Photosynthesis gene expression in *Rhodobacter sphaeroides* is regulated by redox changes which are linked to electron transport

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The effect of the respiratory electron acceptor nitrous oxide on the synthesis of the photosynthetic apparatus of *Rhodobacter sphaeroides* was examined. In phototrophically grown cells, the addition of nitrous oxide caused a reduction in the level of light-harvesting complex I and light-harvesting complex II under conditions of high light intensity (200 W m^{-2}) and low light intensity (16 W m^{-2}). 5-Aminolaevulinate synthase activity was decreased during growth in the presence of nitrous oxide and this limited production of spectral complexes since addition of exogenous 5-aminolaevulinic acid partially suppressed the effect of nitrous oxide. The effect of nitrous oxide on the expression of the operons encoding the pigment-binding proteins of light-harvesting complex I (*puf*), light-harvesting complex II (*puc*) and the two isoenzymes of 5-aminolaevulinate synthase (*hemA* and *hemT*) were measured using transcriptional fusions to *lacZ*. Nitrous oxide caused a decrease in *puf* and *hemA* transcription. However, there was an apparent increase in the expression of *puc* and *hemT* transcriptional fusions. The level of light-harvesting complexes in cells grown in the dark with different electron acceptors was also examined. Cells grown anaerobically with DMSO had a higher level of light-harvesting complexes than those grown anaerobically with nitrous oxide as electron acceptor. Cells grown aerobically had the lowest level of light-harvesting complexes. It is proposed that FnrL-dependent and FnrL-independent expression of photosynthesis genes is modulated by redox changes elicited by nitrous oxide respiration.

Keywords: photosynthesis, gene expression, *Rhodobacter sphaeroides*, anaerobic respiration

INTRODUCTION

Many species of purple non-sulfur phototrophic bacteria exhibit remarkable bioenergetic versatility. These bacteria are able to respire using oxygen as a terminal electron acceptor, while under anaerobic conditions they can catalyse cyclic photosynthetic electron transfer and respiration with a variety of electron acceptors (McEwan, 1994). Since the pioneering experiments of Cohen-Bazire *et al.* (1957) it has been established that oxygen represses the formation of the photosynthetic apparatus while under anaerobic conditions the size of the photosynthetic apparatus is inversely related to the incident light intensity (Aagard & Sistrom, 1972). *Rhodobacter sphaeroides* and

Rhodobacter capsulatus have been used as models for investigations of the regulation of the coordinate synthesis of the photosynthetic apparatus (Kiley & Kaplan, 1988). The photosynthetic apparatus of *R. sphaeroides* contains three distinct pigment-protein complexes involved in the transduction of light energy into electrochemical energy (Kiley & Kaplan, 1988). Two types of light-harvesting (LH) complex, light-harvesting complex I (LH I or B875) and light-harvesting complex II (LH II or B800–850), are identified by the distinctive absorbance maxima of their bacteriochlorophyll *a* (Bch) pigments. The core of the photosynthetic unit consists of a reaction centre (RC) surrounded by 10–15 LH I. The peripheral LH II complex surrounds the LH I/RC core and its level increases to a greater extent than LH I upon a reduction in light intensity (Aagard & Sistrom, 1972).

A cluster of genes on the large chromosome of *R.*

Abbreviations: ALA, 5-aminolaevulinic acid; Bch, bacteriochlorophyll *a*; Crt, carotenoids; LH, light-harvesting complex; RC, reaction centre.

sphaeroides (Pemberton & Bowen, 1981; Wu *et al.*, 1991) encodes most of the information required for the synthesis of pigment-binding proteins, Bch and carotenoids (Crt). Many of these genes, including the *puf* operon (encoding LH I and RC-L and RC-M polypeptides) (Williams *et al.*, 1983, 1984; Kiley *et al.*, 1987) and the *puc* operon (encoding LH II polypeptides and assembly proteins) (Kiley & Kaplan, 1987; Lee *et al.*, 1989) have been sequenced, *cis*-acting regulatory elements have been identified (Hunter *et al.*, 1991; Lee & Kaplan, 1992) and detailed studies of mRNA turnover have been made (reviewed by Klug, 1993). Research efforts in this area are now directed towards understanding the mechanism of the control of photosynthesis (PS) gene expression and recently a number of *trans*-acting factors involved in this process have been identified in *R. capsulatus* (Sganga & Bauer, 1992; Buggy *et al.*, 1994; Buggy & Bauer, 1995) and *R. sphaeroides* (Penfold & Pemberton, 1991, 1994; Eraso & Kaplan, 1994, 1995; Zeilstra-Ryalls & Kaplan, 1995a). Although considerable progress has been made, the mechanism(s) of light and oxygen sensing in *Rhodobacter* and the means by which these environmental signals are linked to changes in gene expression are not fully understood. A key question is whether a sensing system detects oxygen directly or acts via redox-induced changes.

