

Glucose-induced Expression of Carotenoid Biosynthesis Genes in the Dark Is Mediated by Cytosolic pH in the Cyanobacterium *Synechocystis* sp. PCC 6803*

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Jee-Youn Ryu‡, Ji Young Song‡, Jeong Mi Lee‡, Suk Won Jeong‡, Wah Soon Chow§, Sang-Bong Choi¶, Barry J. Pogson||, and Youn-Il Park‡**

From the ‡Department of Biology, Chungnam National University, Daejeon 305-764, Korea, §Photobioenergetics Group, Research School of Biological Sciences, Australian National University, Canberra ACT 2601, Australia, ¶Department of Biological Sciences, Myongji University, Yongin 449-728, Korea, and ||School of Biochemistry and Molecular Biology, Australian National University, Canberra ACT 0200, Australia

The expression of carotenoid biosynthesis genes coding for phytoene synthase (*crtB*), phytoene desaturase (*crtP*), ζ -carotene desaturase (*crtQ*), and β -carotene hydroxylase (*crtR*) is dependent upon light in the cyanobacterium *Synechocystis* sp. PCC 6803 (*Synechocystis*). We have demonstrated that the expression of the above four genes was also elevated in the dark-adapted *Synechocystis* cells upon glucose treatment as a consequence of transcriptional activation. Treatment with glucose analogs such as L-glucose, 3-O-methylglucose, 2-deoxyglucose, and mannose, or inactivation of glucose uptake and phosphorylation by deletion mutation of glucose transporter (*glcP*) and glucokinase (*gk*), respectively, did not induce up-regulation of carotenoid genes. When respiratory electron transport or coupling to oxidative phosphorylation was inhibited, glucose induction was not observed, indicating that respiratory electron transport *per se* is not critical for the expression of these genes. In agreement with this view, the extent of gene expression showed a saturation curve with increasing acridine yellow fluorescence yield, without having a close correlation with the ATP contents or ATP/ADP ratio. The results indicate that glucose induction of carotenoid gene expressions is mediated by an increase in cytosolic pH rather than either redox or glucose sensing.

Carotenoids in all photosynthetic organisms including cyanobacteria participate in the light-harvesting process and function in the protection of the photosynthetic apparatus against photo-oxidative damage (1). The cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter called *Synechocystis*) contains several genes encoding enzymes involved in carotenoid biosynthesis (2): the *crtB* gene for phytoene synthase, *crtP* for phytoene desaturase, *crtQ* for ζ -carotene desaturase, *crtO* for β -carotene ketolase, and *crtR* for β -carotene hydroxylase.¹ Despite an abundance of information about the carotenoid biosynthesis pathway and the genes involved, very little is known about

the regulation of the expression of carotenoid biosynthesis genes (3).

Light seems to play an important role in the expression of carotenoid biosynthesis genes. In higher plants and green algae, photoreceptors like phytochrome (4, 5) and blue light receptors (6) are involved in light signaling. But, in the cyanobacteria, redox-sensing and signaling by photosynthetic electron transport (7, 8) are probably more important than other photosensory systems. This is because the content of carotenoids in *Synechococcus* sp. PCC 7942 is proportional to the photosynthetic rate (9), and the expression of phytoene synthase (*crtB*) and phytoene desaturase (*crtP*) genes in *Synechocystis* depends upon light intensity (10).

To address the redox control over carotenogenesis genes, we took advantage of *Synechocystis* that grows at the expense of an exogenously supplied sole carbon source, glucose (11). *Synechocystis* cells uptake external glucose by means of a glucose transporter (*glcP*, the gene locus *sl0771*; Ref. 12) and metabolize it further via glycolysis and the oxidative pentose phosphate pathway (13). NAD(P)H produced from glucose metabolism is oxidized by NAD(P)H dehydrogenase in the photosynthetic thylakoid membranes; it modulates the redox status of the plastoquinone (PQ)² pool. Thus, glucose has been commonly used for the observation of membrane redox-controlled processes in cyanobacteria (14–16).

However, glucose effects on the expression of some genes could result from so-called glucose-sensing and signaling or from changes in the cytosolic redox state, ATP/ADP ratio, or cytosolic alkalization. In addition to its involvement in redox-sensing by means of respiration, glucose has been known to act as a signaling molecule, which is well documented in higher plants, yeast, and human pancreatic cells. In *Arabidopsis*, for example, at least three different glucose-sensing systems responsible for up- or down-regulation of photosynthesis, carbon metabolism, and pathogenesis-related gene expression are operative: a hexokinase-dependent system, a hexokinase-independent but as yet unidentified glucose sensor or transporter, and a system dependent upon active glycolysis downstream from hexokinase (for reviews, see Refs. 17 and 18). Therefore, the interpretation of glucose induction of gene expression solely

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** To whom correspondence should be addressed: Dept. of Biology, Chungnam National University, Daejeon 305-764, Korea. Tel.: 82-42-821-5493; Fax: 82-42-822-9690; E-mail: yipark@cnu.ac.kr.

¹ Available on the World Wide Web at www.kazusa.or.jp/cyanobase/.

² The abbreviations used are: PQ, plastoquinone; WT, wild-type; DB-MIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; L-Glc, L-glucose; OMG, 3-O-methylglucose; Man, mannose; 2-dGlc, 2-deoxyglucose; RT, reverse transcriptase; Cyt, cytochrome; CtaI, Cyt *c* oxidase; Cyt, quinol oxidase; CCCP, carbonylcyanide-3-chlorophenyl hydrazine; Fo, instantaneous (dark) acridine yellow fluorescence; $\Delta F/F_0$, relative change of acridine yellow fluorescence induced by 10 mM glucose.

in terms of redox-sensing should be made cautiously in higher plants. The cyanobacterium *Synechocystis* is not an exception either, because it contains potential components for glucose signaling cascades, such as hexose transporter, glucokinase (the locus *sll0593*), and other signaling components found in the genomic DNA sequence.

