RegB/RegA, A Global Redox-Responding Two-Component System

ARTICLE *in* ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY · FEBRUARY 2008

Impact Factor: 1.96 · DOI: 10.1007/978-0-387-78885-2_9 · Source: PubMed

2 AUTHORS, INCLUDING:

Carl E Bauer
Indiana University Blooming…

133 PUBLICATIONS **6,110** CITATIONS

SEE PROFILE

CHAPTER 9

RegB/RegA, A Global Redox-Responding Two-Component System

Jiang Wu and Carl E. Bauer*

Abstract

The RegB-RegA regulon from *Rhodobacter capsulatus* and *Rhodobacter sphaeroides* encodes proteins involved in numerous energy-generating and energy-utilizing processes such as photosynthesis, carbon fixation, nitrogen fixation, hydrogen utilization, aerobic and anaerobic respiration, denitrification, electron transport and aerotaxis. The redox signal that is detected by the membrane-bound sensor kinase, RegB, has been identified to be the ubiquinone pool in the membrane. Regulation of RegB autophosphorylation also involves a redox-active cysteine that is present in the cytosolic region of RegB. Both phosphorylated and unphosphorylated forms of the cognate response regulator RegA are capable of activating or repressing a variety of genes in the regulon. Highly conserved homologues of RegB and RegA have been found in a wide number of photosynthetic and nonphotosynthetic bacteria with evidence suggesting that RegB/RegA have a fundamental role in the transcription of redox-regulated genes in many bacterial species.

Introduction

Genetic screens initially identified RegB and RegA as regulators of the photosystem synthesis in *R. capsulatus*.[1,2] In this species, null mutations in *regB* and *regA* are defective in high-level expression of the photosystem which are normally only synthesized under conditions of low oxygen tension.[3] Expression of the *puh*, *puf* and *puc* operons that encode apoproteins for the light harvesting I, light harvesting II and reaction center complexes of the photosystem are significantly reduced in RegB and RegA mutants.[1,2] The similar phenotypes displayed by these mutants led to the hypothesis that they may be cognate *trans*-acting partners constituting a two-component regulatory system. This was confirmed by sequence analysis which demonstrated that RegB exhibits homology to histidine protein kinases[1,4-6] and RegA exhibits homology to DNA-binding response regulators.[2,4-6]

Subsequent to the discovery of RegB/RegA from *R. capsulatus*, homologous two-component regulatory systems were found and genetically characterized in many other species such as the RegB/RegA[7] homologs in *Rhodobacter sphaeroides* (also called PrrB/PrrA),[8,9] RegS/RegR system from *Bradyrhizobium japonicum*,[10] ActS/ActR from *Sinorhizobium meliloti*,[11] RoxS/RoxR from *Pseudomonas aeruginosa*[12] and RegB/RegA from *Rhodovulum sulfidophilum* and *Roseobacter denitrificans*[13] (Table 1). Genome sequence studies have also identified RegA and RegB homologues in many other photosynthetic as well as nonphotosynthetic α- and γ-proteobacterial species (Table 1).

Genetic shuttling studies have demonstrated that RegB and RegA homologues from different species are in vitro and in vivo interchangeable, which means phosphotransfer can be observed between different RegB and RegA homologues,[12,14] and that some RegA homologues can bind to promoters and regulate gene transcription in another species.[12,14] Indeed it is now well established

*Corresponding Author: Carl E. Bauer—Department of Biology, Indiana University, Myers Hall, 915 E. Third St., Bloomington, IN 47405-7170, USA. Email: bauer@indiana.edu

Bacterial Signal Transduction: Networks and Drug Targets, edited by Ryutaro Utsumi.

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

	RegB					RegA			
Organism	**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
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
Jannaschia sp.	YES Q28JY5	51%	YES	YES	YES	YES Q28JX7	81%	YES	YES
uncultured proteobacterium	YES Q8KYV6	53%	YES	YES	YES	YES Q8KYV8	78%	YES	YES
Rhizobium loti	YES Q98C40	38%	YES	YES	YES	YES Q98C39	69%	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
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	NO[1]			
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

continued on next page

Table 1. Continued

	RegB					RegA			
Organism	**RegB**	**Identity**	**H-Box**	**Q-Binding Site**	**Redox-Active Cystein**	**RegA**	**Identity**	**Acid-Box**	**DNA-Binding Domain**
Agrobacterium tumefaciens	YES Q8UJ81	36%	YES	YES	YES	YES Q8UJ82	68%	YES	YES
Nitrosospira multiformis	YES Q2YD54	27%	YES	YES	YES	YES Q2YD55	59%	YES	YES
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
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
Pseudomonas entomophila	YES Q1IEE3	29%	YES	YES	YES	YES Q1IEE2	50%	YES	YES
Sphingopyxis alaskensis	*YES Q1GT06*	*26%*	*YES*	*YES*	*YES*	*YES Q1GT07*	*45%*	*YES*	*YES*
Sphingomonas sp.	YES Q1N6Y2	24%	YES	YES	YES	NO1			
Paracoccus denitrificans	NO[2]					YES Q3PEU7	77%	YES	YES
Uncultured Acidobacteria bacterium	NO[2]					YES Q7X351	66%	YES	YES
Pelagibacter ubique	NO[2]					YES Q4FP64	67%	YES	YES
Psychroflexus torquis	NO					YES Q1VJU0	65%	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
Anaeromyxobacter dehalogenans	NO[2]					ES Q2II97	47%	YES	YES

continued on next page

Table 1. Continued

	RegB					RegA			
Organism	**RegB**	**Identity**	**H-Box**	**Q-Binding Site**	**Redox-Active Cystein**	**RegA**	**Identity**	**Acid-Box**	**DNA-Binding Domain**
Anaeromyxobacter dehalogenans	NO[2]					YES Q2II97	47%	YES	YES
Ralstonia metallidurans	NO[2]					YES Q1LS55	46%	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
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
Dechloromonas aromatica	NO[2]					YES Q47FP7	44%	YES	YES
Hahella chejuensis	NO[2]					YES Q2SM70	42%	YES	YES

continued on next page

Table 1. Continued

	RegB					RegA			
Organism	**RegB**	**Identity**	**H-Box**	**Q-Binding Site**	**Redox-Active Cystein**	**RegA**	**Identity**	**Acid-Box**	**DNA-Binding Domain**
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 homologues to RegA were found in Blast 2complete database. [2]No homologues to RegB were found in Blast2 complete database.

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. Specifically, energy generating and energy utilizing metabolic and bioenergetic processes such as photosynthesis, tetrapyrrole synthesis, carbon fixation, nitrogen fixation, hydrogen oxidation, denitrification, aerobic/anaerobic respiration and electron transport are known members of the RegB/RegA regulon in *R. capsulatus* (Fig. 1).

