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Regulation of Photosynthesis Genes (*puf, puc, puhA, bchC, bchE, bchF*, and *bchI*) in *Rhodobacter sphaeroides*

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Here we examined the expression patterns and regulation of seven photosynthesis (PS) genes (*puf, puc, puhA, bchC, bchE, bchF*, and *bchI*) in the anoxygenic photosynthetic bacterium, *Rhodobacter sphaeroides*, based on *lacZ* reporter gene assay. Expression of the tested PS genes, except *puhA* and *bchI*, were strongly induced in *R. sphaeroides* grown under anaerobic conditions relative to that under aerobic conditions. The *puhA* and *bchI* genes appear to form the operons together with *bchFNBHLM-RSP0290* and *crtA*, respectively. Expression of the *puf, puc*, and *bchCXYZ* operons in *R. sphaeroides* grown photosynthetically was proportional to the incident light intensity, whereas that of *bchFNBHLM(RSP0290 -puhA)* was inversely related to light intensity. Expression of *bchEJG* was lowest under medium-light photosynthetic conditions (10 W/m^2) and highest under high light conditions (100 W/m^2). The regulation of PS genes by the three major regulatory systems involved in oxygen- and light-sensing in *R. sphaeroides* is as following: *puf* and *bchC* are regulated by both the PpsR repressor and the PrrBA two-component system. The *puc* operon is under control of PpsR, FnrL, and PrrBA system. Expression of *bchE* is controlled by FnrL and PrrBA two-component system, whereas *bchF* is regulated exclusively by PpsR. It was demonstrated that the PpsR repressor is responsible for high-light repression of *bchF* and that FnrL might be involved in perceiving the cellular redox state in addition to sensing O_2 itself.

Key words – FnrL, photosynthesis gene, PrrBA two-component system, PpsR repressor, redox sensing, *Rhodobacter sphaeroides*

Introduction

The purple non-sulfur photosynthetic bacterium *Rhodobacter sphaeroides* possesses remarkable metabolic versatility. In the presence of oxygen it grows aerobically by performing aerobic respiration. Under anaerobic conditions it is capable of growing by anaerobic respiration, photosynthesis, or fermentation. When oxygen tensions fall below ~3%, the specialized membrane system housing the photosynthetic apparatus (spectral complexes) or intracytoplasmic membrane (ICM), is formed as the result of invaginations from the cytoplasmic membrane[17,25,32]. The photosynthetic apparatus consists of two light-harvesting complexes (B875 and B800-850) and a photochemical reaction center which forms the photosynthetic electron transport chain together with the cytochrome bc_1 complex as well as the mobile electron carriers such as ubiquinone/ubiquinol pool and cytochrome c_2[17,25,32]. Under anaerobic conditions in the light, the cellular level of the B800-850 complex relative to the B875 complex is inversely proportional to the incident light intensity[11,17,25,32]. The apoproteins of the reaction center and the B875 complex are encoded by the *puhA* gene and *pufKBALMX* operon, while those of the B800-850 complex are encoded by the *pucBAC* operon[1]. The synthesis of the photopigments (bacteriochlorophyll and carotenoid), which serve as chromophores of the spectral complexes, is catalyzed by enzymes encoded by *bch* and *crt* genes, respectively. The aforementioned genes required for the formation of the spectral complexes are referred to as photosynthesis (PS) genes, which are clustered in a contiguous 67-kb DNA region on chromosome I of *R. sphaeroides*[1].

The primary determinant that governs the expression of PS genes in *R. sphaeroides*, is oxygen[25,32]. PS genes are expressed only under oxygen-limiting or anaerobic conditions. PS gene expression is also affected by both the incident light intensity and the cellular redox state[11,14,23,24,31]. The more oxidized the cellular redox state is (or the stronger the incident light intensity is), the less amount of the spectral complexes is formed in *R. sphaeroides*. In *R. sphaeroides* three major regulatory systems (PrrBA two-component system, PpsR repressor, and FnrL) have been shown

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to participate in the regulation of PS genes in response to changes in oxygen tension and light intensity[4-7,9,10,34].