In addition to changes in the redox state of respiratory electron transport, glucose treatment also affects the cytosolic redox state, ATP/ADP ratio, and pH in the cytosol, which are important factors of metabolism (19), glucose repression of yeast genes (17), and hormone signaling (18, 20, 21). Thus, there is a possibility that glucose induction of some of the above-mentioned photosynthesis genes is under the control of the cytosolic redox status (NAD(P)H/NAD(P)⁺), ATP/ADP ratio, or cytosolic pH. In this respect, the translation machinery was suggested to be regulated by photosynthetic electron transport via the proton gradient and/or ATP concentration in higher plants (22) and the cyanobacterium *Synechococcus* (23).

While working on light induction of carotenoid genes with respect to redox-signaling in *Synechocystis*, we found that glucose alone in the dark is enough to stimulate the gene expression. To address whether glucose induction arises from glucose-sensing or whether it is the consequence of respiratory electron transport, we used specific inhibitors of respiratory electron transport, glucose analogs, and various electron transport-, glucose uptake-, and glucokinase-defective mutants. Our results clearly showed that glucose induction of carotenoid synthesis genes is not mediated by either glucose signaling or redox status of respiratory electron transport but is rather mediated by changes in the pH of the cytosol. To our knowledge, this is the first report suggesting that cytosolic pH change is critical for gene regulation in photosynthetic prokaryotic systems.

EXPERIMENTAL PROCEDURES

Cell Strains, Growth Conditions, and Inhibitors—Cells of *Synechocystis* sp. PCC 6803 wild-type (WT) and various mutants defective in respiratory electron-transport chains (M55, *Cyd-less*, and *CtaI-less* strains), glucose uptake (*ΔglcP*), and hexose phosphorylation (*Δgk*) were grown at a light intensity of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in BG-11 inorganic medium buffered with 5 mM TES (*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid)-NaOH, pH 8.0, at 30 °C (24). Chlorophyll content was determined using whole-cell spectra (25). When required, electron-transport inhibitors (10 μM HgCl₂, 10 μM DBMIB, or 0.1 mM KCN) and 10 mM glucose or its analogs, L-glucose (L-Glc), 3-*O*-methylglucose (OMG), mannose (Man), and 2-deoxyglucose (2-dGlc) were added to the cell cultures pre-adapted for 16 h in the dark and incubated in the dark for further periods. The transcription inhibitor rifampicin was added at a final concentration of 300 $\mu\text{g ml}^{-1}$.

Mutagenesis—We isolated genomic DNA using the method of Porter (26). Restriction endonucleases were obtained from MBI Fermentas and used according to the manufacturer's recommendations. The glucose-transport protein (locus *sll0771*, *glcP* in the CyanoBase) and glucokinase (locus *sll0593*, *gk* in the CyanoBase) genes were amplified by PCR from the genomic DNA of WT *Synechocystis* using primers of *glcP* (forward, 5'-ATGAATCCCTCTCTCTCTCCCAATC-3' and reverse, 5'-TCACATTGTGCTCCAGAGTTTACCCTTAGTTCC-3') and *gk* (forward, 5'-GGCCAGGTGGAGGAAGTGC-3' and reverse, 5'-CAATTCCAGGTAAAGGCAAAG-3'). To inactivate *glcP* and *gk* genes, cassettes conferring chloramphenicol resistance (digested with *Sma*I) were blunt end-ligated into the *Msc*I site (positions 451 and 1079 of the insert) and the *Stu*I and *Dra*II site (positions 366 and 977 of the insert), respectively, of PCR products that were cloned into pGEM-T Easy vector (Promega). Vectors containing these disrupted genes were used to transform *Synechocystis* cells, and antibiotic-resistant transformants were selected (26). Genomic DNA was isolated from individual transformants, and PCR analysis was performed to verify that all chromosomal copies carried the disrupted gene.

RNA Isolation and RT-PCR—For analysis of transcript levels, total RNA was isolated using the TRIzol™ reagent (Invitrogen) and treated with RNase-free DNase (Promega). The expression levels of both *crtB* and *crtP* genes in *Synechocystis* were very low, and quantifying the

mRNA level by Northern hybridization was difficult (10). Thus, we used a reverse transcriptase (RT)-mediated PCR technique to analyze the expression of mRNAs levels. RT-PCR analysis was carried out by using total RNA and specific primers of *crtB* (forward, 5'-CTTTACCGTTCCCGTTACCA-3' and reverse, 5'-GGGTACATAGGCACGACGAT-3'), *crtP* (forward, 5'-GGGGAGGGGAAGTACACATT-3' and reverse, 5'-CACGTGGGATTTAAGCAGT-3'), *crtQ* (forward, 5'-ATGGTTCCTCAGCAAAGTG-3' and reverse, 5'-TTCATTTCGTAACCCAAACC-3'), *crtR* (forward, 5'-CGTTTTTGGCTACTGGCAAT-3' and reverse, 5'-AATGGCTTGATGTGAT-3'), and *rrn16Sa* (forward, 5'-CACACTGGGACTGAGACACG-3' and reverse, 5'-CCACGCCTAGTATCCATCGT-3'). One microgram of total RNA was used for each reverse transcription in a 20- μl reaction volume, and 0.8 μl of the reaction mixture was subjected to PCR amplification in an MJ Research Minicycler under the following conditions: initial denaturation at 95 °C for 3 min, followed by 25 (*crtB*, *crtP*, and *crtQ*), 28 (*crtR*), and 8 (*rrnA*) cycles of 95 °C for 30 S, 60 °C for 20 S, and 72 °C for 30 S, and then a final extension step at 72 °C for 7 min. These RNA concentrations were within the linear response range of the PCR amplification (data not shown). The 16S rRNA was included as a positive control. To detect possible DNA contamination, control reactions were performed without RT but with Taq polymerase. The PCR products were routinely analyzed on a 1.4% agarose gel and viewed with a GELDOC2000 densitometer and a computer-aided image analysis system (Bio-Rad). The identities of all RT-PCR products were confirmed by DNA sequencing.