Members of the Reg Regulon

Photosynthesis

The RegB/RegA system was initially discovered by selecting for mutations that exhibited reduced synthesis of the photosystem in *R. capsulatus*.[1,2] An intact copy of *regA* was subsequently shown to be required for *R. capsulatus* to grow photosynthetically under dim light.[2] As is the case for RegA, RegB is also necessary for anaerobic synthesis of the photosystem. Gene expression studies have indicated that expression of the *puc, puf* and *puh* operons that code for apoproteins of the light harvesting and reaction center complexes are significantly reduced when either RegB or RegA are disrupted.[1,2] Mutations in RegB and RegA homologues from *R. sphaeroides* also show similar effects with respect to the control of *puh, puf* and *puc* expression as was reported for *R. capsulatus*.[8]

In addition to controlling synthesis of light harvesting and reaction center apoproteins, RegA also affects tetrapyrrole synthesis whose branched pathways produce compounds such as bacteriochlorophyll and heme that are bound by photosystem and cytochrome apoproteins, respectively. For example, expression of the *bchE* in *R. sphaeroides* that encodes an enzyme in the bacteriochlorophyll biosynthesis pathway was reported to be regulated by PrrB/PrrA system.[15] There are also reports that RegA from *R. sphaeroides* controls expression of *hemA, hemZ* and *hemN*[15,16] that code for enzymes involved in the common branch used by both the heme and bacteriochlorophyll biosynthetic pathways. In *R. capsulatus* it has been demonstrated that *hemA, hemC, hemE, hemH* and *hemZ*, are also components of the RegB/RegA regulon.[17] Putative RegA-binding sites were also revealed in the promoter region of these *hem* genes and two PrrA-binding sites have been identified upstream of *hemA* in *R. sphaeroides*,[18] demonstrating that RegA directly regulates *hem* gene expression.[17]

Electron Transfer System

The RegA homologue from *R. sphaeroides* (PrrA) was found to positively regulate the expression of *cycA*, that encodes cytochrome c_2.[8] In this species, cytochrome c_2 shuttles electrons from the cytochrome bc_1 complex to the photosystem reaction center as well as to respiratory component cytochrome oxidase. Primer extension and in vitro transcription studies indicated that PrrA directly activates *cycA* transcription.[19] Evidence that RegA directly controls *cycA* expression was provided by DNase I protection assays which showed that RegA* (constitutively active variant of RegA) from *R. capsulatus* binds to a region of the *cycA* P2 promoter centered -50 bp from the start site of transcription.[20]

Swem et al[21] demonstrated that RegB/RegA controls synthesis of cytochrome c_2 as well as cytochrome c_y and the cytochrome bc_1 complex in *R. capsulatus*. It was shown that RegA activates biosynthesis of cytochromes bc_1 and c_2 under anaerobic, semi-aerobic and aerobic growth conditions, whereas it only activates cytochrome c_y under semi-aerobic and anaerobic conditions. DNase I protection assays also demonstrated that RegA binds to 2 sites on the promoter of the *pet* (bc_1) operon and to 4 sites on the promoters of the *cycA* and *cycY* genes encoding cytochrome c_2 and cytochrome c_y, respectively.[21]

Like many bacterial species, *R. capsulatus* possesses a branched respiratory chain involving two different terminal oxidases. In one branch, the ubiquinol (ubihydroquinone) oxidase takes electrons directly from the quinone pool to reduce O_2 to H_2O. The second branch, which is similar to the mitochondrial electron transfer chain, is comprised of the cytochrome bc_1 complex, cytochromes c_2 or c_y and a cbb_3-type cytochrome *c* oxidase.[22] RegA has been observed to activate cytochrome cbb_3 oxidase expression semi-aerobically and aerobically while repressing expression anaerobically.[21]

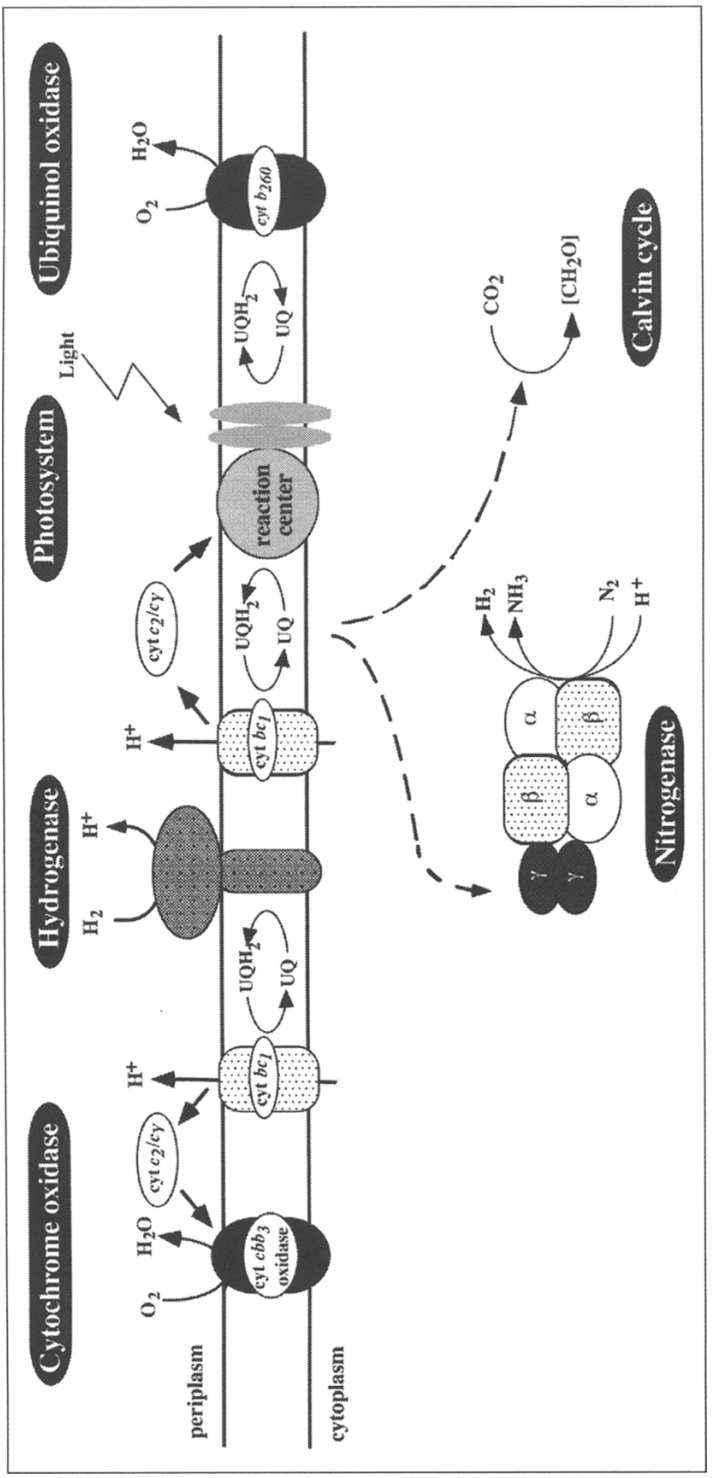

Figure 1. A diagram of various RegB/RegA controlled systems that have been identified in *Rhodobacter capsulatus*.

DNase I footprint analysis revealed that RegA directly controls synthesis of cytochrome cbb_3 oxidase by binding to a site on the *ccoNOQP* promoter located just upstream from the -35 sequence.[21]

As observed for *ccoNOQP* operon, RegB/RegA is also involved in the regulation of *cydAB* operon encoding ubiquinol oxidase, with RegA required for activation of *cydAB* transcription under all growth conditions tested. DNase I footprint assays indicate that RegA binds to two sites upstream from the -35 region of the promoter.[21]

Recently RegB/RegA homologues from *P. aeruginosa* (RoxS/RoxR) were reported to be involved in the control of aerobic respiration in this species.[12] More precisely, RoxS/RoxR controls the induction of the cyanide-insensitive oxidase, which is the terminal oxidase of one aerobic electron transport pathway, in the presence of cyanide. It is proposed that RoxR coregulates the *cioAB* promoter with another anaerobic regulator, ANR, thereby permitting the integration of different stimuli in the control of cyanide-insensitive oxidase expression.

R. capsulatus and *R. sphaeroides* are also both capable of anaerobic respiration using dimethylsulfoxide (DMSO) as a terminal electron acceptor.[23] The reduction of DMSO is catalyzed by a membrane-bound DMSO reductase enzyme that is encoded by the *dorCDA* operon. The *dor* operon is under the transcription control of a two-component signal transduction system, DorS/DorR, that responds to the availability of DMSO.[24-26] The sensor kinase, DorS is known to autophosphorylate in the presence of DMSO with the phosphate transferred to the response regulator, DorR, which then activates *dorCDA* expression. In addition to DorS/DorR, the *dorCDA* operon is also regulated by the RegB/RegA system with RegA acting as a repressor of the *dorCDA* operon during photoheterotrophic growth in the presence of malate as a carbon source.[27] However, RegA seems to lose control of the *dorCDA* operon if the cells are grown on pyruvate rather than malate. This indicates that another unidentified regulator can suppress the *regA* mutant phenotype in cells grown on pyruvate but not in cells grown on malate.

