Chapter 35

Regulation of Gene Expression in Response to Oxygen Tension

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Summary

Purple photosynthetic bacteria control numerous energy-generating and energy-utilizing processes in response to alterations in cellular redox, which is affected by environmental oxygen tension. The list of redox-regulated events includes synthesis of the pigmented and cytochrome components of the photosystem, enzymes for fixation of carbon and nitrogen, the synthesis of several terminal respiratory electron transport complexes, and synthesis of the energy-generating hydrogenase complex. Regulating synthesis of these components involves several well-characterized transcription factors including the sensor kinase RegB and its cognate response regulator RegA. Other redox-responding regulators include CrtJ and Fnr. Mechanisms of redox sensing by these transcription factors are discussed.

I. Introduction

Many metabolic processes such as photosynthesis, respiration, carbon and nitrogen fixation are highly regulated in response to alterations in environmental oxygen tension (redox). Some processes, such as photosynthesis, appear to be predominately regulated by oxygen tension (Cohen-Bazire et al., 1957), while other processes, such as nitrogen and carbon fixation, are regulated by the availability of nitrogen or carbon,

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respectively, as well as by alteration in cellular redox (Madigan, 1995; Tabita, 1995; Bowman et al., 1999). To a large extent, redox control of many different processes occurs by controlling gene expression either directly in response to the presence or absence of molecular oxygen or in response to changes in the overall redox state of the cell. This chapter is focused on both oxygen- and redox-responding master regulators of gene expression in purple bacteria.

Studies on redox-regulation of gene expression in purple photosynthetic bacteria initially focused on photosynthesis because a change in pigment biosynthesis provided a simple visible screen for mutants that either failed to synthesize a photosystem under anaerobic conditions (Sganga and Bauer 1992; Mosley et al., 1994) or aberrantly synthesized photopigments aerobically (Penfold and Pemberton, 1994; Du et al., 1998). These studies resulted in the identification of several conserved transcription factors that are responsible for controlling synthesis of the photosystem in *Rhodobacter* (*Rba.*) *capsulatus* and *Rba. sphaeroides*. The first identified redox regulators were the RegB/RegA two-component transduction system from *Rba. capsulatus* (Sganga and Bauer 1992; Mosley et al., 1994). This two-component system induces the synthesis of nearly all of the components of the bacterial photosystem comprised of the light-harvesting, reaction center and cytochrome apoproteins (Elsen et al., 2004), as well as bacteriochlorophyll, carotenoids and heme (Smart et al., 2004; Willet et al., 2007).

In addition to RegB-RegA, there is a redox-responding aerobic repressor of photopigment, light-harvesting and cytochrome biosynthesis called CrtJ (designated as PpsR in other species) that has also been extensively characterized. Like RegA/RegB, CrtJ controls the synthesis of individual components of the photosystem such as the tetrapyrroles bacteriochlorophyll (Penfold and Pemberton, 1994; Ponnampalam et al., 1995) and heme (Smart et al., 2004), as well as apoproteins for cytochromes (Swem and Bauer, 2002) and the light-harvesting complex (Ponnampalam et al., 1995). Finally, a redox-responding homolog of the *Escherichia* (*E.*) *coli* transcriptional regulator FNR (fumarate-nitrate reduction) is also involved in controlling photosynthetic and respiratory growth in *Rba. capsulatus* (Swem and Bauer, 2002; Smart et al., 2004) and in *Rba. sphaeroides* (Zeilstra-Ryalls and Kaplan, 1995; Mouncey and Kaplan, 1997; Zeilstra-Ryalls and Kaplan, 1998). This chapter is focused on biochemical analysis of redox-responding transcription factors that have been characterized in purple photosynthetic bacteria.

Abbreviations: B. – *Bradyrhizobium*; bp – base pair(s); *E.* – *Escherichia*; NMR – nuclear magnetic resonance; *P.* – *Pseudomonas*; PAS – Per-ARNT-Sim ; *Rba.* – *Rhodobacter*; *Rps.* – *Rhodopseudomonas*

II. RegB/RegA Two-Component Signal Transduction System

RegA and RegB were initially identified in genetic screens designed to isolate mutants defective in oxygen control of photosystem synthesis in *Rba. capsulatus* (Sganga and Bauer, 1992; Mosley et al., 1994). Mutants defective in anaerobic synthesis of the photosystem were isolated that exhibited significantly reduced expression of the *puh*, *puf* and *puc* operons that encode apoproteins for the light-harvesting 1, light-harvesting 2 and reaction center complexes (Sganga and Bauer, 1992; Mosley et al., 1994). Sequence analysis demonstrated that these strains contained mutations in two linked genes, *regB* and *regA*, with RegB exhibiting homology to histidine protein kinase (McCleary and Stocks, 1994; Mosley et al., 1994), and RegA exhibiting homology to a DNA-binding response regulator (Parkinson and Kofoid, 1992; Sganga and Bauer, 1992; McCleary and Stocks, 1994). Subsequent to the discovery of RegB and RegA from *Rba. capsulatus*, homologous two-component systems were found and genetically characterized in many other species such as: RegB/RegA homologs in *Rba. sphaeroides* (called PrrB/PrrA) (Eraso and Kaplan, 1994, 1995; Phillips-Jones and Hunter, 1994); RegS/RegR from *Bradyrhizobium* (*B.*) *japonicum* (Bauer et al., 1998); ActS/ActR from *Sinorhizobium meliloti* (Tiwari et al., 1996); RoxS/RoxR from *Pseudomonas* (*P.*) *aeruginosa* (Comolli and Donohue, 2002); and RegB/RegA from *Rhodovulum sulfidophilum* and *Roseobacter denitrificans* (Masuda et al., 1999). Genome sequence studies have also identified RegA and RegB homologs in many other photosynthetic as well as non-photosynthetic α- and γ-proteobacterial species (>90 species) with a partial list of these homologs present in Table 1.

Genetic shuttling studies demonstrated that RegB and RegA homologs from different species are interchangeable in vitro and in vivo. This indicates that phosphotransfer can be observed between different RegB and RegA homologs (Comolli and Donohue, 2002; Emmerich et al., 2000), and that some RegA

Table 1. RegB and RegA homologs identified by similarity based search

Organism	RegB					RegA			
	RegB	Identity	H-Box	Q-binding site	Redox-active cystein	RegA	Identity	Acid-Box	DNA-Binding domain
Rhodobacter capsulatus	YES Q9L906		YES	YES	YES	YES P42508		YES	YES
Rhodobacter sphaeroides	YES Q3J6C1	58%	YES	YES	YES	YES Q53228	83%	YES	YES
Dinoroseobacter shibae	YES A0VNJ9	57%	YES	YES	YES	YES A0VNJ7	82%	YES	YES
Sulfitobacter sp.	YES A3S9L0	55%	YES	YES	YES	YES A3S9K8	79%	YES	YES
Rhodobacterales bacterium	YES A3VGE1	55%	YES	YES	YES	YES A3VGE3	80%	YES	YES
Silicibacter pomeroyi	YES Q5LLQ5	54%	YES	YES	YES	YES Q1GCP6	82%	YES	YES
Rhodovulum sulfidophilum	YES O82866	54%	YES	YES	YES	YES O82868	83%	YES	YES
Roseobacter denitrificans	YES O82869	55%	YES	YES	YES	YES Q9ZNM4	81%	YES	YES
Oceanicola granulosus	YES Q2CJX1	53%	YES	YES	YES	YES Q2CJX3	84%	YES	YES
Sagittula stellata	YES A3KAZ2	53%	YES	YES	YES	YES A3KAZ4	82%	YES	YES
Roseovarius sp.	YESA3W499	53%	YES	YES	YES	YES Q0FV45	82%	YES	YES
uncultured proteobacterium	YES Q8KYV6	53%	YES	YES	YES	YES Q8KYV8	78%	YES	YES
Loktanella vestfoldensis	YES A3V2H4	52%	YES	YES	YES	YES A3V2H5	83%	YES	YES
Jannaschia sp.	YES Q28JY5	51%	YES	YES	YES	YES Q28JX7	81%	YES	YES
Paracoccus denitrificans	YES A1B5R9	47%	YES	YES	YES	YES Q3PEU7	77%	YES	YES
Parvibaculum lavamentivorans	YES A1H0J9	39%	YES	YES	YES	YES A1H0J8	70%	YES	YES
Uncultured *Acidobacteria bacterium*	YES Q7X352	39%	YES	YES	YES	YES Q7X351	66%	YES	YES
Stappia aggregata	YES A0P3M9	39%	YES	YES	YES	YES A0P3M8	71%	YES	YES
Rhizobium loti	YES Q98C40	38%	YES	YES	YES	YES Q98C39	69%	YES	YES
Mesorhizobium sp.	YES Q11B16	38%	YES	YES	YES	YES Q11B17	70%	YES	YES
Brucella suis	YES Q8G321	37%	YES	YES	YES	YES Q8G319	67%	YES	YES
Brucella abortus	YES Q2YP02	37%	YES	YES	YES	YES Q57FN7	67%	YES	YES
Xanthobacter sp.	YES Q26N86	38%	YES	YES	YES	YES Q26N85	69%	YES	YES
Brucella melitensis	YES Q8YER2	37%	YES	YES	YES	YES Q8YER6	67%	YES	YES
Methylobacterium sp.	YES A5P1X6	37%	YES	YES	YES	YES A5P1X5	YES	YES	YES
Bradyrhizobium japonicum	YES O86124	36%	YES	YES	YES	YES Q89VZ0	69%	YES	YES
Aurantimonas sp.	YES Q1YF90	35%	YES	YES	YES	YES Q1YF91	68%	YES	YES
Nitrobacter winogradskyi	YES Q3SWG3	35%	YES	YES	YES	YES Q3SWG2	70%	YES	YES
Rhizobium meliloti	YES Q92TA1	36%	YES	YES	YES	YES Q52913	70%	YES	YES
Rhodopseudomonas palustris	YES Q6NCA0	36%	YES	YES	YES	YES Q6NCA1	70%	YES	YES
Rhizobium etli	YES Q2KE47	36%	YES	YES	YES	YES Q2KE48	69%	YES	YES
Sinorhizobium medicae	YES Q52912	36%	YES	YES	YES	YES Q0MH93	70%	YES	YES
Nitrobacter hamburgensis	YES Q1QRL7	34%	YES	YES	YES	YES Q1QRL6	70%	YES	YES
Caulobacter crescentus	YES Q9ABH9	38%	YES	YES	YES	YES Q9ABI0	68%	YES	YES
Rhizobium leguminosarum	YES Q1MNA6	36%	YES	YES	YES	YES Q1MNA7	69%	YES	YES
Fulvimarina pelagi	YES Q0FYD3	36%	YES	YES	YES	YES Q0FYD2	68%	YES	YES
Hyphomonas neptunium	YES Q0BWS2	36%	YES	YES	YES	YES Q0BWS1	69%	YES	YES
Agrobacterium tumefaciens	YES Q8UJ81	36%	YES	YES	YES	YES Q8UJ82	68%	YES	YES

