Gene expression under low-oxygen conditions in the cyanobacterium *Synechocystis* sp. PCC 6803 demonstrates Hik31-dependent and -independent responses

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We have investigated the response of the cyanobacterium *Synechocystis* sp. PCC 6803 during growth at very low O_2 concentration (bubbled with 99.9 % N_2/0.1 % CO_2). Significant transcriptional changes upon low-O_2 incubation included upregulation of a cluster of genes that contained *psbA1* and an operon that includes a gene encoding the two-component regulatory histidine kinase, Hik31. This regulatory cluster is of particular interest, since there are virtually identical copies on both the chromosome and plasmid pSYSX. We used a knockout mutant lacking the chromosomal copy of *hik31* and studied differential transcription during the aerobic–low-O_2 transition in this ΔHik31 strain and the wild-type. We observed two distinct responses to this transition, one Hik31 dependent, the other Hik31 independent. The Hik31-independent responses included the *psbA1* induction and genes involved in chlorophyll biosynthesis. In addition, there were changes in a number of genes that may be involved in assembling or stabilizing photosystem (PS)II, and the *hox* operon and the LexA-like protein (Sll1626) were upregulated during low-O_2 growth. This family of responses mostly focused on PSII and overall redox control. There was also a large set of genes that responded differently in the absence of the chromosomal Hik31. In the vast majority of these cases, Hik31 functioned as a repressor and transcription was enhanced when Hik31 was deleted. Genes in this category encoded both core and peripheral proteins for PSI and PSII, the main phycobilisome proteins, chaperones, the ATP synthase cluster and virtually all of the ribosomal proteins. These findings, coupled with the fact that ΔHik31 grew better than the wild-type under low-O_2 conditions, suggested that Hik31 helps to regulate growth and overall cellular homeostasis. We detected changes in the transcription of other regulatory genes that may compensate for the loss of Hik31. We conclude that Hik31 regulates an important series of genes that relate to energy production and growth and that help to determine how *Synechocystis* responds to changes in O_2 conditions.

INTRODUCTION

Cyanobacteria are a diverse and ancient phylum of phototrophic prokaryotes that thrive in terrestrial, marine and freshwater habitats (Rippka *et al.*, 1979). Their ability to adapt to fluctuating environments has contributed to their widespread distribution. Mechanisms involved in sensing and responding to many environmental parameters have been determined through DNA microarray experiments

investigating genome-wide expression patterns. Transcriptional responses to numerous stimuli have been well characterized in the freshwater cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*); stimuli included changes in light conditions, pH, nutrient availability, salt stress and temperature stress (Gill *et al.*, 2002; Hihara *et al.*, 2001; Huang *et al.*, 2002; Singh *et al.*, 2003, 2006; Summerfield & Sherman, 2008; Wang *et al.*, 2004a, b). In addition, we have examined the response to low-oxygen conditions in *Synechocystis* (Summerfield *et al.*, 2008). Cyanobacteria experience low oxygen concentrations in habitats such as hot springs, soils (e.g. rice paddies or estuarine mud) and eutrophic lakes (Stewart & Pearson, 1970). Where the rate of photosynthesis is low and/or oxygen consumption is high, low oxygen levels may occur intracellularly (Paerl

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et al., 2000). Key questions are: how did protocyanobacteria function in a primarily anoxygenic environment, and how did their metabolism change as they began adding oxygen into the atmosphere? An understanding of this anaerobic to aerobic transition may be important for understanding the development and regulation of photosynthesis.

In Synechocystis, the transition to low-oxygen conditions resulted in widespread changes in transcript abundance. This included increased mRNA levels of genes encoding the histidine kinase, Hik31 (Summerfield et al., 2008). A histidine kinase is one of two proteins comprising a two-component regulatory system that senses and responds to environmental fluctuations. For a typical two-component system, a specific environmental change first activates the histidine kinase; in turn, this kinase phosphorylates its cognate response regulator resulting in a specific output that frequently involves altered transcript abundance of a specific group of genes (Stock et al., 2000). The Synechocystis genome contains 47 genes that encode putative histidine kinases, and 45 genes that encode putative response regulators (Los et al., 2008). In Synechocystis, characterization of gene-interruption mutants has identified histidine kinases involved in sensing environmental stimuli, including high light, cold and oxidative stress (Hik33), heat (Hik34), salt stress and hyperosmotic stress (Hik33, Hik34, Hik2, Hik41, Hik16 and Hik10), and phosphate limitation (Hik7) (Hirani et al., 2001; Hsiao et al., 2004; Kanesaki et al., 2007; Mikami et al., 2002; Paithoonrangsarid et al., 2004; Shoumskaya et al., 2005; Suzuki et al., 2005). These data demonstrate that one histidine kinase may respond to more than one type of stimulus and that multiple histidine kinases may respond to any specific environmental cue.

The histidine kinase Hik31 (Sll0790) is the only chromosomally encoded histidine kinase in Synechocystis to have a plasmid-encoded homologue (Slr6041). These proteins share 95.6 % sequence identity, with the first 420 residues being identical, and they are predicted to contain three transmembrane helices supporting the idea that these are membrane-bound sensors. However, the signalling domains have yet to be identified (Ashby & Houmard, 2006). The hik31 gene appears to be part of a duplicated three gene cluster encoding a hypothetical protein, a response regulator and a histidine kinase [chromosomal genes sll0788–sll0790 and plasmid (pSYSX) genes slr6039–slr6041]. Both of the gene clusters are located next to genes encoding probable cation efflux proteins and it has been suggested that these two-component systems may have a role in mediating cation transport (Kaneko et al., 2003).

In addition, Hik31 is involved in regulating glucose metabolism. A strain lacking both the chromosomal (sll0790) and plasmid (slr6041) hik31 homologues was unable to grow in the presence of glucose (Kahlon et al., 2006). Data indicated that this mutant was able to take up glucose, but lacked glucokinase activity to convert glucose into glucose 6-phosphate, so that glucose could not be metabolized. In contrast, elevated glucose-6-phosphate dehydrogenase

activity indicated that the mutant was able to convert glucose 6-phosphate to gluco-1,5-lactone phosphate. This led the authors to suggest that Hik31 is involved in both positive and negative regulation of functions involved in glucose metabolism that enable switching between photoautotrophic and mixotrophic growth.

Upregulation of hik31 under low-oxygen conditions indicates that Hik31 has an additional role in the transition between aerobic and low-oxygen growth. We report on the growth of the wild-type and hik31 mutants under low-O_2 conditions and the way in which gene expression is altered by such growth conditions. We demonstrate that in the absence of Hik31, strains exhibited increased growth compared with the wild-type under low-oxygen conditions and that Hik31 is involved in negative regulation of genes involved in growth in low-oxygen conditions.