In addition to regulating synthesis of a larger number of cytochrome apoproteins, RegA also controls synthesis of the cytochrome cofactor heme. Smart et al[17] demonstrated that expression of *hemA, hemC, hemE, hemZ* and *hemH* genes that code for enzymes in the common heme/bacteriochlorophyll branch of the tetrapyrrole pathway are part of the RegB-RegA regulon. Thus, RegB-RegA are involved in regulating the stoicheometry of heme biosynthesis with synthesis of cytochrome apoproteins that bind this cofactor.

Carbon Fixation

The Calvin-Benson-Bassham (CBB) reductive pentose phosphate pathway allows production of organic carbon via the assimilation of CO_2. Enzymes of the Calvin cycle are encoded by the cbb_I and cbb_{II} operons. Transcription of these operons is regulated in response to carbon by the transcriptional activator, CbbR.[28,29]

An involvement of the RegB/RegA system in the biosynthesis of Calvin cycle enzymes was first discovered in *R. sphaeroides* where it was demonstrated that RegB (PrrB) was required for positive regulation of the *cbb* operons, both anaerobically in the light and aerobically in the dark.[32] Using purified *R. capsulatus* RegA*, Dubbs et al[28,30] demonstrated that RegA directly controls *R. sphaeroides cbb* expression by binding to four sites in the cbb_I promoter and to six sites on the cbb_{II} promoter. The authors hypothesized that the locations of RegA binding could allow direct interactions with CbbR and/or with RNA polymerase. Furthermore, binding of RegA to the two sites located in the upstream activating sequence (UAS) in the cbb_I promoter appears responsible for a RegA-mediated 41-fold enhancement in cbb_I expression.[28]

Gibson et al[31] demonstrated that chemoautotrophically grown *regA* (*prrA*) mutants of *R. sphaeroides* differentially express the two *cbb* operons with expression of the cbb_{II} promoter severely reduced and expression of the cbb_I promoter enhanced in the *prrA* mutant strain. This result indicates that PrrA functions as an activator of cbb_{II} and a repressor of cbb_I. Analysis of promoter mutants suggests that RegA may bind to distinct regions in cbb_{II} and in cbb_I during photoautotrophic and chemoautotrophic growth.

In *R. capsulatus*, the RegB/RegA system also controls expression of the two *cbb* operons that are present in this species.[32] Inactivation of *regA* and *regB* affects cbb_I and cbb_{II} expression with only 14 and 10% of wild-type levels, respectively, in a *regA*-disrupted strain under photoautotrophic growth condition. RegA* was also shown to bind to two DNA-binding sites in both the cbb_I and cbb_{II} promoter regions. There is a major high affinity RegA binding upstream of the cbb_I transcription start site that is assumed to be involved in transcriptional activation in concert with $CbbR_I$. A low affinity RegA binding site overlapping a $CbbR_I$ DNA-binding site is proposed to has a negative role caused by RegA mediated occlusion of $CbbR_I$ binding to this region. On the cbb_{II} promoter, there are 2 high affinity RegA binding sites. The upstream location of these binding sites suggests that they are involved in activation.

It has been reported that RegA homologues from *B. japonicum* (RegR) and from *S. meliloti* (ActR), also function as activators of *cbb* operons in concert with CbbR.[33,34] Thus, as was demonstrated for *R. capsulatus*, RegA homologues appear to control CO_2 fixation in number of photosynthetic and nonphotosynthetic bacteria.

Nitrogen Fixation

Conditions of nitrogen and oxygen limitation are known to activate expression of *nif* genes that are required for biosynthesis of molybdenum nitrogenase (reviewed in ref. 35). Joshi and Tabita[36] observed that nitrogenase synthesis is de-repressed in the presence of excess ammonium in *R. sphaeroides* strains that lacked a functional CO_2 fixation pathway. They proposed that nitrogenase becomes de-repressed to serve as an alternative secondary electron sink in the absence of CO_2 fixation. Interestingly, a functional *regB* gene is required for de-repression of nitrogenase in the absence of carbon fixation, suggesting that RegB/RegA system is involved in the control of nitrogen fixation.

Elsen et al[37] shed light on the mechanism of de-repression of nitrogenase in *R. capsulatus* by showing that the RegB/RegA system indirectly controls expression of the *nifHDK* operon that encodes the molybdenum-containing nitrogenase complex. In *R. capsulatus* and in many other species, nitrogenase expression is regulated by nitrogen limitation through the NtrB/NtrC two-component system. Under nitrogen limiting conditions NtrB phosphorylates NtrC which then activates *nifA* transcription. NifA then activates expression of numerous *nif* genes including *nifHKD* (reviewed in ref. 35). In *R. capsulatus*, there are two functional copies of *nifA*, *nifA1* and *nifA2*, either of which can activate *nifHDK* expression. Elsen et al[37] demonstrated that RegA binds to the *nifA2* promoter and activate the transcription. Interestingly, RegA-mediated activation of *nifA2* transcription requires NtrC thereby indicating that RegA~P (phosphorylated RegA) alone is not sufficient to stimulate *nifA2* expression.[37] Thus, RegA appears to provide an overarching layer of redox control on top of the control of nitrogen availability that is provided by NtrC.

In *B. japonicum*, the RegB/RegA homologues (RegS/RegR) are required for the aerobic and anaerobic expression of the *fixRnifA* operon.[10] Interestingly a mutation that disrupts the response regulator RegR reduces *fixRnifA* expression and consequently nitrogen fixation activity. However, no related phenotype was observed upon disruption of the sensor kinase, RegS. RegR mutants of *B. japonicum* form nodules but the nodules are functionally incapable of fixing nitrogen (a *fix*⁻ phenotype).

Denitrification

Recently, the *R. sphaeroides* RegB/RegA system (PrrB/PrrA) was shown to control expression of nitrite reductase that is a terminal electron acceptor involved in denitrification.[38] Specifically, *regB* and *regA* disrupted strains reduced expression of the nitrite reductase structural gene, *nirK* which resulted in the inability to grow anaerobically on nitrite-containing medium. *nir* expression is also regulated by nitrite availability through the transcription factor NnrR. Thus, RegA presumably acts in concert with NnrR, to coordinate *nirK* expression.

Hydrogen Oxidation

R. capsulatus possesses the *hupSLC* operon that codes for a membrane-bound uptake [NiFe]hydrogenase that catalyses H_2 oxidation. This enzyme allows the bacterium to grow autotrophically with H_2 as the sole electron source (reviewed in ref. 39).

H_2 regulation is mediated by the two-component regulatory system HupT/HupR with the response regulator HupR directly activating *hupSLC* transcription in the presence of H_2.[40] Maximal expression of *hupSLC* also requires the binding of IHF between the HupR and the RNA polymerase DNA-binding sites.[41]

Elsen et al[37] demonstrated that RegA is involved in repressing *hupSLC* expression under both aerobic and anaerobic heterotrophic growth conditions. A major DNA-binding site of RegA was shown to be located close to the −35 promoter recognition sequence with a second lower affinity RegA binding site overlapping the IHF DNA-binding region. At that location it is possible that RegA could prevent either the RNA polymerase, or the IHF protein, or both, from binding to the *hupSLC* promoter.

