RESEARCH LETTER

Role of *mrgA* in peroxide and light stress in the cyanobacterium *Synechocystis* sp. PCC 6803

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Abstract

In the unicellular cyanobacterium *Synechocystis* sp. PCC 6803, the *mrgA* gene is part of the PerR regulon that is upregulated during peroxide stress. We determined that an Δ*mrgA* mutant was highly sensitive to low peroxide levels and that the mutant upregulated a gene cluster (*sll1722-26*) that encoded enzymes involved with exopolymeric substance (EPS) production. We made mutants in this EPS cluster in both a wild type and Δ*mrgA* background and studied the responses to oxidative stress by measuring cell damage with LIVE/DEAD stain. We show that *Synechocystis* sp. PCC 6803 becomes highly sensitive to oxidative stress when either *mrgA* or the *sll1722-26* EPS components are deleted. The results suggest that the deletion of the EPS cluster makes a cell highly susceptible to cell damage, under moderate oxidative stress conditions. Mutations in either *mrgA* or the EPS cluster also result in cells that are more light and peroxide sensitive, and produce significantly less EPS material than in wild type. In this study, we show that in the absence of MrgA, which is known to be involved in the storage or mobilization of iron, cells can be more easily damaged by exogenous oxidative and light stress.

Introduction

Iron is a critical nutrient for the growth of all life, but it is particularly important for photosynthetic organisms. Iron can have two oxidation states (II and III) and it is a key component in those reactions involved with redox cycling, such as respiration and photosynthesis. The more soluble Fe(II) reacts with O_2 to create reactive oxygen species that can damage DNA and proteins, thus inhibiting many physiological processes. This inhibition is particularly critical in photosynthetic organisms such as cyanobacteria where iron is required in photosystem I (PSI), which consumes up to 25% of the cellular iron (Keren *et al.*, 2004). Thus, it is of considerable importance for all organisms to inhibit the deleterious side effects of Fe (II) and to store the excess Fe (II) in a bioavailable form. It is for this reason that most organisms produce ferritin or ferritin-like proteins as Fe storage and detoxifying units.

Synechocystis sp. PCC 6803 (*Synechocystis* 6803) contains two types of ferritin storage complexes: two proteins that resemble bacterioferritin and a protein termed MrgA. The importance of the bacterioferritin proteins was shown by inactivation mutants in either of the two genes, which resulted in the loss of about 50% of the total cellular iron, as well as a reduction in the PSI content and induction of the Fe stress pathway even under Fe-sufficient conditions (Keren *et al.*, 2004). In *Synechocystis* 6803, there is another ferritin-like protein encoded by the gene *slr1894* (*mrgA*, metal regulated gene). MrgA is related to the Dps proteins that are members of the ferritin family, but which lack the fifth C-terminal helix that are present in other ferritins. We have adopted the MrgA designation because the gene was first identified as a peroxide-responsive gene in our study of peroxide sensitivity in *Synechocystis* 6803 (Li *et al.*, 2004). MrgA, as well as the repressor RerR, resembled proteins induced by the peroxide stress response in *Bacillus subtilis*, where MrgA protected against oxidative killing (Chen & Helmann, 1995; Chen *et al.*, 1995; Helmann *et al.*, 2003). The *mrgA* gene also has a strong relationship to *dpsA* in *Synechococcus* sp. PCC 7942 and a strain lacking *dpsA* was more sensitive to peroxide (Dwivedi *et al.*, 1997; Sen *et al.*, 2000).