METHODS

Cyanobacterial strains and growth conditions. The glucose tolerant strain, Synechocystis sp. strain PCC 6803 (Williams, 1988) was grown at 30 ± 2 °C. Cells were grown in blue green algae medium BG11 with 25 mM HEPES pH 7.5, in an airlift bioreactor (BioFlo 3000, 6 l) or in 750 ml Cytolifts (Kontes). The ΔHik31 strain in which the sll0790 gene was interrupted with a spectinomycin resistance cassette at the NcoI site was provided by Julian Eaton-Rye, University of Otago. Thus, 25 μg spectinomycin ml^{-1} was added to the medium during the growth of this mutant. In addition, deletion mutants were constructed in the chromosomal copy of hik31 (Δhik31c, Cm^r), in the plasmid copy (Δhik31p, Sp^r) and in both copies (Δhikcp) (S. Nagarajan and L. A. Sherman, unpublished observations). The culture was illuminated by two panels using alternating arrays of orange (640 nm) and blue (430 nm) light-emitting diodes, yielding an intensity of ~100 μmol photons m^{-2} s^{-1} inside the bioreactor or Cytolift (underwater quantum light meter, LI 192, Li-Cor). For microarray experiments, cultures were grown in the bioreactor to a cell density of ~ 5×10^7 cells ml^{-1} and then bubbled with 99.9 % nitrogen/0.1 % carbon dioxide to achieve low-oxygen conditions, as described by Summerfield et al. (2008).

Whole cell spectra. Spectra were collected on a Perkin-Elmer spectrophotometer (Lambda 40). To correct for scattering, Scotch tape was placed on the front and back of the reference and sample cuvette holders. Spectra were normalized to the peak at 682 nm.

RNA extraction. Total RNA was extracted and purified using phenol/chloroform extraction and CsCl gradient purification as described previously (Reddy et al., 1990; Singh & Sherman, 2002).

Semi-quantitative RT-PCR. DNase I treatment and reverse transcription were performed as described by Summerfield et al. (2008). PCR was carried out at 94 °C 1 min, 20–30 cycles of 94 °C 30 s, 54 °C 30 s and 68 °C 30–120 s (depending on amplicon size), to amplify regions of the genes listed below. Primers (F 5′-gcatgcacatg-caatgga and R 5′-ggtgtacgtaattcgtgg) were used to amplify the three-gene cluster containing hik31. These primers were a 100 % match to the chromosomal and plasmid copies of these genes with the forward primer binding at 116–133 bp of sll0788/slr6039 and the reverse primer binding at 698–681 bp of sll0790/slr6041. This enabled amplification of both the chromosomal and plasmid clusters, resulting in product of 1865 bp. This PCR product was digested with NheI that has a restriction site at nt 144 in the plasmid response regulator gene slr6040, but not in its chromosomal homologue

sll0789. The *Nhe*I digestion of an amplicon derived from the transcript of the plasmid genes would result in two fragments of 352 bp and 1513 bp.

Semi-quantitative RT-PCR was used to validate the microarray data. The genes amplified, the primers used and PCR product size and number of PCR cycles were: *sll1875* (F 5′-ggacttatccggtggtc, R 5′-ctattcacctaccattagg, 367 bp, 25 cycles), *slr1181* (F 5′-ggattacaggaa-caaagc, R 5′-agggccaccatttgg, 1040 bp, 30 cycles) *slr1311* (F 5′-cacttgcttcatcattgc, R 5′-tgcatcaggatgttgtgc, 450 bp, 20 cycles), *sll1514* (F 5′-gataatttccagcagcag, R 5′-gtcaaagttaggataccg, 350 bp, 25 cycles), *ssr2595* (F 5′-ggatttcgcctcgaccaa, R 5′-agagagcaaccaacccac, 195 bp, 25 cycles), *sll1577* (F 5′-ttcccaagctgatgctcg, R 5′-agcactgcaat-caccacg, 439 bp, 20 cycles) and *rnpB* (F 5′-tgtcacagggaatctgagg, R 5′-gagagagttagtcgtaagc, 405 bp, 30 cycles). The *rnpB* gene was included as this transcript is frequently used as a constitutively expressed control for gene expression. Amplification of chromosomal *hik31* (*sll0790*) was performed (primers: F 5′- gctggatcaagagcttac and R 5′- ggtgtacgtaattcgtgg); the forward primer differed from the plasmid *hik31* sequence at three nucleotide positions. This was sufficient to enable PCR of only the chromosomal copy and resulted in a PCR product of 567 bp.

Microarray design. The microarray platform and construction was as described by Postier *et al.* (2003) and the cDNA labelling, prehybridization and hybridization protocols are described in detail in Singh *et al.* (2003). The microarray experiment involved a loop design that compared the wild type and the ΔHik31 strain under aerobic conditions and 1 h following a transition to low oxygen by using an ANOVA model (Li *et al.*, 2004; Singh *et al.*, 2003). The microarray was deposited in the NCBI Gene Expression Omnibus Series under accession number GSE24882.

Data acquisition included an ANOVA model approach to test the null hypothesis that a particular gene's expression level did not differ between the treatments and to calculate a *P*-value (Singh *et al.*, 2003). This experiment contained two genotypes (wild-type and the ΔHik31 strain) and two stimuli (aerobic growth and transition to low-oxygen conditions) for a total of four treatment combinations. The effects of interrupting Hik31 and the transition from aerobic to low-oxygen conditions were examined in an ANOVA essentially as described by Kerr & Churchill (2001a, b), Li *et al.* (2004) and Singh *et al.* (2003). We used the false discovery rate (FDR) of 5 % to control the proportion of significant results that are type I errors (false rejection of the null hypothesis) as described by Summerfield & Sherman (2007). Genes with an FDR=0.05 (corresponding to 5 % expected false positives) and that exhibited a change of at least 1.5-fold were considered interesting and retained for further analysis. The *P*-value of these genes ranged from 3.2×10^{-2} to 9.6×10^{-15}.

RESULTS AND DISCUSSION

Both the chromosomal and plasmid *hik31* copies are in operons upregulated under low-oxygen conditions

The chromosomal and plasmid *hik31* genes (*sll0790* and *slr6041*, respectively) are part of homologous three gene clusters. The other two genes in each of these clusters encode response regulators *sll0789* and *slr6040* and hypothetical proteins *sll0788* and *slr6039*. The homologous gene clusters encode proteins that differ by 19/458, 10/232 and 2/196 amino acids, for the Hik31, Rre34 and hypothetical protein, respectively. Using RT-PCR with a

forward primer to the hypothetical genes *sll0788* and *slr6039* and a reverse primer to *hik31* (both *sll0790* and *slr6041*), we demonstrated that this three gene cluster is co-transcribed and that this operon is upregulated under low-oxygen conditions (Fig. 1a). DNA microarray data indicated that the hypothetical gene was upregulated only 1.3-fold on transfer to low-oxygen conditions, but this is likely to be an underestimate as the abundance of this transcript is at the top of the dynamic range for our detection system. The high level of nucleotide sequence identity between the chromosomal and plasmid genes meant they could not be distinguished in our microarray experiment or by using RT-PCR.

To determine whether the RT-PCR products were derived from the chromosomal and/or plasmid transcripts, we utilized an *Nhe*I restriction site that was present in the gene encoding the plasmid response regulator, but was not present in the gene encoding the chromosomal response regulator. Digestion with *Nhe*I would result in cleavage of the RT-PCR products derived from the plasmid gene, but not the RT-PCR products from the chromosomal gene. The *Nhe*I digestion of RT-PCR products from the wild-type resulted in cleavage of the plasmid-derived RT-PCR product to give a PCR product of ~1.51 kb (and a ~0.35 kb fragment that is not shown in Fig. 1a), and an uncut ~1.86 kb PCR product corresponding to the RT-PCR product from the chromosomal genes. These data indicated both the plasmid and chromosomal copies of the genes are expressed and are upregulated under low-oxygen conditions (Fig. 1a).