Dehydrogenases

Glutathione-dependent formaldehyde dehydrogenase serves an important role in the detoxification of formaldehyde by conversion to formate. Analysis of expression of the glutathione-dependent formaldehyde dehydrogenase gene, *adhI*, demonstrated that *adhI* expression is under control of several effectors that respond to formaldehyde, methanol, or other formaldehyde adduct.[42] This enzyme is absolutely required for growth with carbon sources such as methanol that generates formaldehyde. Formaldehyde oxidation creates reducing power in the form of NADH thereby providing cellular energy as a product. Interestingly, in *R. sphaeroides* RegA (PrrA) was shown to be essential for normal aerobic expression of the *adhI* gene.[42] Analysis of RegA binding to the *adhI* promoter has not been undertaken, so it is not yet certain whether RegA directly or indirectly affects expression of formaldehyde dehydrogenase.

In *S. meliloti* the RegB/RegA homologues, ActS/ActR, control biosynthesis of three dehydrogenases: formaldehyde dehydrogenase, formate dehydrogenase and methanol dehydrogenase as well as CO_2 fixation.[34] The ActS/ActR system is also involved in acid tolerance.[11]

The Sensor Kinase RegB

The *R. capsulatus regB* gene encodes a 460 amino acid (50.1 kDa) histidine protein kinase that is composed of two domains; a N-terminal trans-membrane domain containing six hydrophobic membrane-spanning regions[11,43-44] and a C-terminal cytoplasmic "transmitter" domain. A recent study identified the ubiquinone pool as the redox signal for RegB with a highly conserved quinone binding site found to be located in the trans-membrane domain thereby indicating that this region plays a role in redox-sensing (Fig. 2).[45] 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.[1,6,44] The transmitter domain also contains a conserved redox-active cysteine capable of regulating the activity of RegB through forming an intermolecular disulfide bond in response to the redox state that is located in a conserved "redox box" just downstream of the H-box (Fig. 2).[46]

Kinase Activity

Initial kinase assays demonstrated that a His-tagged cytosolic domain of *R. capsulatus* RegB was capable of autophosphorylation in vitro as well as phosphotransfer to its cognate response regulator, RegA. The rate of autophosphorylation was initially reported to be low with half maximal phosphorylation observed after 45 minutes of incubation with [γ−^{32}P]ATP.[47,48] As the kinetics were not affected by the ATP concentration, it was suggested that the rate-limiting step of RegB autophosphorylation was phosphotransfer from bound ATP to the histidine residue or the dimerization of the protein, rather than binding of ATP.[47] Recent results from Swem et al[46] demonstrate that a nonHis-tagged (truncated) version of RegB exhibits a significantly faster autophosphorylation rate, reaching half maximal phosphorylation within 5 minutes. Similar results were seen with truncated cytosolic forms of RegB homologues from *R. sphaeroides*,[19] *B. japonicum*,[49] as well as with the full-length version from *R. sphaeroides*[50] and *R. capsulatus*.[45] Phosphorylated full-length RegB exhibits decreased stability of the phosphate compared to the truncated version of RegB (half-life of about 34 minutes versus 5.5 to 6 hours), pointing to a role of the transmembrane domain in

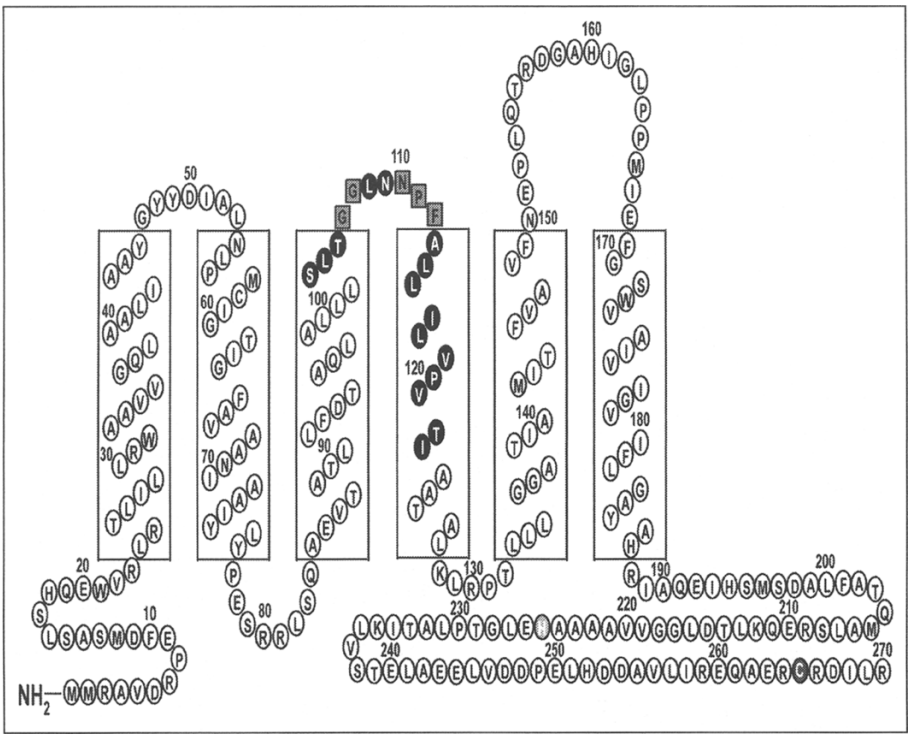

Figure 2. Membrane spanning, histidine autophosphorylation and cysteine regulatory portions of RegB. The photolytic portion of RegB that photo-affinity crosslinks to a quinone analog is indicated in inverse white lettering with universally conserved residues in this fragment shown with a red background. The histindine site of autophosphorylation is indicated in green and the redox active cystein is indicated in blue. A color version of this figure is available online at www.eurekah.com.

regulating the phosphorylation state of RegB.[48,50] As mutations in the N-terminal transmembrane domain led to RegB proteins with constitutive kinase activity in vivo, the "unsignaled" state of the protein has been proposed to be "autophosphorylation dominant".[9,51]

The first demonstration of phosphotransfer from *R. capsulatus* RegB~P (phosphorylated RegB) to RegA was reported by Inoue et al.[48] Phosphotransfer studies showed that the transfer of phosphate is rapid (<1 min) from the cytosolic domain of RegB to RegA in vitro.[47] Similar phosphotransfer kinetics has been reported using truncated cytosolic and full-length versions of *R. sphaeroides* RegB homologues and *B. japonicum* homologue ($RegS_C$).[49] No back-transfer of phosphate from RegA~P to RegB has been observed.[19,50] Since phosphotransfer is very rapid, autophosphorylation of RegB appears to be the rate-limiting step in the phosphorylation of RegA.

Phosphatase Activity

Like many histidine kinases, RegB can modulate the level of phosphorylated RegA, not only through phosphorylation, but also by exerting phosphatase activity on RegA~P. Dephosphorylation of RegA~P in vitro has been shown to be dependent on the amount of unphosphorylated RegB, which is a good indication that RegB possesses phosphatase activity toward RegA.[47] Similar results were observed for the *B. japonicum* RegB homologue (RegS)[49] and for the full-length version of RegB from *R. sphaeroides*.[50] Phosphatase activity has also been characterized for a truncated soluble form of *R. sphaeroides* RegB by measuring the stability of the phosphate on RegA~P in the presence and

absence of RegB. Collectively, these results showed that the presence of RegB resulted in > 16-fold reduction in the stability of the phosphate on RegA~P.[19] 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 domain of RegB.[50] However, further studies will be required to determine if phosphatase activity is redox-regulated.

In the *E. coli* sensor kinase EnvZ, it is apparent that a threonine residue positioned 4 residues downstream of the conserved phosphorylated histidine is important for phosphatase activity.[52] This threonine residue is positioned on the same α-helical face directly below the phosphorylated histidine. It is believed that the histidine residue may deprotonate the threonine hydroxyl group to form a good nucleophile that attacks the phosphoryl group bound to the aspartate residue of the cognate response regulator. Interestingly, RegB homologues also contain a 100% conserved threonine residue located four residues downstream of the phosphorylated histidine, suggesting that RegB may exhibit a similar phosphatase mechanism with RegA. Nonetheless, Potter et al[50] point out that a significant difference between RegB and EnvZ phosphatase activity exists given that EnvZ has a requirement for ATP, or a nonhydrolyzable analogue as a cofactor for phosphatase activity while RegB lacks this nucleotide requirement.

