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Moderate UVB triggers dynamic regulation of *cpc*, *psbA* and *rbcL* transcripts in the cyanobacterium *Synechococcus* sp. PCC 7942

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Introduction

UVB is only about 1% of the solar radiation reaching the Earth's surface, but it is biologically highly active. With declining stratospheric ozone more UVB is penetrating the euphotic zone where it inhibits phytoplankton photosynthesis (Hader et al., 1998). Many studies of UVB have used cells grown under low photosynthetically-active radiation (PAR) and then treated with unrealistic levels or durations of UVB (Castenholtz & Garcia-Pichel, 2000), while natural aquatic UVB exposure is transient and occurs in predictable ratios to PAR. We exposed cyanobacteria to ecophysiological levels of PAR and UV-B and then tracked transcripts encoding three major proteins with different functions in cyanobacterial photosynthesis.

Phycocyanin (PC), encoded by the cpcBA genes, is the major constituent of cyanobacterial light harvesting phycobilisomes. In Synechococcus grown under moderate light and nitrogen repletion phycocyanin accounts for 65% of extractable cellular protein. Phycobilisomes are a major target of UVB radiation in cyanobacteria (Araoz and Hader, 1997) since they absorb in the UVB region. At high doses UVB damages phycobilisome structure and function (Lao and Glazer, 1996; Pandey et al., 1997; Sinha et al., 1997). The D1 proteins of PSII, encoded by the psbA gene family, bind the major redox co-factors of PSII. They turn over rapidly in the light (Aro et al., 1993) and are involved in cyanobacterial UVB tolerance (Campbell et al., 1998a). RUBISCO is the primary CO2 fixation enzyme, whose large catalytic subunit is encoded by the rbcL gene. In our cultures it accounts for 5-10% of extractable cellular protein and is a suspected target for UVB inhibition of cyanobacterial photosynthesis (Castenholtz & Garcia-Pichel, 2000).

Materials and Methods

Axenic cultures of Synechococcus sp. PCC 7942 were grown in flat polystyrene flasks (Costar, USA, transparent to UVB above 290 nm), inoculated to 0.5 µg chl ml^{-1} (Myers et al., 1980) in BG-11 media (pH 7.5) (Rippka et al., 1979) at 37 ºC, bubbled with 5%CO_2 in air, and illuminated at Low (50) or High (300 µmol photons PAR m^{-2} s^{-1}). After about 24 h of exponential growth cultures reached 2 µg chl ml^{-1} and UVB treatments began.

Two treatments applied a PAR:UV-B quantum ratio of 100:0.25 , within the range of shallow temperate aquatic habitats in summer (Scully & Lean, 1994). High PAR cells were exposed to High UVB (0.75 µmol photons m^{-2} s^{-1}) while Low PAR cells were exposed to Low UVB (0.125 µmol photons m^{-2} s^{-1}). A third treatment exposed Low PAR cells to excess High UVB, to compare the response of Low and High PAR cells to the same level of UVB. fPSII (= (Fm'-Fs)/Fm') was measured using an OS1 fluorometer (OptiSciences, USA). fPSII

in cyanobacteria is distorted by phycobiliprotein fluorescence (Campbell et al., 1998b) but does track PSII function.

RNA isolations were performed using the Trizol® method (Life Technologies) followed by blots using Northern Max-Gly™ (Ambion) with Bright Star-Plus™ (Ambion) membranes. cpcBA and rbcL probes were prepared using PCR with primers designed from cpcBA and rbcL from Synechococcus. AlkPhos Direct (APB) was used to label the purified DNA probes, followed by hybridisation and washing at 55 ºC. CDP-Star chemiluminescent reagent (APB) was used to generate a signal from the bound probes which was captured with a BioRad Fluor-S Max™ CCD camera. psbAI and a combined pool of psbAII/AIII transcripts were detected using gene-specific primers and Reverse Transcriptase PCR (Ready-to-Go, APB). The RT-PCR was run for 18 cycles, followed by product separation on agarose gels stained using Gel-Star (BioWhittaker).

Results

After 24 h Low PAR cells accumulated significantly more PC than did High PAR cells (Fig. 1A, T0). Under all treatments PC/cell declined slightly after 45 min of UVB exposure. There was no significant change in the growth rate of the cells exposed to environmentally appropriate UVB. Low PAR cells did suffer growth inhibition when exposed to High UVB; their doubling time slowed from 13.5 h pre-exposure to 20.2 h under High UVB. As expected fPSII dropped significantly under the excess UVB exposure (Fig. 1B). There was no initial change in fPSII in the other treatments although fPSII dropped by 90 min of High PAR:High UVB .

Low PAR cells initially contained significantly more cpcBA transcripts than did High PAR cells (Fig. 2A). The content of cpcBA transcripts was sensitive to UVB exposure, dropping within 15 min under all UVB treatments but then recovering so that by 90 min of exposure cpcBA transcripts returned to control levels (Fig. 2A). There was no consistent difference in the initial abundance of rbcL transcripts in Low or High PAR cells (Fig. 2B), nor did the rbcL transcript pool size change significantly under any of the UVB treatments. The constitutively expressed psbAI transcripts were present in all cells (Fig. 2C), although at lower levels in High PAR cells. Conversely High PAR cells had a higher initial pool of psbAII/AIII transcripts than did cells grown under Low PAR (Fig. 2D). UVB induced additional accumulation of psbAII/AIII within 15 min in Low PAR cells.