Adenylate Contents—*Synechocystis* cell pellets (2 μg of chlorophyll) treated with various electron-transport inhibitors and uncoupler with or without 10 mM glucose for 15 min in the dark were resuspended in adenylate extraction media (1 $\times$ CCLR, Promega) and then incubated at 65 °C for 10 min. After centrifugation at 12,000 $\times g$ for 5 min at 4 °C, the supernatant was used for the measurement of adenylate contents. ATP and ADP contents were measured by luminometry (MicroLUMAT LB96P, E&G Berthold, Bad Wildbad, Germany) using the chemiluminescent ENLITEN™ ATP assay system (Promega). To determine the ADP concentration, ADP was converted to ATP using pyruvate kinase with buffer (30 mM Hepes-NaOH, pH 7.4, 4 mM MgCl₂, 0.125 mM phosphoenol pyruvate) for 1 h at 30 °C. Enzyme was inactivated by heating the reaction mixture for 10 min at 95 °C.

Acridine Yellow Fluorescence—Fluorescence yield of acridine yellow from dark-adapted intact cells which had been suspended in the culture media buffered with 5 mM TES-NaOH, pH 8.0, was measured using Xe-PAM (Walz, Effeltrich, Germany) at 30 °C. Acridine yellow was shown to be the best pH-sensitive indicator for the energization of cytosol of *Synechocystis* (27). For the excitation and detection of acridine yellow fluorescence, the emitter and detector was equipped with band-pass filters (UG11 and BG18 for emitter, and KV418 and BG18 for detector; Walz). The concentration of acridine yellow was 5 μM and that of chlorophyll was 80 μg .

RESULTS

Expression of Carotenoid Biosynthesis Genes in Response to Light and Glucose—Expression of carotenogenesis genes, *crtB* and *crtP*, is up-regulated upon illumination of dark-adapted *Synechocystis* cells (10). To determine the extent of light regulation of the key carotenoid biosynthesis genes, *crtO*, *crtQ*, and *crtR* were analyzed in addition to *crtB* and *crtP*. Photoautotrophically grown cells at the exponential growth phase were subjected to dark treatment for 16 h and then exposed to high light (100 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 30 min. RT-mediated PCR analysis showed that dark-adapted cells had only small amounts of mRNAs of all four *crt* genes, but 30 min of illumination at high light intensity increased significantly the levels of mRNAs accumulated (Fig. 1). *crtO* mRNAs were not easily detectable even by RT-PCR analysis (data not shown) and hence were not characterized further. The promoter analysis of both *crtB* and *crtP* genes showed that the expression of both genes can be photoactivated (10). If this is the case, it would be expected that expression of *crt* genes should be subject to light signaling by means of either the photosynthetic apparatus or photoreceptor systems. To address whether redox status of electron transport is sufficient to induce gene expression, cells were exposed to glucose under dark conditions to exclude any involvement of photoreceptor systems. Upon addition of glucose to dark-

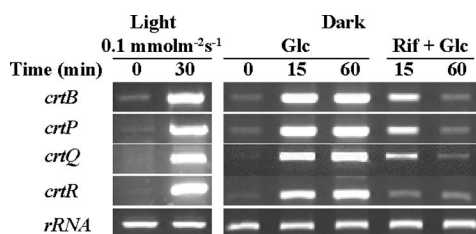


FIG. 1. Light- and glucose-induced expressions of carotenoid biosynthesis genes *crtB*, *crtP*, *crtQ*, and *crtR* in *Synechocystis* wild-type cells. Cells were dark-adapted for 16 h and then illuminated ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 30 min (Light) or treated with 10 mM glucose (Glc) for 15 and 60 min with or without 300 $\mu\text{g/ml}$ Rifampicin (Rif+Glc) under dark conditions (Dark). RT-PCR analysis was performed as described under "Experimental Procedures."

adapted cells, all gene transcripts investigated were clearly observed even after 15 min of glucose treatment (Fig. 1).

Treatment of cells with rifampicin, a transcription inhibitor, inhibited accumulation of mRNAs of all genes, suggesting that glucose induction is a consequence of transcriptional activation. From these results, it is apparent that accumulation of the four carotenoid biosynthesis genes is induced not only by light but also by glucose in the dark.

Further Glucose Metabolism, Rather than Glucose Phosphorylation by Hexokinases, Is Required for the Glucose Induction of Carotenogenesis Genes—Glucose induction for the expression of carotenoid biosynthesis genes is likely to be related to either glucose sensing, changes in membrane or cytosolic redox status, or changes in cytosolic energy status as a consequence of respiratory electron transport. First, we asked whether glucose-induction of *crt* genes is a consequence of glucose signaling as in higher plants (17, 18). A glucose transporter (*slr0771*, *glcP*) was insertionally inactivated (ΔglcP) to see whether glucose uptake is required for glucose induction of *crt* genes. To investigate whether hexokinases act as a sensor, in addition to their catalytic activity, we made an insertional mutant of glucokinase gene *slr0593* (Δgk). Cells defective in glucose uptake and phosphorylation failed to show glucose induction of the four *crt* genes (Fig. 2A), indicating that glucose uptake into the cell and its concomitant phosphorylation is a prerequisite for glucose induction of the four *crt* genes.