Redox Signaling via Quinone

In vivo studies indicated that RegB/RegA-regulated photosynthesis genes was inhibited by growth under aerobic conditions. It was therefore initially presumed that RegB kinase activity was directly inhibited by oxygen. However, this possibility was subsequently excluded because *R. capsulatus* is fully capable of de-repressing pigment biosynthesis under chemiautotrophic growth conditions involving growth in the presence of oxygen, hydrogen and carbon dioxide.[53]

Another signal proposed to regulate RegB was the redox state of the respiratory electron transport chain.[8,36,54-57] This conclusion was based on the observation that mutations of *R. sphaeroides* and *R. capsulatus* cytochrome cbb_3 oxidase lead to elevated aerobic expression of RegB/RegA-regulated genes.[8,58] It was therefore suggested that cytochrome cbb_3 oxidase generates an "inhibitory" signal that represses the RegB/RegA two-component system. Recently Swem et al 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 autophosphorylaton.[45] In the Swem study, full-length RegB kinase activity was inhibited approximately 6-fold in vitro by the presence of oxidized coenzyme Q1 (Q1 is a derivative of ubiquinone) whereas kinase activity was not affected by the presence of reduced Q1. In purple photosynthetic bacteria, there is a large ubiquinone pool that functions as electron carrier of the electron transport chain in the membrane.[59] The oxidization/reduction 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.[60] Ubiquinone is a facile signal given that ubiquinones and RegB are both membrane-associated and the redox state of quinones reflects changes in environmental oxygen tension and changes in the redox state of these cells in general. Ubiquinone as redox signal for controlling RegB activity also correlates well with the observation that mutations in cytochrome cbb_3 oxidase lead to elevated RegB activity. In this case, a mutation in a terminal respiratory electron donor such as a cbb_3 oxidase would result in a more reduced ubiquinone pool, which would lead to elevation of RegB kinase activity.

With the use of ^{14}C-azidoquinone photo affinity cross-linking, a ubiquinone-binding site was identified in a periplasmic loop between transmembrane helices three and four (Fig. 2).[45] In this region there is a heptapeptide sequence of GGXXNPF that is 100% conserved among all known RegB homologs.[45] It has been proposed that oxidized ubiquinone may bind to this heptapeptide through the 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).[45] The binding of oxidized ubiquinone may result in allosteric modification of RegB that leads to the inhibition of autophosphorylation. When the ubiquinone pool is shifted to protonated form under anaerobic conditions, the hydrogen bond between asparagine and ubiquinone could be disrupted, which could trigger structural changes facilitating the autophosphorylation of RegB. Subsequent in vivo mutational study on Phe112

resulted in elevated aerobic synthesis of photosystem, confirming that this heptapeptide is involved in the sensing of the ubiquinone pool redox state and regulating of the RegB activity.[45]

Additional Redox Signals

In addition to the ubiquinone-binding site, there is a fully conserved cysteine that is also involved in redox-sensing.[46] This redox-active cysteine (Cys 265) is located in a "redox-box" that is harbored in the cytosolic dimerization interface located downstream of the H-box (Fig. 2). In vitro analysis using truncated RegB without the transmembrane domain indicates that an intermolecular disulfide bond forms between RegB dimmers under oxidizing conditions, converting active dimmers into inactive tetramers.[46] In vitro disulfide bond formation was shown to require the presence of a divalent metal ion which may help to fold RegB to the functional structure.[46] The involvement of an intermolecular disulphide bridge in the control of RegB activity is also supported by an increase in vitro full-length RegB phosphorylation in the presence of DTT.[50] Furthermore, Western blot has confirmed that the intermolecular disulfide bond formation under aerobic growth conditions regulates the RegB activity in vivo. However, the Western blot also showed only <20% Cys 265 forms disulfide bond in vivo. The reminder of Cys 265 has been proposed to form other derivatives, such as sulfenic acid (Cys-S-OH), to regulate the RegB activity.[46]

Various mutations have been constructed to probe the roles of the ubiquinone and Cys265 redox signals in regulating RegB activity. A Cys265 to alanine 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.[45,46] Similar results were observed with mutations in the ubiquinone binding domain which shows elevated expression which still harbors a level of redox control.[45] These results suggests that ubiquinone pool is a redox signal independent of the redox state of Cys265.[45] Given that ubiquinone binding site is located in transmembrane domain and Cys265 is located in cytosolic domain, they are not likely to directly interact. So, it seems ubiquinone pool and Cys265 function independently and that both contribute to the redox control of the RegB activity.

Genetic studies also implicate a role of SenC (also called PrrC) in transduction of a "redox signal" in *R. sphaeroides* and in *R. capsulatus*. Specifically, inactivation of *senC* (*prrC*), which is cotranscribed with *regA*, results in an oxygen-insensitive phenotype in *R. sphaeroides*.[8,36,54-57] Interestingly, SenC also has sequence similarity to a family of oxidoreductases that are involved in disulfide bond oxidation and reduction.[61] One possibility is that, SenC could be directly involved in modulation of the oxidation and reduction state of a redox-active cysteine residue within RegB.[46]

The Response Regulator RegA

R. capsulatus RegA is a protein (184 amino acid residues, 20.4 kDa) containing conserved residues that are typically found in two-component response regulators including a phosphate accepting aspartate and an "acid pocket" containing two highly conserved aspartate residues in the N-terminal receiver domain. The receiver domain is linked by a four proline hinge to a 50 amino acid C-terminal output domain that contains a three helix bundle helix-turn-helix (H-T-H) DNA-binding motif.[2,62-63] RegA homologues have been found to be *highly* conserved among numerous α-proteobacterial species with an unprecedented complete conservation of the DNA-binding domain.

Effect of Phosphorylation

DNA-binding activity of RegA was initially demonstrated using a constitutively active variant of RegA called RegA*.[62] Subsequent DNase I footprint analysis to the *puc* promoter region revealed that phosphorylated and unphosphorylated wild-type RegA, protect identical regions with varying affinities. This observation indicates that phosphorylation does not affect the points of protein-DNA interaction but rather the affinity for the binding site.[47] Indeed, phosphorylation is reported to increase the DNA-binding affinity of RegA by at least 16-fold.[47] A further 6-fold increase in the DNA binding activity is achieved upon phosphorylation of RegA*.[47] These results are consistent with what was reported for the *B. japonicum* RegA homologue (RegR), whose DNA-binding activity is increased by at least 8-fold upon phosphorylation.[49] Phosphorylation-induced stimulation of DNA-binding activity was also reported for *P. aeruginosa* RoxR.[12]

Mutational analysis has been undertaken at the site of phosphorylation (Asp63) in *R. capsulatus* RegA, as well as with RegA homologues from *B. japonicum* and *R. sphaeroides*. A D63N mutation in RegR of *B. japonicum* rendered the protein unable to be phosphorylated and also unable to bind DNA as demonstrated by gel retardation experiments with a *fixR-nifA* promoter probe.[49] In contrast, experiments involving a D63K RegA mutant from *R. capsulatus* showed that the mutant protein was capable of binding DNA despite an inability to be phosphorylated. This indicated that phosphorylation might not affect DNA-binding ability, but rather, facilitates a conformational change allowing appropriate interaction of RegA with RNA polymerase.[64] This theory was further supported by in vitro transcription assays by Comolli et al[19] involving wild-type and a D63A mutant of PrrA from *R. sphaeroides*. Their studies revealed that both unphosphorylated and phosphorylated wild type PrrA are able to activate in vitro transcription of the *cycA* P2 promoter with phosphorylated PrrA exhibiting greater activity than unphosphorylated PrrA. Interestingly, the D63A form of PrrA was unable to activate any detectable amounts of transcription. These data suggest that Asp63 is essential for function and that its presence in phosphorylated and unphosphorylated states may affect several steps of activation such as the DNA-binding of RegA and interaction with RNA polymerase.

