we suggest that the limitations in enzyme activities probably reflect differences in the amount of enzyme rather than differences in their catalytic properties.

Phosphorylated Intermediates

At 27°C, *Chlorella* grown at HEP exhibited 3- to 4-fold higher levels of both PGA and RuBP than cells grown at LEP (27/150) (Fig. 4, A and B). However, the ratios of RuBP/PGA were comparable (Table III). Triose-P levels were 3-fold higher in cells grown at 27/2200 than in those grown at 27/150 (Fig. 4E), but the ratios of triose-P/RuBP were not different (Table III). Although the levels of Glc-6-P (Fig. 4C) and Fru-1,6-BP (Fig. 4F) were comparable, the level of Fru-6-P was 38% lower in cells grown at 27/2200 than in those grown at 27/150 (Fig. 4D). As a result, the ratio of Fru-1,6-BP/Fru-6-P in cells grown at 27/2200 was

Table II. *Effect of altered PSII excitation pressure on characteristics of the enzymes Rubisco, FBPase, and SPS in C. vulgaris*

Data represent mean ± SE, n = 4.

Enzyme	Growth Conditions			
	27/150 LEP	27/2200 HEP	5/20 LEP	5/150 HEP
		°C/μmol $m^{-2}s^{-1}$		
Rubisco				
Total activity (nmol $[10^7$ cells$]^{-1}$ h^{-1})	649 ± 22	621 ± 41	1348 ± 101	840 ± 48
Carbamylation ratio (%)	47	53	45	49
Amount of Rubisco (pmol $[10^7$ cells$])^{-1}$	4.8 ± 0.4	3.7 ± 0.5	13.5 ± 0.9	7.2 ± 0.7
k_{cat}, s^{-1}	39	47	28	33
FBPase activity				
Stromal (nmol $[10^7$ cells$]^{-1}$ h^{-1})	162 ± 16	97 ± 2	190 ± 19	200 ± 17
Cytosolic (nmol $[10^7$ cells$]^{-1}$ h^{-1})	51 ± 4	29 ± 2	34 ± 1	18 ± 3
Cytosolic (% of total)	24	23	15	8
SPS activity				
V_{max} assay (nmol $[10^7$ cells$]^{-1}$ h^{-1})	37 ± 6	96 ± 10	63 ± 7	8 ± 2
Activation state (%)	54	49	48	62

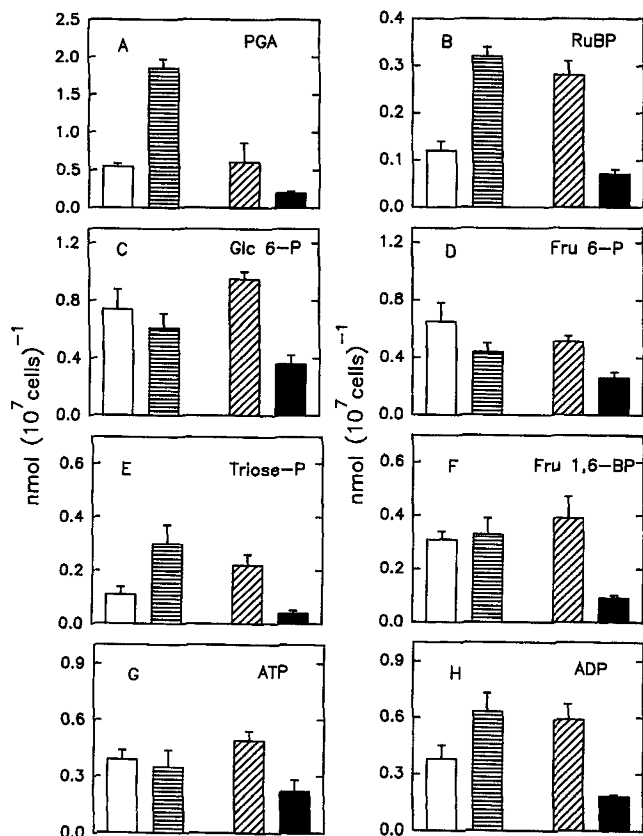

Figure 4. Pool sizes of major metabolites in *C. vulgaris*. Cells were grown at 27/150 (open bars), 27/2200 (horizontally hatched bars), 5/20 (diagonally hatched bars), and 5/150 (solid bars). All data represent the means of four measurements ± SE.

almost double that of cells grown at 27/150 (Table III). Furthermore, the ratio of ATP/ADP in cells grown at 27/2200 was 50% of that observed for cells grown at 27/150 (Table III).