To confirm the aforementioned results and determine whether it is glucose *per se*, its phosphorylation, or its further metabolism that is involved in the gene induction, cells were treated with several glucose analogs: L-Glc, OMG, 2-dGlc, and Man. L-Glc is an isomer of D-glucose and is very poorly transported into cells (28). OMG is a glucose analog that is transported into *Synechocystis*, but is not phosphorylated by hexokinase (11, 29). 2-dGlc and Man are sugars that are substrates of hexokinases, being phosphorylated by hexokinases, but inhibit the production of glucose-6-phosphate, NADPH, and ATP (30). Cells treated with L-Glc, OMG, 2-dGlc, and Man in the dark hardly showed any accumulation of mRNAs of the four *crt* genes (Fig. 2B). Thus, it is clear that glucose uptake and phosphorylation alone is not sufficient for *crt* gene induction, ruling out the possibility of the involvement of glucokinase or a membrane protein as a glucose sensor. Instead, it suggests that further downstream metabolism of phosphorylated glucose is required for the glucose induction of *crt* genes in the dark.

Uncoupling of Respiratory Electron Transport to Proton Translocation Inhibits the Glucose Induction of Gene Expression—We hypothesized that glucose induction of the four *crt* genes may be the consequence of further metabolism of phosphorylated glucose through changes in the redox state of the respiratory electron-transport chain, as is the case for some photosynthetic and non-photosynthetic genes (14–16). To test

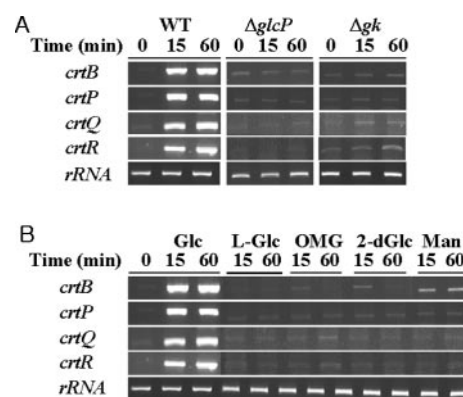


FIG. 2. Expression of carotenoid biosynthesis genes *crtB*, *crtP*, *crtQ*, and *crtR* in *Synechocystis* wild-type (WT) (A and B), glucokinase (Δgk), and glucose transporter (ΔglcP)-defective (A) cells. Cells pre-adapted in the dark for 16 h were treated with 10 mM of glucose (Glc) and glucose analogs, L-glucose (L-Glc), 3-O-methylglucose (OMG), 2-deoxyglucose (2-dGlc), and mannose (Man) for 15 and 60 min in the dark.

this, we investigated the expression levels of the four genes in various respiratory electron-transport-defective mutants or WT cells treated with respiratory electron-transfer inhibitors or with a proton uncoupler. Cyanobacterial respiratory electron transport shares some electron carriers with photosynthesis, including the PQ and cytochrome (Cyt) *b₆f* complex. The cyanobacterial PQ pool is mainly reduced by NADPH dehydrogenase (31, 32) and succinate dehydrogenase (33), and oxidized by the terminal Cyt *c* oxidase (CtaI) and quinol oxidase (Cyd) (33).

When glucose was fed in the dark to various respiratory electron-transfer-defective mutants, M55 (NADPH dehydrogenase-defective strain), cytochrome *c* oxidase-defective strain, and quinol oxidase-defective strain, accumulation of transcripts of the four genes was not observed in M55 strains, but was comparable with WT cells in both cytochrome *c* oxidase-defective and quinol oxidase-defective strains (data not shown). Loss of glucose induction of four *crt* genes was also observed in WT cells treated with HgCl_2 (NADPH dehydrogenase inhibitor), KCN (inhibitor of terminal oxidases, CtaI and Cyd) or CCCP (an uncoupler), but not with DBMIB (inhibitor of electron flow to CtaI, not Cyd), which only partially inhibited glucose induction (Fig. 3). This partial inhibition was also observed when Cyd-defective cells were treated with DBMIB together with glucose (data not shown). These data imply that glucose induction of the four *crt* genes is mediated by electron flow through the respiratory electron-transport chain, because the full inhibition of respiratory electron-transport chains by KCN and HgCl_2 abolished the accumulation of gene products. But despite having opposite effects on the redox state of the PQ pools, both inhibitors, KCN and HgCl_2 , failed to show opposite effects upon the gene expression, indicating redox changes in the PQ pools cannot be the glucose signal for gene accumulation. Furthermore, the cytosolic redox state, as measured by the NAD(P)H/NADP⁺ ratio, is not a likely sensor for gene expression because the NAD(P)H/NADP⁺ ratio induced by glucose treatment hardly changed under uncoupled conditions. Instead, the glucose effect could be closely related to oxidative phosphorylation or cytosolic pH.