Superimposed on the effect of phosphorylation are numerous in vivo observations that unphosphorylated RegA is also capable of affecting transcription. Specifically, mutational analysis indicates that phosphorylated RegA functions as an anaerobic repressor of cytochrome cbb_3 oxidase expression, while unphosphorylated RegA functions as an aerobic activator.[21,65] In addition, both phosphorylated and unphosphorylated RegA are involved in activation and repression of ubiquinol oxidase expression,[65] *cbb* (carbon fixation) operon expression,[32] and regulation of *hupSLC* expression.[37] Additional DNA-binding studies with both phosphorylated and unphosphorylated RegA are clearly needed to obtain an understanding of the mechanism of activation or repression by unphosphorylated RegA.

DNA-Binding Sites

DNase I footprint analysis initially demonstrated that purified wild-type RegA and RegA* bind to identical specific sites in the *puf* and *puc* promoters.[62,67] Subsequent DNase I footprint analysis demonstrated that numerous other operons are under direct regulation of the RegB/RegA system, including *nifA2*,[37] *hupSLC*,[37] *regB*,[68] *senC-regA-hvrA*,[68] *petABC*,[21] *cycA*,[21] *cycY*,[21] *cydAB*,[21] *ccoNOPQ*,[21] *cbb*[32]*I and cbbII*,[32] and to *cheOp2*.[32] DNA-binding activity has also been reported for the phosphorylated forms of RegR in *B. japonicum*[49] and RoxR in *P. aeruginosa*[12] using gel mobility retardation experiments.

The number of RegA DNA-binding sites to target promoters ranges between 1 and 6, based on DNase I protection assays. Individual sites in the promoter regions have varying affinities as determined by the amount of RegA needed to obtain half maximal protection of individual sites.[21] These different binding locations may allow RegA to interact with RNA polymerase in more than one manner at these promoters.

The alignment of 21 RegA binding sites from *R. capsulatus* and *R. sphaeroides* revealed that RegA indeed binds to 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'.[21] For RegR from *B. japonicum*, a related consensus "RegR box" 5'-GNG(A/G) C(A/G)TTNNGNCGC-3' on the *fixRnifA* promoter, was also identified.[69] More insights into the DNA recognition and binding ability of RegA was provided by an recently solved NMR structure of the DNA-binding output domain of RegA bound to DNA.[63] This structure demonstrated that the RegA DNA binding structure is comprised of a three-helix bundle encompassing a helix-turn-helix motif.[63] The NMR structure also confirmed that the consensus RegA recognition sequence consists of YGCGRCRx(T/A)(T/A)xGNCGC(x = a variable number of bases).[63] The three helices within the three-helix bundle can be labeled α6, α7 and α8, where α7 and α8 compose the predicted helix-turn-helix motif. α8 is the recognition helix, which binds specifically to the GCG inverted repeat sequence within the major DNA groove, while α6 seems to make nonspecific interaction with the phosphate backbone of the DNA.[63] A subsequent mutational analysis of the

helix-turn-helix motif identified several residues critical for the function of PrrA, which support the proposed DNA-recognition site from the NMR structure.[70]

Concluding Remarks

Genetic and biochemical analyses have revealed that the RegB/RegA system is a major global regulator of numerous energy-generating and energy-utilizing cellular processes. The systems controlled by RegB/RegA in *R. capsulatus* and in *R. sphaeroides* include such fundamental and diverse processes as photosynthesis, tetrapyrrole synthesis, CO_2 fixation, N_2 assimilation, hydrogen utilization, denitrification, dehydrogenases, electron transport and aerotaxis (Fig. 1). The Reg regulon is continuously growing and it is likely that there are many more, yet to be discovered, target genes under the control of RegB/RegA in these metabolically diverse bacteria.

Inspection of known members of the Reg regulon, as shown in Figure 1, exhibits an interesting interrelationship between the various regulated components. Specifically, photosynthesis, respiration (oxygen and DMSO mediated) and hydrogen oxidation all directly affect the oxidation/reduction state of the ubiquinone pool. Processes such as carbon fixation, nitrogen assimilation and formaldehyde dehydration all can function as electron sinks. In addition, formation of hydrogen by nitrogenase can be used as a substrate by the uptake hydrogenase system. Likewise, carbon generated by dehydration of formaldehyde can be used by the Calvin cycle during carbon fixation. Indeed evidence suggests that RegA can function as a "master controller" that is responsible for coordinating these various redox-responding systems.[71] For example, the carbon fixing Calvin cycle becomes de-repressed under photoheterotrophic conditions involving light plus organic compounds. Under this growth condition, carbon fixation is thought to function as an electron sink that bleeds off excess reducing power. If carbon fixation is incapacitated, such as when RubisCO is mutated, then nitrogenase becomes de-repressed, even in the presence of excess ammonium so that nitrogenase can take over their role of functioning as an electron sink in the absence of carbon fixation. Importantly, de-repression of carbon fixation and nitrogen fixation under conditions of excess carbon and ammonium are RegB/RegA-dependent events.[36] This clearly underscores the importance of RegB/RegA in controlling the overall cellular redox poise.

The observation that highly conserved RegB and RegA homologues exist in many other bacterial species also indicates that the RegB/RegA system constitutes an important redox control element that is not easily replaced by other regulators. Although many questions regarding the

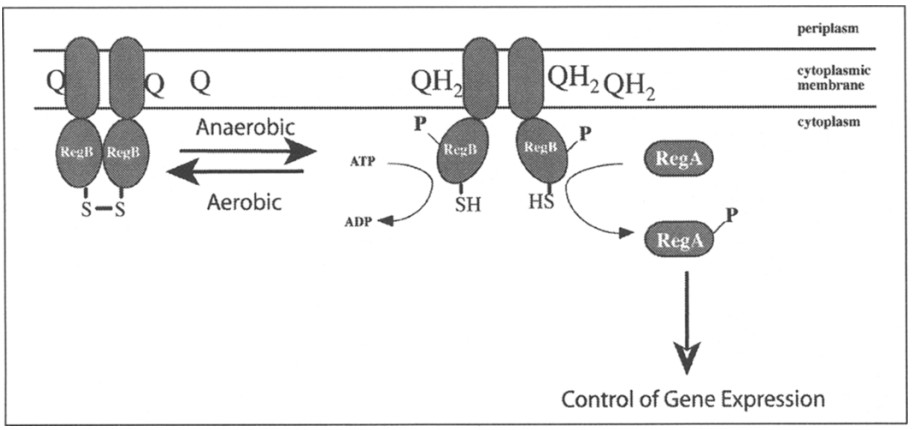

Figure 3. RegB kinase activity is controlled by at least two redox inputs. One is the redox state of the quinone pool (Q and QH_2) that interacts with the membrane spanning portion of RegB. A second input is the redox state of a conserved Cys (designated as SH or S-S) located downstream of the site of autophosphorylation (P).

function of RegB and RegA in other systems still need to be addressed, evidence is mounting that they control a similar set of target genes in a number of bacterial species.

Finally, there are several questions regarding the molecular mechanism of redox sensing by RegB. As depicted in Figure 3, there are at least two established redox sensing inputs in this membrane spanning kinase. One input is the redox state of the quinone pool that interacts with membrane spanning portion of RegB. The second input is the redox state of a conserved Cyst that is located downstream of the site of phosphorylation. In both cases, the inhibition of kinase activity by these different input signals remains to be established. There also remain many outstanding questions about the mechanism of transcription activation and repression by phosphorylated and dephosphorylated RegA as well as the nature of interactions that RegA may have with other transcription factors at target promoters.