Using *lacZ* transcriptional fusions, we systematically investigated the regulation of PS genes that encode the apoproteins of the spectral complexes and the enzymes catalyzing the biosynthesis of bacteriochlorophyll in response to changes in oxygen tension, light intensity, and cellular redox state and here report the regulation patterns of those genes.

Materials and Methods

Strains, plasmids, and growth conditions

The bacterial strains and plasmids used in this study are listed in Table 1. *R. sphaeroides* and *Escherichia coli* strains were grown as described previously[22].

DNA manipulations and conjugation techniques

Standard protocols[27] or manufacturer's instructions were followed for recombinant DNA manipulations. Mobilization of plasmids from *E. coli* strains into *R. sphaeroides* strains was performed as described elsewhere[2].

Table 1. Bacterial strains and plasmids used in this work

Strain or plasmid	Relevant phenotype or genotype	Source or reference
	R. sphaeroides	
2.4.1	Wild type	[30]
PRRBCA2	2.4.1 derivative, Δ*prrBCA*::ΩTp^r	[22]
PPSR1	2.4.1 derivative, Δ *ppsR*::ΩKm^r	[11]
	E. coli	
DH5α	(Φ80d*lacZ*ΔM15)Δ*lacU169 recA1 endA1 hsdR17 supE44 thi1 gyrA96 relA1*	[15]
S17-1	Pro$^-$ Res$^-$ Mob$^+$ *recA*; integrated plasmid RP4-Tc::Mu-Km::Tn7	[28]
	plasmid	
pUI8461	Tc^r; pLA2917-derived cosmid from *R. sphaeroides* cosmid library	[3]
pUI8714	Tc^r; pLA2917-derived cosmid from *R. sphaeroides* cosmid library	[3]
pCF1010	Sp^r St^r Tc^r; IncQ, promoterless *lacZ*	[19]
pUI1663	Sp^r St^r Km^r; IncQ, *puf*::*lacZYA'*	[7]
pCF200Km	Sp^r St^r Km^r; IncQ, *puc*::*lacZYA'*	[18]
pBCHE	Sp^r St^r ; IncQ, *bchE*::*lacZYA'*	[22]
pLX200	Sp^r St^r ; IncQ, *bchF*::*lacZYA'*	[9]
pBCHCLAC	Sp^r St^r Tc^r; IncQ, *bchC*::*lacZYA'*	This study
pBCHILAC	Sp^r St^r Tc^r; IncQ, *bchI*::*lacZYA'*	This study
pPUHALAC	Sp^r St^r Tc^r; IncQ, *puhA*::*lacZYA'*	This study

Construction of lacZ transcriptional fusion plasmids

(i) pBCHCLAC: To construct the *bchC*::*lacZ* transcriptional fusion, the *bchC* promoter region was amplified with primers 5'-CTGCTCCTGCAGCGGGTGCGCGAGGCG-3' (*Pst*I site is underlined) and 5'-GGATGGTCTAGAGGCCAAGGT CCCGCG-3' (*Xba*I site is underlined) and pUI8461 as the template to generate a 480-bp product. The PCR product comprising the 422-bp upstream and 58-bp coding sequences of *bchC*, was digested with *Pst*I and *Xba*I and cloned into promoterless *lacZ* vector pCF1010 digested with the same enzyme, yielding plasmid pBCHCLAC. (ii) pBCHILAC: the *bchI*::*lacZ* transcriptional fusion plasmid, pBCHILAC, was constructed in the same way as pBCHCLAC except that the primers 5'-ATCTGCTCTAGACCGACGATG GCCGAG-3' and 5'-TTCGTCCTGCAGGTTACCTTCTCGAT C-3' were used for PCR. The 525-bp PCR product consists of the 478-bp upstream and 47-bp coding sequence of *bchI*. (iii) pPUHALAC: to construct the *puhA*::*lacZ* transcriptional fusion, the *puhA* promoter region was amplified with primers 5'-CCATCCTCATGGGCGGCG-3' and 5'-GGAAGATC TAGAAGCTATAGATCGCCGG-3' and pUI8714 as the template to generate a 321-bp product. The PCR product was digested with *Pst*I and *Xba*I and a 250-bp restriction fragment was cloned into pCF1010, resulting in pPUHALAC. The transcriptional fusion contains the 180-bp upstream and 70-bp coding sequences of *puhA*.