Oxidative Phosphorylation Is Not Involved in the Glucose Induction of Gene Expression—Under coupled conditions, ATP is produced through ATP synthase and there is an increase in pH in the cytosol. Because both cytosolic pH and cytosolic ATP/AMP levels are closely involved in the signaling cascades and translational activation in yeast cells (17), higher plants (22, 34) and the cyanobacterium *Synechococcus* (23), we hypothesized that either adenylate levels or cytosolic pH may

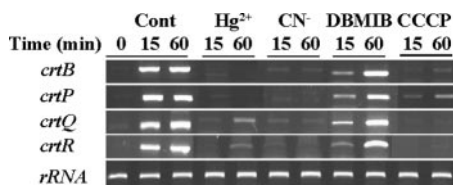


FIG. 3. Effects of various respiratory electron-transport inhibitors (Hg^{2+} , CN^- , and DBMIB) and an uncoupler (CCCP) upon the expression of carotenoid biosynthesis genes *crtB*, *crtP*, *crtQ*, and *crtR* in *Synechocystis* wild-type cells. Cells pre-adapted in the dark for 16 h were treated with 10 μM of HgCl_2 , 0.1 mM KCN, 10 μM DBMIB, or 50 μM CCCP for 15 or 60 min in the presence of 10 mM glucose in the dark.

contribute to regulation of *crt* gene expression. We measured ATP, ADP contents, and ATP/ADP ratio in cells treated with glucose with or without respiratory electron-transport inhibitors or an uncoupler. Glucose treatment for 15 min in the dark reduced both cellular ATP content and ATP/ADP ratio, but electron-transport inhibitors (HgCl_2 , KCN), and an uncoupler (CCCP) treatment inhibited this glucose effect (Fig. 4). When *crt* gene transcript abundance were compared with [ATP] and the ATP/ADP ratio, there was no clear linear correlation (data not shown). Therefore, we suggest that the ATP content or ATP/ADP ratio is not closely related to glucose induction of genes expression.

The Increase in Cytosolic pH Is Involved in the Glucose Induction of crt Gene Expression—To correlate changes in cytosolic pH with glucose-induced gene expression, we measured the kinetics of acridine yellow fluorescence, an indicator for the cytosolic pH of *Synechocystis* (27). When dark-adapted cells were treated with 10 mM glucose, acridine yellow fluorescence reached a transient peak about 8 min after a 2-min lag period and then remained relatively constant (Fig. 5A). This fluorescence change of acridine yellow was observed only in glucose-treated wild-type cells; it was not induced by other glucose analogs such as L-Glc, OMG, 2-dGlc, and Man, nor in glucose transporter- and glucokinase-defective cells (Fig. 5A).

When wild-type cells were exposed to the chemical inhibitions of either input of electrons to or output from the PQ pool by Hg^{2+} and CN^- treatment, respectively, or to an uncoupler (CCCP) ~10 min after glucose treatment, acridine yellow fluorescence decreased rapidly to that of dark-adapted cells, abolishing almost fully the glucose effect. However, inactivation of respiratory electron flow to Cyt *b₆f* complex resulted in an ~50% decrease in the steady-state level of acridine yellow fluorescence (Fig. 5B). These data strongly indicate that glucose can modulate cytosolic pH via respiratory electron transport at the expense of NAD(P)H produced from glucose metabolism.

The fluorescence yield of acridine yellow was investigated as a function of CCCP concentration. At 5 μM CCCP, acridine yellow fluorescence decreased to 25% of the control and then decreased further with increasing CCCP concentration (Fig. 6A). The dose-dependant inhibitory effect of CCCP also reduced expression of the four *crt* genes, with a 50% reduction in mRNA abundance at concentrations of about 15 μM (Fig. 6, B and C). When the gene expression levels were plotted against the acridine yellow fluorescence yields, expression of the four genes increased with increasing acridine yellow fluorescence, showing increasing saturation above 25% of acridine yellow fluorescence (Fig. 6D).

DISCUSSION

Light induction of carotenoid biosynthesis is a widespread phenomenon, but the light regulation mechanism of carotenoid

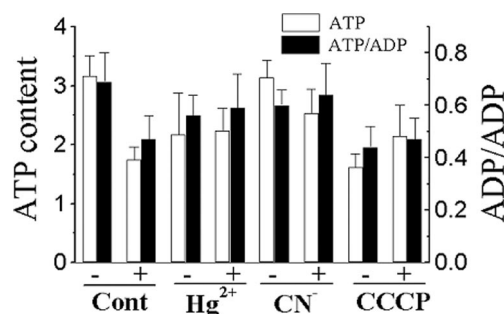


FIG. 4. Effects of respiratory electron-transport inhibitors (Hg^{2+} , CN^-) and an uncoupler (CCCP) on the ATP content (mol ATP/gChl) and ATP/ADP ratio in *Synechocystis* wild-type cells. Wild-type cells dark-adapted for 16 h were treated with 10 μM of HgCl_2 , 0.1 mM KCN, 10 μM DBMIB, or 50 μM CCCP for 15 min in the presence (+) or absence (–) of 10 mM glucose.

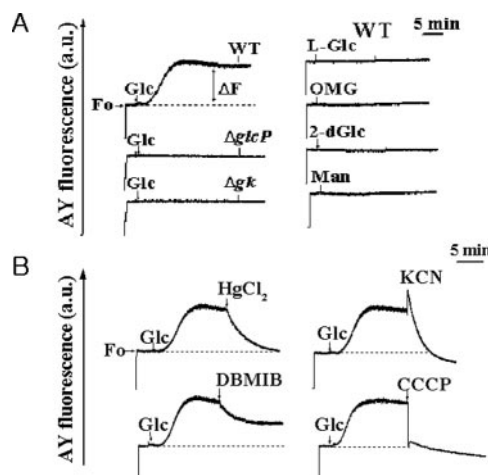


FIG. 5. A, changes in acridine yellow (AY) fluorescence in *Synechocystis* wild-type (WT), glucokinase-defective (Δgk) and glucose transporter-defective ($\Delta glcP$) cells in response to glucose (Glc) or its analogs: L-glucose (L-Glc), 3-O-methylglucose (OMG), 2-deoxyglucose (2-dGlc), and mannose (Man). B, effects of various respiratory electron-transport inhibitors (HgCl_2 , KCN, and DBMIB) and uncoupler (CCCP) on the glucose-induced acridine yellow (AY) fluorescence in *Synechocystis* wild-type cells. Concentration was 10 mM for glucose and its analogs, 10 μM for HgCl_2 and DBMIB, 50 μM for CCCP, and 0.1 mM for KCN. F_0 is the acridine yellow fluorescence yield before glucose treatment, and ΔF is the increase in the steady-state fluorescence yield induced by glucose treatment, respectively.

