Transcriptional control of several aerobically induced cytochrome structural genes in *Rhodobacter sphaeroides*

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To decipher how the synthesis of energy-transducing enzymes responds to environmental cues, the response of three *Rhodobacter sphaeroides* aerobic cytochrome gene promoters was analysed under different conditions. Two of these promoters are upstream of structural genes (ctaD and coxII) for individual subunits of the cytochrome aa₃ respiratory complex. The third promoter is that for the cycFG operon, which encodes two c-type cytochromes of unknown function, cytochrome c₅₅₉ and CycG. Primer extension analysis identified a single oxygen-responsive transcription start site for each gene. Utilizing operon fusions to *Escherichia coli* lacZ as a measure of promoter activity, transcription from the ctaD, coxII and cycFG promoters was approximately twofold higher when cells were grown at high (30%) oxygen tensions than under low (2%) oxygen or anaerobic (photosynthetic) conditions. Analysis of promoter function using specific host mutations indicated that loss of the *R. sphaeroides* FNR homologue, Fnrl, causes a small, but reproducible, increase in cycFG and coxII transcription when cells are grown at 2% oxygen. However, neither the ΔFnrl mutation nor alterations in sequences related to a consensus target site for the *E. coli* FNR protein increased function of any of these three promoters to that seen under aerobic conditions in wild-type cells. From this we conclude that Fnrl is not solely responsible for reduced transcription of these three aerobic cytochrome genes under low oxygen or anaerobic conditions. When activity of these three promoters was monitored after cells were shifted from anaerobic (photosynthetic) conditions to a 30% oxygen atmosphere, it took several cell doublings for LacZ levels to increase to those found in steady-state 30% oxygen cultures. From these results, it appears that activity of these promoters is also regulated by a stable molecule whose synthesis or function responds slowly to the presence of high oxygen tensions.

**Keywords**: cytochromes, gene expression, transcriptional control, anaerobic regulation, oxygen regulation

INTRODUCTION

Most facultative bacteria contain multiple energy-generating pathways whose synthesis is often controlled by overlapping global regulatory networks. By coordinating expression of specific respiratory components, these networks help direct electron flow through particular energy-generating pathways under different environmental conditions. This study sought to define metabolic and genetic elements that control expression of cytochromes known to either function or to be induced under aerobic respiratory conditions in the facultative phototroph *Rhodobacter sphaeroides*. While genes encoding components of the *R. sphaeroides* photosynthetic apparatus are known to respond to oxygen, light and tetrapyrrole availability (Lee & Kaplan, 1992; Schilke & Donohue, 1992; Eraso & Kaplan, 1994; Penfold & Pemberton, 1994; Gomelsky & Kaplan, 1995), factors governing expression of

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**Abbreviations**: cyt aa₃, cytochrome aa₃ oxidase; LacZ, β-galactosidase.
aerobic electron carriers in this facultative phototroph have received relatively little attention.

Like most bacterial species, *R. sphaeroides* contains a branched aerobic respiratory chain (García-Horsman *et al*., 1994a). The cytochrome aa₃ oxidase (cyt aa₃) of this bacterium is a well-characterized prokaryotic homologue of the mitochondrial cytochrome oxidase and a member of the haem-copper oxidases superfamily (Cao *et al*., 1991, 1992; Shapleigh & Gennis, 1992). From studies on many haem-copper oxidases, a paradigm has developed in which the cyt aa₃ family members are maximally expressed in highly aerated bacteria because they have a lower affinity for oxygen than most of the alternative cytochrome oxidases (García-Horsman *et al*., 1994a; Trumpower & Gennis, 1994). To test if such differential expression of aerobic cytochromes occurs in *R. sphaeroides*, we examined transcription of two cyt aa₃ genes (ctaD and coxII), both in steady-state cultures grown at different oxygen tensions and when photosynthetic (anaerobic) cultures were shifted to high (30%) oxygen conditions. Since previous work suggested that the elevated levels of cytochrome c₅₅₄ found under high (30%) oxygen growth conditions reflects increased *cycFG* transcription (Flory & Donohue, 1995), we also analyzed function of this promoter. In considering how these genes might be regulated by changes in oxygen tension, the existence of sites similar to the consensus promoter activity when oxygen is added to anaerobic cultures (Flory & Donohue, 1995). This plasmid has unique *KpnI* and *StuI* sites for directional cloning of DNA fragments upstream of *lacZ*. Similar IncQ plasmids exist at 4-10 copies per *R. sphaeroides* cell (Donohue & Kaplan, 1991).