Although the presence of anaerobic respiratory pathways in *R. sphaeroides* and *R. capsulatus* has been established for a number of years (Ferguson *et al.*, 1987), there is very little information about the effect of anaerobic oxidants on PS gene expression. It was shown that in a denitrifying strain of *R. sphaeroides* (strain IL106) the presence of nitrate during phototrophic growth caused an approximately twofold decrease in the level of Bch (Michalski & Nicholas, 1987). A major problem with the interpretation of these data is the complexity of denitrification; nitrate reduction in strain IL106 proceeds via two ubiquinol-oxidizing nitrate respiratory pathways while the intermediates of denitrification [nitrite, nitric oxide and nitrous oxide (N_2O)] are reduced via pathways involving the cytochrome *bc₁* complex and cytochrome *c₂* (McEwan, 1994). Furthermore, it is known that nitrite, an intermediate in denitrification, affects both Crt and Bch biosynthesis at the enzymic level (Michalski *et al.*, 1985). N_2O is an attractive tool to overcome the above problems since it is reduced to dinitrogen gas by N_2O reductase in the last step of denitrification. In this paper the effects of N_2O on the synthesis of the photosynthetic apparatus are compared to the effect of oxygen and possible mechanisms of electron-transport-dependent control of PS gene expression in *R. sphaeroides* are discussed.

METHODS

Bacterial strains and culture conditions. *R. sphaeroides* strain RS630 (Pemberton & Bowen, 1981) was grown on RCV minimal medium containing vitamins required for growth of this species (Weaver *et al.*, 1975). For phototrophic growth, cells were grown in completely filled 30 ml screw-cap bottles between banks of tungsten lights. Incident light intensity was calculated as described by Clayton (1970). Addition of DMSO or

potassium ferricyanide was made, as required, from a sterile stock solution, to a final concentration of 30 mM and 2.5 mM, respectively. For growth in the presence of N_2O , two screw-cap bottles half-filled with RCV medium were sparged with filter-sterilized N_2O from a gas cylinder (Richardson *et al.*, 1991). After inoculation of one screw-cap bottle with a bacterial culture, the second bottle was used to completely fill the first. This procedure was carried out for phototrophic and anaerobic dark growth of *R. sphaeroides*. For aerobic growth of *R. sphaeroides*, 25 ml of cells were shaken (200 r.p.m.) in 250 ml flasks. A 5% (v/v) inoculum was used for all experiments and cells were precultured under conditions identical to the experimental conditions. Cells were harvested in late-exponential phase of the bacterial growth curve. Filter-sterilized 5-aminolaevulinic acid (ALA) was added to a final concentration of 5 mM as required. *Escherichia coli* was grown at 37 °C in LB medium (Sambrook *et al.*, 1989). Spectinomycin was added where necessary to a final concentration of 25 µg ml⁻¹.

Genetic manipulations. Plasmids pCF200 (Lee & Kaplan, 1992) (Sm^r/Sp^r Tc^r IncQ, *puc* (URS+DRS)::*lacZYA'*), pUI1830 (Gomelsky & Kaplan, 1995) (Sm^r/Sp^r Tc^r IncQ, *pufB*::*lacZYA'*), pUI1063 (Zeilstra-Ryalls & Kaplan, 1995a) (Sm^r/Sp^r Tc^r IncQ, *hemA*::*lacZYA'*) and pUI1098 (Zeilstra-Ryalls & Kaplan, 1995b) (Sm^r/Sp^r Tc^r IncQ, *hemT*::*lacZYA'*) were gifts from Professor S. Kaplan (University of Texas Health Science Center at Houston, USA). These plasmids were transferred from *Escherichia coli* strain S17-1 (Pro⁻ Res⁻ Mod⁺ RecA⁻ integrated plasmid RP4-Tc::Mu-Km::Tn7) (Simon *et al.*, 1983) into *R. sphaeroides* by a patch mating method described by Bonnett *et al.* (1995).