In this present study, we are interested in understanding the role of MrgA in peroxide resistance in *Synechocystis* 6803. Previously, it has been shown that *mrgA* was induced by peroxide in differential transcription studies using a full-genome microarray (Li *et al*., 2004). In the presence of 1.5 mM hydrogen peroxide (H_2O_2), the *mrgA* gene was induced approximately twofold, and although it had a putative *perR* box, it was still upregulated by twofold in a Δ*perR* mutant (Li *et al*., 2004). However, a knockout mutant in *mrgA* was highly sensitive to peroxide and currently represents the most H_2O_2-sensitive strain of *Synechocystis* yet identified (Li *et al*., 2004). Previous studies have shown that in comparisons of Δ*mrgA* and wild type in the presence and absence of 75 μM H_2O_2, there are a number of significant transcriptional changes in the mutant (Singh *et al*., 2004). These differentially regulated genes included one major cluster (*sll1722-26*) that appeared to be involved with the biosynthesis of exopolysaccharides (e.g. glycosyltransferases) and several other proteins destined for the periplasm or the cell wall (Singh *et al*., 2004). Here, we examine the physiological alterations of mutants defective in Δ*mrgA* and *sll1722-24* (construct A) and *sll1722-26* (construct B) during oxidative and light stress, so that we can determine some of the characteristics that lead to peroxide sensitivity in this strain. Based on these results and DNA microarray studies (Singh *et al*., 2004), we propose that the production of exopolysaccharides in the *Synechocystis* 6803 acts as an extracellular protective mechanism to potential stress damage by agents such as exogenous H_2O_2 and light. Furthermore, we postulate that Δ*mrgA* cells are less sensitive to H_2O_2 damage as the culture approaches stationary phase than while it is growing exponentially. In this study, we test these predictions and discuss the potential cellular strategies for protection against oxidative stress.

Materials and methods

Growth conditions

Synechocystis sp. PCC 6803 (hereafter *Synechocystis* 6803) wild-type (WT) and Δ*mrgA* were grown at 30 °C in the chemically defined media BG-11 (Rippka *et al*., 1981). Cultures were inoculated with 1×10^8 cells mL^{-1} of media and shaken at 125 r.p.m. under three light regimes: low (30 μmol photons m^{-2} s^{-1}), medium (50 μmol photons m^{-2} s^{-1}), and high (100 μmol photons m^{-2} s^{-1}). All light treatments utilized cool white fluorescent lights (Phillips 30 W). Cell growth was monitored using a spectrophotometer at $A_{750\,nm}$ and direct cell counting using a Petroff–Hausser cell counter. Where appropriate, cells were also cultivated in BG-11 media with variable levels of iron. Cells were grown in 1× (20 μM Fe), 0.1× (2 μM Fe), 0.01× (0.2 μM Fe), or 0.001× (20 nM Fe) BG-11 media. All chemicals for reagents supplied by Sigma-Aldrich (St. Louis, MO) unless otherwise noted.

Construction of *Synechocystis* 6803 mutants

The cluster *sll1722-26* was PCR amplified using Taq Polymerase (Takara Bio, Shiga, Japan) using following primers (forward primer, ATGCACTCCATTCAATCACC and reverse primer, CTAATTTGCTTCCATCCCAAG). The amplified product was cloned in pGEM-T vector (Promega, Madison, WI). The *sll1722-24* genes were replaced by Sp^r cassette following digestion with AflII and blunting of the cohesive ends with T4 DNA polymerase (A construct). The *sll1722-26* genes were replaced by Sp^r cassette following digestion with AflII and blunting of the cohesive ends with T4 DNA polymerase (B construct). The resultant constructs are diagrammed in Fig. 1. Because the results with both A and B mutations in either the WT or the Δ*mrgA* background were very similar, we show the results for the WT-A and Δ*mrgA*-A mutants only.

Bacterial membrane integrity staining

To assess the effects of H_2O_2 and light stress on membrane permeability, *Synechocystis* 6803 WT, WT-A, Δ*mrgA* and Δ*mrgA*-A were stained with the LIVE/DEAD Bacterial Viability kit (Molecular Probes, Invitrogen, Carlsbad, CA) as described previously (Foster & Kolenbrander, 2004). Briefly, the viability kit visualizes the integrity of the plasma membrane and consists of two fluorescence markers: (1)

