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ORIGINAL ARTICLE

# Transcriptional response of the photoheterotrophic marine bacterium *Dinoroseobacter shibae* to changing light regimes

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**Bacterial aerobic anoxygenic photosynthesis (AAP) is an important mechanism of energy generation in aquatic habitats, accounting for up to 5% of the surface ocean's photosynthetic electron transport. We used *Dinoroseobacter shibae*, a representative of the globally abundant marine *Roseobacter* clade, as a model organism to study the transcriptional response of a photoheterotrophic bacterium to changing light regimes. Continuous cultivation of *D. shibae* in a chemostat in combination with time series microarray analysis was used in order to identify gene-regulatory patterns after switching from dark to light and vice versa. The change from heterotrophic growth in the dark to photoheterotrophic growth in the light was accompanied by a strong but transient activation of a broad stress response to the formation of singlet oxygen, an immediate downregulation of photosynthesis-related genes, fine-tuning of the expression of ETC components, as well as upregulation of the transcriptional and translational apparatus. Furthermore, our data suggest that *D. shibae* might use the 3-hydroxypropionate cycle for $CO_2$ fixation. Analysis of the transcriptome dynamics after switching from light to dark showed relatively small changes and a delayed activation of photosynthesis gene expression, indicating that, except for light other signals must be involved in their regulation. Providing the first analysis of AAP on the level of transcriptome dynamics, our data allow the formulation of testable hypotheses on the cellular processes affected by AAP and the mechanisms involved in light- and stress-related gene regulation.**

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## Introduction

Aerobic anoxygenic photoheterotrophic bacteria (AAPB) represent a diverse group of proteobacteria capable of transforming light energy through bacteriochlorophyll-*a* (BChl-*a*)-based photosystems into a proton gradient that is used for the generation of biochemical energy in the form of ATP (Yurkov and Beatty, 1998). AAPB are widely distributed in marine plankton where they account for less than 1% to 25% of the total microbial community, depending on the site of study (Beja *et al.*, 2002; Oz *et al.*, 2005; Cottrell *et al.*, 2006, 2010; Lami *et al.*, 2007; Jiao *et al.*, 2010). An early study by Kolber *et al.* (2000) estimated the contribution of aerobic anoxygenic photosynthesis (AAP) to the surface ocean's electron transport to be as high as 5%, a finding that has been doubted (Goericke, 2002). In a recent global survey Jiao *et al.* found that AAP contributes 2% and 5.7%, respectively, to the phototrophic energy flow in shelf waters and oligotrophic oceans. They concluded that BChl-*a*-based AAP activity—supplementing chlorophyll-*a*-based phototrophy—might be a factor that determines whether a marine region functions as a source or sink of atmospheric $CO_2$ (Jiao *et al.*, 2010).

In contrast to the closely related phototrophic purple bacteria performing anaerobic anoxygenic photosynthesis, AAPB synthesize BChl-*a* exclusively in the presence of oxygen (Yurkov and Beatty, 1998) that is strictly required for the functioning of the photosystems (Yurkov and Beatty, 1998; Koblizek *et al.*, 2010). Light-excited BChl-*a* can transfer energy to oxygen, leading to the formation

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of toxic singlet oxygen ($^1O_2$) (Borland *et al.*, 1989). To mitigate the negative effects of this AAP by-product, the synthesis of pigments and the photosynthetic apparatus are shut down in response to light exposure (Yurkov and van Gemerden, 1993; Biebl and Wagner-Döbler, 2006). In all sequenced proteobacteria using light as a source of energy, the vast majority of photosynthesis-related genes are organized in a ~45-kb gene cluster, indicating the need for tight regulation of their expression (Elsen *et al.*, 2005).

Energy and biomass yield resulting from AAP have been demonstrated for several strains (Yurkov and van Gemerden, 1993; Biebl and Wagner-Döbler, 2006; Koblizek *et al.*, 2010). Although all AAPB sequenced so far lack genes encoding ribulose bisphosphate carboxylase/oxygenase (RuBisCO), an enzyme required in the Calvin cycle for carbon fixation, as well as genes for other autotrophic $CO_2$ fixation pathways (Fuchs *et al.*, 2007; Swingley *et al.*, 2007; Newton *et al.*, 2010; Wagner-Döbler *et al.*, 2010), light-dependent $CO_2$ fixation has been demonstrated in some *Erythrobacter* strains (Koblizek *et al.*, 2003) and in *Roseobacter denitrificans* (Tang *et al.*, 2009). The latter uses the anaplerotic pathway mainly through the malic enzyme in order to fix 10–15% of the protein carbon from $CO_2$.

Because of their putative importance for marine ecosystems and global nutrient cycles, the ecology and physiology of marine AAPB have been well studied. However, it has not yet been shown how the bacterial cell responds to the process of AAP on the transcriptional level. This study aimed to fill this gap by using *Dinoroseobacter shibae* DFL12 as a model organism for analysing the impact of light on the transcriptome of this photoheterotrophic bacterium. *D. shibae* DFL12 belongs to the globally abundant marine *Roseobacter* clade whose representatives are frequently found among marine AAPBs (Wagner-Döbler and Biebl, 2006; Brinkhoff *et al.*, 2008). It has been isolated from the phototrophic dinoflagellate *Prorocentrum lima* and is thought to live in a symbiotic relationship with its host (Biebl *et al.*, 2005). The genome of *D. shibae* DFL12 is fully sequenced and the automatic annotation has been verified manually (Wagner-Döbler *et al.*, 2010). This strain can be cultivated on a defined mineral medium with a single carbon source (Fürch *et al.*, 2009) and it is accessible to genetic modification (Piekarski *et al.*, 2009). For these reasons, *D. shibae* DFL12 is a well-suited candidate for transcriptome analysis. Furthermore, a relatively high BChl-*a* content as compared with that in other AAPB as well as an impact of light on the growth rate, biomass formation and BChl-*a* synthesis of this organism have been reported (Biebl and Wagner-Döbler, 2006). Steady-state chemostat cultivation in combination with time series microarray analysis was used to monitor the transcriptome dynamics in *D. shibae* after the transition from heterotrophic–dark to photoheterotrophic–light growth and vice versa. Clustering of genes according to the shape of time-dependent expressional changes and biological function was performed in order to identify the cellular processes affected by changing light regimes as well as their putative regulation.

## Materials and methods

Additional details on materials and methods can be found in Supplementary Material S1.