Table 1. Continued

Organism	RegB					RegA			
	RegB	Identity	H-Box	Q-binding site	Redox-active cystein	RegA	Identity	Acid-Box	DNA-Binding domain
Maricaulis maris	YES Q0AKP8	36%	YES	YES	YES	YES Q0AKP9	67%	YES	YES
Parvularcula bermudensis	YES A3VQ48	36%	YES	YES	YES	YES A3VQ47	68%	YES	YES
Oceanicaulis alexandrii	YES A3UI09	36%	YES	YES	YES	YES A3UI10	65%	YES	YES
Myxococcus xanthus	YES Q1CZ19	31%	YES	YES	YES	NO[1]			
Nitrosospira multiformis	YES Q2YD54	27%	YES	YES	YES	YES Q2YD55	50%	YES	YES
Blastopirellula marina	YES A4A0N7	27%	YES	YES	YES	NO[1]			
Pseudomonas syringae	YES Q4ZNL1	30%	YES	YES	YES	YES Q87WJ3	52%	YES	YES
Chromohalobacter salexigens	YES Q1QWH9	26%	YES	YES	YES	YES Q1QWI0	47%	YES	YES
Pseudomonas fluorescens	YES Q3KI43	29%	YES	YES	YES	YES Q3KI42	52%	YES	YES
Azotobacter vinelandii	YES Q4IY97	28%	YES	YES	YES	YES Q4IY98	50%	YES	YES
Methylobacillus flagellatus	YES Q1GZ69	27%	YES	YES	YES	YES Q1GZ68	51%	YES	YES
Anaeromyxobacter dehalogenans	YES Q2II98	27%	YES	YES	YES	YES Q2II97	47%	YES	YES
Pseudomonas putida	YES Q2XJG8	28%	YES	YES	YES	YES Q88PG2	50%	YES	YES
Oceanospirillum sp	YES Q2BQZ7	26%	YES	YES	YES	YES Q2BQZ8	49%	YES	YES
Nitrosomonas europaea	YES Q82V00	25%	YES	YES	YES	YES Q820M1	50%	YES	YES
Pseudomonas aeruginosa	YES Q9HVS7	28%	YES	YES	YES	YES Q9HVS8	50%	YES	YES
Pelagibacter ubique	YES Q4FNH0	28%	YES	YES	YES	YES Q4FP64	67%	YES	YES
Stigmatella aurantiaca	YES Q08V74	28%	YES	YES	YES	YES Q08V73	51%	YES	YES
Candidatus Pelagibacter ubique	YES Q1V1K9	29%	YES	YES	YES	YES Q1V0V8	67%	YES	YES
Pseudomonas entomophila	YES Q1IEE3	29%	YES	YES	YES	YES Q1IEE2	50%	YES	YES
Psychroflexus torquis	YES Q1VI62	28%	YES	YES	YES	YES Q1VJU0	65%	YES	YES
Dechloromonas aromatica	YES Q47FP6	27%	YES	NPF>NPL	YES	YES Q47FP7	44%	YES	YES
Sphingopyxis alaskensis	YES Q1GT06	26%	YES	YES	YES	YES Q1GT07	45%	YES	YES
Sphingomonas sp.	YES Q1N6Y2	24%	YES	YES	YES	NO[1]			
Mariprofundus ferrooxydans	YES Q0EZG0	24%	YES	YES	YES	NO[1]			
Nitrococcus mobilis	NO[2]					YES A4BPC8	50%	YES	YES
Nitrosomonas eutropha	NO[2]					YES Q3N6K1	49%	YES	YES
Thiobacillus denitrificans	NO[2]					YES Q3SFG7	49%	YES	YES
Alcaligenes eutrophus	NO[2]					YES Q476X9	48%	YES	YES
Plesiocystis pacifica	NO[2]					YES A6G990	48%	YES	YES
Ralstonia metallidurans	NO[2]					YES Q1LS55	46%	YES	YES
Alkalilimnicola ehrlichei	NO[2]					YES Q0A850	45%	YES	YES
Saccharophagus degradans	NO[2]					YES Q21GP2	39%	YES	YES
Pseudoalteromonas haloplanktis	NO[2]					YES Q3IBV7	42%	YES	YES
Shewanella frigidimarina	NO[2]					YES Q3NW53	42%	YES	YES
Burkholderia cenocepacia	NO[2]					YES Q44X66	43%	YES	YES
Chromobacterium violaceum	NO[2]					YES Q7NZM5	47%	YES	YES
Burkholderia vietnamiensis	NO[2]					YES Q4BNG8	43%	YES	YES

Table 1. Continued

Organism	RegB					RegA			
	RegB	Identity	H-Box	Q-binding site	Redox-active cystein	RegA	Identity	Acid-Box	DNA-Binding domain
Ralstonia solanacearum	NO[2]					YES Q8Y3E0	45%	YES	YES
Burkholderia pseudomallei	NO[2]					YES Q3JXA5	43%	YES	YES
Burkholderia thailandensis	NO[2]					YES Q2T278	43%	YES	YES
Burkholderia mallei	NO[2]					YES Q62F01	51%	YES	YES
Burkholderia ambifaria	NO[2]					YES Q3FK41	43%	YES	YES
Colwellia psychrerythraea	NO[2]					YES Q47UR4	42%	YES	YES
Methylococcus capsulatus	NO[2]					YES Q602T5	46%	YES	YES
Idiomarina loihiensis	NO[2]					YES Q5QWI7	38%	YES	YES
Shewanella amazonensis	NO[2]					YES Q3QI67	42%	YES	YES
Shewanella denitrificans	NO[2]					YES Q3P3L2	37%	YES	YES
Shewanella putrefaciens	NO[2]					YES Q2ZVU1	38%	YES	YES
Shewanella oneidensis	NO[2]					YES Q8E9U1	38%	YES	YES
Shewanella baltica	NO[2]					YES Q3Q7V3	37%	YES	YES
Hahella chejuensis	NO[2]					YES Q2SM70	42%	YES	YES
Marinobacter aquaeolei	NO[2]					YES Q36SD4	38%	YES	YES
Rhodopirellula baltica	NO[2]					YES Q7UHV2	40%	YES	YES
Bordetella pertussis	NO[2]					YES Q7VUY1	42%	YES	YES
Bordetella bronchiseptica	NO[2]					YES Q7WMV8	42%	YES	YES
Bordetella parapertussis	NO[2]					YES Q7WBD8	42%	YES	YES
Bordetella avium	NO[2]					YES Q2KWQ0	42%	YES	YES

1. No homologs to RegA were found in Blast2 complete database
2. No homologs to RegB were found in Blast2 complete database

homologs can bind to promoters and regulate gene transcription in other species (Emmerich et al., 2000; Comolli and Donohue, 2002).

It is now well established that RegB and RegA constitute a highly conserved global regulatory system that provides an overlying layer of redox-control on a variety of energy-generating and energy-utilizing biological processes in many diverse species of bacteria (Fig. 1A) (Elsen et al., 2004). Furthermore, recent genome array experiments indicate that nearly 20% of *Rba. sphaeroides* genes are part of the RegB-RegA regulon (Kaplan et al., 2005). Predictive hierarchical clustering analysis of the *Rba. sphaeroides* genome, for putative RegA binding sites, also indicates the presence of a large number of putative RegA binding sites well beyond that of photosynthesis and respiratory genes (Mao et al., 2005). These results indicate that RegB-RegA provide redox control of many cellular processes.

A. The Sensor Kinase RegB

The *Rba. capsulatus regB* gene encodes a 50.1 kDa histidine protein kinase composed of 460 amino acids. The N-terminal region comprises a transmembrane domain containing six hydrophobic membrane-spanning regions which is followed by a C-terminal cytoplasmic 'transmitter' domain (Tiwari et al., 1996; Ouchane and Kaplan, 1999; Chen et al., 2000). A recent study identified the ubiquinone pool as a redox signal for RegB with a highly conserved quinone binding site (Table 1) found to be located in the transmembrane domain thereby indicating that this region plays a role in redox-sensing (Swem et

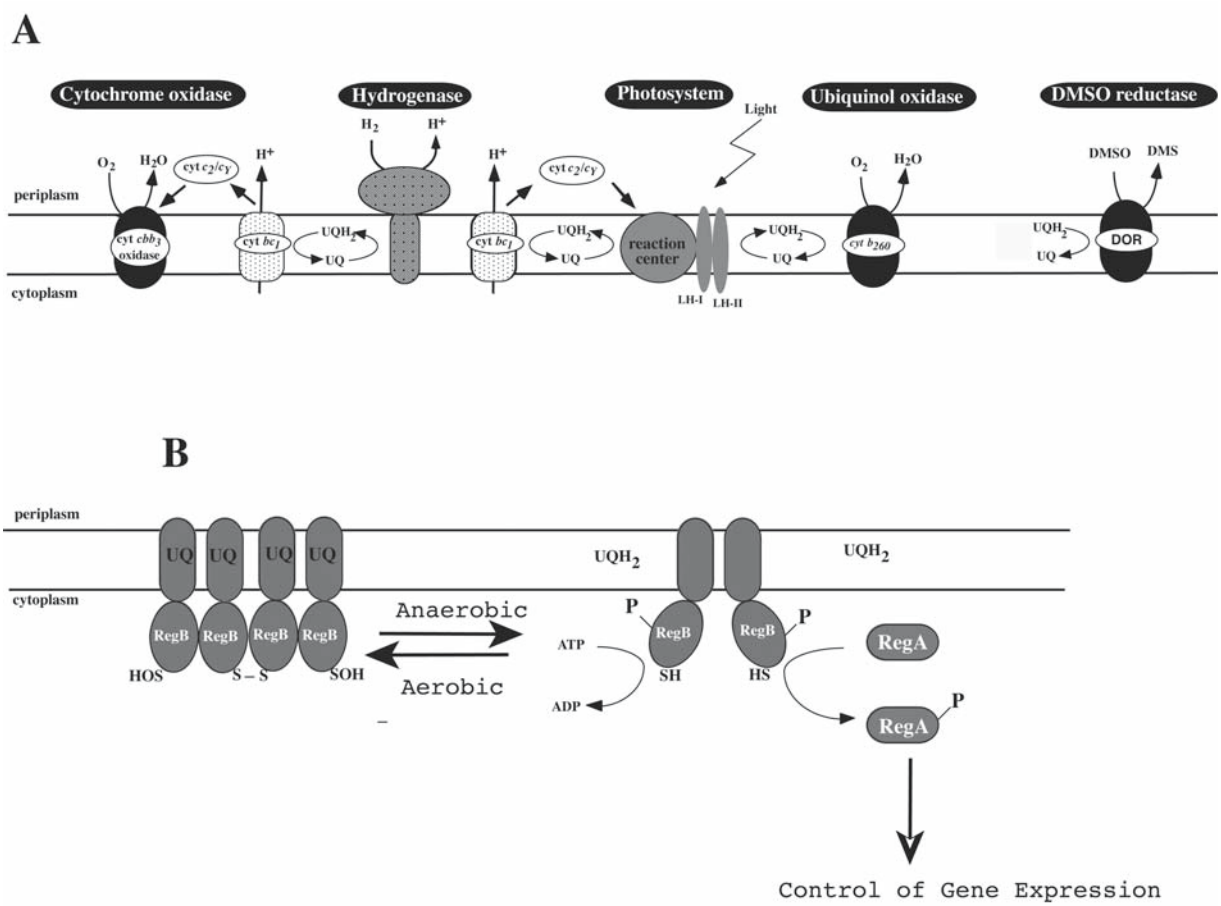

Fig. 1. A) Biological processes regulated by the RegB/RegA system through a direct interaction with the ubiquinone pool. B) Oxidized redox sensors of RegB include direct interaction with oxidized ubiquinones (UQ) that inhibits kinase activity, the formation of a disulfide bond (S-S) that stimulates tetramerization, and formation of a putative cysteine sulfonate (SOH).

al., 2006). The transmembrane domain is followed by a cytosolic domain that contains an H-box site of autophosphorylation (His225), and the N, G1, F, and G2 boxes that define the nucleotide binding cleft (McCleary and Stocks, 1994; Mosley et al., 1994; Ouchane and Kaplan, 1999). The cytosolic domain contains a conserved redox-active cysteine located in a conserved 'redox box' just downstream of the H-box (Fig. 1B; Table 1) (Swem et al., 2003). This Cys is capable of regulating the activity of RegB through forming an intermolecular disulfide bond in response to the redox state (Swem et al., 2003).