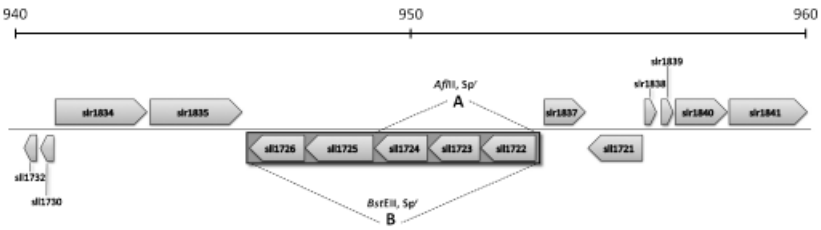

Fig. 1. Gene map and construction of WT and Δ*mrgA* mutants defective in genes *sll1722-26*. In mutants denoted with the letter A, the *sll1722–24* genes were replaced with a spectinomycin cassette (Sp^r) using the restriction enzyme AflII. Mutants with the entire gene segment of *sll1722–26* removed using the restriction enzyme BstEII are denoted with the letter B. In all cases, the deleted genes were replaced with a spectinomycin cassette (Sp^r). Because the results from both mutant strains were comparable, we only show results from the mutant designated A.

SYTO 9, a green stain that is indicative of intact membranes; and (2) propidium iodide (PI), a red stain that permeates cells that have undergone cell death or membrane damage. Cells undergoing peroxide treatment were cultivated under medium light levels (50 μmol photons m^{-2} s^{-1}) exposed to 2 mM H_2O_2 for 30 min and then stained for 5 min with a LIVE/DEAD solution containing 1.67 μM of SYTO 9 and 10 μM of PI. In those cells exposed to light stress, cells cultivated at medium light were incubated for 30 min at low (30 μmol photons m^{-2} s^{-1}) or high (100 μmol photons m^{-2} s^{-1}) light levels and stained for 5 min in the LIVE/DEAD solution. In both treatments, the stained cells were examined with an epifluorescence Zeiss Axioscope microscope (Carl Zeiss, Jena, Germany). Because cyanobacterial autofluorescence (650 nm), overlaps with the emission spectrum of PI, a 515-nm-long pass filter (Zeiss) was used to simultaneously visualize the emission spectra of SYTO 9 and PI, thereby removing autofluorescence.

Peroxide activity assay

To ensure the H_2O_2 remained active throughout the treatments and was not rapidly degraded by the *Synechocystis* 6803 cells, H_2O_2 levels in the surrounding growth medium were measured. WT and Δ*mrgA* cells that were in either logarithmic (3 days) or stationary (6 days) phase growth were incubated with 2 mM of H_2O_2 for 0, 30, 60, 120, and 240 min under high- and low-light conditions. After treatment, the cells were removed from the media via centrifugation and the media was assayed for peroxide activity using the Amplex Red Peroxide Assay Kit (Invitrogen) according to the manufacturer's instructions. The fluorescence emission of the samples was measured at 590 nm using a Synergy HT microplate reader (BioTek, Winooski, VT). The readings were correlated to a standard curve containing known concentrations of H_2O_2.

Changes in cell diameter during growth cycle

Cultures of *Synechocystis* 6803 WT, WT-A, and Δ*mrgA*, and Δ*mrgA-A* were monitored for changes in cell diameter during growth at the exponential and stationary phase growth. Cells diameters were measured and compared at logarithmic growth phase (3 days) and in stationary growth phase (6 days). The cell diameters (μm^2) were measured using the Zeiss MicroImaging software package (Carl Zeiss). For each strain, the diameters of 10 cells from three replicate cultures were measured and statistically compared (MiniTab, State College, PA).