gene expression is not clear. Photosynthetic redox control (35) or control by red- and blue-light photoreceptor systems (4, 6) over carotenoid biosynthesis genes have been reported in green algae and higher plants. In *Synechocystis*, expression of both *crtB* and *crtP* genes is light-dependent, but little is known about light signaling (10). In the present study, in addition to *crtB* and *crtP* genes (10), we observed light induction of another two carotenogenesis genes, *crtQ* and *crtR*, in a dark-to-light shift (Fig. 1). Thus, all four carotenoid biosynthesis genes are light-regulated; this is atypical, as often only a few genes, such as *crtB*, are tightly regulated at the level of transcript abundance, with *crtQ* mRNA often showing little variation (3).

However, light is not the sole signal for the accumulation of *crt* gene mRNA because glucose alone in the dark results in a similar increase (Fig. 1). The glucose induction is apparently related to the transcriptional activity rather than to the stability of mRNAs as indicated by rifampicin treatment. Glucose induction in yeast, higher plants, and human pancreatic cells of a range of genes is mediated by a membrane-bound transporter homolog, a hexokinase, or extensive metabolism of phosphorylated glucose (18, 36). For *Synechocystis*, metabolism of phos-

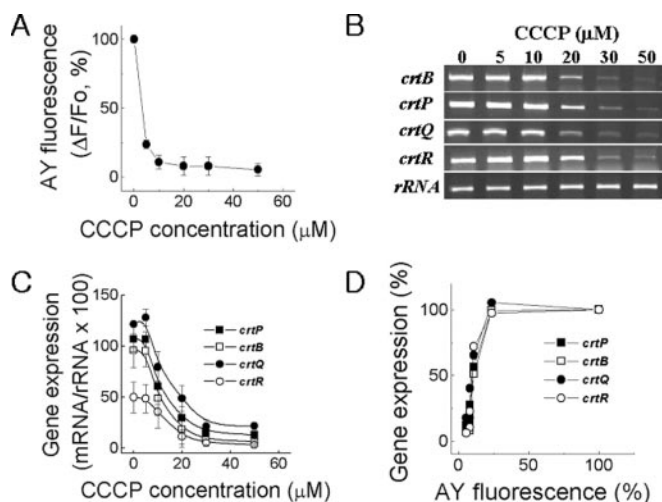


FIG. 6. Effects of various concentrations of CCCP (μM) on the acridine yellow (AY) fluorescence yield ($\Delta F/F_0$ = % of control) (A) and the expression of carotenoid biosynthesis genes *crtB*, *crtP*, *crtQ*, and *crtR* in wild-type *Synechocystis* cells (B). C, relative gene expression levels were plotted by comparison with rRNA expression level according to the increasing CCCP concentrations. The band intensities in B were quantified using Adobe Photoshop version 6.0. D, correlation between the expression levels of carotenoid biosynthesis genes (taken from C) and the acridine yellow (AY) fluorescence (taken from A), of which both reflect the RNA expression levels and AY fluorescence against variable CCCP concentrations. Cells adapted in the dark for 16 h were treated with various concentration of CCCP in the presence of 10 mM glucose for 15 min. In panel B, the relative value at each point was calculated using the intensity of each gene divided by that of rRNA. The mean $\pm$ S.E. was calculated in three independent experiments.

phorylated glucose is required for glucose induction of *crt* genes (Fig. 2): the glucose effect was not reproduced by glucose analogs that are non-transportable (L-Glc), transportable but not phosphorylated (3-OMG), or phosphorylated without further metabolism (2-dGlc and Man) (Fig. 2B). Further, the inactivation of a glucose transporter or glucokinase resulted in the loss of the glucose effect (Fig. 2A). These results rule out the possibility of either (i) a glucose transporter (or a homolog) or (ii) glucokinase of *Synechocystis* acting in a regulatory role in addition to its catalytic activity, as in higher plants and yeast (17). Instead, they suggest that metabolic activity downstream of glucose phosphorylation is required.

In this respect, cyanobacterial glucose-sensing is analogous to insulin secretion in pancreatic cells, where ATP generation with a concomitant decrease in ADP concentration by glycolytic and oxidative events from NADH represent a key transduction phenomenon (19, 20). If this is the case in cyanobacteria, the inhibition of respiratory electron flow or oxidative phosphorylation would diminish the glucose effect, because NAD(P)H produced by means of the oxidative pentose phosphate pathway and glycolysis donates electrons to the component of respiratory electron transport in cyanobacteria (PQ) (13) to drive ATP formation. Indeed, chemical inhibition of either inputs (Hg^{2+} treatment) or outputs (CN^- treatment) of electrons to the PQ pool or uncoupling of proton gradient (for CCCP treatment, see Ref. 37) fully diminished the glucose effect (Fig. 3). Partial blockage of the electron flow (to Cyt *c* oxidase) by DBMIB (27) inhibited the early induction (at 15 min) of the four genes by glucose (Fig. 3), which could have resulted from the partial blockage of proton pumping. In line with this view, genetic inhibition of electron input into the PQ pool (in NADPH dehydrogenase-defective cells) or output to CtaI- and Cyd-defective cells fully or partially inhibited the glucose induction of the four genes (data not shown). Further, the fact that uncoupling conditions, where oxidation of NAD(P)H still occurred