1. Kinase Activity

Initial kinase assays demonstrated that a His-tagged cytosolic domain of *Rba. capsulatus* RegB was capable of autophosphorylation in vitro as well as phosphotransfer to its cognate response regulator, RegA (Inoue et al., 1995; Bird et al., 1999). As the kinetics were not affected by ATP concentration, it was suggested that the rate-limiting step of RegB autophosphorylation was phosphotransfer from ATP to the histidine residue, rather than binding of ATP (Bird et al., 1999). Similar results have been obtained with cytosolic versions of RegB homologs from *Rba. sphaeroides* and from *B. japonicum* (Emmerich et al., 1999; Comolli et al., 2002). It was shown that phosphorylated full-length RegB exhibits decreased stability of phosphate compared to the truncated version of RegB (half-life of about 34 min versus 5.5 to 6 h), pointing to a role of the transmembrane domain in regulating the phosphorylation state of RegB (Masuda et al., 1999; Potter et al., 2002).

The first demonstration of phosphotransfer from *Rba. capsulatus* RegB~P to RegA was reported by Inoue et al., (1995). Phosphotransfer studies showed that the transfer of phosphate is rapid (<1 min) from the cytosolic domain of RegB to RegA in vitro (Bird et al., 1999). Since autophosphorylation of RegB is slow and phosphotransfer from RegB~P to RegA is very rapid, it appears that autophosphorylation of RegB is a rate-limiting step in controlling the phosphorylation level of RegA (Bird et al., 1999). No back-transfer of phosphate from RegA~P to RegB has been observed (Comolli and Donohue, 2002; Potter et al., 2002).

2. Phosphatase Activity

Histidine kinases can modulate the level of phosphorylation of a cognate phosphorylated response regulator not only through phosphorylation, but also by exerting phosphatase activity on the receiver's Asp~P. Dephosphorylation of RegA~P in vitro was shown to be dependent on the amount of unphosphorylated RegB, which indicates that RegB can dephosphorylate RegA (Bird et al., 1999). Results from the *B. japonicum* RegB homolog (RegS) (Emmerich et al., 1999), and both full-length version and truncated forms of RegB from *Rba. sphaeroides* (Potter et al., 2002), showed that the presence of RegB resulted in >16-fold reduction in the stability of the phosphate on RegA~P (Comolli and Donohue, 2002). Because both truncated and full-length RegB exhibit the same phosphatase activity, it is assumed that modulation of phosphatase activity does not require the N-terminal region of RegB. However, further studies are required to determine whether phosphatase activity is redox-regulated.

3. Redox Sensing

In vivo studies indicated that expression of many RegB-RegA-regulated photosynthesis genes were inhibited by growth under aerobic conditions (Bauer et al., 1988; Sganga and Bauer, 1992; Mosely et al., 1994). It was therefore presumed that the kinase activity of RegB was directly inhibited by oxygen (Mosely et al., 1994). However, this possibility was subsequently excluded because *Rba. capsulatus* is fully capable of inducing pigment biosynthesis under chemoautotrophic growth conditions in the presence of oxygen, hydrogen and carbon dioxide (Madigan and Gest, 1979).

Another signal proposed to regulate RegB was the redox state of the respiratory electron transport chain (O'Gara and Kaplan, 1997; Eraso and Kaplan, 2000; Oh and Kaplan, 2000; Roh and Kaplan, 2000). This conclusion was based on the observation that mutations of cytochrome cbb_3 oxidase in *Rba. sphaeroides* and *Rba. capsulatus* lead to elevated aerobic expression of RegB-RegA-regulated genes (Eraso and Kaplan, 1994; Buggy and Bauer, 1995). It was suggested that cytochrome cbb_3 oxidase generates an 'inhibitory signal' that represses the RegB/RegA two-component system. Recently, Swem et al., (2006) demonstrated that the redox state of the ubiquinone pool, which is known to be affected by respiration and photosynthesis, is a direct signal controlling RegB autophosphorylation. In this study, autophosphorylation activity of full-length RegB

was significantly inhibited in vitro by the presence of oxidized, but not reduced, ubiquinone. A highly conserved ubiquinone-binding site was identified in a short periplasmic loop between transmembrane helices three and four with the use of ^{14}C-azidoquinone photo-affinity cross-linking (Swem et al., 2006). There is a heptapeptide sequence of GGXXNPF that is 100% conserved among all known RegB homologs in the same region (Table 1) (Swem et al., 2006). It has been proposed that oxidized ubiquinone binds to this heptapeptide through π-π interactions between its para-hydroxybenzoate ring and the aromatic side group of the conserved phenylalanine (Phe112), as well as hydrogen bond interaction between ubiquinone and the conserved asparagine (Asp111) (Swem et al., 2006). The binding of oxidized ubiquinone presumably results in allosteric modification of RegB that leads to inhibition of autophosphorylation. When the ubiquinone pool is shifted to a protonated form under anaerobic conditions, a hydrogen bond between asparagine and ubiquinone could be disrupted triggering a structural change that allows autophosphorylation of RegB (Fig. 1B). Subsequent in vivo mutational study on Phe112 found elevated aerobic synthesis of the photosystem, confirming that this amino acid is involved in the sensing of the redox state of the ubiquinone pool and regulating RegB kinase activity (Swem et al., 2006).

There is a large ubiquinone pool in membranes from purple photosynthetic bacteria that provides electron carriers for photosynthesis and respiration (Bolton, 1978). The redox state of the ubiquinone pool varies in response to changes in oxygen tension, being predominantly oxidized under aerobic conditions and predominantly reduced under anaerobic conditions (Parson, 1978). Ubiquinone is a good redox signal given that the redox state of ubiquinones reflects changes in the redox state of cells in general. Ubiquinone as a redox signal for controlling RegB activity also correlates well with the observation that mutations in cytochrome *cbb$_3$* oxidase lead to elevated RegB activity (Buggy and Bauer, 1995). In this case, a mutation in a terminal respiratory electron acceptor such as a cytochrome *cbb$_3$* oxidase would result in a more reduced ubiquinone pool, which would subsequently lead to an elevation of RegB kinase activity.

In addition to the ubiquinone-binding site, there is a fully conserved cysteine (Cys 265) that is involved in redox-sensing (Table 1) (Swem et al., 2003). This redox-active cysteine is located in a 'redox-box' that is harbored in a cytosolic dimerization interface downstream of the H-box (Fig. 2 and Table 1). In vitro analysis using truncated RegB without the transmembrane domain indicates that an intermolecular disulfide bond forms between RegB dimers under oxidizing conditions, converting active dimers into inactive tetramers (Fig. 1B) (Swem et al., 2003). In vitro disulfide bond formation was shown to require the presence of a divalent metal ion which may help to fold RegB into a functional structure (Swem et al., 2003). The involvement of an intermolecular disulfide bond in the control of RegB activity is also supported by an increase in vitro of full-length RegB phosphorylation in the presence of DTT (Potter et al., 2002). Furthermore, Western blot analysis has confirmed that the RegB intermolecular disulfide bond can form under aerobic growth conditions in vivo (Swem et al., 2003). However, Western blot analysis also showed that <20% of wild type RegB forms a disulfide bond in vivo when cells are shifted from anaerobic to aerobic growth conditions. The remainder of the RegB dimers have been proposed to form other oxidized derivatives of Cys 265 such as sulfonic acid (Cys-S-OH), to regulate kinase activity (Swem et al., 2003).

Several mutations have been constructed to probe the roles of ubiquinone and Cys265 redox signals in regulating RegB activity. A Cys265 to Ala mutation (C265A) in full-length RegB led to attenuated, but not absent redox control by coenzyme Q1 in vitro and reduced redox control in vivo (Swem et al., 2006; Swem et al., 2003). Similar results were observed with mutations in the ubiquinone binding domain, which show elevated aerobic expression but still harbor a low level of redox control (Swem et al., 2006). These results indicate that the ubiquinone pool is a redox signal independent of the redox state of Cys265 (Swem et al., 2006). Given that the ubiquinone binding site is located in the transmembrane domain, and Cys265 is located in the cytosolic domain, they are not likely to directly interact. So, it seems that ubiquinone-binding and Cys265 function independently and that they both contribute to redox control of RegB autophosphorylation activity.

B. The Response Regulator RegA

RegA is a 20.4 kDa response regulator comprised of 184 amino acid residues, that consists of a receiver domain and a DNA binding domain that are linked by a four proline hinge (Elsen et al., 2004). The N-

terminal receiver domain is similar to that of other two-component response regulators, containing an aspartate residue (Asp63) where phosphorylation occurs and a highly conserved 'acid pocket' consisting of two aspartate residues. The C-terminal DNA binding domain is made up of a 3 α-helices (α-6, α-7, and α-8). The α-7 and α-8 helices comprise a wing-turn-helix DNA binding motif (Sganga and Bauer, 1992; Du et al., 1998; Laguri et al., 2003; Elsen et al., 2004). This binding domain was found to be 100% conserved in RegA homologs from a variety of α-proteobacterial species (Elsen et al., 2004).

1. Effect of Phosphorylation

For response regulators to bind DNA, there typically needs to be an interruption of intramolecular forces between the receiver and DNA-binding domains to allow access of DNA binding helices to the DNA surface. The structural change that mediates this opening typically occurs upon phosphorylation of a conserved aspartate (Du et al., 1998; Laguri et al., 2003). A recent crystal structure of a RegA homolog from *Mycobacterium tuberculosis* provides evidence that domain interface is indeed destabilized upon phosphorylation, yielding a more extended confirmation (Nowak et al., 2006).

The affinity of *Rba. capsulatus* RegA for the *puc* promoter, and the effect of phosphorylation on DNA binding, has been determined (Bird et al., 1999). The DNA binding affinity of wild type RegA increases 16-fold upon phosphorylation. Similar phosphorylation-induced increases in binding affinities have been reported for *B. japonicum* RegR and for *P. aeruginosa* RoxR (Emmerich et al., 1999; Comolli and Donohue, 2002).

