STATE 1-STATE 2 TRANSITIONS IN *SYNECHOCOCCUS* 6301 ARE INFLUENCED BY RESPIRATORY ELECTRON FLOW; EVIDENCE FOR THE CONTROL OF EXCITATION ENERGY DISTRIBUTION BY THE REDOX LEVEL OF PLASTOQUINONE IN CYANOBACTERIA.

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1. INTRODUCTION
       Cyanobacteria can adapt rapidly to changes in the spectral quality of actinic light by regulating the distribution of absorbed excitation energy between photosystems I and II so as to maximise the efficiency of light-energy utilisation (1). The mechanism by which state transitions are controlled in this class of organisms remains controversial.
       Here we report results consistent with the control of state transitions in the cyanobacterium *Synechococcus* 6301 by the redox level of plastoquinone. We suggest that some of the apparent differences in the control of state transitions between cyanobacteria and higher plants result from the intersection of the photosynthetic and respiratory electron transport chains in cyanobacteria (2-4), and propose that excitation energy distribution in the cyanobacteria may, as in green plants (5), be modified by protein phosphorylation catalysed by a kinase which is activated by reduced plastoquinone.

2. MATERIALS AND METHODS
       Synechococcus 6301 (*Anacystis nidulans*) was grown photoautotrophically at $35^{0}C$ in medium C of Kratz and Myers (6). Cells were subcultured to a concentration of about 1 ug chlorophyll a ml^{-1} and grown for 4-6 hours after subculturing. Where appropriate, the cells were then starved by aerobic incubation in the dark in a stirred flask at $32^{0}C$.
       For modulated fluorescence measurements the cell suspension was diluted with medium C where necessary to 2 ug chlorophyll a ml^{-1}. Fluorescence measurements were made in a stirred cuvette at $25^{0}C$. Fluorescence was generated using a weak yellow light (light 2) at 1 Wm^{-2} and modulated at 870 Hz. This was provided by an array of yellow LEDS screened by a 650 nm short pass optical filter (Hansatech, King's Lynn). Fluorescence was detected by a Hansatech photodiode which was screened by a 700 nm interference filter and which was connected to an amplifier locked-in to the frequency of the modulated light 2. Light 1 at 6 Wm^{-2} was defined by a Corning 5-60 blue filter.
       For 77 K fluorescence spectroscopy the cells were harvested by centrifugation and resuspended to 100 ug chlorophyll a ml^{-1}. Fluorescein was added to 5uM and the cells were incubated for 30 minutes in the dark in the presence of DBMIB (50 uM) or methyl viologen (2 mM), or without added reagents. They were then illuminated, where appropriate, with light 1 (defined by a Corning 5-60 filter) or light 2 (defined by a combination of 660 nm short pass and 560 nm long pass filters) for 10 minutes. The cells were then diluted to 10 ug chlorophyll a ml^{-1} with 50% glycerol and immediately frozen in liquid nitrogen. Fluorescence emission spectra were

recorded using a Perkin-Elmer LS-5, with an excitation wavelength of 435 nm.
The spectra were normalised to the fluorescein emission peak at 490 nm, or
to the phycocyanin peak at 654 nm if DBMIB was present.

3. RESULTS AND DISCUSSION

The fluorescence emission of PS II is dependent in part on
absorption cross-section and hence may be used as an indicator of light-
state adaptation (7). Figure 1 shows the effect of starvation on state
transitions in *Synechococcus* 6301 cells observed using a modulated
fluorescence measurement system as in (8). The lock-in amplifier resolves
only the fluorescence generated by the modulated light 2; only the indirect
effects of the non-modulated light 1 are observed. The addition of light
1 always induced a slow rise in fluorescence which we interpret as a state
1 transition. When light 1 was extinguished, there was a rapid rise in
fluorescence due to the reduction of the plastoquinone pool and the
consequent net closure of PS II reaction centres. This was followed by a
slow falling phase most easily interpreted as a state 2 transition.

When cells were starved by 21 hours dark aerobic incubation, there
was no measurable change in the level of either chlorophyll a or phycocyan-
in (data not shown). Starved cells gave an initial fluorescence yield
close to Fm (figure 1b), indicating greatly reduced PS I turnover due to
depletion of reductive pentose phosphate pathway intermediates which are
synthesised only in the hight.m However, following a period of light
adaptation, normal levels of PS I turnover are restored, as evidenced by
the steady state fluorescence yield (figure 1b). The respiration rate
(measured in the dark at $25^{o}C$ in a Clark-type oxygen electrode) was,
however, decreased from 7,6 to 1.0 umoles O_2 (mg chlorophyll)$^{-1}$ hour^{-1} by
21 hours starvation. It thus seems likely that the differences which
result from 21 hours starvation result from a deceeased rate of respiratory
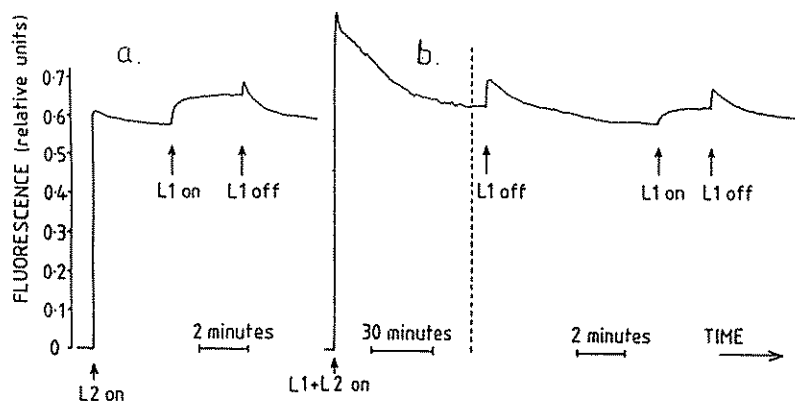electron flow into the plastoquinone pool.
These differences are:

FIGURE 1 Effect of starvation on cells of *Synechococcus* 6301
a. Photoautotrophically grown cells b. cells starved for 21 hours.
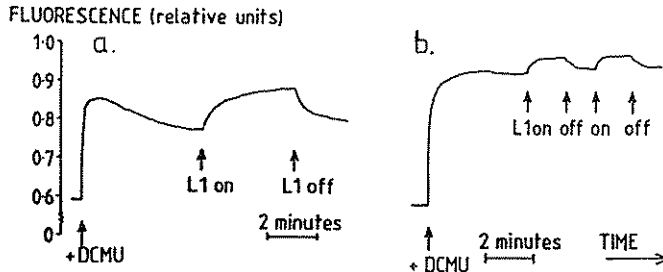
FLUORESCENCE (relative units)

FIGURE 2 State transitions in the presence of DCMU (traces continued from figure 1). a. Photoautotrophically grown cells. b. Cells starved for 21 hours.

a. An increase in the half-time for the state 2 transition from about 30 s (figure 1a) to about 50 s (figure 1b).

b. An altered response to DCMU addition. In unstarved cells the addition of DCMU induces a fast-phase fluorescence rise due to the closure of PS II reaction centres followed by a small slow-phase rise which is subsequently reversed (figure 2a). The addition of light 1 then induces a fluorescence rise which is reversed when light 1 is extinguished. Since DCMU at 50 uM was sufficient completely to suppress electron transport from PS II to plastoquinone, and since any fluorescence generated by the non-modulated light 1 is not detected by the lock-in amplifier, this fluorescence rise can only be interpreted as a state 1 transition. In starved cells (figure 2b) the addition of DCMU induces a large slow-phase fluorescence rise, and the subsequent addition of light 1 has only a small further effect.

These effects may be accounted for if it is assumed that excitation energy distribution in this organism is governed by the redox state of plastoquinone, or of another component common to the photosynthetic and respiratory electron transport pathways. In unstarved cells, rapid respiratory electron flow maintains the plastoquinone pool in a reduced state even in the presence of DCMU, so that the cells remain in state 2 when DCMU is added. The subsequent additoon of light 1 partially oxidises the plastoquinone pool, inducing a shift towards state 1 which is seen as a
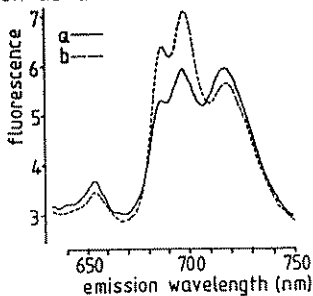

FIGURE 3 77K fluorescence emission spectra of *Synechococcus* 6301 cells. a. dark-adapted b. light 1 adapted

TABLE 1 Effect of pretreatments on 77K fluorescence emission of *Synechococcus* 6301 cells. (Averaged from 3 samples).

Pretreatment	F697/F720
Dark*	1.02 ± 0.02
Light 1	1.27 ± 0.06
Light 1+DBMIB	1.13 ± 0.01
Light 2	1.22 ± 0.01
Light 2+methyl viologen	1.32 ± 0.04
Dark + methyl viologen	1.03 ± 0.01

fluorescence rise (figure 2a). In starved cells, however, the plastoquinone pool becomes oxidised following the addition of DCMU, since the rate of respiratory electron flow into the plastoquinone pool is lower than the rate of PS I turnover induced by the modulated light 2. The addition of DCMU therefore induces a state 1 transition, and the subsequent addition of light 1 has only a small further effect (figure 2b).

Further evidence for the control of excitation energy distribution by the redox state of plastoquinone comes from 77 K fluorescence emission spectroscopy. The ratio of PS II to PS I fluorescence emission is an indicator of light energy distribution between the two photosystems (7). Figure 3 shows spectra for dark-adapted cells and cells illuminated with light 1 prior to freezing. The dark adapted cells show an increased F720 and a decreased F695 and F697, indicating that the dark state is state 2. Table 1 shows the ratio F697/F720 for cells subjected to a variety of pretreatments. DBMIB decreases the ratio F697/F720 in light 1-adapted cells, indicating that it induces a state 2 transition. Since DBMIB blocks photochemical oxidation of plastoquinone (9), the addition of this reagent will cause reduction of the plastoquinone pool in the light. Methyl viologen, by contrast, should cause oxidation of the plastoquinone pool in the light, since it is a powerful PS I electron acceptor. Table 1 shows that methyl viologen induces a state 1 transition in light 2-adapted cells, but has no significant effect in the dark.

We conclude that excitation energy distribution in *Synechococcus* 6301 is controlled by the redox state of plastoquinone, or of another component whose position in the electron transport chain lies between the sites of action of DCMU and DBMIB, and which is common to the photosynthetic and respiratory electron transport chains. In view of the light-dependent phosphorylation of a number of membrane polypeptides in *Synechococcus* 6301 (10), including a phycobilisome component (11), we suggest that state transitions in this organism result from protein phosphorylation catalysed by a kinase which is activated by reduced plastoquinone.

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