**METHODS**

**Cell growth, media and genetic techniques.** *R. sphaeroides* cells (Table 1) were grown on solid media or in liquid cultures of Sistrom’s minimal medium A at 32 °C (Donohue *et al*., 1986). Aerobic cultures (100 ml) were sparged either with a gas mixture known to repress photosynthetic membrane synthesis (30% O₂, 69% N₂, 1% CO₂; Chory *et al*., 1984) or at low oxygen tensions (2% O₂, 97% N₂, 1% CO₂). Steady-state anaerobic (photosynthetic) cells were grown in filled tubes in front of a tungsten light bank (Donohoe *et al*., 1986).

For shifting cultures from anaerobic (photosynthetic) to respiratory (30% oxygen) growth conditions, illuminated 500 ml cultures were sparged for several doublings with 95% N₂ and 5% CO₂. When illuminated, the gas mixture was changed to 30% O₂, 69% N₂, 1% CO₂ and illumination terminated. Cultures were diluted into fresh medium, as necessary, to maintain exponential growth during the course of the experiment.

*E. coli* cells were grown in LB on a rotary shaker or on solid media at 37 °C (Sambrook *et al*., 1989). To maintain plasmids (Table 1) in *E. coli*, ampicillin (50 μg ml⁻¹), kanamycin (25 μg ml⁻¹), spectinomycin (25 μg ml⁻¹) or tetracycline (10 μg ml⁻¹) were used. For *R. sphaeroides*, spectinomycin and kanamycin were used at 25 μg ml⁻¹ and tetracycline was added to 1 μg ml⁻¹. After diparental mating between *E. coli* S17-1 (Simon *et al*., 1983) and *R. sphaeroides* recipients (Donohue *et al*., 1988), cells were plated aerobically on media containing spectinomycin and kanamycin to select for exconjugants.

**DNA manipulations, cloning and sequencing techniques.** Restriction enzymes, T4 DNA ligase, T4 DNA polymerase and Klenow fragment DNA polymerase were used according to suppliers’ specifications and standard techniques (Sambrook *et al*., 1989). Plasmid DNA was prepared with Qiagen kits; DNA was isolated from agarose gels using Prep A Gene kits (Bio-Rad). *Tag* DNA polymerase was used for dideoxy DNA sequencing with deanzaucleotide triphosphates (Promega) and either vector (Strategene) or *R. sphaeroides*-specific primers (Genosys Biotechnologies). Sequence analysis was aided by University of Wisconsin Genetics Computer Group software (Devereux *et al*., 1984).

**Use of prKK200 to construct lacZ operon fusions.** Plasmid pRK200 (R. K. Karls & T. J. Donohue, unpublished) is a kanamycin-resistant *pKT231* derivative (Bagdasarian & Timmis, 1982) that contains a promoterless *E. coli lacZ* gene downstream of a strong transcription terminator on the *psp* cassette (Frentk & Krish, 1984). This plasmid has unique *KpnI* and *StuI* sites for directional cloning of DNA fragments upstream of *lacZ*. Similar IncQ plasmids exist at 4-10 copies per *R. sphaeroides* cell (Donohue & Kaplan, 1991).

**cycFG operon fusions.** To construct a *cycFG::lacZ* fusion, a 140 bp *Mscl* restriction fragment of *cycFG* promoter DNA (Flory & Donohue, 1995) was cloned into the *Stul* site of pRK200. DNA sequencing identified a plasmid in which the *cycFG* promoter was fused to *lacZ* (pRKcycF).