Exopolymeric substance (EPS) extraction and quantification

To examine the effect of light stress on EPS production, the EPS material was isolated and quantified from WT, WT-A, Δ*mrgA*, and Δ*mrgA*-A mutants. The EPS extraction was performed in triplicate using a modified procedure of Kawaguchi & Decho (2000). Cultures were grown to stationary phase for 6 days under either low (30 μmol photons m^{-2} s^{-1}) or high (100 μmol photons m^{-2} s^{-1}) light and centrifuged to pellet the cells. Pellets were then vortexed for 30 min in a 1.5% NaCl solution then recentrifuged. Subsequently, the supernatant was removed and heated at 80 °C for 15 min, and then briefly centrifuged to remove any remaining cells. To precipitate the EPS, four volumes of 95% ethanol was added and placed at − 80 °C for 1 h. The EPS material was collected by centrifugation, washed once with 95% ethanol, and then allowed to air dry. The dry pellet was then resuspended in sterile water and the carbohydrate content evaluated by the anthrone quantification method (Laurentin & Edwards, 2003). EPS material from each extraction (40 μL) was added to an untreated 96-well microtiter plate and chilled for 10 min. A solution (100 μL) of 10.3 mM (2 mg mL^{-1}) of anthrone solubilized in sulfuric acid was added to each well. The plate was then sealed and briefly vortexed, then heated for 3 min at 90 °C. After heating, the plate was cooled for 5 min at room temperature and incubated at 45 °C for 15 min. The absorbance of each well was measured using a Synergy HT microtiter plate reader (BioTek) at 630 nm. The absorption of the EPS material was compared with a glucose standard curve. The glucose standard curve ranged from 0 to 400 mg mL^{-1} at 50 mg mL^{-1} increments.

Results and discussion

Changes in membrane integrity to peroxide stress at different growth phases

Growth of the four strains (WT, WT-A, Δ*mrgA*, and Δ*mrgA*-A) was examined under a variety of conditions, including different light intensities and in media with 0.1, 0.01, and 0.001 of the normal levels of iron. The strains displayed comparable growth rates in regular BG-11 at low light intensities, but the WT-A mutant had slower growth rates when the light intensity was increased to 100 μmol photons m^{-2} s^{-1}. In addition, under Fe-deficient conditions, WT-A grew at only 30–40% the WT level, even at 0.1 × Fe. Δ*mrgA* (and Δ*mrgA*-A) responded as described previously (Shcolnick *et al.*, 2007) and grew poorly under iron deficiency, also growing to about 30% of the level seen in normal BG-11 medium (data not shown).

To examine the sensitivity of WT and Δ*mrgA* to oxidative stress throughout the growth cycle, *Synechocystis* 6803 cells were grown at 50 μmol photons m^{-2} s^{-1} and exposed to H_2O_2 during logarithmic (3 days) and stationary (6 days) phase for 30 min. To visualize the effects of H_2O_2 on *Synechocystis* 6803, cells were stained with the membrane permeability stain LIVE/DEAD Viability kit (Fig. 2). Although originally intended to assess viability of target cells, evidence suggests that PI stained cells are not always dead and can recover from the imposed