Spectroscopic and biochemical analyses. Cultures for spectral analysis were harvested by centrifugation (7000 *g* for 20 min at 4 °C) then resuspended in 5 ml 20 mM sodium dihydrogen phosphate, 1 mM EDTA sodium salt, pH 7.0. The cells were broken by two passages through a French pressure cell (16000 p.s.i.; 110.4 MPa). Cell debris was removed by centrifugation (7000 *g* for 15 min at 4 °C) and the supernatant membranes were pelleted by centrifugation (145000 *g* for 60 min at 4 °C). The membrane fraction was then resuspended in 1 ml phosphate/EDTA buffer. The protein content of membranes was determined by the bicinchoninic acid reagent with BSA as a standard (Smith *et al.*, 1985). Absorbance spectra of membranes from *R. sphaeroides* were collected on a Hitachi U-3000 spectrophotometer. From the resulting spectra the concentration of Bch in LH complexes was determined as described by Meinhardt *et al.* (1984) using $\epsilon_{mM} = 73$ for LH I at 878–820 nm and $\epsilon_{mM} = 96$ for LH II at 849–900 nm. The LH content of membranes was calculated assuming 2 mol Bch per mol LH I and 3 mol Bch per mol LH II. The total Bch values obtained by this spectroscopic method were within 15% of values for Bch determination after extraction of pigment from membranes using acetone/methanol by the method of Clayton (1966) (data not shown). ALA synthase activity and β -galactosidase activity were measured in cell-free extracts as described by Burnham (1970) and Clark & Switzer (1977), respectively. All measurements of enzyme activity and LH content in membranes were performed on three samples for each growth condition. All experiments were performed at least twice. Results are presented as means $\pm$ SE.

RESULTS

A number of species of phototrophic bacteria are known to respire using N_2O even though they do not catalyse complete denitrification (McEwan *et al.*, 1985). This was found to be the case for *R. sphaeroides* strain RS630, which

Fig. 1. Absorbance spectra of membranes from *R. sphaeroides* grown phototrophically in the absence (A) and presence (B) of N_2O . Both samples contained 195 μ g protein.

Table 1. Level of LH complexes and photosynthetic pigments in membranes from cells grown phototrophically in the presence and absence of N_2O

Growth conditions	Amount [nmol (mg protein) ⁻¹] of	
	LH I	LH II
200 W m ⁻²	10.2 ± 0.2	9.3 ± 0.1
200 W m ⁻² + N_2O	6.7 ± 0.3	3.5 ± 0.3
200 W m ⁻² + ferricyanide	3.8 ± 0.2	6.0 ± 0.5
16 W m ⁻²	20.5 ± 1.2	23.2 ± 0.6
16 W m ⁻² + N_2O	7.0 ± 0.2	5.4 ± 0.2
16 W m ⁻² + N_2O + ALA	10.6 ± 1.2	10.0 ± 0.4

grew anaerobically in the dark with a doubling time of approximately 8.5 h on the non-fermentable carbon source malate with N_2O as sole electron acceptor. The ability of strain RS630 to use N_2O was confirmed by the demonstration of a reduced methylviologen: N_2O oxidoreductase activity in cell-free extracts (data not shown). Since it is known that phototrophically growing rhodobacters also utilize respiratory electron acceptors (Jones *et al.*, 1990) a series of experiments was performed to determine whether electron transfer to N_2O during phototrophic growth influenced PS gene expression.

Effect of N_2O and ferricyanide on the level of LH complexes in phototrophically grown cells

Fig. 1 shows the spectra of membranes from cells grown at 200 W m⁻² (high light intensity). In the sample from cells grown in the absence of N_2O , the 875 nm Bch absorbance band of LH I can be seen as a poorly defined shoulder on the 850 nm Bch absorbance band of the LH II complex. In membranes from cells grown at the same light intensity in the presence of N_2O the LH I peak at 875 nm was almost equal in magnitude to the LH II peak at 850 nm, indicating a marked decrease in the LH II content of these membranes. Quantification of the level of LH II and LH I complexes in the photosynthetic membranes from cells grown at high light intensity is shown in Table 1. An approximately 30% decrease and a

60% decrease in the level of LH I and LH II complexes, respectively, was caused by the presence of N_2O . As a result, in membranes from N_2O -grown cells the LH I/LH II ratio was about double that found in membranes from cells grown in the absence of N_2O .