Kahlon *et al.* (2006) used a strain lacking both the plasmid and chromosomal *hik31* to examine the role of this histidine kinase in glucose metabolism. Their data indicated that Hik31 is involved in switching between photoautotrophic and mixotrophic growth by acting as both a positive and a negative regulator of functions involved in glucose metabolism. However, they reported that investigating the role of Hik31 using a mutant lacking both the plasmid and chromosomal copies presented complications, including serious difficulty with the stability of phenotypes. We first examined a strain (ΔHik31) lacking only the chromosomal copy of *hik31*, *sll0790*. As expected, only the plasmid copy of the gene was detected in the ΔHik31 strain. However, expression of this copy was altered in the mutant compared with the wild-type, as levels of the plasmid transcripts were reduced in the mutant at 1 h compared with the wild-type, but increased by 2 h (Fig. 1a). These data indicated that chromosomally encoded Hik31 regulates plasmid *hik31* expression.

Growth and whole-cell spectra of the wild-type and ΔHik31 strains

We constructed strains lacking each of the *hik31* genes and also a double deletion mutant lacking both the chromosomal and plasmid *hik31* genes. These strains have been tested for growth under diverse photoautotrophic and
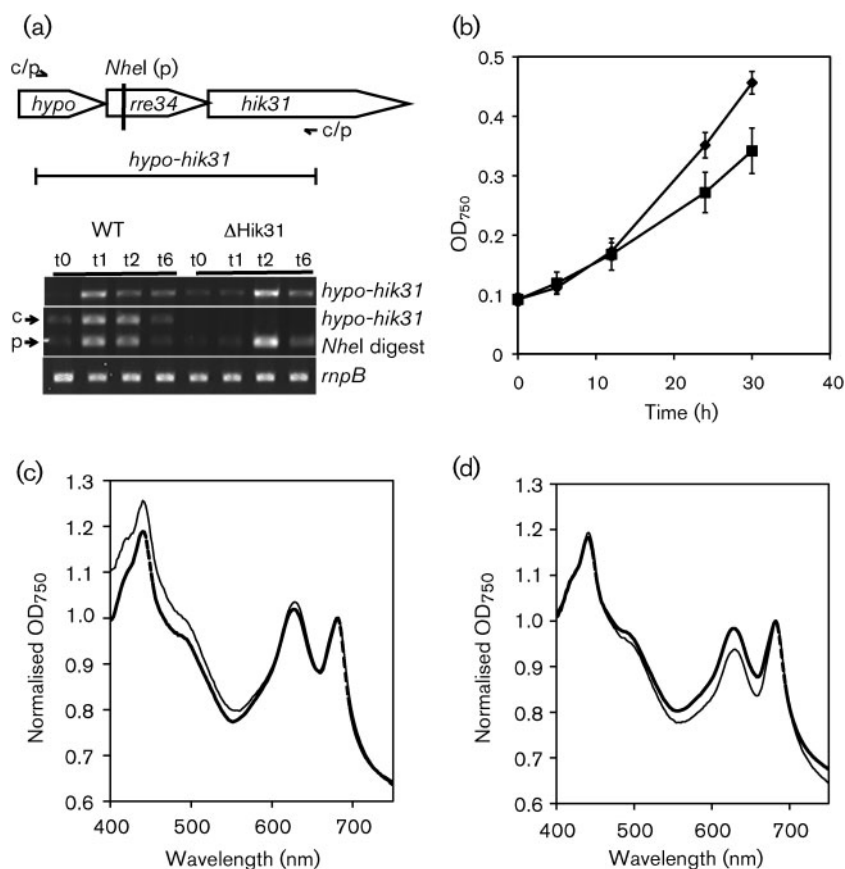
Fig. 1. The wild-type and ΔHik31 strain exhibit different responses to low-oxygen conditions. (a) The organization of the three gene cluster of chromosomal/plasmid genes: *hypo* (*sll0788/slr6039*), *rre34* (*sll0789, slr6040*) and *hik31* (*sll0790/slr6041*). Small arrows indicate primer binding positions in the chromosomal (c) and plasmid (p) genes. *Nhe*I (p) indicates the location of an *Nhe*I restriction site in the plasmid copy of *rre34* but not the chromosomal copy. RT-PCR using the primers indicated generated the PCR product, labelled *hypo–hik31*, corresponding to part of the *hypo–rre34–hik31* sequence from both the chromosomal and plasmid copies. These results are from one experiment that is representative of three independent experiments. (b) Growth of the wild-type (■) and ΔHik31 strain (◆) under low-oxygen conditions as measured by OD_{750} (n=3; data are ±SD). (c) Whole-cell absorption spectra of wild-type cells before (thick line) and 24 h after (thin line) exposure to low-oxygen conditions. (d) Whole-cell absorption spectra of ΔHik31 cells before (thick line) and 24 h after (thin line) exposure to low-oxygen conditions.

mixotrophic conditions and these results will be described separately (S. Nagarajan and L. A. Sherman, unpublished results). In addition, each strain was grown under identical conditions in the bioreactor (bubbled with 99.9 % nitrogen/0.1 % carbon dioxide) and cultures were sampled at regular intervals for cell number and absorption spectra for 2 days prior to the low oxygen shift and 2 days thereafter (Table 1). These data indicated that strains lacking the chromosomal *hik31* (Δ*hik31c*), the plasmid *hik31* (Δ*hik31p*) and both *hik31* copies (Δ*hikcp*) all grew better than the wild-type under low-O_2 conditions. This type of experiment has been repeated over 12 times and, in each case, the *hik* mutant grew as well as, or better than, the wild-type. The Δ*hik31c* and Δ*hikcp* strains always grew better than the wild-type. We concluded that the original ΔHik31 mutation was representative of the expression changes that would occur during the transition from aerobic to low-oxygen conditions and that the double mutant would only lead to further quantitative, but not qualitative, differences in gene expression. Therefore, we used the chromosomal ΔHik31 strain to investigate the role of Hik31 in the response to low-oxygen conditions. In addition, use of the ΔHik31 strain avoided any potential problems of phenotype stability of the double mutant that have previously been reported (Kahlon *et al.*, 2006), the stable phenotype of the ΔHik31 strain under low oxygen conditions is shown in Fig. 1(b).

The wild-type and ΔHik31 strain had similar doubling times under aerobic conditions in flasks (13±1 h), consistent with the growth of the Hik31 mutant reported by Kahlon *et al.* (2006). Generation times were typically slower at low cell density in the 6 l bioreactor and aerobic doubling times were 20–30 h for all of the strains (Table 1). The mutants all grew faster than the wild-type under low-O_2 conditions with doubling times up to 50 % faster (Table 1). Cultures of the wild-type grown in a 750 ml Cytolift for 30 h under low-oxygen conditions showed a decreased growth rate (19 h) compared with the ΔHik31 strain (12 h) (Fig. 1b). The whole-cell spectra of the two strains differed in cells grown under aerobic conditions, as the peak with absorption maxima at 630 nm, originating from the phycobilins, was higher in the wild-type (Fig. 1c and d). Incubation under low-oxygen conditions altered the whole-cell spectra of both strains. The wild-type had a decreased 440 nm chlorophyll *a* peak relative to the 680 nm peak (Fig. 1c), whereas the ΔHik31 strain showed an increased phycobilin peak relative to the chlorophyll *a* peaks (Fig. 1d).