Protein determination and enzyme assay

Protein concentration was determined by the bicinchoninic acid protein assay (Pierce, Rockford, IL) using bovine serum albumin as the standard protein. Preparation of crude cell extracts and determination of β-galactosidase activities were performed as described previously[23].

Results and Discussion

Regulation of PS gene expression in response to changes in oxygen tension and incident light intensity

Expression patterns of all the PS genes in response to changes in oxygen tension and light intensity were reported by Roh *et al.*[26] using a DNA chip of *R. sphaeroides* 2.4.1. However, some results obtained from the DNA chip contradicted those reported by Happ *et al.*[13] especially with regard to the regulation of PS gene expression in response to changes in the incident light intensity under anaerobic conditions.

We here reexamined the regulation patterns of the PS genes that encode the apoproteins of the spectral complexes and the enzymes involved in the biosynthesis of bacteriochlorophyll, using the *R. sphaeroides* 2.4.1 strains containing the corresponding transcriptional *lacZ* fusions. Since the *bch* genes involved in bacteriochlorophyll biosynthesis are clustered into four transcriptional units (*bchFNBHLM, bchEJG, bchIOD,* and *bchCXYZ*)[1], we investigated the expression patterns of the first genes of the operons.

The expression of the *puf* operon, which codes for the apoproteins (PufA and PufB) of the B875 complex as well as L- and M-subunits of the reaction center, was shown to be strongly induced in *R. sphaeroides* 2.4.1 strain grown under anaerobic photosynthetic conditions relative to that in the *R. sphaeroides* 2.4.1 strain grown aerobically (Table 2). Under photosynthetic conditions the extent of *puf* expression was highest at 100 W/m^2 of light intensity and proportional to the incident light intensity. The expression patterns of the *puc* operon and *bchC* were similar to those of the *puf* operon. The *puc* operon encodes the apoproteins (PucA and PucB) of the B800-850 complex. The *bchC* gene forms an operon (*bchCXYZ*) together with *bchX, bchY,* and *bchZ* and their products as well as BchF and BchG are involved in conversion of chlorophyllide *a* to bacteriochlorophyll *a* during bacteriochlrophyll biosynthesis[29].

The expression level of the *bchE* gene, which encodes the enzyme catalyzing the conversion of Mg-protoporphyrin monomethyl ester to divinyl-protochlorophyllide in the bacteriochlorophyll biosynthetic pathway[29], was significantly increased under photosynthetic conditions as compared with that under aerobic conditions. However, the expression of *bchE* was more induced at 3 W/m^2 of light intensity than was that at 10 W/m^2, which is different from the expression patterns of *puf, puc,* and *bchC.*

Interestingly, the expression level of *bchF*, whose product catalyzes the conversion of chlorophyllide *a* to 2-hydroxyethyl bacteriochlorophyllide *a*[29], was shown to be inversely related to the incident light intensity, and the strong induction of its expression was observed under anaerobic photosynthetic conditions as compared with the expression level of *bchF* under aerobic conditions. Only the basal expression level of the *puhA* gene encoding the H subunit of the reaction center was observed under both aerobic and photosynthetic conditions although expression of *puhA* was slightly induced under photosynthetic conditions. The ORF *RSP0290* is located 19 bp upstream of *puhA* and *RSP0290* overlaps with *bchM*, the last gene of the *bchFNBHLM* operon, by 3 bp, implying that *puhA* does not have its own promoter and forms an operon with *bchFNBHLM* and *RSP0290.*

With regard to *bchI* coding for subunit I of protoporphyrin IX chelatase[29], expression of the gene was not induced under photosynthetic conditions when compared with that under aerobic conditions, which is not in line with previous finding that expression of *bchI* was induced under anaerobic photosynthetic conditions[26]. The *bchI* gene is located 5 bp downstream of the *crtA* gene whose product catalyzes the conversion of spheroidene to spheroidenone, carotenoids found in the spectral complexes of *R. sphaeroides*[31]. Therefore, it appears that the *bchI* gene forms a transcriptional unit with *crtA* whose expression was shown to be strongly induced under photosynthetic conditions[31].