A lowering in light intensity triggers an increase in the levels of LH complexes, particularly LH II (Aagard & Sistrom, 1972). To determine whether N_2O affected the induction of LH complexes in response to a decrease in light intensity, *R. sphaeroides* was grown at 16 W m⁻² (low light intensity) and spectra of membranes prepared from these cells were collected. The increased level of LH complexes in membranes from low light grown cells compared to those from cells grown at high light is illustrated in Table 1. The results in Table 1 also show that, as for the cells grown at high light intensity, N_2O caused a dramatic lowering in the level of LH complexes in cells grown at low light intensity, with an approximately 65% decrease in LH I and a 75% decrease in LH II being observed. It can also be seen that membranes from low light grown cells had twice the level of LH I compared to those from high light grown cells but in membranes from cells grown with N_2O there was virtually no change in the level of LH I as a function of light intensity (Table 1). Cells grown at low light intensity had about 2.5 times the level of LH II compared to cells grown at high light intensity, but the presence of N_2O resulted in the level only being about 1.5-fold higher. Inspection of the spectra in Fig. 1 shows that Crt levels (absorbance peaks between 450 nm and 530 nm) also changed in parallel with those of the Bch component of the LH complexes. Although the Crt content of LH complexes was not analysed in detail, this is taken to indicate that N_2O was affecting the level of the LH complexes in photosynthetic membranes and was probably not causing a major change in their composition.

It is established that N_2O reductase takes electrons from cytochrome c_2 (Richardson *et al.*, 1991) and this led us to test whether other oxidants of cytochrome c_2 could affect PS gene expression. Ferricyanide was used, and it was found that the presence of this oxidant during phototrophic growth caused a lowering of both LH I and LH II levels in cells grown at high light intensity, with the level of LH I being lowered to a greater degree than that of LH II. However, it should be noted that ferricyanide

Table 2. β -Galactosidase activity in cell extracts of *R. sphaeroides* bearing *lacZ* transcriptional fusions grown phototrophically at high light intensity in the presence and absence of N_2O

Growth conditions	β -Galactosidase activity [nmol ONPG hydrolysed min ⁻¹ (mg protein) ⁻¹]			
	<i>puf-lacZ</i>	<i>puc-lacZ</i>	<i>hemA-lacZ</i>	<i>hemT-lacZ</i>
200 W m ⁻²	4000 $\pm$ 200	4800 $\pm$ 500	4300 $\pm$ 90	180 $\pm$ 20
200 W m ⁻² + N_2O	3400 $\pm$ 200	7800 $\pm$ 300	1400 $\pm$ 240	310 $\pm$ 20

caused *R. sphaeroides* to grow more slowly and to a lower culture density than in the other growth conditions shown in Table 1. At low light intensity the effect of ferricyanide on growth was even more severe and this prevented measurement of LH levels.

Partial suppression of the N_2O effect by ALA

The first step in haem and Bch biosynthesis, catalysed by ALA synthase, has been recognized for many years as a potential control point in the Bch biosynthetic pathway and it is known that the addition of ALA to the culture medium can restore photosynthetic competence to mutants that are unable to synthesize ALA (Lascelles & Altshuler, 1969; Neidle & Kaplan, 1993a). Table 1 also shows the effect of ALA on LH levels in *R. sphaeroides* grown phototrophically at low light intensity in the presence of N_2O . There was a decrease in the level of LH complexes in cells grown with N_2O but the presence of ALA partially suppressed this effect, elevating the level of both LH I and LH II (Table 1). The presence of ALA had no effect on the level of LH I and only slightly raised the level of LH II in cells grown in the absence of N_2O (data not shown).