*Cultivation*

Continuous cultivation of *D. shibae* DFL12T was performed in a defined minimal medium in a Biostat B-Reactor (Sartorius, Göttingen, Germany) at 30 °C (pH 8.0), with aeration of 0.55 l per minute and a stirring speed of 250 r.p.m. The reactor volume was 1 l and the working volume was 500 ml. The pH was adjusted automatically with 0.1 M HCl and 0.1 M NaOH. The oxygen saturation of the culture adjusted itself to ~85% in the steady state. $CO_2$ in the off-gas was analysed with a Maihak S710 gas analyser. The chemostat was covered with aluminium foil to avoid disturbance of the experiment by external light sources. Illumination with a 35-W halogen bulb (Osram, Munich, Germany) resulted in a photon flow of 100 $\mu E\,m^{-2}\,s^{-1}$ as measured at the inner surface of the bioreactor. The bioreactor was inoculated with 2% of a pre-culture grown in a flask in the same medium as the main culture at 30 °C in darkness to an $OD_{650}$ of ~0.7. Feeding with fresh medium was started after approximately 20 h when the oxygen saturation in the culture had reached a minimum. The dilution rate was 0.1 $h^{-1}$, corresponding to approximately the half-maximum growth rate of *D. shibae* in the exponential phase.

*Determination of BChl-*a *content*

A 10-ml volume of the bioreactor outflow was centrifuged at 6000 *g* for 20 min. The supernatant was removed completely and the pellet was resuspended in 50 µl of artificial sea water. Pigments were extracted for 1 h with 1 ml acetone/methanol (7:2); BChl-*a* absorption was determined at 772 nm and BChl-*a* content was calculated using an extinction coefficient of 75 $mmol\,l^{-1}\,cm^{-1}$ (Biebl and Wagner-Döbler, 2006).

*Determination of dry weight*

A 10-ml volume of the bioreactor outflow was centrifuged at 6000 r.p.m. for 20 min. For all washing steps the supernatant was not removed completely to reduce the osmotic shock of the cells. Pellets were washed three times with MilliQ-$H_2O$ and dried at 80 °C until the weight of the pellet remained constant.

*Microarray experiment and data analysis*
Microarrays for the first time series monitoring the dark–light transition were performed in three to four biological replicates. Microarrays for the second time series monitoring the light–dark transition were performed in only two biological replicates as the statistical power was still high for this number of replicates, and we found the results highly consistent with the data from the first time series. A 2-μg weight of total RNA was labelled with Cy3 using the ULS-system (Kreatech, Amsterdam, The Netherlands) according to the manufacturer's manual. A 600-ng weight of the labelled RNA was fragmented and hybridized to the microarray according to Agilent's one-colour microarray protocol. The microarrays were scanned using a GenePix Pro 4001 scanner and the GenePix 4.0 software. Data processing was performed in the R environment (http://www.cran.r-project.org/) using the LIMMA package (Smyth, 2005) of the BioConductor project (http://www.bioconductor.org/). In addition, the R-script ComBat was used to eliminate batch-specific effects from the data (Johnson *et al*., 2007). Only genes with a *P*-value $<0.001$ and an absolute log2 fold change (FC) $>0.58$ were considered in subsequent analyses.

*Microarray data*
Raw and processed microarray data have been deposited in the GEO database under accession number GSE25591.

## Results and discussion

*Physiological changes of* D. shibae *during the shift between heterotrophic–dark and photoheterotrophic–light growth*
A continuous chemostat strategy was chosen for the cultivation of *D. shibae*. Two time series were analysed: For the first time series, aiming to monitor the switch from a dark-adapted culture to growth in the light, the bacteria were grown for four residence time periods in the dark to ensure that the culture was in a steady state before the experiment was started (Figure 1a). An adaptation of this strategy for the second time series, aiming to monitor the switch of a light-adapted culture to growth in the dark, was not possible as *D. shibae* cells tended to clog heavily and attach to the glass surface of the cultivation vessel when cultivated for more than 12 h in the light. For this reason, *D. shibae* was cultivated in 12-h dark–light cycles for the second time series. (Figure 1b). During growth in the light, a reproducible decrease in both oxygen consumption and $CO_2$ evolution was measured for each of the cultivations (Figures 1a and b). Furthermore, the biomass increased during growth in the light and decreased again in the following dark period (Figures 1c and d).

These findings indicate that light-driven cyclic electron transport led to a reduction of linear electron transport towards the terminal oxidases and thus reduced oxygen consumption. The respiration of the carbon source succinate was reduced when *D. shibae* used photophosphorylation to gain energy, as indicated by the lower level of $CO_2$ in the offgas and the increase in biomass. As the energy driving cyclic electron flow is not available in the dark, the carbon source is used again as the electron donor to feed the respiratory chain. This is shown by the decrease in biomass and $O_2$, as well as by the increase in $CO_2$.

During growth in the light, an immediate and continuous decrease in BChl-*a* concentration was observed, indicating a fast shutdown of the biosynthesis pathway for this pigment and its subsequent washout (Figure 1c). The reduction of photoactive pigments and thus the decrease in energy generation through cyclic electron transport also explains the continuous increase in the respiratory electron flow during growth in the light as indicated by the slight diminution of dissolved $O_2$. When the culture was deprived of light after 12 h of photoheterotrophic growth, the BChl-*a* concentration remained constant for 4 h before starting to increase (Figure 1d).

*Transcriptional dynamics in* D. shibae *following a change in the light regime*
For both time series a control sample before and samples 15 min, 30 min, 1 h, 2 h, 4 h and 8 h after the change in the light regime were analysed. When a dark-grown, strongly pigmented culture is exposed to light, cyclic electron transport leads to a decrease in the respiration rate and an increase in the rate of ATP synthesis, and the $^1O_2$ concentration in the cell can be assumed. In light of these manifold physiological changes, a broad response on the transcriptional level is expected. By contrast, in light-grown, weakly pigmented cultures photophosphorylation is low and small amounts of $^1O_2$ are generated. The deprivation of light is assumed to cause only minor changes in the physiological state of the cell. Therefore, only genes directly regulated by light are expected to show major changes in expression. Indeed, both time series support these two assumptions: 1358 and 386 genes, respectively, were expressed differentially during the transition from light to dark and vice versa (Supplementary Table S4). Despite the high number of genes, the number of distinct expression profiles was rather small. Based on the shape of the expression curves, six different categories could be distinguished for the transition from dark to light (Figure 2a): One group of 52 genes was permanently downregulated (Cluster-1); one putative operon of five genes was immediately and permanently upregulated in the light (Cluster-2); 331 genes reached a maximum in expression 15 min after the shift, followed by a sharp decrease in their expression (Cluster-3); 118 genes showed an upregulation, with a maximum within the first 30 min after the shift, followed by a

