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Light influences *Synechococcus* response to oxidative stress

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Light history influences the response of the marine cyanobacterium
*Synechococcus* sp. WH7803 to oxidative stress

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Abstract

Marine *Synechococcus* undergo a wide range of environmental stressors, especially high and variable irradiance, which may induce oxidative stress through the generation of reactive oxygen species (ROS). While light and ROS could act synergistically on the impairment of photosynthesis, inducing photodamage and inhibiting photosystem II repair, acclimation to high irradiance is also thought to confer resistance to other stressors. To identify the respective roles of light and ROS in the photoinhibition process and detect a possible light-driven tolerance to oxidative stress, we compared the photophysiological and transcriptomic responses of *Synechococcus* sp. WH7803 acclimated to low (LL) or high light (HL) to oxidative stress, induced by hydrogen peroxide (H$_2$O$_2$) or methylviologen. While photosynthetic activity was much more affected in HL than in LL cells, only HL cells were able to recover growth and photosynthesis after addition of 25 µM H$_2$O$_2$. Depending upon light conditions and H$_2$O$_2$ concentration, the latter oxidizing agent induced photosystem II inactivation through both direct damages to the reaction centres and inhibition of its repair cycle. Although the global transcriptome response appeared similar in LL and HL cells, some processes were specifically induced in HL cells that seemingly helped them withstand oxidative stress, including enhancement of photoprotection and ROS detoxification, repair of ROS-driven damages and regulation of redox state. Detection of putative LexA binding sites allowed the identification of the putative LexA regulon, which was down-regulated in HL compared to LL cells, but up-regulated by oxidative stress under both growth irradiances.
Marine cyanobacteria must cope with a wide range of fluctuating environmental factors which, by affecting the cellular redox status, may lead to oxidative stress (Asada, 1994; Hirayama et al., 1995; Latifi et al., 2005; Schwarz and Forchhammer, 2005; Takahashi and Murata, 2008; Latifi et al., 2009). For instance, absorption of solar radiations, especially ultraviolet (UV) wavelengths, by dissolved organic matter in aquatic environments leads to the photochemical production of various reactive transients, including reactive oxygen species (ROS ; Mopper and Kieber, 2000). These compounds are partially reduced forms of oxygen, between atmospheric oxygen ($O_2$) and its fully reduced form $H_2O$, and constitute powerful oxidizing agents. Environmental stress usually induces an increase of ROS intracellular production in photosynthetic organisms, particularly when photosynthetic electron transport outpaces the rate of electron consumption during CO$_2$ fixation (Knox and Dodge, 1985; Latifi et al., 2009; Rastogi et al., 2010). This may occur when cells are exposed to excess light or UV radiations but also under nutrient and salt stress, which induce a slowing down of cell metabolism. For instance, Latifi et al. (2005) have shown that cells of _Anabaena_ sp. PCC 7120 subjected to iron limitation exhibited a 100-fold increase in the amount of ROS compared to replete cells. Similarly, high salinity induces the release of $H_2O_2$ from estuarine _Microcystis aeruginosa_ cells (Ross et al., 2006). Thus, as environmental stress is intimately linked to oxidative stress, disentangling the direct effects of environmental stressors from indirect effects of stress-induced ROS formation is not an easy task.

In cyanobacteria and other oxygenic phototrophs, the major source of ROS is the photosynthetic apparatus, although respiratory machinery also contributes to the generation of ROS compounds (Asada, 1999; Mittler, 2002; Ledford and Niyogi, 2005; Nishiyama et al., 2006; Latifi et al., 2009). Within photosystem I (PSI), transfer of electrons from reduced Fe-S centres to $O_2$ leads to the formation of superoxide radicals ($O_2^-$) that undergo further dismutation into hydrogen peroxide ($H_2O_2$) and hydroxyl radicals (OH$^-$; (Asada, 1999). Photosystem II (PSII) also contributes to ROS formation: at PSII acceptor side, excessive reduction of electron acceptors, particularly during limitation of electron transport between PSII and PSI, may result in reduction of molecular oxygen into $O_2^-$, which subsequently results in the formation of $H_2O_2$ and OH$^-$. At PSII donor side, incomplete oxidation of $H_2O$ at the water-splitting manganese complex induces the formation of $H_2O_2$, which can be further oxidized into $O_2^-$ by manganese complex radicals or reduced into OH$^-$ by free metals released by damaged PSII (Chen et al., 1992; Vass et al., 1992; Keren et al., 1997; Pospísil, 2009). Energy transfer from photoexcited pigments to $O_2$ in the light-harvesting antenna also leads to the formation of singlet oxygen ($^1O_2$; Knox and Dodge, 1985; Zolla and Rinalducci, 2002).

Excess ROS may severely affect the cell by damaging proteins, cleaving nucleic acids, peroxidizing unsaturated fatty acids, and inhibiting protein synthesis; this can ultimately lead to
growth inhibition and cell death (Asada, 1999; Storz and Imlay, 1999; Ledford and Niyogi, 2005; Nishiyama et al., 2006). In particular, ROS have been reported to induce serious deleterious effects on phytoplankton by affecting cell membranes and photosynthesis (Lesser, 2006). Thus, the photosynthetic machinery is not only a major source of ROS but also constitutes a major target for these compounds. ROS are indeed thought to be involved in PSII photoinactivation, a process that has been intensively studied in freshwater cyanobacteria (Tichy and Vermaas, 1999; Nishiyama et al., 2001; Nishiyama et al., 2004; Nishiyama et al., 2006; Song et al., 2006; Kanesaki et al., 2007; Murata et al., 2007). Although PSII photoinactivation continually occurs under regular and stable growth conditions, it may increase under environmental stress conditions that induce an increase in the rate of ROS formation (Long et al., 1994; Hirayama et al., 1995; Asada, 1999; Latifi et al., 2005; Nishiyama et al., 2006; Takahashi and Murata, 2008). Furthermore, photoinhibition induced by ROS seems to be higher in cyanobacteria than in other phytoplanktonic organisms (Drabkova et al., 2007).

PSII photoinactivation is thought to occur primarily at the level of the oxygen-evolving complex, immediately followed by the destruction of the PSII core protein D1 (Hakala et al., 2005; Nishiyama et al., 2006). To maintain photosynthesis, photoinactivation is countered by a rapid repair cycle that includes the proteolytic removal of photodamaged D1 protein, the coordinated high rate of de novo D1 protein synthesis and its insertion into the PSII (Park et al., 1995; Tyystjarvi and Aro, 1996; Nixon et al., 2005; Ohnishi et al., 2005; Nishiyama et al., 2006). Thus, cells must modulate PSII repair cycle activity to avoid outrun of repair by PSII photoinactivation, which would result in net photoinhibition of photosynthesis (Aro et al., 2005; Six et al., 2008). The role of ROS in PSII photoinactivation has been much debated over the past 15 years and remains controversial (Adir et al., 2003; Edelman and Mattoo, 2008; Latifi et al., 2009; Nixon et al., 2010). ROS have been suggested to be involved in the direct inactivation of PSII reaction centre through direct D1 degradation and by increasing D1 susceptibility to proteases (Vass et al., 1992; Aro et al., 1993; Miyao et al., 1995; Okada et al., 1996; Keren et al., 1997; Lupinkova and Komenda, 2004; Hideg et al., 2007). Another nonexclusive hypothesis proposed that ROS primarily affect PSII by inhibiting the synthesis of D1 and therefore impairing the repair cycle (Nishiyama et al., 2004; Nishiyama et al., 2006; Takahashi and Murata, 2008). Strengthening this idea, Kojima et al. (2007) have shown that the translation elongation factor EF-G is a primary target of H2O2, thus suggesting that the synthesis of the D1 protein may be impaired at the elongation step of the protein translation machinery (Nishiyama et al., 2001; Nishiyama et al., 2004; Nishiyama et al., 2006).

Taken together, these data show the close interactions between light and ROS in photosynthetic organisms. On the one hand, the photosynthetic machinery constitutes a major source of ROS, with ROS generation particularly promoted when light absorption and the
concomitant electron transport exceeds consumption of photochemically generated reductants. On the other hand, light and ROS seem to act synergistically in PSII photoinhibition, either through direct damage to PSII and/or by inhibiting repair. The influence of growth irradiance on the sensitivity to reactive oxygen stressors remains unclear. In particular, it is not well understood whether low light- (LL-) and high light- (HL-) acclimated cells, which show very different photophysologies (Kana and Glibert, 1987a, b; Kana et al., 1988; Moore et al., 1995; Six et al., 2004, Six, et al., 2005) and different tolerance to stress factors (MacDonald et al., 2003; Bouchard et al., 2005; Garczarek et al., 2008), are equally able to withstand oxidative stress. Cells acclimated to LL have a slow electron transport rate, presumably low rates of primary ROS generation, but would not be expected to maintain full induction of ROS detoxification paths. Cells acclimated to high light have a faster electron transport rate, and presumably higher rates of primary ROS generation, but are in parallel expected to maintain stronger induction of ROS detoxification paths. The net cellular response to additional ROS therefore depends upon the relative magnitudes of the underlying effects of primary cellular ROS generation and the counteracting effects of ROS detoxification.

In order to address this question, we used *Synechococcus* sp. WH7803, a physiologically well characterized marine cyanobacterial isolate (see e.g. Kana and Glibert, 1987a, b; Toledo et al., 1999; Lindell and Post, 2001; Garczarek et al., 2008; Jia et al., 2010) for which the complete genome sequence (Dufresne et al., 2008) and a whole-genome microarray are available. We monitored PSII photophysiology and the global transcriptome response of LL- and HL-acclimated cells of this strain upon exposure to various concentrations of a direct reactive oxygen stressor, hydrogen peroxide (H$_2$O$_2$), and a cellular-dependent O$_2^-$ producing agent, methylviologen (MV).

RESULTS

**Effect of H$_2$O$_2$ and MV on PSII function of LL and HL cells**

*Synechococcus* sp. WH7803 cells acclimated to low light (18 μmol photons m$^{-2}$ s$^{-1}$, hereafter LL cells) and high light (250 μmol photons m$^{-2}$ s$^{-1}$, hereafter HL cells) are clearly in different initial states. In particular, the initial PSII activity, as indicated by the F$_{V}$/F$_{M}$ values before the application of any treatment, is lower in HL-acclimated cells than in LL-acclimated ones (Fig. 1). Incubation of LL- and HL-acclimated *Synechococcus* in the presence of different concentrations of H$_2$O$_2$ and MV for 2 h under their respective growth light irradiances indicated that the higher the concentration of oxidizing agent, the stronger the decrease of PSII activity (F$_{V}$/F$_{M}$, Fig. 1). LL and HL cells however showed quite distinct photoinactivation patterns in response to oxidizing stress. For LL-acclimated
cells, increasing concentrations up to 1,250 µM H₂O₂ induced a progressive PSII photoinactivation, but PSII of HL-grown cells were already completely inactivated at 250 µM H₂O₂ (Fig. 1A). This suggests a synergistic effect of light and H₂O₂ on PSII photoinactivation. Concentrations of MV higher than ca. 25 µM led to a stable PSII photoinactivation rate within 2 h, suggesting that 25 µM MV is a saturating concentration, with no additional ROS production above this level in both LL and HL cells (Fig. 1B). The saturating level of 25 µM MV induced a stress equivalent to exogenous application of about 125 µM H₂O₂, as at these concentrations both stressors induced a similar decrease of PSII quantum yield of about 20% for LL cells and 90% for HL cells.