R. sphaeroides possesses two ALA synthase enzymes which both catalyse the condensation of succinyl-CoA and glycine (Neidle & Kaplan, 1993b). The total ALA synthase activity was 2.5 ± 0.2 nmol ALA formed h⁻¹ (mg protein)⁻¹ and 2.2 ± 0.3 nmol ALA formed h⁻¹ (mg protein)⁻¹ in extracts from cells grown phototrophically in the presence of N_2O at low light and high light, respectively. This compared to 9.2 ± 0.1 nmol ALA formed h⁻¹ (mg protein)⁻¹ (low light) and 5.2 ± 0.1 nmol ALA formed h⁻¹ (mg protein)⁻¹ (high light) in extracts from cells grown phototrophically without the electron acceptor.

Effect of N_2O on expression of the *puf*, *puc*, *hemA* and *hemT* operons

The results described above clearly showed that levels of both LH complexes were lower during phototrophic growth of cells in the presence of N_2O . This raised the question of whether the effect of N_2O was exerted at the level of transcription of the *puf* and *puc* operons or at other stages in the formation of LH complexes. To address this,

Table 3. Level of light-harvesting complexes in membranes from cells grown in the dark with oxygen or DMSO or N_2O as electron acceptor

Electron acceptor	Amount [nmol (mg protein) ⁻¹] of	
	LH I	LH II
Oxygen	1.3 $\pm$ 0.3	0.4 $\pm$ 0.1
N_2O	2.8 $\pm$ 0.5	1.9 $\pm$ 0.4
DMSO	9.1 $\pm$ 0.5	9.5 $\pm$ 0.5

the effect of N_2O on the activity of *puf-lacZ* and *puc-lacZ* fusions in *R. sphaeroides* grown phototrophically at high light intensity was investigated. The β -galactosidase activity of the *puf-lacZ* fusion was lower in extracts from cells grown in the presence of N_2O than in those grown in its absence (Table 2). This correlates positively with the level of LH I complex in cells grown under the same conditions (Table 1). The pattern of β -galactosidase activity in cells expressing the *puc-lacZ* fusion was quite different and was higher in the extracts from cells grown in the presence of N_2O (Table 2). The expression of the *hemA-lacZ* fusion was reduced by about threefold by the presence of N_2O during phototrophic growth of *R. sphaeroides* (Table 2). In contrast, the pattern of expression of the *hemT-lacZ* fusion resembled that of the *puc-lacZ* fusion and the level of β -galactosidase activity was higher in cells grown with N_2O (Table 2).

Level of LH complexes in cells grown in the dark with oxygen, N_2O and DMSO as electron acceptors

Gratuitous synthesis of the photosynthetic apparatus occurs under anaerobic dark conditions and this has proved to be particularly valuable for analysis of photosynthetically incompetent mutants (Eraso & Kaplan, 1995). DMSO has been the electron acceptor most widely used and it was found that strain RS630 could grow anaerobically in the dark with DMSO as electron acceptor and malate as carbon source. The levels of LH complexes in membranes from cells grown under anaerobic dark conditions with DMSO or N_2O as electron acceptor are shown in Table 3. Cells grown on N_2O contained about

threefold less LH I and about fourfold less LH II than cells grown with DMSO. This resulted in LH I/LH II ratios of 0.9 for DMSO-grown cells and 1.5 for N₂O-grown cells. Although the level of LH complexes was low in cells grown with N₂O, it was higher than that found for cells grown aerobically in the dark (Table 3).

DISCUSSION

A problem with investigating the effects of oxygen on gene expression is that this oxidant can exert an effect via a variety of direct and indirect mechanisms (reviewed by Unden *et al.*, 1995). In order to overcome this problem we used two electron acceptors of anaerobic respiration, N₂O and DMSO. The data described in this paper show that the presence of the respiratory electron acceptor N₂O during phototrophic (anaerobic) growth of *R. sphaeroides* causes a marked reduction in the level of LH complexes. The simplest explanation for the data is that a redox change induced by N₂O respiration acts as a signal which leads to a reduced level of LH complexes.