Differential transcript abundance in the wild-type following transition to low-oxygen conditions

Based on the growth data, ΔHik31 was used to investigate the role of Hik31 in modulating gene expression under

Table 1. Growth of *Synechocystis* sp. PCC 6803 wild-type and *hik31* mutants with air bubbling for 48 h (aerobic) followed by 99.9 % N_2 and 0.1 % CO_2 (low O_2) for a subsequent 48 h

Cultures were grown in a 6 l bioreactor and cells were counted in a hemocytometer. The data presented are for individual experiments representative of three or more experiments for each strain.

Strain	$10^{-7} \times$ Cells ml^{-1}			Low O_2 growth mean fold increase*
	0 h	48 h, air	96 h, low O_2	
Wild-type	1.2	3.5	5.3	1.5
Δ*hikc*†	0.9	3.8	7.6	2.0
Δ*hikp*‡	0.9	4.3	7.2	1.7
Δ*hikcp*§	1.5	4.8	11.7	2.4

*Mean growth increase for three or more experiments for each strain under low O_2, low growth increase was reproducible within ±15 % of the mean for each strain.
†Strain lacking chromosomal *hik31*.
‡Strain lacking plasmid *hik31*.
§Strain lacking chromosomal and plasmid *hik31*.

low-oxygen conditions. *Synechocystis* cultures were grown in continuous light and bubbled with air to a density of $\sim 5 \times 10^7$ cells ml^{-1}. Cultures were subjected to low-oxygen conditions by bubbling with 99.9 % nitrogen and 0.1 % carbon dioxide. Samples were taken at t0, prior to bubbling with the nitrogen/carbon dioxide mix, and at t1, after 1 h of bubbling with nitrogen/carbon dioxide. As described in Methods, we considered genes to be differentially regulated if they showed a fold change ⩾1.5 and with an FDR of 0.05. Genes that met these criteria represented 13 and 8 % of chromosomal genes for the wild-type and ΔHik31 strain, respectively. These differentially expressed genes were divided into functional categories, according to the Cyanobase designation (http://genome.kazusa.or.jp/cyanobase/) and the number of these genes in each functional category (excluding hypothetical, other or unknown genes) is shown in Fig. 2, all gene categories are shown in Supplementary Fig. S1 (available with the online version of this paper).

In the wild-type, a similar number of genes were upregulated and downregulated following transition to low oxygen. There were 211 downregulated genes, more than a third of these belonged to either the hypothetical, other or unknown gene categories (81 genes). Additional functional categories containing large numbers of downregulated genes included translation (36 genes), photosynthesis and respiration (20 genes), regulatory functions (15 genes) and energy metabolism (14 genes) (Fig. 2). A total of 209 genes were upregulated in the wild-type under low-oxygen conditions, over half of which were designated hypothetical, other or unknown genes (115 genes) (data not shown). Additional genes with increased mRNA levels under low-oxygen conditions included those encoding transport and binding proteins (28 genes), regulatory proteins (16 genes) and energy metabolism proteins (11 genes).

A major component of the transcriptional response to low oxygen was the decreased transcript levels for many genes

essential for growth; e.g. genes encoding ribosomal proteins, ATP synthase and phycobilisome components. Decreased mRNA levels of these genes were observed in conditions associated with decreased growth; e.g. during the linear growth phase, after the transition from light to dark, under sulfur deprivation and during acclimation to decreased inorganic carbon (Foster *et al.*, 2007; Summerfield & Sherman, 2007; Wang *et al.*, 2004a; Zhang *et al.*, 2008). Response to low-oxygen conditions involved the upregulation of genes that encoded proteins involved in sensing and responding to environmental change and maintaining cellular homeostasis, including some stress-responsive proteins, regulatory proteins and transport and binding proteins.

A comparison with previous microarray data generated similar results (Supplementary Fig. S1) and especially included all of the downregulated genes with functions in translation, and photosynthesis and respiration. In addition, semiquantitative RT-PCR was used to validate a subset of microarray data and we observed good agreement between these two techniques (see Supplementary Fig. S2, available with the online version of this paper).

The absence of Hik31 alters transcriptional response to low oxygen conditions

We compared the transcriptional response of the wild-type and ΔHik31 strain to the transition from aerobic to low-oxygen conditions (see Tables 2 and 3 and Fig. 2). Following this transition, only 32 genes were down-regulated in ΔHik31 compared with >200 genes down-regulated in the wild-type. The two strains had similar numbers of genes with increased mRNA levels under low-oxygen conditions (209 and 230 genes in the wild-type and ΔHik31 strain, respectively), approximately half these genes were common to both strains.
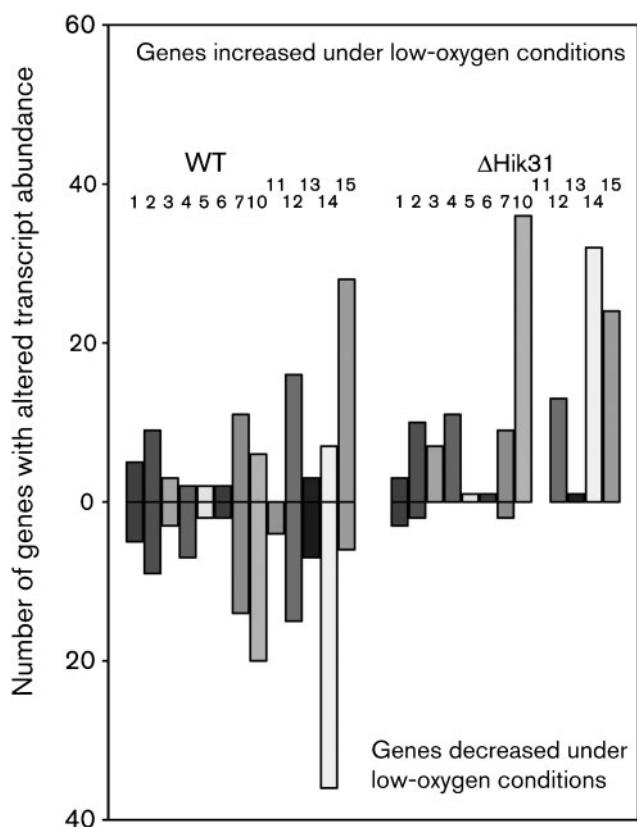
Fig. 2. Histogram of genes differentially expressed 1.5-fold or more (FDR=0.05) in the wild-type (WT) and the ΔHik31 strain. The numbers of differentially expressed genes for different functional categories are plotted, with genes upregulated under low-oxygen conditions shown above the zero line on the *y* axis and genes downregulated shown beneath the zero line. Genes are presented as Cyanobase functional categories, which are labelled according to the Cyanobase designation: 1, amino acid biosynthesis; 2, biosynthesis of cofactors, prosthetic groups and carriers; 3, cell envelope; 4, cellular processes; 5, central intermediary metabolism; 6, DNA replication, restriction, modification, recombination and repair; 7, energy metabolism; 10, photosynthesis and respiration; 11, purines, pyrimidines, nucleosides and nucleotides; 12, regulatory functions; 13, transcription; 14, translation; 15, transport and binding proteins.