Following this first experiment, two H₂O₂ concentrations inducing a comparable 50% PSII photoinactivation after 2 h were selected; 750 µM H₂O₂ for LL and 25 µM H₂O₂ for HL cells (Fig. 1A) to monitor the effect of oxidative stress on PSII activity over 3 days (Fig. 2A). For MV, since the saturating level was reached quickly and this oxidizing agent induced a very different photoinhibition level depending on the growth irradiance at which the culture was acclimated, comparable photoinactivation levels in LL and HL cells could not be reached within 2 h. Thus, two concentrations of MV, a sub-saturating one (1 µM) and a saturating one (50 µM), were chosen to monitor their kinetic effect on PSII inactivation (Fig. 2B). As expected from the previous experiment, HL cells, under their growth irradiance, exhibited much faster and stronger photoinhibition than did LL cells in response to both H₂O₂ and MV. At both MV concentrations tested, HL cells reached full PSII inactivation about 2.5 h earlier than LL cells. However, all cultures submitted to MV eventually died, independently of their growth irradiance. This is probably due to the inability of cells to get rid of MV, which continued to produce ROS as long as the MV redox cycle was maintained and MV⁺ was continuously re-oxidized (Supplemental Fig. S1, Bus and Gibson, 1984).

The response to 25 µM H₂O₂ differed tremendously between HL- and LL-acclimated cultures. While LL cells did not show any change in their PSII quantum yield for more than 10 h, HL ones exhibited fast and dramatic PSII inactivation under their growth irradiance once exposed to 25 µM H₂O₂ (Fig. 2A). During the first 3 h, the rate of PSII inactivation was similar between LL cells incubated with 750 µM H₂O₂ and HL cells with 25 µM H₂O₂. After this initial period, the evolution of PSII activity however markedly differed between the two sets of cultures: while in LL cells subjected to 750 µM H₂O₂ photoinactivation led to total shutdown of PSII activity, HL cells exposed to 25 µM H₂O₂ exhibited an increase of PSII function until full recovery after 3 days. Moreover, as measured by flow cytometry, the growth rate observed after this recovery was very similar to that observed before stress initiation (data not shown). Taken together, these data demonstrate the capacity of HL cells to recover after exposure to a low dose of H₂O₂ by completely
detoxifying the cells from this oxidizing agent and restoring photosynthetic activity, and most probably other affected cellular processes.

**Effect of light acclimation and electron transport rate on MV-mediated ROS production and PSII inactivation**

If it is correlated with photosynthetic electron flow at PSI acceptor side, MV-mediated ROS production is expected to differ between LL- and HL-grown cells under their respective growth irradiance. To check this hypothesis, HL-acclimated cultures were either maintained under HL or shifted to LL conditions (inducing a sharp decrease in photosynthetic electron flow) 10 min before addition of either sub-saturating (1µM) or saturating concentrations (50 or 250 µM) of MV (Fig. 3). For all MV concentrations tested, the shift to LL led to a much lower PSII inactivation after 2 h (Fig. 3A) and an interruption of H₂O₂ production, as assessed by the quasi constancy of the fluorescence of scopoletin, a compound sensitive to H₂O₂ (Fig. 3B), compared to cells maintained under HL. This demonstrates that the MV-mediated PSII photoinactivation rate is highly dependent upon the photosynthetic electron transport rate. Surprisingly, the relative PSII photoinactivation in HL cells shifted to LL was only slightly lower than that of LL cells maintained in LL in the presence of MV (Fig. 3A), even though the H₂O₂ production was significantly higher in the LL cultures (Fig. 3B).

**Relation between D1 protein turnover and ROS-dependent photoinactivation of PSII**

In order to compare the PSII repair capacities of LL and HL cultures exposed to 25 µM H₂O₂, we plotted the time course variations of the relative D1 protein cell content (Fig. 4). While both LL and HL cells were able to increase their D1 protein content in response to oxidative stress during the first 8 h, only the HL cells sustained a high level of D1 protein over two days, thus enabling sufficient D1 protein turnover until complete recovery of PSII function. In contrast, PSII activity of LL cells dropped sharply and ceased after 30 hours, suggesting that even a low dose of H₂O₂ provoked an irreversible impairment of PSII repair cycle within about one day, and likely subsequent cell death.

To assess the initial D1 repair capacities of HL and LL cells and the effect of H₂O₂ on the balance between PSII inactivation and repair, HL and LL *Synechococcus* cells were either maintained under their initial growth irradiance or transferred to dark, and exposed to H₂O₂ and/or lincomycin, an inhibitor of protein synthesis. The results showed that no PSII damage was detectable after 2 hours in LL-acclimated cells without addition of any oxidant, neither in the dark nor under light, independently of the presence of lincomycin (Fig. 5A). In contrast, HL cells suffered a strong progressive light-dependent PSII inactivation in the presence of lincomycin (Fig.
5B), reaching 70% inactivation after two hours compared to control cultures without lincomycin. This demonstrates that, in contrast to LL cells, PSII complexes from HL *Synechococcus* undergo considerable photoinactivation under their growth irradiance, which must be counteracted by the induction of a fast PSII repair cycle at a much higher rate than in LL-acclimated cells.

Secondly, the stronger effect on LL cells of 750 µM H₂O₂ plus lincomycin compared to lincomycin alone (Fig. 5C) suggested that, at this concentration and under light exposure, H₂O₂ induces direct damage on PSII complexes. This effect is seemingly light-dependent since no significant decrease of the Fv/Fm occurred upon addition of H₂O₂ plus lincomycin in the dark. A similar photoinactivation under light with or without lincomycin indicated that the D1 repair rate was too low to be measured, either because it was initially low, due to the lack of light-dependent photodamages, and/or because of an inhibitory effect of H₂O₂. The absence of detectable light-driven damage in LL cells, which would necessitate an active repair (Fig. 5A), did not allow us to discriminate between these two hypotheses.

In HL cells, addition of 25 µM H₂O₂ and lincomycin provoked a drop in Fv/Fm equal to the HL plus lincomycin only treatment, suggesting that this amount of oxidizing agent did not induce additional direct damages to PSII above those induced by HL alone, when repair was fully blocked (Fig. 5D). In contrast, the 25 µM H₂O₂ treatment induced a modest decrease in PSII quantum yield compared to control HL cells, suggesting some direct inhibitory effect of H₂O₂ on PSII repair. Notably, the inhibition was only partial at 25 µM H₂O₂ since Fv/Fm did not drop to the level obtained with the lincomycin treatment to fully block PSII repair. This H₂O₂ effect on repair was confirmed by the absence of significant effect of 25 µM H₂O₂ alone in the dark, when no light-induced PSII damage occurred.

The stronger effect under light of 750 µM H₂O₂ plus lincomycin compared to lincomycin alone indicated that at this concentration, the oxidant also induced direct damage to PSII (Fig. 5D). Furthermore, 750 µM H₂O₂ induced the same level of PSII photoinactivation with and without lincomycin indicating that the HL cultures also underwent a complete inhibition of PSII repair at this oxidant concentration. Incubation of the culture in the dark furthermore indicated that, in contrast to cells submitted to 25 µM H₂O₂, HL cultures exposed to 750 µM also underwent light-independent damages, since inactivation occurred with or without lincomycin in the dark.

Altogether, these results suggest that high concentrations of H₂O₂ affect photosynthetic activity by acting on PSII repair as well as by inducing both light-dependent and light-independent damages on PSII itself.

**Global transcriptomic response of *Synechococcus* sp. WH7803 to oxidative stress**
The global gene expression of *Synechococcus* sp. WH7803 was determined in response to H$_2$O$_2$ and MV doses leading to 50 % of PSII photoinactivation (note that the time needed to reach this state varies between treatments; see Fig. 2). Microarray analyses were performed either by expressing the data with regard to the common reference hybridized on all arrays or by pairwise comparisons between non-stressed and stressed conditions (*i.e.* LL-Ct vs. LL+MV, LL-Ct vs. LL+H$_2$O$_2$, HL-Ct vs. HL+MV, HL-Ct vs. HL+H$_2$O$_2$) or between HL- and LL cultures (*i.e.* LL-Ct vs. HL-Ct). Overall, 1,202 out of the 2,623 genes present on the array were significantly differentially expressed (false discovery rate (FDR) ≤ 0.05 using t-test and/or LIMMA) in at least one of the pairwise comparisons. As expected, a higher number of genes were identified as differentially expressed in response to oxidative stress (450, 715, 530 and 585 genes in LL+H$_2$O$_2$ vs. LL-Ct , LL+MV vs. LL-Ct, HL+H$_2$O$_2$ vs. HL-Ct, HL+MV vs. HL-Ct respectively when considering only FDR cut-off) than between the two light acclimation conditions (121 genes). The complete set of gene expression data is available as supplemental material (Supplemental Table S1).

Principal Component Analysis (PCA, Fig. 6A) as well as hierarchical clustering dendrograms of the set of genes significantly differentially expressed in at least one of the pairwise comparisons (Fig. 6B) indicated good reproducibility among biological replicates used for microarrays analyses since all replicates grouped together but for the HL stressed ones. This suggests a similar effect of H$_2$O$_2$ and MV on HL cells (Fig. 6AB). The most highly differentiated datasets were the oxidative stressed cells vs. control ones, independently of the reactive oxidizing agent used. The contribution of the stress factor seems to be dominant in the PCA component 1, responsible for 35.4 % of the variability of the data (Fig. 6A). The second major factor appears to be light acclimation, which segregated LL and HL datasets within both control and stressed conditions. This effect would be mainly represented by the PCA component 2, responsible for 18.08 % of the data variability. Furthermore, the higher distance between HL and LL datasets in stressed conditions compared to control ones suggests that this component 2 takes into account the synergistic effect of oxidative stress and HL acclimation.

In order to identify the proportion of genes responding to the different treatments, Venn diagrams were performed using the same datasets (Fig. 7). These analyses showed that MV and H$_2$O$_2$ induced a similar effect on both LL- and HL cells (Fig. 7A). Indeed, 82% of the genes affected by H$_2$O$_2$ in HL cells (434 out of 530 genes) were also affected by MV and 74% (434 out of 585) the opposite way around. Very similar results were obtained for LL-acclimated cells, 71% and 44% respectively. Furthermore, among the genes common to MV and H$_2$O$_2$ induced stresses, 100 % showed concordance between LL and HL acclimated cells, meaning that no gene was activated in
one condition and repressed in the other. This indicates that both oxidative treatments induced similar response mechanisms.

To be able to directly compare the response of LL and HL cells, genes responding to MV and/or H2O2 were pooled per light condition and the resulting gene sets were called LL+Ox and HL+Ox. Pairwise comparison of LL+Ox vs. LL-Ct and HL+Ox vs. HL-Ct confirmed that, generally speaking, LL and HL cells responded similarly to oxidative stress. Indeed, Fig. 6B shows that about half of the genes responding to oxidative stress in one light condition were also differentially expressed in the other (366 out of 847 genes for LL+Ox vs LL-Ct, 366 out of 681 for HL+Ox vs HL-Ct) and that the transcriptomic response of these genes was concordant in 93% of the cases.