On the basis of the results presented in Table 2 as well as DNA sequence analysis, it can be assumed that the

Table 2. Promoter activities of PS genes related to the synthesis of the apoproteins of the spectral complexes and bacteriochlorophyll

	Apoproteins of the spectral complexes			Bacteriochlorophyll biosynthesis			
Growth condition	*puf*	*puc*	*puhA*	*bchC*	*bchE*	*bchF*	*bchI*
30% O_2	14± 1	72± 4	7±0	150± 10	4±1	7±1	58±3
PS 100W	1561±79	1344±33	22±1	2520± 60	101±3	52±0	98±9
PS 10W	796± 9	558±30	15±1	1380±150	35±2	110±4	45±5
PS 3W	395±92	359±16	15±0	1270± 70	64±9	153±4	15±3

R. sphaeroides 2.4.1 strains harboring the correspond *lacZ*-transcriptional fusions were grown aerobically by sparging with 30% O_2, 69% N_2, 1% CO_2 to an A_{600} of 0.4 to 0.5 and photosynthetically (PS) at low (3 W/m^2), medium (10 W/m^2), and high (100 W/m^2) light intensity in completely filled screw-cap glass tubes to an A_{600} of 0.4 to 0.5. The unit of β-galactosidase activity are expressed as nmole/min/mg of protein. All values are the averages of two independent determinations.

puhA and *bchI* genes are transcribed from the promoters of *bchFNBHLM* and *crtA*, respectively. According to the regulation patterns of PS genes in response to changes in the incident light intensity, the genes encoding the apoproteins of the spectral complexes and the enzymes catalyzing bacteriochlorophyll biosynthesis, can be divided into three groups. The *puf*, *puc*, and *bchCXYZ* operons belong to the first group. The genes of the first group are strongly induced under anaerobic conditions in the same manner as other PS genes and their expression levels under photosynthetic conditions are proportional to the incident light intensity. The second group contains those genes comprising the *bchFNBHLM* operon and probably the *puhA* gene. The genes of this group are also strongly expressed when oxygen is removed from the growth condition. In contrast to the genes of the first group, the lower the incident light intensity is, the more strongly induced are the genes of the second group. High-light repression of *bchFNBHLM* probably leads to a decrease in bacteriochlorophyll synthesis, which in turn results in the decrease in the cellular levels of the spectral complexes in *R. sphaeroides* grown anaerobically under high light conditions. Indeed, the amount of the spectral complexes synthesized in *R. sphaeroides* grown anaerobically is inversely related to the incident light intensity[11]. The *bchE* gene was shown to be regulated differently from the PS genes belonging to the first and second groups. Its expression was lowest under medium-light photosynthetic conditions (10 W/m^2) and highest under high light conditions (100 W/m^2).

Regulation of PS gene expression by the PrrBA two-component system and the PpsR repressor

To ascertain whether the PS genes studied in Table 2 are regulated by the PrrBA two-component system, the transcriptional activities of *puf*, *puc*, *bchC*, *bchE*, and *bchF* were determined in the wild-type strain 2.4.1 and the Prr null mutant strain PRRBCA2 grown under anaerobic conditions. Since the Prr null mutant strain was not able to grow under photosynthetic conditions[5], both *R. sphaeroides* strains were grown anaerobically in the dark with DMSO as a terminal electron acceptor for anaerobic respiration.