At low temperatures, growth under HEP resulted in a general decrease in the level of all measured phosphorylated intermediates relative to cells grown under LEP (5/20) (Fig. 4). However, the ratios of PGA/triose-P and ATP/ADP were 1.8- and 1.5-fold higher, respectively, in cells grown at 5/150 than in those grown at 5/20 (Table III). In contrast, RuBP/PGA, triose-P/RuBP, and Glc-6-P/Fru-6-P were about 1.4-fold lower in cells grown at 5/150 than the same ratios calculated for cells grown at 5/20. Furthermore, growth at 5/150 exerted a 2-fold decrease in Fru-1,6-BP/Fru-6-P relative to cells grown at 5/20 (Table III).

Chlorella grown under LEP at 5°C exhibited a similar level of PGA but a 63% higher Glc-6-P/Fru-6-P ratio, a 1.6-fold higher Fru-1,6-BP/Fru-6-P ratio, and a 2-fold higher triose-P level than cells grown at LEP but at 27°C (Fig. 4; Table III). It appears that growth of *Chlorella* under light-limited conditions at low temperatures resulted in a restriction in Suc biosynthesis.

Thus, increasing excitation pressure at either 27 or 5°C resulted in comparable 1.3-fold decreases in the ratio of RuBP/PGA and 1.3- to 1.8-fold increases in PGA/triose-P (Table III). However, increasing excitation pressure at 27°C caused the ratios of triose-P/RuBP, Glc-6-P/Fru-6-P, and Fru-1,6-BP/Fru-6-P to either remain constant or to increase by 1.3- to 1.7-fold, whereas the same ratios decreased from 1.4- to 2-fold upon an increase in excitation pressure at 5°C (Table III). In addition, increasing excitation pressure at 27°C resulted in a 50% decrease in the ratio of ATP/ADP, even though the total adenosine phosphate pool remained unchanged. In contrast, increasing excitation pressure at 5°C caused a 47% increase in the ratio of ATP/ADP and a 60% decrease in the total adenosine phosphate pool (Table III; Fig. 4). Thus, it appears that growth at HEP but at low temperatures results in different restrictions in photosynthetic carbon metabolism than growth at HEP and high temperatures.

PSII Excitation Pressure

Based on previous data (Maxwell et al., 1994, 1995a), we predicted that growth of *Chlorella* at either 5/20 or 27/150 would result in equivalent LEP (1 − qP = 0.10) and that cells grown at either 5/150 or 27/2200 would exhibit equivalent HEP (1 − qP = 0.75) (Table IV). The results for PSII excitation pressure measured for log phase cells after growth at either 5/20 or 27/150 (Table IV) indicated that these growth conditions did indeed result in values of 1 − qP close to our predicted value of 0.10. Although log phase cells grown at either 5/150 or 27/2200 did exhibit higher PSII excitation pressures than either population grown at LEP (Table IV), PSII excitation pressure for *Chlorella* grown at 27/2200 was about 3-fold higher than for cells grown at 5/150 (Table IV). Furthermore, PSII excitation pressure for

Table III. *Effect of altered PSII excitation pressure on changes of metabolite ratios in C. vulgaris*

Ratio	Growth Conditions			
	27/150 LEP	27/2200 HEP	5/20 LEP	5/150 HEP
		mol/mol		
RuBP/PGA	0.22	0.17	0.46	0.35
PGA/triose-P	5.00	6.50	2.78	5.00
Triose-P/RuBP	0.92	0.91	0.78	0.57
Glc-6-P/Fru-6-P	1.14	1.40	1.86	1.38
Fru-1,6-BP/Fru-6-P	0.47	0.80	0.76	0.35
ATP/ADP	1.03	0.55	0.83	1.22

Table IV. *Measuring temperature and growth temperature effects on PSII excitation pressure ($1 - q_p$) in C. vulgaris*

Measuring temperature data were estimated from Maxwell et al. (1995a). $1 - q_p$ was measured at either 5 or 27°C as a function of irradiance using cells grown at 27/150. Growth temperature data were obtained by measuring $1 - q_p$ in log phase cells after growth at either 5/20, 5/150, 27/150, or 27/2200.

Temperature	$1 - q_p$			
	LEP		HEP	
	5/20	27/150	5/150	27/2200
Measuring	0.10	0.10	0.75	0.75
Growth	0.14 ± 0.02	0.12 ± 0.02	0.21 ± 0.01	0.57 ± 0.03

cells grown at either 5/150 or 27/2200 was significantly lower than the predicted value of 0.75 (Maxwell et al., 1995a).