For creating *cycFG* promoter mutations, the same DNA was cloned into the *Smal* site of pUC119 (pTC119F) and transformed into RZ1032 (Kunkel, 1985). Single-stranded DNA (Kunkel, 1985) was hybridized to mutagenic oligonucleotides *cycFFNR* (CGATCACATTAAAGCTACATTTTTCTAGT-GTAGTTCGTCG) to create a mutant FNR site (*cycFG-1* allele), or *cycFREP* (CCAGTATGTCTGTCGTAGTTAG-CGGGCTAGATTC) to destroy an inverted repeat in the *cycFG* promoter spacer (*cycFG-2* allele). After confirming individual mutations (indicated by underlining) by DNA sequencing, *KpnI* and *HincII* were used to isolate mutant *cycFG* promoter fragments for cloning into *KpnI* and *StuI*-digested pRK200. This mutagenesis scheme added 2 bp of upstream DNA and 17 bp before the start of *lacZ* compared to the wild-type *cycFG::lacZ* fusion. The additional downstream DNA accounts for the size difference in primer extension products from a wild-type *cycFG::lacZ* fusion and those from analogous *cycFG-1* or *cycFG-2* reporter genes (see Results).

**coxII operon fusions.** A 282 bp *coxII* DNA fragment (Cao *et al*., 1991) was amplified by PCR using the primers *coxII*PCR1...
Table 1. Bacterial strains and plasmids

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<td>pTJ7718 + ctaD coding sequence</td>
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(GCTGGTACCGCTCGGAGGCATCCTAAC) and coxIPC-R2 (AGAGTCCGTCGCCGCTCTCGAACC). The ~300 bp product was digested with KpnI and HimII (underlined), ligated into KpnI and Stul-digested pRKK200, and sequenced to identify a wild-type coxII::lacZ reporter plasmid (pRKKcoxII).

For mutagenesis, this PCR product was cloned into KpnI and HimII-digested pBSIIKS—. After finding a plasmid where no mutations were introduced into the coxII promoter by PCR (pBScoxII), the Fnr site was mutagenized (Kunkel, 1985) with the coxII FNR primer (CATGACTACTGTCCCT &TTCA- GACJAGATGATGACGCTTCG) and sequenced to identify a wild-type coxII::lacZ reporter plasmid (pRKKIIFNR).

**ctaD operon fusions.** A 226 bp restriction fragment of the ctaD upstream region was isolated as a SmaI–MscI fragment from pJS3 (Shapleigh & Gennis, 1992) and ligated into the Stul site of pRKK200. DNA sequencing identified a plasmid with lacZ under ctaD promoter control (pRKKctaD).

**RNA techniques.** Cellular RNA was prepared (Zhu & Kaplan, 1983), quantified (Donohue et al., 1986) and used in primer extension assays (Karls et al., 1989) with avian myeloblastosis virus reverse transcriptase (Molecular Genetic Resources). Primers were: cycF10 (AGGCGTGGAGCGCTTGAGAT), coxII-1 (AGGTCGTTGAAGTCTCATG), and ctaD-1 (CTGGCTGAGGCATCGTCCT). Products were separated on denaturing urea/polyacrylamide gels alongside DNA sequencing ladders.

**LacZ assays.** R. sphaeroides cells containing individual transcriptional fusions were assayed in triplicate for LacZ activity (Schilke & Donohue, 1992). Cells were harvested in the exponential phase of growth to minimize unwanted secondary effects on promoter activity. Data presented are the mean ± SD of at least three independent cultures. The Student’s t test was used to assess if LacZ levels in individual cultures were significantly different from each other.

**Bacteriochlorophyll analysis.** For these assays, 25 ml culture samples were harvested, stored at −20 °C and resuspended in 0.2 ml deionized water to determine the specific bacteriochlorophyll content. Half of the sample was used for protein determination (Markwell et al., 1978); the remainder was assayed for bacteriochlorophyll (Cohen-Bazire et al., 1957).