The major difference in the transcriptional response of the two strains to low-oxygen conditions involved genes associated with growth that were downregulated in the wild-type and/or upregulated in the ΔHik31 strain. This included genes categorized as cellular processes, energy metabolism, photosynthesis and respiration, regulatory functions, and translation (Fig. 2), as well as 69 genes belonging to hypothetical, other and unknown categories that were downregulated in the wild-type, but not in ΔHik31 (Supplementary Fig. S1). These differences in gene expression may explain the enhanced growth of the ΔHik31 strain compared with the wild-type under low-oxygen conditions. Genes involved in the response to

low-oxygen conditions were divided into two categories: those that responded similarly in the two strains and those that responded differently in the two strains. Representatives of both of these categories are discussed in more detail below.

Genes showing similar expression patterns in the wild-type and the ΔHik31 strain under low-oxygen conditions (Hik31-independent changes)

Transcriptional responses shared by both strains involved adaptation to low oxygen conditions that included unique changes to the photosynthetic electron transport chain. This included a low-oxygen-induced gene cluster containing a divergent copy of the photosystem II (PSII) *psbA* gene (*psbA1*) and an alternative copy of the gene encoding the Rieske iron–sulfur protein of the cytochrome b_6f complex (*petC2*) that we previously reported to be upregulated in the wild-type (Summerfield *et al.*, 2008). Other genes involved in photosynthetic electron transport were upregulated e.g. *ctpA* (*slr0008*), a gene that encodes a protease that processes the D1 protein into its mature form during turnover of PSII (Anbudurai *et al.*, 1994; Jansèn *et al.*, 2003) and *ctpB* (*slr0257*) encoding a related carboxyl-terminal endoprotease thought to have a minor role in processing D1 (Komenda *et al.*, 2007). Transcripts of both of these proteases are elevated under stress conditions (e.g. high-light and high-salt) where PSII damage is increased (Jansèn *et al.*, 2003; Komenda *et al.*, 2007). The gene encoding ClpB1 (Slr1641) was upregulated, this molecular chaperone has a role in acquisition of thermotolerance in the cyanobacterium *Synechococcus* sp. strain PCC 7942 (Eriksson & Clarke, 1996).

In addition, genes *sll0217–sll0220* (including two genes encoding A-type flavoproteins, *sll0217*, *flv4* and *sll0219*, *flv2*) and a third flavoprotein (*sll0550*, *flv3*) were upregulated in both strains. The sequences of these proteins are indicative of functions in electron transfer (Helman *et al.*, 2003). The proteins Flv2 and Flv4 are thought to protect PSII from photoinhibition (Zhang *et al.*, 2009) and our results may suggest that these proteins have a greater role in PSII assembly. In contrast, Flv3 appears to be involved in the flux of electrons from photosystem I (PSI) to O_2 in the Mehler reaction (Helman *et al.*, 2003). Genes downregulated under low-oxygen conditions included *slr0074–0077*, genes homologous to the *sufBCDS* operon of *Synechococcus* sp. PCC 7002, and the *suf* operon of *Escherichia coli* that functions in iron–sulfur cluster biogenesis (Takahashi & Tokumoto, 2002; Wang *et al.*, 2004b) (Table 2). In *Synechococcus* sp. PCC 7002, this operon is believed to have a role in PSI biogenesis (Wang *et al.*, 2004b).

Low-oxygen conditions altered the transcript levels of genes involved in chlorophyll synthesis. In both strains, we observed a large increase in transcript abundance for *ho2* (*sll1875*) and *hemN1* (*sll1876*), genes that encode an oxygen-independent haem oxygenase (HO) and coproporphyrinogen III oxidase (CPO), respectively. Upregulation of these genes is thought to enable maintenance of haem

Table 2. Selected genes showing similar regulation in *Synechocystis* sp. strain PCC 6803 wild-type and a ΔHik31 strain on transition from aerobic to low-oxygen condition

Genes (grouped in the table) were considered differentially regulated when FDR=0.05, fold change >1.5 (bold type). The fold changes were calculated by dividing the normalized mean intensities obtained for: the wild-type under low-oxygen conditions by those for the wild-type under aerobic conditions (WT); the ΔHik31 strain under low-oxygen conditions by those for the ΔHik31 strain under aerobic conditions (ΔHik31); the wild-type under aerobic conditions by those for the ΔHik31 strain under aerobic conditions (WT/ΔHik31, Air); and the wild-type under low-oxygen conditions by those for the ΔHik31 strain under low-oxygen conditions (WT/ΔHik31, Low O_2). transp, Transport; hypo, hypothetical; unk, unknown.

Gene no.	Name	WT	ΔHik31	WT/ΔHik31	
				Air	Low O_2
Biosynthesis of cofactors, prosthetic groups					
Chlorophyll and porphyrin synthesis					
sll1875	*ho2*	**10.0**	**11.1**	1.1	1.0
sll1876	*hemN1*	**9.8**	**15.4**	1.1	−1.4
slr0056	*chlG*	**2.6**	**1.5**	1.3	**2.2**
slr0506	*por*	**−2.1**	**−1.5**	1.1	−1.2
slr0750	*chlN*	**−2.6**	**−1.5**	1.3	−1.4
Other					
Adaptations					
ssr2595	*hliB*	**3.5**	**1.5**	−1.2	**1.9**
ssl1633	*hliC*	**1.5**	**1.7**	−1.4	**−1.6**
slr1641	*clpB1*	**1.8**	**1.7**	−1.4	−1.4
Regulatory functions					
sll0792	*ziaR*	**−2.4**	**−1.5**	**−1.8**	**−2.9**
sll1626	*lexA*	**1.5**	**1.5**	1.1	1.1
slr0947	*rpaB*	**2.1**	**1.6**	1.1	1.4
Transport and binding proteins					
slr1295	*futA1*	**−2.4**	**−2.2**	**1.6**	**1.5**
slr0513	*futA2*	**−2.3**	**−2.0**	**2.1**	**1.8**
slr1490	*fhuA*	**−2.2**	**−1.8**	**−1.9**	**−2.2**
sll1428	transp	**2.1**	**8.8**	**4.7**	1.1
slr1149	transp	**2.3**	**1.5**	1.0	**1.6**
sll0759	transp	**2.1**	**2.6**	**1.5**	1.2
sll0778	transp	**1.9**	**2.4**	**2.9**	**2.2**
Translation					
Proteases					
slr0008	*ctpA*	**1.8**	**2.0**	1.3	1.2
slr0257	*ctpB*	**2.6**	**3.8**	**3.3**	**2.3**
Gene clusters					
Hydrogenase					
sll1220	*hoxE*	**1.9**	**1.8**	1.0	1.0
sll1221	*hoxF*	**2.5**	**2.4**	1.2	1.3
sll1222	hypo	**2.7**	**2.0**	−1.3	1.1
sll1223	*hoxU*	**2.5**	**2.0**	1.0	1.3
sll1224	*hoxY*	**2.0**	**1.6**	−1.1	1.2
sll1225	unk	**1.7**	**1.7**	−1.3	−1.4
sll1226	*hoxH*	1.4	1.4	−1.3	−1.2
Flavoproteins					
sll0217	*flv4*	**2.7**	**2.5**	1.0	1.1
sll0218	hypo	**3.3**	**2.9**	1.3	**1.5**

Table 2. cont.