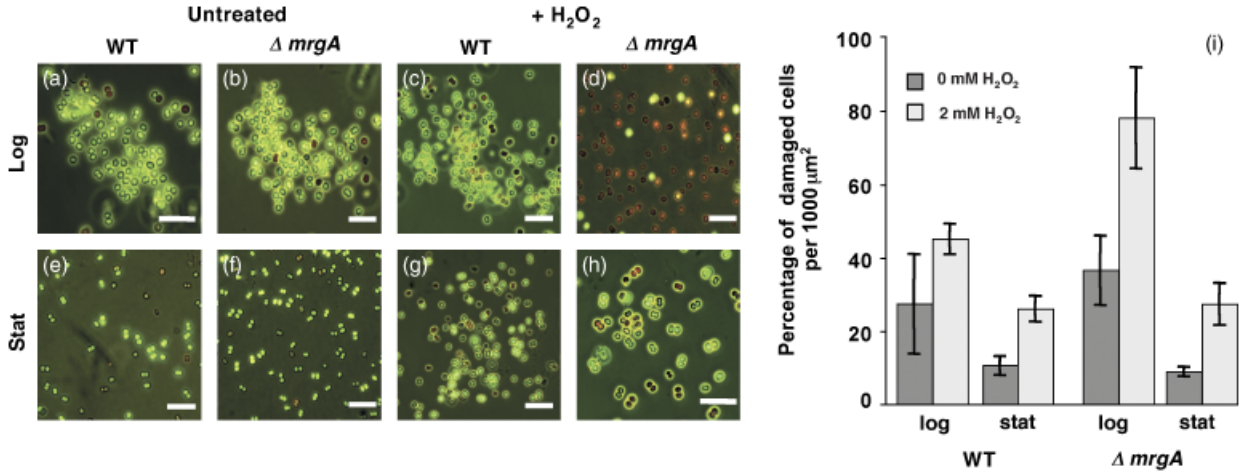

Fig. 2. *Synechocystis* 6803 cells exposed to peroxide stress throughout the growth cycle. (a, b) Epifluorescent micrograph of untreated wild type (WT) and Δ*mrgA* cells. (c) WT cells exposed to 2 mM H_2O_2. (d) Mutants defective in *mrgA* exposed to 2 mM H_2O_2 in logarithmic growth. (e, f) Micrographs depicting WT and Δ*mrgA* cells in stationary phase (6 days). (g, h) Stationary phase WT and Δ*mrgA* cells treated with 2 mM H_2O_2. Scale bar = 10 μm. (i) Graphical representation of epifluorescent micrographs depicting the increased levels of membrane damage and cell death during logarithmic (log) and stationary (stat) growth in wild type (WT) and Δ*mrgA* mutants under peroxide stress. Bars represent the SD within the ten replicate cultures examined for each strain.

treatment (Foster *et al.*, 2003). Therefore, the staining method is often used to detect cell damage where the membrane integrity has been compromised (Hentzer *et al.*, 2001; Alonso *et al.*, 2002; Foster *et al.*, 2003), thereby allowing the PI stain to penetrate the cells even under oxidative stress conditions (Ramachandran *et al.*, 2007). In untreated cells, both the parent and the mutant exhibited a twofold higher background level of cell damage in logarithmic growth than in stationary phase growth. The mean percentage of damaged cells per 1000 μm^2 was 27% (± 13.6) in WT and 36% (± 9.5) in Δ*mrgA* cultures (Fig. 2a, b and i), whereas in stationary phase, the mean percentage of dying or damaged cells was 11% (± 3.2) in WT and 8.7% (± 1.4) in Δ*mrgA* (Fig. 2e, f and i). Although differences were detected in the two growth phases in untreated cells, there was no statistical difference in the number of dead or damaged cells between the parent WT and mutant Δ*mrgA* strains during logarithmic ($P < 0.25$) or stationary ($P < 0.15$) phase growth (Fig. 2).

In cultures treated with 2 mM H_2O_2, there were significant differences between the two strains in the number of PI-stained cells during logarithmic phase growth. In peroxide-treated WT cultures, an average of 45% of the cells exhibited signs of cell membrane damage or death, whereas 77% of the Δ*mrgA* treated exhibited signs of cell death or damage (Fig. 2c, d and i). Although significant differences between the parent and mutant strain were detected at logarithmic growth ($P < 0.01$), these differences did not persist into stationary phase growth. The average number of peroxide-sensitive cells significantly decreased during stationary phase in both the WT (26%) and Δ*mrgA* (27%) cells and were not significantly different from each other ($P < 0.74$; Fig. 2g, h and i). The staining results indicated that, during logarithmic growth, mutants defective in *mrgA* were more susceptible to peroxide stress than in stationary phase growth. To ensure that these results were not due to H_2O_2 degradation, H_2O_2 activity was measured in the surrounding growth media and shown to be active in all treatments and conditions after the 30-min exposure (data not shown). Even after 240 min of peroxide exposure to Δ*mrgA* cells, there was no degradation of peroxide in logarithmic or stationary phase in high or low light. In WT cells at stationary phase, however, peroxide degradation was detected after 60 min only in low light. In high light, WT cells were unable to degrade H_2O_2 even after 240 min regardless of growth phase (data not shown). These results are consistent with the hypothesis that Δ*mrgA* is more easily damaged than the WT in the logarithmic growth phase and that this stress is relieved as the culture proceeds toward stationary phase growth. These results also strongly correlate to our previous study in which there was an upregulation of the PerR regulon, including *mrgA*, under peroxide stress (Li *et al.*, 2004).