**RESULTS AND DISCUSSION**

Activity of the ctaD, coxII and cycFG promoters is increased under aerobic conditions

By primer extension assays the start site for coxII transcription maps 80 nt upstream of the initiator methionine, while that for ctaD is 54 nt upstream of the start of translation (summarized in Fig. 1). Each gene appears to be transcribed from a single promoter because the primer extension product is the same when RNA from aerobically or anaerobically grown cells is used (Fig. 2 for coxII, data not shown for ctaD). Both primer extension assays and Northern blot analysis suggested that the ctaD and coxII promoters were oxygen-regulated since there was more mRNA in aerobic and anaerobic cells than in those grown under anaerobic (photosynthetic) conditions (see Fig. 2 for coxII primer extension assays, data not shown for ctaD).

To facilitate the study of oxygen-dependent control of ctaD, coxII and cycFG transcription, individual lacZ operon fusions were constructed. To verify that these low-copy reporter genes were valid indicators of pro-
motor activity, primer extension assays showed that the lacZ-specific transcripts had the same 5' end as their genomic counterparts (data not shown). Cells containing any of these three reporter genes produced LacZ activity well over the background observed from a control strain containing a promoterless lacZ gene (pRK200, <6 Miller units). In addition, LacZ levels in cells containing the ctaD, coxII or cycFG reporter genes were approximately twofold higher when cells were grown at high (30%) oxygen levels rather than under anaerobic (photosynthetic) conditions (Fig. 3). This suggests that a portion of the increased mRNA abundance is due to elevated transcription. While a contribution of additional cis-acting sequences to promoter function is still possible, these data indicate that the amount of each promoter DNA which is fused to lacZ (268 bp for coxII, 149 bp for ctaD or 89 bp for cycFG) is sufficient to increase transcription at 30% oxygen.

To further evaluate the effects of oxygen tension on ctaD, coxII and cycFG expression, LacZ levels were measured in cells grown under a 2% oxygen atmosphere, a condition sufficient to induce photosynthetic membrane assembly (Cohen-Bazire et al., 1957). Activity of the ctaD, coxII and cycFG promoters in cells grown in a 2% oxygen environment was similar to that found in anaerobic (photosynthetic) cultures (Fig. 3). Such a reduction in ctaD and coxII promoter function at reduced oxygen tensions was the response expected given the low affinity of cyt aa₃ complexes for oxygen (Trumpower & Gennis, 1994). Indeed, the E. coli equivalent to cyt aa₃ (cytochrome bo oxidase) is also transcribed less at low oxygen (Iuchi et al., 1990; Minagawa et al., 1990). Thus, these estimates of coxII...
and ctaD promoter function provide a provisional indication that cyt aa₃ levels are lower at reduced oxygen tensions. However, the modest reduction in ctaD and coxlI promoter activity at 2% oxygen or anaerobic conditions suggests these cells contain a considerable pool of cyt aa₃ complexes. If R. sphaeroides regulates synthesis of its high affinity oxidase(s) as in other bacteria (García-Horsman et al., 1994a, b) they should be maximally expressed at low oxygen tensions (luchi et al., 1990; Fu et al., 1991; Moshiri et al., 1991).

Given that CtaD and CoxII are found in equimolar amounts within the cyt aa₃ complex, we were surprised that ctaD promoter activity was approximately ninefold higher than its coxlI counterpart (Fig. 3). This difference could reflect deletion of important cis-acting sequences on either reporter gene. However, differences in stability of ctaD and coxlI transcripts could also explain both the apparent strengths of the coxlI and ctaD promoters and our difficulty in reproducibly obtaining ctaD primer extension products (see above). Alternatively, ctaD transcription could generate a stable pool of CtaD for assembly of the cyt aa₃ complex. A similar role as a membrane-bound nucleation site for newly synthesized enzyme complexes has been proposed for the H subunit of the R. sphaeroides reaction centre complex (Chory et al., 1984).