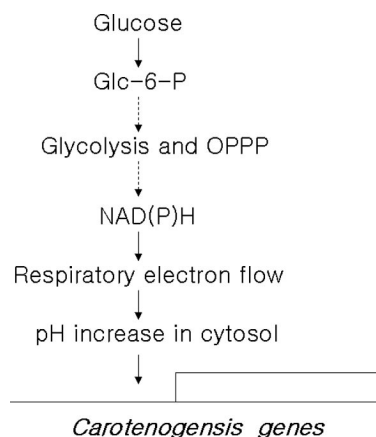


FIG. 7. A working model for glucose induction of carotenogenesis gene expression in *Synechocystis* sp. PCC 6803. Exogenously supplied glucose enters cells via a glucose transporter and is then phosphorylated by glucokinase. Phosphorylated glucose-6-phosphate was further metabolized into NAD(P)H by means of glycolysis and an oxidative pentose phosphate pathway. The reducing power of NADPH was oxidized by means of respiratory electron transport, resulting in the alkalization of the cytosol. Increase in cytosolic pH up-regulates carotenoid biosynthesis-related genes through an unknown mechanism.

without ATP generation, inhibited glucose induction (Fig. 3), strongly suggesting that both the redox state of the cytosol (indicated by the NAD(P)H/NA(D)P⁺ ratio) and of the PQ pool of photosynthetic membranes is not likely to be an important factor for glucose induction of *crt* genes. Moreover, the observation that changes in ATP content and the ATP/ADP ratio are not closely related to the extent of gene expression (data not shown) indicates that energy charge or adenylate status is not likely to be involved in the transcriptional activation of the four genes; this is different from the case of yeast cells, where selective gene expression induced by glucose is sensed via adenylate contents (17).

If cytosolic, membrane redox state, ATP content, or ATP/ADP ratio are not determining factors for gene expression, then alkalization of the cytosol could be a decisive factor, as respiratory electron transport can provide not only ATP but also an alkaline cytosolic environment for a variety of cellular processes. Indeed, glucose treatment in the dark induced alkalization of cytosol via respiratory electron transport; the alkalization was D-glucose-specific (Fig. 5A) and required further metabolism of glucose phosphorylation via respiratory electron transport (Fig. 5B). The extent of expression of the four genes showed a saturation curve with an increasing acridine yellow fluorescence yield (Fig. 6D). Although it is complicated to draw a simple relationship between fluorescence yield and intracellular pH because of unspecific indicator binding to DNA, proteins, and lipids (27), one may expect that a slight change of cytosolic pH is enough to saturate gene expression. It was suggested that illumination of cyanobacterial cells with saturating light increased cytosolic pH by about 0.5 units (27). Because the extent of the fluorescence increase by glucose treatment ($\Delta F/F_0 = 0.85$) was about half of that by saturating light intensity ($\Delta F/F_0 = 1.75$) in this experiment, glucose treatment could increase the cytosolic pH by about 0.25 units. Above 25% of acridine yellow fluorescence, the extent of gene expression was saturated (Fig. 6D). To confirm that the changes in the intracellular pH would affect the expression of *crt* genes, we had tried other uncouplers such as nigericin and NH_4Cl (37). In the presence of 10 mM KCl, nigericin indeed inhibited the alkalization of cytosol. But the concentration required to fully abolish the cytosolic pH increase to the same extent as that obtained by CCCP was over 1 mmol (data not shown). As this concentration is expected to be high enough to evoke some

harmful side effects, we did not follow up further. For the NH_4Cl , we did not use it either, as NH_4Cl treatment of the intact wild-type cells in the dark increased the cytosolic pH (contrary to our expectations; data not shown), probably because of its being utilized as a nitrogen source instead of acting as an uncoupler.

Based on our results, we propose a working model for the glucose induction of carotenoid biosynthesis genes in *Synechocystis* as shown in Fig. 7. When dark-adapted cells are exposed to exogenously supplied glucose, up-regulation of *crt* genes are induced. This glucose induction is mediated by respiratory electron flow coupled to cytosolic alkalization, instead of respiratory electron redox, cytosolic redox status, or ATP/ADP ratio. In animals, it has been known for quite some time that cytosolic pH changes are associated with changes in cellular metabolism and development, such as fertilization, spore germination, cell cycle, and insulin stimulation (21). In plants, cytosolic pH is known to act on a wide range of cellular enzymes, membrane transport, and regulatory agents (21). As a regulatory agent, ΔpH has been considered to be a synergistic messenger that acts as a master metabolic variable through which the actions of other effectors, such as calcium signaling in differentiation and cellular proliferation (21), are integrated. In this respect, cytosolic pH is established as a second messenger of ABA signaling in guard cells of higher plants (34). For the gene expression of chloroplasts, the proton gradient formed during electron transport is known to regulate translation elongation in chloroplasts (22). Thus, it is expected that the cytosolic pH of *Synechocystis* will activate gene transcription through either activation of transcriptional control proteins or the deactivation of transcriptional repressors. However, the precise mechanism by which cytosolic alkalization regulates the expression of carotenoid genes remains unclear.