The expression of PS genes in *R. sphaeroides* is now very well documented. For many years a key regulatory step in Bch synthesis has been known to be ALA synthase and it is established that this can occur both at the level of enzyme activation/deactivation (Clement-Metral, 1979) and at the level of gene expression (Neidle & Kaplan, 1993b). The situation in *R. sphaeroides* is complicated by the presence of two genes encoding ALA synthase, *bemA* and *bemT* (Neidle & Kaplan, 1993a, b). The effect of N₂O on phototrophically grown cells was to significantly reduce the activity of ALA synthase in the cell but this experiment did not identify whether one or both types of ALA synthase was affected. The reduction in total ALA synthase activity limited production of Bch pigment complexes since addition of ALA at least partially restored the level of Bch pigment to that observed in the absence of N₂O. The production of ALA is a highly sensitive control point and this probably arises because the *K_m* of ALA dehydratase is 700 µM (Shemin, 1972) and as a result the flux via the Bch pathway may be highly dependent on the intracellular level of ALA.

Our results indicate that a decrease in ALA synthase activity caused by the presence of N₂O during phototrophic growth is correlated with a decrease in *bemA* expression under the same conditions. *bemA* expression has been shown to be under control of FnrL, a homologue of the regulator of anaerobic gene expression in *E. coli* (Zeilstra-Ryalls & Kaplan, 1995a). This leads us to suggest that redox changes elicited by N₂O respiration may affect FnrL activity. It has also been shown that Fnr-binding consensus sequences are present in upstream sequences of *bchE* and other genes which may be involved in PS gene expression (Zeilstra-Ryalls & Kaplan, 1995a). Hence, the failure of ALA to completely restore the Bch levels to that seen in cells grown in the absence of N₂O can be explained if redox changes induced by N₂O exert an effect on the FnrL-dependent expression of genes encoding enzymes in the Bch pathway in addition to *bemA*. Since N₂O reductase takes electrons from peri-

plasmic cytochrome *c*₂ (Richardson *et al.*, 1991) and ferricyanide, a membrane-impermeable oxidant, is also thought to oxidize cytochrome *c*₂ then it seems likely that in *R. sphaeroides* a redox signal elicited by these oxidants needs to be transduced across the cytoplasmic membrane. Support for the view that FnrL may respond to redox changes comes from the observation that *R. sphaeroides cco* mutants, which lack a *cb*-type cytochrome oxidase, exhibit increased expression of FnrL-dependent genes in the presence of oxygen (Zeilstra-Ryalls & Kaplan, 1996). This result is consistent with our observations since it is expected that the *cb*-type cytochrome oxidase will oxidize periplasmic cytochrome *c*₂.

Recent results of Kiley and co-workers have established that Fnr from *E. coli* probably contains one 4Fe-4S cluster per monomer (Lazazzera *et al.*, 1996). The active form of Fnr is a dimer (Ziegelhoffer & Kiley, 1995) and there is now evidence that the extreme oxygen lability of the Fe-S cluster is associated with monomer formation and decreased ability to bind DNA (Lazazzera *et al.*, 1996). Although these results support the view that Fnr responds to an oxygen species directly, there is evidence that even in *E. coli* redox reactions also influence its activity. Unden *et al.* (1990) showed that, under anoxic conditions, positive redox potentials (> +440 mV at pH 7.0) were sufficient to decrease Fnr-dependent expression of an *frd-lacZ* fusion in *E. coli*. Although *E. coli* has rather different pathways of aerobic respiration compared to *R. sphaeroides* (Garcia-Horsman *et al.*, 1994) the two bacterial species do possess some homologous redox proteins. *R. sphaeroides* possesses two genes, *rdxA* and *rdxB*, which each encode a predicted transmembrane protein containing four 4Fe-4S clusters (Neidle & Kaplan, 1992; Zeilstra-Ryalls & Kaplan, 1996). Rdx proteins are homologous to NapG in *E. coli* and proteins of this family are found in a number of facultative aerobes (Berks *et al.*, 1995).

The *rdxBHIS* operon, *bemA* operon and *cco* operon in *R. sphaeroides* all have Fnr-binding consensus sequences and are located in a gene cluster which also contains *fnrL* (Zeilstra-Ryalls & Kaplan, 1996). The recent observation that FnrL-dependent gene expression was increased in cells lacking the *cb*-type cytochrome oxidase has led to a proposal that redox activation of FnrL occurs under conditions of low oxygen tension and that this process may involve Rdx (Zeilstra-Ryalls & Kaplan, 1996). The only established function of RdxA in *R. sphaeroides* is in the reduction of tellurite to tellurium metal (Neidle & Kaplan, 1992). Since this process is also dependent upon cytochrome *c*₂ and cytochrome *bc*₁ it suggests that Rdx may interact with the respiratory chain via these components (Moore & Kaplan, 1992). If a pathway of electron transport involving cytochrome *c*₂, the cytochrome *bc*₁ complex and Rdx was involved in a direct or indirect redox interaction with FnrL then the effect of respiratory oxidants such as oxygen and N₂O on FnrL activity could be explained as a diversion of electrons away from the Rdx pathway. This model would also explain why *R. sphaeroides* growing under phototrophic or anaerobic dark conditions with DMSO as electron acceptor synthesizes higher levels of LH complex than those grown with N₂O