**Function of the aerobic cytochrome promoters increases slowly after exposure to 30% oxygen**

To examine how oxygen controls ctaD, coxlI and cycFG promoter function, the kinetics of LacZ accumulation from individual reporter genes was monitored after photosynthetic cells were exposed to 30% oxygen (Fig. 4). Photosynthetically grown R. sphaeroides cells contain considerable cytochrome oxidase activity (García-Horsman et al., 1994a, b), so it is not surprising that cell growth continues when these cells are exposed to 30% oxygen. When oxygen was introduced into steady-state anaerobic (photosynthetic) cultures, there was a slow, progressive increase in LacZ activity from each operon fusion. Indeed, about 24 h (six cell doublings) after the introduction of 30% oxygen were required for LacZ levels from each reporter fusion to increase to that found in steady-state cells grown under these conditions. The slow increase in activity of these three promoters seems to preclude function of either a pre-existing oxygen sensor or a rapidly metabolized intermediate as negative transcriptional regulators. Rather, the slow increase in transcription could reflect time required for an activator or co-inducer of ctaD, coxlI and cycFG promoter function to accumulate after photosynthetic cells are shifted to a 30% oxygen atmosphere. Another equally plausible alternative is that ctaD, coxlI and cycFG promoter function is repressed by an oxygen-insensitive transcription factor that uses a component of the photosynthetic apparatus or a stable compound that is accumulated under low oxygen conditions as a co-repressor. In either of these two latter cases, coxlI, ctaD and cycFG promoter function would only increase after the putative repressor, pre-existing photosynthetic units, or a potential co-repressor was diluted by growth at 30% oxygen.

The rapid cessation of photosynthetic membrane synthesis that occurs when R. sphaeroides is exposed to high oxygen tensions (Cohen-Bazire et al., 1957) allowed us to test if the increased activity of these three promoters under these conditions could be correlated with dilution of a regulatory factor from photosynthetic cultures. When we assayed the specific bacteriochlorophyll content of these cells (as a marker for photosynthetic membrane content), we observed the expected time-dependent decrease in this parameter (Fig. 4) as pre-existing photosynthetic membranes were diluted by cell division after oxygen was introduced (Cohen-Bazire et al., 1957). When all the data are considered together, it appears that increased function of these three promoters parallels the loss of pre-existing components from photosynthetic cells.

**The R. sphaeroides FNR homologue, Fnrl, is not sufficient to mediate oxygen regulation of ctaD, coxlI and cycFG expression**

In considering potential molecular explanations for the increased activity of these three promoters at high oxygen tensions, we noted that sequences related to a
Fig. 4. The effect of oxygen on anaerobic cultures of cells containing individual LacZ operon fusions. Photosynthetic cultures of each strain were shifted to aerobic, dark conditions at time zero (dotted line) and were later diluted to fresh medium for continued aerobic growth (arrow). Top panel: □, culture density (OD600). Bottom panel: ○, LacZ activity in Miller units; ▲, specific bacteriochlorophyll content [μg bacteriochlorophyll (mg protein)].

Fig. 5. Effect of mutations in fnrL or the putative FnrL target sites on expression from cycFG, coxII or ctaD reporter genes. LacZ activities of wild-type R. sphaeroides (WT) or an FnrL null strain (ΔfnrL; JZ1670) containing the indicated reporter plasmids grown under 30% oxygen (■), 2% oxygen (□) or anaerobic (photosynthetic) (△) conditions. Reported values are the mean±SD of at least three independent assays and are corrected for activity from the vector alone under the same conditions (pRKK200, <6 Miller units). (a) Wild-type (WT) cycFG::lacZ fusion (plasmid pRKKcycF); cycFG-1::lacZ fusion (plasmid pRKKF-FNR). (b) Wild-type (WT) coxII::lacZ fusion (plasmid pRKKcoxII), cycFG-1::lacZ fusion (plasmid pRKKIIFNR). (c) Wild-type ctaD::lacZ fusion (plasmid pRKKctaD).