Gene no.	Name	WT	ΔHik31	WT/ΔHik31	
				Air	Low O_2
sll0219	*flv2*	**2.0**	**2.8**	**1.5**	1.0
sll0220	*glmS*	**3.0**	**5.9**	**5.6**	**2.8**
sll0550	*flv3*	**1.5**	**1.5**	1.0	1.0
Photosynthesis cluster					
slr1181	*psbA1*	**2.1**	**2.9**	−1.3	**−1.7**
slr1182	hypo	**1.6**	**3.6**	1.1	**−2.1**
slr1183	hypo	**1.5**	**3.0**	−1.3	**−2.6**
slr1184	hypo	**1.7**	**2.4**	**−1.5**	**−2.1**
slr1185	*petC2*	**1.5**	**2.4**	1.3	−1.2
slr1186	hypo	1.2	**1.5**	−1.1	−1.4
sufBCDS operon					
slr0074	*ycf24*	−1.4	−1.1	1.1	−1.2
slr0075	*ycf16*	**−1.7**	−1.3	1.0	−1.3
slr0076	hypo	**−1.9**	**−1.5**	1.0	−1.3
slr0077	*nifS*	**−1.6**	**−1.6**	1.2	1.2

and chlorophyll *a* biosynthesis under low-oxygen conditions (Minamizaki *et al.*, 2008). In addition, we observed upregulation of *chlG* (*slr0056*), encoding chlorophyll *a* synthase and downregulation of *chlN* (*slr0750*) and *por* (*slr0506*) in both strains (Table 2).

Upregulation of genes encoding transport and binding proteins constituted a major response to low-oxygen conditions in both strains. Transcript abundance was increased for 20 genes that encoded a diverse range of transport and binding proteins, including cation transporters and metal permeases in both strains. Only three genes in this category were downregulated in both strains, these encoded the two ferric iron-binding subunits of the iron transport system, Fut (*futA1*, *slr1295* and *futA2*, *slr0513*) and a putative ferrichrome-iron receptor (*slr1490*) (Badarau *et al.*, 2008; Katoh *et al.*, 2001).

Nine regulatory genes were upregulated in both strains, including four histidine kinases, two response regulators, a hybrid sensor–regulator, a transcription regulator and the LexA-like protein (*sll1626*). The LexA-like protein binds two sites in the hydrogenase (*hox*) operon and may function as a transcription activator (Gutekunst *et al.*, 2005; Oliveira & Lindblad, 2005). Consistent with this, we observed that transcript levels of the *hox* operon (*sll1220–sll1226*) increased in both strains under low oxygen (Table 2).

Genes with altered expression in the ΔHik31 strain compared with the wild-type under low-oxygen conditions (Hik31-dependent changes)

Low-oxygen conditions altered transcript abundance of many genes involved in photosynthesis and respiration (26 and 36 genes in the wild-type and mutant, respectively). Twenty genes had decreased mRNA levels in the wild-type and one had decreased mRNA levels in the ΔHik31 strain. Many of the genes downregulated in the wild-type and

Table 3. Selected differentially regulated genes in *Synechocystis* sp. strain PCC 6803 in the wild-type and ΔHik31 strain on transition from aerobic to low-oxygen conditions

Genes were considered differentially regulated when FDR=0.05, fold change >1.5 (bold type). The fold changes were calculated by dividing the normalized mean intensities obtained for: the wild-type under low-oxygen conditions by those for the wild-type under aerobic conditions (WT); the ΔHik31 strain under low-oxygen conditions by those for the ΔHik31 strain under aerobic conditions (ΔHik31); the wild-type under aerobic conditions by those for the ΔHik31 strain under aerobic conditions (WT/ΔHik31, Air); and the wild-type under low-oxygen conditions by those for the ΔHik31 strain under low-oxygen conditions (WT/ΔHik31, Low O_2). unk, Unknown; reg, regulatory function.

Gene no.	Name	WT	ΔHik31	WT/ΔHik31	
				Air	Low O_2
Cellular processes					
Chaperones					
sll0058	dnaK1	1.1	**2.2**	**1.6**	−1.3
sll0170	dnaK2	−1.2	**1.5**	**1.5**	−1.2
sll0416	groEL2	−1.7	1.3	1.0	−2.2
sll0430	htpG	−2.0	1.0	1.0	−2.0
slr2075	groES	−1.5	**1.6**	**1.5**	−1.6
slr2076	groEL1	−1.7	1.4	**1.6**	−1.5
sll1514	hspA	−1.1	1.2	**−2.3**	**−3.0**
Biosynthesis of cofactors, prosthetic groups					
Chlorophyll and porphyrin synthesis					
sll1184	ho1	1.0	**1.7**	1.3	−1.2
sll1185	hemF	**−1.9**	**2.1**	**5.0**	1.3
Energy metabolism					
Pyruvate/acetyl-CoA metabolism					
sll0920	ppc	**2.1**	1.3	**1.5**	**2.3**
slr1096	pdhD	**−1.6**	−1.1	−1.1	**−1.5**
sll1721	pdhB	**−1.6**	−1.3	−1.2	**−1.5**
sll0741	nifJ/pfo	**1.5**	1.0	−1.1	**−1.5**
slr0301	ppsA	**3.4**	**2.0**	**2.8**	**4.6**
Photosynthesis and respiration					
Phycobilisome					
sll1577	cpcB	1.2	**1.6**	**1.7**	1.3
sll1578	cpcA	1.2	**1.6**	**1.7**	1.2
sll1579	cpcC2	1.0	**1.5**	**1.5**	1.0
sll1580	cpcC1	1.1	**1.6**	**1.7**	1.2
ssl3093	cpcD	**−2.1**	1.1	1.3	**−1.7**
slr2051	cpcG1	1.2	**1.6**	**1.5**	1.1
sll1471	cpcG2	**−1.9**	−1.2	**−2.1**	**−3.4**
slr2067	apcA	1.2	**1.5**	**1.5**	1.3
slr1986	apcB	1.2	**1.5**	**1.7**	1.4
ssr3383	apcC	**−2.2**	1.2	1.1	**−2.3**
slr1459	apcF	**−1.6**	1.1	1.0	**−1.8**
PSI					
slr1835	psaB	1.2	**1.6**	1.4	1.0
ssl0563	psaC	1.0	**1.5**	1.4	1.0
slr0737	psaD	1.1	**1.6**	**1.6**	1.0
sll0819	psaF	1.1	**1.6**	**1.5**	1.1
slr1655	psaL	1.1	**1.6**	1.4	1.0

Table 3. cont.