As shown in Fig. 1, the expression levels of *puf*, *puc*, *bchC*, and *bchE* were significantly lower in the PRRBCA2 mutant strain grown under anaerobic conditions than those detected in the wild-type strain 2.4.1, indicating that the PrrBA two-component system is required for anaerobic induction of *puf*, *puc*, *bchC*, and *bchE* and that these genes are under the control of the PrrBA two-component system. By contrast, the promoter activity of the *bchF* gene in the wild-type strain was similar to that in the PRRBCA2 strain. The expression of the *bchF* gene was induced in both the PRRBCA2 and the wild-type strains grown under anaerobic conditions when compared with its expression levels in the strains grown aerobically (Table 2 and Fig. 1). These results indicate that expression of the *bchF* gene is not regulated by the PrrBA two-component system.

It was previously demonstrated that anaerobic induction of *puf*, *puc*, and *bchE* is mediated by the PrrBA two-component system[5,22]. Our results confirmed the previous results and demonstrated the regulation of *bchC* by the PrrBA system at the first time.

PpsR is a soluble, redox-responding repressor protein with a homotetrameric quaternary structure[8]. It functions as a repressor under aerobic conditions as well as under anaerobic conditions in a light-dependent manner to repress the expression of some PS genes. PpsR contains two PAS (Per-ARNT-Sim) domains in its central region, which have been shown to affect PpsR repressor activity, and a helix-turn-helix motif in the C-terminal region, which binds to the conserved palindromic DNA sequence motif (TGT-N_{12}-ACA)[8,25,32]. We next examined whether expression of *puf*, *puc*, *bchC*, *bchE*, and *bchF* is regulated by

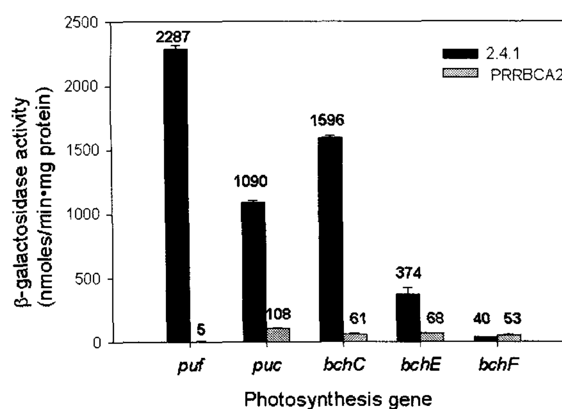

Fig. 1. Promoter activities of several PS genes in the wild-type (2.4.1) and Prr-null mutant (PRRBCA2) strains of *R. sphaeroides* grown anaerobically. The *R. sphaeroides* strains harboring the corresponding transcriptional fusion plasmids were grown anaerobically with DMSO as a terminal electron acceptor in the dark. Promoter activities were quantitatively determined by measuring the β-galactosidase activity. The activity is expressed as nmole/min/mg of protein. All values are the averages of two independent determinations.

the PpsR repressor. The wild-type strain and the PpsR null mutant strain (PPSR1) harboring the corresponding transcriptional fusion plasmids were grown under 30% O_2 conditions and the promoter activity of each PS gene was measured by β-galactosidase assay. As shown in Fig. 2, *puf, puc, bchC,* and *bchF* were strongly derepressed in the PPSR1 mutant strain as compared with their expression rates in the wild-type strain. By contrast, the *bchE* gene was not derepressed in the PPSR1 mutant strain grown aerobically. These results suggest that the expression of *puf, puc, bchC,* and *bchF* are under the control of PpsR, and that *bchE* is not regulated by the PpsR system. There is a PpsR-binding site 17-bp upstream of the *bchE* translation start codon[20,22], which led us to postulate that the *bchE* gene is under the control of PpsR. However, we demonstrate here that expression of *bchE* is not repressed by PpsR under aerobic conditions despite of the presence of the PpsR-binding on the upstream sequence of *bchE.*

It is worthwhile to note that the *puf* operon is derepressed in the PpsR null mutant grown aerobically although no PpsR-binding site is present on the upstream regions of the *puf* operon. At present we cannot explain this observation. There is a possibility that the *puf* operon might be regulated by an additional unknown regulator whose gene is regulated by PpsR.