There are exceptions to the need of phosphorylation for promoting DNA-binding activity of RegA. One exception is the isolation of a hyperactive mutant of RegA called RegA* that contains an Ala95 to Ser mutation, which is near the hinge region in the receiver domain. This mutation allows RegA to promote photosynthesis gene expression even in the absence of RegB, indicating that this substitution increases protein dynamics in favor of an open pseudo-phosphorylated state even under conditions of nonphosphorylation (Du et al., 1998). Nonphosphorylated RegA* has a similar DNA binding affinity as that of phosphorylated wild type RegA. RegA* can also be phosphorylated resulting in an additional six-fold increase in binding activity beyond that of phosphorylated wild type RegA. Furthermore, the phosphate bound to RegA*~P was shown to be significantly more stable with a much longer half-life than that observed with wild type RegA~P (Bird et al., 1999).

Both phosphorylated and nonphosphorylated RegA have been observed to play a role in regulating gene expression. For example, while phosphorylated RegA is an anaerobic repressor of cytochrome cbb_3 oxidase, nonphosphorylated RegA also functions as an aerobic activator of cytochrome cbb_3 oxidase (Swem et al., 2001; Swem and Bauer, 2002a). Additionally, both phosphorylated and nonphosphorylated RegA participate in activation and repression of ubiquinol oxidase expression as well as expression of the *cbb* and *hupSLC* operons (Qian and Tabita, 1996; Elsen et al., 2000; Swem and Bauer, 2002a). It remains unclear how phosphorylated and nonphosphorylated forms of RegA are capable of selectively binding and repressing to different promoters.

Mutational studies have been performed on the phosphorylation site of RegA from *Rba. capsulatus*, as well as RegA homologs from *B. japonicum* and *Rba. sphaeroides*. An Asp65 to Lys mutant (D63A) of RegA from *Rba. capsulatus* retained the ability to bind DNA despite being unable to be phosphorylated (Hemschemeier et al., 2000). Comolli and Donohue (2002) also demonstrated that both the nonphosphorylated and phosphorylated versions of wild-type *Rba. sphaeroides* PrrA activated transcription in vitro, but that the D63A mutant could not. This suggests that Asp63 is also involved in transcriptional activation in some manner, perhaps involving interaction with RNA polymerase. This conclusion must be tempered by the conflicting observation that a Asp65 to Asn mutant of RegR from *B. japonicum* was incapable of DNA-binding to the *fixR-nifA* promoter (Emmerich et al., 1999).

2. DNA Binding Sites

DNA recognition sequences bound by RegA have been identified by DNase I footprinting and oligonucleotide retention assays. RegA binding sites have been defined at numerous *Rba. capsulatus* promoters including the *puf*, *puc*, *nifA2, hupSLC*, *regB*, *senC-regA-hvrA*, *petABC*, *cycA*, *cycY*, *cydAB*, *ccoNOPQ*, *cbbI*, *cbbII*, *cheOp2, bchE* and *crtI-crtA* (Du et al., 1999; Elsen et al., 2000; Vichivanives et al., 2000; Swem et al., 2001). Alignment of RegA binding sites from *Rba. capsulatus* yields a consensus

sequence of 5′-G(C/T)G(G/C)(G/C)(G/A)NN(T/A)(T/A)NNC(G/A)C-3′ (Swem et al., 2001). The alignment reveals a partially conserved GCG…CGC palindromic binding sequence separated by three to nine bp. A similar inverted repeat is also seen in the *B. japonicum* RegR consensus sequence that was identified by oligonucleotide affinity trapping. (5′-GNC(A/G)C(A/G)TTNNGNCGC-3′) (Emmerich et al., 2000). Two RegA molecules bind each half of the palindromic sequence in gel shift assays indicating that binding is highly cooperative (Du et al., 1998; Laguri et al., 2003). The variable distances between the GCG…CGC palindrome suggests that RegA proteins bound to palindrome half-sites may interact differently, depending on spacing. The AT-rich region in the middle of the consensus sequence is presumably flexible, and may facilitate interaction of RegA molecules with each other or with the promoter DNA sequence. It was proposed that RegA may recognize the shape of the DNA rather than just the sequence (Swem et al., 2001; Elsen et al., 2004). This suggestion is supported by nuclear magnetic resonance (NMR) studies of RegA bound to DNA, which show that RegA binds to kinks formed by pyrimidine-purine steps approximately one helix turn apart (Laguri et al., 2003). This may provide a structural recognition element to aid RegA in finding correct DNA sequences that vary slightly in composition.

The NMR experiments (Laguri et al., 2003) also confirmed that RegA binds to each half of the palindromic sequence in a symmetric fashion. The structure revealed that the DNA binding domain consists of the three predicted helices α-6, α-7, and α-8. The α-8 helix is the DNA recognition helix and lies within the major groove interacting with the base pairs of the palindrome half site. The α-6 helix interacts non-specifically with the phosphate backbone of DNA to increase binding affinity. These NMR studies also allowed a refinement of the consensus sequence to 5′Y**GCG**TCRxTATAxGN**CGC**-3′ (where x is a variable number of nucleotides). The full-length NMR structure of *Rba. sphaeroides* RegA reveals that the interaction between the receiver and DNA-binding domains only involves the α-6 helix (Laguri et al., 2003). Therefore, the inactive, nonphosphorylated state does not directly block the entire DNA recognition helix, but rather, just the helix that is involved in stabilizing DNA-binding. Mutational analysis of the C-terminal DNA-binding domain identified several residues critical for both binding and transcription. The critical residues correlate well with the proposed recognition site from the NMR analysis (Jones et al., 2005). Additionally, Laguri et al. (2006) demonstrated through NMR diffusion times, gel filtration, and analytical ultracentrifugation that dimerization of PrrA occurs when a BeF_3^- generated phosphate analog of PrrA is constructed. This may indicate that phosphorylation induces dimerization and may be important for correct alignment and binding.

C. Global Significance

As more RegB/RegA homologs and Reg-regulated metabolic processes are discovered, it is becoming evident that RegB/RegA act as a global regulatory system that provides an overlying layer of redox regulation on many important biological processes. Unraveling the redox control mechanism of RegB will provide insight into how a large number of α and γ proteobacteria bacteria balance synthesis of many different redox-responding cellular processes. Analysis of redox control by RegB/RegA also can have practical application in medicine and agriculture. This regulon is present in a number of pathogenic bacteria including *P. aeruginosa, Brucella melitensis, Brucella suis,* and several species with agricultural importance, such as *Agrobacterium tumefaciens, Rhizobium leguminosarum, Sinorhizobium meliloti,* and *B. japonicum*.

III. Aerobic repression by CrtJ

Present in the photosynthesis gene cluster of several purple photosynthetic bacteria is a redox-responding transcription factor called CrtJ or PpsR, depending on the species. Studies have indicated that CrtJ/PpsR is an aerobic repressor of bacteriochlorophyll (*bch*), heme (*hem*) and carotenoid (*crt*) biosynthesis genes in *Rba. sphaeroides* and *Rba. capsulatus* (Penfold and Pemberton, 1994; Gomelsky and Kaplan, 1995a; Ponnampalam and Bauer, 1997; Elsen et al., 1998; Cho et al., 2004; Smart et al., 2004; Kovacs et al., 2005; Moskvin et al., 2005). CrtJ also aerobically represses synthesis of light-harvesting 2 polypeptides (PucBA) that bind bacteriochlorophyll, and carotenoids in these species (Gomelsky and Kaplan, 1995a; Ponnampalam et al., 1995; Moskvin et al., 2005). As discussed above, anaerobic induction of bacteriochlorophyll, carotenoid and light-harvesting genes also requires phosphorylated RegA. So together, CrtJ and RegA regulate synthesis of the photosystem by coordinating aerobic repression, and anaerobic activation of

photosystem genes, respectively. A good example of coordinate regulation by CrtJ and RegA occurs in the *Rba. capsulatus puc* operon. DNase footprint analysis demonstrated that CrtJ and RegA~P have binding sites that overlap near the -35 promoter recognition sequence (Bowman et al., 1999). Oxidized CrtJ was shown to effectively out-compete RegA~P for binding to this promoter region, based on alteration of footprint protection patterns during titration experiments. Oxidized CrtJ also repressed RegA~P-mediated in vitro transcription of *puc* expression (Bowman et al., 1999).

Rba. capsulatus CrtJ and RegA also coordinately regulate synthesis of the respiratory terminal electron acceptors, quinol oxidase (*cydAB*) and cytochrome cbb_3 oxidase (*ccoNOPQ*) (Swem and Bauer, 2002). Quinol oxidase has a high affinity for oxygen and is maximally expressed under low oxygen tension. This is contrasted by cytochrome cbb_3 oxidase that has a low affinity for oxygen, but a high turnover rate and is maximally expressed under high oxygen conditions. Together CrtJ and RegA regulate expression of these oxidases by a coordinating activation and repression. For example, under conditions of high oxygen tension, expression of cytochrome cbb_3 oxidase is activated by dephosphorylated RegA while expression of quinol oxidase is repressed by CrtJ. Under low oxygen tension, phosphorylated RegA represses expression of cytochrome cbb_3 oxidase and also stimulates expression of quinol oxidase (Swem and Bauer, 2002). The mechanism by which phosphorylated and nonphosphorylated RegA functions as both an activator and repressor of these respiratory promoters remains to be addressed.

Protein domain analysis indicates that there are two putative Per-ARNT-Sim motifs called PAS domains in the central region of CrtJ/PpsR, followed by a highly conserved helix-turn-helix DNA binding domain at the C-terminal end (Gomelsky et al., 2000). Gel retardation analysis indicates that PpsR is present in solution as a tetramer (Gomelsky et al., 2000; Masuda and Bauer, 2002) suggesting that at least one of the PAS domains may be involved in tetramerization of CrtJ, however ligand binding to one or both PAS sites cannot be ruled out.

In all cases where it has been examined, CrtJ cooperatively binds to two copies of the palandromic sequence TGT-N_{12}-ACA (Ponnampalam and Bauer, 1997; Elsen et al., 1998; Ponnampalam et al., 1998). The palandromic sequence is found either eight base pairs apart, or at sites that are distantly separated. For example, the *Rba. capsulatus bchC* promoter region has a CrtJ recognition palindrome that spans the –35 promoter region, and a second CrtJ palindrome located 8 bp away that spans the –10 promoter region (Ponnampalam et al., 1998). Binding to these two palindromes is cooperative, so if the 8 bp space between the two palindromes in the *bchC* promoter region is altered by the addition or deletion of just a few bp, then CrtJ is unable to bind to either palindrome effectively (Ponnampalam et al., 1998).