References

1. Mosley CS, Suzuki JY, Bauer CE. 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 1994; 176:7566-7573.
2. Sganga MW, Bauer CE. Regulatory factors controlling photosynthetic reaction center and light-harvesting gene expression in Rhodobacter capsulatus. Cell 1992; 68:945-954.
3. Cohen-Bazire G, Sistrom WR, Stanier RY. Kinetic studies of pigment synthesis by nonsulfur purple bacteria. J Cell Physiol 1957; 49:25-68.
4. Parkinson JS, Kofoid EC. Communication modules in bacterial signaling proteins. Annu Rev Genet 1992; 26:71-112.
5. Stock JB, Ninfa AJ, Stock AM. Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol Rev 1989; 53:450-490.
6. Stock AM, Robinson VL, Goudreau PN. Two-component signal transduction. Annu Rev Biochem 2000; 69:183-215.
7. Phillips-Jones MK, Hunter CN. Cloning and nucleotide sequence of regA, a putative response regulator gene of Rhodobacter sphaeroides. FEMS Microbiol Lett 1994; 116:269-275.
8. Eraso JM, Kaplan S. prrA, a putative response regulator involved in oxygen regulation of photosynthesis gene expression in Rhodobacter sphaeroides. J Bacteriol 1994; 176:32-43.
9. Eraso JM, Kaplan S. Oxygen-insensitive synthesis of the photosynthetic membranes of Rhodobacter sphaeroides: a mutant histidine kinase. J Bacteriol 1995; 177:2695-2706.
10. Bauer E, Kaspar T, Fischer HM et al. Expression of the fixR-nifA operon in Bradyrhizobium japonicum depends on a new response regulator, RegR. J Bacteriol 1998; 180:3853-3863.
11. Tiwari RP, Reeve WG, Dilworth MJ et al. Acid tolerance in Rhizobium meliloti strain WSM419 involves a two-component sensor-regulator system. Microbiology 1996; 142:1693-1704.
12. Comolli JC, Donohue TJ. Pseudomonas aeruginosa RoxR, a response regulator related to Rhodobacter sphaeroides PrrA, activates expression of the cyanide-insensitive terminal oxidase. Mol Microbiol 2002; 45:755-768.
13. Masuda S, Matsumoto Y, Nagashima KV et al. Structural and functional analyses of photosynthetic regulatory genes regA and regB from Rhodovulum sulfidophilum, Roseobacter denitrificans and Rhodobacter capsulatus. J Bacteriol 1999; 181:4205-4215.
14. Emmerich R, Hennecke H, Fischer HM. Evidence for a functional similarity between the two-component regulatory systems RegSR, ActSR and RegBA (PrrBA) in alpha-Proteobacteria. Arch Microbiol 2000; 174:307-313.
15. Oh JI, Eraso JM, Kaplan S. Interacting regulatory circuits involved in orderly control of photosynthesis gene expression in Rhodobacter sphaeroides 2.4.1. J Bacteriol 2000; 182:3081-3087.
16. Oh JI, Kaplan S. Generalized approach to the regulation and integration of gene expression. Mol Microbiol 2001; 39:1116-1123.
17. Smart JL, Willett JW, Bauer CE. Regulation of hem gene expression in Rhodobacter capsulatus by redox and photosystem regulators RegA, CrtJ, FnrL and AerR. J Mol Biol 2004; 342:1171-1186.
18. Ranson-Olson B, Jones DF, Donohue TJ et al. In vitro and in vivo analysis of the role of PrrA in Rhodobacter sphaeroides 2.4.1 hemA gene expression. J Bacteriol 2006; 188:3208-3218.
19. Comolli JC, Carl AJ, Hall C et al. Transcriptional activation of the Rhodobacter sphaeroides cytochrome c(2) gene P2 promoter by the response regulator PrrA. J Bacteriol 2002; 184:390-399.
20. Karls RK, Wolf JR, Donohue TJ. Activation of the cycA P2 promoter for the Rhodobacter sphaeroides cytochrome c2 gene by the photosynthesis response regulator. Mol Microbiol 1999; 34:822-835.
21. Swem LR, Elsen S, Bird TH et al. The RegB/RegA two-component regulatory system controls synthesis of photosynthesis and respiratory electron transfer components in Rhodobacter capsulatus. Mol Microbiol 2001; 309:121-138.

22. Gray KAaD, F. Mutational studies of the cytochrome bc_1 complexes. In: Blankenship RE, Madigan MT, Bauer CE, eds. Anoxygenic Photosynthetic Bacteria. Dordrecht: Kluwer Academic Publishers, 1995:747-774.
23. Zannoni D. Aerobic and anaerobic transport chains in anoxygenic phototrophic bacteria. In: Blankenship RE, Madigan MT, Bauer CE, eds. Anoxygenic Photosynthetic Bacteria. Dordrecht: Kluwer Academic Publishers, 1995:949-971.
24. Mouncey NJ, Kaplan S. Redox-dependent gene regulation in Rhodobacter sphaeroides 2.4.1(T): effects on dimethyl sulfoxide reductase (dor) gene expression. J Bacteriol 1998; 180:5612-5618.
25. Mouncey NJ, Choudhary M, Kaplan S. Characterization of genes encoding dimethyl sulfoxide reductase of Rhodobacter sphaeroides 2.4.1T: an essential metabolic gene function encoded on chromosome II. J Bacteriol 1997; 179:7617-7624.
26. Shaw AL, Leimkuhler S, Klipp W et al. Mutational analysis of the dimethylsulfoxide respiratory (dor) operon of Rhodobacter capsulatus. Microbiology 1999; 145:1409-1420.
27. Kappler U, Huston WM, McEwan AG. Control of dimethylsulfoxide reductase expression in Rhodobacter capsulatus: the role of carbon metabolites and the response regulators DorR and RegA. Microbiology 2002; 148:605-614.
28. Dubbs JM, Bird TH, Bauer CE et al. Interaction of CbbR and RegA* transcription regulators with the Rhodobacter sphaeroides cbbI promoter-operator region. J Biol Chem 2000; 275:19224-19230.
29. Dubbs JM, Tabita FR. Two functionally distinct regions upstream of the cbbI operon of Rhodobacter sphaeroides regulate gene expression. J Bacteriol 1998; 180:4903-4911.
30. Dubbs JM, Tabita FR. Interactions of the cbbII promoter-operator region with CbbR and RegA (PrrA) regulators indicate distinct mechanisms to control expression of the two cbb operons of Rhodobacter sphaeroides. J Biol Chem 2003; 278:16443-16450.
31. Gibson JL, Dubbs JM, Tabita FR. Differential expression of the CO_2 fixation operons of Rhodobacter sphaeroides by the Prr/Reg two-component system during chemoautotrophic growth. J Bacteriol 2002; 184:6654-6664.
32. Vichivanives P, Bird TH, Bauer CE et al. Multiple regulators and their interactions in vivo and in vitro with the cbb regulons of Rhodobacter capsulatus. J Mol Biol 2000; 300:1079-1099.
33. Emmerich R, Strehler P, Bauer E et al. Functional analysis of the Bradyrhizobium japonicum RegSR two-component regulatory system. In: Pedrosa FO, Hungria M, Yates G, Newton WE, eds. Nitrogen Fixation: From Molecules to Crop Productivity. London: Kluwer, 2000:89-90.
34. Fenner BJ, Tiwari RP, Reeve WG et al. ActR is a global genetic regulator in Sinorhizobium meliloti. In: Pedrosa FO, Hungria M, Yates G, Newton WE, eds. Nitrogen Fixation: From Molecules to Crop Productivity. London: Kluwer, 2000:488.
35. Masepohl BKW. Organization and regulation of genes encoding the molybdenum nitrogenase and the alternative nitrogenase in Rhodobacter capsulatus. Arch. Microbiol 1996; 165:80-90.
36. Joshi HM, Tabita FR. A global two component signal transduction system that integrates the control of photosynthesis, carbon dioxide assimilation and nitrogen fixation. Proc Natl Acad Sci USA. 1996; 93:14515-14520.
37. Elsen S, Dischert W, Colbeau A et al. Expression of uptake hydrogenase and molybdenum nitrogenase in Rhodobacter capsulatus is coregulated by the RegB-RegA two-component regulatory system. J Bacteriol 2000; 182:2831-2837.
38. Laratta WP, Choi PS, Tosques IE et al. Involvement of the PrrB/PrrA two-component system in nitrite respiration in Rhodobacter sphaeroides 2.4.3: evidence for transcriptional regulation. J Bacteriol 2002; 184:3521-3529.
39. Vignais PM, Toussaint B, Colbeau A. Regulation of hydrogenase gene expression. In: Blankenship RE, Madigan MT, Bauer CE, eds. Anoxygenic Photosynthetic Bacteria. Dordrecht: Kluwer Academic Publishers, 1995:1175-1190.
40. Dischert W, Vignais PM, Colbeau A. The synthesis of Rhodobacter capsulatus HupSL hydrogenase is regulated by the two-component HupT/HupR system. Mol Microbiol 1999; 34:995-1006.
41. Toussaint B, de Sury d'Aspremont R, Delic-Attree I et al. The Rhodobacter capsulatus hupSLC promoter: identification of cis-regulatory elements and of trans-activating factors involved in H2 activation of hupSLC transcription. Mol Microbiol 1997; 26:927-937.
42. Barber RD, Donohue TJ. Pathways for transcriptional activation of a glutathione-dependent formaldehyde dehydrogenase gene. J Mol Biol 1998; 280:775-784.
43. Chen W, Jager A, Klug G. 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 2000; 182:818-820.
44. Ouchane S, Kaplan S. Topological analysis of the membrane-localized redox-responsive sensor kinase PrrB from Rhodobacter sphaeroides 2.4.1. J Biol Chem 1999; 274:17290-17296.
45. Swem LR, Gong X, Yu CA et al. Identification of a ubiquinone-binding site that affects autophosphorylation of the sensor kinase RegB. J Biol Chem 2006; 281:6768-6775.