Gene no.	Name	WT	ΔHik31	WT/ΔHik31	
				Air	Low O_2
PSII					
slr1311	psbA2	1.2	**1.7**	**1.5**	1.1
sll1867	psbA3	1.1	**1.5**	1.4	1.0
slr0906	psbB	1.2	**1.6**	**1.5**	1.1
sll0849	psbD	1.2	**1.5**	1.4	1.1
sll0851	psbC	1.1	**1.6**	**1.5**	1.1
slr0927	psbD	1.1	**1.5**	1.4	1.0
sll1281	psbZ	**−1.7**	1.0	−1.2	**−2.0**
Cytochrome b6f complex					
sll1317	petA	**−1.8**	1.1	1.1	**−1.8**
slr0342	petB	1.2	**1.5**	1.4	1.1
slr0343	petD	1.2	**1.6**	1.3	1.0
ATP synthase					
sll1322	atpI	−1.2	1.2	**1.6**	1.2
sll1323	atpG	**−2.1**	1.0	1.3	**−1.5**
sll1324	atpF	**−2.3**	1.0	1.2	**−1.9**
sll1325	atpD	**−2.0**	1.1	1.3	**−1.8**
sll1326	atpA	**−1.8**	1.1	**1.6**	−1.2
sll1327	atpC	**−2.2**	1.1	**1.5**	**−1.6**
slr1329	atpB	−1.3	**1.5**	**1.8**	−1.1
slr1330	atpE	**−2.6**	−1.3	1.0	**−2.1**
Soluble electron carriers					
sll0199	petE	1.2	**1.5**	1.4	1.1
slr1643	petH	−1.1	**1.6**	**1.5**	−1.2
ssl0020	petF	1.2	**1.6**	1.4	1.1
Regulatory proteins					
slr0312	rre	**−2.3**	−1.1	−1.1	**−2.2**
sll0797	rre	**−2.0**	−1.1	1.1	**−1.6**
sll0798	hik30	**−1.7**	1.1	−1.1	**−2.1**
slr1285	hik34	**−1.5**	1.1	1.0	**−1.7**
Gene cluster					
slr1852	unk	**−1.8**	−1.4	−1.1	−1.3
slr1853	other	**−2.0**	−1.3	−1.1	−1.3
slr1854	unk	**−1.9**	−1.1	1.3	−1.4
slr1855	unk	**−1.8**	−1.2	1.2	−1.2
slr1856	icfG	**−2.5**	−1.1	**1.5**	**−1.7**
slr1857	amylase	**−2.3**	−1.0	**1.5**	−1.4
slr1859	reg	**−2.5**	−1.3	1.0	**−2.0**
slr1860	icfG	**−1.8**	1.0	**1.6**	1.1

upregulated in the mutant encoded proteins involved in light harvesting and photosynthetic electron transport. For example, genes encoding phycobilisome components were upregulated in the ΔHik31 strain and downregulated in the wild-type (Table 3). This parallels the changes observed in phycobilisome content in the whole cell spectra shown in Fig. 2. In addition to the genes encoding HO and CPO that were dramatically upregulated in both strains, a second copy of genes encoding HO (*ho1*, *sll1184*) and CPO (*hemF*, *sll1185*) had increased mRNA levels in the mutant strain, but not in the wild-type.

Genes encoding core proteins of PSII were upregulated in ΔHik31, but not in the wild-type under low-oxygen

conditions (Table 3). The gene *psbZ*, encoding a PSII protein involved in modulating electron transfer (Bishop *et al.*, 2007) had decreased mRNA levels in the wild-type, but not in the mutant. Similarly, genes encoding cytochrome b_6f subunits (*petB*, *slr0342* and *petD*, *slr0343*) were upregulated in the mutant and the gene encoding subunit PetA (*sll1317*) was downregulated in the wild-type. Several genes encoding PSI subunits, as well as soluble electron carriers ferredoxin NADP-oxidoreductase (*petH*, *slr1643*), plastocyanin (*petE*, *sll0199*) and ferredoxin I (*petF*, *ssl0020*) had increased mRNA levels in the ΔHik31 strain but not the wild-type (Table 3). In addition, under low-oxygen conditions, the genes encoding ATP synthase subunits were downregulated in the wild-type (Table 3).

In ΔHik31, the gene *slr0228*, encoding an FtsH metalloprotease, was upregulated; this protein is involved in the maintenance of PSII activity under stress conditions (Komenda *et al.*, 2006). Two genes downregulated in the wild-type, *desA* (*slr1350*) and *desD* (*sll0262*), encode acyl-lipid desaturases that introduce double bonds at defined positions in fatty acids that form membrane glycerolipids (Wada & Murata, 1990). The presence of double bonds in membrane lipids has been implicated in protecting PSII from photoinhibiton under stress conditions (Allakhverdiev *et al.*, 2001; Tasaka *et al.*, 1996). These data suggest that there were modifications to photosynthetic performance under low oxygen and that these modifications were mediated by Hik31.

Molecular chaperones play important roles in preventing the misfolding and aggregation of proteins and increased transcript levels of these genes are frequently associated with stresses, such as heat, high light or oxidative stress (Hihara *et al.*, 2001; Li *et al.*, 2004; Rupprecht *et al.*, 2007; Singh *et al.*, 2006). Low-oxygen conditions did not induce this stress response in the wild-type, in fact four chaperones (*groEL2*, *groES*, *groEL1* and *htpG*) exhibited decreased transcript abundance under low-oxygen conditions (Table 3). In contrast, in ΔHik31, transcript levels of several chaperones were increased (*htpG*, *dnaK1*, *dnaK2* and *groES*) or unchanged (*groEL2* and *groEL1*) following the transition to low-oxygen conditions (Table 3). In addition, *sll1514*, encoding the chaperone HspA, exhibited substantially higher mRNA levels in the ΔHik31 strain than the wild-type under both aerobic and low oxygen conditions.

Twelve regulatory genes had decreased mRNA levels in the wild-type, but the mutant. These genes included *sll0797* and *sll0798* that encode a two-component system with multiple regulatory roles responding to Ni^{2+}, cellular redox and inorganic carbon availability (Li & Sherman, 2000; López-Maury *et al.*, 2002; Wang *et al.*, 2004a). The gene *slr1285* that encodes Hik34 was also downregulated in the wild-type. This histidine kinase mediates the response to numerous stresses, including upregulation of transcript abundance of a number of chaperone-encoding genes. This is consistent with our observation of downregulation of *hik34* and genes encoding chaperones (*groEL2*, *htpG*, *groES* and *groEL1*) in the wild-type under low oxygen conditions

(Table 3). A further four response regulators (*sll0039*, *slr0312*, *slr1042* and *slr1693*) and one hybrid sensor–regulator (*slr0322*) were downregulated in the wild-type.

Under low-oxygen conditions, genes encoding ribosomal proteins showed decreased mRNA abundance in the wild-type (23 genes) and increased mRNA levels in the ΔHik31 strain (20 genes). Downregulation of ten genes involved in tRNA synthesis and modification provided a further indication that translation is suppressed in the wild-type (Supplementary Table S1, available with the online version of this paper). This response appeared to be accompanied by a decrease in transcription, as we observed the downregulation of seven genes involved in transcription in the wild-type, including genes encoding RNA polymerase subunits, and increased mRNA level of the sigma factor, SigH, associated with stationary-phase growth (Foster *et al.*, 2007).