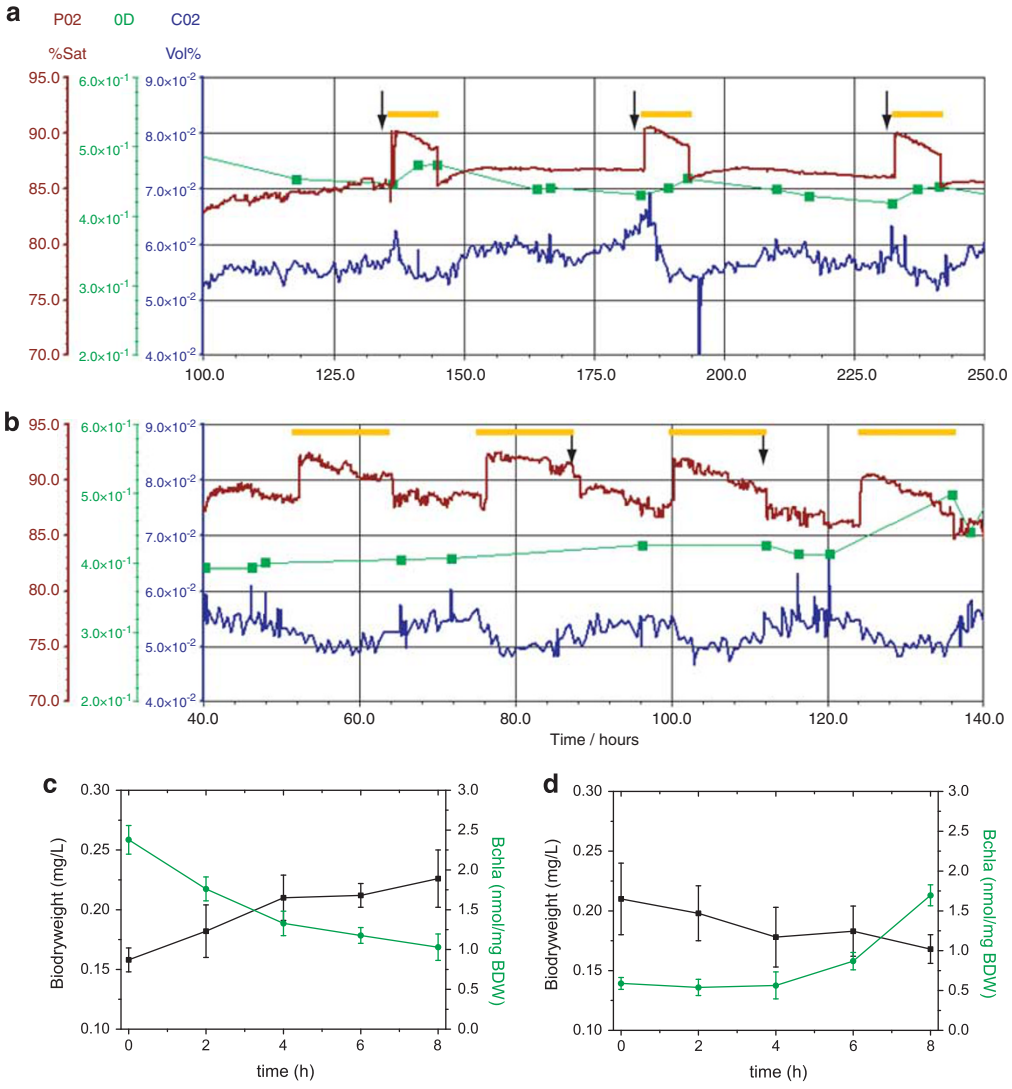


**Figure 1** Cultivation of *D. shibae* under changing light regimes. (**a**, **b**) Change of dissolved oxygen (upper red line), $CO_2$ evolution (lower blue line) and $OD_{650}$ (green squares) under changing light regimes (yellow bar: light). The starting points for sampling are indicated by black arrows. (**a**) Continuous cultivation from which samples were taken for the dark–light transition. (**b**) Continuous cultivation from which samples were taken for the light–dark transition. (**c**) Changes in bio-dry-weight (BDW) and BChl-*a* content when a dark-adapted culture is exposed to light. (**d**) Changes in BDW and BChl-*a* content when a light-adapted culture is deprived of light. The mean values and standard deviations from at least three biological replicates are shown. BChl-*a*, bacteriochlorophyll-*a*.

slow decrease in expression (Cluster-4); 172 genes reached a maximum in expression 2 h after the shift (Cluster-5); and 670 genes showed a sharp down-regulation, with a minimum at 15 min after the shift, before recovering slowly to pre-shift levels (Cluster-6). For the light–dark transition, only three different categories could be distinguished according to the shape of expression curves (Figure 2b). A total of 386 genes in total were changed, of which 58 were unique in this second data set. A group of 52 genes, identical to those in Cluster-1 of the first data set, were strongly upregulated 4 h after the shift from light to dark (Cluster-7); 43 genes, most of them also present in Clusters 2 and 3, were immediately and permanently downregulated (Cluster-8). The largest group comprising 288 genes showed an initial moderate upregulation followed by a downregulation 4 h after the transition (Cluster-9). For all clusters from both time series we found an enrichment of KEGG functions (Supplementary Table S5). Furthermore, it was possible to assign expression patterns to specific biological processes that will be discussed in the following sections.