CrtJ also cooperatively binds to palindrome pairs at other promoters, but these palindromes are separated by more than 100–150 base pairs (Elsen et al., 1998). An example of this type of binding occurs in the intergenic region between *crtA* and *crtI* which contains two promoters, one that is responsible for driving expression of the *crtA-bchI-bchD* operon, and a second divergent promoter >100 bp away that is responsible for expression of the *crtI-crtB* operon (Elsen et al., 1998). The promoter for the *crtA-bchI-bchD* transcript has a single CrtJ binding site that spans the –10 promoter sequence, while the *crtI-crtB* promoter also has a single CrtJ recognition sequence that spans the -35 recognition sequence. Cooperative binding of CrtJ to these two palindromes coordinately represses expression of both the *crtA-bchI-bchD* and *crtI-crtB* operons (Elsen et al., 1998). This affects synthesis of both bacteriochlorophyll and carotenoids since BchI and BchD are subunits of Mg-chelatase, which is the first committed enzyme of the bacteriochlorophyll branch of the tetrapyrrole biosynthetic pathway (Bollivar et al., 1994). In addition, *crtI* and *crtB* code for phytoene dehydrogenase and phytoene synthase, respectively, which are enzymes for the first two committed steps of carotenoid biosynthesis (Armstrong et al., 1990). A similar example of distantly removed CrtJ binding sites occurs in the *Rba. capsulatus puc* operon that has one CrtJ binding site overlapping the –35 promoter recognition sequence and a second site located 240 bp upstream (Elsen et al., 1998). Like that of the *crtA* and *crtI* promoters, binding of CrtJ to the two distant binding sites in the *puc* promoter region occurs cooperatively. Presumably, binding of CrtJ to distant sites involves looping of the DNA so that tetrameric CrtJ can bind cooperatively to both of the recognition palindromes (Elsen et al., 1998).

A. Redox and Light Regulation

Redox regulation of CrtJ/PpsR repression in *Rba. capsulatus* and *Rba. sphaeroides* has been shown to involve the oxidation and reduction of an intramolecular disulfide bond (Fig. 2) (Masuda et al.,

2002). In the case of *Rba. capsulatus* CrtJ, the redox potential (E_m) of the disulfide bond is –180 mV at pH 7.0, which means that CrtJ would be predominately reduced in the cytosol, that has a redox state of –220 mV (Masuda et al., 2002). It is therefore assumed that disulfide bond formation may be directly stimulated by molecular oxygen in this species (Masuda et al., 2002). Once oxygen is depleted, the redox poise of the cytosol would effectively reduce the disulfide in CrtJ to promote derepression of photosystem gene expression. In this model, CrtJ would be a direct sensor of the presence of molecular oxygen. Interestingly, the disulfide bond in *Rba. sphaeroides* PpsR has an E_m= –320 mV at pH 7.0, which would make the cysteines predominately oxidized in the cytosol.

There is also a redox- and light-responding anti-repressor called AppA in *Rba. sphaeroides*, which is required to keep the cysteines in PpsR reduced in the absence of oxygen (Fig. 2) (Kim et al., 2006). *Rba. sphaeroides* strains that are disrupted in AppA thus constitutively have PpsR in its active oxidized state resulting in constitutive repression by PpsR (Gomelsky and Kaplan, 1995b; Masuda and Bauer, 2002). The redox potential of the disulfide in AppA

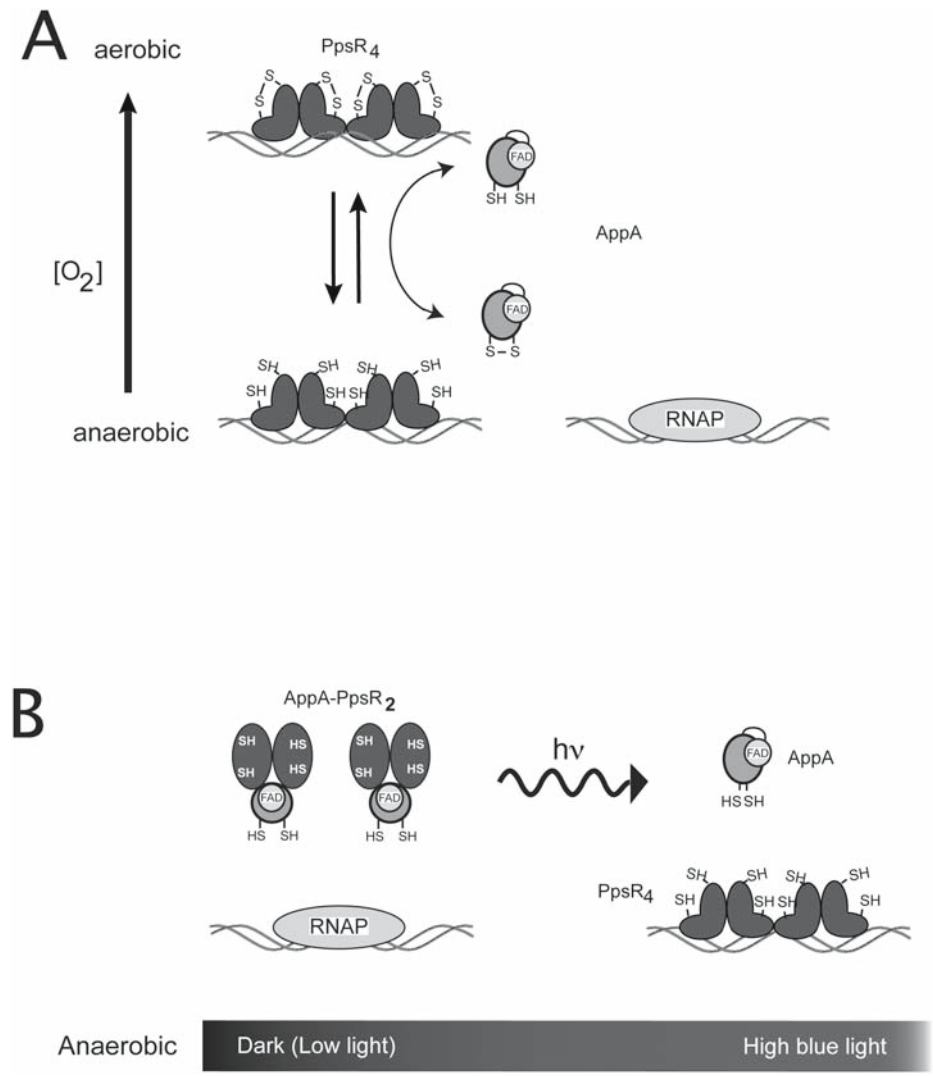

Fig. 2. The role of AppA in the regulation of PpsR repressor activity. As discussed in the text, AppA has two activities: one is to reduce a disulfide bond in PpsR which partially disrupts DNA binding activity of the repressor; the second activity of AppA is to convert the PpsR tetramer into an AppA-$(PpsR)_2$ complex that is incapable of binding DNA. This latter reaction is inhibited by blue light excitation of a flavin in AppA.

has been determined to be from –315 to –325 mV, which is isopotential with the disulfide in PpsR (–320 mV), thereby allowing efficient electron transfer from AppA to PpsR (Kim et al., 2006). AppA has recently been shown to bind heme although the function of heme in redox sensing, or in disulfide bond formation, is not yet clear (Han et al., 2007; Moskvin et al., 2007).

AppA is also a blue light photoreceptor that contains a photoactive flavin with a conserved BLUF domain (Gomelsky and Kaplan, 1998; Masuda and Bauer, 2002, 2005; Gomelsky and Klug, 2002). The flavin undergoes a photocycle (for details of the BLUF photocycle see Chapter 36, Klug and Masuda) upon light excitation where a conformational change occurs in AppA, disrupting the interaction between AppA and PpsR (Fig. 2). Under dark conditions, AppA is able to convert PpsR from an active tetramer into an inactive AppA$(PpsR)_2$ complex (Masuda and Bauer, 2002). Under high intensity blue light AppA releases PpsR, which then is able to repress the expression of the photosystem.

Even though the function of CrtJ/PpsR as a repressor of photosystem synthesis is well supported in *Rba. capsulatus* and in *Rba. sphaeroides*, the function of CrtJ/PpsR in other species has some significant differences. For example, PpsR from *Rubriviax gelatinosus* is capable of activating light-harvesting 2 (*puc*) gene expression while also repressing bacteriochlorophyll and carotenoid gene expression (Steunou et al., 2004). In *Bradyrhizhobium* there are two PpsR genes *ppsR1* and *ppsR2* (Giraud et al., 2002; Jaubert et al., 2004; Elsen et al., 2005). PpsR1 has only one Cys and yet is still able to respond to redox through the formation of an intermolecular disulfide bond (Jaubert et al., 2004). PpsR1 functions as an activator of photosystem gene expression, and contrary to its homologs, its DNA-binding affinity is higher under reducing conditions, thus promoting expression of the photosystem under low oxygen levels (Jaubert et al., 2004). PpsR2 is active as a repressor that binds to the same target DNA sequence as PpsR1. However PpsR2 does not respond to changes in redox and instead responds to light via interaction with a light-responding antirepressor not unlike the light-dependent interaction of AppA with PpsR in *Rba. sphaeroides*. A significant difference is that the antirepressor of PpsR2 in *Bradyrhizhobium* is not a member of the blue light-absorbing BLUF family of flavin photoreceptors like AppA. Instead, the *Bradyrhizhobium* antirepressor of PpsR2 is a red-light absorbing bacteriophytochrome photoreceptor that uses a bilin as a photoreceptor (Jaubert et al., 2004). The mode of interaction between the phytochrome antirepressor and PpsR2 is unclear.

Rhodopseudomonas (*Rps.*) *palustris* also has two PpsR transcriptional regulators; however, in contrast to *Bradyrhizhobium,* both function as transcriptional repressors in response to oxygen (Braatsch et al., 2006). PpsR1 contains three cysteines, two of which would potentially be capable of forming intramolecular disulfide bonds in response to oxygen like that shown for *Rba. capsulatus* and *Rba. sphaeroides*, while PpsR2 contains only one cysteine. Like *Bradyrhizhobium*, *Rps. palustris* contains a bacteriophytochrome, BphP1, which upon illumination at 750 nm triggers derepression of the photosynthetic genes by one of its two PpsR repressor proteins, PpsR2 (Braatsch et al., 2007; see Chapter 40, Evans et al. for a review).

B. Phylogenetic Analysis

Phylogenetic analysis of the available CrtJ/PpsR protein sequences reveal three distinct clusters (Fig. 3). Cluster III contains *Rba. sphaeroides* PpsR and *Rba. capsulatus* CrtJ. All members of this group have the two well conserved cysteines, (Cys-420 and Cys-249 as defined in *Rba. capsulatus* CrtJ) that have been shown to be involved in disulfide bond formation (Masuda and Bauer, 2002; Masuda et al., 2002; Kim et al., 2006;). The two closely related Cluster III CrtJ/PpsR proteins from *Rba. capsulatus* and *Rba. sphaeroides* have been shown to form disulfides in vitro with disulfide reduction potentials determined (Kim et al., 2006; Masuda et al., 2002). These CrtJ/PpsR proteins are most likely able to respond to redox via formation of a intramolecular disulfide bond.