as electron acceptor; electron transfer to DMSO does not involve the cytochrome *bc₁* complex or cytochrome *c₂* but branches from the cyclic photosynthetic electron transfer pathway at the level of the ubiquinone pool (Q-pool) (McEwan, 1994). As a result, cytochrome *c₂* would be expected to be more reduced in cells growing with DMSO than in those cells growing in the presence of N₂O, and this has been observed for dark anaerobic cells of *R. capsulatus* (Jones *et al.*, 1990). The situation in *E. coli* is slightly different from that of *R. sphaeroides* but since the Fnr-regulated *naf/ccm* gene cluster, formerly known as the *aeg-46.5* cluster, encodes *c*-type cytochromes and periplasmic nitrate reductase, as well as NapG (Groves *et al.*, 1996), the possibility that respiratory electron transport is in redox communication with Fnr needs to be considered.

The effect of N₂O expression on the *puf-lacZ* and *puc-lacZ* fusions in phototrophically grown cells shows that both of these operons are subject to redox control. The mechanisms which control *puf* operon expression are still unclear but certainly its expression is highly responsive to Bch availability and this may arise as a result of decreased ALA synthase activity (Neidle & Kaplan, 1993a). The increase in the level of *puc-lacZ* expression in cells grown in the presence of N₂O may seem surprising in view of the fact the level of mature LH II was lower under the same conditions. However, the relationship between the level of *puc* transcripts and LH II level in *Rhodobacter* is known to be complex. Zucconi & Beatty (1988) have shown that the level of the 0.5 kb *puc* mRNA was about four times higher in *R. capsulatus* cells grown at 140 W m⁻² compared to those grown at 2 W m⁻², which is in contrast to the level of mature LH II. It is interesting that Neidle & Kaplan (1993b) reported that in a *hemT* mutant both *puc* and *puf* transcripts were higher than in wild-type cells, even though the level of LH complexes was lower. The control of *hemT* expression is quite different from *hemA* (Neidle & Kaplan, 1993a, b) and this was also reflected in the change in expression of *hemT* in response to N₂O. The apparent increase in the expression of *hemT* cannot be explained but the data suggest that the expression of *hemT* and *puc* may be subject to a similar control mechanism.

Although we can explain some of the effects of N₂O respiration on PS gene expression in terms of regulation by FnrL, the unusual regulation of *hemT* and *puc* expression suggests that another signal transduction pathway may be affected by N₂O. A key observation regarding the effect of N₂O on phototrophically growing cells was that, although N₂O affected the level of LH I, one of its major effects was to prevent the increase in the level of LH II that occurs when cells are grown at low light intensity. This situation resembles that of a *reg* mutant in *R. capsulatus* which makes reduced amounts of LH complex compared to wild-type cells and cannot increase the synthesis of LH complexes in response to low light intensities (Sganga & Bauer, 1992). The Reg system of *R. capsulatus* and its counterpart, Prr, in *R. sphaeroides* is a sensor histidine kinase response regulator which acts as an activator of PS gene expression (Eraso & Kaplan, 1994, 1995), although its action is probably indirect since

the response regulator, PrrA, is not a DNA-binding protein. A third protein, PrrC, seems to modulate the activity of the PrrB sensor kinase (Eraso & Kaplan, 1995) and it is known that this membrane protein has a periplasmic domain and a predicted Fe-S cluster (Buggy & Bauer, 1995). We suggest that the PrrB kinase is influenced by changes in the redox state of cytochrome *c₂*, possibly working via PrrC, and that this affects PS gene expression. In this model, the maintenance of cytochrome *c₂* in a relatively oxidized state during phototrophic growth in the presence of N₂O would result in lowered PrrB activity and decreased PS gene expression.

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