consensus target site for the E. coli global anaerobic transcription factor, FNR, were present upstream of each gene (Fig. 1). While the site in the cycFG promoter (centred at −42.5) has only 5/10 matches to the E. coli FNR consensus, those in the coxII (−71.5) and ctaD (−60.5) promoters are identical to this consensus in both half sites. E. coli FNR represses transcription of several aerobic respiratory enzymes (Sharrocks et al., 1991). In the case of the E. coli ndh promoter, FNR represses transcription of this aerobic respiratory enzyme under anaerobic conditions by binding to independent sites centred at −50.5 and −94.5 bp upstream of the start of transcription (Green & Guest, 1994). Thus, it would not be surprising to find that...
transcription of \( \text{cycFG} \) or genes encoding individual subunits of the \( R. \text{sphaeroides} \) \( \text{cttD} \), \( \text{cotll} \), and \( \text{cycFG} \) promoters was measured in the \( R. \text{sphaeroides} \)  \( \text{FnrL} \) null mutant, JZ1678 (Fig. 5a–c). Because the  \( \text{FnrL} \) null mutant is photosynthetically incompetent (Zeilstra-Ryalls & Kaplan, 1995),  \( \text{LacZ} \) levels were only measured from cultures grown in the presence of 30% or 2% oxygen (a condition which approximates the effects of anaerobic growth on \( \text{cttD} \), \( \text{cotll} \) and \( \text{cycFG} \) promoter function, Fig. 3). At high (30%) oxygen tensions,  \( \text{LacZ} \) levels from the \( \text{cycFG-I} \) (Fig. 5a) and \( \text{cotll-1} \) (Fig. 5b) fusions were nearly identical to their wild-type counterparts in both wild-type cells and the \( \text{FnrL} \) null mutant. Normal activity of the \( \text{cotll-1} \) and \( \text{cycFG-I} \) mutant promoters in the \( \text{FnrL} \) null mutant at 30% oxygen is a clear indication that this global regulator is not functioning under these conditions. Similarly,  \( \text{LacZ} \) levels produced from the \( \text{cotll-1} \) promoter at 2% oxygen in the  \( \Delta \text{FnrL} \) mutant were indistinguishable from those measured in cells containing a wild-type \( \text{cotll} \) reporter gene (Fig. 5b). This provides an indication that this target site is not solely responsible for reducing function of the \( \text{cotll} \) promoter under these conditions. In contrast, there was a slight, but reproducible, increase in activity of the \( \text{cycFG} \) (Fig. 5a) and \( \text{cotll-II} \) (Fig. 5b) promoters at 2% oxygen in the  \( \Delta \text{FnrL} \) null mutant compared to wild-type cells (Fig. 5). While this reproducible increase in \( \text{LacZ} \) levels suggests that \( \text{FnrL} \) has some role in reducing transcription from the \( \text{cycFG} \) and \( \text{cotll-II} \) promoters at low oxygen tension, the magnitude of the effect is below the twofold increase expected if this protein were the sole negative regulator of these genes under these conditions.

When the above results are considered together, it appears likely that \( \text{FnrL} \) is not solely responsible for reducing \( \text{cttD} \), \( \text{cotll} \) and \( \text{cycFG} \) promoter activity under the conditions we have tested (2% oxygen and anaerobic (photosynthetic) growth). However, it is still possible that \( \text{FnrL} \) or a related protein contributes to reduced transcription of these \( R. \text{sphaeroides} \) aerobic cytochrome genes under these or other conditions we did not test. For example, action of \( E. \text{coli} \) \( \text{Fnr} \) can often require
additional proteins (Spiro & Guest, 1991), so the failure of the target site or host mutations to fully increase cycFG, coxII or ctaD transcription at low oxygen tensions could reflect the action of additional transcription factors. E. coli FNR interacts at two sites within the ndh promoter (Green & Guest, 1994), so it is possible that additional non-consensus FNR sites in these promoters might prevent us from observing a strong effect of the mutations we made. R. sphaeroides is also one of a growing number of eubacteria which have been found to contain more than one protein in the FNR family (Cuypers & Zumft, 1993; Tosques et al., 1996). Thus, the potential for related proteins, such as NnrR from R. sphaeroides strain 2.4.3 (Tosques et al., 1996), to alter ctaD, coxII or cycFG promoter function in the presence or absence of FnrL can not be ruled out at this time (if this protein exists in strain 2.4.1).