Clusters I and II CrtJ/PpsR proteins are clearly different in that they do not contain the same set of conserved cysteines. Specifically, the cysteine that is located between the internal PAS domains (equivalent to Cys249 in *Rba. capsulatus*) are replaced by hydrophobic residues such as leucine or valine. Furthermore, only a subset of CrtJ/PpsR proteins in Cluster I (*Rhodospirillum centenum, Rubrivivax gelatinosus, Thiocapsa roeopersicina, Rhodospirillum rubrum, Bradyrhizobium* sp. ORS278, *Bradyrhizobium* sp. BTAi1, *Erythrobacter* sp. NAP1, Gamma proteobacterium KT 71, Uncultured proteobacterium AAL76373, Uncultured proteobacterium AAM48620) contains the redox active cysteine near the helix-turn-helix (equivalent to Cys420 in *Rba.*

capsulatus). Presumably this subset of PpsR/CrtJ proteins could respond to redox through a intermolecular disulfide interaction between Cys420 as has been documented to occur for *Bradyrhizobium* (Jaubert et al., 2004). The remaining proteins in Cluster I (*Rps. palustris* BisB5, *Rps. palustris* HaA2, *Rps. palustris* CGA009, *Rps. palustris* BisB18, *Rps. palustris* BisA53,) have a serine at the Cys420 equivalent position suggesting that these proteins may be redox-active. Interestingly, *Rps. palustris* BisB5, *Rps. palustris* HaA2, *Rps. palustris* CGA009 all have a conserved cysteine at position 377 which is 52 amino acids before Cys420 (relative to the amino terminus), as well as a cysteine at position 240 which is 16 amino acids before redox-active Cys249 in *Rba. capsulatus*/*Rba. sphaeroides* CrtJ/PpsR. No mutational studies have been reported for these additional Cys in these other species so it is unclear if they are involved in controlling DNA-binding activity.

Cluster II CrtJ/PpsR proteins (Fig. 3) also are lacking the corresponding Cys at position 249 and all but one (*Thiocapsa roseopersicina* PpsR2) are lacking Cys at position 420. These proteins would most likely be responding to another mechanism

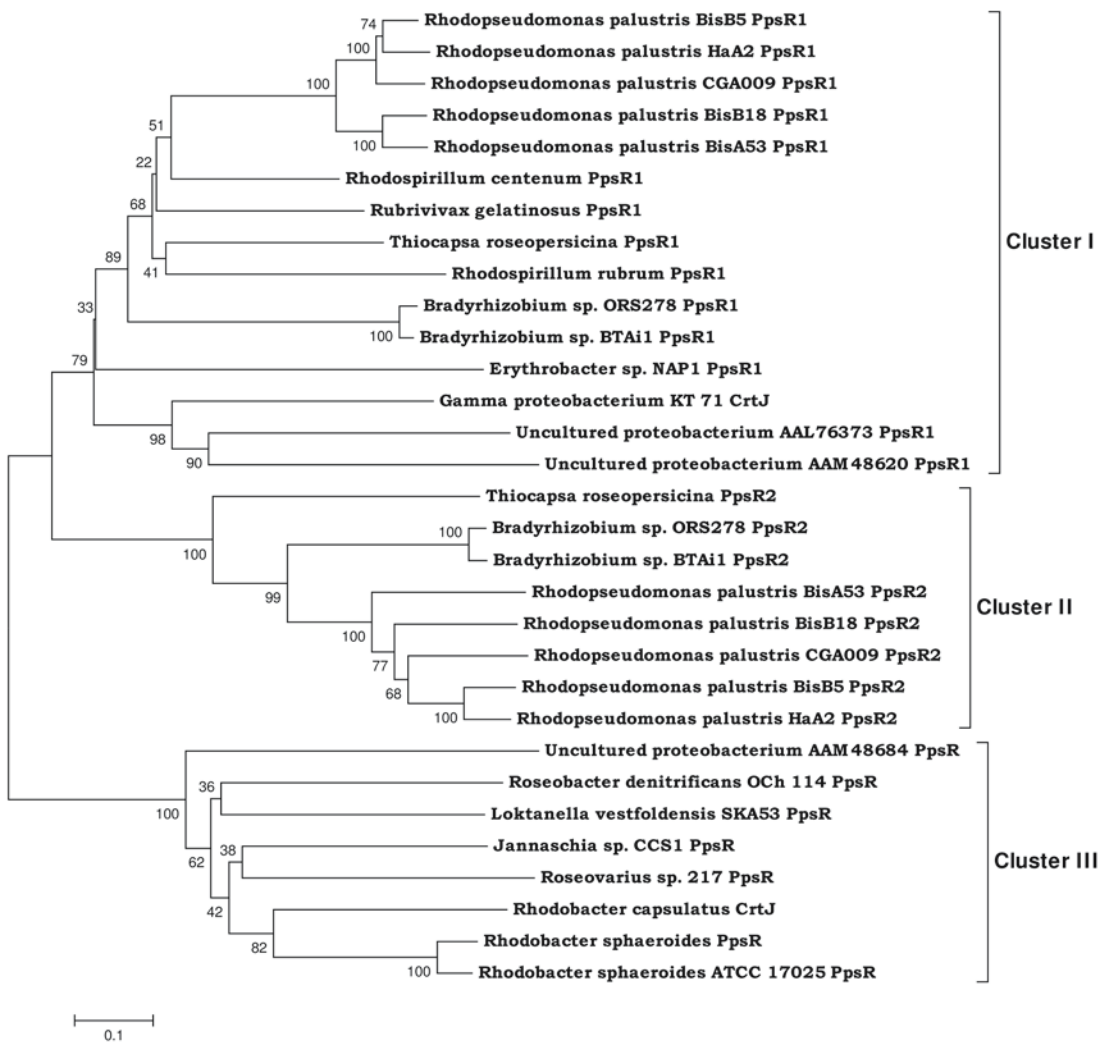

Fig. 3. Phylogenetic analysis of CrtJ/PpsR peptide sequences. Sequences were aligned using Neighbor-Joining method with 1000 replicates. For Cluster I peptide sequences, only half contain the C-terminal cysteine required for redox activity and do not contain the middle cysteine. Cluster II proteins do not contain either cysteine. Cluster III proteins contain both cysteines. Horizontal branch lengths represent relative evolutionary distances with the scale bar corresponding to 10 amino acid substitutions per 100. Bootstrap values (%) are indicated at each node except basal nodes.

such as in response to light as is the case of PpsR2 from *Bradyrhizobium*, and PpsR2 from *Rps. palustris* CGA009, which is regulated by a light responding phytochrome (Giraud et al., 2002, 2004; Braatsch et al., 2007). The presence of PAS domains also raises the possibility that proteins lacking Cys may bind a photoactive or redox active ligand though no evidence has been published as of yet for ligand binding to these domains.

IV. Regulation by Fnr

FnrL in *Rba. sphaeroides* and *Rba. capsulatus* is a homolog of the *E. coli* anaerobic regulatory protein, Fnr (Zeilstra-Ryalls and Kaplan, 1995). In *E. coli*, Fnr regulates *f*umarate and *n*itrate *r*eduction as well as a host of other cellular processes such as respiration (reviewed in Bauer et al., 1999). FnrL is a member of the Crp family of transcription factors that bind to a palindrome target sequence as a dimer. The amino terminal region of FnrL contains a ferredoxin-like cysteine cluster (Cys-X_3-Cys-X_2-Cys-X_5-Cys). For *E. coli* Fnr, it is known that an Fe-S center is formed with three of these clustered Cys, as well as with a fourth conserved Cys located ~100 amino acid residues away. Exposure of the FeS cluster to oxygen leads to disassembly of the cluster and subsequent loss of DNA binding activity of Fnr (Fig. 4). *Rba. capsulatus* and *Rba. sphaeroides* FnrL have not been isolated and biochemically characterized, but they do contain the same conserved Cys, so it is likely that they also form an oxygen sensitive Fe-S cluster not unlike that of *E. coli* Fnr. Fnr and FnrL also contain a highly conserved helix-turn-helix motif near the carboxyl terminus, so it is also likely that they bind to a similar DNA sequence of TTGAT-N_4-ATCAA (Zeilstra-Ryalls and Kaplan, 1998). In *Rba. sphaeroides* this sequence is found upstream of several tetrapyrrole and nitrogen regulatory genes such as *fnrL*, *hemA* (ALA synthase), *hemN* (coproporphyrinogen III oxidase), *hemZ* (coproporphyrinogen III oxidase), *bchE* (Mg^{2+}-protoporphyrin monomethyl esterase), *ccoNOQP* (*cbb_3* cytochrome oxidase), *rdxBHIS*/*ccoGHIS* (membrane-localized redox complex), *ctaD* (subunit of the *aa_3* cytochrome *c* terminal oxidase), *ctaABC* (subunit of the *aa_3* cytochrome *c* terminal oxidase), *cycP* (cytochrome *c′*), *dorS* (sensor protein of the *dor* operon), *pucBAC* (α and β structural polypeptides of LHII), *nnrR* (activator of *nir* and *nor*), *nirK* (nitrite reductase structural gene), and *norCBQD* (nitric oxide reductase operon)(Zeilstra-Ryalls et al., 1997; Mao et al., 2005). However, this sequence is found in only a few promoter regions in *Rba. capsulatus* such as *hemZ*, porphobilinogen synthase (*hemB*), *fnrL*, and *ccoNOQP* (Zeilstra-Ryalls et al., 1997; Swem and Bauer, 2002b; Smart et al., 2004; Choi et al., 2005; Chapter 39, Zeilstra-Ryalls).

A *fnrL* mutant of *Rba. sphaeroides* cannot grow photosynthetically (Mouncey and Kaplan, 1998). For cultures grown in 2% oxygen, the expression of *ccoN* is increased greatly in an *fnrL* knockout, yet under 30% oxygen growth conditions the expression is much reduced (Mouncey and Kaplan, 1998). FnrL has been shown to activate the expression of *cbb_3* cytochrome oxidase and ALA synthase under anaerobic conditions in *Rba. sphaeroides* (Mouncey and Kaplan, 1998; Zeilstra-Ryalls and Kaplan, 1995;

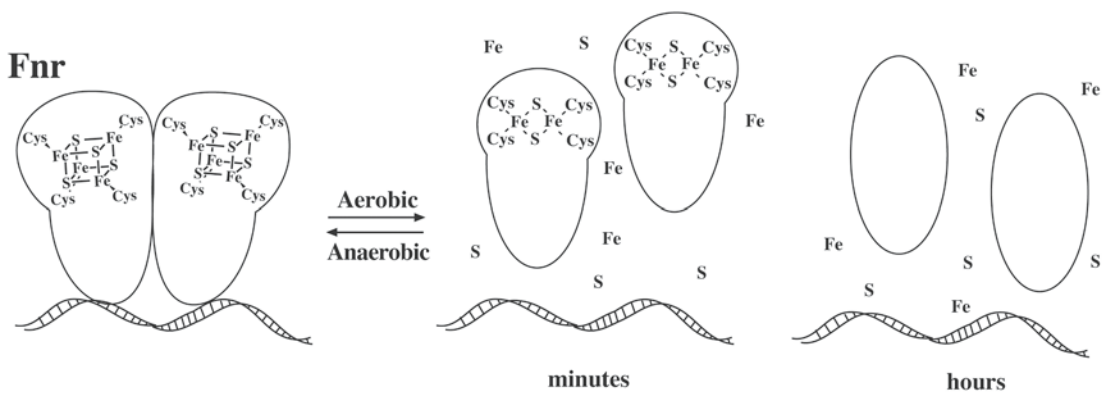

Fig. 4. Model of oxygen inactivation of FnrL activity. *E. coli* Fnr has a Fe-S center that is disrupted by molecular oxygen. Based on results from the Fnr homolog in *E. coli*, we propose that oxygen-dependent disruption of the Fe-S center in FnrL from *Rba. capsulatus* and *Rba. sphaeroides* leads to inhibition of DNA-binding activity.