Loss of *mrgA* and *sll1722-26* causes increased sensitivity to oxidative and light stress

In our DNA microarray analyses of *Synechocystis* 6803 cells exposed to low levels of peroxide, we discovered that the gene cluster was *sll1722-26* was upregulated in Δ*mrgA* cells under both treated and untreated conditions (Singh *et al.*, 2004). Genes within this cluster exhibited homology to genes associated with the biosynthesis of exopolysaccharides and proteins designated for the periplasm or cell wall. Mutants defective in these genes (WT-A, B and Δ*mrgA*-A, B)

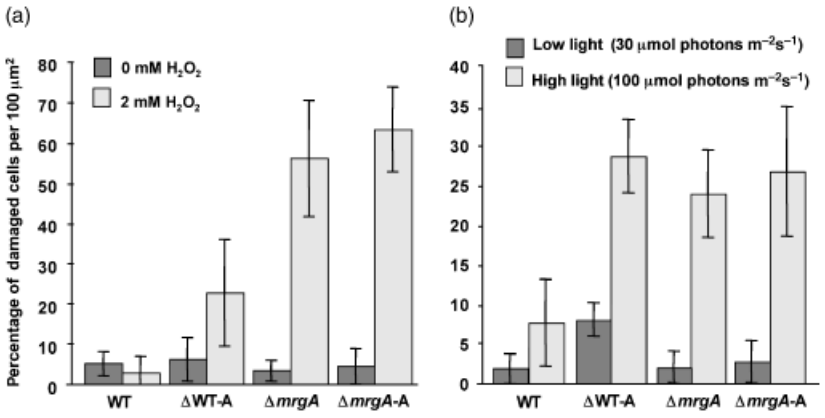

Fig. 3. Effects of peroxide and high light stress on *Synechocystis* 6803 WT and Δ*mrgA* mutants defective in *sll1722–24* (ΔWT-A and Δ*mrgA-A*) using the LIVE/DEAD fluorescent staining kit. (a) Cells exposed to 2 mM H_2O_2 at 50 μmol photons m^{-2} s^{-1}. (b) Cells exposed to low (30 μmol photons m^{-2} s^{-1}) and high (100 μmol photons m^{-2} s^{-1}) light for 30 min. Bars represent the SD of the 10 representative cultures examined.

were generated and tested under different oxidative stress conditions. Results from both A and B mutants were comparable and only mutant A (*sll1722-24*) is being shown. The results in Fig. 3a demonstrated that both Δ*mrgA* and Δ*mrgA-A* were highly sensitive to 2 mM H_2O_2 and that *c.* 60% of cells was damaged after only a 30-min exposure. The two strains showed similar sensitivity to this stress. On the other hand, the control WT cells had very few damaged cells, whereas WT-A showed a significant increase, up to 25% damaged cells. The WT-A strain manifested greater variability under all stress conditions, suggesting that they are more easily damaged than WT even under modest stresses.

Based on these results, cells were grown to stationary phase (6 days) and then exposed to low- (30 μmol photons m^{-2} s^{-1}) and high-light (100 μmol photons m^{-2} s^{-1}) conditions. LIVE/DEAD fluorescent staining indicated that, under low-light conditions, all tested strains had low background levels of damaged or dying cells (Fig. 3b). Of the four tested strains, only WT-A had a significantly higher number of damaged or dying cells compared with its parent strain WT ($P < 0.0001$) in low light. Strains Δ*mrgA* and Δ*mrgA*-A showed no difference in the numbers of dead or damaged cells ($P < 0.18$). However, under high light, all three of the mutant strains had significantly more damaged cells than the WT parent strain after 30 min. In WT cultures, only 8% of the cells exhibited signs of cell damage. This was significantly lower than 29% of the WT-A ($P < 0.0001$), 24% of the Δ*mrgA* ($P < 0.0001$), and 27% of the Δ*mrgA*-A cells ($P < 0.0001$). These results clearly indicate that mutants defective in *mrgA* and *sll1722-26* have a decreased tolerance to high light stress. The results also suggest that the deletion of the *sll1722-26* cluster makes the cells highly sensitive to cell damage, even under moderate oxidative stress conditions, such as 100 μmol photons m^{-2} s^{-1} of light. This cluster is an important upregulated gene clusters in the Δ*mrgA* strain and may act, in conjunction with other gene products, to protect the cell. The WT-A strain did not have the benefit of additional differential gene transcription that occurs after the deletion of Δ*mrgA* (Singh & Sherman, 2007) and became hypersensitive to both peroxide and the higher light used for growth. We conclude that *sll1722-26* is an important gene cluster for maintaining cellular integrity. High light may trigger an increase in the Fenton reactions in cells that lack MrgA, thus increasing the extent of oxidative damage in the cell membranes.

