Effects of Oxygen and Light Intensity on Transcriptome Expression in Rhodobacter sphaeroides 2.4.1

REDOX ACTIVE GENE EXPRESSION PROFILE*

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The roles of oxygen and light on the regulation of photosynthesis gene expression in Rhodobacter sphaeroides 2.4.1 have been well studied over the past 50 years. More recently, the effects of oxygen and light on gene regulation have been shown to involve the interacting redox chains present in R. sphaeroides under diverse growth conditions, and many of the redox carriers comprising these chains have been well studied. However, the expression patterns of those genes encoding these redox carriers, under aerobic and anaerobic photosynthetic growth, have been less well studied. Here, we provide a transcriptional analysis of many of the genes comprising the photosynthesis lifestyle, including genes corresponding to many of the known regulatory elements controlling the response of this organism to oxygen and light. The observed patterns of gene expression are evaluated and discussed in light of our knowledge of the physiology of R. sphaeroides under aerobic and photosynthetic growth conditions. Finally, this analysis has enabled us to go beyond the traditional patterns of gene expression associated with the photosynthesis lifestyle and to consider, for the first time, the full complement of genes responding to oxygen, and variations in light intensity when growing photosynthetically. The data provided here should be considered as a first step in enabling one to model electron flow in R. sphaeroides 2.4.1.

Rhodobacter sphaeroides 2.4.1 is a purple nonsulfur photosynthetic bacterium that can grow aerobically, anaerobically in the light, or anaerobically in the dark in the presence of external electron acceptors such as dimethyl sulfoxide. When grown anaerobically in the light or dark, R. sphaeroides synthesizes an intracytoplasmic membrane (ICM)3 system, which constitutes the photosynthetic apparatus and possesses the structural components necessary for light energy capture, subsequent electron transport, and energy transduction (1). The composition and assembly of the ICM are known to be tightly regulated, with light intensity and oxygen tension being among the most prominent environmental stimuli that serve to control the synthesis and function of the ICM (2, 3).

The role of oxygen in regulating the presence or absence of the ICM in R. sphaeroides has been well documented in numerous publications over the past 50 years (2–5). Recently, studies have revealed that the role of oxygen in ICM formation is the result of the actions of key regulatory pathways, including the cbb3 terminal oxidase-PrrBA pathway (6–10), the PpsR-AppA repressor-anti-repressor pathway (11–14), and the FnrL pathway (15, 16). Light intensity has a somewhat more subtle effect on photosynthetically growing cells, whereby the cellular levels of ICM are inversely related to light intensity and the composition of the ICM is differentially altered with changing light intensity (1, 17). Recent studies have implicated the cbb3-Prr system (18) and the PpsR-AppA system (14, 19) as being factors in light control of PS gene expression. Very recent work has further implicated the AppA protein as being involved in blue light control of PS gene expression (13, 20).

What has been obvious from all of these studies is that they have been by necessity incremental, i.e. one gene at a time. With the advent of the DNA sequence, assembly and annotation of the R. sphaeroides 2.4.1 genome (21), as well as the construction of an Affymetrix GeneChip, we are in the unique position of being able not only to validate the earlier described observations, but more importantly we are in the position to extend these observations to the expression of all genes comprising the photosynthesis regulon including N2 and CO2 fixation, and taxis (22–25), as well as other physiologic activities for which genetic elements are regulated by oxygen and light intensity. Finally, we are in the position of being able to follow the expression of well studied regulatory elements controlling the photosynthesis lifestyle, as these respond to oxygen- and light-induced regulation. For the first time, we are able to observe the expression of many genes for which encoded proteins are responsible for the flow of redox in R. sphaeroides, with the goal being to model these complex interacting pathways.

In the present work we have extended our studies of oxygen control of gene expression as well as the role of high (100 W/m2) versus low (3 W/m2) light intensity on gene expression in R. sphaeroides 2.4.1. We directly address the hypothesis that oxygen and light regulation of gene expression in R. sphaeroides extend well beyond those systems, giving rise to the photosynthetic lifestyle.

EXPERIMENTAL PROCEDURES

R. sphaeroides Growth Conditions—R. sphaeroides strains were grown at 30 ± 0.5 °C on Sistrom’s minimal medium A containing...
succinate as a carbon source. Aerobic cultures were grown sparging with a gas mixture of 20% O2, 69% N2, and 1% CO2, and harvested at an A600 of 0.2 ± 0.02 to ensure oxygen saturation. Photosynthetic cultures were grown at a light intensity of either 3 or 100 W/m² measured at the surface of the growth vessel and sparging with 95% N2, 5% CO2 and harvested at A600 of 0.45 ± 0.05, which insures that self-shading is not problem. Light intensity was measured using an YSI-Kettering model 555A radiometer (Simposion Electric Co.).