Additionally, these pooled sets were also compared to the effect of photoacclimation (HL-Ct vs. LL-Ct) to check whether acclimation to HL and oxidative stress may lead to a common response. As shown in Fig. 7C, only 42 genes (corresponding to about 5% of the LL+Ox vs. LL-Ct) were differentially regulated in both LL+Ox vs. LL-Ct and HL-Ct vs. LL-Ct comparisons. Furthermore, 50% of these genes showed an opposite transcriptomic response (11+8 vs. 15+8 genes). In contrast, although 61 genes were differentially regulated in both HL+Ox vs. HL-Ct and HL-Ct vs. LL-Ct sets, most of them (58 out of 61) were discordant, indicating a different expression behavior between LL and HL cells in our analyses. The full list of genes showing concordant or opposite transcriptomic patterns between the effects of acclimation to HL and ROS stress, is available in Table 1. Among discordant genes in HL cells, the few ones which are also discordant in LL cells are retrieved. Noteworthy, these common genes include *lexA* and *recA* that play a key role in the control of DNA repair (Kuzminov, 1999; Butala et al., 2009; Kolowrat et al., 2010). The expression of these two genes was activated by oxidative stress but repressed by HL acclimation. Most of the other discordant genes encode ribosomal subunits or proteins without attributed function.

**Identification of functional groups responding to oxidative stress and light acclimation**

Two-dimension hierarchical clustering analysis allowed the identification of 5 gene clusters behaving similarly in the different treatments (Fig. 8): two clusters corresponding to transcripts mainly activated by ROS under LL (group A) or HL (group C) conditions only, a third one consisting in oxidant induced genes under both light conditions (group B), and two clusters including transcripts mainly repressed by oxidative stress in HL cells (group D) or in both LL and HL (group E). These five clusters highlighted the behavior of coherent functional groups. The two most obvious ones included ribosomal and photosynthesis genes that were strongly down-regulated by oxidants in both light conditions (group E), suggesting a decrease of photosynthetic and protein translation activities. Indeed, most of the genes encoding the subunits of the complexes driving
these processes (ATP synthase, photosystems I and II, phycobilisomes, Calvin cycle, ribosomes) exhibited a similar response. This group E also includes numerous genes involved in phosphate uptake (*pstB, pstSII, phnDE, etc*), storage (*ppx, ppa*) and regulation (*phoB*) as well as DNA repair genes such as *mutM* and most *rec* genes (except *recA*, located in group B). Another functional group with a characteristic behavior consists in genes involved in photoprotection, which are mainly induced by oxidative stress in LL and/or HL conditions (groups A to C). Among those, genes encoding HL inducible proteins (HLIP) are particularly well represented in group B corresponding to transcripts induced in both light conditions, which also include the *ocp* gene coding for the so-called orange caroteno-protein (Kerfeld et al., 2005; Wilson et al., 2006; Kirilovsky, 2007). In contrast, genes involved in the synthesis of other carotenoids (*crtDEHQR* genes), including zeaxanthin, are located in the group C of genes induced by ROS in HL cells only. As expected, chaperones and proteases encoding genes (*clpBCPX, dnaJK, groLS, etc.*) were induced by ROS and were also mainly represented in groups A to C.

**DISCUSSION**

**Are HL acclimation and oxidative stress cumulative?**

The physiological experiments performed in this study revealed that under their growth irradiance, HL-acclimated cells were more affected than LL ones by H$_2$O$_2$ stress with regard to PSII photoinactivation, and this was verified across the whole range of H$_2$O$_2$ concentrations tested (Fig. 1 and 2). A possible hypothesis explaining this interesting observation could be that cells grown at 250 μmol photons m$^{-2}$ s$^{-1}$ were already facing a stress situation, so that they could not handle an additional stress. However, marine *Synechococcus* exhibit a large physiological flexibility that allows them to grow under a large range of irradiance in laboratory conditions (Kana and Glibert, 1987a, b; Six et al., 2004; Six et al., 2005) and with maximum growth rates usually observed between 200-300 μmol photons m$^{-2}$ s$^{-1}$ (Kana and Glibert, 1987a; Moore et al., 1995; Six et al., 2004). Thus, the HL irradiance used in this study should not represent highly stressful growth conditions. Furthermore, microarray analyses revealed that only 121 genes were differentially expressed in the HL-Ct vs. LL-Ct pairwise comparison, while 4 to 6 times more genes responded to addition of oxidizing agents. None of the 121 genes seemed to be involved in ROS defense or detoxification mechanisms, except for a ferredoxin (*petF_1580*) and a peroxiredoxin (*prxQ_1236*) encoding genes that were both significantly upregulated in HL compared to LL cells. Interestingly, *lexA* and *recA* genes, which are involved in SOS response (Butala et al., 2009; Kolowrat et al.,
2010), were downregulated in HL cells, supporting the interpretation that those cells were not under high light stress that would require activation of this DNA repair system.

**Why are the HL cells more sensitive than LL cells to MV-mediated oxidative stress?**

MV is a very low potential redox compound that primarily accepts electrons from the F$A$ and F$B$ iron-sulfur clusters of PSI centres at the expense of ferredoxin reduction (Supplemental Fig. S1; Fujii et al., 1990). By reacting very quickly with O$_2$, the reduced MV$^{++}$ radical is spontaneously re-oxidized back to MV with formation of the membrane impermeable superoxide anion. The latter compound undergoes further dismutation to form H$_2$O$_2$ that can readily pass through biological membranes (Bus and Gibson, 1984; Goldstein et al., 2002). PSI electron transport includes both linear electron flow (LEF) from PSII through PSI to NADP$^+$, and cyclic electron flow (CEF) from the PSI acceptor side back to cytochrome $b$_6$f$ complex (Supplemental Fig. S1; Bendall and Manasse, 1995; Joliot and Joliot, 2002). By reacting with any electrons coming from PSI, MV inhibits CEF and diverts LEF (Yu et al., 1993; Herbert et al., 1995) towards ROS production (Jia et al., 2008; Fan et al., 2009). In the presence of MV, the ROS production in a cell is thus mostly dependent upon the absolute number of electrons passing through PSI complexes per time unit, which results from two parameters that are both strongly influenced by growth irradiance: the electron transport rate at PSI complexes, and the number of PSI per cell. LEF is thus a critical parameter when considering the differential response of LL and HL cells to MV-mediated oxidative stress.

At their growth irradiance, HL cells mediate much faster LEF than do LL cells, thereby producing more ROS per PSI. For PSI content, previous studies have shown that in cyanobacteria, acclimation to high irradiance is accompanied by a strong decrease of the thylakoid surface and chlorophyll $a$ content per cell (Kana and Glibert, 1987a; Moore et al., 1995; Six et al., 2004). In cyanobacteria chlorophyll $a$ is almost exclusively bound to reaction centers (especially to PSI complexes), and HL cells must therefore contain fewer photosystems than LL cells. Thus, HL cells exhibit a high PSI electron transport rate favoring a large MV-mediated ROS production rate, but a lower photosystem cell content, and therefore a lower number of potential ROS formation sites than LL cells.

Addition of MV concentrations higher than 25 µM induced a saturation of PSII photoinactivation in both LL and HL cells (Fig. 1B), most likely originating from a saturation in the ROS production rate from PSI centres. Such a saturating effect has previously been observed in the freshwater *Synechococcus* sp. PCC 7942 albeit at a somewhat higher MV concentration (100 µM; Roncel et al., 1988). While the MV concentration leading to saturation of PSII photoinactivation rate was similar between LL and HL cells, the remaining PSII quantum yield at the saturating MV
concentration was in contrast almost 10-fold lower in HL cells than in LL cells (Fig. 1B). If the number of PSI per cell was a major factor influencing ROS production, a severe PSII photoinactivation would have been expected in LL cells, given their high PSI cell content. Since this is not the case, this rather suggests that the LEF rate is the major factor controlling the amplitude of the MV-induced oxidative stress. Furthermore, when HL cells were suddenly shifted to LL conditions, lowering LEF without affecting the PSI cell content, the production of H2O2 almost stopped (Fig. 3B), with a consequent slowing down of PSII photoinactivation compared to the cells maintained in HL conditions (Fig. 3A). This clearly highlights the importance of LEF in the amplitude of MV-induced oxidative stress in HL cells. Still, it is worth noting that this only partially explains the higher sensitivity of HL cells to oxidative stress. Firstly, despite the sharp reduction in MV-dependent ROS production, the HL cells shifted to LL still exhibited a 25% photoinhibition (Fig. 3AB). Moreover, even though this stronger PSII inactivation could, at least in part, be due to a higher rate of endogenous ROS formation in the presence of MV, this justification does not hold true for H2O2, since the same oxidant concentration, added exogenously, also produces a much stronger photoinhibition in HL cells than in LL ones (Fig. 1A). Thus, besides inducing a higher MV-dependent ROS production, HL acclimation also seems to result in a higher sensitivity to ROS-driven damages.

**Effect of ROS on PSII damage and repair**

The PSII repair cycle is a crucial process in the response of photosynthetic organisms to various stressful conditions (Park et al., 1995; Nishiyama et al., 2006; Six et al., 2007a). Several hypotheses have been proposed to explain the detrimental effect of ROS on PSII function, either through direct damage to PSII reaction centers with consequent degradation of the D1 protein (Hideg et al., 2007; Vass and Cser, 2009), or through effects on the de novo D1 protein synthesis through ROS inhibition of protein translation {Nishiyama, 2004 #26; Takahashi, 2008 #6; Kojima, 2007 #51}. Our results suggest that H2O2 induces both direct PSII damage and inhibition of PSII repair, with the respective importance of these processes being dependent upon both photoacclimation and oxidant concentration.

In HL cells, a treatment with lincomycin and 25 µM H2O2 induced the same level of photoinactivation as the lincomycin only treatment. This shows that at this concentration and under this growth irradiance, H2O2 does not induce damage beyond that observed when the PSII repair cycle is inhibited (Fig. 5D). The moderate decrease of PSII function in the presence of 25µM H2O2 and absence of lincomycin therefore suggests that H2O2 - at this concentration and under this growth irradiance - acts by partially inhibiting PSII repair rather than by directly damaging PSII. Despite the inhibitory effect of H2O2 in these conditions, a strong induction of D1 synthesis
occurred right after stress initiation (Fig. 4). This *de novo* synthesis of D1 was not sufficient to maintain PSII quantum yield indicating that most of these D1 proteins were not included in functional PSII centers (Garczarek et al., 2008).

While PSII repair cycle inhibition was partial at 25 µM, it seemed to be complete in presence of 750 µM H₂O₂, since this oxidant had the same effect on HL cells with and without lincomycin (Fig. 5D). Furthermore, this H₂O₂ concentration also induced strong direct PSII damage since the level of photoinactivation was about twice higher than when the repair cycle was blocked by lincomycin alone. These ROS-driven damages seemed mostly light-independent, since the photoinactivation in the presence of 750 µM H₂O₂ alone was similar in the dark. Such damages could not be restricted to PSII only but could be attributed to a more global degradation of cellular components by ROS (Imlay, 2003), including translation machinery (Kojima et al., 2007). At high H₂O₂ concentrations, the decrease of PSII quantum yield in HL cells reflected both an impairment of PSII repair cycle and direct damages to PSII complexes.