The FnrL protein, which normally acts as an anaerobic activator, binds to the DNA consensus sequence that has been established as TTGAT-N_4-ATCAA[33,34]. The FnrL-binding motif are located 229 and 54-bp upstream of *pucB* and *bchE*[20], respectively. It was previously demonstrated that induction of the *pucBAC* and *bchEJGP* operons requires FnrL[22]. By means of sequence analysis no FnrL-binding motif was identified on the upstream sequences of *puf, bchC, bchF,* and *crtA*[20].

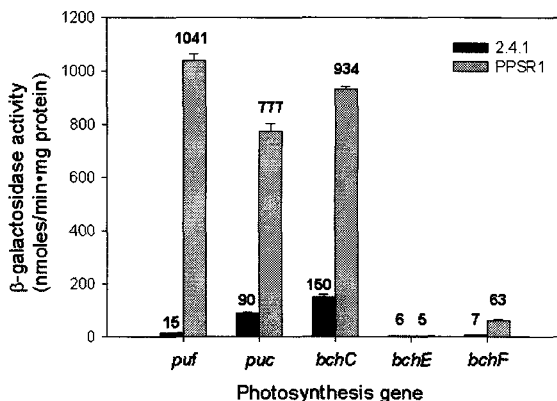

Fig. 2. Promoter activities of several PS genes in the wild-type (2.4.1) and PpsR-null mutant (PPSR1) strains of *R. sphaeroides* grown aerobically. The *R. sphaeroides* strains harboring the corresponding transcriptional fusion plasmids were grown aerobically by sparging with 30% O_2, 69% N_2, 1% CO_2 to an A_{600} of 0.4 to 0.5. Promoter activities were quantitatively determined by measuring the β-galactosidase activity. The activity is expressed as nmole/min/mg of protein. All values are the averages of two independent determinations.

Taken together, the data presented in Fig. 1 and Fig. 2 as well as sequence analyses of the upstream regions of PS genes allowed us to postulate the regulation of the PS genes by three major regulatory systems that are implicated in anaerobic expression of PS genes: *puf* and *bchC* are regulated by both the PpsR repressor and the PrrBA two-component system. The *puc* operon is under control of PpsR, FnrL, and PrrBA system. Expression of *bchE* is controlled by FnrL and PrrBA two-component system, whereas *bchF* is regulated exclusively by PpsR.

PpsR is responsible for high-light repression of PS gene expression

Expression of *bchF* was shown to be regulated exclusively by the PpsR repressor. As shown in Table 2, the expression level of *bchF* in *R. sphaeroides* grown under photosynthetic conditions is inversely related to the incident light intensity. This implies that high-light repression of *bchF* is mediated by PpsR. To examine whether PpsR is involved in light-dependent repression of *bchF*, the *R. sphaeroides* PPSR1 strain (a PpsR null mutant) harboring the *bchF::lacZ* transcriptional fusion plasmid was grown under photosynthetic conditions at 100 and 3 W/m^2 of light intensity and the activity of β-galactosidase was measured to determine the promoter activity of *bchF*. As a control, the wild-type strain 2.4.1 containing the same plasmid was included in the experiment. When grown under high-light conditions (100 W/m^2), the promoter activity of *bchF* in the wild-type strain was decreased to 34% of the activity detected in the wild-type strain grown under low-light conditions (3 W/m^2) (Fig. 3). The high-light repression observed in the wild type was abolished in the PPSR1 mutant strain. This result indicates that PpsR is responsible for high-light repression of PS genes. It was suggested that light-dependent induction of the *puc* and *puf* operons in the absence of oxygen is mediated by the cbb_3-PrrBA signal transduction pathway[13]. Since the *bchF* gene is not regulated by the PrrBA system, the *bchF* gene is repressed under high-light