**DNA Manipulation**—To prepare hybridization probes for Northern blot analysis, genes encoding the translation elongation factor G (RSP2248) and two encoding cold shock proteins (RSP2346 and RSP3621) were PCR-amplified using chromosomal DNA of *R. sphaeroides* 2.4.1 as template. RSP2248 was amplified with primers 5'-GAT CTT CGA CGG CCG CAA GCT GGT CTT-3' and 5'-GAA GCT CGG CCA TCA GGT C GT CTA-3'. RSP2346 and 3621 were amplified with primers 5'-GGT TGA GAC GAA GAC GGC CTC GAG CAG-3' and 5'-GGC CGG TTG CTG ATG CTG ACG CGC TGC-3', and with primers 5'-ACA GGA GAG ATC ACG AGC GGC AAT GGC AGC ATT GGC-3' and 5'-GGC GAG AAC GAG GTT CGT CGC CGA CTC GCG GC-3', respectively. PCR-amplified DNA fragments were subcloned in pGEM-T Easy vector (Promega), and the DNA sequence was confirmed. Each subclone was digested with NotI, and the DNA fragments containing the PCR-amplified DNA were isolated following agarose gel electrophoresis, and labeled with [α-32P]dATP using the RadPrime DNA labeling system (Invitrogen). Hybridization and washing was performed with QuickHyb hybridization solution following the instructions from the manufacturer (Stratagene). Quantitation of specific signals from the Northern blot analyses was performed by subtracting background for each lane from the values for the specific band using the spot density tools of the AlphaEaseFC™ imaging system (Alpha Innotech).

**RNA Manipulation**—A previously described RNA isolation procedure was modified to optimize the isolation of intact mRNA for DNA microarray analysis. We modified the earlier procedure by eliminating cell collection by centrifugation. A volume of cells grown as described was directly pipetted into an equal volume of 2 × lysis buffer (100 °C). After thorough mixing, lysis cells were immediately transferred to an equal volume of hot phenol solution (66 °C). Total time required for the procedure from the culture vessel to hot phenol was kept to less than 1 min to minimize mRNA degradation and to maximize the yield of intact mRNA. The remainder of the RNA purification procedure was identical to that described previously (26). Each isolated RNA sample was treated with 50 μl of RNase-free DNase I (1 unit/μl, Promega) and 50 μl of 10 × buffer in a total volume of 500 μl. Samples were incubated for 1 h at 37 °C, extracted with acidic phenol, acidic phenol/chloroform, and chloroform; and then precipitated by 1 ml of ethanol. The pellet was washed with 75% ethanol and suspended in diethylpyrocarbonate-treated water. Chromosomal DNA contamination was tested by PCR amplification using the mdR-specific primers (a and b) as described previously (26).

**Microarray Experiment and Data Analysis**—The transcriptome profiles from three independent cultures grown and processed independently under high and low light intensities, as well as under aerobic conditions, were analyzed. The present/absent/marginal percentage and the reliability between triplicate samples are summarized in Table I. “Present” calls by MAS5 for both 100 W/m² and aerobic growth conditions were approximately the same percentage values; these calls were considerably greater than those observed for cells grown at 3 W/m². Conversely, the percentage “absent” was greater at 3 W/m² than observed for aerobic or high light grown cells. “Marginal” calls were approximately the same regardless of growth condition. The experimental reproducibility between samples of the same data set is indicated by the r value (Table I). There are two different forms of control genes on the *R. sphaeroides* 2.4.1 GeneChip. In addition to genes from other organisms, namely *E. coli*, *Bacillus subtilis*, and bacteriophage P1, as well as nine ORF from *R. sphaeroides* 2.4.1 which are represented by five copies each. The 24 control probe sets from the different organisms were all called as “absent,” and the standard deviation for the five copies each of nine ORF from *R. sphaeroides* 2.4.1 did not exceed 25% of the average value for the set. The mean intensity levels for each probe set were cross-compared using dChip 1.2.
software, and the -fold change between the group means was calculated using the standard deviation of the group means (27, 28). Those genes/ORFs that showed a greater than 1.5-fold change with 90% confidence boundaries were used for further analysis.