Increase in cell diameter in mutants defective in *mrgA* and *sll1722-26*

Mutants defective in *mrgA* and *sll1722-26* were compared with WT to determine if there were any growth or morphological phenotypes. Growth curves for WT and all mutant strains showed no statistically significant differences in growth rate (data not shown). However, the size properties of the WT and the mutants differed in three important ways. First, the cell diameter of the WT (Fig. 4a), Δ*mrgA* (Fig. 4b) and *sll1722-26* mutants were quite different from each other. When comparing strains in the logarithmic growth phase, the cell diameter of WT was smaller than all of the other tested strains (Fig. 4c, $P < 0.001$). Second, there was a significant increase in the cell diameter of each *Synechocystis* 6803 strain between logarithmic (3 days) and stationary phase growth (6 days; $P < 0.002$) in all tested strains. Moreover, Δ*mrgA* was the strain that became the largest after 6 days of growth (although it was also the most variable). Third, there were no statistical differences between the WT, WT-A, and WT-B strains in the stationary phase and WT cells were the smallest after 6 days of growth ($P < 0.002$). These results suggest that mutants defective in the EPS cluster increase in cell size during logarithmic growth when compared with the normal WT cells and that cells that are also defective in Δ*mrgA* become even larger, especially after long-term growth.

Production of EPS under low and high light

The levels of extracellular EPS were compared in the WT, WT-A, and Δ*mrgA* mutant strains cultivated under low and

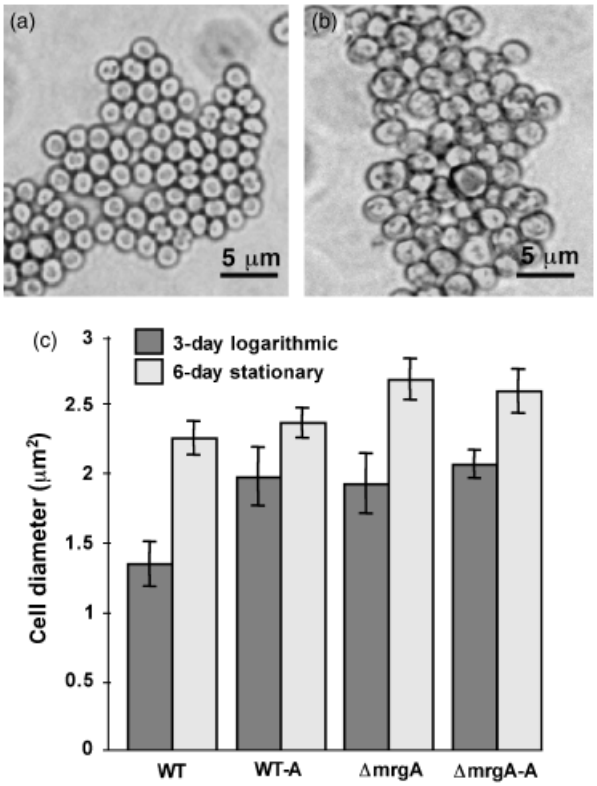

Fig. 4. Changes in cell size of *Synechocystis* 6803 wild type (WT) and Δ*mrgA* mutants throughout the growth cycle. Light micrographs of (a) WT and (b) Δ*mrgA* cells in logarithmic growth. (c) Graphical representation of the differences in cell diameter in WT, Δ*mrgA*, and mutants defective in the genes *sll1722-24* (WT-A and Δ*mrgA-A*) in logarithmic and stationary-phase growth. Error bars reflect the SD within the 10 replicate samples.