Kahlon *et al.* (2006) reported that the absence of Hik31 resulted in glucose sensitivity. This sensitivity was likely to result from accumulation of glucose due to decreased glucokinase activity and not due to transcriptional changes. In fact, the authors reported that the presence of glucose resulted in few transcriptional changes for genes involved in glucose metabolism in the wild-type compared with a Hik31 mutant. Similarly, our data show little change in transcripts involved in glucose metabolism, or the oxidative pentose phosphate pathway (Supplementary Table S1). However, several genes encoding proteins involved in pyruvate metabolism were altered in the wild-type, but not in the mutant, following transition to low oxygen (Table 3). This included downregulation of genes *phdB* (*sll1721*) and *phdD* (*slr1096*) involved in converting pyruvate to acetyl-CoA and upregulation of genes encoding proteins phosphoenolpyruvate synthase (*ppsA*, *slr0301*) and phosphoenolpyruvate carboxylase (*ppc*, *sll0920*) that catalyse the conversion of pyruvate to phosphoenolpyruvate (PEP) and PEP to oxaloacetate, respectively. Oxaloacetate is a precursor in numerous biosynthetic reactions (e.g. production of amino acids) and it has been suggested that PEP carboxylase plays a role in maintaining the availability of carbon compounds for many pathways (Owttrim & Colman, 1986). The role of oxaloacetate under low-oxygen conditions is not clear, but our data indicate that Hik31 is involved in regulating its production.

The gene cluster *slr1852–slr1860* was downregulated in the wild-type, but not in the mutant. The gene *icfG* (*slr1860*) encodes a protein, serine/threonine phosphatase, that is vital for growth in the presence of glucose and this gene is upregulated in the wild-type in the presence of glucose, but not in a ΔHik31 strain (Beuf *et al.*, 1994; Kahlon *et al.*, 2006; Shi *et al.*, 1999). In our experiment, *icfG* was part of an eight-gene cluster that exhibited low transcript levels in both strains, but was more abundant in the wild-type than the mutant under aerobic conditions. Other genes in this cluster may have roles in regulation, including *slr1856* and

slr1859 that encode proteins with similarities to phospho-protein substrates for protein kinases (Shi *et al.*, 1999). Our data indicated that Hik31 is involved in regulating this *icfG* cluster and this was suggested to be one of the roles of Hik31 in glucose metabolism (Kahlon *et al.*, 2006).

Conclusion

Synechocystis sp. PCC 6803 demonstrated a low-oxygen-specific response that included upregulation of the *psbA1* gene cluster, genes involved in chlorophyll biosynthesis, and the hydrogenase operon, as well as the transient upregulation of *hik31* (Summerfield & Sherman, 2008). The interruption of the chromosomal copy of *hik31* resulted in increased growth under low-oxygen conditions. However, the wild-type and ΔHik31 strain shared some transcriptional responses to low oxygen conditions. These responses indicated that additional regulators are involved in the response to low-oxygen conditions. One correlation is between the gene encoding the LexA-like protein (Sll1626) and the *hox* operon, these genes were upregulated after the transition to low O_2. This was consistent with the finding that Sll1626 interacts with the *hox* promoter and positively regulates its expression (Oliveira & Lindblad, 2005). The genes encoding Sll0359 and Sll0882 are also regulators of the *hox* operon (Ishii & Hihara, 2008; Oliveira & Lindblad, 2008), these genes are not upregulated under low-oxygen conditions, but may play a role in regulation.

Differences in the transcriptional response of the wild-type and mutant to low-oxygen conditions included: (i) the downregulation of many genes associated with decreased growth in the wild-type, but not in the ΔHik31 mutant and (ii) the upregulation of many stress responsive genes in the mutant, but not the wild type. These data suggest that the chromosomally encoded Hik31 is involved in negatively regulating gene expression in response to low oxygen. This is consistent with the growth observed under low-oxygen conditions where, in the absence of Hik31, there was no downregulation of genes involved in growth and the ΔHik31 strain grew faster than the wild-type (Fig. 3). It is unclear whether Hik31 is sensing low-oxygen conditions or responding to intracellular changes resulting from low-oxygen conditions, for example changes in cellular redox, which have been shown to induce transcription of *hik31* (Hihara *et al.*, 2003). The plasmid-encoded Hik31 may be involved as well, but the growth data suggest that the two Hik31 copies have similar effects under low-oxygen conditions and that any gene expression changes will only show quantitative alterations. However, the role of Hik31 in switching between photoautotrophic and mixotrophic growth suggests a wider role of this histidine kinase. We will identify other conditions in which the Hik31 proteins control different, but related, processes and highlight the overall importance of this two-component regulator (S. Nagarajan and L. A. Sherman, unpublished results).
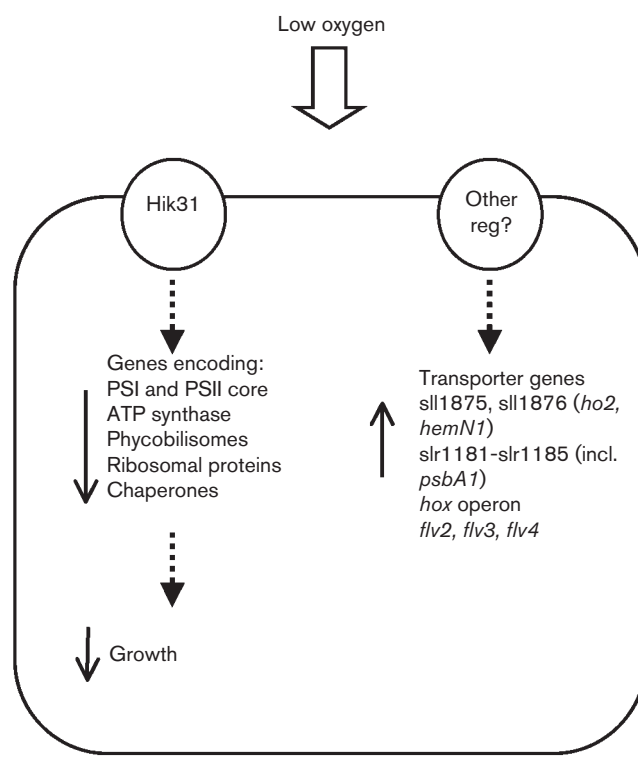

Fig. 3. Model of the transcriptional response of a *Synechocystis* sp. PCC 6803 cell to low-oxygen conditions. The histidine kinase Hik31 is involved in upregulation of genes encoding PSI and PSII core proteins, ATP synthase, phycobilisomes, ribosomal proteins and a number of chaperones. An unidentified regulator(s) is involved in upregulating genes encoding a number of transporters, *sll1875* and *sll1876*, the *hox* operon, *slr1181–slr1185* and the flavoproteins (Flv2, 3 and 4, encoded by *sll0219*, *sll0550* and *sll0217*, respectively).

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