In Fig. 1, we have depicted the numbers of genes/ORFs that either increased or decreased relative to one of the other culture conditions, with all combinations being provided. Several points are noteworthy. There is the greatest degree of variability in gene expression when the 3 W/m² condition is viewed relative to aerobic growth as opposed to when high light (100 W/m²) is similarly compared with aerobic growth. In the former comparison, there were 543 up-regulated and 911 down-regulated genes. This observation is consistent with earlier, although much more limited observations that high light grown photosynthetic cells are more similar to aerobic grown cells, at least with regard to the percentage of genes up- or down-regulated. However, such cells contain 3–4-fold higher levels of photosynthetic membrane, as well as a greater differential increase in the B800–850 spectral complex relative to the B875 spectral complex, and a volume nearly 70% greater than high light grown cells (1). Second, the data reveal that the median range in increased -fold change in gene/ORF expression is in the 3–5 range for aerobic versus 3 W/m² or 100 W/m², declining to the 2–3-fold range for 3 W/m² versus 100 W/m². Similarly, when one looks at those genes/ORFs showing a decline in expression, the median values decrease from −2 to −3 range to −3 to −5 range as one goes from aerobic versus 3 W/m² to 3 W/m² versus 100 W/m². A comparison between the two photosynthetically grown data sets shows 636 genes/ORFs up-regulated and 427 down-regulated. Because there are between 1200–1500 genes/ORFs showing changes under these conditions, it implies that most of the genes/ORFs affected by oxygen and light intensity have never been observed. If we are overly generous in compiling those genes known to be part of the photosynthetic lifestyle, we come up with −150–175.

A detailed list of genes/ORFs that comprise the data described in Fig. 1 can be found at our web site (www.rhodobacter.org) and has been included here as supplementary data, available in the on-line version of this article. As described above there are many published studies that have followed, individually, the expression of a few, but not most of the genes encoding the photosystem in R. sphaeroides (32–37), or genes encoding enzymes involved in CO₂ fixation (38, 39), nitrogen fixation (40), electron transport (41–46), taxis (47, 48), or assorted transcriptional regulators (16, 49, 50), etc., using more traditional approaches such as Northern hybridization, lacZ fusions, and even phoA fusions. However, these studies have been by necessity selective, and for the most part the overwhelming majority of these genetic elements comprising the photosynthetic lifestyle have never been investigated under the diverse environmental conditions described here.
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Photosynthesis Gene Cluster—As previously described for those genes encoding the apoproteins of the spectral complexes (19, 32, 33), there is a wealth of expression data, but for the most part the majority of the genes of the PS gene cluster of *R. sphaeroides* 2.4.1 have never been studied, except to note that their expression is higher under photosynthetic growth conditions relative to aerobic growth. In Fig. 2, in the left panel we show the expression profiles for those genes encoding the apoproteins of the reaction center (RC, *pueBA*, *psFL*, *M*), the B875 complex (*pufBA*), and the B800–850 complex (*pucBA*) in the presence of oxygen and high and low light intensity under anaerobic growth. Although these data are a repeat of many earlier studies, they serve a very useful and unique purpose, namely helping to validate the use of the GeneChip. When comparisons are made between gene expression profiles for cells grown at 3 W/m² versus 100 W/m², we also observe, for those genes encoding the apoproteins of the spectral complexes, that these are expressed to much greater levels at low relative to high light. Note that cells grown aerobically constitute our baseline for this analysis. Although there are a few genes encoding enzymes in the pigment biosynthetic pathways, which are expressed at moderately high levels and therefore appear on the left panel of Fig. 2, most of the genes in this category are represented in the right panel where expression levels are greatly decreased. The data reveal that for almost all of the pigment encoding genes, even those appearing in the left panel, their expression levels decline at 3 W/m² relative to 100 W/m². The consistency of this observation, when coupled to the fact that there are higher cellular pigment levels at low light by a factor of −3 (1), is unexpected and appears paradoxical. However, we suggest that these data may be explained by concluding that post-transcriptional controls, at the level of enzyme activity, may play a more prominent role in pigment production than previously suggested (51, 52). Such a conclusion is also supported by some earlier studies in *R. sphaeroides* on PucC (25, 53), CrtA (54), and TspO (55). Throughout these investigations, we caution that we are cognizant that posttranscriptional analyses, such as an analysis of the proteome, corresponding to most of these genes is lacking. However, many of the pigment-encoding genes that appear to reside in the same transcription unit show similar levels of expression and behavior. Nonetheless, there are a few genes that defy this general trend, e.g. *bchM*, *bchP*, *crtE*, *crtA*, etc. Evidence exists that for certain of these genes there are additional regulatory factors (19, 56). The CrtA protein, which has been observed to be highly expressed, is under redox control (56); the *bchE* gene is regulated by FnrL (19), which itself is under post-transcriptional regulation; and for the *tspO* gene (56), *phoA* fusion data support the expression profile depicted here (see Fig. 3). We have also included expression data for the *hemA* gene, which encodes the first step in heme biosynthesis as well as a critical late step in heme biosynthesis, coproporphyrinogen III oxidase (*hemN* and *hemZ*), expression levels of which are carefully controlled (19, 57, 58). All these gene products are essential to providing intermediates, as the result of the greatly increased demand of both the Bchl and heme biosynthetic pathways, in photosynthetic cells. Expression of the *hemA* gene as a function of light intensity is what we would expect in that
hemA is expressed well aerobically and derepressed photosynthetically (59), but here we show it is further derepressed under low light. Similarly, hemV was expressed aerobically at a low level with hemZ being off, but both showed increased expression when cells were shifted to anaerobic conditions as a result of the fact that both are under FnrL control (19, 56). The expression level of pacC relative to pacBA is $\sim 4\%--8\%$, which is entirely consistent with the Northern hybridization results (35) and lacZ fusion studies (55) reported earlier, despite the fact that all of these genes reside in the pacBAC operon. Further, the expression of puhA (60) as well as pufBA (32) shows low expression under aerobic conditions as observed here, but both loci show increased expression as light intensity decreases.