In contrast to HL cells, LL cells initially showed a nearly constant D1 pool in the presence of 25 µM of H₂O₂, which appeared sufficient to maintain PSII quantum yield at its initial level during 6 h (Figs. 2 and 4). This suggests that 25 µM H₂O₂ did not provoke additional PSII damage beyond the very low photoinactivation rate at this light level, which was below detection. However, an effect leading to PSII damage was clearly noticed at 750 µM of H₂O₂ (Fig. 5C). In contrast to HL cells, these damages were light-dependent, as almost no damage occurred when H₂O₂ was added in the dark (Fig. 5C-D). A possible explanation for the light-dependency of these damages relies on the formation of reactive hydroxyl radicals OH⁺ through oxidation by H₂O₂ of reduced metals, such as the Fe or Mn atoms located in reaction centres II (Imlay, 2003). Indeed, PSII non-heme iron, one of the prosthetic group of the D1 protein, has been reported to be oxidized by H₂O₂ and is thought to be involved in a specific OH⁺-induced cleavage of this protein (Diner and Petrouleas, 1987; Lupinkova and Komenda, 2004). Such reactions are expected to be light-dependent since the redox state of the non-heme iron depends on photosynthetic electron transfer (Ishikita and Knapp, 2005). Similarly, at PSII donor side, the redox status of the Mn cluster, that can also be oxidized by H₂O₂ (Lupinkova and Komenda, 2004), is also dependent on PSII electron transport. In darkness, the oxidised state of such PSII-bound metals might prevent a reaction with H₂O₂ and therefore the resulting formation of OH⁺ leading to D1 protein cleavage.

Interestingly, a cumulative effect of light and oxidative stress on PSII function does not agree with the results obtained on *Synechocystis* sp. PCC 6803 for which oxidative stress induced by H₂O₂ did not accentuate light-induced damages but only affected the rate of PSII repair (Allakhverdiev and Murata, 2004). It should however be noted that there are major experimental differences between those results and our study. Indeed, the *Synechocystis* experiments were
mainly performed by monitoring the recovery of PSII activity after cultures were briefly exposed to very high light conditions (from 250 to 2,000 µmol photons m\(^{-2}\) s\(^{-1}\)), inducing strong PSII photoinactivation that the cells were only able to withstand for a short time. In contrast, in our study, *Synechococcus* sp. WH7803 cells were fully acclimated to the different growth irradiances. Furthermore, a large range of H\(_2\)O\(_2\) concentrations was tested, as the dose proved to be important to differentiate between ROS-induced direct damage and inhibition of repair. For instance, the effect of H\(_2\)O\(_2\) on PSII repair activity could only be detected at 25 µM and on HL cells, *i.e.* when some light- but no ROS-mediated damages were generated. Although our approach did not allow the assessment of the PSII repair cycle when no detectable photodamage had occurred, it had the advantage of taking into account the differential damage resistance/repair capacities of these cells that cannot be appreciated through a light shift. For instance, one could expect that HL cells, which possess a higher ratio of D1:2 to D1:1 protein isoforms than LL cells (Garczarek et al., 2008), also possess a higher PSII resistance to photoinactivation (Campbell et al., 1995; Campbell et al., 1998; Tichy et al., 2003). The fact that HL cells were in contrast even more sensitive to photoinhibition indicates that oxidative stress has a strong effect on direct PSII damages independently of the dominant D1 isoform present in the PSII. Altogether, these data suggest that the cumulative effect of light- and ROS-driven damages in HL cells and the resulting stronger dependence on D1 repair, are probably responsible for the higher PSII sensitivity of those cells to oxidative stress. For cells shifted from HL to darkness we observed a moderate, progressive decline in F\(_v/F_M\) (Fig. 5B). Addition of lincomycin prevented this decline, which therefore depends upon protein synthesis in the dark. This decline in F\(_v/F_M\) in the dark resulted mostly from an increase in the F\(_0\) level of fluorescence in cells shifted from HL to darkness (Supplemental Figure S2). Similar increases in F\(_0\) occurred in the absence or presence of H\(_2\)O\(_2\).

**Transcriptome response to oxidative stress**

Effects of oxidative stress on PSII do not alone determine the survival capacities of the cells. Indeed, while HL cells were able to recover from a 25 µM H\(_2\)O\(_2\) stress, LL ones eventually died even though their photosynthetic activity seemed initially less affected (Fig. 1). We performed transcriptome analyses to observe the global response of the cells and to understand why HL cells were capable of better resistance and recovery than LL cells when facing oxidative stress.

Microarray analyses showed that H\(_2\)O\(_2\) altered the expression of functional groups of genes (*e.g.* photosynthesis, NADPH dehydrogenase, ribosomal proteins, some chaperones) in the marine *Synechococcus* sp. WH7803, in much the same way as what has been observed in the freshwater *Synechocystis* sp. PCC 6803 in response to ca. millimolar H\(_2\)O\(_2\) concentrations (Li et al. 2004; Houot al., 2007). Moreover, global transcriptomic data clearly indicated that H\(_2\)O\(_2\) and MV induced
similar transcriptomic responses in both LL and HL *Synechococcus* sp. WH7803 cells (Figs. 6 and 7A). Pairwise comparison between pooled genes responding to H$_2$O$_2$ or MV resulted in 93% concordance between LL and HL cells, meaning that most genes differentially expressed in both light conditions responded similarly in HL and LL cells (Fig.7B). Hierarchical clustering analyses (Fig. 8) however, allowed highlighting some differences associated with one or the other light condition.

The photosynthetic genes are among the most affected by oxidative stress in both LL and HL cells (Fig. 8 and Supplemental Table S1), including the four *psbA* gene copies encoding the D1 protein of the PSII core in *Synechococcus* sp. WH7803. At the transcriptomic level, microarray data showed that the two transcripts making up most of the *psbA* mRNA pool in this strain (Garczarek et al., 2008), are inversely regulated in response to oxidative stress, indicating an exchange between D1:1 (encoded by *psbA* _0784) and D1:2 (encoded by *psbA* _0790) isoforms in both HL and LL cells. The resulting D1 pool would then be dominated by the D1:2 isoform, which is thought to provide resistance to photoinhibition (Krupa et al., 1991; Clarke et al., 1993a; Clarke et al., 1993b; Campbell et al., 1995; Campbell et al., 1998; Tichy et al., 2003). Accordingly, *ftsH* _1216_, the ROS-induced direct ortholog of *Synechocystis* sp. PCC 6803 slr0228 gene (Li et al. 2004; Houot al., 2007), encoding the FtsH2 protease involved in the clearance of damaged D1 proteins from inactivated PSII (Komenda et al., 2006; Komenda et al., 2010), belongs to the cluster of genes mainly activated in response to ROS in *Synechococcus* sp. WH7803 (cluster B; Fig. 8). It is worth noting that, as previously described, the D1 pool in the PSII of HL cells is dominated by the D1:2 isoform (Garczarek et al., 2008). HL cells would then be better prepared to sustain ROS-driven photoinhibition not only thanks to the initial dominance of the transcript coding for D1:2 isoform but also by completing the isoform switch in response to additional stress. Most other photosynthetic genes belong to some of the main gene clusters inhibited by oxidative stress (clusters D and E; Fig. 8). This includes most of the ATP synthase, photosystems I and II subunits as well as the genes involved in phycobilisome synthesis and Calvin cycle, suggesting a decrease in energy supply in response to stress.

Although most of the photosynthetic genes were not significantly differentially affected by oxidative stress in LL and HL cells, some differences in gene induction however gave us hints to explain the survival of HL cells in oxidative stress conditions that were lethal for LL cells. This includes a number of genes involved in photoprotection mechanisms such as genes encoding the orange carotenoid protein (*ocp*, Kerfeld et al., 2005; Wilson et al., 2006; Kirilovsky, 2007), whose induction was significantly higher in HL than in LL cells (Supplemental Table S1). In addition, the induction of the *crtDEHQR* genes involved in carotenoid synthesis (Masamoto et al., 1998; Klassen, 2010) mainly belong to the cluster C of genes more activated in HL cells than in LL ones.
(Fig. 8), with $crtR$, a gene involved in the conversion of from $\beta$-carotene into zeaxanthin, being clearly the most up-regulated. Carotenoids are thought to dissipate energy from photosensitized chlorophyll or from singlet oxygen and may have intrinsic antioxidant properties (Edge et al., 1997). In particular, zeaxanthin-deficient mutants of *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002 are more sensitive than their respective wild types to HL and oxidative stress, and it has been shown that such mutants indeed accumulate ROS (Schafer et al., 2005; Zhu et al., 2010). The genes differentially regulated by oxidative stress also include the high-light-inducible proteins (HLIPs), three of which being induced at higher levels in HL cells than in LL ones ($hli_{0937}$, $hli_{0797}$, $hli_{1441}$). HLIPs have been shown to be involved in protecting cells against photodamages either by direct or indirect dissipation of excess absorbed light energy (Montané and Kloppstech, 2000; He et al., 2001; Havaux et al., 2003), or by binding and storage of free chlorophylls (Funk and Vermaas, 1999; Vavilin et al., 2007; Kufryk et al., 2008).

Among the genes clearly induced by oxidants in HL cells (cluster C, Fig. 8), those encoding the NADPH dehydrogenase subunits are highly represented. This complex is involved in a variety of cellular processes including respiratory electron flow, CEF around PSI, indirect control of redox state of the plastoquinone pool, as well as in CO$_2$ uptake (Cooley and Vermaas, 2001; Ogawa and Mi, 2007; Battchikova et al., 2010). The active induction of *ndh* genes would allow maintaining energy production of HL cells under oxidative stress. Notably, a direct effect of NDH-dependent CEF in alleviating photo-oxidative stress has also been suggested in tobacco chloroplast by compensating the stromal over-reduction that induces formation of ROS (Wang et al., 2006).

Tolerance to the deleterious effects of ROS may result from the activity of a number of mechanisms that maintain ROS at a harmless level, or that actively repair ROS-induced damages. ROS scavenging enzymes such as catalases, peroxidases and superoxide dismutases, as well as antioxidants such as glutathione, tocopherol and ascorbic acid, are involved in the maintenance of low levels of ROS (Noctor and Foyer, 1998; Storz and Imlay, 1999; Mittler, 2002; Masip et al., 2006). Such antioxidant activities involved in ROS scavenging and redox homeostasis have been reported in freshwater cyanobacteria such as *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942 (Regelsberger et al., 2002; Yousef et al., 2003; Kobayashi et al., 2004; Hosoya-Matsuda et al., 2005; Kanesaki et al., 2007). Most of the genes involved in antioxidant activities have also been reported in the genome of *Synechococcus* sp. WH7803, suggesting that similar detoxification systems occur in this marine cyanobacterium (Scanlan et al., 2009). Among these genes, some, like the catalase/peroxidase encoding gene *katG*, were induced in response to oxidative stress in both LL and HL cells, but others showed an obvious difference between HL and LL. While the expression of the cytosolic [Fe] superoxide dismutase (Fe-SOD) encoding gene *sodB* was down-regulated in both LL and HL cells submitted to oxidative stress, the response of the periplasmic [Cu/Zn]
superoxide dismutase (Cu/Zn-SOD) gene sodC was upregulated in HL cells only (Supplemental Table S1; Langford et al., 2002; Culotta et al., 2006). In contrast, in Synechocystis sp. PCC 6803, that only carries the sodB gene, the latter seemed to be induced in response to H₂O₂ (Li et al. 2004; Houot et al., 2007). In Synechococcus sp. PCC 7942, the cytosolic Fe-SOD does not protect the cells from ROS generated within the thylakoid membrane (Thomas et al., 1998), suggesting that other SOD might confer such a protection. Thus, the increase in periplasmic Cu/Zn-SOD activity would confer a resistance to the light-driven ROS production in HL cells. Another gene involved in ROS detoxification, dpsA, encoding a DNA binding protein, was also upregulated in response to oxidative stress in HL cells only. DpsA has been shown to be involved in the detoxification of Fe²⁺ ions and H₂O₂ and to more specifically protect DNA from oxidative damages (Chiancone and Ceci, 2010). In Synechococcus sp. PCC7942, while an insoluble DpsA fraction would bind DNA, a soluble faction, localized into the thylakoid membranes, would allow maintaining metal homeostasis of the photosynthetic apparatus (Durham and Bullerjahn, 2002). Thus, as the Cu/Zn-SOD, DpsA would confer a better resistance of HL cells to the ROS generated within the thylakoid membrane. The genes ftrC and ftrV, encoding the subunits of the ferredoxin-thioredoxin reductase (FTR) complex are clearly differentially regulated in response to oxidative stress between LL and HL cells: both genes are downregulated in LL cells but not in HL cells, for which ftrC is upregulated. The FTR complex links PSI redox status with the regulation of numerous photosynthetic processes and is required for the growth of plants that are submitted to oxidative stress (Buchanan and Balmer, 2005; Schürmann and Buchanan, 2008). Thus, the activation of the FTR system in HL cells, while it is inhibited in LL ones, may partially prevent HL cells from cellular damages due to reactive oxygen stressors, by the means of a better retro-control of the redox status of the cells.