### *Transcription-, translation- and anabolism-related genes*

Most of the genes encoding the RNA–polymerase complex, subunits of the ribosome and enzymes loading the tRNAs with amino acids showed a transient upregulation in the light and could be found in Clusters 4 and 5 (Supplementary Table S5). The coordinated expression of operons encoding ribosomal proteins has been studied in detail (Asato,

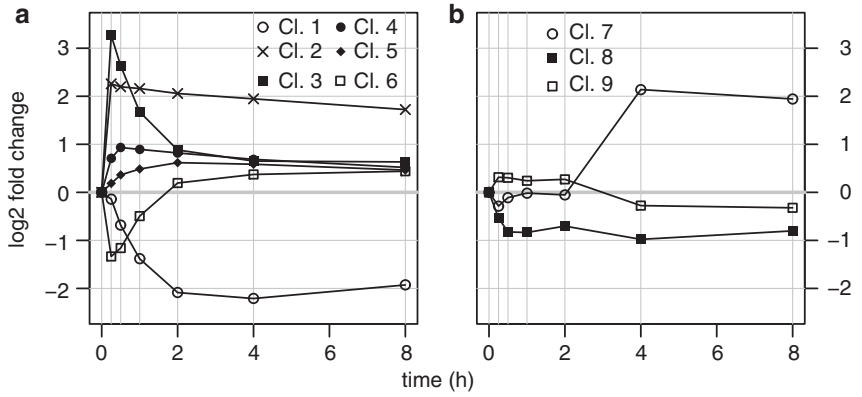


**Figure 2** An overview of *D. shibae* transcriptome dynamics. The log2 FCs of various time points as compared with the control condition are plotted for the means of dominating clusters. The KEGG pathways enriched in each cluster are found in Supplementary Table S5. (**a**) The time series of samples from a dark-adapted culture in the light. (**b**) The time series of samples from a light-grown culture deprived of light. The symbols represent clusters (Cl.) mentioned in the text. FC, fold change.

**Table 1** *C. auraticus* genes of the 3-hydroxypropionate cycle with homologues in *D. shibae*

| C. auraticus[a] | D. shibae[a] | *Description*[b] | *% Identity*[c] | *E-value*[c] | *Reaction*[d] |
|---|---|---|---|---|---|
| Caur_1648 | Dshi_0131 | Acetyl-CoA carboxylase, β-subunit | 43.85 | 1.E−59 | 1 |
| Caur_1647 | Dshi_3146 | Acetyl-CoA carboxylase, α-subunit | 53.85 | 7.E−75 | 1 |
| Caur_2614 | Dshi_2182 | Malonyl-coenzyme-A reductase (NADP) | 35.03 | 5.E−20 | 2 |
| Caur_0613 | Dshi_3553 | 3-Hydroxypropionyl-CoA synthetase | 31.86 | 1.E−57 | 3 |
| Caur_2034 | Dshi_1300 | Propionyl-CoA carboxylase | 63.01 | 0.E+00 | 4 |
| Caur_3037 | Dshi_2630 | Methylmalonyl-CoA epimerase | 34.81 | 3.E−13 | 5 |
| Caur_0179 | Dshi_1057 | Succinyl-CoA:L-malate-CoA transferase sub.-A | 32.03 | 4.E−52 | 7 |
| Caur_0178 | Dshi_1057 | Succinyl-CoA:L-malate-CoA transferase sub.-B | 32.59 | 2.E−56 | 7 |
| Caur_0174 | Dshi_2490 | L-erythro-3-methylmalyl-CoA lyase | 35.19 | 5.E−41 | 10 |
| Caur_0173 | Dshi_2871 | L-erythro-3-methylmalyl-CoA dehydratase | 56.61 | 1.E−107 | 11 |
| Caur_0175 | Dshi_2494 | Mesaconyl-CoA C1–C4 CoA transferase | 54.05 | 2.E−125 | 12 |
| Caur_0180 | Dshi_2493 | Mesaconyl-C4-CoA hydratase | 43.88 | 2.E−48 | 13 |

[a]Locus tags of *C. auraticus* genes and homologues in *D. shibae*.
[b]Description according to the *C. auraticus* annotation.
[c]Percent identities and E-values of protein alignments.
[d]The number of the reaction in the *C. auraticus* 3-hydroxypropionate cycle according to Zarzycki *et al.* (2009).

2005). Other genes found within Cluster-4 encode the NADP transhydrogenase (Dshi_1233, Dshi_1234) transferring electrons from NADH to NADP+. Several enzymes of amino-acid and fatty acid anabolism were found in this cluster, too. These findings strengthen the hypothesis that reducing power is redirected from the respiratory chain towards biosynthesis of cellular compounds. Only one gene of the thiamine biosynthesis pathway passed the applied filtering criteria. ThiC encoded on a plasmid (Dshi_3877) was upregulated during photoheterotrophic growth, with a maximum activation of 2.4-fold (log2 FC 1.15) after 1 h. Other genes in this pathway show a similar expression profile, although a lower level of induction. For most of the genes in Clusters 4 and 5 there was no shift in expression when a light-adapted culture of *D. shibae* was deprived of light. Therefore, we assume that they reached pre-shift levels after 12 h of growth in the light. In summary, these findings show that the energy gained from photophosphorylation is immediately used to build up biomass, and that the genes necessary for anabolic process are transcribed in a highly coordinated manner.

*Putative $CO_2$ fixation pathways*

The search for enriched KEGG pathways showed that genes belonging to a proposed reductive carboxylate cycle for $CO_2$ fixation (Evans *et al.*, 1966) were enriched in Clusters 3 and 6 (Supplementary Table S5). Furthermore, our attention was drawn to the putative operon Dshi_1989–Dshi_1993 owing to its unique expression profile in response to light (Cluster-2). A BLAST (Altschul *et al.*, 1990) search of the corresponding but poorly annotated genes showed a high similarity to some of the genes involved in the 3-hydroxypropionate cycle, a mechanism of $CO_2$ fixation in *Chloroflexus auraticus* (Zarzycki *et al.*, 2009). In a subsequent BLAST search, putative homologues of the remaining genes involved in this mechanism in *C. auraticus* were found in the genome of *D. shibae* (Table 1). The

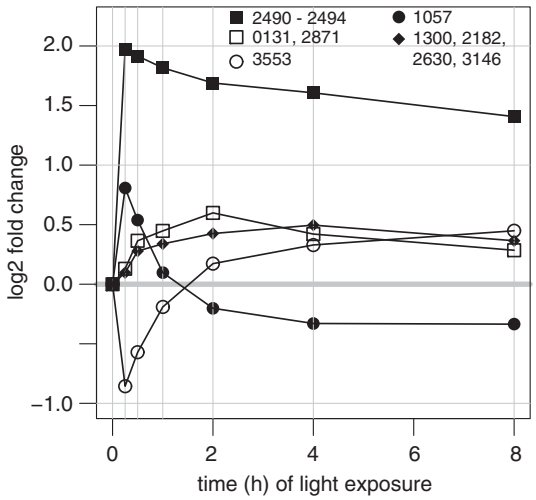


**Figure 3** Light-dependent regulation of putative $CO_2$ fixation genes. The log2 FCs of putative 3-hydroxypropionate cycle genes as compared with the control sample are plotted against the time the culture has been subjected to light. The symbols represent single genes or the mean of expression change for genes with a similar profile. Locus tags are indicated in the graphical legend. FC, fold change.