An inverted repeat overlapping the cycFG promoter is not essential for oxygen regulation

We also tested whether a previously noted inverted repeat (centred at −24.5) regulated cycFG promoter function (Flory & Donohue, 1995). When a mutation that destroyed the downstream half-site (without changing the upstream half-site that overlaps a potential −35 element; Fig. 1) was generated (cycFG-2), LacZ levels from this mutant reporter gene increased approximately twofold in cells grown at 30% oxygen (Fig. 7). The cycFG-2 allele appears to have created a new promoter since these cells contain a second primer extension product that is 14 nt longer than the wild-type transcript (Fig. 8). Even though the cycFG-2::lacZ fusion probably contains multiple promoters, transcription under 2% oxygen and anaerobic conditions was reduced to an extent comparable to its wild-type counterpart. Thus, we can conclude that the downstream half-site is not essential for decreased cycFG promoter function at low oxygen tensions. Finally, changes in oxygen tension must affect both potential promoters since the abundance of both primer extension products is lowered under photosynthetic conditions (data not shown).

Is oxygen regulation of the coxII, ctaD and cycFG promoters independent of systems which control photosynthesis genes?

In summary, our data show that transcription of genes for cyt aa3 subunits as well as the cycFG operon is reduced at low oxygen tension. The slow, gradual

**Fig. 7.** Effect of the cycFG-2 mutation on promoter activity. LacZ activities of wild-type R. sphaeroides 2.4.1 or an FnrL null strain (ΔFnrL; JZ1678) containing either a wild-type (WT) cycFG::lacZ fusion (plasmid pRKKcycF) or the cycFG-2::lacZ fusion (plasmid pRKKF-REP) grown under 30% oxygen (●), 2% oxygen (□) or anaerobic (photosynthetic) (△) conditions are shown. Reported values are the mean ± SD of at least three independent assays and are corrected for activity from the vector alone under the same conditions (pRKK200, <6 Miller units). Note that under 2% oxygen conditions, the SD in LacZ levels from wild-type cells carrying the wild-type cycFG plasmid and ΔFnrL cells carrying the promoter mutant plasmid were each <5 Miller units.

**Fig. 8.** The cycFG-2 mutation creates a new promoter. Primer extension assays of 20 μg RNA from photosynthetically grown wild-type cells containing: lane 1, a wild-type cycFG::lacZ fusion (plasmid pRKKcycF); or lane 2, a cycFG-2::lacZ fusion (plasmid pRKKF-REP) are shown. Primer extension product 1 is the wild-type 5' end; product 2 depicts the wild-type transcript displaced 17 nt by addition of vector sequence during construction of the cycFG-2 allele (Methods); product 3 denotes the new 5' end created by the cycFG-2 allele.
increase in ctaD, coxII and cycFG transcription after oxygen is added to photosynthetic cultures contrasts dramatically with the rapid induction of photosynthetic gene expression seen when respiratory cultures of purple bacteria like R. sphaeroides are shifted to low oxygen or anaerobic conditions that trigger intracytoplasmic membrane formation (Cohen-Bazire et al., 1957; Chory et al., 1984). Thus, it will be interesting to define the regulators of these aerobic cytochrome genes, explore if these transcription factors interact with proteins or products accumulated under photosynthetic conditions and determine if the similar patterns of ctaD, coxII and cycFG promoter function reflects their existence in a regulon of genes with similar metabolic functions.

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