cycA encoding cytochrome $c_2$ is transcribed at very high levels under aerobic conditions increasing $2$-fold at 3 W/m$^2$ photosynthetic conditions, which is entirely consistent with independently obtained results (61--63). Nonetheless, the regulation of cycA by light intensity adds significantly to our knowledge concerning this obligatory photosynthetic electron carrier.

Thus, for the major structural protein encoding genes involved in photosynthetic membrane structure and function, the results reported here are consistent with early studies when examined through a comparison of aerobic versus photosynthetic growth. However, the role of light intensity on those genes encoding the enzyme of the pigment biosynthetic pathways is quite unexpected.

Finally, there still remain a number of ORFs, 479, 128, 213, etc., the functions of which are still not well understood, but expression levels of which are clearly under light regulation and in some instance under both light and oxygen control. The data provided here make it important to assess the function of these ORFs and their role(s) in the processes described above.

Transcriptional Regulators—In studies of the regulation of photosynthesis gene expression in *R. sphaeroides*, a number of regulatory elements and their cognate genetic loci have been identified and their site of action has been the subject of numerous publications (8, 10, 14, 16, 49, 50, 56, 64--69). However, the transcriptional regulation of these regulators by oxygen and light has not, with very few exceptions, been examined. In Fig. 3, we have depicted the relative expression levels for the genes we know to be involved in the regulation of PS gene expression in *R. sphaeroides*. Several of the regulatory genes show little variation in expression levels regardless of the growth conditions, e.g. osp (64), ppsR (14, 65), mgpS (66), ihfA (67), ihfB (67), prrA (49), and fnrL (16). On the other hand, prrB (8, 10), prrC (25), appA (50), ppaA (68), and tspO (56) reveal significant changes in expression levels, depending on growth condition. Genes prrB (histidine kinase) and prrC (signal transducer), part of the cbb$\_3$ regulon together with the response regulator PrrA, are expressed to a greater extent under aerobic conditions with the expression levels diminishing as cells are grown at progressively lower light intensities. These two genes are believed to be divergently transcribed from the same promoter region (25). The fact that the expression of the response regulator prrA is severalfold higher, but relatively constant regardless of growth conditions emphasizes the importance of the prrB and prrC gene products in this signal transduction pathway, which serves to maintain PS gene expression at basal levels under aerobic conditions. As light intensity declines it would appear that PrrB activity becomes limiting, but so does the ability of the cell to inhibit PrrB kinase function as the inhibitory signal transducer prrC expression also declines. Because PrrA is relatively abundant this result might also suggest that “other” histidine kinases become a factor in activating PrrA (70) under low light as the cognate histidine kinase (PrrB) decline. Although prrA is down-stream of prrC in this operon, there is a second promoter for prrA buried within the prrC structural gene, which is available to increase the expression of prrA (25). Thus, the expression pattern for these genes can differ. AppA expression progressively increases as cells move from oxygenic conditions to decreasing light intensity under photosynthetic conditions. Because AppA serves as an anti-repressor of PpsR, for which gene expression levels are constant, the transcriptome data fit with the physiological observations of increased PS gene expression as cells move to lower light intensities, because the appA gene product is an anti-repressor of PpsR. A similar pattern of