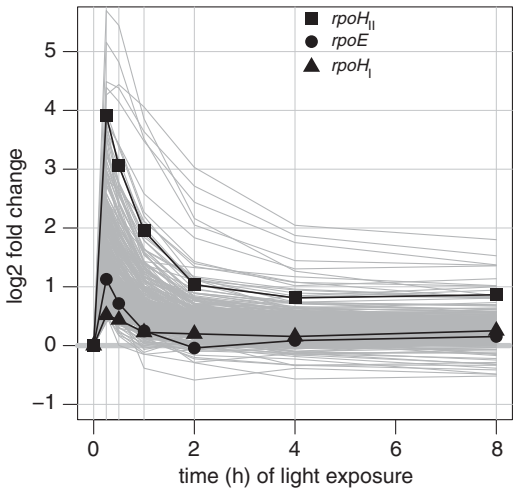


**Figure 4** Transcriptional response to $^1O_2$ stress in *D. shibae*. The log2 FCs of putative $^1O_2$ stress response genes as compared with the control sample are plotted against the time the culture has been subjected to light. The grey lines represent expression profiles. Genes encoding sigma factors are highlighted. FC, fold change.

key enzyme mesaconyl coenzyme-A hydratase (Caur_0173) was highly conserved in *D. shibae* (Zarzycki *et al.*, 2008). However, the similarities between the malonyl-coenzyme-A reductases and methylmalonyl-CoA epimerases of both organisms were rather weak (Table 1). The expression profiles of the putative $CO_2$ fixation-related genes were quite different from each other, but with the exception of the putative 3-hydroxypropionyl-CoA synthetase (Dshi_3553) all genes were upregulated in the light (Figure 3). The $CO_2$ fixation mechanisms in *D. shibae* need to be verified experimentally.

*Singlet oxygen stress response—regulation*

During the process of photophosphorylation, light energy-excited electrons are transferred to ubiquinone, which is reduced to ubiquinol. Electrons in the BChl-*a* molecule are restored through a back-cycling mechanism involving cytochrome *c*. Light-excited BChl-*a* electrons can transfer their energy to $^3O_2$, thereby forming $^1O_2$ (Borland *et al.*, 1989). This highly reactive form of oxygen damages proteins, lipids and DNA (Halliwell, 2006), and is therefore a major challenge for all bacteria exposed to photosensitizers (Dufour *et al.*, 2008), especially for AAP bacteria (Berghoff *et al.*, 2010). In the anaerobic anoxygenic photosynthetic bacterium *Rhodobacter sphaeroides* three alternative sigma factors are involved in the transcriptional response to this toxic by-product of photosynthesis: The heterodimer consisting of the sigma factor RpoE and its corresponding anti-sigma factor ChrR dissociates in the presence of $^1O_2$ (Anthony *et al.*, 2004) so that freed RpoE can direct the RNA polymerase complex to its own and to other specific promoters, thereby activating more than 180 genes, either directly or indirectly (Anthony *et al.*, 2005). Likewise, the sigma factor genes $rpoH_I$ (Nuss *et al.*, 2010) and $rpoH_{II}$ (Nuss *et al.*, 2009) are activated, whereas only the latter is a direct target of RpoE.

Homologues to all three *R. sphaeroides* sigma factors are present in the genome of *D. shibae* and are upregulated immediately but transiently in response to light exposure, therefore belonging to Cluster-3 (Figures 2a and 4). Taking into account the vast number of *R. sphaeroides* genes dependent on RpoE activity, it could be argued that virtually all 331 genes in Cluster-3 might be controlled by RpoE, $RpoH_{II}$ or $RpoH_I$, and thus have a role in the $^1O_2$ stress response. The putative biological function of selected genes within this cluster is discussed in the following sections.

It should be noted that the action of RpoE might explain the observation of a general downregulation of genes in *D. shibae* within the first 30 min after the shift from dark to light (Supplementary Material S2). Assuming that RpoE and ChrR are permanently present in the cell and light-driven formation of $^1O_2$ leads to instant activation of RpoE, the ratio of the various active sigma factors present in the cell will be altered drastically. As sigma factors compete for binding to the RNA polymerase core complex, the activation of an alternative sigma factor leads to a displacement of all others, including the 'housekeeping' sigma factors, and hence to a reduction in the expression of the genes they control (Mooney *et al.*, 2005). The upregulation of RpoE-controlled genes must therefore occur at the expense of downregulation all other genes.

*Singlet oxygen stress response—cryptochromes*
The genome of *D. shibae* harbours four genes with a conserved photolyase domain, among which are three cryptochromes (Dshi_0599, Dshi_1225, Dshi_1389) and the deoxyribodipyrimidine photolyase *phrB* (Dshi_2318), which mediates the light-driven repair of the pyrimidines in the DNA that have been dimerized by UV light (Sancar *et al.*, 1984). These four genes showed a strong upregulation in the light, highly similar to that of *rpoE* and $rpoH_{II}$, suggesting that they are part of the response to singlet oxygen. Indeed, *phrB* has been shown to be a target of RpoE in *R. sphaeroides* (Hendrischk *et al.*, 2007).

Cryptochromes are blue light sensors that can be found in all kingdoms of life. They have a role in circadian regulation in plants and animals (Cashmore, 2003), although their function in prokaryotes is still under debate (Purcell and Crosson, 2008). The involvement of a cryptochrome in the transcriptional regulation of photosynthesis-related genes has been shown recently in *R. sphaeroides*, although the mechanism of this control could not been clarified yet (Hendrischk *et al.*, 2009).