A number of chaperone and protease encoding genes were also affected by ROS in only one of the light acclimation condition. Among those, the 3 genes encoding the GroEL/GroES system (groS, groL1, groL2) and htpG were particularly activated in LL cells. These two major chaperone systems in bacteria are known to be activated in response to cold stress, high irradiance and MV in Synechococcus sp. PCC 7942 (Hossain and Nakamoto, 2002; Hossain and Nakamoto, 2003; Sato et al., 2010). In contrast, clpP genes mostly responded to oxidative stress in HL cells, with three being activated (clpP2, clpP4, clpP3), while the clpP1 gene was repressed in both LL and HL cells (Fig. 8 and Supplemental Table S1). The expression of these genes may also be related to clpX, which encodes a regulatory ATPase/chaperone interacting with ClpP, and was surprisingly inhibited by ROS. Indeed, despite the fact that clpX and clpP are typically induced by heat, high salt, oxidation and glucose deprivation in bacteria, such an inhibitory effect by H₂O₂ on clpX expression has already been observed in Synechococcus sp. PCC7942 (Schelin et al., 2002) and may be linked to a
possible exchange of Clp proteolytic core complexes in response to stress (Stanne et al., 2007; Adrian Clarke, pers. com.). Finally, *degQ*, which encodes a periplasmic protease known to confer some stress resistance (Barker et al., 2006), was also somewhat more induced in HL cells than in LL ones.

An element which could contribute to the better ROS resistance of HL cells is the capacity to induce an SOS response, which is involved in protecting the cells from damaging effect of increased mutation rates. Although some authors have suggested that cyanobacteria could lack an SOS response similar to that of *Escherichia coli* (Domain et al., 2004; Michel, 2005; Patterson-Fortin et al., 2006), it seems that *Synechococcus* sp. WH7803, like *Prochlorococcus* (Kolowrat et al., 2010), could well possess such an inducible pathway involved in DNA repair. SOS response is under control of the two regulatory components RecA and LexA, the latter being a transcriptional regulator of SOS genes, including itself. The consensus sequence motif for LexA binding site in cyanobacteria has been proposed to be RGTACNNNDGTWCB (Mazon et al., 2004). Among the 43 genes belonging to the LexA regulon in *E. coli* (Courcelle et al., 2001), 10 seem to be present in *Synechococcus* sp. WH7803 genome, namely *lexA, recAN, umuCD, uvrAD, ssb* and *ruvAB*, but only *lexA, recA*, and *umuD* show potential LexA binding sites in their regulatory regions, independently on their orientation (Table 2). In order to identify other putative SOS genes, *Synechococcus* sp. WH7803 intergenic regions were screened with the consensus motif RRTACNNNDGTWYB that integrates one degenerated position from the LexA box of *lexA* gene. Five other putative LexA binding sites have been detected downstream *wcaG, dprA* (or *smf*), *WH7803_0096, WH7803_0814, WH7803_0843* and *WH7803_1500* genes (Table 2). These findings slightly differ from those of Li et al. (2010), which by using another cyanobacterial consensus, failed to detect significant sites in the regulatory regions of *lexA* and *umuCD*, leading them to conclude that the SOS response regulation might be attenuated in *Synechococcus* sp. WH7803. According to our analysis, all genes from the LexA regulon, *i.e.* that carry a putative LexA box in their regulatory region, were very similarly regulated in response to HL acclimation and to oxidative stress, strengthening the hypothesis that LexA may indeed regulate these genes and that the gene products with unknown function might be involved in the SOS response. In contrast, genes that are thought to be part of the LexA regulon in *E. coli*, but that do not have a LexA binding motif in their promoter region in *Synechococcus* sp. WH7803, are not similarly regulated and are most probably not under the control of LexA. Using the WebLogo tool (Crooks et al., 2004), the nine putative binding sites found in *Synechococcus* sp. WH7803 allowed to define the consensus of the LexA-Box in this strain, which seems to be degenerated from the palindromic TACAN₂TGTA consensus (Supplemental Fig. S3). Interestingly, the expression of *lexA* was more induced in HL than in LL cells, suggesting that the SOS response may indeed play a role in the better resistance of
HL cells to oxidative stress. Although it might appear surprising that both repressor- (*lexA*) and derepressor- (*recA*) encoding genes are simultaneously induced, the constant production of LexA would allow its re-accumulation to repress SOS genes once DNA damages have been repaired (Michel, 2005). At last, the expression of *uvrA* which was clearly differently regulated compared to all other SOS genes, was strongly repressed in response to oxidative stress in HL cells, and that may be due to the fact that *uvrA* is only expressed in very early stages of the SOS response (Kuzminov, 1999; Michel, 2005).

CONCLUSION

Our data showed that the susceptibility of *Synechococcus* cells to oxidative stress varies depending on culture light history. HL cells are more immediately affected by oxidant exposure than are LL cells, but HL cells then show better capacity to ultimately acclimate and recover from low concentration of hydrogen peroxide. By testing a range of oxidant concentrations and treatment durations on LL and HL cultures, we show that, depending upon the oxidant concentration, PSII damage observed in oxidant-stressed HL cells results both from cumulative effects of direct ROS damage to PSII, and ROS inhibition of the rapid PSII repair needed to counter the high photoinactivation rate. These findings largely reconcile earlier findings that ROS directly inactivates PSII with other studies showing ROS inhibition of PSII repair. Even though the active PSII repair cycle of HL cells is initially inhibited by oxidative stress, it nonetheless allowed them to fully recover when H$_2$O$_2$ exposure did not exceed 25 µM. Although the oxidative stress induced a similar global transcriptomic response in HL and LL cells, in HL cells we detected the induction of other cellular processes including more efficient photoprotection mechanisms, some ROS detoxification and a better redox/energy homeostasis, that most likely helped prevent the death of HL cells. Thus, in HL cells, physiological changes induced by ROS were reversible when the intracellular level of ROS decreased, while in LL ones, similar ROS treatment led to cell death. More generally, identification of the factors that set the threshold at which a cell makes the transition from successful acclimation/resistance to oxidative stress-induced cell death will be of major interest to understand the cumulative effect of environmental stress conditions.

MATERIAL AND METHODS

Culture and oxidative stress conditions
Synechococcus sp. WH7803 (Roscoff Culture Collection strain RCC752) was grown at 22°C in 0.2 μm filtered PCR-S11 medium (Rippka et al., 2000) supplemented with 1 mM NaNO₃. Cultures were acclimated during many generations to continuous LL (18 μmol photons m⁻² s⁻¹) and HL (250 μmol photons m⁻² s⁻¹) provided by Sylvania Daylight 58W/154 fluorescent bulbs. Growth rates for this strain grown at several irradiances have been published elsewhere (Kana and Glibert, 1987; Garczarek et al. 2008). For all stress experiments performed in this study, exponentially growing cultures (1 to 3 x 10⁷ cells mL⁻¹), were split into subcultures and incubated in the presence of different concentrations (cf. results) of oxidising agent under various light conditions: the subcultures were either set back to their growth light conditions, or shifted to lower light to investigate the influence of photosynthetic electron flux on ROS production, or shifted to dark to differentiate direct ROS-induced damages from light-driven damages. The oxidative stress induced by H₂O₂ was compared with the one provoked by the herbicide methylviologen (MV, Sigma Aldrich, St. Louis, MO, USA). H₂O₂ stock solutions were titrated before each experiment by absorption at 240 nm (Nanodrop ND-1000, NanoDrop Technologies, Wilmington, USA) using a molar extinction coefficient of 43.6 M⁻¹ cm⁻¹ (Hildebrandt and Roots, 1975), and adjusted at 100 mM.

Synechococcus sp. WH7803 growth was monitored by flow cytometry using a BD FACS Canto flow cytometer (Becton Dickinson Biosciences, San Jose, CA, USA) as previously described (Marie et al., 1997; Marie et al., 1999) and by phycoerythrin fluorescence emission maximum (excitation at 530 nm) using a spectrofluorometer (LS-50B Perkin Elmer, Waltham, MA).

**Photosystem II quantum yield and repair**

Photosystem II fluorescence quantum yield (Fv/FM) was measured using a Pulse Amplitude Modulated (PAM) fluorometer (PhytoPAM, Walz, Effeltrich, Germany) as previously described (Six et al., 2007b; Garczarek et al., 2008).

In order to measure PSII damage and repair, 500 μg mL⁻¹ lincomycin (Sigma Aldrich), an inhibitor of protein synthesis, was added or not to the culture immediately before submitting the cells to oxidative stress, following a procedure similar to the one described by Six et al. (2007a). When cultures were shifted from one light condition to another, cells were shifted to the new light condition 10 min prior to addition of oxidising agent, immediately after the lincomycin.

**Measurement of H₂O₂ production during MV stress**

Hydrogen peroxide production by cells upon MV-induced oxidative stress was monitored by the decrease of scopoletin fluorescence (Patterson and Myers, 1973; Tichy and Vermaas, 1999), as this molecule is degraded by H₂O₂ in the presence of horseradish peroxidase. 0.5 to 1 x 10⁸ cells...
from exponentially growing cultures were pelleted (6,000 x g for 10 min, Eppendorf 5424R), resuspended in 3 ml of 25 mM HEPES (pH 7.0) and incubated in a cuvette for 30 min under their initial HL- or LL-acclimation conditions. After addition of 1.33 µM scopoletin (Sigma Aldrich) and 25 U mL⁻¹ horseradish peroxidase (Sigma Aldrich), the cells where incubated for 10 min under their initial irradiance or shifted to LL irradiance and 0, 50 or 250 µM of MV were then added. After 30 min the scopoletin fluorescence emission was monitored at 460 nm (excitation at 350 nm) using a spectrofluorometer (LS-50B Perkin Elmer, Waltham, MA). After subtracting the dye-free background signal, fluorescence was expressed as percentage of initial value.