expression is observed for tspO, the outer membrane protein product of which is involved in the efflux of intermediates of the porphyrin biosynthetic pathway under photosynthetic growth (71). Because the flow of intermediates through the heme and bacteriochlorophyll pathways is increased as light intensity decreases (see above), the role of TspO becomes more critical at lower light fluxes. The family of genes designated spbA, -B, -C, and -D is similar to the R. capsulatus hvrA gene (72), as well as the originally identified spb gene (73) (designated spbA) in R. sphaeroides, which is suggested to be involved in light regulation of PS gene expression. Although all genes in this family are expressed under all growth conditions, expression is both variable, depending upon the member of the gene family, and also higher under aerobic growth, which would appear to run counter to their presumed role in light regulation (73). The answer to this paradox is not obvious at this time. The expression pattern for ppaA, which is transcriptionally coupled to ppsR, is very interesting, because a recent study of ppaA function in R. sphaeroides appears to be consistent with the observation reported here (68). Nonetheless, this gene, which is immediately upstream of ppsR and transcriptionally coupled to ppsR, expression levels of which are low and constant, shows low aerobic expression but increased expression as cells are grown photosynthetically at decreasing light intensities. This expression pattern strongly resembles that of appA, suggesting that both of these gene products play a role in affecting PpsR function. It should be noted that the presumed PpaA protein shows homology with the central portion of AppA. There is evidence (74) that TspO is part of the PspR/AppA regulon, and, together with ppaA, all these genes reveal similar expression patterns and all appear to be intimately involved in maintaining precise control over pigment levels during photosynthetic growth. The genes encoding the subunits of the IHF are highly expressed and generally independent of growth conditions, which might be expected as the result of the varied physiological roles of this DNA-binding protein. This expression pattern is similar to that observed for E. coli IHF (for review, see Ref. 75). Finally, fnrL is strongly expressed and generally its expression levels are independent of growth conditions, which is also true in E. coli (for review, see Ref. 75).

Electron Transport Chain—When R. sphaeroides undergoes the metabolic changes that accompany a shift from aerobic to anaerobic photosynthetic conditions, it is anticipated that those genes and their cognate proteins involved in aerobic redox activities should undergo change. Very few of these changes have been documented in the literature (41, 42, 61, 76). Similar changes as a function of alteration in light intensity have also not been investigated. Thus, we chose to examine the expression levels of those genes for which gene products have been well studied, and which are essential for aerobic growth, and in some cases photosynthetic growth. Cytochromes c2 and c5 have been documented (77) to be involved in aerobic respiration in R. sphaeroides, and in the case of cytochrome c2, it is essential for photosynthetic electron transport (61, 78). The transcription profile depicted in Fig. 4 fits with the increase demand for cytochrome c2 as the levels of photosynthetic membrane increase. In the case of cytochrome c5, it does not serve as a carrier in photosynthetic electron transport (42, 79), unlike cytochrome c2: hence it is primarily but not exclusively as shown here, an aerobic component of redox flow. The fact that it can move electrons from the bc1 complex to the cbb3 terminal oxidase aerobically (77) also suggests that it plays a similar role in the movement of electrons over the same pathway under anaerobic conditions, because there is evidence of anaerobic redox flow through the cbb3 (7, 19). Therefore, the expression of cycY appears to fit with the presumed physiological demand for this carrier under these diverse growth conditions. The genes encoding the subunits of the bc1 complex to the cbb3 terminal oxidase are expressed under all growth conditions as expected, with their levels of expression being lowest aerobically and increasing as light intensity decreases under photosynthetic conditions (Fig. 4). Not only do all of these genes behave similarly, but the expression levels match the anticipated physiological demand, i.e. increased RC levels and light gathering components at lower light intensities (1). Interestingly, the expression of cycA precisely matches the expression of the