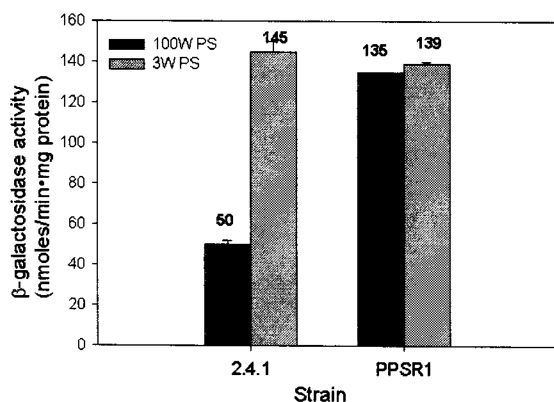

Fig. 3. Promoter activities of the *bchF* gene in the wild-type (2.4.1) and PpsR-null mutant (PPSR1) strains of *R. sphaeroides* grown photosynthetically at low (3 W/m^2) and high (100 W/m^2) light intensity. The *R. sphaeroides* strains containing the *bchF::lacZ* transcriptional fusion plasmid were grown photosynthetically in completely filled screw-cap glass tubes to an A_{600} of 0.4 to 0.5. Promoter activities were quantitatively determined by measuring the β-galactosidase activity. The activity is expressed as nmole/min/mg of protein. All values are the averages of two independent determinations.

conditions. However, *puc, puf,* and *bchC* are under the control of the PrrBA two-component system in addition to PpsR. Therefore, high-light repression of these genes, which is exerted by PpsR, might be compensated with the inductive effect of the PrrBA two-component system on their expression under high light conditions (see Table 1).

The PpsR protein is a redox-responsive protein that contains two conserved cysteine residues (Cys251 and Cys424). When exposed to oxygen, two cysteines form the intramolecular disulfide bond, which makes PpsR functional as an active repressor[21]. PpsR cannot sense light by itself. The AppA protein containing FAD as the chromophore has been demonstrated to act as an antirepressor of PpsR [12,21]. Under anaerobic conditions in the dark AppA is capable of both breaking the intramolecular disulfide bond within PpsR and forming a stable AppA-PpsR complex, antagonizing the PpsR repressor activity. The FAD molecule within AppA absorbs blue light, which leads to conformational change in AppA. Light-excited AppA cannot form the AppA-PpsR complex, which renders the PpsR regulon repressed under high-light conditions.

Recent results obtained from microarray analysis showed that *bchF* gene expression in *R. sphaeroides* 2.4.1 was proportional to the incident light intensity under photosynthetic conditions[26], which is in contrast with our result. Since PpsR is known as the high-light repressor and the *bchF* gene is regulated exclusively by PpsR, our result appears to be correct.

Regulation patterns of PS genes in response to changes in the cellular redox state

It was previously demonstrated that the addition of DMSO, a terminal electron acceptor, to photosynthetic cultures of *R. sphaeroides* led to a substantial decrease in levels of the synthesized spectral complexes[24]. The DMSO reductase is synthesized in *R. sphaeroides* grown anaerobically in the presence of DMSO. The redox state of components of the photosynthetic electron transport chain is affected by the rate at which electrons are removed to the terminal reductase such as DMSO reductase that receives electrons from the ubiquinone/ubiquinol pool of the electron transport chain and reduces DMSO to DMS. Therefore, the cellular redox state of *R. sphaeroides* grown photosynthetically is more oxidized in the presence of DMSO than is that in the absence of DMSO.

We examined the effect of DMSO on PS gene expression in *R. sphaeroides* grown photosynthetically at medium light (10 W/m^2). As shown in Fig. 4, the addition of DMSO to photosynthetic cultures of *R. sphaeroides* 2.4.1 strain led to