DISCUSSION

Photosynthetic acclimation to HEP, induced either by low temperature and moderate irradiance (5°C/150 μmol m^{-2} s^{-1}) or moderate temperature and high irradiance (27°C/2200 μmol m^{-2} s^{-1}), is similar when examined at the levels of pigment content and composition, LHCII abundance, susceptibility to photoinhibition (Table I) (Maxwell et al., 1994, 1995a), *cab* mRNA abundance (Maxwell et al., 1995b), as well as cell ultrastructure (Fig. 1). However, adjustment of photosynthetic carbon metabolism to HEP clearly is dependent on the manner in which the HEP is created. First, upon growth at HEP at 27°C (27/2200 versus 27/150), the Suc/starch ratio increased 25-fold due to a 18-fold increase in Suc and a concomitant 25% decrease in starch content. In contrast, upon growth of *Chlorella* at HEP at 5°C (5/150 versus 5/20), the Suc/starch ratio decreased 23-fold as a consequence of a 7-fold decrease in Suc and a concomitant doubling in the starch content. Second, growth at HEP at 27°C caused the ratios of triose-P/RuBP, Glc-6-P/Fru-6-P, and Fru-1,6-BP/Fru-6-P to remain constant or to increase by 1.3- to 1.7-fold, whereas the same ratios decreased from 1.4- to 2-fold upon growth of *Chlorella* at HEP at 5°C. Third, growth at HEP at 27°C resulted in a 50% decrease in the ratio of ATP/ADP, even though the total adenosine phosphate pool remained unchanged. In contrast, a 47% increase in this ratio was accompanied by a 60% decrease in the total adenosine phosphate pool when *Chlorella* was grown at HEP at 5°C. Thus, we conclude that increasing PSII excitation pressure at 27°C appeared to result in a significant restriction at the level of stromal FBPase, whereas increasing PSII excitation pressure at 5°C appeared to result in major limitations at the level of cytosolic FBPase and SPS. We suggest that the HEP observed after growth at either 27/2200 or 5/150 is, at least in part, a consequence of these restrictions in carbon metabolism that feedback on photosynthetic electron transport and consequently increase PSII excitation pressure. *Chlorella vulgaris* grown at either 5/150 or 27/2200 appears unable to compensate for HEP through adjustments in carbon metabolism, so *Chlorella* responds by reducing its capacity to absorb light through down-regulation of *cab* genes and increased nonradiative dissipation through zeaxanthin to protect the photosynthetic apparatus from excessive radiation (Maxwell et al., 1995a, 1995b).

In the present report, we demonstrate that *Chlorella* grown at low temperature exhibited rates of gross photosynthesis that were 7- to 10-fold lower than the rates for cells grown at high temperature (Table I). This occurred even though 5°C-grown cells exhibited 2- to 3-fold higher amounts of Rubisco associated with high extractable Rubisco activities (Table II), 2-fold higher ratios of RuBP/PGA (Table III), and comparable levels of ATP (Fig. 4G) relative to 27°C-grown cells. Such discrepancies in *Chlorella* have been noted previously (Kerimov et al., 1980; Demidov et al., 1978; Krupenko et al., 1978). The glycolate pathway has been shown to exist in green algae (Bidwell, 1983), so we suggest that the discrepancy among the low rate of photosynthesis, the high levels of ATP, and the high RuBP/PGA ratio, with no apparent restriction at the level of Rubisco, may reflect stimulation of the glycolate pathway in *Chlorella* grown at low temperatures. This is presently under investigation.

Why is there such a large discrepancy between the predicted and the actual HEP experienced by *Chlorella* (Table IV)? Cells grown at 27/150 and exposed to measuring temperature of 27°C and an irradiance of 2200 μmol m^{-2} s^{-1} exhibited a PSII excitation pressure of about 0.75. To exhibit the same PSII excitation pressure at a measuring temperature of 5°C, *Chlorella* grown at 27/150 had to be exposed to an irradiance of only 150 μmol m^{-2} s^{-1} (Table IV). Thus, the comparable PSII excitation pressure elicited under measuring conditions of 5/150 and 27/2200 is the result of a thermodynamic constraint imposed upon intersystem electron transport and photosynthetic carbon metabolism. We suggest that the discrepancy between the predicted excitation pressure estimated through a specific temperature/light measuring regime and the excitation pressure actually observed after exposure to a specific temperature/light growth regime (Table IV) may be the result of differential photosynthetic restrictions at the level of carbon metabolism. The Suc/starch ratio for *Chlorella* grown under nonlimited conditions (27/150) is low, so this indicates that the starch biosynthetic pathway may be the major carbon sink in this green alga. Restrictions in this pathway may exert a greater negative feedback on photosynthetic electron transport than cytosolic restrictions in Suc metabolism. Thus, restrictions in the starch pathway would cause a greater PSII excitation pressure than restrictions in the Suc pathway. As a consequence, cells grown at 27/2200 would be expected to exhibit a higher PSII excitation pressure than those grown at 5/150. Based on the data for Rubisco, stromal and cytosolic FBPase, as well as SPS activities (Table II), we suggest that these restrictions probably reflect differential restrictions in the amounts of these enzymes due to gene repression rather than major changes in their kinetic properties. Further work on the regulation of ADP-Glc pyrophosphorylase, as well as a detailed examination of the regulation of this enzyme and other photosynthetic enzymes at the mRNA and protein levels, is required to substantiate this hypothesis.