*Singlet oxygen stress response—detoxification*
One of the major threats to the $^1O_2$-exposed cell is the formation of partially oxidized products such as protein and lipid peroxides (Davies, 2004; Hayes *et al.*, 2005). Several mechanisms of detoxification have evolved throughout the kingdoms of life, including superoxide dismutases, catalases and glutathione peroxidases (Margis *et al.*, 2008). The glutathione peroxidase (Dshi_2055) catalysing the reduction of $H_2O_2$ or organic hydroperoxides (EC 1.11.1.9) was found in Cluster-3, with a maximum log2 3.3-fold upregulation. Both superoxide dismutase (Dshi_1067), detoxifying superoxide radicals, and catalse (Dshi_3801), catalysing the degradation of hydrogen peroxide, showed an upregulation in response to light. The latter was found in Cluster-5 and therefore might not be regulated by RpoE/$RpoH_{II}$ or $RpoH_I$. As both enzymes are known to be damaged by $^1O_2$, their upregulation might be necessary in order to replace the inactivated enzymes (Kim *et al.*, 2001, 2002). It is noteworthy that superoxide dismutase has been shown to have a protective role against $^1O_2$ in *Agrobacterium tumefaciens* cells (Saenkham *et al.*, 2008).

Another threat to the $^1O_2$-exposed cell is the oxidation of guanine to 8-oxo-guanine and the incorporation of the oxidized dGTP derivative into DNA (Cadet *et al.*, 2009). Inactivation of oxidized dGTP by dephosphorylation occurs through enzymes belonging to the NUDIX (Nucleotide Diphosphate linked to X) hydrolase family catalysing the hydrolysis of organic pyrophosphates with varying degrees of specificity (McLennan, 2006). The genome of *D. shibae* contains five genes belonging to this family, two of them being probably under the control of the $^1O_2$ regulators. According to their expression profile, they might have a role in the detoxification of oxidized dGTP.

*Singlet oxygen stress response—protein folding and turnover, membrane proteins*
Oxidation of proteins through $^1O_2$ might affect their folding as well as their functionality; therefore, an activation of chaperones and proteases in response to light can be expected. Ten genes involved in proteolysis showed an expression pattern strongly correlated with the RpoE/$RpoH_{II}$- and $RpoH_I$-controlled operons. By contrast, the chaperone DnaK and both subunits of the chaperonin complex were found in Cluster-4. $^1O_2$-induced activation of protein folding- and proteolysis-related proteins has already been shown in other bacteria such as *R. sphaeroides*, whereas activation of iron–sulphur assembly complexes has not yet been reported. Many bacteria have two different machineries for iron–sulphur cluster assembly: the ISC (iron–sulphur cluster) system, mainly involved in *de novo* synthesis (Zheng *et al.*, 1993; Schwartz *et al.*, 2000), and the SUF (Sulphur mobilization) system, mainly involved in the repair of ISCs (Djaman *et al.*, 2004; Fontecave *et al.*, 2005). Whereas ISC genes are scattered throughout the genome of *D. shibae*, the SUF genes are organized in a single operon. In contrast to the ISC genes that remained unchanged, the SUF genes were transiently upregulated threefold (log2 1.4–1.6) in response to light exposure, suggesting that ISCs might be damaged either by $^1O_2$ itself or by products formed from this reactive oxygen species. Two genes encoding fasciclin domain proteins and an operon containing three predicted membrane proteins with unknown function were the highest-upregulated genes in the entire data set, showing, respectively, 52-fold (log2 FC 5.7) and 16-fold (log2 FC 4) induction in the light. The fasciclin domain proteins were identified as glycoproteins mediating cell attachment in the neurons of grasshopper (Bastiani *et al.*, 1987). The upregulation of these cell attachment-related genes might explain the finding that *D. shibae* cells tend to clog when exposed to light for several hours, but also raise question on the role these genes might have in the response to singlet oxygen stress.

*Porphyrin biosynthesis node*
As the BChl content in *D. shibae* varies under changing light regimes, we examined the possibility of transcriptional regulation in the early steps of its biosynthesis. As illustrated in Figure 5a, all porphyrins are synthesized from a shared precursor pathway with branching points, leading to distinct pathways for cobalamin, BChl and heme synthesis (Beale, 2005). Our data showed that the light-mediated regulation of the porphyrin biosynthesis node in *D. shibae* occurred mainly at the entry to

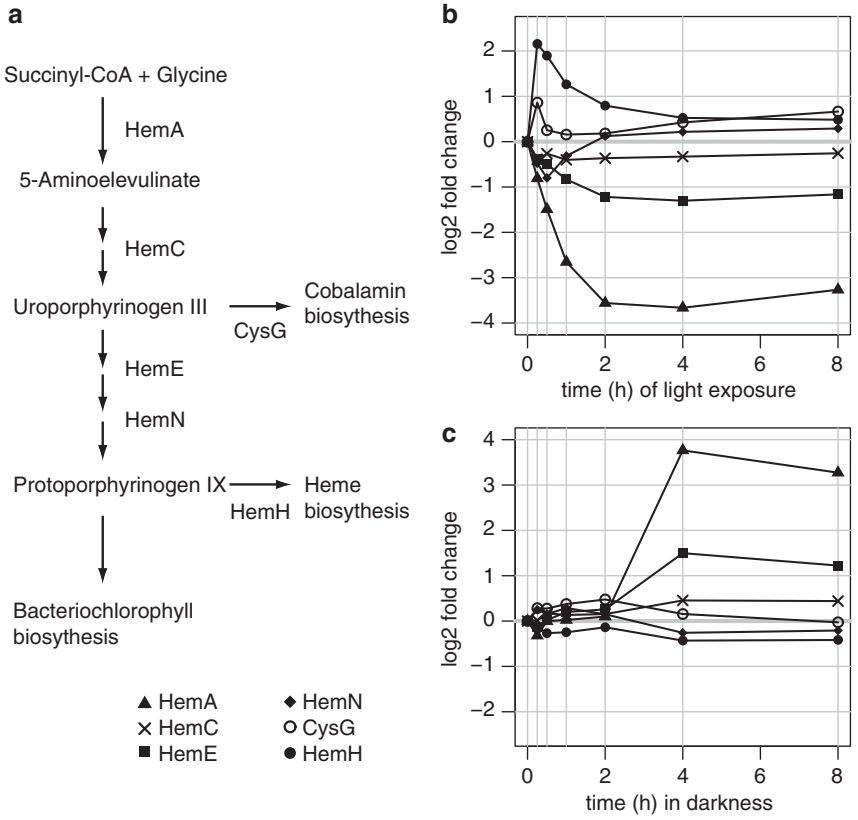


**Figure 5** Changes in the expression of porphyrin biosynthesis node genes. (**a**) A simplified representation of the biosynthesis pathway shared by all porphyrins. (**b**, **c**) The log2 FCs of genes of the porphyrin-biosynthesis genes are plotted against time. The symbols represent the genes encoding the enzymes shown in the representation in panel **a**. (**b**) The time series from dark to light. (**c**) The time series from light to dark.