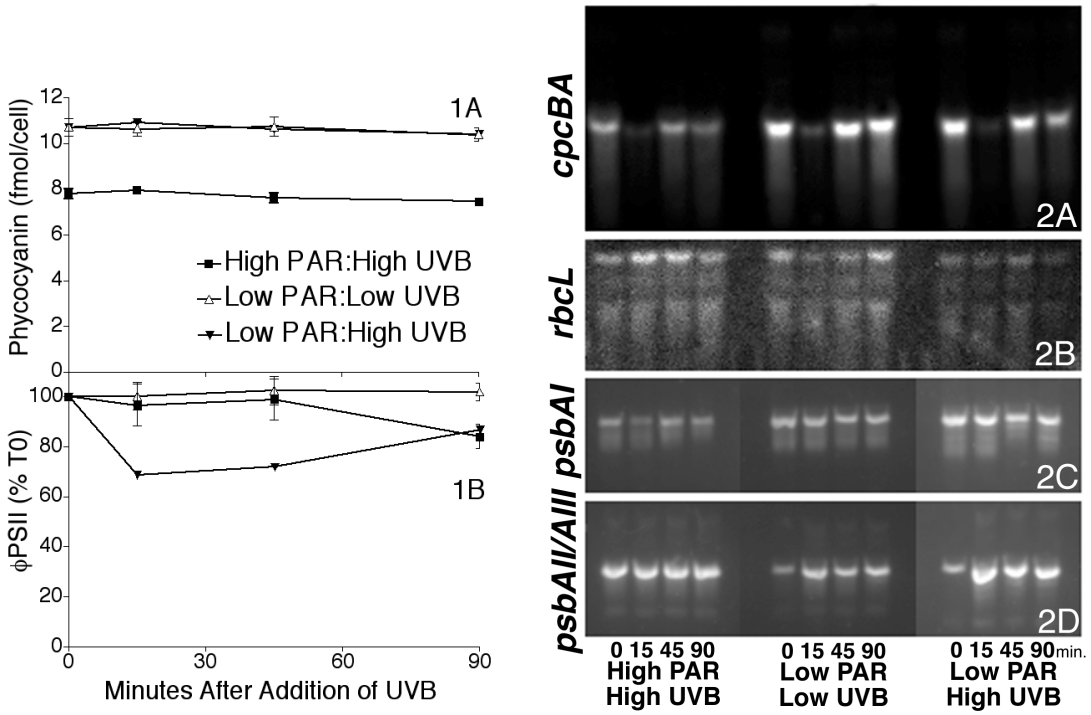

Fig. 1. (A) nmol of Phycocyanin/ cell (B) fPSII under UVB treatments.

Fig. 2. (A) cpcBA transcripts under UVB treatments detected with a hybridization probe spanning the transcribed non-translated spacer region between cpcB and cpcA.
(B) rbcL transcripts under UVB treatments detected with a hybridization probe internal to the coding sequence.
(C) RT-PCR products amplified from psbAI transcripts under UVB treatments.
(D) RT-PCR products amplified from pooled psbAII and psbAIII under UVB treatments.
These blots and gels are representative of 3-4 independent determinations.

Discussion

The response of cells to UVB depended on their light acclimation history. High PAR cells tolerated a dose of UVB that inhibited both the fPSII and growth of Low PAR cells. High PAR cells initially had lower phycocyanin content, fewer cpcBA transcripts and a larger pool of psbAII/AIII transcripts. Since psbAII/AIII expression is important for short-term resistance to UVB (Campbell et al., 1998a) this pre-accumulation of the transcripts potentiated the High PAR cells to tolerate an environmentally appropriate dose of UVB. Similarly Low PAR cells were able to rapidly alter their gene expression to tolerate an environmentally appropriate dose of UVB with no short-term inhibition of fPSII or growth.

In all the cultures UVB induced a rapid but transient loss of cpcBA transcripts, concomitant with an accumulation of psbAII/AIII transcripts. We suggest that in these nitrogen replete cells luxury expression of cpcBA and accumulation of PC is a dispensable activity from which transcriptional resources can be rapidly re-allocated for the short-term expression of the psbAII/AIII important for UVB resistance. The transient drop in cpcBA transcripts upon UVB exposure could also represent a rapid down-regulation of phycocyanin expression to lower the UVB absorbance by the cell. Phycocyanin content did drop slightly by the end of the treatment but by that time the cpcBA transcript pool had fully recovered, suggesting the initial drop in cpcBA transcripts is too transient to account for the longer term decline in PC. We detected no change in rbcL expression even in the Low PAR cells exposed to excess High UVB, so of the three major transcript classes rbcL is least sensitive to moderate short-

term UVB exposure. The cells in this study were growing exponentially under nutrient replete conditions where they accumulate high levels of phycocyanin, which can be rapidly sacrificed under nitrogen limitation. Their transcriptional, physiological and compositional responses to UVB may be quite distinct under nutrient limitation, an area for future work.

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