Gene expression in green algae such as *Chlorella* and *Euglena* has been shown to be regulated by carbon metabolites (Schiff and Schwartzbach, 1982; Hilgarth et al., 1991). Exposure of autotrophically grown *C. kessleri* to Glc results in increased expression of at least 10 genes involved in carbon and nitrogen metabolism (Cho et al., 1981; Hilgarth et al., 1991). Glc or its nonmetabolizable analog 6-deoxyglucose can act as the inducer, so it has been suggested that the sugar itself acts as the primary signal for the increase in gene expression (Hilgarth et al., 1991). In contrast, Glc can also act to repress the expression of genes in *Chlorella* as well as higher plants (McCullough and John, 1972; Semenenko, 1978; Kuptsova and Semenenko, 1984, 1987; Krapp et al., 1991, 1993; Krapp and Stitt, 1995; Sheen 1994; Jang

and Sheen, 1994; Graham et al., 1994). It has been suggested that hexokinase may be the principal sensor/signal component involved in Glc/hexose-mediated gene repression in higher plants. *C. vulgaris* grown at HEP that is either at 27/2200 or 5/150 exhibits a disruption in chloroplast ultrastructure (Fig. 1), a significant reduction in LHCII apoprotein content, and a 5-fold decrease in Chl content per cell (Table I). In addition, growth of *D. salina* at HEP results in an 8-fold reduction in *cab* mRNA levels (Maxwell et al., 1995b). Thus, it appears that growth at HEP mimics the "Glc bleaching effect" reported in *Chlorella* and higher plants (Kuptsova and Semenenko, 1984; Krapp et al., 1991).

However, our carbohydrate and metabolite data indicate that the proposed Glc/hexose sensing/signaling mechanism cannot account for photosynthetic acclimation to HEP for the following reasons. First, although *Chlorella* grown at 27/2200 and 5/150 exhibit comparable ultrastructure and pigmentation, endogenous Glc and Fru contents differed by at least 10-fold (Fig. 3), triose-P levels differed by 6-fold, and Glc-6-P and Fru-6-P contents were lower than for cells grown at LEP (Fig. 4). Second, Suc content can be ruled out as a signal in our case, because Suc content was at least 100-fold higher in cells grown at 27/2200 than in those grown at 5/150 (Fig. 3), even though both populations of *Chlorella* exhibited similar ultrastructure and pigmentation. Thus, we conclude that the results of this report support our previous contention that photosynthetic acclimation of *Chlorella* can be rationalized best on the basis of chloroplastic redox control (Maxwell et al., 1995a, 1995b).

We have suggested that photosynthetic adjustment to temperature and light is similar in *Chlorella* (Maxwell et al., 1995a) and *Dunaliella* (Maxwell et al., 1995b) because both may reflect a common redox sensing/signaling mechanism that can be activated either thermodynamically or photodynamically. This is consistent with a recent report by Escoubas et al. (1995). However, *Chlorella* grown at 27/2200 exhibited a PSII excitation pressure that was almost 3-fold higher than that of cells grown at 5/150, even though both populations were similar with respect to chloroplast ultrastructure (Fig. 1), pigment content, Chl *a/b* (Table I), susceptibility to photoinhibition, and LHCII apoprotein abundance (Maxwell et al., 1995a). Thus, if we assume a redox sensing/signaling mechanism, the redox state of Q_A cannot be the primary signal regulating photosynthetic adjustment to the combination of light and temperature. However, we suggest that the redox state of Q_A as reflected by PSII excitation pressure may be one component of a multicomponent redox sensing/signaling pathway. Furthermore, our approach to understanding photosynthetic acclimation through a comparison of organisms grown at either HEP or LEP has allowed us to separate clearly acclimation processes regulated through redox poise from those regulated either solely by temperature, solely by light, or regulated by light and temperature in an additive but independent manner (G.R. Gray, L.P. Chauvin, F. Sarhan, and N.P.A. Huner, unpublished results). Clearly, further work is required to elucidate the nature of the redox signal and the transduction pathway as well as their integration into other cellular signal-transduction pathways.

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