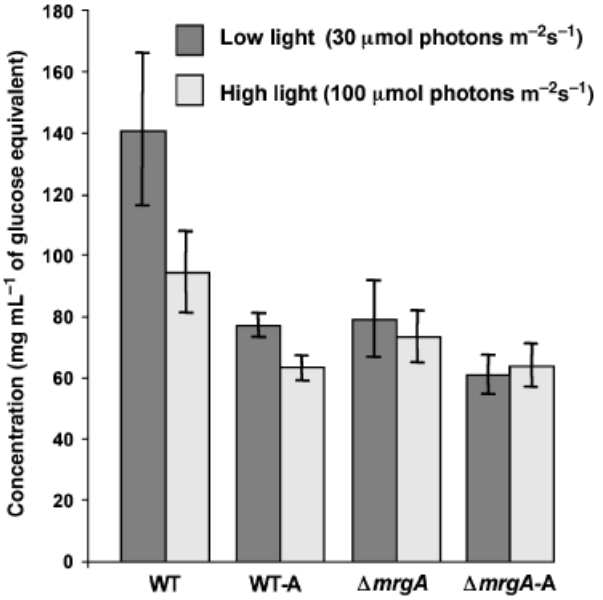

Fig. 5. Levels of extracellular EPS detected in cultures of *Synechocystis* 6803 wild type (WT), Δ*mrgA*, and mutants defective in the *sll1722-1724* (ΔWT-A and Δ*mrgA-A*) grown for 1 week under low- and high-light growth conditions. The EPS was extracted from each culture as described in the Materials and methods and then compared with a glucose standard curve. Bars represent the SD in the 10 replicate extractions of EPS material from each treatment.

high light levels. Anthrone colorimetric staining revealed that in WT cells there was a twofold higher level of extracellular EPS compared with mutants WT-A, Δ*mrgA*, and Δ*mrgA-A* at low light levels ($P < 0.0001$). At high light levels, the EPS production was less in WT cells; however, it still was significantly higher than in the other tested mutants ($P < 0.0001$). When WT-A was cultivated under high light there was less extracellular EPS material than in the WT-A cultures grown under low light ($P < 0.01$). In all the Δ*mrgA* mutants, there was no statistical difference between the strains when grown at low or high light levels ($P < 0.30$). These results demonstrated the importance of these EPS components for the WT strain and the drop in EPS concentration was consistent with the sensitivity of the WT-A strain to oxidative stresses. The levels of EPS in WT vs. the other three strains was also consistent with the results in Figs 3 and 4 and suggest the importance of this EPS for maintaining cell size, integrity, and resistance to oxidative stress. It is evident that the enzymes encoded by the *sll1722-26* cluster are only responsible for less than half of the extracellular EPS (Fig. 5) and that the Δ*mrgA* is already deficient in this other component. Other EPS genes known to have homologues in *Synechocystis* 6803 (e.g. *rfb* cluster; Eisenhut *et al.*, 2007) will need to be examined in detail to generate a comprehensive understanding of the role of EPS in the *Synechocystis* 6803 oxidative stress response.

In conclusion, our results indicate that *Synechocystis* sp. PCC 6803 becomes highly sensitive to oxidative stress when either *mrgA* or the EPS components produced by *sll1722-26* are deleted. Both the MrgA and the EPS material could be serving as protective mechanisms in *Synechocystis* 6803 against light and oxidative stress. EPS has been has been shown to scatter and absorb solar radiation (Elasri & Miller, 1999; Decho *et al.*, 2003), scavenge reactive oxygen species from the surrounding environment (Bylund *et al.*, 2006), and alter the structural integrity or surface electrical charge of microbial community structure to reduce oxidative stress (Wai *et al.*, 1998; Chen *et al.*, 2004). The increased sensitivity of Δ*mrgA* mutants may be a direct result of the inability of the cell to mobilize or utilize iron. Previous studies have shown that MrgA is required to access and mobilize the stored Fe within the cells (Shcolnick *et al.*, 2007). Further studies will be necessary to characterize EPS material and the mechanisms by which it may work synergistically with MrgA as a protectant against oxidative stress.

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