Zeilstra-Ryalls and Kaplan, 1998).

A *fnrL* disruption in *Rba. capsulatus* is somewhat different in that loss of FnrL does not significantly affect the ability of these cells to grow photosynthetically (Swem and Bauer, 2002). Expression of ubiquinol oxidase in a *Rba. capsulatus fnrL* knockout was significantly increased when grown semiaerobically (Swem and Bauer, 2002). Expression studies of cbb_3 oxidase in an *fnrL* knockout strain showed a decrease in expression under anaerobic and semi-aerobic conditions (Swem and Bauer, 2002). In *Rubrivivax gelatinosus*, a null mutant of *fnrL* was unable to grow under anaerobic conditions; however photosynthetic complexes were produced under high oxygen levels. FnrL was shown to regulate the expression of *hemN* (oxygen dependent coproporphyrin III dehydrogenase), and *bchE* (Mg^{2+}-protoporphyrin monomethyl cyclase) in response to oxygen tension (Ouchane et al., 2007). Clearly much more research needs to be undertaken on this important regulator, to better understand its role in controlling the synthesis of the photosystem and respiration in response to alterations in oxygen tension.

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References

Armstrong GA, Schmidt A, Sandmann G and Hearst JE (1990) Genetic and biochemical characterization of carotenoid biosynthesis mutants of *Rhodobacter capsulatus.* J Biol Chem 265:8329–8338

Bauer CE, Young DA and Marrs BL (1988) Analysis of the *Rhodobacter capsulatus puf* operon: Location of the oxygen regulated promoter region and the identification of an additional *puf* encoded gene. J Biol Chem 263: 4820–4827

Bauer CE, Elsen S and Bird TH (1999) Mechanisms for redox control of gene expression. Ann Rev Microbiol 53: 495–523

Bauer E, Kaspar T, Fischer HM and Hennecke H (1998) Expression of the *fixR-nifA* operon in *Bradyrhizobium japonicum* depends on a new response regulator, RegR. J Bacteriol 180: 3853–3863

Bird TH, Du S and Bauer CE (1999) Autophosphorylation, phosphotransfer, and DNA-binding properties of the RegB/RegA two-component regulatory system in *Rhodobacter capsulatus.* J Biol Chem 274: 16343–16348

Bollivar DW, Suzuki JY, Beatty JT, Dobrowlski JD and Bauer CE (1994) Directed mutational analysis of bacteriochlorophyll *a* biosynthesis in *Rhodobacter capsulatus.* J Mol Biol 237: 622–640

Bolton JR (1978) Primary electron acceptor. In: Clayton RK and Sistrom WR (ed) The Photosynthetic Bacteria, pp 419–429. Plenum Press, New York

Bowman WC, Du S, Bauer CE and Kranz RG (1999) In vitro activation and repression of photosynthesis gene transcription in *Rhodobacter capsulatus*. Mol Microbiol 33: 429–437

Bratsch S, Gomelsky M, Kuphal S and Klug G (2002) A single flavoprotein, AppA, integrates both redox and light signals in *Rhodobacter sphaeroides*. Mol Microbiol 45: 827–836

Braatsch S, Bernstein JR, Lessner F, Morgan J, Liao JC, Harwood CS and Beatty JT (2006) *Rhodopseudomonas palustris* CGA009 has two functional *ppsR* genes, each of which encodes a repressor of photosynthesis gene expression. Biochemistry 45:14441–14451

Braatsch S, Johnson JA, Noll K and Beatty JT (2007) The O_2-responsive repressor PpsR2 but not PpsR1 transduces a light signal sensed by the BphP1 phytochrome in *Rhodopseudomonas palustris* CGA009. FEMS Microbiol Lett 272: 60–64

Buggy J and Bauer CE (1995). Cloning and characterization of *senC*, a gene involved in both aerobic respiration and photosynthesis gene expression in *Rhodobacter capsulatus.* J Bacteriol *177*, 6958–6965

Chen W, Jager A and Klug G (2000) Correction of the DNA sequence of the *regB* gene of *Rhodobacter capsulatus* with implications for the membrane topology of the sensor kinase RegB. J Bacteriol 182: 818–820

Cho SH, Youn SH, Lee SR, Yim HS and Kang SO (2004) Redox property and regulation of PpsR, a transcriptional repressor of photosystem gene expression in *Rhodobacter sphaeroides.* Microbio 150: 697–706

Cohen-Bazire G, Sistrom WR and Stanier RY (1957) Kinetic studies of pigment synthesis by nonsulfur purple bacteria. J Cellular Comp Physiol 49: 25–68

Comolli JC and Donohue TJ (2002) *Pseudomonas aeruginosa* RoxR, a response regulator related to *Rhodobacter sphaeroides* PrrA, activates expression of the cyanide-insensitive terminal oxidase Mol Micro 45: 755–768

Dong C, Elsen S, Swem LR and Bauer CE (2002) AerR, a second aerobic repressor of photosynthesis gene expression in *Rhodobacter capsulatus*. J Bacteriol 184: 2805–2814

Du S, Bird TH and Bauer CE (1998) DNA binding characteristics of RegA. A constitutively active anaerobic activator of photosynthesis gene expression in *Rhodobacter capsulatus*. J Biol Chem 273:18509–18513

Du S, Kouadio J-L K and Bauer CE (1999) Regulated expression of a highly conserved regulatory gene cluster is necessary for controlling photosynthesis gene expression in response to anaerobiosis in *Rhodobacter capsulatus*. J Bacteriol 181: 4334–4341

Elsen S, Ponnampalam SN and Bauer CE (1998) CrtJ bound to distant binding sites interacts cooperatively to aerobically repress photopigment biosynthesis and light harvesting II gene expression in *Rhodobacter capsulatus*. J Biol Chem 273: 30762–30769

Elsen S, Dischert W, Colbeau A and Bauer CE (2000) Expression of uptake hydrogenase and molybdenum nitrogenase in *Rhodobacter capsulatus* is coregulated by the RegB-RegA

two-component regulatory system. J Bact 182: 2831–2837
Elsen S, Swem LR, Swem DL and Bauer CE (2004) RegB/RegA, a highly conserved redox-responding global two-component regulatory system. Microbio Molec Biol Rev 68: 263–279
Elsen S, Jaubert M, Pignol D and Giraud E (2005) PpsR: A multifaceted regulator of photosynthesis gene expression in purple bacteria. Mol Microbiol 57: 17–26
Emmerich R, Panglungtshang K, Strehler P, Hennecke H and Fischer H-M (1999) Phosphorylation, dephosphorylation and DNA-binding of the *Bradyrhizobium japonicum* RegSR two-component regulatory proteins. Eur J Biochem 263: 455–463
Emmerich R, Strehler P, Hennecke H and Fischer H-M (2000a) An imperfect inverted repeat is critical for DNA binding of the response regulator RegR of *Bradyrhizobium japonicum*. Nuc Acids Res 28: 4166–4171
Emmerich R, Hennecke H and Fischer H-M (2000b) Evidence for a functional similarity between the two-component regulatory systems RegSR, ActSR and RegBA (PrrBA) in α-proteobacteria. Arch Microbiol 174:307–313
Eraso JM and Kaplan S (1994) prrA, a putative response regulator involved in oxygen regulation of photosynthesis gene expression in *Rhodobacter sphaeroides*. J Bacteriol 176: 32–43
Eraso JM and Kaplan S (1995) Oxygen-insensitive synthesis of the photosynthetic membranes of *Rhodobacter sphaeroides*: A mutant histidine kinase. J Bacteriol 177: 2695–2706
Eraso JM and Kaplan S (2000) From redox flow to gene regulation: Role of the PrrC protein of *Rhodobacter sphaeroides* 2.4.1. Biochem 39: 2052–2062
Giraud E, Fardoux J, Fourrier N, Hannibal L, Genty B, Bouyer P, Dreyfus B and Verméglio A (2002) Bacteriophytochrome controls photosystem synthesis in anoxygenic bacteria. Nature 417: 202–205
Giraud E, Zappa S, Jaubert M, Hannibal L, Fardoux J, Adriano JM, Bouyer P, Genty B, Pignol D and Verméglio A (2004) Bacteriophytochrome and regulation of the synthesis of the photosynthetic apparatus in *Rhodopseudomonas palustris*: Pitfalls of using laboratory strains. Photochem Photobiol Sci 3: 587–591
Gomelsky M and Kaplan S (1995a) Genetic evidence that PpsR from *Rhodobacter sphaeroides* 2.4.1 functions as a repressor of *puc* and *bchF* expression. J Bacteriol 177: 1634–1637
Gomelsky M and Kaplan S (1995b). *appA*, a novel gene encoding a trans-acting factor involved in the regulation of photosynthesis gene expression in *Rhodobacter sphaeroides.* J Bacteriol 177: 4609–4618
Gomelsky M and Klug G (2002) BLUF: A novel FAD-binding domain involved in sensory transduction in microorganisms. Trends Biochem. Sci. 27: 497–500
Gomelsky M, Horne IM, Lee HJ, Pemberton JM, McEwan AG, Kaplan S (2000) Domain structure, oligomeric state, and mutational analysis of PpsR, the *Rhodobacter sphaeroides* repressor of photosystem gene expression. J Bacteriol 182: 2253–2261
Han Y, Meyer MH, Keusgen M, Klug G (2007) A haem cofactor is required for redox and light signalling by the AppA protein of *Rhodobacter sphaeroides*. Mol Microbiol 64:1090–1104
Hemschemeier SK, Ebel U, Jager A, Balzer A, Kirndorfer M and Klug G (2000) In vivo and in vitro analysis of RegA response regulator mutants of *Rhodobacter capsulatus.* J Mol Microbiol Biotech 2: 291–300
Inoue K, Mosley C, Kouadio J-L and Bauer C (1995) Isolation and in vitro phosphorylation of sensory transduction components controlling anaerobic induction of light harvesting and reaction center gene expression in *R. capsulatus.* Biochemistry 34: 391–396
Jaubert M, Zappa S, Fardoux J, Adriano J M, Hannibal L, Elsen S, Lavergne J, Verméglio A, Giraud E and Pignol D (2004) Light and redox control of photosynthesis gene expression in *Bradyrhizobium*: Dual roles of two PpsR. J Biol Chem 279: 44407–44416
Jones DF, Stenzel RA and Donohue TJ (2005) Mutational analysis of the C-terminal domain of the *Rhodobacter sphaeroides* response regulator PrrA. Microbiol 151: 4103–4110
Joshi HM and Tabita FR (1996) A global two component signal transduction system that integrates the control of photosynthesis, carbon dioxide assimilation, and nitrogen fixation. Proc Natl Acad Sci USA 93: 14515–14520
Kaplan S, Eraso J and Roh JH (2005) Interacting regulatory networks in the facultative photosynthetic bacterium, *Rhodobacter sphaeroides* 2.4.1. Biochem Soc Trans 33: 51–55
Kim, SK, Mason, JT, Knaff, DB, Bauer, CE and Setterdahl, AT (2006) Redox properties of the *Rhodobacter sphaeroides* transcriptional regulatory proteins PpsR and AppA. Photosynth Res 89: 89–98
Kovacs AT, Rakhely G and Kovacs KL (2005) The PpsR regulator family. Res Microbiol 156: 619–625
Laguri C, Phillips-Jones MK and Williamson MP (2003) Solution structure and DNA binding of the effector domain from the global regulator PrrA (RegA) from *Rhodobacter sphaeroides*: Insights into DNA binding specificity. Nucl Acids Res 31: 6778–6787
Laguri C, Stenzel RA, Donohue TJ, Phillips-Jones MK and Williamson MP (2006) Activation of the global gene regulator PrrA (RegA) from *Rhodobacter sphaeroides.* Biochem 45: 7872–7881
Laratta WP, Choi PS, Tosques IE and Shapleigh JP (2002) Involvement of the PrrB/PrrA two-component system in nitrite respiration in *Rhodobacter sphaeroides* 2.4.1: Evidence for transcriptional regulation. J Bact 184: 3521–3529
Madigan MT (1995) The microbiology of nitrogen fixation by anoxygenic photosynthetic bacteria, In: Blankenship RE, Madigan MT and Bauer CE (ed) Anoxygenic Photosynthetic Bacteria (Advances in Photosynthesis and Respiration, Vol 2), pp 915–928. Kluwer Academic Publishing, Dordrecht
Madigan MT and Gest H (1979) Growth of the photosynthetic bacterium *Rhodopseudomonas capsulata* chemoautotrophically in darkness with H_2 as the energy source. J Bacteriol 137: 524–530
Mao L, Mackenzie C, Roh JH, Eraso JM, Kaplan S and Resat H (2005) Combining microarray and genomic data to predict DNA binding motifs. Microbiology 151: 3197–3213
Masuda S and Bauer CE (2002) AppA is a blue light photoreceptor that antirepresses photosynthesis gene expression in *Rhodobacter sphaeroides.* Cell 110: 613–623
Masuda S and Bauer CE (2005) The antirepressor AppA uses the novel flavin-binding BLUF domain as blue-light-absorbing photoreceptor to control photosystem synthesis. In: Briggs W and Spudich J (eds) Handbook of Photosensory Receptors, pp 433–446. Wiley-VCH publishing, Weinheim
Masuda S, Matsumoto Y, Nagashima KVP, Shimada K, Inoue K, Bauer CE and Matsuura K (1999) Structural and functional