3 M. Gomelsky and S. Kaplan, unpublished result.
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5. Expression patterns for many of the genes encoding enzymes involved in CO₂ fixation. Each point on the graph is derived from the mean values for the microarray experiments conducted in triplicate, and the standard deviations did not exceed 20% of the average value calculated for each gene in the triplicate set except when expression levels were very low. Expression of the form I and form II Calvin cycle gene clusters are represented on the left and right panel, respectively. Gene symbol (RSP number); cbbZ (RSP1278), cbbY (RSP1279), cbbX (RSP1280), cbbS (RSP1281), cbbL (RSP1282), cbbA (RSP1283), cbbP (RSP1284), cbbF (RSP1285), cbbR (RSP1286), cbbF₆ (RSP3266), cbbP₆ (RSP3267), cbbT₆ (RSP3268), cbbG₆ (RSP3269), cbbA₆ (RSP3270), and cbbM₆ (RSP3271).

genes encoding the bc₁ complex from which it accepts electrons under both aerobic and photosynthetic growth conditions. These findings have not been previously documented, but fit our expectation and add to our knowledge of redox flow in R. sphaeroides. Given the expression patterns of these genes, it makes the need for the corresponding proteomic analysis more important than ever. Conversely, and as anticipated from the physiological response, those genes encoding the major aerobic terminal oxidase, namely the aa₃ terminal oxidase, which is responsible for more than 70% of the electron flow under aerobic conditions (18), are expressed to the greatest extent aerobically and shift to decreasingly lower levels of expression as light intensity decreases, and are only minimally transcribed under very low light conditions. On the other hand, encoding the cbb₃ terminal oxidase, which is a critical redox component under microaerobic growth (76), reveal a rather unique response to changing growth conditions. Expression of each of these genes is generally lowest, but similar, under high O₂ aerobic growth and low light photosynthetic growth and highest under high light photosynthetic conditions. Although the data are not provided here, microaerobic growth, e.g. 2% O₂, results in the greatest level of cco operon expression and the analysis of either lacZ fusion data (76) or microarray data are similar (not reported here). Because expression of the cco operon in high light photosynthetic growth is more similar to microaerobic expression (76), it suggests that the FnrL protein, shown to be a major regulator of cco expression microaerobically, is also likely to play a similar role photosynthetically in high light and raises the possibility of a light-induced regulation of FnrL activity. Further, the ccoNOQP operon when examined for the expression levels of each gene, as provided here, is completely consistent. These results should be contrasted with those obtained for genes encoding the aa₃ terminal oxidase, namely as cells become anaerobic the aa₃ becomes less important as an electron carrier, whereas the cbb₃ remains as an important component of redox flow. This observation is enhanced further by virtue of the relatively poor affinity of the aa₃ oxidase for O₂ as compared with the cbb₃ oxidase (80). When the expression pattern depicted for the cco operon (Fig. 4) is compared with the expression pattern for the prrB gene encoding the histidine kinase as well as the prrC gene encoding a component of the cbb₃ signal transduction pathway, we observe an important consistency. The cbb₃ is known to generate a signal that inhibits the autophosphorylation activity of the PrrB membrane-bound histidine kinase, but increases the intrinsic phosphatase activity. The fact that expression of cco is increased at high light is in line with the observation that there is less demand for the photosynthetic apparatus at high light, and increased amounts of cbb₃ would lead to enhanced inhibition of the PrrB histidine kinase. However, because there is no oxygen present under these growth conditions, electron flow through the cbb₃ would help counter that trend. We have previously showed (7) that extra copies of the cco operon in trans decreases the expression of photosynthesis genes, presumably by increased levels of the cbb₃ oxidase. These results support our previous observations of continued electron flow through the cbb₃ under anaerobic conditions.

When genes encoding the bc₁ complex are deleted, R. sphaeroides is still capable of aerobic growth (this is also true when both cycA and cycY are deleted), employing the Qxt quinol oxidase (43). Here, we show that the gene pair qxtAB is expressed under all growth conditions and the results are similar to the lacZ fusion data (81). Scanning the genome of R. sphaeroides reveals the coding potential for a second quinol oxidase, Qox, (81) as well as a caa₃ type terminal oxidase. Repeated efforts involving Northern analysis, lacZ fusions and alterations in growth conditions have yet to reveal conditions under which either of the loci encoding these redox systems is expressed (81). Similarly, the array data reported here have shown that the expression of these same loci was consistently described as absent. As pointed out earlier, confirming the inferences derived from the transcriptome data described above, by analysis of the proteome, would go a long way to being able to model the flow of redox in R. sphaeroides.