and the branching points of this pathway. The genes encoding enzymes *en route* to BChl-*a* synthesis were downregulated immediately in response to light exposure and were, in turn, upregulated after 4 h in darkness (Figures 5b and c). This expression profile could also be found for the photosynthesis genes (Figures 6c and d). Binding sites for the transcription factor PpsR were identified in the promoter regions of the *hemA* homologue Dshi_3546 located in the photosynthesis gene cluster (PGC), and the predicted operon containing *hemE* (Dshi_2705) and *hemC* (Dshi_2704). These findings highlight the close coordination between the transcriptional activity of the shared porphyrin and the BChl-*a* biosynthesis pathway. Whereas *hemN* (Dshi_0541) was downregulated only temporarily in the light and expressed at a virtually constant level in the dark, other genes in this pathway were not expressed differentially. *cysG* (Dshi_1155) and *hemH* (Dshi_3498), encoding, respectively, the first enzyme in the cobalamin and heme biosynthesis pathway, were upregulated transiently in the light but did not change when the culture was set to darkness (Figures 5b and c). Thus, those genes encoding enzymes withdrawing precursors from BChl-*a* biosynthesis showed an expression profile suggesting their control in response to $^1O_2$. Their activation in response to light might be explained by the necessity of a rapid withdrawal of precursors from BChl-*a* biosynthesis, a crucial step in reducing $^1O_2$ formation.

*Photosynthesis gene cluster*

In all anoxygenic photo(hetero)trophic proteobacteria sequenced so far, the genes for BChl (*bch*) and carotenoid (*crt*) synthesis, structural proteins of the reaction centre (*pufLM*, *puhA*) and light-harvesting complex-I (*pufAB*), as well as transcriptional regulators (*ppsR*) and modulators (*tspO*, *ppaA*), are located within a single gene cluster of ~47 kb. If present, the genes encoding structural proteins of the light-harvesting complex-II (*pucABC*) are clustered in the puc operon and located elsewhere in the genome (Liotenberg *et al*., 2008). The structures of the PGC and the *puc* operon of *D. shibae* are shown in Figures 6a and b, respectively. The PGC ranges from Dshi_3501 to Dshi_3547; the *puc* operon consists of the genes Dshi_2897 to Dshi_2900. The conserved binding sequence for the transcriptional regulator PpsR is shown in Figure 6c. Light exposure of *D. shibae* led to an immediate shutdown of all non-regulatory genes in both regions

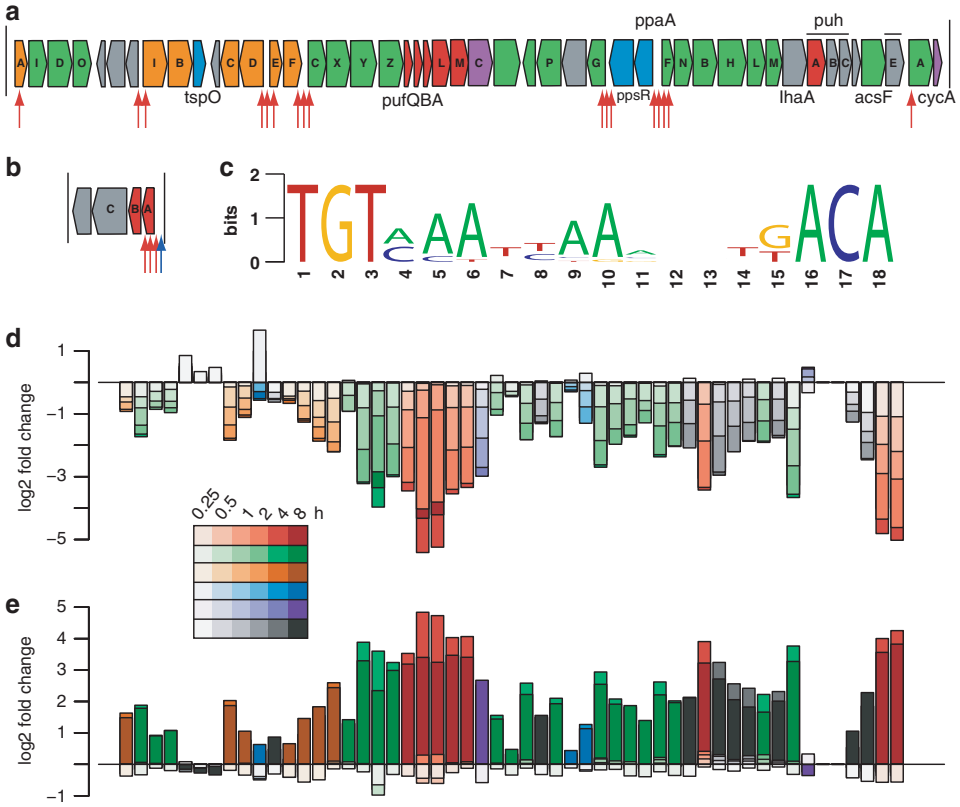


**Figure 6** The transcriptional dynamics of the PGC after a change in the light regime. (**a**, **b**) A representation of the PGC (Dshi_3501–Dshi_3547) and the puc operon encoding the LHII complex genes (Dshi_2897–Dshi_2900). Red, structural components of the photosystem; green, BChl-*a* biosynthesis; orange, sphaeroidenone biosynthesis; blue, regulatory proteins; velvet, cytochromes; grey, assembly proteins or no function assigned. The red arrows indicate identified binding sites for the transcription factor PpsR. The blue arrows indicate binding sites for the transcription factor FnrL. (**c**) The consensus sequence of the PpsR-binding sites identified in the genome of *D. shibae*. (**d**) The log2 FCs of the genes in the PGC in the light as compared with that in the dark control. Every bar stack is a superimposition of the log2 FCs compared with the pre-shift expression of one gene as it appears in the PGC and the puc operon. (**e**) The same representation for the log2 FCs in the dark as compared with the light control. In both plots the colour indicates the function of the genes as described in the representation in panels **a** and **b**, and the shading indicates the time after the shift as represented in the legend between the plots in panels **d** and **e**. BChl-*a*, bacteriochlorophyll-*a*; FC, fold change; LHII, light-harvesting complex-II; PGC, photosynthesis gene cluster.