In conclusion, we have demonstrated that expression of the four carotenoid biosynthesis genes, *crtB*, *crtP*, *crtQ*, and *crtR*, in *Synechocystis* is dependent upon exogenously supplied glucose. Glucose metabolism is involved in the glucose induction of these four genes, which is presumably sensed by pH changes in cytosol. Direct evidence for the dependence of the expression of carotenoid biosynthesis genes upon the cytosolic pH and the gene activation mechanisms remains to be determined.

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REFERENCES

- Frank, H. A., and Cogdell, R. J. (1996) *Photochem. Photobiol.* **63**, 257–264
- Largade, D., Beuf, L., and Vermaas, W. (2000) *Appl. Environ. Microbiol.* **66**, 64–72
- Cunningham, F. X., and Gantt, E. (1998) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49**, 557–583
- von Lintig, J., Welsch, R., Bonk, M., Giuliano, G., Batschauer, A., and Kleinig, H. (1997) *Plant J.* **12**, 625–634
- Welsch, R., Beyer, P., Hugueney, P., Kleinig, H., and von Lintig, J. (2000) *Planta* **211**, 846–854
- Bohne, F., and Linden, H. (2002) *Biochim. Biophys. Acta* **1579**, 26–34
- Mullineaux, C. W. (2001) *Mol. Microbiol.* **41**, 965–971
- Pfannschmidt, T. (2003) *Trends Plant Sci.* **8**, 33–41
- Reddy, K. J., Masamot, K., Sherman, D. M., and Sherman, L. A. (1989) *J. Bacteriol.* **171**, 3486–3493
- Fernandez-Gonzalez, B., Martinez-Ferez, I. M., and Vioque, A. (1998) *Biochim. Biophys. Acta* **1443**, 343–351
- Flores, E., and Schmetterer, G. (1986) *J. Bacteriol.* **166**, 693–696
- Schmetterer, G. R. (1990) *Plant Mol. Biol.* **14**, 697–706
- Schmetterer, G. R. (1994) in *The Molecular Biology of Cyanobacteria* (Bryant, D. A., ed) pp. 409–435, Kluwer, Dordrecht, The Netherlands
- Alfonso, M., Perewoska, I., and Kirilovsky, D. (2000) *Plant Physiol.* **122**, 505–516
- Li, H., and Sherman, L. A. (2000) *J. Bacteriol.* **182**, 4268–4277
- Kujat, S. L., and Owttrim, G. W. (2000) *Plant Physiol.* **124**, 703–713
- Rolland, F., Winderickx, J., and Thevelein, J. M. (2001) *Trends Biochem. Sci.* **26**, 310–317
- Rolland, F., Moore, B., and Sheen, J. (2002) *Plant Cell* **14**, S185–S205
- Salgado, A. P., Pereira, F. C., Seica, R. M., Fernandes, A. P., Platt, P. R., Santos, R. M., Rosario, L. M., and Ramasamy, R. (1999) *Mol. Cell. Endocrinol.* **154**, 79–88
- Isfort, R. J., Cody, D. B., Stuard, S. B., Ridder, G. M., and LeBoeuf, R. A. (1996) *Exp. Cell Res.* **226**, 363–371
- Felle, H. (1989) in *Second Messengers in Plant Growth and Development* (Boss, W. F., Morre, D. J., and Alan, R., eds) pp. 145–166, Liss Inc., New York
- Muhlbauer, S. K., and Eichacker, L. A. (1998) *J. Biol. Chem.* **273**, 20935–20940
- Schmitz, O., Tsinoremas, N. F., Schaefer, M. R., Anandan, S., and Golden, S. S. (1999) *Photosyn. Res.* **62**, 261–271
- Ryu, J. Y., Suh, K. H., Chung, Y.-H., Park, Y.-M., Chow, W. S., and Park, Y.-I. (2003) *Mol. Cells* **16**, 74–77
- Myers, J., Graham, J. R., and Wang, R. T. (1980) *Plant Physiol.* **66**, 1144–1149
- Porter, R. (1988) *Methods Enzymol.* **167**, 703–712
- Teuber, M., Rogner, M., and Berry, S. (2001) *Biochim. Biophys. Acta* **1506**, 31–46
- Gogarten, J. P., and Bentrup, F. W. (1989) *Planta* **178**, 52–60
- Joset, F., Buchou, T., Zhang, C. C., and Jeanjean, R. (1988) *Arch. Microbiol.* **149**, 417–421
- Klein, D., and Stitt, M. (1998) *Planta* **205**, 223–234
- Mi, H., Endo, T., Schreiber, U., Ogawa, T., and Asada, K. (1992) *Plant Cell Physiol.* **33**, 1233–1237
- Ryu, J. Y., Suh, K. H., Chung, Y.-H., Park, Y.-M., Chow, W. S., and Park, Y.-I. (2003) *Mol. Cells* **15**, 240–244
- Cooley, J. W., and Vermaas, W. F. J. (2001) *J. Bacteriol.* **183**, 4251–4258
- Giraudat, J. (1995) *Current Opin. Cell Biol.* **7**, 232–238
- Steinbrenner, J., and Linden, H. (2000) *Plant Physiol.* **125**, 810–817
- Moore, B., Zhou, L., Rolland, F., Hall, Q., Cheng, W.-H., Liu, Y.-X., Hwang, I. D., Jones, T., and Sheen, J. (2003) *Science* **300**, 332–336
- McCarty, R. E. (1980) *Methods Enzymol.* **69**, 719–730

Metabolism and Bioenergetics:
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Jee-Youn Ryu, Ji Young Song, Jeong Mi Lee,
Suk Won Jeong, Wah Soon Chow, Sang-Bong
Choi, Barry J. Pogson and Youn-Il Park
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