CO₂ Fixation Genes—The expression of the photosynthesis genes as well as genes encoding the redox carriers described

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4 J. I. Oh and S. Kaplan, unpublished data.
above is ultimately manifest in the production of ATP and reduced pyridine nucleotides (see supplemental data available in the on-line version of this article) to be used for a variety of cellular activities, but especially those involved in carbon assimilation through the reductive Calvin cycle. Therefore, it was important to follow the expression of those genes encoding crucial components of the photosynthetic CO₂ fixation cycle. The expression of these genes is virtually absent under standard aerobic growth conditions (Fig. 5). The genes encoding the subunits of the form I carboxylase \((cbb_{S}c, cbb_{L})\) show a generally increased level of expression under low light as compared with high light. Conversely, the gene encoding the form II carboxylase \((cbb_{M}_{p})\) is expressed to a greater extent under high light as opposed to low light conditions. In these experiments, CO₂ concentrations were saturating and thus should not be a factor in the differential regulation of form I and form II gene expression as previously described (38, 39, 82). The genes encoding other components of the form I Calvin cycle pathway also increased under low light conditions, whereas genes encoding the form II pathway were increased under high light conditions in the same coordinate fashion as were the Rubisco-encoding genes. With few exception most of these genes have not been studied in detail, and the differences reported here for the form I and form II gene clusters as a function of light intensity raise new and important questions as to their regulation. A recent publication (23) has revealed a difference in gene expression patterns between these two pathways as they relate to the cbb₃ signal transduction pathway. Whereas the results show that both the cbb₃ and cbb₄ promoters are affected similarly by the cbb₃-Prr system under phototrophic growth, the cbb₄ promoter was severely affected by the loss of PrrA, but not the cbb₃ promoter, under chemotrophic growth. Whether or not the results reported here, depicting differences observed under low versus high light, relate to the results cited above remains to be determined. However, it is clear that these two regulons can show differences as well as similarities in their expression pathways.

We might add that all of the genes encoding enzymes in N₂ fixation are also designated absent under the conditions of growth employed here. This is to be expected because of the presence of NH₃ in the growth medium. However, the result is clearly satisfying and serves as an important internal control. Growth of R. sphaeroides grows severalfold faster under high versus low light conditions with aerobic growth rate being similar to that observed for high light (1). Thus, we might anticipate a greater overall level of metabolic activity under conditions of more rapid growth. In Tables II and III, we have excerpted some of these findings from the overall transcriptome analysis depicted in Fig. 1. Genes encoding activities associated with flagella biosynthesis were down-regulated by \(-1.7\)-fold. As expected, the GeneChip as described above has revealed previously unknown expression patterns, apparently regulated by oxygen and light intensity. Several such genes were chosen for more detailed analysis because their physiological role as well as their expression levels proved to be interesting, namely the translation elongation factor G (EF-G, RSP2248), and the cold shock proteins (CSP, RSP2346, and 3621). Further, these genes appear to be physiologically unrelated to the generalized effects of light and oxygen as those relate to redox flow, and therefore we felt it important to determine if the GeneChip data could be further validated. As shown in Fig. 6 and in the supplemental data, expression of RSP2248 encoding EF-G increased substantially under photosynthetic conditions relative to aerobic growth and appeared to be independent of growth rate. The physiological role of the anaerobic photosynthetic induction of elongation factor G is not clear at present, but similar anaerobic induction of a gene encoding the mitochondrial EF-G was reported in the dimorphic yeast, Arxula adeninivorans (83). In contrast, transcript
Signal level of triplicate 3 W/m² condition was used as baseline and compared with that from 100 W/m² condition. Positive and negative effect indicate increased and decreased expression in the 100 W/m² condition compared to 3 W/m² condition, respectively.