analyses of photosynthetic regulatory genes *regA* and *regB* from *Rhodovulum sulfidophilum, Roseobacter denitrificans,* and *Rhodobacter capsulatus*. J Bacteriol 181: 4205–4215

Masuda S, Dong C, Swem D, Setterdahl AT, Knaff DB and Bauer CE (2002) Repression of photosynthesis gene expression by formation of a disulfide bond in CrtJ. Proc Natl Acad Sci USA 99: 7078–7083

McCleary WR and Stocks JB (1994) Acetyl phosphate and the activation of two-component response regulators. J Biol Chem 269: 31567–31572

Moskvin OV, Gomelsky L and Gomelsky M (2005) Transcriptome analysis of the *Rhodobacter sphaeroides* PpsR regulon: PpsR as a master regulator of photosystem development J Bacteriol 187: 2148–2156

Moskvin OV, Kaplan S, Gilles-Gonzalez MA and Gomelsky M (2007) Novel heme-based oxygen sensor with a revealing evolutionary history. J Biol Chem 282: 28740–28748

Mosley CS, Suzuki JY and Bauer CE (1994) Identification and molecular genetic characterization of a sensor kinase responsible for coordinately regulating light harvesting and reaction center gene expression in response to anaerobiosis. J Bacteriol 176: 7566–7573

Mouncey N J and Kaplan S (1998) Oxygen regulation of the *ccoN* gene encoding a component of the cbb_3 oxidase in *Rhodobacter sphaeroides* 2.4.1: Involvement of the FnrL protein. J Bacteriol 180: 2228–2231

Nowak E, Panjikar S, Konarev P, Svergun DI and Tucker PA (2006) The structural basis of signal transduction for the response regulator PrrA from *Mycobacterium tuberculosis.* J Biol Chem 281: 9659–9666

O'Gara JP and Kaplan S (1997) Evidence for the role of redox carriers in photosynthesis gene expression and carotenoid biosynthesis in *Rhodobacter sphaeroides.* 2.4.1. J Bacteriol 179: 1951–1961

Oh JI and Kaplan S (2000) Redox signaling: Globalization of gene expression EMBO J 19: 4237–4247

Ouchane S and Kaplan S (1999) Topological analysis of the membrane-localized redox-responsive sensor kinase PrrB from *Rhodobacter sphaeroides* 2.4.1. J Biol Chem 274: 17290–17296

Ouchane S, Picaud M, Therizols P, Reiss-Husson F and Astier C (2007) Global Regulation of Photosynthesis and Respiration by FnrL: The first two targets in the tetrapyrrole pathway. J Biol Chem 282: 7690–7699

Parkinson JS and Kofoid EC (1992) Communication modules in bacterial signaling proteins. Ann Rev Genet 26: 71–112

Parson W (1978) Quinones as secondary electron acceptor, In: RK Clayton and Sistrom WR (ed) The Photosynthetic Bacteria, pp 455–469. Plenum Press, New York

Penfold RJ and Pemberton JM (1994) Sequencing, chromosomal inactivation, and functional expression in *Escherichia coli* of *ppsR*, a gene which represses carotenoid and bacteriochlorophyll synthesis in *Rhodobacter sphaeroides.* J Bacteriol 176: 2869–2876

Pfenning N (1978) General physiology and ecology of photosynthetic bacteria, In: RK Clayton and Sistrom WR (ed) The Photosynthetic Bacteria, pp 1–18. Plenum Press, New York

Phillips-Jones MK and Hunter CN (1994) Cloning and nucleotide sequencing of RegA, a putative response regulator gene of *Rhodobacter sphaeroides*. FEMS Microbiol Lett 116: 269–275

Ponnampalam SN and Bauer CE (1997) DNA binding characteristics of CrtJ A redox-responding repressor of bacteriochlorophyll, carotenoid, and light harvesting-II gene expression in *Rhodobacter capsulatus*. J Biol Chem 272: 18391–18396

Ponnampalam SN, Buggy JJ, Bauer CE (1995) Characterization of an aerobic repressor that coordinately regulates bacteriochlorophyll, carotenoid, and light harvesting-II expression in *Rhodobacter capsulatus.* J Bacteriol 177: 2990–2997

Ponnampalam SN, Elsen S and Bauer CE (1998) Aerobic repression of the *Rhodobacter capsulatus bchC* promoter involves cooperative interactions between CrtJ bound to neighboring palindromes. J Biol Chem 273: 30757–30761

Potter CA, Ward A, Laguri C, Williamson MP, Henderson PJ and Phillips-Jones MK (2002) Expression, purification and characterization of full-length histidine protein kinase RegB from *Rhodobacter sphaeroides.* J Mol Biol 320: 201–213

Qian, Y and Tabita FR (1996) A global signal transduction system regulates aerobic and anaerobic CO_2 fixation in *Rhodobacter sphaeroides.* J Bact 178: 12–18

Roh JH and Kaplan S (2000) Genetic and phenotypic analyses of the *rdx* locus of *Rhodobacter sphaeroides* 2.4.1. J Bacteriol 182: 3475–3481

Sganga MW and Bauer CE (1992) Regulatory factors controlling photosynthetic reaction center and light-harvesting gene expression in *Rhodobacter capsulatus*. Cell 68: 945–954

Smart JL, Willett JW and Bauer CE (2004) Regulation of *hem* gene expression in *Rhodobacter capsulatus* by redox and photosystem regulators RegA, CrtJ, FnrL, and AerR. J Mol Biol 342: 1171–1186

Steunou AS, Astier C and Ouchane S (2004) Regulation of photosynthesis genes in *Rubrivivax gelatinosus*: Transcription factor PpsR is involved in both negative and positive control. J Bacteriol 186: 3133–3142

Swem DL and Bauer CE (2002) Coordination of ubiquinol oxidase and cytochrome cbb_3 oxidase expression by multiple regulators in *Rhodobacter capsulatus.* J Bacteriol 184: 2815–2820

Swem LR, Elsen S, Bird TH, Swem DL, Koch HG, Myllykallio H, Daldal F and Bauer CE (2001) The RegB/RegA two-component regulatory system controls synthesis of photosynthesis and respiratory electron transfer components in *Rhodobacter capsulatus*. J Mol Biol 309:121–138

Swem LR, Kraft BJ, Swem DL, Setterdahl AT, Masuda S, Knaff DB, Zaleski JM and Bauer CE (2003) Signal transduction by the global regulator RegB is mediated by a redox-active cysteine. EMBO J 22: 4699–4708

Swem LR, Gong X, Yu CA and Bauer CE (2006) Identification of a ubiquinone-binding site that affects autophosphorylation of the sensor kinase RegB. J Biol Chem 281: 6768–6775

Tabita FR (1995) The biochemistry and metabolic regulation of carbon metabolism and CO_2 fixation in purple bacteria. In: Blankenship RE, Madigan MT and Bauer CE (ed) Anoxygenic Photosynthetic Bacteria (Advances in Photosynthesis and Respiration, Vol 2), pp 885–914. Kluwer Academic Publishing, Dordrecht

Tiwari RP, Reeve WG, Dilworth MJ and Glenn AR (1996) Acid tolerance in *Rhizobium meliloti* strain WSM419 involves a two-component sensor-regulator system Microbiol 142: 1693–1704

Vichivanives P, Bird TH, Bauer CE and Tabita FR (2000) Multiple regulators and their interactions in vivo and in vitro with the *cbb* regulons of *Rhodobacter capsulatus*. J Mol Biol 300: 1079–1099

Willett J, Smart JL and Bauer CE (2007) RegA controls synthesis of bacteriochlorophyll and carotenoid photopigments in *Rhodobacter capsulatus*. J Bacteriol, In Press

Zeilstra-Ryalls JH, Gabbert K, Mouncey NJ, Kaplan S and Kranz RG (1997) Analysis of the *fnrL* gene and its function in *Rhodobacter capsulatus.* J Bacteriol 179: 7264–7273

Zeilstra-Ryalls JH and Kaplan S (1995) Aerobic and anaerobic regulation in *Rhodobacter sphaeroides* 2.4.1: The role of the *fnrL* gene. J Bacteriol 177: 6422–6431

Zeilstra-Ryalls JH and Kaplan S (1998) Role of the *fnrL* gene in photosystem gene expression and photosynthetic growth of *Rhodobacter sphaeroides* 2.4.1. J Bacteriol 180: 1496–1503