D1 protein quantification by immunoblotting

A culture volume of 50 mL was centrifuged in presence of 10 mg L⁻¹ of the non-ionic surfactant Pluronic F-68 (Sigma Aldrich) to avoid cell adhesion at the surface of the tubes and the pellet was flash frozen in liquid nitrogen and stored at -80°C until analysis. Total protein assays, polyacrylamide gel electrophoresis, protein transfer onto a polyvinylidene fluoride membrane, immunoreactions and chemoluminescence detection were performed as described by Garczarek et al. (2008). D1 protein amounts were expressed as a percentage of initial conditions.

RNA sampling and extraction

For the transcriptomic analyses, *Synechococcus* sp. WH7803 cultures were submitted to oxidative stress by addition of H₂O₂ or MV and harvested when PSII quantum yield fell to half of the initial value. For H₂O₂ experiments, this level of PSII photoinactivation was reached 2 h after submitting LL and HL cultures to 750 µM and 25 µM respectively (Fig. 2). Because of the large divergence in dose and kinetics responses to MV between LL- and HL cells, it was not possible to find MV concentrations leading to 50 % decrease of quantum yield at the same time for both light acclimations. Thus, array analyses for MV were performed on HL and LL cultures incubated at the same MV concentration (50 µM) but harvested once PSII quantum yield was halved, *i.e.* after 1 and 3.5 h of stress respectively (Fig. 2).

For every experiment, a 300 mL volume of stressed culture was harvested by centrifugation (10,000 x g for 7 min, Eppendorf 5417R) in presence of 10 mg L⁻¹ of the nonionic surfactant Pluronic F-68 (Sigma Aldrich). Pellets were then flash-frozen in liquid nitrogen and stored at -80°C until analysis. Four independent biological replicates of the experiments were carried out.

Total RNA was extracted with acid pH-guanidinium thiocyanate/phenol/chloroform (TRIzol reagent, Invitrogen) following a modified procedure from Millican and Bird (Millican and Bird, 1998) and purified on column (QIAGen RNEasy Mini Kit). Briefly, frozen cells were resuspended in 500 µL of TRIzol (Invitrogen, Carlsbad, CA, USA), and submitted to three cycles of 2 min heating
at 65°C and flash-freezing in liquid nitrogen. Following 10 min incubation at 65°C with vortexing, the clear lysate was vigorously mixed with 100 µL of chloroform and incubated at room temperature for 5 min in microtubes containing an interphase gel barrier (Phase Lock Gel Heavy, 5 PRIME). After centrifugation at 9,000 x g for 15 min, upper aqueous fraction was collected and the total RNA was purified following the QIAgen RNeasy Mini Kit procedure. Two consecutive DNase treatments (QIAgen RNase-Free DNase Set) were performed on the RNeasy column during the purification step, as recommended by the manufacturer. RNA was retrieved by two consecutive elutions with 30 µl of RNA-free water (Ambion) and its quality checked by capillary electrophoresis on a Bioanalyzer 2100 using the Procyote total RNA nano Chips (Agilent, Santa Clara, CA, USA).

**Microarray analysis – Array design, cDNA labelling and hybridization**

Microarray experiments were performed using a homemade array targeting 2,497 protein coding genes out of the 2,586 genes identified so far in the *Synechococcus* sp. WH7803 genome (Dufresne et al., 2008), as well as 126 potential small RNA (WR Hess, personal communication). The 60-mer oligonucleotides were designed and synthesized by Eurogentec (Liege, Belgium), resuspended at a final concentration of 20 µM into a 1X spotting buffer (Schott Nexterion, Jena, Germany) and spotted in duplicates on Schott Nexterion slides using facilities of the Rennes transcriptomic platform (France).

cDNA synthesis from 5 µg of total RNA and indirect CyDye cDNA labelling were performed using the ChipShot Indirect Labeling and Clean-Up System (Promega, Madison, USA) following the manufacturer procedure. After resuspension, labeled cDNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and vacuum concentrated to use equal amounts of Cy3- and Cy5-labelled probes (20 to 100 pmol) for hybridization. All hybridizations were performed on 4 independent biological replicates. A pool of RNA from all samples investigated in this study was used as reference sample. This allowed to maximize the number of spots with a significant signal. Furthermore, in order to minimize bias due to differential dye bleaching or unequal incorporation of the Cy3 and Cy5 dyes during RT reactions, 2 out of the 4 replicate samples for each condition were hybridized in dye swap experiments.

Printed slides were rinsed and blocked by 5 min incubation in 0.1% Triton X-100 followed by 2 min in HCl 0.0037%, 10 min in 100 mM KCl and finally 15 min at 55°C in a blocking solution (0.1% SDS, 0.1 M Tris pH 9, 0.03% ethanolamine). The slides were then prehybridized during 60 min at 55°C in prehybridization buffer (150 µM BSA, 3.5% SSC, 0.7% SDS). Labeled cDNA was denatured at 90°C for 3 min and immediately spun down and kept at 55°C until loading onto the slide. The slides were then covered with a cover slip (Corning, Glendale, USA), placed in a
hybridization chamber (Telechem, Atlanta, USA) and hybridized for 17 h in a water bath at 55°C. The washing consisted in steps of 5 min at 55°C in 4 X SSC, 5 min in the same buffer at room temperature, 1 min in 0.2 X SSC at room temperature and finally 1 min in 0.1 X SSC at room temperature. Immediately after washing, slides were briefly rinsed with distilled water before being dried by centrifugation at 300 × g for 90 s. Scanning was performed immediately after this step.

**Microarray analysis - Image Acquisition and statistical analysis**

Scanning of the arrays was performed using a Genepix 4000A scanner (Molecular Devices, Sunnyvale, USA). Photomultiplier gain at 570 nm (Cy3) and 660 nm (Cy5) was adjusted automatically using a threshold saturation of 0.001%, as implemented in Genepix 6.0 (Molecular Devices, Sunnyvale, USA). Addressing and segmentation of spots were automatically detected, manually corrected and intensities were quantified using the Genepix 6.0 software. All microarray experiments were MIAME compliant and raw data were deposited under accession number E-MTAB-681 at the ArrayExpress database of the EMBL-EBI (http://www.ebi.ac.uk/microarray-as/ae/).

Data treatments were done using custom-designed scripts written under R environment (R_Development_Core_Team, 2009). Normalization of the Cy3 and Cy5 signal intensities within arrays was performed by loess normalisation (Yang and Thorne, 2003) followed by quantile normalisation between arrays, as implemented in the Bioconductor package, LIMMA (Smyth and Speed, 2003; Gentleman et al., 2004). The value of each sampling time point is an average of the technical replicates on a slide. Student t-test and linear modelling features and empirical Bayes test statistics of the LIMMA package (Smyth, 2004) were used to perform pairwise comparison of the stress induced by either H2O2 or MV on both LL- and HL cultures (i.e. LL-Ct vs. LL+MV, LL-Ct vs. LL+H2O2, HL-Ct vs. HL+MV, HL-Ct vs. HL+H2O2) as well as comparing the steady state acclimation to different light conditions (i.e. LL-Ct vs. HL-Ct). To take into account multiple testing, p-values were adjusted using the Benjamini and Hochberg algorithm (Thissen et al., 2002). We particularly focused our attention on genes which were statistically significant using the Student t-test and/or LIMMA with discovery rate (FDR) lower or equal to 0.05, also taking into account a fold change (FC) cut-off between two conditions (log2(FC) < -1 or > 1).

Finally, hierarchical clustering analyses (HCA, (Bolstad et al., 2004) and Principal Component Analyses (PCA, (Raychaudhuri et al., 2000) were performed to investigate the technical and biological reproducibility of our results and highlight the main groups of genes, excluding control oligonucleotides, that shared similar expression patterns over multiple conditions. The clustering was performed using the hclust function from the stats R package (R_Development_Core_Team, 2009), using the clustering method “ward” and a Pearson
correlation, and PCA analysis was carried out using the FactoMineR package (Husson et al., 2008). Analyses were performed on a subset of genes corresponding to the genes significantly differentially expressed (FDR ≤ 0.05 using t-test and/or LIMMA) in at least one of the pairwise comparisons (1,202 genes left), except for two dimension (genes vs. arrays) HCA analysis where no FDR restriction had been applied but where oligonucleotides targeting unknown proteins, hypothetical proteins and small RNA were removed (1548 genes left).

**Real time quantitative PCR**

To validate microarray data, real time quantitative PCR (hereafter qPCR) was performed on ten *Synechococcus* sp. WH7803 genes. This set includes genes that were either differentially expressed in microarray analyses or representative of key processes, including ROS detoxification processes, and photosystem II turnover. Gene specific primers for both reverse transcription and qPCR were designed using PrimerExpress™ software V2.0 (Applied Biosystems ; Supplemental Table S2) and the linearity of the cDNA content to C_T (cycle at threshold) ratio was checked for every set of primers within the dilution range used. Reverse transcription was carried out on 100 ng RNA using SuperScriptII reverse transcriptase (Gibco-BRL, Gaithersburg, MD) as previously described (Six et al., 2007b). qPCR was performed on the 1:100 diluted cDNA obtained, using the DNA Engine/Chromo4 Real Time PCR-Detector (BioRad, Hercules, CA, USA) and using absolute SYBR Green ROX Mix (Abgene, Epsom, UK), as previously described (Garczarek et al., 2008), in the presence of 200 nM primers, except for the *sodB* primers which were used at 500 nM. The relative expression of each gene between two conditions was calculated using the 2^{-\Delta\Delta C_T} method (Livak and Schmittgen, 2001), using the *rnpB* gene as an internal standard (Mary and Vaulot, 2003). All genes and pairwise comparisons tested showed a similar response (up- or downregulation) in qPCR and microarray experiments (Supplemental Table S2; Pearson's correlation coefficient of 0.85).
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FIGURES LEGENDS

Figure 1
Effect of photoacclimation on PSII photochemistry. PSII maximal quantum yield (Fv/Fm) of *Synechococcus* sp. WH7803 cells acclimated to low light (LL, closed triangles), or high light (HL, open squares), was measured 2 h after the addition of a range of concentrations of H2O2 (A) or methylviologen (MV, B). These data are based on 3 to 5 independent experiments and are expressed as mean ± standard deviation.

Figure 2
PSII activity in response to H2O2 (A) and methylviologen (MV, B). The time-course of changes in PSII maximal photochemical yield (Fv/Fm) of *Synechococcus* sp. WH7803 cells acclimated to low light (LL, triangles), or high light (HL, squares), was measured after the addition of 25 µM (grey) or 750 µM (black) H2O2 and 1 µM (grey) or 50 µM (black) MV. Control cultures without stress induction are indicated by white symbols. Broken vertical lines indicate a change of time scale on the x-axis. These data are based on 3 to 5 independent experiments and are expressed as mean ± standard deviation.