46. Swem LR, Kraft BJ, Swem DL et al. Signal transduction by the global regulator RegB is mediated by a redox-active cysteine. EMBO J 2003; 22:4699-4708.
47. Bird TH, Du S, Bauer CE. Autophosphorylation, phosphotransfer and DNA-binding properties of the RegB/RegA two-component regulatory system in Rhodobacter capsulatus. J Biol Chem 1999; 274:16343-16348.
48. Inoue K, Kouadio JL, Mosley CS et al. Isolation and in vitro phosphorylation of sensory transduction components controlling anaerobic induction of light harvesting and reaction center gene expression in Rhodobacter capsulatus. Biochemistry 1995; 34:391-396.
49. Emmerich R, Panglungtshang K, Strehler P et al. Phosphorylation, dephosphorylation and DNA-binding of the Bradyrhizobium japonicum RegSR two-component regulatory proteins. Eur J Biochem 1999; 263:455-463.
50. Potter CA, Ward A, Laguri C et al. Expression, purification and characterisation of full-length histidine protein kinase RegB from Rhodobacter sphaeroides. J Mol Biol 2002; 320:201-213.
51. Oh JI, Ko IJ, Kaplan S. The default state of the membrane-localized histidine kinase PrrB of Rhodobacter sphaeroides 2.4.1 is in the kinase-positive mode. J Bacteriol 2001; 183:6807-6814.
52. Inouye M, Dutta R, Zhu Y. Regulation of porins in Escherichia coli by the osmosensing histidine kinase/Phosphatase EnvZ. In: M. Inouye, ed. Histidine Kinases in Signal Tranduction. Burlington: Academic Press, 2003:25-46.
53. Madigan MT, Gest H. Growth of the photosynthetic bacterium Rhodopseudomonas capsulata chemoautotrophically in darkness with H_2 as the energy source. J Bacteriol 1979; 137:524-530.
54. Eraso JM, Kaplan S. From redox flow to gene regulation: role of the PrrC protein of Rhodobacter sphaeroides 2.4.1. Biochemistry 2000; 39:2052-2062.
55. O'Gara JP, Kaplan S. Evidence for the role of redox carriers in photosynthesis gene expression and carotenoid biosynthesis in Rhodobacter sphaeroides 2.4.1. J Bacteriol 1997; 179:1951-1961.
56. Oh JI, Kaplan S. Redox signaling: globalization of gene expression. EMBO J 2000; 19:4237-4247.
57. Roh JH, Kaplan S. Genetic and phenotypic analyses of the rdx locus of Rhodobacter sphaeroides 2.4.1. J Bacteriol 2000; 182:3475-3481.
58. Buggy J, Bauer CE. Cloning and characterization of senC, a gene involved in both aerobic respiration and photosynthesis gene expression in Rhodobacter capsulatus. J Bacteriol 1995; 177:6958-6965.
59. Bolton JR. In: Clayton RK, Sistrom WR, eds. The Photosynthetic Bacteria. New York: Plenum Press, 1978:419-429.
60. Parson W. In: Clayton RK, Sistrom WR, eds. The Photosynthetic Bacteria. New York: Plenum Press, 1978:455-469.
61. McEwan AG, Lewin A, Davy SL et al. PrrC from Rhodobacter sphaeroides, a homologue of eukaryotic Sco proteins, is a copper-binding protein and may have a thiol-disulfide oxidoreductase activity. FEBS Lett 2002; 518:10-16.
62. Du S, Bird TH, Bauer CE. DNA binding characteristics of RegA. A constitutively active anaerobic activator of photosynthesis gene expression in Rhodobacter capsulatus. J Biol Chem 1998; 273:18509-18513.
63. Laguri C, Phillips-Jones MK, Williamson MP. Solution structure and DNA binding of the effector domain from the global regulator PrrA (RegA) from Rhodobacter sphaeroides: insights into DNA binding specificity. Nucleic Acids Res 2003; 31:6778-6787.
64. Hemschemeier SK, Ebel U, Jager A et al. In vivo and in vitro analysis of RegA response regulator mutants of Rhodobacter capsulatus. J Mol Microbiol Biotechnol 2000; 2:291-300.
65. Swem DL, Bauer CE. Coordination of ubiquinol oxidase and cytochrome cbb(3) oxidase expression by multiple regulators in Rhodobacter capsulatus. J Bacteriol 2002; 184:2815-2820.
66. Qian Y, Tabita FR. A global signal transduction system regulates aerobic and anaerobic CO_2 fixation in Rhodobacter sphaeroides. J Bacteriol 1996; 178:12-18.
67. Hemschemeier SK, Kirndorfer M, Hebermehl M et al. DNA binding of wild type RegA protein and its differential effect on the expression of pigment binding proteins in Rhodobacter capsulatus. J Mol Microbiol Biotechnol 2000; 2:235-243.
68. Du S, Kouadio JL, Bauer CE. 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 1999; 181:4334-4341.
69. Emmerich R, Strehler P, Hennecke H et al. An imperfect inverted repeat is critical for DNA binding of the response regulator RegR of Bradyrhizobium japonicum. Nucleic Acids Res 2000; 28:4166-4171.
70. Jones DF, Stenzel RA, Donohue TJ. Mutational analysis of the C-terminal domain of the Rhodobacter sphaeroides response regulator PrrA. Microbiology 2005; 151:4103-4110.
71. Tichi MA, Tabita FR. Interactive control of Rhodobacter capsulatus redox-balancing systems during phototrophic metabolism. J Bacteriol 2001; 183:6344-6354.