### Table III

| Reaction/pathway | Effect | Changes | RSP number |
|------------------|--------|---------|------------|
| Protein secretion system | Positive | 2.4–4.9 | RSP1589, -1905, -1909, and -1910 |
| Type II | Positive | 2.4–38.2 | RSP0034, -0053, -0055, -0056, and -0061 |
| Type III | Positive | 2.4–160.6 | RSP0032, -0034, -0036, -0040, -0052, -0056, -0060, -0061, -0063, -0065 |
| Flagella synthesis | Positive | 2.0–20.8 | RSP0042, -0044, -0046–0049, -1584–1588, -3083, -3303, -3708 |
| Chemotaxis | Positive | 1.7–5.2 | RSP0385, -0425, -0568, -0584, -0585, -0615, -0675, -1761, -1856, -1989 |
| Aminoacyl-tRNAs | Positive | 1.8–3.1 | RSP0375, -1568, -1768, -1894, -2099, -2100, -2103, -3916 |
| Peptidoglycan biosynthesis | Positive | 1.8–3.7 | RSP1035-1038, -2297-2300 |
| ATP synthase | Positive | -3.7 to -7.4 | RSP3929, -3930, -3933-3935 |
| NADH dehydrogenase | Positive | 1.6–3.6 | RSP0992, -0994, -2082, -2515, -2517, -2518, -2521, -2524, -2526-2531 |
| CO dehydrogenase | Negative | -1.9 to -3.3 | RSP2876-2879, -2172 |
| Quorum sensing system | Negative | -2.5 to -3.4 | RSP0122-0124 |
| Cell division | Positive | 1.7-20.0 | RSP0045, -1062, -1233, -1495, -1744, -2112, -2113 |
| Cold shock protein | Negative | -1.8–80.6 | RSP0386, -0591, -1485, -1952, -2024, -2346, -3620, -3621 |
| Conjugative transfer | Positive | 2.1–7.7 | RSP3905, -4224, -4226, -4230, -4233, -4235, -4237, -4238 |

### Fig. 6

**Northern blot analysis using total RNA isolated from R. sphaeroides 2.4.1 grown aerobic (O₂) and photosynthetic at 3 W/m² (3W), and 100 W/m² (100W).** Approximately 10 µg of total RNA was loaded in each lane. Each RSP number represents a specific gene that was used to construct a probe for the hybridization. Transcript levels from aerobic conditions were designated 100%, and other values are relative to that value. Quantitation was performed as described under “Experimental Procedures.” Microarray signal level is the mean signal value from triplicate microarray results. Standard deviations are less than 20% for the given values.

The sequencing of *R. sphaeroides* 2.4.1 was performed by the Joint Genome Institute of the Department of Energy (DOE), and annotation with the aid of Dr. Frank Larimer at Oak Ridge National Laboratory. We also thank Dr. Mark Gomelsky at the University of Wyoming for collaboration in constructing the *R. sphaeroides* GeneChip as well as the DOE Genomes to Life Consortium for helpful discussions.

These observations reveal new approaches to the study of oxygen and light regulation in *R. sphaeroides* and reveal a broader view of the cellular changes that accompany the photosynthesis lifestyle. Just as we had previously shown that the microarray data validated many years of results already in the literature, we show here that these data in themselves are validated by the use of more traditional methodologies as shown in Fig. 6.

**Conclusions**—The results reported here build significantly on our ongoing studies of the control of gene expression in *R. sphaeroides* as mediated through changes in growth conditions involving the environmental parameters, oxygen, and light intensity under photosynthetic conditions. Some of the results reported here are validated by earlier observations, although the broad sweep of the data reported here have never been previously reported from either a single experiment or from the totality of experiments conducted earlier. We therefore have a more complete view of the transcriptome of *R. sphaeroides* under aerobic and photosynthetic growth conditions, especially for those physiological functions represented by electron transport, photosynthesis and CO₂ fixation. We have made physiological extrapolations from these expression results, but we caution that, until there is a corresponding proteomics profile of these same gene products, we cannot know for certain that our analysis is reflected in cellular metabolic function. Further, we have for the first time assessed the transcriptional activities of genes encoding key regulators of these processes. Gene products involved in the cold shock response, as well as protein synthesis, represent fundamental cellular processes that have the potential to be important regulators. The fact that the expression of such genes is also under oxygen and light regulation extends such studies. This is best typified by the fact that nearly 25–30% of the genome shows changes in gene expression profile as the result of alteration in oxygen levels and light intensity.

In work currently under way, we show, using mutants of *R. sphaeroides* defective in one or more of the key regulators described above, that we can both validate earlier results and expand these to encompass a greater understanding of gene expression and regulation in *R. sphaeroides*. 

**Acknowledgments**—The sequencing of *R. sphaeroides* 2.4.1 was performed by the Joint Genome Institute of the Department of Energy (DOE), and annotation with the aid of Dr. Frank Larimer at Oak Ridge National Laboratory. We also thank Dr. Mark Gomelsky at the University of Wyoming for collaboration in constructing the *R. sphaeroides* GeneChip as well as the DOE Genomes to Life Consortium for helpful discussions.
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