(Figure 6d). Genes encoding structural proteins of the photosystem reaction centre and light-harvesting complexes, as well as genes found together with these *puf* and *puh* genes in putative super-operons (Liotenberg *et al.*, 2008), showed the strongest downregulation. Surprisingly, the PGC remained repressed after light deprivation. A significant reactivation of the photosynthesis genes was observed not before 4 h of growth in darkness (Figure 6e). Only for *cycA*, *ppsR* and *tspO* located in the PGC were the expression profiles distinct from this general trend: *cycA* (Dshi_3547) encoding a soluble cytochrome *c* involved in photosynthetic electron transport was upregulated in the light and downregulated in the dark. The transcription factor encoding the gene *ppsR* could be found in the PGC of all sequenced photo(hetero)trophic proteobacteria. In several strains its role as a redox- and light-dependent master regulator has been identified, either repressing or activating the expression of the PGC (Elsen *et al.*, 2005; Moskvin *et al.*, 2005). *ppsR* (Dshi_3531) expression in *D. shibae* remained virtually constant throughout the entire cultivation period. Thus, whether PpsR functions as repressor or activator could not be deduced from its expression profile. The conserved PpsR-binding site TGT-$N_{12}$-ACA (Figure 6c) was found in either one, two or three copies in the promoters of the corresponding *D. shibae* genes (Figures 6a and b). Although the results of binding experiments in *R. sphaeroides* showed that PpsR requires two neighbouring palindromes for binding (Masuda and Bauer, 2002; Masuda *et al.*, 2002), we found that the *crtA-bchIDO* operon and the *bchA* gene in the PGC were downregulated despite the presence of only one PpsR-binding site in the promoter (Figure 6a). The transient threefold (log2 FC 1.66) activation of *tspO* (Dshi_3510) in response to light suggests its regulation through RpoE/$RpoH_{II}$ or $RpoH_{I}$. TspO has been characterized as a membrane protein facilitating the export of porphyrin molecules and modulating PpsR activity in *R. sphaeroides* (Zeng and Kaplan, 2001). Therefore, the PpsR-mediated regulation of photosynthesis-related genes in the light might be

modulated through the activation of *tspO* in the presence of $^1O_2$.

*Electron transport chain*

Genes coding for parts of the electron transport chain (ETC) (Supplementary Figure S3A) of *D. shibae* showed distinct expression patterns in response to changing light regimes. The major expression patterns for the dark–light transition are shown in Supplementary Figure S3B: Genes coding for components of the entry and exit points of the ETC, and one of the two ATP-synthases, were grouped in Cluster-6. By contrast, both, *ubiG* encoding the terminal enzyme of the ubiquinone biosynthesis pathway and *cydA*/*B* encoding the ubiquinol-oxidase were found in Cluster-3. Other genes of the ubiquinone biosynthesis pathway that were upregulated, as well, could be found in Cluster-5. The genes encoding the cytochrome *bc1* complex, a mobile and a membrane-attached cytochrome *c* were also assigned to Cluster-5 (Supplementary Figure S3B). Hence, the ETC components involved only in linear electron transport were transiently downregulated in the light, whereas those ETC components involved in cyclic electron transport were upregulated. After switching from light to dark, changes in the expression of ETC-related genes were comparatively weak, and except for *ubiG* and *cydA*/*B*, both grouped in Cluster-8, all ETC genes were found in Cluster-9 (Supplementary Figure S3C).

Three genes, *regA* and *regB* encoding a two-component system sensing the electron flow towards terminal oxidases (Elsen *et al*., 2004), and *fnrL*, a global oxygen-sensitive regulator (Roh and Kaplan, 2002), showed the same expression pattern as the genes for the entry and exit points of the ETC (Supplementary Figure S3D), making both systems likely candidates for the regulation of ETC gene expression. This hypothesis is further supported by the light-induced reduction of the respiration rate of *D. shibae* resulting in reduced linear electron flow and increased oxygen saturation in the medium. According to the similarity in their expression patterns, *ubiG* and *cydA*/*B* might be regulated by RpoE/$RpoH_{II}$ or $RpoH_I$. In addition, the concentration of the ubiquinol oxidase was increased in $^1O_2$-exposed *R. sphaeroides* cells (Nuss *et al*., 2010). Adjustment of the ubiquinone redox state might be necessary for full functioning of the cyclic and linear ETC under $^1O_2$ stress.

## Conclusion

AAP has been shown to be an additional source of energy for marine bacteria when grown under laboratory conditions. The distribution and abundance of AAP bacteria in the world's oceans suggests that this form of energy generation from light has an important role in marine ecosystems. Our study sheds light on the transcriptional basis underlying the process of AAP in a marine bacterium. We could show that exposure of pigmented cells to light leads to changes in the gene expression level of approximately 33% of all the genes encoded in the genome of *D. shibae*, whereas exposing light-grown cells to darkness results in a much weaker response of only 9% of all the genes. Remarkably, transcriptional changes occur in a highly coordinated manner as shown by the limited number of distinct expression profiles, and, in accordance with the decline of AAP-generated energy during cultivation in the light, most of the changes are only transient. One surprising finding was the rapid activation and subsequent inactivation of more than 300 genes in the light, which are likely to be under the control of RpoE/$RpoH_{II}$ or $RpoH_I$. This finding suggests that sources of $^1O_2$ and the harmful $^1O_2$ itself are quickly depleted in *D. shibae* cells. Another surprising finding was the extremely slow reactivation of the PGC and related genes in the dark, as compared with their quick inactivation in response to light. The difference in response to the same parameter only depending on the direction of the change strongly suggests that at least two different mechanisms regulate the expression of those genes. Based on the similarity of expression patterns, we propose that the transcriptional modulator TspO might be involved in the rapid shutdown of the PGC in response to $^1O_2$. This difference in regulation might be important for AAP being an advantage for *D. shibae*. Whereas the fast shutdown of $^1O_2$-evolving photosynthesis pathways is crucial for the survival of the bacterium in the light, their immediate reactivation in the dark might be rather disadvantageous as too much energy would be used for the biosynthesis of photosystems at the expense of other anabolic processes, resulting in slower cell division. In addition, our data showed quick recovery of BChl-*a* levels in *D. shibae* cells after activation of the PGC. So, if *D. shibae* maintain a high cell division rate during the first few hours of growth in the dark, a greater number of cells can later activate their photosystems for the light period that follows.

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