Figure 3
Effect of photosynthetic electron transport on the methylviologen (MV)-mediated photoinhibition and H2O2 production. A. PSII maximal photochemical yield (Fv/Fm) was measured 2 h after the addition of 0, 1, 50 and 250 µM MV (white, dashed, crossed and dark bars respectively) to *Synechococcus* sp. WH7803 cells acclimated to high light (HL) and maintained in HL (HL → HL), acclimated to HL but shifted to low light (LL) 10 min before the stress initiation (HL → LL), or acclimated to LL and maintained under LL (LL → LL). The percentage of photoinactivation of stressed cells compared to untreated ones for the same light treatment is indicated above the bars. B. Scopoletin fluorescence was measured 0.5 h after the addition of MV in the same conditions as in A. The decrease of fluorescence is due to the scopoletin degradation by H2O2 from the cells. All these data are based on 3 independent experiments and are expressed as mean ± standard deviation.

Figure 4
D1 protein content in response to 25 µM H2O2. The D1 protein content (filled symbols) in low light- (LL, A) and high light- (HL, B) acclimated *Synechococcus* sp. WH7803 cultures was measured by western-blot analysis after addition of 25 µM H2O2 and expressed as percents of initial
D1 content. The evolution of the PSII quantum yield after the stress initiation, expressed as percent of initial values, is indicated by open symbols. These data are based on 3 independent experiments and are expressed as mean ± standard deviation.

Figure 5

Effect of H₂O₂ on the balance between PSII damage and repair. To estimate the initial PSII repair capacity of *Synechococcus* sp. WH7803, cells acclimated to low light (LL, A) and high light (HL, B) were maintained under their initial growth irradiance (triangle and square for LL and HL cells respectively) or shifted to dark (upside down triangles and diamonds) in the presence (Lin, filled symbols) or absence (open symbols) of lincomycin. PSII maximal photochemical yield (FV/FM) was then followed for 2 h. The effect of H₂O₂ on PSII damage and repair in LL- (C) and HL cells (D) was estimated on cells maintained under their initial growth irradiance or shifted in the dark, by measuring the FV/FM 2 h after the addition of lincomycin (dashed bars) or water (open bars) and of 0 µM (white bars), 25 µM (light grey bars) or 750 µM H₂O₂ (dark grey bars). These data are based on 3 independent experiments and are expressed as mean ± standard deviation.

Figure 6

Multivariate analyses of microarray data. Principal Component Analysis (PCA, A) and hierarchical clustering dendrograms (B) were performed using the R Stats package on a subset identified as significantly differentially expressed (FDR ≤ 0.05) in at least one of the pairwise comparisons. Experiment with low light (LL)- and high light (HL)-acclimated cells are indicated by filled triangles and open squares, respectively. Treatments using 25 µM H₂O₂, 750 µM H₂O₂ and 50 µM methylviologen (MV) are indicated by light blue, dark blue and dark green symbols respectively. Non-treated controls are in orange. In PCA, each replicate is indicated by one symbol and percentages of variability accounted by the two main components are shown nearby the axes.

Figure 7

Pairwise comparisons of microarray datasets. Venn diagrams were performed with genes showing a statistically significant (FDR ≤ 0.05, see material and methods) differential expression in response to the different treatments. The number of genes differentially expressed in each dataset is indicated near its representing symbol (see legend on the right). The percentage of concordance is related to genes that were significantly differentially expressed between the two compared conditions (represented by overlapping symbols) and that are additionally regulated in the same way (up- or down-regulation) in both conditions. A. Comparison of the transcriptomic responses to H₂O₂ and methylviologen (MV) treatments for both low light- (LL-) and high light- (HL-)
acclimated cells. B. Comparison of the pooled responses to oxidative stress (+/- oxidizing agent, i.e. genes responding to H₂O₂ and/or MV) between LL- and HL- acclimated cells. C. Comparison of the pooled responses to oxidative stress (+/- reactive oxygen stressors) in LL (upper signs/values) and HL (lower signs/values) cells and of the genes differentially expressed in HL- compared to LL-acclimated conditions. Concordant and discordant genes (i.e. common genes regulated in opposite way between two conditions) of this diagram are listed in Table 1.

Figure 8
Two-way cluster analysis diagram of gene expression data. This analysis is based on 1,548 genes, corresponding to all genes present on the array but microarray controls, small RNA oligonucleotides as well as unkown and hypothetical protein encoding genes. Each row in the diagram represents a gene and each column a light acclimation or a stress condition. The dendrogram at the top is similar to Fig. 6 except that each column was obtained from the mean of the 4 biological replicates performed in each condition. The color saturation represents differences in gene expression across the samples; red indicates higher than the median expression (black), and green indicates lower than median expression. The color intensity indicates degree of gene regulation. The boxes to the right of the diagram represent gene clusters A–E and show some of the genes present in these clusters and belonging to specific functional categories. Genes showing a statistically significant differential expression in at least one condition (Student’s t-test and/or LIMMA with FDR≤0.05) are indicated in blue.
DESCRIPTION OF ADDITIONAL DATA FILES

Supplemental Figure S1
Effect of methylviologen on ROS production through photosynthetic electron transport.
In the absence of methylviologen (MV), linear electron flow (LEF) from water to NADP through Photosystems (PS) II and I, and cyclic electron flow (CEF) around PSI, merge at the Cytochrome (Cyt) $b_6f$ complex. In the presence of MV, CEF is inhibited (plain purple cross) and only LEF remains. MV is reduced by components of the photosynthetic chain, chiefly by FeS center $F_b$ and to a lesser extent by FeS center $F_A$ of the electron acceptor complex of PSI. At saturating concentration, MV would channel out all electrons from PSI, thus blocking electron transfer to FNR (dotted purple cross). A one-electron reaction between reduced MV (MV$^{+}$) and O$_2$ results in the generation of the membrane impermeable superoxide anion $O_2^-$, which is disproportionated by superoxide dismutase (SOD) to the membrane impermeable H$_2$O$_2$ and to molecular oxygen O$_2$. The resulting H$_2$O$_2$ is reduced into water by catalases, peroxidases and/or catalase-peroxidases. An alternate reaction of $O_2^-$ with ferrous iron (Fe$^{3+}$) may lead to the reduction of H$_2$O$_2$ resulting in the formation of OH$^{-}$ via Fenton-type reactions.

Supplemental Figure S2
Effect of dark adaptation on PSII basal fluorescence in HL cells. *Synechococcus* sp. WH7803 cells acclimated to high light (HL) were shifted in the dark in the presence of lincomycin (open bars) or water as a control (filled bars). PSII basal fluorescence intensity (F$_0$), i.e. when all PSII centers are in the open state, was measured using modulated light before or 2 h after the addition of 0, 25 or 750 µM H$_2$O$_2$. These data are based on 3 independent experiments, given in mean ± the standard deviation.

Supplemental Figure S3
Graphical representation of the predicted LexA motif identified in *Synechococcus* sp. WH7803. The overall height of stacked symbols indicates the sequence conservation at the corresponding position, while the height of each symbol indicates the relative frequency of nucleotide. The logo has been obtained from the WebLogo application (http://weblogo.berkeley.edu).

Supplemental Table S1
Complete set of gene expression data as measured by microarray analyses. This table includes locus tags, gene names, probe names, product description as well as COG functional categories for all 2,497 genes and 126 potential small RNA present on the *Synechococcus* sp. WH7803 array.
Expression data are shown as log₂(FC) calculated for each experimental sample (blue background) as well as for the 5 pairwise comparisons performed in this study (HL-Ct vs. LL-Ct, LL+H₂O₂ vs. LL-Ct, LL+MV vs. LL-Ct, HL+H₂O₂ vs. HL-Ct and HL+MV vs. HL-Ct; green background). For the latter, p-values and adjusted p-values were calculated using LIMMA and t-test (beige background). Values highlighted in red correspond to genes and pairwise comparisons for which adjusted p-values (FDR) was ≤ 0.05 and/or log₂(FC) > 1. The last columns show p-values and adjusted p-values calculated with one-way and two-way ANOVA where group 1 corresponds to light acclimation and group 2 to oxidative stress treatment (purple background).

**Supplemental Table S2: Validation of Microarray data using qPCR analyses** on selected genes from *Synechococcus* sp. WH7803. Expression data for both qPCR and microarray are shown as log₂(FC) calculated for each pairwise comparison. Standard deviation (sd) for three biological replicates are shown for qPCR data and Microarray p-values indicated in this table were calculated using LIMMA. All genes and pairwise comparisons tested led to concordant results between qPCR and microarray data, *i.e.* that genes were detected as up- or down-regulated using both methods (Pearson's correlation coefficient : 85 %).
Table 1: List of genes showing concordant or opposite transcriptomic patterns between the effects of acclimation to HL and the effect of oxidative stress in low light-(LL) and high light- (HL) acclimated cells, as represented in Fig. 7C. Put.: Putative; Con.: Conserved; Un.: Uncharacterized; Hyp.: Hypothetical; Alt.: Alternative; * down-regulated by HL-acclimation also means up-regulated by LL acclimation.

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**Up-regulated by ROS in HL cells and down-regulated by HL-acclimation***

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**Down-regulated by ROS in HL cells and up-regulated by HL-acclimation**

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<tr>
<td>pstSII_1045</td>
<td>ABC transporter, substrate binding protein, phosphate</td>
<td></td>
</tr>
</tbody>
</table>

*0095, 0096, 0934, 1953: Hypothetical proteins
1796, 1798, 1799: Uncharacterized conserved secreted proteins, pil subunit superfamily
1732: Sulfate ion transporter
1115: 50S ribosomal protein L32
2002: Conserved hypothetical proteins
2198: Inositol monophosphatase family protein
Table 2. Putative LexA regulon and DNA binding sites in *Synechococcus* sp. WH7803

<table>
<thead>
<tr>
<th>Gene</th>
<th>Putative LexA binding site</th>
<th>Position from ATG (^a)</th>
<th>Log(_2) (fold change) from microarray data (^b)</th>
<th>Gene cluster (^c)</th>
<th>Gene product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HL-Ct/LL-Ct</td>
<td>LL+H(_2)O(_2)/LL-Ct</td>
<td>LL+MV/LL-Ct</td>
<td>HL+H(_2)O(_2)/HL-Ct</td>
</tr>
<tr>
<td><strong>genes with a putative LexA binding site in their upstream intergenic region</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>lexA</em></td>
<td>GGTACACATGTATT</td>
<td>-21</td>
<td>-0.1</td>
<td>0.9</td>
<td>(0.2)</td>
</tr>
<tr>
<td><em>recA</em></td>
<td>AGTACAGATGTACG</td>
<td>-137</td>
<td>-1.1</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td><em>umuD</em></td>
<td>AGTACAGATGTTCCT</td>
<td>-40</td>
<td>(-0.3)</td>
<td>2.6</td>
<td>1.2</td>
</tr>
<tr>
<td><em>umuC</em></td>
<td>AGTACACATGATT</td>
<td>+1</td>
<td>-0.5</td>
<td>0.6</td>
<td>(0.2)</td>
</tr>
<tr>
<td><em>dprA</em></td>
<td>AGTACACATGATT</td>
<td>-8</td>
<td>(-0.3)</td>
<td>(-0.1)</td>
<td>0.5</td>
</tr>
<tr>
<td><em>wcaG</em></td>
<td>AGTACAAACGTATT</td>
<td>-8</td>
<td>(-0.3)</td>
<td>(-1.1)</td>
<td>0.5</td>
</tr>
<tr>
<td>0096(^e)</td>
<td>AATACAGTTGATT</td>
<td>-88</td>
<td>-2.1</td>
<td>2.2</td>
<td>(0.6)</td>
</tr>
<tr>
<td>0814(^e)</td>
<td>GGTACGCCTGTTCCT</td>
<td>-96</td>
<td>(-0.6)</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>0843(^e)</td>
<td>AGTACAGATGTACT</td>
<td>-20</td>
<td>-0.7</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>1500(^e)</td>
<td>AGTACAGATGTACT</td>
<td>-13</td>
<td>-1.5</td>
<td>1.5</td>
<td>(0.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>consensus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RRTACRNNYGTWYK</td>
</tr>
</tbody>
</table>

| **genes with no LexA binding site found but whose orthologs are thought to be part of the *E. coli* LexA regulon** |
|---|----------------|
| *recN*    | (-0.4)         | (-0.2)         | (-0.5)         | (0.1)         | (-0.3)       | D | ATPase involved in DNA repair |
| *ruvB*    | (-0.3)         | (-0.2)         | (0.7)          | 0.9           | (0.4)        | B | Holliday ATP-dependent DNA helicase |
| *ssb*     | -0.8           | (-0.1)         | (0.5)          | 1.0           | 1.0          | A | Single-stranded DNA-binding protein |
| *uvrA*    | (0.1)          | (0.1)          | -0.7           | -1.2          | -1.2         | D | Excinuclease ATPase subunit |
| *uvrD*    | (-0.4)         | (-0.4)         | (-0.1)         | (-0.4)        | (-0.2)       | A | DNA-dependent ATPase I and helicase II |