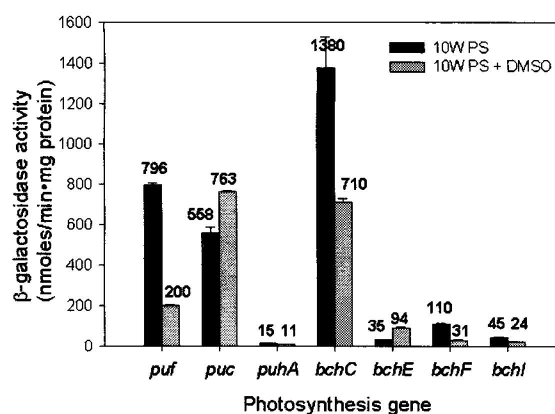

Fig. 4. Effect of DMSO on PS gene expression in *R. sphaeroides* under photosynthetic conditions. The wild-type strains (2.4.1) of *R. sphaeroides* harboring the corresponding *lacZ* transcriptional fusion plasmids were grown photosynthetically at medium light intensity (10 W/m^2; 10WPS). To examine DMSO effect, the strains were grown photosynthetically with supplementation of DMSO to a final concentration of 0.5% (v/v) at 10 W/m^2 (10WPS+DMSO). Cells were harvested at an A_{600} of 0.4 to 0.5. Promoter activities were quantitatively determined by measuring the β-galactosidase activity. The activity is expressed as nmole/min/mg of protein. All values are the averages of two independent determinations.

a decrease in promoter activities of *puf*, *bchC*, and *bchF*, which accounts for the reduction of spectral complex levels in the presence of DMSO. By contrast, expression of *puc* and *bchE* was increased by the addition of DMSO to photosynthetic cultures. The common feature that *puc* and *bchE* share is that they are regulated by FnrL. The FnrL protein, a homologue of the global anaerobic regulator Fnr of *E. coli*, is indispensable for spectral complex formation and photosynthetic growth in *R. sphaeroides*. FnrL serves as a transcriptional activator for several PS genes including *puc*, *bchE*, and *cycA*[22,25,34]. The active form of FnrL was suggested to be a homodimer containing two [4Fe-4S] centers. FnrL appears to sense oxygen directly by means of the [4Fe-4S] center[16]. The [4Fe-4S] center was suggested to be converted to a [2Fe-2S] center upon exposure to oxygen, thereby affecting its activity.

It remains elusive why expression of *puc* and *bchE*, which are under control of FnrL, is upregulated in the presence of DMSO. FnrL might also be able to perceive the cellular redox state besides oxygen itself. The other possibility is that an unknown regulatory factor, which can sense the cellular redox state, might be involved in the regulation of *puc* and *bchE*. To address this question, further study is required.

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초록 : *Rhodobacter sphaeroides*에서의 광합성유전자(*puf, puc, puhA, bchC, bchE, bchF*와 *bchI*)의 발현조절

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본 연구에서는 *lacZ* transcriptional fusion plasmid를 이용하여 광합성 세균인 *Rhodobacter sphaeroides*에서의 7가지 광합성유전자 (*puf, puc, puhA, bchC, bchE, bchF, bchI*) 발현의 경향과 조절을 조사하였다. *R. sphaeroides*에서 *puhA*와 *bchI*를 제외한 모든 광합성유전자들이 호기적 조건과 비교했을 때 혐기적 조건에서 더욱 강하게 발현되었다. *puhA* 유전자는 *bchFNBHLM-RSP0290*과 operon을 형성하며, *bchI* 유전자는 *crtA*와 operon을 이루는 것으로 나타났다. 광합성 조건에서 자란 *R. sphaeroides*의 *puf, puc, bchCXYZ* operon의 발현은 빛의 세기에 비례하는 반면, *bchFNBHLM(RSP0290 puhA)* operon의 발현은 빛의 세기에 반비례 하였다. *bchEJG*의 발현은 10 W/m^2의 빛이 조사된 광합성 조건에서 제일 낮았으며, 100 W/m^2의 빛의 광합성 조건에서 가장 높았다. *R. sphaeroides*의 산소인지와 빛 인지에 관련된 세 가지 주요 조절기작에 의한 광합성유전자 조절은 다음과 같다. *puf*와 *bchC*는 PpsR repressor와 PrrBA two-component system에 의해 조절된다. 그리고 *puc* operon은 PpsR, FnrL, PrrBA system에 의해 조절된다. *bchE*의 발현은 FnrL과 PrrBA system에 의해 조절되는 반면, *bchF*는 오로지 PpsR에 의해서만 조절된다. PpsR repressor는 강한 세기의 빛 조건에서 *bchF* 발현억제의 원인이 되며, FnrL은 그 자체가 산소를 인지하는 기능 이외에도 세포질의 산화/환원 상태의 인지에 관련될 것으로 보인다.