\(^a\) positions have been calculated between the 1\(^{st}\) nucleotide of the start codon and the middle of the putative binding site  
\(^b\) values in parenthesis are not statistically significant, i.e. with FDR > 0.05  
\(^c\) see hierachical clustering analysis in Fig. 8, ND : not determined (hypothetical protein were excluded from the analysis)  
\(^d\) *umuD* and *umuC* genes are likely organized in operon  
\(^e\) the complete ORF number is SynWH7803_XXXX
Figure 1. Effect of photoacclimation on PSII photochemistry. PSII maximal quantum yield ($F_{v}/F_{m}$) of *Synechococcus* sp. WH7803 cells acclimated to low light (LL, closed triangles), or high light (HL, open squares), was measured 2 h after the addition of a range of concentrations of $H_{2}O_{2}$ (A) or methylviologen (MV, B). These data are based on 3 to 5 independent experiments and are expressed as mean ± standard deviation.
Figure 2. PSII activity in response to $H_2O_2$ (A) and methylviologen (MV, B). The time-course of changes in PSII maximal photochemical yield ($F_{v}/F_{m}$) of *Synechococcus* sp. WH7803 cells acclimated to low light (LL, triangles), or high light (HL, squares), was measured after the addition of 25 µM (grey) or 750 µM (black) $H_2O_2$ and 1 µM (grey) or 50 µM (black) MV. Control cultures without stress induction are indicated by white symbols. Broken vertical lines indicate a change of time scale on the x-axis. These data are based on 3 to 5 independent experiments and are expressed as mean ± standard deviation.
Figure 3. Effect of photosynthetic electron transport on the methylviologen (MV)-mediated photoinhibition and H$_2$O$_2$ production. **A.** PSII maximal photochemical yield ($F_{V}/F_{M}$) was measured 2 h after the addition of 0, 1, 50 and 250 µM MV (white, dashed, crossed and dark bars respectively) to *Synechococcus* sp. WH7803 cells acclimated to high light (HL) and maintained in HL (HL → HL), acclimated to HL but shifted to low light (LL) 10 min before the stress initiation (HL → LL), or acclimated to LL and maintained under LL (LL → LL). The percentage of photoinactivation of stressed cells compared to untreated ones for the same light treatment is indicated above the bars. **B.** Scopoletin fluorescence was measured 0.5 h after the addition of MV in the same conditions as in A. The decrease of fluorescence is due to the scopoletin degradation by H$_2$O$_2$ from the cells. All these data are based on 3 independent experiments and are expressed as mean ± standard deviation.
Figure 4. D1 protein content in response to 25 µM H₂O₂. The D1 protein content (filled symbols) in low light- (LL, A) and high light- (HL, B) acclimated Synechococcus sp. WH7803 cultures was measured by western-blot analysis after addition of 25 µM H₂O₂ and expressed in percent of the initial D1 content. The evolution of the PSII quantum yield after the stress initiation, expressed in percent of initial activity, is indicated by open symbols. These data are based on 3 independent experiments and are expressed as mean ± standard deviation.
Figure 5. Effect of H$_2$O$_2$ on the balance between PSII damage and repair. To estimate the initial PSII repair capacity of *Synechococcus* sp. WH7803, cells acclimated to low light (LL, A) and high light (HL, B) were maintained under their initial growth irradiance (triangle and square for LL and HL cells respectively) or shifted to dark (upside down triangles and diamonds) in the presence (Lin, filled symbols) or absence (open symbols) of lincomycin. PSII maximal photochemical yield ($F_{V}/F_{M}$) was then followed for 2 h. The effect of H$_2$O$_2$ on PSII damage and repair in LL- (C) and HL cells (D) was estimated on cells maintained under their initial growth irradiance or shifted in the dark, by measuring the $F_{V}/F_{M}$ 2 h after the addition of lincomycin (dashed bars) or water (open bars) and of 0 µM (white bars), 25 µM (light grey bars) or 750 µM H$_2$O$_2$ (dark grey bars). These data are based on 3 independent experiments and are expressed as mean ± standard deviation.
Figure 6. Multivariate analyses of microarray data. Principal Component Analysis (PCA, A) and hierarchical clustering dendrograms (B) were performed using the stats R package on a subset identify as significantly differentially expressed (FDR ≤ 0.05) in at least one of the pairwise comparisons. Experiment with low light (LL)- and high light (HL)-acclimated cells are indicated by filled triangles and open squares, respectively. Stress induced by 25 µM H₂O₂, 750 µM H₂O₂ and 50 µM methylviologen (MV) are indicated by light blue, dark blue and dark green symbols respectively. Non-stressed controls are in orange. In PCA, each replicate is indicated by one symbol and percentages of variability accounted by the two main components are shown nearby the axes.
Figure 7. Pairwise comparisons of microarray datasets. Venn diagrams were performed genes showing a statistically significant (FDR ≤ 0.05, see material and methods) differential expression in response to the different treatments. The number of genes differentially expressed in each dataset is indicated near its representing symbol (see legend on the right). The percentage of concordance is related to genes that are significantly differentially expressed between the two compared conditions (represented by overlapping symbols) and that are additionally regulated in the same way (up- or down-regulation) in both conditions. A. Comparison of the transcriptomic responses to H$_2$O$_2$ and methylviologen (MV) treatments for both low light- (LL) and high light- (HL) acclimated cells. B. Comparison of the pooled responses to oxidative stress (+/- oxidizing agent, i.e. genes responding to H$_2$O$_2$ and/or MV) between LL- and HL-acclimated cells. C. Comparison of the pooled responses to oxidative stress (+/- reactive oxygen stressors) in LL (upper signs/values) and HL (lower signs/values) cells and of the genes differentially expressed in HL compared to LL acclimation condition. Concordant and discordant genes (i.e. common genes regulated in opposite way between two conditions) of this last diagram are listed in Table 1.
A. Up-regulated in LL + oxidant

- Photosynthesis: PSII (psbA_0784)
- Photoprotection & pigment synthesis: chlGLMN, hli_2059
- Redox & ROS detoxification: katG, ferredoxin (petf_1242/2014, petH, 2053), glutathione (gst_2216)
- DNA Repair: phr, recO, ssb, uvrD
- Chaperones & proteases: clpB_0748/0749, clpS_0369, dnaJ_0023, dnaK_2514, gcp, gro1L2, hsp_2403
- Others: phosphate uptake & metabolism (phoHR, phnDE, dnaX_2100, divB_2237, ftsH(ftsH_0355/2216/1699), fur_1382/2447, division (ftsQ, ftsW), nblS

B. Up-regulated in LL & HL + oxidant

- Photosynthesis: PSII (psbA_0790/2084, psbD_1647/2239, psbB_2080)
- NADH dehydrogenase: ndhAGJ, ndhD_0686/0772
- Photoprotection & pigment synthesis: chlB, hli_0054
- Redox & ROS detoxification: sodC[Fe], thioredoxin (txlA, 0264, 0593, 1634), glutaredoxin (2322), glutathione (gloB, gor, gshB, 1529, 2089)
- DNA Repair: dpsA, dprA, lexA, mutSY, nth, recA, sbcD, umuCD, xseB
- Chaperones & proteases: clpC, clpP2, clpX, degG, dnaJ_2100, grpe, gro1, htsO, htrN, pepNP, sppA
- Others: phosphate uptake & metabolism (phoC, phoD, ptrA, som_1178, division (ftsQ, ftsW), nblS

C. Up-regulated in HL + oxidant

- Photosynthesis: PSII (psbA_0366)
- NADH dehydrogenase: ndh_2175, ndhBHKM, ndhD_2280, ndhf_0695/2278
- Photoprotection & pigment synthesis: chlI, hli_0054
- Redox & ROS detoxification: ftrC, ferredoxin (nrfA), peroxidoxin (pxQ_1042/1236), thioredoxin (txIB)
- DNA Repair: radA, recF, uvrBC
- Others: phosphate uptake & metabolism (pskB, psr_2513, FtsH(ftsH_0355/2216/1699), fur_1382/2447, division (ftsQ, ftsW), nblS

D. Down-regulated in LL + oxidant

- Ribosomal proteins: rplIS, rpmJ, rpsKL
- Photosynthesis: phycobilisome (cpeC, cpeF), Calvin cycle (pgk_2357), ATP synthase (atpH)
- Photoprotection & pigment synthesis: ctp, hli_0054
- Redox & ROS detoxification: sodB[Fe], ferredoxin (0625), glutathione (gloA)
- DNA Repair: recBN, uvrA
- Others: phosphate uptake & metabolism (phoHR, phnDE, DNA polymerase (rpoAC2Z), stress (usp)

E. Down-regulated in LL & HL + oxidant

- Ribosomal proteins: rplA2, rplM, rpsKLMNOU
- Photosynthesis: phycobilisome (apoABCDEF, apoG1G2, apoABERSTUZ, mpeABDEV, rpcAB, pebAB, hli_1646, PSII (psbCBE1KMNOTUVXYZ, psb28/29), Calvin cycle (prk, rbcLRS, rbsK, ppe, lktA), ATP synthase (atpACDEFGH)
- NADH dehydrogenase: ndh_0345, ndhCEL
- Photoprotection & pigment synthesis: chlHP, crtB
- Redox & ROS detoxification: ftrV, ruh, ferredoxin (petf_1580/1979, 1624, 1659), glutathione (gst_0820, 0151), peroxidoxin (pxQ_2172, 2-Cvs-prx, glutaredoxin (1375), thioredoxin (trxA)
- DNA Repair: mutM, recDFJG, xthA, mcrA_0404, sbcD, xseA
- Chaperones & proteases: clpP1, clpS_1210, clpX, dnaJ_1247
- Others: phosphate uptake & metabolism (phoB, ppa_0605/0624, pppK_1710/2287, ppk, ppx, pstB, pstS_1045, som_0993/2235/2236), DNA polymerase (rpoBC1), division (ftsZ, maf), stress (uspA), fur_1162 (perR homolog), ntcA